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Molecular phylogenetics and biogeography of Neotropical tanagers in the genus *Tangara*

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Abstract

Species in the genus *Tangara* are distributed throughout the New World tropics and vary in their morphology, behavior, and ecology. We used data from the cytochrome *b* and ND 2 genes to provide the first phylogenetic perspective on the evolution of this diversity. Reconstructions based on parsimony, maximum likelihood, and Bayesian approaches were largely congruent. The genus is monophyletic and consists of two main clades. Within these clades, DNA sequence data confirm the monophyly of most previously recognized species groups within *Tangara*, indicating general concordance between molecular data and impressions based on geographic distribution, morphology, and behavior. Within some currently recognized species, levels of DNA sequence variation are larger than expected, suggesting multiple taxa may be involved. In contrast, some currently recognized species are only weakly differentiated from their sister species. Biogeographic analyses indicate that many early speciation events occurred in the Andes. More recently, dispersal events followed by subsequent speciation have occurred in other geographic areas of the Neotropics. Assuming a molecular clock, most speciation events occurred well before Pleistocene climatic cycles. The time frame of *Tangara* speciation corresponds more closely to a period of continued uplift in the Andes during the late Miocene and Pliocene.

Keywords: *Tangara*; Biogeography; Systematics; Birds; Andes; Tanager; Neotropics

1. Introduction

The avian genus *Tangara* contains 49 species (Sibley and Monroe, 1990), more than any other genus of Neotropical birds (Isler and Isler, 1999). Species in this genus show substantial variation in plumage coloration, geographic distribution, habitat preference, and foraging behavior. Of all these characteristics, their complex and elaborate plumage patterns are perhaps their best known and most striking feature. The different species have a variety of contrasting color patches on regions such as the crown, face, throat, back, rump, belly, wing, and tail. *Tangara* species are found throughout tropical and subtropical America from sea level to near tree line; thus, these birds are an important part of one of the most diverse regions in the world. Although many species have restricted distributions and distinct habitat preferences (Isler and Isler, 1999), the degree of sympatry is exceptional. For example, as many as 10 species can be found in the same Andean cloud forest (Isler and Isler, 1999; Naoki, 2003). Where syntopic, *Tangara* species show ecological segregation in the way they forage on insects (Hilty cited in Ridgely and Tudor, 1989; Isler and Isler, 1999; Naoki, 2003; Snow and Snow, 1971). Each species tends to specialize on a particular foraging behavior such as foliage gleaning, searching bark on larger branches or smaller twigs, searching moss-covered branches, and aerial foraging for flying insects (Isler and Isler, 1999). The lack of a phylogeny for *Tangara* has hindered the study of the evolution of this behavioral, ecological, and morphological diversity. In addition, a phylogeny for the group is needed to understand the biogeographic history of the group.

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Several recent studies have examined the biogeography of Neotropical birds using molecular data. Most have involved taxa confined to either highland (e.g., Chesser, 2000; García-Moreno et al., 1998, 2001; García-Moreno and Fjeldså, 1999; Roy et al., 1999) or lowland areas of the Neotropics (e.g., Bates et al., 1999; Hackett, 1996; Hackett and Lehn, 1997; Marks et al., 2002). The genus *Tangara* as a whole is widespread in both these areas, but with a few exceptions, individual species have distributions restricted to particular montane or lowland areas (Isler and Isler, 1999). Many species are endemic to the Andes, but some are restricted to other areas such as Amazonia, the Atlantic Forest of eastern Brazil, the Chocó lowlands, and the Chiriquí-Darién highlands (Parker et al., 1996). Of the 22 zoogeographic regions defined by Parker et al. (1996) for the Neotropics, species of *Tangara* are found in all but eight. Thus, a biogeographic analysis of *Tangara* could provide an opportunity to examine historical connections among these different areas. Specifically, a study of *Tangara* biogeography could address whether montane Andean taxa are the result of more recent speciation events (Bates and Zink, 1994; Fjeldså, 1994; Roy et al., 1997) or whether their lowland counterparts are more recently derived (Voelker, 1999).

Although no previous phylogenetic work has explored relationships within *Tangara*, recent molecular phylogenies (Burns, 1997; Burns et al., 2002; Yuri and Mindell, 2002) support the placement of the genus with other genera of tanagers and tanager-finches; this group has been ranked as either a family (Thraupidae; American Ornithologists' Union, 1998; Dickinson, 2003), a subfamily (Thraupinae; Storer, 1970), or a tribe (Thraupini; Sibley and Monroe, 1990). Within *Tangara*, subdivisions based largely on plumage colors and patterns have long been recognized (Sclater, 1857). In the absence of specific phylogenies for *Tangara* species, several linear classifications (e.g., Hellmayr, 1936; Isler and Isler, 1999; Sclater, 1886; Storer, 1970) have been proposed in which species are listed according to their presumed evolutionary relationships. Isler and Isler (1999) largely followed Storer's (1970) arrangement except for the position of two species and the addition of one newly described species. Isler and Isler (1999) also classified each species into one of 13 different groups (Fig. 1) based on geographic distributions, morphology, plumage features, behavior, vocalizations, and nest sites. These species groups of Isler and Isler (1999) represent the most recent evolutionary hypothesis concerning *Tangara*, and provide specific predictions against which phylogenies can be tested.

Using data from the cytochrome *b* (cyt *b*) and NADH dehydrogenase subunit 2 (ND 2) genes, this study presents the first phylogenetic hypotheses concerning relationships among species within the genus *Tangara*. This phylogeny is compared to previous taxonomic treatments of the genus, including the species groups of Isler and Isler (1999). In this study, we also use these phylogenies to study the biogeographic history of the group, and elsewhere (Naoki, 2003; Naoki and Burns, in prep.), the phylogenies are used to study behavioral and ecological characters and patterns of community evolution.

