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MOLECULAR PHYLOGENETICS OF THE GENUS *PIRANGA*: IMPLICATIONS FOR BIOGEOGRAPHY AND THE EVOLUTION OF MORPHOLOGY AND BEHAVIOR

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ABSTRACT.—Species in the genus *Piranga* vary in degree of sexual dimorphism, plumage coloration, morphology, song, migratory patterns, and geographic distribution. To study these aspects of *Piranga* biology in an evolutionary context, I constructed a phylogeny for this genus using cytochrome-*b* sequence data. Parsimony and maximum-likelihood analyses of DNA data reveal three possible phylogenies for species in this genus. All three trees identify a clade containing *P. rubriceps, P. leucoptera,* and *P. erythrocephala* and a clade containing *P. rubra, P. ludoviciana, P. olivacea, P. bidentata,* and *P. flava.* The trees differ in the placement of *P. roseogularis.* Morphology, song, and plumage data did not agree with these phylogenies. Levels of sequence divergence and the phylogeny of haplotypes are consistent with the idea that *P. flava* as currently described contains more than one evolutionary unit. Mapping the evolution of seasonal migration onto the DNA trees indicates that migration evolved once within *Piranga.* *Received 15 November 1996, accepted 20 November 1997.*

STUDYING PATTERNS OF CHARACTER VARIATION in conjunction with a phylogeny provides a powerful method for understanding how characters evolve and for uncovering evolutionary associations among traits (Brooks and McLennan 1991, Harvey and Pagel 1991). The tanager genus *Piranga* (Passeriformes: Thraupidae) provides an excellent model for studying the evolution of different types of characters. Species in this genus vary in the degree of sexual dimorphism, plumage coloration, morphology, song, migratory patterns, and geographic distribution. None of these aspects of *Piranga* biology has been examined in a historical, evolutionary context because of the lack of a rigorous phylogeny for the group.

The nine species of *Piranga* have been placed in this genus and arranged in a linear classification (Storer 1970) based on plumage characteristics, a stoutly shaped bill, geographic distribution, and the presence of sexual dimorphism. One of the more striking features of this genus is the conspicuous plumage coloration of males. Males typically are more colorful than females, and males of all species have at least some red plumage. Females are more cryptic, with mainly brown, yellow, or olive-colored plumage. One exception to this marked sexual dichromatism is *P. rubriceps,* in which males and females differ only in the extent of red coloration on the upper breast.

Brush (1967) and Hudon (1990) characterized the chemical basis underlying some of the dramatic plumage color in four species of *Piranga.* In three of these species (*rubra, flava,* and *olivacea*), several common carotenoids were responsible for red plumage (Hudon 1990). *Piranga ludoviciana* was unique among the four species in that the only red pigment found was rhodoxanthin, a dietary-based carotenoid. The relative significance of these findings and patterns of plumage-color evolution need to be addressed in a phylogenetic context. Although plumage color and pattern often are used to define genera and to determine species status, their relevance as phylogenetic characters has not been thoroughly investigated.

Body size and shape vary considerably among species of *Piranga.* Two species, *P. leucoptera* and *P. erythrocephala,* average 33% smaller than the other seven species. These smaller-sized *Piranga* were considered by Howell and Webb (1995) to belong to a separate genus, *Spermagra* (Swainson 1827). In addition, some species of *Piranga* (*leucoptera, erythrocephala,* and *roseogularis*) have shorter and less-pointed wings than the other species in the genus (Ridgway 1902). Whether there is a phylogenetic compo-

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nent to this variation cannot be addressed without a phylogeny for this group.

Shy (1983, 1984a, b, c) detailed intraspecific and interspecific song variation in *P. olivacea, P. ludoviciana, P. rubra,* and *P. flava.* In a comparison of song characteristics of the four species, Shy (1984c) provided data on nine song variables: maximum frequency, minimum frequency, average maximum frequency, average minimum frequency, average interval between figures, average duration of figures, frequency range, average frequency range of figures, and number of figure types per bird. In conjunction with a phylogeny for *Piranga,* Shy's information could address the usefulness of song characteristics as systematic data. Vocal information has proven important to systematic questions at and below the species level, yet few studies have used song data to establish relationships among species (see Payne 1986).

The geographic distributions of *Piranga* are centered in Mexico and Central America. Three species (*erythrocephala, bidentata,* and *roseogularis*) are endemic to this region, and all but one occur there during at least some time of the year. *Piranga flava* and *P. leucoptera* are widespread in Mexico, Central America, and South America, and the distribution of *P. flava* also extends northward to North America during the boreal summer. Three other species (*rubra, ludoviciana,* and *olivacea*) are seasonal migrants that spend the breeding season in North America and the nonbreeding season in parts of Mexico, Central America, and South America. The one species not found in Mexico or Central America during at least part of the year is *P. rubriceps,* which occurs solely in the Andes of Colombia, Ecuador, and Peru. Many authors have hypothesized that closely related species of North American birds (Rand 1948, Mengel 1964, Hubbard 1973) and closely related species of South American birds (Haffer 1985) diverged from each other as a result of isolation in refugia during Pleistocene glaciation. The biogeographic history of *Piranga* remains unexplored due to lack of knowledge about evolutionary relationships within this group.

Here, I use data from the cytochrome-*b* gene of the mitochondria to reconstruct the first phylogenetic hypothesis for the genus *Piranga.* I then use the phylogeny to assess how aspects of *Piranga* biology mentioned above have changed during the evolutionary history of the group. Although no single character defines the genus, two molecular studies confirm that *Piranga* is monophyletic with respect to other tanagers. A study of allozyme variation (McDonald 1988) showed that three morphologically similar species (*rubra, olivacea,* and *ludoviciana*) form a monophyletic group. Furthermore, a comprehensive cytochrome-*b* phylogeny of Thraupidae (Burns 1997) showed that two morphologically distinct species (*olivacea* and *erythrocephala*) form a clade with respect to the 48 other tanager genera sampled. Thus, an additional goal of this study is to further clarify the limits of this genus.

