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PHYLOGEOGRAPHIC PATTERNS OF DIFFERENTIATION IN THE ACORN WOODPECKER

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ABSTRACT.—Acorn Woodpecker (*Melanerpes formicivorus*) populations were sampled to evaluate geographic patterns of differentiation and connectivity across the species' range. We observed patterns of differentiation generally coincident with geographic patterns in plumage patterns with distinct subpopulations in Baja California Sur, northern Central America, southern Central America, and mainland Mexico north into the southwestern United States. We confirmed the existence of geographic genetic structuring of populations of this species, although shared haplotypes between Baja California Sur and mainland Mexico suggest that lineage sorting is not yet complete. The process of geographic differentiation and speciation is likely still underway in this group. *Received 31 January 2007. Accepted 26 October 2007.*

The montane forests of North and Central America have had a complex history and geography over the past 100,000 years (Graham 1975, Wells 1983). During the Pleistocene, montane areas, particularly in the northern part of the region, appear to have been largely covered by ice or tundra and, thus, uninhabitable for forest birds, whereas desert basins filled with what are presently 'montane' coniferous forests (Wells 1983). Pleistocene and Holocene climatic shifts must have had important implications for avian biogeography in terms of population connectivity and isolation, and likely affected the species inhabiting these biomes profoundly.

Studies have now addressed the climatic and biogeographic implications of Pleistocene-Holocene climate shifts (Hugall et al. 2002, Martínez-Meyer et al. 2004, Martínez-Meyer and Peterson 2006, Ruegg et al. 2006), but surprisingly few detailed phylogeographic studies of birds have been conducted to illustrate how climatic changes and habitat shifts influenced the evolution and differentiation of birds. Only *Aphelocoma* jays (Peterson 1992, Rice et al. 2003), *Sphyrapicus* sapsuckers (Cicero and Johnson 1995), MacGillivray's Warbler (*Oporornis tolmiei*) (Milá et al. 2000), and Hutton's Vireos (*Vireo huttoni*) (Cicero and Johnson 1992) have been studied in North American pine-oak (*Pinus-Quercus*) woodlands and forests. Patterns of genetic differentiation and the extent to which they do—or do not—relate to Pleistocene patterns of connection and disjunction of habitats are only beginning to be understood.

The objective of this paper is to present the results of molecular genetic studies of 98 individuals from 15 populations of Acorn Woodpeckers (*Melanerpes formicivorus*) across North and Central America. An earlier contribution based on many of the same samples as in this paper (Benítez-Díaz 1993) identified a series of morphologically distinct populations with major units including populations in California, Baja California Sur, mainland Mexico, Central America, and Colombia. Samples are lacking to represent the distinctive populations of northwestern South America, but sampling of the remainder of the distribution of the species is more or less intensive. This study, based on sequences of two mitochondrial genes, offers a first view of geographic patterns of genetic differentiation among populations of the Acorn Woodpecker.

METHODS

Samples and Sequencing.—Samples of muscle, heart, and liver collected from 98 individual Acorn Woodpeckers across most of the species' range (the distinct Colombian

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populations, and those of lowland areas in Belize and the remainder of the Petén region were not included for lack of access to samples; Fig. 1). We included sequences from 10 individuals of seven related species, including *Melanerpes lewis*, *M. aurifrons* (3 individuals), *M. uropygialis*, *M. pygmaeus* (2 individuals), *M. pucherani*, *Sphyrapicus nuchalis*, and (more distantly) *Coracias spatulatus* (Appendix). These samples were obtained from field collections by several of the authors; full specimen voucher specimens are deposited in the Museo de Zoología ''Alfonso L. Herrera'' of the Universidad Nacional Autónoma de México (UNAM), Field Museum of Natural History, and the University of Kansas Natural History Museum, supplemented by tissue samples associated with specimens kindly provided by the Barrick Museum of Natural History (University of Nevada–Las Vegas) and the Museum of Vertebrate Zoology (University of California–Berkeley). Data were obtained from GenBank for two outgroup individuals.

Total tissue DNA was extracted via DNEasy Extraction Kits (Qiagen, Valencia, CA, USA). Specific fragments were amplified via polymerase chain reaction (PCR) using primers spanning 334 bp of the mitochondrial gene ND2 segment (L5215 TAT CGG GCC CAT ACC CCG AAA AT; H5578 CCT TGA AGC ACT TCT GGG AAT CAG A) (Hackett 1996) and a 608 bp fragment of the cytochrome *b* gene (L15413 CTG ACA AAA TTC CAT TTC ACC C; H16064 CTT CAG TTT TTG GTT TAC AAG ACC) (Kocher et al. 1989 and Sorenson et al. 1999, respectively). All numbers refer to the 3-prime end of the primer reference of the complete mtDNA sequence of the domestic chicken (*Gallus gallus*) (Desjardins and Morais 1990).

A typical ND2 amplification involved 35 cycles of 95° C for 1 min, 48° C for 2 min, 72° C for 3 min, and a final 10 min extension period at 72° C. Cyt*b* amplification involved 27 cycles of 94° C for 1 min, 50° C for 1 min, and 72° C for 2 min, followed by a 7 min extension period at 72° C. PCRs were conducted on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Products were verified on a 1% agarose gel with added ethidium bromide and cleaned using a QiaQuick Kit (Qiagen, Valencia, CA, USA), obtaining a final volume of 15–30 μL of PCR products.

We purified PCR products using Geneclean® (Qbiogene, Bio101® Systems, Krackeler Scientific Inc., Albany, NY, USA) and Millipore purification kits following manufacturers' protocols. Purified PCR products were sequenced on a Perkin-Elmer ABI 373 automatic sequencing machine. Sequences were cleaned using Chromas 1.45 (McCarthy 1996), and aligned using ClustalX (Thompson et al. 1997). We corroborated the origin of our sequences by combining at least two of the following: amplifying overlapping gene segments, sequencing both DNA strands, and/or using multiple individuals of single populations.