2. Materials and Methods

2.1. Taxon Sampling

Outgroups. When choosing among outgroups for a particular study, the sister taxon to the ingroup has been shown to provide the most reliable root (Smith, 1994). Unfortunately, the sister taxon to *Tangara* is unclear and relationships among tanager genera are ambiguous in many cases (Burns, 1997; Klicka et al., 2000; Yuri and Mindell, 2002). Therefore, we included a number of potential outgroups in our study to maximize the probability that our tree of *Tangara* relationships was rooted properly. Based on morphological similarities such as unusual, club-shaped feathers (Innes, 1979; Storer, 1969), the genus *Chlorochrysa* is often considered the closest living relative to *Tangara*. Therefore, we included samples of two of the three species in this genus. A genus-level analysis of tanager relationships using partial sequences of cyt *b* (Burns, 1997) indicated that other genera of tanagers besides *Chlorochrysa* might be more closely related to *Tangara*. Depending on the weighting scheme used, the topologies of the trees of Burns (1997) indicated that either *Chlorochrysa*, *Neothraupis*, or *Iridosornis* is the sister taxon to *Tangara*. Burns et al. (2002, 2003) included better taxon sampling, but were not able to unambiguously identify the sister taxon to *Tangara*. However, these studies showed that *Tangara* belongs to a clade containing the genera *Chlorochrysa*, *Thraupis*, *Iridosornis*, *Pipraeidea*, *Neothraupis*, *Delothraupis*, *Dubusia*, *Chlorornis*, *Anisognathus*, *Buthraupis*, *Cissopis*, *Schistochlamys*, and *Calochaetes*. Yuri and Mindell (2002) found a similar clade; however, they also showed that the genus *Paroaria* (not sampled by Burns et al., 2002, 2003) belonged to this clade as well. As a conservative approach to identifying the closest living relative to *Tangara*, we included all the aforementioned genera (Table 1). For ease of discussion, we will refer to these genera as the "core" tanager clade because they include species often thought to represent typical tanagers. To root the relationships of these taxa, we included six species of "Tholospiza" (*sensu* Burns et al., 2002): *Geospiza fortis*, *Coereba flaveola*, *Tiaris olivacea*, *Tiaris bicolor*, *Loxigilla violacea*, and *Loxipasser anoxanthus*. Tholospiza was chosen to root the tree because it was identified as the sister taxon to the core tanagers (Burns et al., 2002, 2003; Yuri and Mindell, 2002).

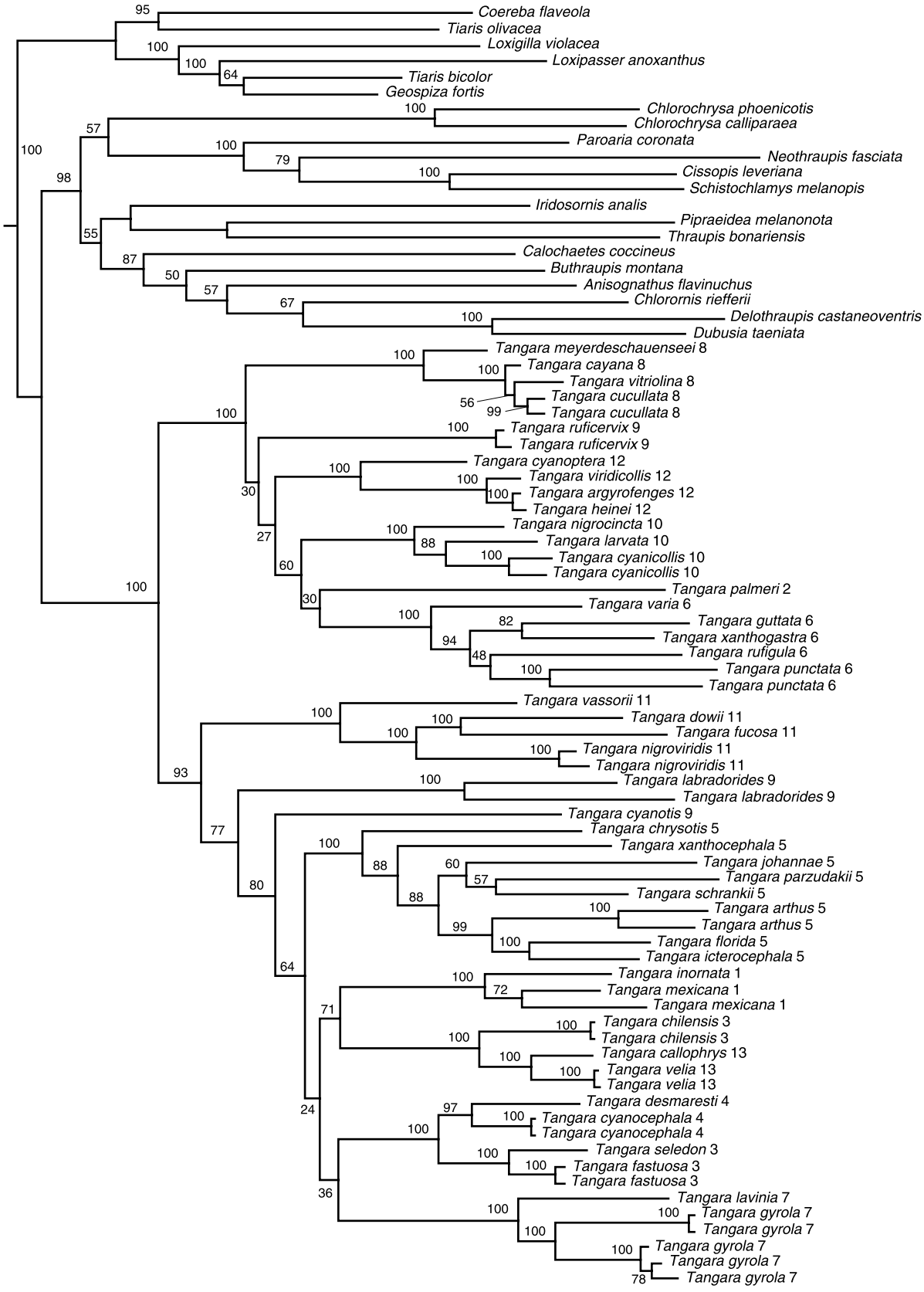

Fig. 1. Consensus tree of the 75,000 trees resulting from the Bayesian analyses with the data partitioned by gene region. Numbers on nodes indicate the posterior probability of a particular clade. Numbers following species names indicate the species group assignment of Isler and Isler (1999).

Table 1
Species names, voucher numbers, and locality information of sequences not previously reported