METHODS

Taxon sampling and outgroup choice.—I used individuals representing all nine species currently assigned to *Piranga* (Storer 1970). For all species except *P. rubriceps,* more than one individual was used to reduce problems associated with using only a single exemplar per taxon (Table 1). Tanager outgroups used included representatives of the genera *Habia, Chlorothraupis, Calochaetes,* and *Ramphocelus* (GenBank Accession numbers AF006213, AF006219, AF006233, and U15717 to U15724). *Habia* and *Chlorothraupis* were chosen based on a close relationship to *Piranga* identified by a molecular phylogenetic study of tanager genera (Burns 1997). I also included *Calochaetes* and *Ramphocelus* because the traditional linear arrangement of tanagers (e.g. Storer 1970) places them near *Piranga.* Multiple sequences of *Ramphocelus* (from Hackett 1996) were included in order to increase tree balance and to reduce the branch length leading to this genus (Smith 1994). As an outgroup to these tanager sequences, I used sequence of a non-emberizid passerine, *Pomatostomus temporalis* (from Edwards et al. 1991; GenBank Accession number X60936).

DNA isolation and sequencing.—DNA extracts were prepared from liver or muscle tissue preserved in 95% ethanol or frozen at −80°C. Extractions were performed using either a 5% Chelex solution (Walsh et al. 1991) or by NaCl extraction (Miller et al. 1988). Using standard protocols (Burns 1997), specific fragments of the cytochrome-*b* gene were then amplified using the polymerase chain reaction (PCR) and seven different primers: L14987, L15236, H15304, H15706 from Cicero and Johnson (1995); H15916 from Edwards et al. (1991); H16065 from Smith and Patton (1993); and L15661 (ACCTCCTAGGAGA[C/T]CCAGA[C/A/T]AA[C/T]T), which is new to this study. Fragments were sequenced using manual and ABI dye-terminator methods following standard procedures (Burns 1997). Sequence Navigator Version 1.0.1 (Applied Biosystems, Perkin Elmer) was

TABLE 1. Species names, voucher numbers, and locality information of individuals sequenced for this study. Subspecies names are given when known. AMNH = American Museum of Natural History; LSUMNS = Louisiana State University Museum of Natural Science, MZECOSUR = Museo de Zoologia, Colegio de la Frontera Sur; MZFC = Museo de Zoologia, Facultad de Ciencias, Universidad Nacional Autonoma de Mexico; USNM = Laboratory of Molecular Systematics of the U.S. National Museum (Smithsonian Institution).

Species	Museum	Number	Locality
P. bidentata 1	MZFC	BMM722	Mexico: Queretaro, 7 km S Tres Lagunas
P. bidentata 2	MZFC	BMM678	Mexico: Hidalgo, 5 km E Tlanchinol
P. erythrocephala canida 1	MZFC	BMM497	Mexico: Sinaloa, La Laguna, 1 km E Loberas
P. erythrocephala canida 2	MZFC	BMM496	Mexico: Sinaloa, La Laguna, 1 km E Loberas
P. flava flava (*flava* group)	LSUMNS	B-18650	Bolivia: Dept. Santa Cruz, Costillera, Estancia Perforacion, ca. 130 km E Charagua
P. flava rosacea (*flava* group)	LSUMNS	B-15408	Bolivia: Dept. Santa Cruz, Serrania de Huanchaca, 45 km E Florida
P. flava (*hepatica* group) 1	LSUMNS	B-3417	USA: Louisiana, Cameron, Peveto Beach Woods
P. flava (*hepatica* group) 2	MZFC	BMM735	Mexico: Queretaro, 7 km S Tres Lagunas
P. flava hepatica (*hepatica* group)	MZFC	BMM493	Mexico: Sinaloa, La Laguna, 1 km E Loberas
P. flava lutea (*lutea* group)	LSUMNS	B-5400	Peru: Dept. San Martin, 20 km NE Tarapoto
P. flava testacea (*lutea* group)	USNM	608235	Panama: Prov. Bocas del Toro, 24 km N Los Planes, Gualaca-Chiriqui Grande Rd.
P. leucoptera ardens 1	LSUMNS	B-12087	Ecuador: Prov. Pichincha, Mindo
P. leucoptera ardens 2	LSUMNS	B-7783	Ecuador: Prov. Pichincha, W slope of Andes, SW side of Cerro Pichincha
P. leucoptera leucoptera	MZFC	OMVP560	Mexico: Oaxaca
P. ludoviciana 1	LSUMNS	B-10195	USA: New Mexico, Colifax, ca. 3 km N Highway 64 at Palo Felchado Pass
P. ludoviciana 2	MZFC	OMVP294	Mexico: Oaxaca
P. ludoviciana 3	LSUMNS	B-10343	USA: Washington, Douglas, Douglas Creek, 8 km N Palisades
P. olivacea 1	LSUMNS	B-19750	USA: Louisiana, Iberville Parish, 6.5 km N St. Gabriel, 435 Pecan Drive
P. olivacea 2	LSUMNS	B-3419	USA: Louisiana, Cameron Parish, Peveto Beach Woods
P. olivacea 3	AMNH	PRS064	USA: New Jersey, Morris Co., Great Swamp
P. roseogularis 1	MZECOSUR	ADAB95110	Mexico: Quintana Roo, Carrilo P. 32 km S desv. Glorieta-Est. Sta. Teresa, Sian Ka'an
P. roseogularis 2	MZECOSUR	ADAB95243	Mexico: Quintana Roo, Carrilo P. 32 km S desv. Glorieta-Est. Sta. Teresa, Sian Ka'an
P. rubra rubra 1	LSUMNS	B-3319	USA: Louisiana, Cameron Parish, Garner Ridge, ca. 5 km W Johnson's Bayou School
P. rubra rubra 2	LSUMNS	B-16276	Costa Rica: Prov. Heredia, 5 km by road S. Puerto Viejo
P. rubriceps	LSUMNS	B-0265	Peru: Dept. Cajamarca, Machete on Sapalache-Carmen Trail

used to reverse complement opposing directions, to align different fragments from the same individual, and to translate complete sequences into amino acids.

Accuracy of DNA sequencing was verified in four ways: (1) sequencing both heavy and light strands of most PCR fragments, (2) using overlapping fragments of cytochrome *b* (approximately 30% of the total sequence is overlapped by two fragments), (3) sequencing some individuals more than once, and (4) comparing levels of sequence divergence separately for the three fragments sequenced in each individual (see Hackett et al. 1995).