Statistical Analyses.—We used MEGA 2.0 (Kumar et al. 2004) to derive basic statistics regarding sequences, and their variation and diversity. We used Arlequin (Schneider et al. 2000) to calculate Nei's pairwise differences (raw distances corrected following Nei [1987]) among populations, as well as F_{st} values. DnaSP Version 4.10 (Rozas et al. 2003) was used to calculate nucleotide diversity (π) and haplotype diversity (κ). We used TCS Version 1.13 (Clement et al. 2000) to estimate networks summarizing mutational differences among haplotypes. We compared matrices of Nei's corrected genetic distances with matrices of straight-line geographic distances separating populations using a Mantel test; we plotted the ratio of genetic to geographic distances on maps to visualize spatial patterns of genetic differentiation on a per kilometer basis.

Only informative characters and unique haplotypes were used for parsimony searches using *Coracias* as the only designated outgroup to avoid problems of non-monophyly of in-group taxa. Maximum parsimony trees were constructed for ND2 and cyt*b* sequences both separately and combined, using heuristic search options in PAUP 4.0 (Swofford 1999) with TBR and ACCTRAN optimization options. We used character-based bootstrap analysis (100 replicates) to estimate support for each node in the resulting tree.

ModelTest 3.0 (Posada and Crandall 1998) was used to identify appropriate models of sequence evolution for haplotypes of *Melanerpes formicivorus*. Bayesian inference (BI) ap-

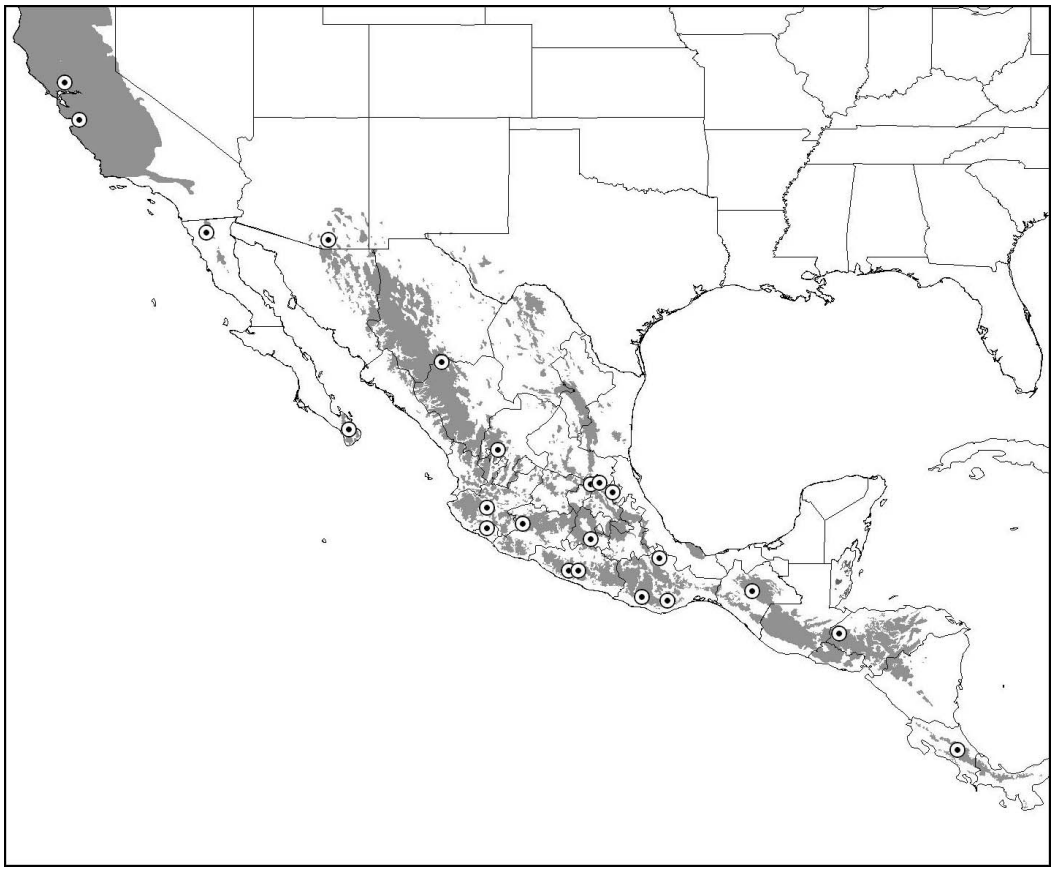

FIG. 1. Geographic distribution of *Melanerpes formicivorus* (shaded) showing localities where samples were obtained.

proaches, as implemented in Mr.Bayes Version 3.1 (Huelsenbeck and Ronquist 2001) used the substitution model GTR (nset = 6) for the number of rate parameters and a gamma distribution for rates at each site. We ran four Markov Chains (random starting trees) for 10^7 generations, each sampling every 250 generations and identifying stationarity visually. We allowed an initial ''burn-in'' of 250 trees to avoid non-optimal solutions and computed a majority-rule consensus tree, as well as posterior probabilities for each node (Huelsenbeck et al. 2002).

RESULTS

Genetic Variation.—We obtained a total of 942 base pairs across the two genes. Of these sites, 586 were conserved, 356 were variable, and 231 were parsimony-informative. The transition/transversion ratio was 3.4 and nucleotide composition was T = 0.26, C = 0.36, A = 0.27, and G = 0.11. Nucleotide diversity was 0.00482 and haplotype diversity was 0.851 with lowest nucleotide diversity values in Baja California Norte and Oaxaca populations. Overall, we found 44 haplotypes (Fig. 2) among the 98 sequences that were distinguishable by 66 polymorphic sites. Almost all (41) haplotypes were restricted to single populations. Haplotype H4 was present in single individuals from population samples from Guerrero and Hidalgo, Mexico; haplotype H35 occurred in seven individuals from Baja California Norte, Mexico, and California, USA, and (most impressively) H29 was found in 33 individuals from 10 localities from Arizona south to Honduras.