Species	Museum	Number	Locality
Anisognathus flavinuchus	LSUMNS	B-566	Peru: Dept. Puno, Abra de Maruncunca, 10 km SW San Juan del Oro
Buthraupis montana	LSUMNS	B-365	Peru: Dept. Cajamarca, Cerro Chinguela, 5 km NE Sapalache
Calochaetes coccineus	LSUMNS	B-6134	Ecuador: Prov. Morona-Santiago, W Slope de Cutucci Yapitya
Chlorochrysa calliparaea	LSUMNS	B-8103	Peru: Dept. Pasco, Playa Pampa, about 8 km NW Cushi on trail to Chaglla
Chlorochrysa phoenicotis	LSUMNS	B-34873	Ecuador: Prov. Pichincha, 30 km SE Santo Domingo de los Colorados; 00°16′N, 78°53′W
Chlorornis riefferii	LSUMNS	B-1859	Peru: Dept. Pasco, Cumbre de Ollon, about 12 km E Oxapampa
Cissopis leveriana	LSUMNS	B-1143	Bolivia: Dept. La Paz, Rio Beni, ca 20 km by river N. Puerto Linares
Coereba flaveola	UMMZ	225179	Jamaica: Trelawny Par., Cornwall, Good Hope Plantation
Delothraupis castaneoventris	LSUMNS	B-6931	Peru: Dept. Huanuco, Quebrada Shugush, 30 km on Huanuco-La Union road
Dubusia taeniata	LSUMNS	B-7710	Peru: Dept. Huanuco, Unchog Pass NNW Acomayo
Iridosornis analis	LSUMNS	B-1706	Peru: Dept. Pasco, Santa Cruz, about 9 km SSE Oxapampa
Loxigilla violacea	AMNH	25433	Dominican Republic: Prov. Independencia, Parque Nacional Sierra de Baoruco, Zapoten, Sawmill Clearing
Loxipasser anoxanthus	FMNH	331107	Jamaica: Surrey, Portland, Hollywell Park
Neothraupis fasciata	LSUMNS	B-13914	Bolivia: Dept. Santa Cruz, Serrania de Huanchaca, 45 km E Florida
Pipraeidea melanonota	LSUMNS	B-12070	Ecuador: Prov. Pinchincha, Mindo
Schistochlamys melanopis	LSUMNS	B-9669	Bolivia: Dept. Pando, Nicolas Suarez, 12 km by road S of Cobija, 8 km W on road to Mucden
Tangara argyrofenges	ANSP	4482	Ecuador: Zamora-Chinchipe, Panguri about 12 km NE San Francisco del Vergel, 4°37′S, 78°58′W
Tangara arthus	LSUMNS	B-34876	Ecuador: Prov. Pichincha, 35 km SE Santo Domingo de los Colorados; 00°16′N, 78°50′W
Tangara arthus	LSUMNS	B-22591	Bolivia: Dept. La Paz, Prov. B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata
Tangara callophrys	LSUMNS	B-34961	Ecuador: Prov. Napo, about 20km SSW Loreto; 00°52′N, 77°23′W
Tangara cayana	LSUMNS	B-15414	Bolivia: Dept. Santa Cruz, Serrania de Huanchaca, 45 km E Florida
Tangara chilensis	LSUMNS	B-34947	Ecuador: Prov. Napo, 40 km NNE Tena; 00°44′N, 77°42′W
Tangara chilensis	MVZ	169699	Peru: Dept. Cajamarca, 1 mi N San Jose de Lourdes, Cordillera del Condor
Tangara chrysotis	LSUMNS	B-34927	Ecuador: Prov. Napo, 40 km NNE Tena; 00°44′N, 77°42′W
Tangara cucullata	STRI	GR-TCU2	Grenada: 6.5 km SW Grenville
Tangara cucullata	STRI	SV-TCU2	St. Vincent: Cumberland Valley
Tangara cyanicollis	LSUMNS	B-34904	Ecuador: Prov. Pichincha, 5 km NE Puento Quito; 00°09′N, 79°12′W
Tangara cyanicollis	LSUMNS	B-15352	Bolivia: Dept. Santa Cruz, Serrania de Huanchaca, 45 km E Florida
Tangara cyanocephala	FMNH	427278	Brazil: Pernambuco, Taquaritinga
Tangara cyanocephala	FMNH	427279	Brazil: Pernambuco, Taquaritinga
Tangara cyanoptera	LSUMNS	B-7436	Venezuela: Amazonas Territory, Cerro de la Neblina Camp VII
Tangara cyanotis	LSUMNS	B-22708	Bolivia: Dept. La Paz, Prov. B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata
Tangara desmaresti	FMNH	395478	Brazil: Alagoas, Ibateouara, Envenho Ceimba, Usina Serra Grande
Tangara dowii	LSUMNS	B-16020	Costa Rica: Prov. Heredia, 4 km SE Virgen del Socorro
Tangara fastuosa	FMNH	427276	Brazil: Alagoas, Ibateouara, Envenho Ceimba, Usina Serra Grande
Tangara fastuosa	FMNH	427277	Brazil: Alagoas, Ibateouara, Envenho Ceimba, Usina Serra Grande
Tangara florida	LSUMNS	B-34982	Ecuador: Prov. Esmeraldas, 2 km W Alto Tambo; 00°55′N, 78°35′W
Tangara fucosa	LSUMNS	B-1398	Panama: Prov. Darien, about 9 km NW Cana on slopes Cerro Pirre
Tangara guttata	LSUMNS	B-2190	Panama: Prov. Darien, about 6 km NW Cana
Tangara gyrola	LSUMNS	B-2149	Panama: Prov. Darien, about 6 km NW Cana
Tangara gyrola	LSUMNS	B-14862	Bolivia: Dept. Santa Cruz, Serrania de Huanchaca, 21 km SE Catarata Arco Iris
Tangara gyrola	LSUMNS	B-22850	Bolivia: Dept. La Paz, Prov. B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata
Tangara gyrola	LSUMNS	B-27281	Costa Rica: Prov. Cartago, 28 km ESE Turrialba
Tangara gyrola	LSUMNS	B-4258	Peru: Loreto, Lower Napo region, E bank Rio Yanayacu, ca 90 km N Iquitos
Tangara heinei	LSUMNS	B-34896	Ecuador: Prov. Pichincha, 5 km S Nanegalito; 00°01′N, 74°41′W
Tangara icterocephala	LSUMNS	B-16032	Costa Rica: Prov. Heredia, 4 km SE Virgen del Socorro
Tangara inornata	LSUMNS	B-28766	Panama: Prov. Colon, Achitoe Road, about 2 km Bridge at Rio Providencia
Tangara johannae	LSUMNS	B-29956	Ecuador: Prov. Imbabura, about 20 km N Pedro Vicente Maldonado; about 0°15.63′N, 78°59.70′W

Table 1 (*continued*)