The resulting sequences include 1,045 base pairs of the cytochrome-*b* gene and part of the threonine t-RNA with the intergenic spacer region. These sequences begin at base 14,991 and end at base 16,064 relative to the published sequence of *Gallus gallus* (Desjardins and Morais 1990). Sequences obtained in this study have been deposited into GenBank (Accession numbers AF011759 to AF011781, AF006247, AF006248). Percent sequence divergence was calculated as the number of nucleotide differences between two sequences divided by the total number of nucleotides compared (*p*-distance of Nei [1987]). Distance between two sequences was also calculated using Kimura's (1980) two-parameter model of sequence evolution. This measure of distance is a maximum-likelihood estimate of sequence difference that corrects for multiple substitutions at sites and the higher rate of transition relative to transversion changes.

Phylogenetic analysis of DNA data.—Phylogenetic analyses were carried out using parsimony and maximum-likelihood approaches as implemented in test version 4.0.0d55 of PAUP* provided by D. L. Swofford. For the parsimony analysis, sequences were analyzed using the heuristic option with 100 random-addition replicates for each analysis. Data were analyzed with all characters given equal weight and using a weighting scheme designed to correct for multiple substitutions at a given site. Relative support for different nodes was assessed using 100 bootstrap replicates (Felsenstein 1985) and by calculating Bremer indices (i.e. decay indices; Bremer 1988).

To explore the possibility that some types of base substitutions have become saturated, I plotted *p*-distance versus Kimura's two-parameter distances for first-, second-, and third-position sites. Saturation was judged to have taken place if the resulting scatter plots did not indicate a linear relationship between the two types of distances and if the data did not fall on the line $y = x$ (see Berbee et al. 1995). A nonlinear relationship would indicate that the Kimura estimate has adjusted the original distance estimate based on the differences in substitution rates between transitions and transversions.

After determining that third-position sites were saturated for transitions (see below), I performed an additional analysis to correct for noise at third-position sites by giving third-position transitions a weight one-sixth of that given to other characters. This was accomplished using the stepmatrix option of PAUP to assign third-position transitions a weight of one, and third-position transversions a weight of six. All first- and second-position changes were assigned a weight of six. The ratio of 6:1 was obtained empirically by examining multiple sequences from each of two genera used in this study: *Ramphocelus* (Hackett 1996) and *Piranga*.

TABLE 2. Plumage characters and character states of species of *Piranga*.[a]

Species	Character number 111111 123456789012345
Piranga roseogularis	022320003331213
Piranga bidentata	112241111111111
Piranga flava	112220002111112
Piranga rubra	122220002221122
Piranga olivacea	112210002221112
Piranga ludoviciana	112111214221112
Piranga leucoptera	012211112241132
Piranga erythrocephala	012130005241122
Piranga rubriceps	012110205242132

[a] Key for characters and character states: (1) juvenal plumage, 1 = streaked, 0 = unstreaked; (2) bill color, 1 = black, 2 = yellow; (3) male crown and throat, 1 = olive green, 2 = red; (4) male breast, 1 = yellow, 2 = red, 3 = buffy gray; (5) male wing and tail, 1 = black, 2 = reddish, 3 = green/yellow, 4 = brownish; (6) greater covert tips, 0 = same, 1 = white, 2 = yellow; (7) median covert tips, 0 = same, 1 = white, 2 = yellow; (8) tertial tips, 0 = same, 1 = white; (9) male back color, 1 = streaked black or red-brown, 2 = red, 3 = olive brown, 4 = black, 5 = yellowish/greenish; (10) male ear coverts, 1 = dusky red, 2 = red, 3 = gray; (11) male lores and ocular, 1 = dusky red, 2 = red, 3 = gray, 4 = black; (12) female crown and throat color, 1 = olive green/yellow, 2 = red; (13) female breast, 1 = olive gray, 2 = gray; (14) female wing and tail, 1 = dark olive green, 2 = light olive green, 3 = black/gray; (15) female back, 1 = streaked, 2 = olive green/yellow, 3 = olive brown.

I performed maximum-likelihood analyses using the heuristic search option and 10 random addition replicates. Empirical base frequencies were used and the transition-to-transversion ratio was estimated from a neighbor-joining tree of Kimura distances. Log-likelihood values of the maximum-likelihood tree were compared with likelihood values of other topologies found in this study. I used Kishino-Hasegawa (1989) tests to determine if the likelihood tree was significantly more likely than the parsimony trees found. A topology was judged to be significantly different from the maximum-likelihood tree if the range of values within ± 1.96 SD of the tree being tested did not include the log-likelihood value of the maximum-likelihood tree.

Parsimony analysis of plumage data.—In addition to DNA sequence data, plumage characters (Table 2) were used to provide information about evolution within *Piranga*. Plumage characters were analyzed

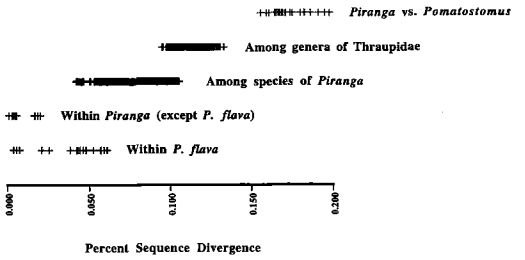
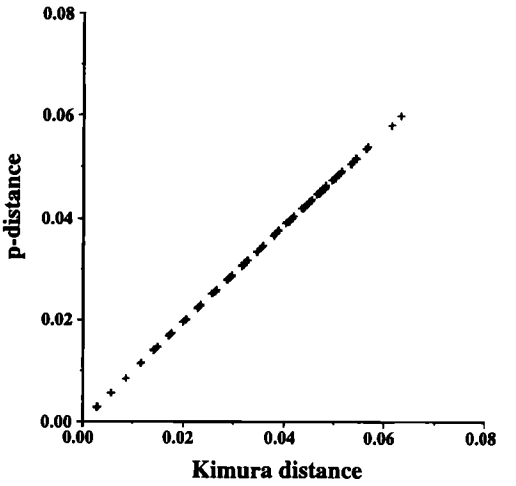
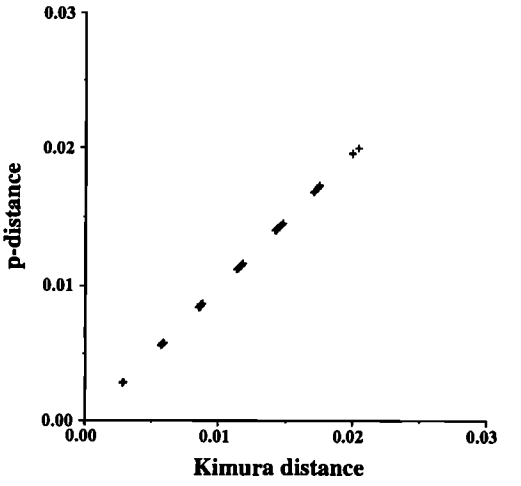


FIG. 1. Uncorrected percent sequence divergence (*p*-distance; Nei 1987) among individuals included in this study. Each cross represents a pairwise comparison between two individuals.

cladistically by coding different body regions as characters and assigning different colors of these regions as unordered character states (see Cracraft and Prum 1988, Hackett and Rosenberg 1990). The resulting data matrix was subjected to parsimony analysis as implemented in PAUP 3.1.1 (Swofford 1993).