Pairwise average population differences (Table 1) ranged from 0 to 8.07 within the Arizona sample, and 0 to 8.35 in the Baja Cal-

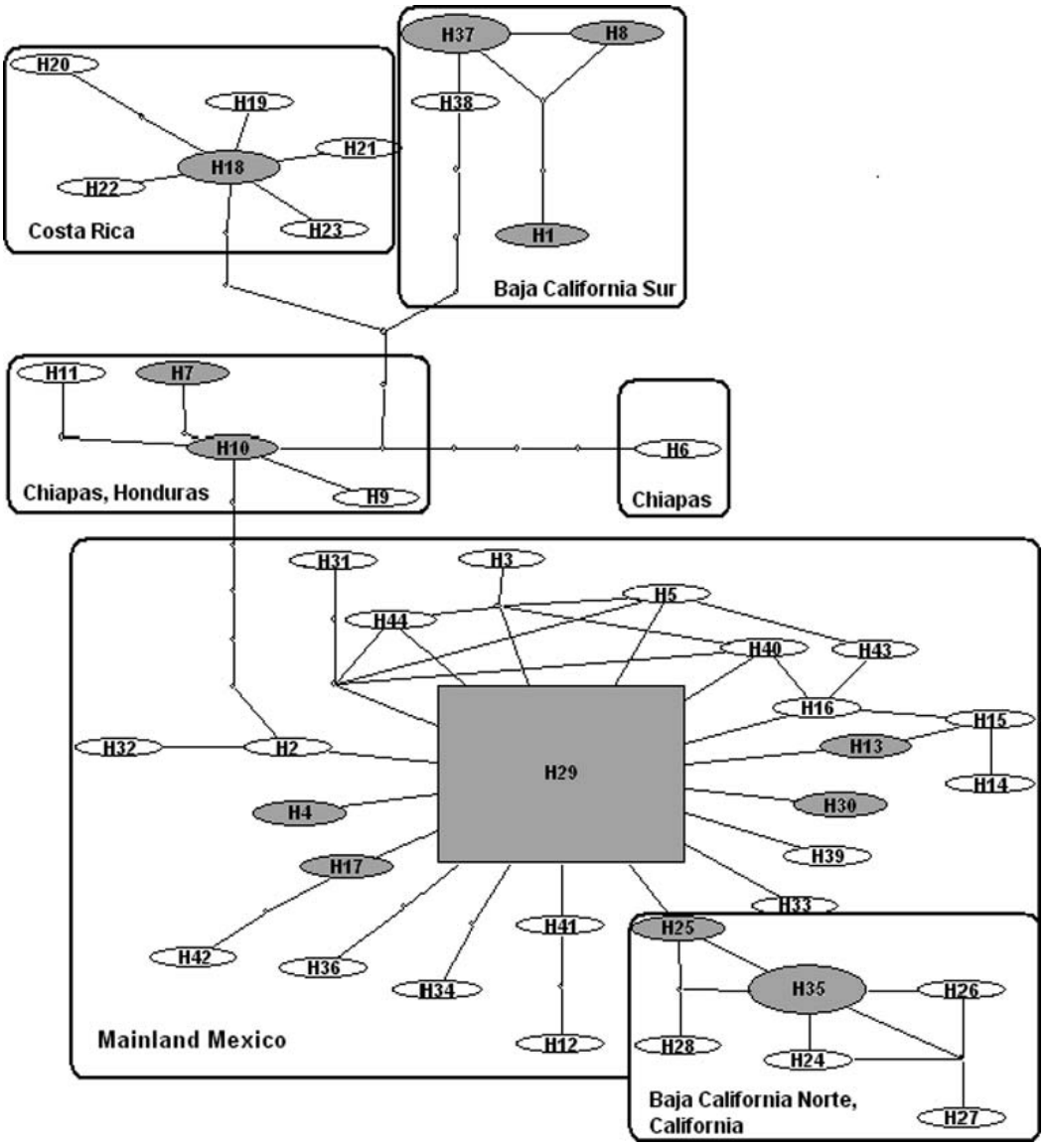

FIG. 2. Haplotype network of the 44 haplotypes of *Melanerpes formicivorus*. Mutational steps are indicated by the number of line segments connecting haplotypes. The size of the ovals represents the number of samples with that haplotype; shaded ovals are haplotypes with more than one sample.

ifornia Norte versus Baja California Sur, Mexico samples. The overall F_{st} statistic for the species was 0.484. Pairwise values between population samples ranged from 0 (several population pairs) to >0.7, most related to the Baja California Sur and Baja California Norte populations and, to a lesser extent, with the Central American populations.

The haplotype network (Fig. 2) had several features. One haplotype (H29) was common, occurring in about one-third of all individuals. Closely associated to this haplotype were 22 other haplotypes that differed by ≤3 mutations from H29; overall, this group of haplotypes generally corresponds to populations of mainland Mexico and Arizona (with one representative from as far south as Honduras). Closely associated to the mainland Mexico

TABLE 1. Pairwise average differences (below the diagonal) and pairwise F_{st} values (above the diagonal) for each population analyzed. Significant values are in italics and the cells along the diagonal show the average pairwise differences within each population. The population of QRO was grouped with HGO to avoid biased results, as it consisted of only one sample. Negative F_{st} values are considered equivalent to zero.