Species	Museum	Number	Locality
Tangara labradorides	LSUMNS	B-32686	Peru: Dept. Cajamarca, Quebrada Las Palmas, about 13 km WSW Chontali; 5°40.0′S, 79°12.2′W
Tangara labradorides	LSUMNS	B-34976	Ecuador: Prov. Pinchincha, 4 km NE Mindo, 00°01′N, 78°44′W
Tangara larvata	LSUMNS	B-34909	Ecuador: Prov. Imbabura, 15 km N Pedro Vicente Maldonado; 00°13′N, 79°02′W
Tangara lavinia	LSUMNS	B-34987	Ecuador: Prov. Esmeraldas, 30 km SE San Lorenzo: 01°05′N, 78°35′W
Tangara mexicana	LSUMNS	B-18465	Bolivia: Dept. Santa Cruz, Velasco; Parque Nacional Noel Kempff Mercado, 86 km ESE of Florida
Tangara mexicana	LSUMNS	B-35572	Brazil: Bahia, about 16 km W Porto Seguro RPPN Vera Cruz
Tangara meyerdeschauenseei	LSUMNS	B-43111	Peru: Dept. Puno, 9.5 km N of Sándia
Tangara nigrocincta	LSUMNS	B-9758	Bolivia: Dept. Pando, Nicolas Suarez, 12 km by road S of Cobija, 8 km W on road to Mucden
Tangara nigroviridis	LSUMNS	B-1627	Peru: Dept. Pasco, Santa Cruz, about 9 km SSE Oxapampa
Tangara nigroviridis	LSUMNS	B-34857	Ecuador: Prov. Pinchincha, 5 km S Nanegalito; 00°01′N, 78°41′W
Tangara palmeri	LSUMNS	B-11999	Ecuador: Prov. Esmeraldas, el Placer; 0°52′N, 78°33′W
Tangara parzudakii	LSUMNS	B-30007	Ecuador: Prov. Esmeraldas, about 2.7 km E Alto Tambo; 00°53′47.1′′N, 78°32′05.2′′W
Tangara punctata	LSUMNS	B-34931	Ecuador: Prov. Napo, about 40km NNE Tena; 00°44′N, 77°42′W
Tangara punctata	LSUMNS	B-35552	Brazil: Para, Fazenda Morelandia, 8 km N. de Santa Barbara, do Para; 1°12′40′′S, 48°14′4′′W
Tangara ruficervix	LSUMNS	B-33410	Peru: Dept. Cajamarca, Las Juntas, 16 km NE junction of Rios Tabaconas and Chinchipe
Tangara ruficervix	LSUMNS	B-8190	Peru: Dept. Pasco, Playa Pampa, about 8 km NW Cushi on trail to Chaglla
Tangara rufigula	LSUMNS	B-11930	Ecuador: Prov. Esmeraldas, el Placer; 0°52′N, 78°33′W
Tangara schrankii	LSUMNS	B-34932	Ecuador: Prov. Napo, 20 km SSW Loreto; 00°52′N, 77°23′W
Tangara seledon	LSUMNS	B-16942	Brazil: Sao Paulo, Salesopolis, E. B. Boraceia
Tangara varia	LSUMNS	B-28010	Peru: Dept. Loreto, about 77 km WNW Contamana; 7°05′S, 75°39′W
Tangara vassorii	LSUMNS	B-1711	Peru: Dept. Pasco, Santa Cruz; about 9 km SSE Oxapampa
Tangara velia	LSUMNS	B-9725	Bolivia: Dept. Pando, Nicolas Suarez, 12 km by road S of Cobija, 8 km W on road to Mucden
Tangara velia	FMNH	390060	Brazil: Rondonia, Cachoeira Nazare, W bank Rio Jiparana
Tangara viridicollis	LSUMNS	B-8090	Peru: Dept. Pasco, Playa Pampa, about 8 km NW Cushi on trail to Chaglla
Tangara vitriolina	LSUMNS	B-34921	Ecuador: Prov. Pichincha, Tumbaco, Avenal, Buena Esperanza; 00°13′N, 78°24′W
Tangara xanthocephala	LSUMNS	B-34922	Ecuador: Prov. Napo, 5 km SE Baeza; 00°30′N, 77°52′W
Tangara xanthogastra	LSUMNS	B-34934	Ecuador: Prov. Napo, 20 km SSW Loreto; 00°52′N, 77°23′W
Thraupis bonariensis	LSUMNS	B-3587	Peru: Dept. Huanuco: Nuevas Flores (Cullquish) on Rio Maranon.
Tiaris bicolor	MVZ	179402	Aviary of Luis F. Baptista, California Academy of Sciences Accn. 5067
Tiaris olivacea	AMNH	25429	Dominican Republic: Prov. Independencia, Parque Nacional Sierra de Baoruco, El Aceitillar, Alcoa Rd.

LSUMNS, Louisiana State University Museum of Natural Science; MVZ, Museum of Vertebrate Zoology at the University of California at Berkeley; FMNH, Field Museum of Natural History; STRI, Smithsonian Tropical Research Institute; ANSP, Academy of Natural Sciences of Philadelphia; UMMZ, University of Michigan Museum of Zoology; and AMNH, American Museum of Natural History.

Ingroup. The genus *Tangara* consists of 49 species (Sibley and Monroe, 1990). Some authorities (e.g., Isler and Isler, 1999; Storer, 1970) considered *Iridophanes pulcherrima* to belong to *Tangara* based on plumage similarities between this species and *Tangara cyanoptera*. However, a recent genetic study (Burns et al., 2003) indicated that this species is not closely related to *Tangara*; thus, we did not include this species in the current study. We were able to include 43 of the 49 recognized species. For six species (*Tangara cabanisi*, *Tangara cyanoventris*, *Tangara peruviana*, *Tangara phillipsi*, *Tangara preciosa*, and *Tangara rufigenis*) tissues were unavailable, and collecting expeditions were beyond the scope of those already planned for this study. All the missing species are morphologically similar to species included in this study, and two (*T. cyanoventris* and *T. preciosa*) are known to hybridize with species we included in the study. Several species of *Tangara* show geographic variation in plumage patterns and colors. Differences within some species are significant enough that multiple subspecies have been described, suggesting that these species may include more than one distinct evolutionary unit. Thus, individuals from multiple subspecies were included for several species. In addition, sequencing multiple individuals from a species also helped verify the accuracy of our sequencing. In total, our ingroup and

outgroup sampling included 80 individuals representing 64 species. Some sequences were obtained from GenBank (AF489901, AF489899, AF489888, AF489887, AF108772, AF447371, AF447282, AF447294, and AF447264). The rest of the sequences are new to this study (Table 1, GenBank Accession Nos. AY383089–AY383239).

2.2. DNA isolation and sequencing

Two mitochondrial genes (cyt *b* and ND 2) were used to infer *Tangara* relationships. These markers were chosen because they have resolved relationships within other closely related species of tanagers (Burns, 1998; García-Moreno et al., 2001; Hackett, 1996). DNA extractions were performed either with a 5% Chelex solution (Walsh et al., 1991) or using the QIAmp DNA MiniKit (Qiagen, Valencia, CA). For ND 2, the first 330 base pairs were amplified using primers L5215 and H5578 (Hackett, 1996). The entire cyt *b* gene (1143 base pairs) was amplified in three overlapping segments using primer pairs H15297/L14851, H15710/L15206, and H16058/L15656 (Groth, 1998). Reactions were performed in 10 μl capillary tubes and typically involved 40 amplification cycles in a hot-air thermocycler (3 s at 94 °C, 0 s at 43–50 °C, and 30 s at 71 °C). Agarose plugs were taken and diluted in 250 μl of water. Plugs were then melted and 3 μl of this solution was re-amplified in a 40 μl total reaction volume. Typical re-amplification involved 41 cycles (12 s at 94 °C, 4 s at 52 °C, and 26 s at 71 °C). Final PCR product was purified using the GeneClean Kit (Bio101) and cycle sequenced (96 °C for 1 min, 96 °C for 30 s, 50 °C for 15 s, 60 °C for 4 min—28 cycles) using Big Dye terminator reaction mix (Applied Biosystems, Foster City, CA). Samples were passed through spin columns containing Sephadex beads before being sequenced on an ABI 377 DNA sequencer (Applied Biosystems). Sequencher (Gene Codes, Ann Arbor, MI) was used to reverse complement opposing directions, to align different fragments from the same individual, and to translate complete sequences into amino acids. Precautions against nuclear copies include sequencing both heavy and light strands, using overlapping fragments of cyt *b* (approximately 12% of the total sequence is overlapped by two fragments), checking that amino acid translation is possible without stop codons or gaps, and comparing levels of sequence divergence separately for the three cyt *b* fragments (as suggested by Hackett et al., 1995).