Song and morphological similarity.—To study morphological evolution, I measured a series of museum skins of males representing each species of *Piranga* (see Appendix). All characters (length of exposed culmen, height of bill at base, width of bill at base, length of closed wing, length of tail, and length of tarsometatarsus) were measured following Baldwin et al. (1931). In addition, mean body mass of each species was obtained from Isler and Isler (1987). Prior to the analysis, the mean body mass of each species was linearized by dividing by its cube root. To scale the characters equally, each character was divided by the variance of that particular character among species of *Piranga*. Means of each character measured were used to calculate a matrix of taxonomic distances (Sneath and Sokal 1973). These distances were used to construct an UPGMA phenogram (using NTSYS-pc; Rohlf 1988) summarizing overall similarity in morphology (see Hackett and Rosenberg 1990 and Johnson and Marten 1992). These distances were also used to build a neighbor-joining tree using PHYLIP version 3.5c (Felsenstein 1993), which, unlike UPGMA, does not assume equal lengths of branches. Because phenetic analyses such as these do not distinguish between shared-derived and shared-primitive characters, trees resulting from neighbor-joining and UPGMA analyses are interpreted as diagrams depicting overall similarity and not as phylogenetic hypotheses of the species in





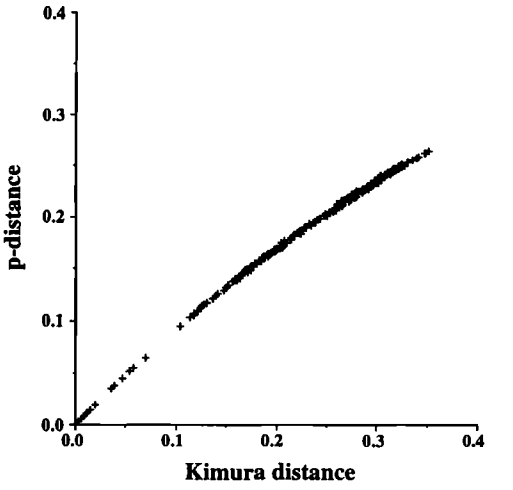


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FIG. 2. Levels of saturation among sequences for first- (top), second- (middle), and third-position sites (bottom).

question. Data on song characteristics of four species of *Piranga* were obtained from Shy (1984c: table 2). These quantitative characters were compared with the phylogeny of *Piranga* to determine which (if any) of the song traits tracked the phylogeny.

RESULTS

Sequence variation.—The portion of cytochrome *b* sequenced for this study aligned easily without gaps and insertions. However, within the intergenic spacer region between cytochrome *b* and the tRNA, all seven individuals of *Piranga flava* had a deletion relative to the other species sequenced and to the chicken sequence (Desjardins and Morais 1990). These seven sequences were aligned by hand by adding a gap at base position 16,038 relative to the chicken sequence.

Of the 1,073 sites, 484 (45%) are variable. Levels of uncorrected sequence divergence are structured hierarchically (Fig. 1). Specifically, divergences between *Pomatostomus temporalis* and the tanager sequences range from 15.6 to 19.8%, divergences among tanager genera range from 9.6 to 13.3%, and divergence among species of *Piranga* range from 4.2 to 10.5%. For most species, levels of intraspecific sequence divergence are relatively low. For species other than *Piranga flava*, levels of divergence within species range from 0.1 to 2.0%. Within *P. flava*, there is a greater range of sequence divergence. The lowest level (0.7%) was observed between an individual from Mexico and one from the United States, whereas the highest level (6.1%) was observed between an individual from Mexico and one from Bolivia.

Base composition (adenine 27.4%, cytosine 34.3%, guanine 13.1%, thymine 25.2%) was similar to that observed in other species in the Thraupidae (Burns 1997) and that reported in other birds (Edwards et al. 1991, Kornegay et al. 1993, Hackett 1996). Changes at third-position sites were more common than changes at second- and first-position sites (Fig. 2). Among the *Piranga* sequences, transitions were approximately six times more common than transversions. Plots of Kimura distances versus uncorrected sequence divergence are linear and roughly fall along the line $y = x$ for first- and second-position sites (Fig. 2). For third-position sites, transitions are saturated relative to transversions (Fig. 2).

Phylogenetics.—Of the 484 variable sites, 294 were phylogenetically informative for parsimony analyses. Giving all characters equal weight resulted in two most-parsimonious trees (one shown in Fig. 3) of 1,098 steps (consistency index, excluding uninformative characters [CI] = 0.44, retention index [RI] = 0.71). The two trees differed only in the arrangement of individual sequences of *P. olivacea*. All species were retained as monophyletic units, and the genus *Piranga* was monophyletic as well. Within *Piranga*, there was a clade of taxa that occurs at high elevations: *P. erythrocephala* (highlands of Mexico), *P. rubriceps* (northern and central Andes), and *P. leucoptera* (widespread in highlands of Middle America, the Andes, and Tepuis of South America). *Habia* together with *Chlorothraupis* formed a monophy-