	DGO	GRO	HGO	JAL	MICH	OAX	ZAC	ARI	BCS	HON	CHIS	CR	CAL	BCN
DGO	**0.8585**	−0.0031	0.0113	*0.1835*	0.0072	−0.0110	0.0185	0.2239	*0.7603*	*0.6602*	*0.8047*	*0.8424*	*0.4987*	*0.6242*
GRO	0.0001	**0.6675**	−0.0608	*0.1819*	−0.0046	0.0001	−0.0272	0.1741	*0.7487*	*0.6406*	*0.7966*	*0.8407*	*0.5124*	*0.6993*
HGO	0.0001	−0.0371	**0.4450**	*0.2273*	−0.0007	−0.0082	0.0312	*0.3197*	*0.7893*	*0.7278*	*0.8543*	*0.8717*	*0.5628*	*0.7494*
JAL	*0.2784*	*0.2784*	*0.2782*	**1.4469**	0.0695	0.1938	*0.1835*	*0.2816*	*0.7390*	*0.6510*	*0.7874*	*0.8200*	*0.4724*	*0.5267*
MICH	0.0179	0.0178	−0.0102	0.1154	**1.7179**	−0.0119	0.0320	0.1762	*0.7411*	*0.6043*	*0.7580*	*0.8006*	*0.4172*	*0.4286*
OAX	0.0001	0.0001	*0.0001*	0.2781	0.0179	**0.3337**	−0.0007	0.2238	*0.7653*	*0.6830*	*0.8258*	*0.8636*	*0.5464*	*0.8254*
ZAC	0.0279	−0.0093	0.0278	*0.3065*	0.0459	0.0277	**1.2805**	0.2106	*0.7447*	*0.6396*	*0.7882*	*0.8175*	*0.4599*	*0.5005*
ARI	−0.0050	−0.1184	−0.0061	0.2768	−0.0657	−0.0034	−0.1260	**8.0693**	*0.5824*	0.0345	0.2889	*0.6094*	*0.4159*	*0.3032*
BCS	*7.3404*	*7.0305*	*7.3378*	*6.8020*	*7.3656*	*7.3367*	*6.7947*	*4.8973*	**3.1555**	*0.6182*	*0.6772*	*0.7196*	*0.7813*	*0.7691*
HON	*3.5340*	*3.2796*	*3.5324*	*3.8177*	*3.5244*	*3.5317*	*3.3949*	0.0000	*5.3618*	**4.0302**	0.1108	*0.6413*	*0.7105*	*0.6927*
CHIS	*7.0862*	*6.7454*	*7.0834*	*7.3741*	*7.1119*	*7.0823*	*6.8917*	*2.5947*	*6.9574*	0.5021	**4.7028**	*0.7311*	*0.8217*	*0.8159*
CR	*6.0534*	*5.7148*	*6.0515*	*6.3384*	*6.0767*	*6.0507*	*5.8588*	*3.3625*	*6.0424*	*3.4981*	*5.0522*	**1.3361**	*0.8504*	*0.8775*
CAL	*1.0028*	*1.0027*	*1.0024*	*1.1458*	*0.9942*	*1.0027*	*1.0188*	*0.9327*	*8.0524*	*4.4636*	*7.9960*	*6.9576*	**1.1127**	−0.1166
BCN	*1.0018*	*1.0018*	*1.0016*	*1.2807*	*1.0201*	*1.0015*	*1.0299*	*1.0011*	*8.3542*	*4.5430*	*8.1008*	*7.0641*	−0.0002	**0.0000**

haplotype assemblage are six haplotypes restricted to Baja California Norte and California. Less closely associated with the main haplotype mass, however, are four haplotypes from Chiapas and northern Central America (7 mutational steps from H29, one sample an additional 6 steps distant) and one from Arizona (H11). More removed from H29 are clusters corresponding to Baja California Sur individuals (4 haplotypes, 14 mutational steps from H29) and Costa Rican individuals (6 individuals, 13 mutational steps from H29). One Baja California Sur haplotype (H36) grouped with the mainland Mexico assemblage of haplotypes, two mutational steps from H29, the most common haplotype.

A generally positive relationship was observed in plots of genetic distances versus geographic distances. The relationship is not tight (Fig. 3), but is statistically significant (Mantel's test, observed $r = 0.792$, $P < 0.001$). The impressive genetic disconnection of the Baja California Sur populations can be appreciated by standardizing genetic distances to geographic distances and plotting these indices of differentiation per kilometer on maps (Fig. 3). Central American populations are also disconnected from northern populations genetically.

Phylogenetic Patterns.—The MP analysis yielded ≥500 equally parsimonious 532-step trees (CI = 0.594, RI = 0.812; Fig. 4). These trees grouped all *Melanerpes formicivorus* populations as a monophyletic group with high bootstrap support (100% of bootstrap replicates). Subclades corresponding to individuals from Baja California Sur (84% support), northern Central America (Chiapas, Honduras; 80% support), and southern Central America (Costa Rica; 73% support) were found within this clade, although none had solid branch support in the bootstrap analyses. The remaining individuals in the study were grouped in one large, but poorly supported clade (51% bootstrap support) of individuals from mainland Mexico, Arizona, California, and Baja California Norte. Two individuals (from Guerrero and Zacatecas) were not connected with any of the subclades within the species, one Baja California Sur individual (haplotype H36) grouped with the mainland Mexico assemblage, and one Arizona individual (haplotype H11) grouped with the Chiapas-Honduras clade.

The BI analyses were based on the TVM + I + G model of substitution and showed a topology (Fig. 5) generally close to that of the MP tree. The clade corresponding to all *Melanerpes formicivorus* populations was well-defined and subclades with intriguing but inconclusive constitution were encountered. In particular, we recovered the Baja California Sur (0.97 posterior probability), northern Central America with the single Arizona sample (0.99 posterior probability), and southern Central America (0.78 posterior probability) nodes. We encountered a weakly supported node corresponding to the California and Baja California Norte samples (0.63 posterior probability); the mainland Mexico and Arizona and single Baja California Sur samples formed a large and poorly-defined assemblage.

DISCUSSION

An earlier morphological analysis (Benítez-Díaz 1993), in many cases of precisely the same individuals as were analyzed in this study, found marked subdivision of the species into seven groups, two of which (Belize and Colombia) were not analyzed in this study. These groups were supported by the distribution of genetic variation found in our study, albeit not strongly or with marked genetic differentiation. Recalculating F_{st} statistics hierarchically, we found that 73.7% of overall genetic variation was assorted among these five groups, as opposed to 26% within them, suggesting these groups have explanatory power regarding population differentiation in the overall complex.