2.3. Phylogenetics

Phylogenetic analyses were performed using Bayesian, maximum likelihood, and parsimony approaches. Two sets of Bayesian analyses were performed: one with data partitioned by gene and one with data partitioned by codon position as well as by gene. We used ModelTest, vers. 3.06 (Posada and Crandall, 1998) to choose the best fit model via a likelihood ratio test for each gene separately, in case different models were optimal for the different gene regions. We then used the chosen model ($GTR + I + \Gamma$ was identified for each gene) in conjunction with MrBayes 3.0b3 (Huelsenbeck and Ronquist, 2001) to perform Bayesian analyses on the data set. Our analyses did not specify values for specific nucleotide substitution model parameters. Thus, parameters were treated as unknown variables with uniform prior values and estimated as part of the analysis. We partitioned the data separately by gene and allowed the parameters to be determined separately for each gene by unlinking them prior to analysis. This was done to account for potential differences in optimal model parameters for the two genes. This analysis was run for two million generations and sampled every 100 generations, resulting in 20,000 samples. Four Markov Chain Monte Carlo chains were run for each analysis. Resulting log-likelihood scores were plotted against generation time to identify the point at which log-likelihood values reached a stable equilibrium value. Sample points prior to this point of stationarity were discarded as "burn-in" samples. The remaining samples were used to produce a majority rule consensus tree, with the percentage values indicating the percentage of samples that identified a particular clade (the clade's posterior probability). In Bayesian analyses, posterior probabilities are true probabilities of clades such that values of 95% or greater deemed significantly supported. We repeated the analysis five times to ensure that results were not dependent on the initial random starting tree used. For these repeated analyses, we compared log-likelihood values and posterior probabilities of each repeated analysis to confirm that using a different starting tree did not alter our results significantly.

Our sequences are protein-coding; therefore, rates of substitution are expected to vary among different codon positions. Therefore, we performed an additional set of Bayesian analyses in which we divided the data into six partitions (one for each codon position in each gene region) and allowed parameters to vary independently for each partition. These analyses were run for one million generations, sampling every 100 generations. Thus, each analysis resulted in 10,000 samples. Other conditions for this set of Bayesian analyses were as described above.

To explore the robustness of our data to different phylogenetic methods, we also analyzed the data using maximum likelihood and parsimony as implemented in PAUP* 4.0b10 (Swofford, 2002). To choose our maximum likelihood model and parameters, we again used ModelTest, vers. 3.06 (Posada and Crandall, 1998) to choose the best fit model for the combined data set (maximum likelihood does not allow mixed-model analyses). Using a starting tree constructed using

neighbor joining and Kimura 2-parameter distances, we chose the best fit model by a likelihood ratio test. The chosen model (TVM + I + Γ) and parameters were then used in a maximum likelihood analysis with 10 random addition replicates.

For parsimony analyses, we used the heuristic search option with 1000 random addition replicates and the tree-bisection-reconnection branch swapping algorithm. Scatterplots of pairwise sequence divergence for each codon position indicate that third position sites may be saturated for transitions. Therefore, in addition to equally weighted analyses, we performed additional parsimony analyses in which third position transitions were downweighted relative to the other sites. To explore the sensitivity of the data to different degrees of downweighting, we downweighted third position transitions by a factor of five and 50. Equally weighted analyses were bootstrapped (Felsenstein, 1985) with 100 random addition replicates for each of 1000 bootstrap replicates. Downweighted analyses were also bootstrapped for 1000 replicates, but with 10 random addition replicates for each bootstrap replicate.

2.4. Biogeography

To infer the biogeographic history of *Tangara* species, we used two different methods that allow for the consideration of both dispersal and vicariant events: ancestral area analysis of Bremer (1992, 1995) and dispersal–vicariance analysis of Ronquist (1997). These methods adopt a parsimony approach and use a phylogeny and the current geographic range of the taxa to infer the historical areas of origin of ancestors of a monophyletic clade of organisms. These methods do not assume vicariance as the sole divergence method, but instead allow that some members of a group may have dispersed from smaller centers of origin. Both methods were used in conjunction with all of our Bayesian and likelihood trees. We used the distributional data in Parker et al. (1996) to assign zoogeographic regions to different species. Bremer's ancestral area analysis (Bremer, 1992, 1995) compares the number of gains under forward Camin–Sokal parsimony relative to the number of losses under reverse Camin–Sokal parsimony. Areas having a higher number of gains relative to losses for a particular clade (a higher gain/loss quotient) have a higher probability of being part of the ancestral area of that clade. We used ancestral area analysis to identify the geographic distribution of the ancestor of *Tangara*. Dispersal–vicariance analysis (Ronquist, 1997) reconstructs ancestral distributions and dispersal events on a phylogeny such that the number of dispersal and extinction events are minimized. We used DIVA vers. 1.1 (Ronquist, 1996) to reconstruct the history of dispersal and vicariant events throughout the phylogeny of *Tangara* using Ronquist's (1997) method. We limited the number of ancestral areas for each node to no more than two using the "max areas" option.

3. Results

3.1. Sequence variation

As expected for protein coding mitochondrial genes, all sequences aligned without gaps or insertions. For cyt *b*, of the 1143 sites, 452 (40%) were variable. Of the 330 bases of ND 2, 156 (47%) were variable. Levels of uncorrected sequence divergence ("*p*"-distance of Nei (1987)) among all taxa ranged from 0 to 12.8% (mean = 8.9%) for cyt *b* and 0–18.8% (mean = 12.8%) for ND 2. Individuals that had identical sequences were from the same species. Maximum observed divergence represent comparisons between outgroups and species in the genus *Tangara*. When only members of the genus *Tangara* were included, the maximum divergence was 12.2% for cyt *b* and 16% for ND 2. Although ND 2 was on average more variable and showed greater levels of maximum divergence than cyt *b*, this was not true of comparisons within species. For several species (*Tangara chilensis*, *Tangara fastuosa*, *Tangara labradorides*, *Tangara nigroviridis*, and *Tangara velia*), levels of genetic variation were actually lower for ND 2 than for cyt *b*.