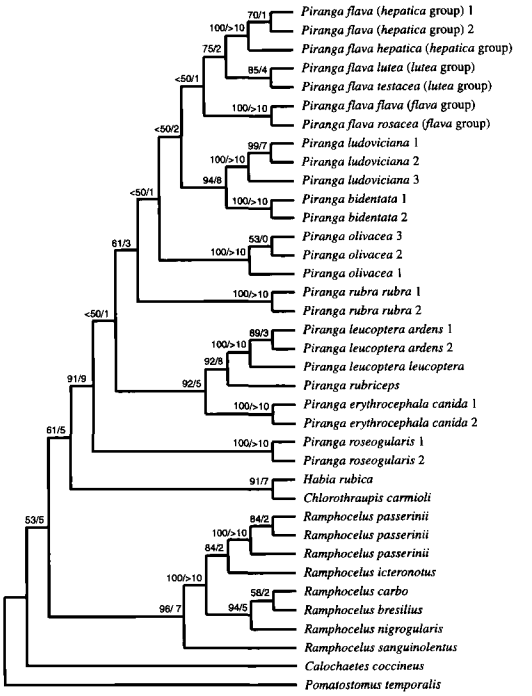

FIG. 3. One of two most-parsimonious trees resulting from a parsimony analysis in which all characters were given equal weight. The other tree resulting from this analysis is identical except that *P. olivacea* 1 and *P. olivacea* 2 are sister taxa. Tree is rooted at *Pomatostomas temporalis*. Numbers on nodes refer to relative support of each clade. The first number indicates the number of bootstrap replicates (out of 100) in which that clade is found. The second number is the Bremer (or decay) index indicating the number of additional steps needed before a particular clade breaks down.

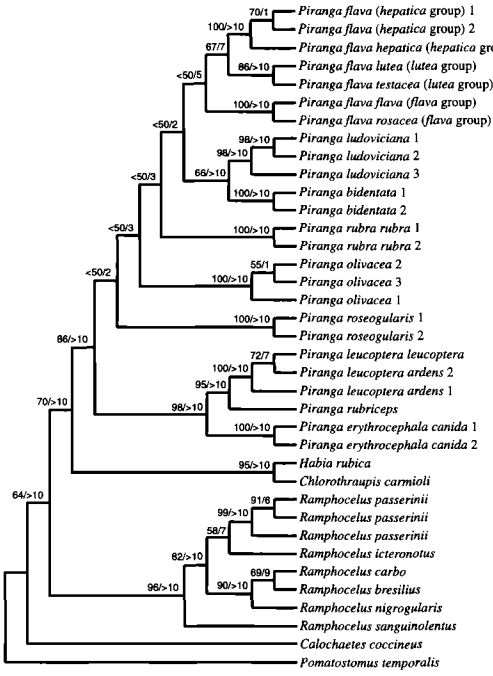

FIG. 4. Most-parsimonious tree resulting from the transversion-weighted analysis described in the text. Tree is rooted at *Pomatostomas temporalis*. Numbers on nodes refer to relative support of each clade. The first number indicates the number of bootstrap replicates (out of 100) in which that clade is found. The second number is the Bremer (or decay) index indicating the number of additional steps needed before a particular clade breaks down.

letic sister group to *Piranga* (see also Burns 1997). Species of *Ramphocelus* formed a monophyletic sister group to the clade containing the *Piranga, Habia,* and *Chlorothraupis* sequences. Relationships among the *Ramphocelus* sequences found in all analyses of the current study were identical to those found by Hackett (1996). As found by Burns (1997), the sequence of *Calochaetes* did not cluster with either *Ramphocelus* or *Piranga,* suggesting that plumage similarities of this species and members of the genus *Ramphocelus* are the result of convergence, not shared evolutionary history.

Because of evidence of saturation at third-position sites (Fig. 2), I performed another parsimony analysis in which third-position transitions were given a weight one-sixth that of other types of changes. This transversion-weighted analysis resulted in a single most-parsimonious tree (3,219 steps, CI = 0.46, RI = 0.75; Fig. 4) that largely agrees with the equally weighted trees (one shown in Fig. 3). Two differences distinguish the transversion-weighted tree from the equally weighted tree. In the equally weighted tree, *P. roseogularis* is the most basal *Piranga*; however, in the transversion-weighted tree, *P. roseogularis* falls within *Piranga* as the sister taxon to the clade containing *P. rubra, P. olivacea, P. flava, P. bidentata,* and *P. ludoviciana.* The two trees also differ in that the positions of *P. rubra* and *P. olivacea* are reversed.

Levels of support of parsimony trees as measured by Bremer indices and bootstrap values varied among nodes (Figs. 3, 4). The monophyly of each species (except *P. flava*) had a relatively high degree of support. In addition, the monophyly of *Piranga* was strongly supported, as was the highland clade containing *P. erythrocephala, P. leucoptera,* and *P. rubriceps.* Lower bootstrap values and smaller Bremer indices were found on branches indicating relationships among *P. rubra, P. olivacea, P. flava,* and the clade containing *P. bidentata* and *P. ludoviciana.*

In all 10 random-addition replicates of the maximum-likelihood analysis, the same most-likely tree was found (−ln likelihood = 7,241.46). This tree was similar to the transversion-weighted parsimony tree (Fig. 4) and to results of the equally weighted analyses (Fig. 3). The same two nodes in contention between the two different parsimony-weighting schemes also differed between the maximum-likelihood analysis and the parsimony analyses. Like the equally weighted trees, the maximum-likelihood tree placed *P. roseogularis* basal to all other *Piranga.* However, both the maximum-likelihood and transversion-weighted trees agreed in the position of *P. rubra* and *P. olivacea.* Other than the placement of these three taxa, the trees obtained from all analyses of this study showed complete agreement in their depiction of relationships among species.

Log-likelihood values of the equally weighted trees are similar to that of the maximum-likelihood tree (Table 3). Although the transversion-weighted tree differs more substantially in log-likelihood value, none of the parsimony trees has likelihood values that are significantly different from the likelihood value of the maximum-likelihood tree. The maximum-likelihood tree and equally weighted trees are also more similar to each other in terms of the

TABLE 3. Log-likelihood (−ln L) values and number of steps required for each of the four topologies obtained using maximum-likelihood and parsimony analyses. Difference in log likelihood (Δ −ln L ± SD) reported relative to the maximum-likelihood tree.

Topology	Maximum likelihood		Parsimony (length)	
	−ln L	Δ −ln L	Equally weighted	Transversion-weighted
Maximum-likelihood tree	7,241.46	—	1,099	3,229
Equal weight tree 1	7,241.69	0.23 ± 3.73	1,098	3,233
Equal weight tree 2	7,241.47	0.01 ± 5.91	1,098	3,233
Transversion-weighted tree	7,247.19	5.73 ± 9.67	1,104	3,219

number of steps required by the tree's particular topology.