The five groups included in this study, with one exception, were distinct from one another in terms of mutational steps in a haplotype network. The exception was that of the California/Baja California Norte populations, which, although they grouped together, were only one mutational step from the mainland Mexico haplotype group. Other groups were more distinct; each was ≥6 mutational steps removed from all other groups. Thus, the haplotypes of the plumage-based groups appear to differ markedly from group to group. Given the high number of unique haplotypes, additional sampling may prove necessary for the details of the situation to be completely clear.

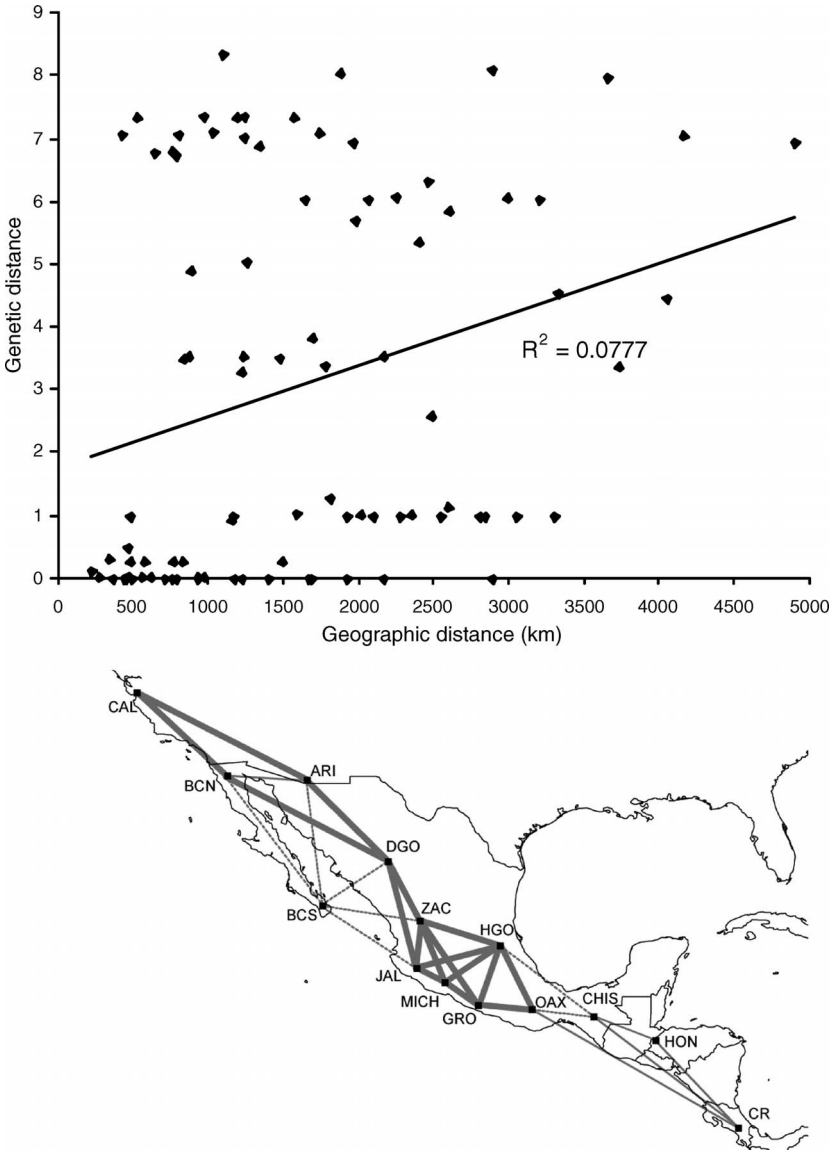

FIG. 3. Geographic patterns of genetic differentiation: (top) relationship between genetic distance and geographic distance based on 15 collecting localities; (bottom) map of genetic distance/km illustrating patterns of genetic connectivity among Acorn Woodpecker population samples. Thick continuous lines indicate rates of <1 genetic distance unit/km, thin continuous lines indicate rates of 1–5 genetic distance unit/km, and thin broken lines indicate rates of >5 genetic distance unit/km.

Our results clearly indicate lack of full establishment of reciprocal monophyly among the various populations in spite of the overall picture of differentiation. This muted differentiation is visible in both the relatively unresolved and poorly supported trees that were recovered, and in the mixture of one Baja California Sur haplotype among the ''mainland Mexico'' haplotypes and the presence of one (H29, the most common haplotype) in Honduras in both the phylogenetic analyses and the haplotype network.