Base composition (guanine 13.6%, adenine 27.8%, thymine 23.8%, and cytosine 34.8%) was similar to that reported in other studies of mitochondrial DNA in passerine birds (Burns et al., 2002; Edwards et al., 1991; Helm-Bychowski and Cracraft, 1993). Changes at third position sites were more common than changes at second and first position sites. Of the 782 variable sites, 124 were first positions, 33 were second positions, and 451 were third positions. Using pairwise comparisons of uncorrected distance among species, the average transition to transversion ratio was 4.2 when considering all taxa and 4.7 when considering only species within *Tangara*.

3.2. Phylogenetics

In the Bayesian analyses with data partitioned by gene, log-likelihood values reached a stable equilibrium well before 500,000 generations. Thus, we chose a burn-in value of 5000 samples for each analysis. The five repeated analyses converged on similar posterior probabilities and likelihood values, indicating insensitivity to initial starting tree. Thus, we combined the trees of all five analyses to construct a majority rule consensus tree of 75,000 trees (Fig. 1). The genus *Tangara* was identified as a monophyletic group with a posterior probability of 100%. In addition, all species for which we included more than one individual were also

monophyletic. The earliest divergence within *Tangara* is a split between two main clades. One clade contains all members of species groups 2, 6, 8, 10, and 12 as well as one member of species group 9 (*Tangara ruficervix*). The other clade contains all members of species groups 1, 3, 4, 5, 7, 11, and 13 as well as two species from species group 9 (*T. labradorides* and *T. cyanotis*). Two of the species groups of Isler and Isler (1999) are not monophyletic (9 and 3; Fig. 1), and only one species from group 2 was included in this study. The other 10 species groups are all monophyletic and well supported (Fig. 1, Table 2). Nine of the groups have a posterior probability of 100%, and the remaining group (group 4) has a 97% posterior probability.

In the Bayesian analysis partitioned by codon position as well as by gene, log-likelihood values converged on a stable equilibrium well before 150,000 generations. Thus, we chose a burn-in value of 1500 samples for each analysis. All five analyses converged on similar posterior probabilities and likelihood values; thus, results of the five analyses were combined to construct a majority-rule consensus tree of the 42,500 trees (Fig. 2). This tree was

Table 2
Level of support for strongly supported clades in Bayesian (partitioned by gene and partitioned by codon and gene) and parsimony (equal weight (EW), 5:1, and 50:1) analyses

Node	Bayesian		Parsimony		
	Gene (%)	Codon and gene (%)	EW	5:1	50:1
Nodes not involving Tangara					
"Core" tanagers	100	100	97%	99%	98%
Coereba and *Tiaris olivacea*	95	85	—	—	52%
Loxigilla, *Loxipasser*, *Tiaris bicolor*, and *Geospiza*	100	100	64%	83%	83%
Loxipasser, *Tiaris bicolor*, and *Geospiza*	100	100	—	76%	84%
Tiaris bicolor amd *Geospiza*	64	79	77%	95%	98%
All "core tanagers" except *Tangara*	98	86	—	—	—
Chlorochrysa	100	100	100%	100%	100%
Paroaria, *Neothraupis*, *Cissopis*, and *Schistochlamys*	100	100	72%	65%	54%
Cissopis and *Schistochlamys*	100	100	99%	98%	97%
Calochaetes, *Anisognathus*, and *Buthraupis*, *Chlorornis*, *Delothraupis*, and *Dubusia*	87	99	M	M	M
Anisognathus and *Calochaetes*	—	—	71%	—	—
Delothraupis and *Dubusia*	100	100	96%	100%	100%
Nodes involving Tangara					
Tangara	100	100	96%	99%	99%
Species groups 2, 6, 8, 10, 12, and *T. ruficervix*	100	100	83%	95%	92%
Species group 8	100	100	100%	100%	99%
T. cayana, *T. vitriolina*, and *T. cucullata*	100	100	100%	100%	98%
Species group 12	100	100	94%	99%	95%
T. viridicollis, *T. argyrofenges*, and *T. heinei*	100	100	100%	100%	100%
T. argyrofenges and *T. heinei*	100	100	98%	100%	99%
Species group 10	100	100	100%	99%	95%
Species group 6	100	100	91%	99%	97%
T. guttata, *T. rufigula*, *T. xanthogastra*, and *T. punctata*	94	97	—	M	—
T. palmeri and *T. ruficervix*	—	—	—	79%	70%
Species groups 1, 3, 4, 5, 7, 11, 13, *T. labradorides* and *T. cyanotis*	93	97	M	53%	62%
Species group 11	100	100	99%	100%	100%
T. dowii, *T. fucosa*, and *T. nigroviridis*	100	100	97%	80%	56%
T. dowii and *T. fucosa*	100	100	55%	59%	58%
Species group 5	100	100	M	M	M
T. arthus, *T. florida*, and *T. icterocephala*	99	100	73%	M	M
T. florida and *T. icterocephala*	100	99	69%	79%	M
Species group 1	100	100	100%	99%	92%
Species group 13 and *T. chilensis*	100	100	100%	100%	99%
Species group 13	100	98	89%	96%	93%
Species group 4, *T. seledon*, and *T. fastuosa*	100	100	100%	100%	99%
Species group 4	97	79	95%	97%	91%
T. seledon and *T. fastuosa*	100	100	99%	100%	96%
Species group 7	100	100	100%	100%	100%

All clades with either >70% bootstrap support or >95% posterior probability are reported, except for those identifying the monophyly of a species. M = clade is monophyletic in the most parsimonious trees, but not recovered in >50% of bootstrap replicates. Dash indicates the clade was not recovered in the analysis.