Plumage.—In contrast to the DNA data, plumage data were much less successful in resolving relationships in *Piranga*. Analysis of plumage characters produced 12 most-parsimonious trees (35 steps, CI = 0.71, RI = 0.57), the strict consensus of which showed little resolution. Only two nodes were recovered by all plumage trees (the monophyly of *erythrocephala* and *rubriceps*, and of *rubra* and *roseogularis*). Neither of these nodes was found in any of the analyses of the DNA data. However, half of the 12 plumage trees show the highland clade (*erythrocephala, rubriceps,* and *leucoptera*) that was recovered in the DNA phylogenies.

Morphology.—Both the UPGMA phenogram and the neighbor-joining tree of the external morphological data (Table 4) resulted in the same topology (Fig. 5). Species of *Piranga* are divided into three main groups. The small-bodied *P. erythrocephala* and *P. leucoptera* form a group; the more medium-sized *P. roseogularis, P. ludoviciana,* and *P. olivacea* form another cluster; and the larger *P. bidentata, P. flava, P. rubriceps,* and *P. rubra* form yet a third group. The phenogram produced from morphological data does not share any nodes in common with the analyses of the cytochrome-*b* data.

Song.—None of the song characteristics described by Shy (1984c: table 2) shows similarities to the phylogeny of *Piranga* (Fig. 6). Species with more similar values of song traits are not each other's closest relative. For example, of the species studied by Shy (1984c), *P. flava* and *P. ludoviciana* are the most closely related (Figs. 3, 4); however, these two species do not have similar song values relative to the other *Piranga* studied.

DISCUSSION

Previous phylogenetic hypotheses.—Few previous hypotheses have been made concerning relationships within *Piranga*. Based on hybrids between *P. ludoviciana* and *P. olivacea* (Tordoff 1950, Mengel 1963), Mengel (1963) argued that these two species were closely related and shared a recent common ancestor. However, these two species are not closely related in any of the DNA phylogenies. Thus, the ability for very limited hybridization to occur between these species represents the retention of a primitive characteristic and should not be used as a

TABLE 4. Mean external measurements (mm) and body mass (g). Values for body mass obtained from Isler and Isler (1987).

Species	Bill length	Bill height	Bill width	Wing	Tail	Tarsus	Body mass
P. bidentata (*n* = 9)	17.22	9.44	10.29	95.37	83.87	21.45	34.0
P. erythrocephala (*n* = 10)	13.30	7.89	8.64	72.15	72.42	20.07	22.0
P. flava (*n* = 21)	17.73	9.64	10.37	95.30	82.81	21.81	35.3
P. leucoptera (*n* = 8)	12.96	7.43	8.33	68.67	64.79	18.72	16.0
P. ludoviciana (*n* = 10)	15.14	7.98	9.08	94.70	75.14	20.59	30.0
P. olivacea (*n* = 11)	14.98	8.45	9.56	96.71	75.64	19.72	25.0
P. roseogularis (*n* = 7)	15.31	8.91	9.25	79.02	74.50	21.85	24.0
P. rubra (*n* = 20)	19.10	9.62	10.35	96.77	82.61	19.74	26.0
P. rubriceps (*n* = 15)	15.33	9.04	10.60	96.51	85.33	22.79	35.0

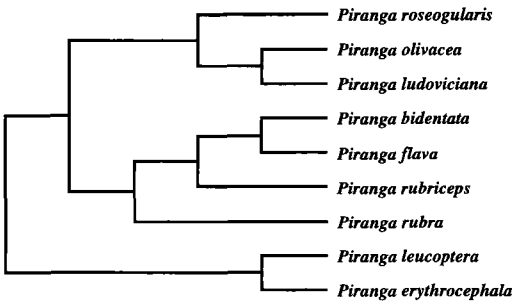

FIG. 5. Morphological similarity among species of *Piranga* as determined by UPGMA and neighbor-joining analyses. Branch lengths are not proportional.

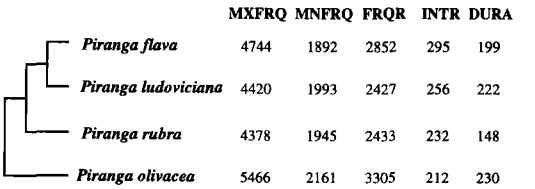

FIG. 6. Song characters and phylogeny of *Piranga*. Phylogeny shown is the transversion- weighted tree and maximum-likelihood tree. The song characters shown (MXFRQ = maximum frequency, MNFRQ = minimum frequency, FRQR = frequency range, INTR = average interval, DURA = average duration) represent five of the nine characters of Shy (1983c). Shy's other four characters also do not show agreement with the phylogenies of this study. Frequency values are in Hz, and duration and interval are in ms.

guideline for relationships (Zink and McKitrick 1995). *Piranga olivacea* has also hybridized with *P. rubra* (McCormick 1893), and *P. ludoviciana* has hybridized with *P. bidentata* (Morse and Monson 1985). The hybridization of *P. olivacea* and *P. rubra* does not agree with phylogeny; however, the hybridization of *P. ludoviciana* and *P. bidentata* is in agreement with the close phylogenetic relationship of these two species. Nevertheless, hybridization is not necessarily a good indicator of phylogenetic relationships. Even species with extensive hybrid zones often are not each other's closest relatives (e.g. Fox Sparrows [*Passerella iliaca*], Zink 1994; orioles, Freeman and Zink 1995; and flickers, Moore et al. 1991; see Zink and McKitrick 1995).

In contrast to current systematic treatments of taxa within *Piranga* (Storer 1970, AOU 1983, Sibley and Monroe 1990), Howell and Webb (1995) placed *P. leucoptera* and *P. erythrocephala* in the genus *Spermagra* apart from other *Piranga* that occur in Mexico. Given the DNA phylogenies resulting from the current study, the use of the genus *Spermagra* solely for these two species is unwarranted. For this genus to be monophyletic, *P. rubriceps* would need to be included in *Spermagra* as well. In addition, if *P. roseogularis* is basal to all other *Piranga* (as suggested by the equally weighted and maximum-likelihood trees), use of the genus *Spermagra* would make *Piranga* paraphyletic. Because taxonomic names should reflect monophyletic groups, the genus *Spermagra* should not be used.