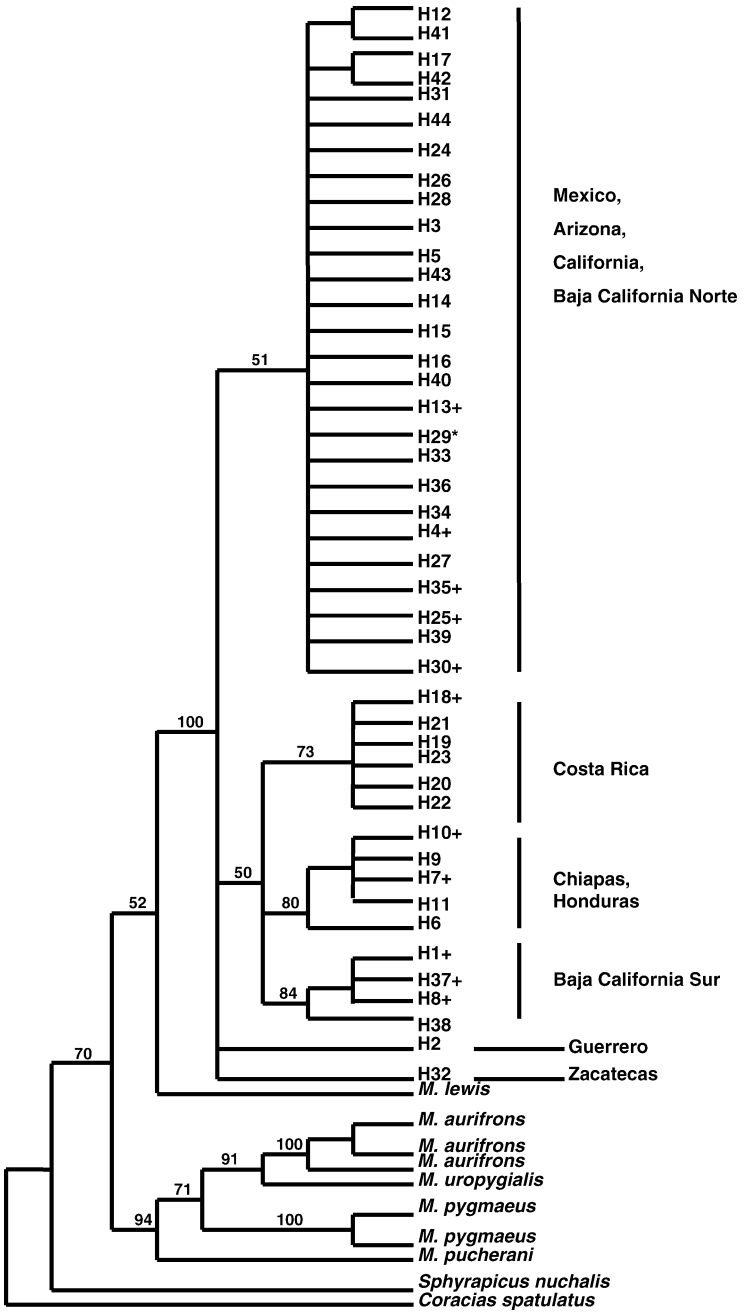

FIG. 4. Maximum parsimony tree (50% majority rule consensus) of the 44 haplotypes of Acorn Woodpeckers and 10 outgroup samples. Numbers on branches indicate bootstrap support. + = haplotypes with more than one sample; * = the most common haplotype (which was represented in a single sample from Honduras). Note that some branches have relatively low bootstrap support and may not be robust hypotheses of relationships.

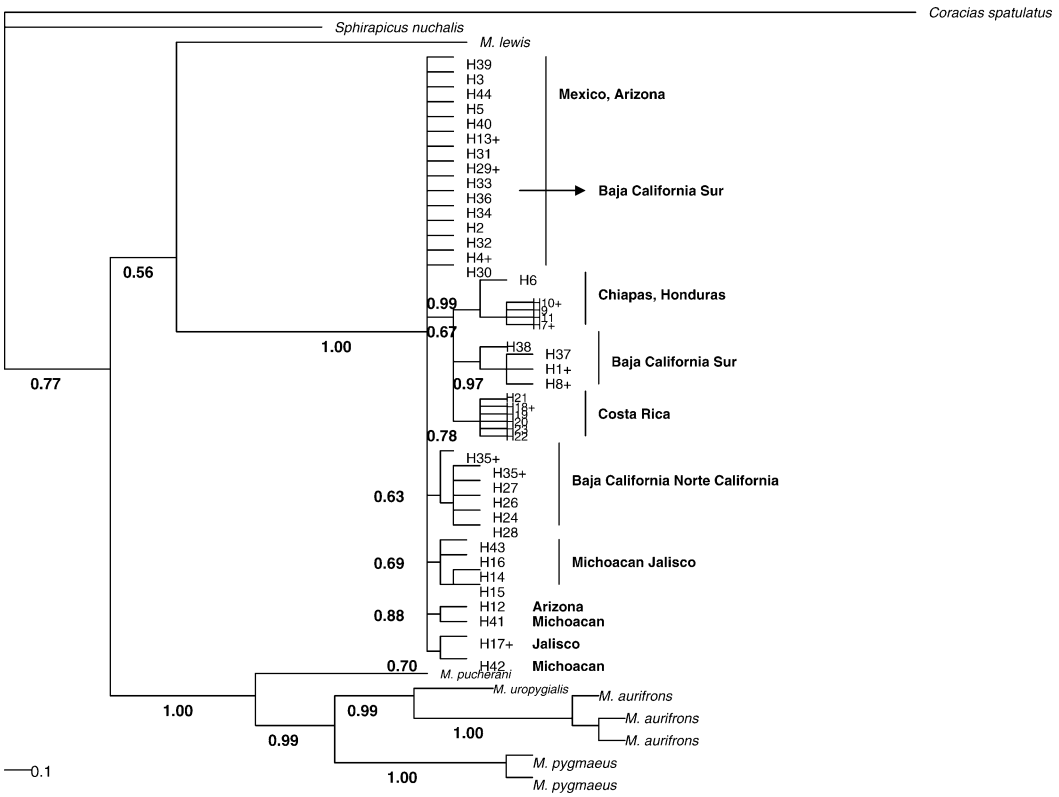

FIG. 5. Bayesian inference tree of the 44 haplotypes and 10 outgroups. Numbers below the branches show the values of the posterior probability of each branch. + = haplotypes with more than one sample; * = the most common haplotype (which was represented in a single sample from Honduras). Note that some of the branches have relatively low probabilities associated and may not be robust hypotheses of relationships.

Benítez-Díaz (1993) documented the existence of seven subgroups within *Melanerpes formicivorus* on the basis of external phenotype. These groups should be considered for formal taxonomic recognition (Navarro and Peterson 2004), at least under the Evolutionary Species Concept (Wiley 1978) and the Phylogenetic Species Concept (Zink and McKitrick 1995, Zink 1996). Benítez-Díaz (1993) recommended recognition of *Melanerpes bairdi* of California and Baja California Norte, *M. angustifrons* of Baja California Sur, *M. formicivorus* of the southwestern United States and mainland Mexico, *M. lineatus* of northern Central America, *M. striatipectus* of southern Central America, *M. albeolus* of Belize, and *M. flavigula* of Colombia. *M. albeolus* and *M. flavigula* were not available to us for molecular analysis and we did not find marked differentiation between populations in California and Mexico. Hence, we focus attention on *M. formicivorus* (including California populations of the *bairdi* group), *M. angustifrons*, *M. lineatus*, and *M. striatipectus* in the rest of our discussion.