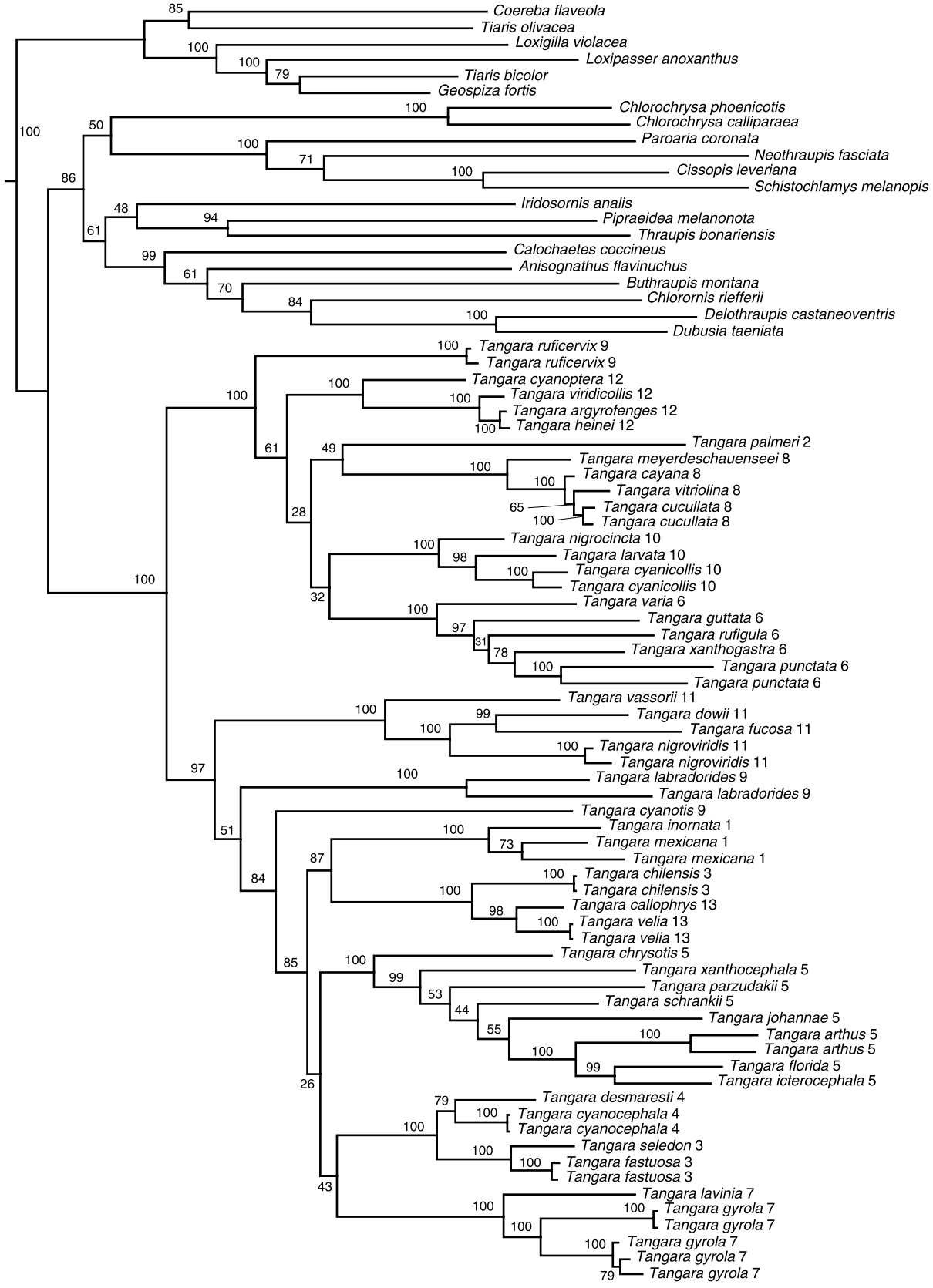

Fig. 2. Consensus tree of the 42,500 trees resulting from the Bayesian analyses with the data partitioned by gene region as well as by codon position. Numbers on nodes indicate the posterior probability of a particular clade. Numbers following species names indicate the species group assignment of Isler and Isler (1999).

similar to our other Bayesian tree (Fig. 1) in topology and posterior probabilities. *Tangara* monophyly is again recovered with 100% posterior probability, the same two major clades are identified, and 10 of the 13 species groups are again monophyletic. Monophyly of the species groups is 98% or greater in nine of these species groups, and the remaining group (4) had a posterior probability of 79%. Differences between the trees include the placement of *T. ruficervix*, the placement of *Tangara palmeri*, the relative positions of species groups 12 and 8, and the position of species group 5 relative to the clade containing species groups 1, 13, and *T. chilensis*.

Our maximum likelihood tree (not shown, −ln likelihood = 19491.55) was similar to the Bayesian tree partitioned by gene only (Fig. 1). The only difference between the two trees was that maximum likelihood identifies the relationship between species group 6, species group 10, and *T. palmeri* as an unresolved trichotomy.

For the parsimony analyses, 544 (89%) of the 608 variable sites were phylogenetically informative. Number of trees found, consistency indices excluding uninformative characters, and number of bootstrap nodes above 50% for each of the three analyses are as follows: equal weighting (78 trees, 0.21, 51 nodes), 5:1 weighting (2 trees, 0.26, 51 nodes), and 50:1 weighting (1 tree, 0.29, 51 nodes). In general, the consistency index increased and fewer most-parsimonious trees were found in the analyses which downweighted third position transitions to a greater degree. Results of the parsimony analyses are consistent with the Bayesian and maximum likelihood analysis. Nodes that are resolved and show bootstrap support typically have high posterior probabilities, and only a few nodes show strong support in only the Bayesian or parsimony analyses (Table 2). In agreement with Bayesian trees, in all parsimony analyses *Tangara* is recovered as a monophyletic group, two major clades are identified, monophyly was recovered for 10 species groups, and all species were monophyletic. Although monophyletic in all most-parsimonious trees, species group 5 was not supported by bootstrap analysis. The remaining nine groups had bootstrap values of 91% or greater regardless of weighting scheme. Agreement among methods of analysis indicates general insensitivity to model of evolution.

3.3. Biogeography

Reconstructing the distributional history of *Tangara* indicates that the group as a whole originated in the northern Andes (Table 3, Fig. 3). Ancestral area analysis (Bremer, 1992, 1995) using either Bayesian tree (Table 3) or the maximum likelihood tree identifies the northern Andes as the most probable region where the ancestor to *Tangara* lived. Although DIVA indicates that many nodes have multiple equally likely distributions (Fig. 3), several patterns emerge that are consistent regardless of the exact reconstruction. As in the ancestral area analysis, DIVA also reconstructs the distribution of the oldest node in the group as being the Northern Andes (Fig. 3). In addition, tracing the dispersal and vicariance history of *Tangara* using DIVA shows that many early nodes were northern Andean as well. The two major clades resulting from the first split within the group are both reconstructed as northern Andean. Furthermore, many of the older speciation events within these two major clades involved the northern Andes. Clearly, the Andes were important in the early evolution of *Tangara*. However, subsequent dispersal into other Neotropical regions resulted in further diversification in the group. These include recent dispersal into and subsequent speciation in Amazonia (groups 1, 13, and *T. chilensis*), the central Andes (group 12), the Chiriquí-Darién Highlands (group 11), and the Atlantic Forest (groups 3

Table 3
Probability that different geographic regions were part of the distribution of the ancestor to *Tangara*