McDonald (1988) surveyed allozyme variation in several warblers and tanagers (including *P. rubra, P. olivacea,* and *P. ludoviciana*) to determine the closest relatives of the genus *Phaenicophilus*. In both an UPGMA phenogram and a Distance-Wagner tree, *rubra* and *olivacea* were sister taxa with *ludoviciana* outside of these two species. This set of species relationships was not identified in any of the trees resulting from my study. It is difficult to determine whether these differences are the result of different patterns of evolution of different characters, variation in the ability of different methods to recover relationships among taxa, or artifacts of taxon sampling. Many studies have shown that incomplete taxon sampling can have profound effects on final topological structure (Gauthier et al. 1988, Weller et al. 1992). I sampled all species of *Piranga* and used cladistic-based character-state data, which resulted in a greater capability to recover phylogenetic relationships.

Species limits of P. flava.—The Hepatic Tanager (*P. flava*), with 15 described subspecies (Storer 1970), exhibits more geographic variation than any other *Piranga*. Some authors (AOU 1983, Isler and Isler 1987) have divided these 15 subspecies into three groups based on plumage differences, disjunct distributions, and ecological differences. Although an early review of variation in *P. flava* (Zimmer 1929) concluded that only a single species was involved, differences among these subspecies groups are profound enough to suggest that *P. flava* as presently defined consists of three species (Ridgely and Tudor 1989). Distributions of these subspecies groups are largely nonoverlapping. Subspecies in the *hepatica* group occur from the southwestern United States to northern Nicaragua; the *lutea* group occurs mostly at higher elevations from Costa Rica to Bolivia, as well as in parts of Venezuela and northern Brazil; and

the *flava* group occurs mainly in southern Brazil, Paraguay, southeastern Bolivia, and northern Argentina. Plumage coloration of males varies across their distribution from deep red to dusky scarlet. Plumage differences between the *flava* and *lutea* groups often are greatest where individuals of these forms co-occur (Ridgely and Tudor 1989). Habitat preferences of *P. flava* change across its range with the three subspecies groups. Representatives of the *hepatic* group are found mostly in pine or pine-oak forest, the *lutea* group occurs in broad-leaved woodlands, and individuals of the *flava* group are commonly found in savanna and semiopen situations (Isler and Isler 1987).

Levels of sequence divergence and phylogenetic relationships observed among *P. flava* correspond well with the three subspecies groups of *P. flava*. Sequence-divergence values between some individuals of *P. flava* are much greater than those found within other species of *Piranga* (Fig. 1). Levels of sequence divergence within *P. flava* at 2.5% and below represent comparisons between representatives of the same subspecies group. Levels of sequence divergence at 3.8% and greater represent comparisons between representatives of different subspecies groups. Thus, levels of sequence divergence among subspecies groups of *P. flava* are as great as levels of divergence observed among other species of *Piranga* (Fig. 1). For example, an individual of *P. flava* representing the *hepatica* group differs by 6.1% from an individual representing the *flava* group. Individuals of *P. ludoviciana* and *P. olivacea* show, on average, a similar level of sequence divergence.

In all phylogenetic analyses in this study, members of the same subspecies group cluster together into monophyletic groups. These monophyletic groups show strong support both in terms of bootstrap values and Bremer indices. Thus, both levels of sequence divergence and the phylogeny of sequences are consistent with the idea that *P. flava* as currently described contains more than one evolutionary unit. The different subspecies groups are monophyletic and diagnosably distinct based on plumage characters. The DNA data of this study add to the morphological, distributional, and ecological evidence that suggest that the three subspecies groups of *P. flava* represent different phylogenetic, if not biological, species.

Evolution of plumage colors.—Parsimony analyses of plumage data failed to recover phylogenetic relationships among the species of *Piranga*. Two similar studies (Christidis et al. 1988, Hackett and Rosenberg 1990) that investigated relationships among closely related avian species also found discrepancies between plumage data and genetically based phylogenies. The fact that plumage characters show more homoplasy than DNA data may indicate that plumage patterns and colors are not as useful in systematic studies as are other types of characters.

One problem with using plumage characters in phylogenetic reconstruction is that sexual selection may cause rapid plumage divergence among taxa and obscure phylogenetic relationships. A more fundamental issue is that the complex mechanisms and multiple sources responsible for plumage color make the identification and coding of homologous characters difficult. Little is known about how the plumage colors and patterns that we see are produced. The color itself may be structurally based or may come from a variety of pigments, most notably melanins and carotenoids. Birds cannot produce carotenoid pigments on their own and must obtain them from their diets. Birds can either deposit these pigments directly, or the pigment may be modified into another pigment prior to being deposited in the feathers.

The carotenoid pigments underlying plumage color (i.e. red, orange, and yellow) in four species of *Piranga* (*flava, rubra, olivacea,* and *ludoviciana*) have been studied in detail (Brush 1967, Hudon 1990). One conclusion of these studies is that colors that appear identical may come from completely different sources. For example, *P. ludoviciana* and *P. olivacea* have a red head that is indistinguishable to the human eye under natural, artificial, and near-ultraviolet illumination and also is indistinguishable using reflectance spectrophotometry (Hudon 1990). However, isolation of the carotenoid pigments responsible for the red coloration in these two species revealed that different carotenoid pigments are responsible for producing the identical head color (Hudon 1990). In *olivacea,* several common carotenoid pigments produce the red coloration. In contrast, the carotenoid responsible for the red coloration in *P. ludoviciana* is rhodoxanthin, which is deposited directly into the feathers in an unmodified form (Hudon

1990). When I coded plumage characters in the parsimony analysis, I assigned the same character state for head color in both *P. olivacea* and *P. ludoviciana*. Because the ultimate source of the red coloration of the head is quite different in these two species, my original coding is an oversimplified depiction of plumage color in these species. Unfortunately, not enough pigments and not enough taxa have been studied for these data to be incorporated into a more thorough phylogenetic analysis. The results of this study suggest that plumage color (especially in ignorance of its chemical basis) may be less useful than other characters for phylogenetic analysis.