Benítez-Díaz's (1993) general picture of differentiation of Acorn Woodpecker populations was supported, but decisions regarding species limits were less clear. From the perspective of the Biological Species Concept (AOU 1998), these populations can be interpreted either as (1) exchanging few genes after a relatively recent separation, or (2) still exchanging genes (which may cause the intermixing of haplotypes), which would probably point to caution in splitting populations under this concept. The Phylogenetic Species Concept would clearly recognize these different forms as species in view of their distinctiveness in plumage, but would hold back from recognition using molecular

characters on the basis of intermixing of haplotypes from Baja California Sur, mainland Mexico, and Central America. Finally, under the Evolutionary Species Concept, one would most likely accord them species status, given that not only are populations apparently in the process of diverging, but unique phenotypic characters are now fixed in at least some populations.

The patterns of genetic variation and differentiation identified would appear to correspond closely to known Pleistocene geography of pine-oak woodlands at the Last Glacial Maximum (LGM, ca. 20,000 years ago). That is, at LGM, montane woodlands moved on large spatial scales, broadly invading the southwestern North American deserts (Lanner and Van Devender 1981, Spaulding et al. 1983, Wells 1983). The major zones of genetic differentiation in *Melanerpes formicivorus* are between the southern tip of Baja California and the California/Mexico portion of the range, and across the Isthmus of Tehuantepec—the lack of differentiation across the Mohave Desert may reflect the Pleistocene connectivity of populations of this species.

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APPENDIX. Data for each of the samples used in this study. Haplotype number shows the corresponding haplotype to the sample sequence (Fig. 2).

Standard ID	Museum/catalog number	Haplotype number	Field catalog number	Country	State	Specific locality
ARI –1		H11	DHB3438	USA	Arizona	Santa Rita Mountains, Garner Canyon
ARI–2		H29	DHB3588	USA	Arizona	Santa Rita Mountains, Temporal Gulch
ARI–3		H12	DHB3570	USA	Arizona	Santa Rita Mountains, Garner Canyon
BCN–1	FMNH 356616	H35	MXJ274	Mexico	Baja California Norte	La Rosa de Castilla, 4 km N
BCN–2	FMNH 393841	H35	MXJ270	Mexico	Baja California Norte	La Rosa de Castilla, 4 km N
BCN–3	FMNH 393842	H35	MXJ272	Mexico	Baja California Norte	La Rosa de Castilla, 4 km N
BCN–4	FMNH 395782	H35	MXJ271	Mexico	Baja California Norte	La Rosa de Castilla, 4 km N
BCS–1	FMNH 356617	H37	MXJ313	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–2	FMNH 356618	H38	MXJ314	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–3	FMNH 356619	H1	MXJ318	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–4	FMNH 395783	H36	MXJ312	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–5	FMNH 395784	H37	MXJ315	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–6	FMNH 395785	H37	MXJ316	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–7	FMNH 395787	H37	MXJ319	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–8	FMNH 395788	H37	MXJ320	Mexico	Baja California Sur	La Burrera, 6 km N, 18 km E Todos Santos, Sierra de la Laguna
BCS–9		H8	CONACYT708A	Mexico	Baja California Sur	Rancho San Dionisio
BCS–10		H8	CONACYT708B	Mexico	Baja California Sur	Rancho San Dionisio
BCS–11		H1	B4481	Mexico	Baja California Sur	Rancho San Dionisio
CAL–1	MVZ179855	H28		USA	California	4 km E of Jamesburg, Hastings Natural History Reservation

APPENDIX. Continued.

Standard ID	Museum/catalog number	Haplotype number	Field catalog number	Country	State	Specific locality
CAL–2	MVZ179821	H26		USA	California	4 km E of Jamesburg, Hastings Natural History Reservation
CAL–3	MVZ179853	H25		USA	California	4 km E of Jamesburg, Hastings Natural History Reservation
CAL–4	MVZ179857	H35		USA	California	4 km E of Jamesburg, Hastings Natural History Reservation
CAL–5	MVZ178440	H25		USA	California	4 km E of Jamesburg, Hastings Natural History Reservation
CAL–6	MVZ179852	H27		USA	California	Jamesburg
CAL–7	MVZ178120	H24		USA	California	Hwy. 12 at Oakmont, Santa Rosa
CAL–8	MVZ179856	H35		USA	California	ca. mile marker 18, Carmel Valley Rd.
CAL–9	MVZ179854	H35		USA	California	0.8 km N and 1.6 km W Red Rock
CHIS–1	IHNE1	H6	CHIS03	Mexico	Chiapas	San Cristóbal de las Casas
CHIS–2	IHNE2	H7	CHIS06	Mexico	Chiapas	San Cristóbal de las Casas
CHIS–3	IHNE3	H7	CHIS08	Mexico	Chiapas	San Cristóbal de las Casas
CR–1	FMNH 394306	H18	HBD14	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–2	FMNH 394308	H19	HBD16	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–3	FMNH 394309	H20	HBD17	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–4	FMNH 394310	H21	HBD18	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–5	FMNH 394311	H22	HBD19	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–6	FMNH 394312	H23	HBD20	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–7	FMNH 394313	H18	HBD21	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–8	FMNH 394314	H18	HBD22	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte
CR–9	FMNH 394315	H18	HBD23	Costa Rica	San Jose	Villa Mills, Cerro de la Muerte

APPENDIX. Continued.