Sexual dimorphism in plumage.—The phylogenetic position of *P. rubriceps* (Figs. 3, 4) indicates that the relatively smaller degree of sexual dimorphism in plumage of this species is a derived condition. In this species, females have evolved more brightly colored plumage, whereas male plumage has remained brightly colored. Such changes in female plumage rather than male plumage are common in tanagers (Burns 1996) and often cause changes in the degree of sexual dimorphism. This suggests that the evolution of sexual dimorphism in plumage is affected by other factors (such as social selection on females; Irwin 1994) as well as sexual selection for brightly colored males. Unfortunately, little is known of the habits and nothing is known of the breeding biology of *P. rubriceps* (Isler and Isler 1987). Thus, the reasons for the relatively small degree of plumage dimorphism in *P. rubriceps* remain unknown.

External morphology and song variation.—As in other lineages of birds (e.g. Hackett and Rosenberg 1990, Zink and Blackwell 1996), morphometric variation among *Piranga* is not correlated with genetic variation. Overall similarity in morphology (Fig. 5) shows no similarities to phylogenies derived from DNA sequence data. Thus, size and shape have changed frequently in *Piranga*. For example, the clade of *P. erythrocephala, P. leucoptera,* and *P. rubriceps* contains the two smallest *Piranga* and one of the largest (i.e. *rubriceps*).

Recent studies have shown a relationship between phylogeny and certain vocal characteristics. For example, fundamental frequency corresponds with phylogeny in herons (McCracken and Sheldon 1997), and duration of notes is associated with phylogeny in warblers

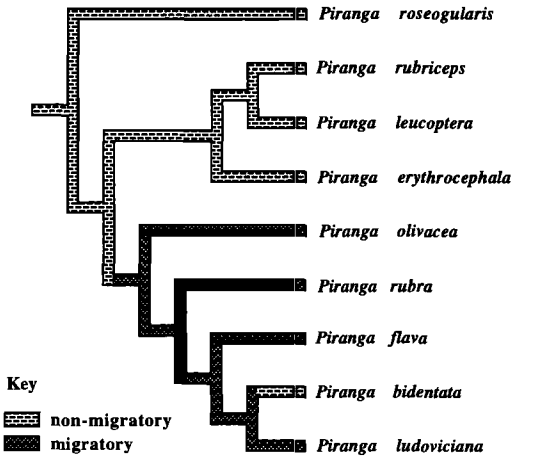

FIG. 7. Evolution of seasonal migration mapped onto the maximum likelihood tree of *Piranga* relationships. Key: nonmigratory = species does not migrate seasonally, migratory = species migrates seasonally in at least part of its range.

(Van Buskirk 1997). In contrast, none of the individual song characters of *Piranga* tracks phylogeny (Fig. 6). In addition to phylogeny, other factors can influence song. Body size constrains song features such as frequency, and physical characteristics of the habitat can have selective influences on song because some types of vocalizations transmit better than others in a given habitat (Morton 1975, Badyaev and Leaf 1997). These factors have probably had a strong influence on shaping song variation among species of *Piranga*. Habitat openness is correlated with vocal characteristics among populations of *P. rubra* (Shy 1983). Additional analyses may show that this relationship exists among other species of *Piranga* as well.

Biogeography and the evolution of seasonal migration.—The phylogeny of *Piranga* presented in this study provides a framework for addressing distributional patterns among species, including the evolution of seasonal migration. Four of the nine species of *Piranga* (*flava, ludoviciana, olivacea,* and *rubra*) are seasonal migrants that breed in North America. Some *P. flava* also migrate seasonally in southern South America. Mapping the evolution of seasonal migration onto any of the DNA trees (e.g. Fig. 7) indicates that the most-parsimonious explanation for the evolution of this trait is that migration evolved once on the branch leading to the monophyletic group of *P. rubra, P. olivacea, P. flava, P. bidentata,*

and *P. ludoviciana*. Thus, these five taxa probably evolved from a migratory ancestor, and later migratory behavior was lost in *P. bidentata* and in some populations of *P. flava*.

The phylogeny of *Piranga* also provides a perspective on relationships among areas of endemism in North America. As mentioned above, *P. ludoviciana* (breeds in western North America) and *P. olivacea* (breeds in eastern North America) previously were thought to represent an eastern/western sister-species pair (Mengel 1963). However, the cytochrome-*b* data clearly show that these two species are not closely related. *Piranga ludoviciana* is more closely allied to *P. bidentata* (a Mexican and Central American species that has recently occurred in the southwestern United States) and *P. flava* (which occurs from the southwestern United States to southern South America). In addition to *Piranga*, many other taxa have closely related forms in eastern, western, and southwestern North America (Zink and Hackett 1988). The phylogeny of *Piranga* indicates that western North America has a more recent association with southwestern North America than it has with eastern North America. Two other molecular phylogenies of the 11 taxa listed by Zink and Hackett (1988) also indicate that relationships among these areas follow the pattern ([west + southwest] + east). Phylogenies of orioles (Freeman and Zink 1995), and flickers (Moore et al. 1991) support the same pattern of area relationships indicated by the phylogeny of *Piranga*. Additional phylogenetic studies of co-distributed taxa are needed to fully address the strength of this area cladogram.

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APPENDIX. Voucher numbers of specimens used in morphological analyses. See Table 1 for institutional abbreviations. Abbreviations after MZFC refer to collector's initials.

AMNH: 198831, 36413, 40857, 46739, 63942, 65943, 66787, 66789, 66790, 66791, 66792, 71972, 110274, 110469, 113084, 154512, 176342, 180863, 183006, 235319, 235320, 235321, 235322, 364065, 364094, 364106, 364114, 387961, 392574, 392578, 392586, 510309, 510350, 510355, 510356, 510357, 808804

MVZ: 294, 5950, 13415, 13425, 14935, 16130, 36713, 36715, 40110, 41619, 54355, 76563, 84139, 86476, 86478, 86481, 86483, 96637, 105072, 105076, 105077, 105078, 108844, 114467, 114469, 114470, 114475, 121933, 124536, 126289, 126290, 133086, 135780, 139019, 139021, 139023, 139024, 142971, 147449, 147478, 150084, 153529, 156375, 161190, 161191, 161192, 161651, 162131, 191198, 105083, 105086, 135776, 139022, 5299, 64883, 78761

MZFC: AGNS 0300, AGNS 478, BMM 496, BMM 820, FMNH 7901, JEMP 0002, JEMP 044, JEMP 0067, JEMP 0079, JEMP 0128, JEMP 0285, JEMP 0354, JEMP 0364, JEMP 0418, OMVP 552