Standard ID	Museum/catalog number	Haplotype number	Field catalog number	Country	State	Specific locality
DGO–1	FMNH 356609	H29	MXJ207	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–2	FMNH 356614	H34	MXJ231	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–3	FMNH 393836	H29	MXJ205	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–4	FMNH 393838	H33	MXJ227	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–5	FMNH 393839	H29	MXJ228	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–6	FMNH 395776	H29	MXJ208	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
DGO–7	FMNH 395777	H29	MXJ213	Mexico	Durango	Villa Ocampo, 3 km N, 2 km E
GRO–1	FMNH 356624	H4	MXJ469	Mexico	Guerrero	Toro Muerto, Sierra de Atoyac
GRO–2	FMNH 356626	H29	MXJ475	Mexico	Guerrero	Nueva Delhi, Sierra de Atoyac
GRO–3	FMNH 393849	H29	MXJ472	Mexico	Guerrero	Nueva Delhi, Sierra de Atoyac
GRO–4	FMNH 394194	H2	BMM049	Mexico	Guerrero	El Falsanal
GRO–5	FMNH 394195	H29	BMM050	Mexico	Guerrero	El Falsanal
GRO–6	FMNH 395792	H29	MXJ470	Mexico	Guerrero	Toro Muerto, Sierra de Atoyac
HGO–1	FMNH 394196	H3	BMM103	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–2	FMNH 394197	H29	BMM104	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–3	FMNH 394199	H29	BMM106	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–4	FMNH 394201	H4	BMM109	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–5	FMNH 394204	H29	BMM392	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–6	FMNH 394205	H5	BMM393	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–7	FMNH 394208	H29	BMM396	Mexico	Hidalgo	Tlanchinol, 5 km E
HGO–8		H29	HGSL23	Mexico	Hidalgo	Cerro Jarros, 1 km E El Sótano
HGO–9	FMNH 394317	H29	MXJ060	Mexico	Hidalgo	Jacala, 4 km N, 2 km E
HON–1		H10	DHB2893	Honduras	Copán	Ruinas de Copán, 10 km ENE
HON–2		H9	DHB2890	Honduras	Copán	Ruinas de Copán, 15 km ENE

APPENDIX. Continued.

Standard ID	Museum/catalog number	Haplotype number	Field catalog number	Country	State	Specific locality
HON–3		H10	DB3169	Honduras	Copán	Ruinas de Copán, 15 km ENE
HON–4		H29	DB3576	Honduras	Copán	Ruinas de Copán, 15 km ENE
JAL–1	FMNH 394297	H13	HBD02	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–2	FMNH 394298	H13	HBD04	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–3	FMNH 394299	H14	HBD05	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–4	FMNH 394300	H16	HBD07	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–5	FMNH 394302	H17	HBD09	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–6	FMNH 394303	H17	HBD10	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–7	FMNH 395807	H29	HBD01	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–8	FMNH 395808	H29	HBD03	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
JAL–9	FMNH 395809	H15	HBD06	Mexico	Jalisco	Las Joyas, Sierra de Manantlan
MICH–1	FMNH 356620	H41	MXJ345	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–2	FMNH 356621	H42	MXJ346	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–3	FMNH 356622	H43	MXJ348	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–4	FMNH 356623	H29	MXJ350	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–5	FMNH 395789	H29	MXJ347	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–6	FMNH 393844	H39	MXJ340	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–7	FMNH 393845	H40	MXJ341	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
MICH–8	FMNH 393847	H29	MXJ343	Mexico	Michoacan	Periban, 5 km S, Cerro de Tancitaro
OAX–1	FMNH 356627	H29	MXJ604	Mexico	Oaxaca	El Zacatal

APPENDIX. Continued.

Standard ID	Museum/catalog number	Haplotype number	Field catalog number	Country	State	Specific locality
OAX–2	FMNH 393850	H29	MXJ602	Mexico	Oaxaca	El Zacatal
OAX–3	FMNH 393851	H29	MXJ603	Mexico	Oaxaca	El Zacatal
OAX–4	FMNH 393853	H29	MXJ611	Mexico	Oaxaca	El Zacatal
OAX–5	FMNH 395795	H44	MXJ608	Mexico	Oaxaca	El Zacatal
OAX–6	FMNH 394192	H29	BMM319	Mexico	Oaxaca	Santa Rosa, 2 km S
QRO–1		H29	QRO104	Mexico	Queretaro	Santa Inés, 2 km W
ZAC–1	FMNH 356603	H30	MXJ172	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–2	FMNH 356604	H31	MXJ173	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–3	FMNH 356605	H32	MXJ187	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–4	FMNH 356606	H29	MXJ192	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–5	FMNH 356607	H29	MXJ194	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–6	FMNH 356608	H29	MXJ195	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–7	FMNH 393835	H29	MXJ168	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–8	FMNH 395772	H29	MXJ170	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
ZAC–9	FMNH 395773	H30	MXJ171	Mexico	Zacatecas	Valparaiso, 9 km N, 6 km W
Outgroup	MZFC	*Melanerpes pucherani*	CHIMA068	Mexico	Oaxaca	San Isidro La Gringa, a 1 km SE de San Francisco, La Paz
Outgroup	MZFC	*Melanerpes aurifrons*	CHIMA506	Mexico	Oaxaca	Chalchijapa, 2.3 km antes del pueblo en Galera
Outgroup	MZFC	*Melanerpes pygmaeus*	CONACYT 99-109	Mexico	Campeche	Tzotzil a 17 km W
Outgroup	MZFC	*Melanerpes pygmaeus*	CONACYT 99-160	Mexico	Campeche	Tachich a 7 km W
Outgroup	MZFC	*Melanerpes aurifrons*	CONACYT 99-036	Mexico	Campeche	Estación de Vida Silvestre Hampolol, 12 km N Campeche
Outgroup	MZFC	*Melanerpes aurifrons*	MEX095	Mexico	Veracruz	Sierra de Santa Martha, El Bastonal
Outgroup	MZFC	*Melanerpes uropygialis*	MEX185	Mexico	Sinaloa	Rancho Bequillos, Mocorito
Outgroup	MZFC	*Melanerpes lewis*	DHB2274	USA		
Outgroup	MZFC	*Sphyrapicus nuchalis*	QRO196	Mexico	Queretaro	Laguna de la Cruz
Outgroup	Genbank AY274057	*Coracias spatulatus*	CORSPA			
Outgroup	Genbank AF082060	*Coracias spatulatus*	CORSPA			