Zoogeographic region	Gene partitioned tree				Gene and codon partitioned tree			
	Gains	Losses	Gains/losses	Ancestral area	Gains	Losses	Gains/losses	Ancestral area
Northern Andes	18	14	1.29	1.00	19	14	1.34	1.00
Central Andes	15	16	0.94	0.72	15	17	0.88	0.66
Northern Amazonia	9	19	0.47	0.36	8	18	0.44	0.33
Southern Amazonia	9	19	0.47	0.36	9	19	0.47	0.35
Northern South America	3	16	0.19	0.14	3	14	0.21	0.15
Tepuis	5	15	0.33	0.26	5	15	0.33	0.25
Central South America	2	8	0.25	0.19	2	9	0.22	0.19
Lesser Antilles	1	5	0.20	0.15	1	8	0.13	0.09
Atlantic Forest	3	9	0.33	0.26	3	9	0.33	0.25
Chiriquí-Darién Highlands	5	18	0.28	0.22	5	19	0.26	0.20
Gulf-Caribbean Slope	5	21	0.24	0.19	5	21	0.24	0.18
Chocó Lowlands	5	16	0.31	0.24	5	17	0.29	0.22

Ancestral area (Bremer, 1992, 1995) refers to the probability that an area is ancestral and is equal to the gains/losses value divided by the largest gains/losses value of all areas. Highest ancestral area value indicates the area most likely to be part of the ancestral distribution.

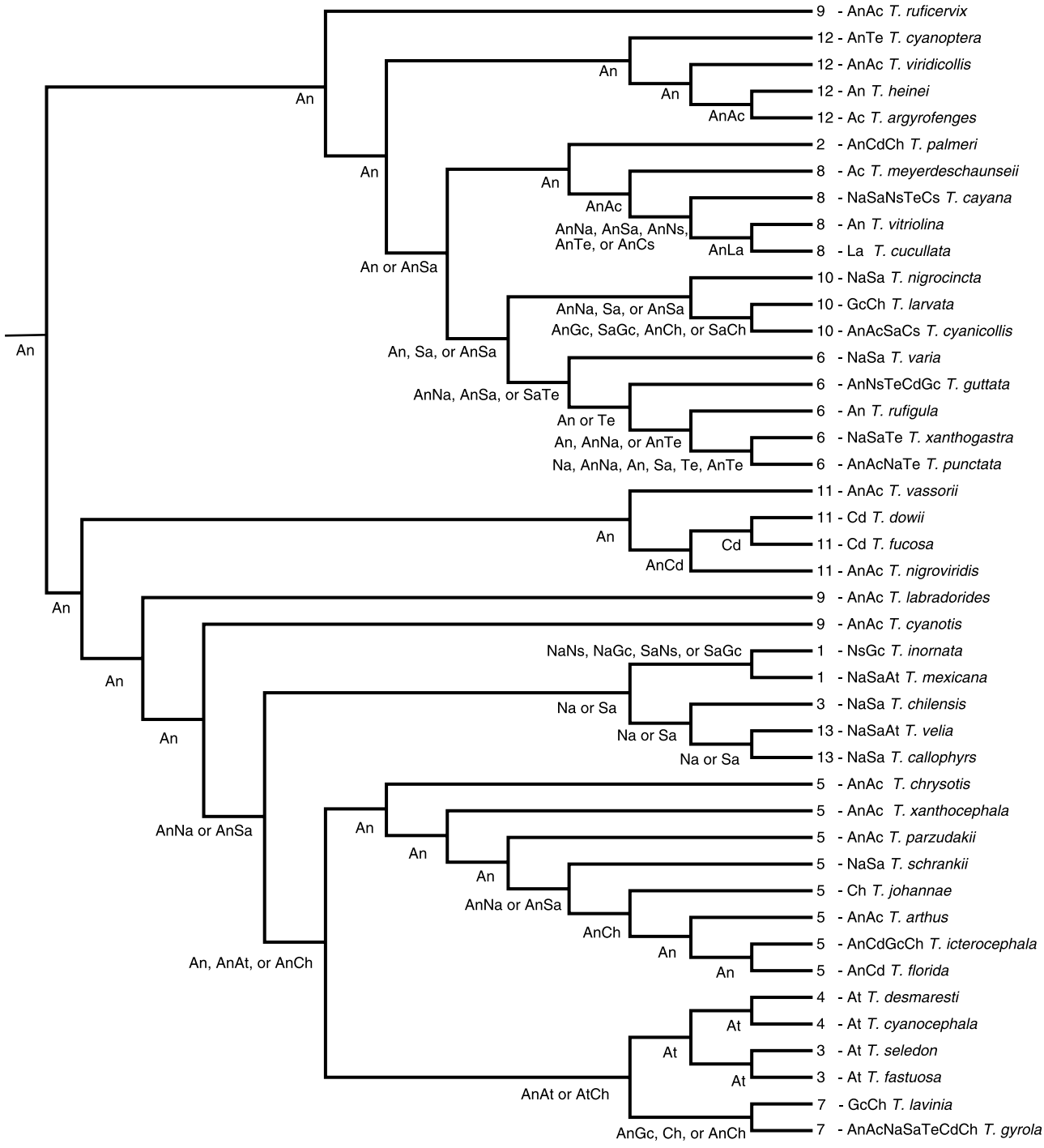

Fig. 3. Reconstruction of the biogeographic history of *Tangara* using Dispersal Vicariance Analysis. Numbers at terminals indicate the species group assignment of Isler and Isler (1999). Letters indicate zoogeographic region: An, Northern Andes; Ac, Central Andes; Mh, Madrean Highlands; Na, Northern Amazonia; Sa, Southern Amazonia; Ns, Northern South America; Te, Tepuis; Cs, Central South America; La, Lesser Antilles; At, Atlantic Forest; Cd, Chiriquí-Darién Highlands; Gc, Gulf-Caribbean Slope; and Ch, Chocó Lowlands. Tree shown is Bayesian analyses with data partitioned by gene region and codon position.

and 4, excluding *T. chilensis*). For example, the close relationship of the Atlantic Forest species *Tangara seledon*, *T. fastuosa*, *Tangara desmaresti*, and *Tangara cyanocephala* indicates that these four species likely descended from an ancestor that also lived in the Atlantic Forest, and their speciation is not the result of repeated colonization into the area. Although many recent speciation events involve areas outside the Andes, the northern Andes continued to play a role in recent speciation events as well (e.g., group 5).

4. Discussion

4.1. Comparisons with previous data sets—outgroups

In agreement with previous studies (Burns et al., 2002, 2003; Yuri and Mindell, 2002), all analyses supported a monophyletic clade of "core" tanagers that include *Tangara* and a variety of other species traditionally considered representative of tanagers (Figs. 1 and 2, Table 2). Within this "core" tanager clade, analyses differed in the identification of the closest living relative to *Tangara*. Both sets of Bayesian analyses and the maximum likelihood analysis identified the sister group to *Tangara* as a clade containing all other species of "core" tanagers (Figs. 1 and 2, Table 2). Support for this relationship was stronger in the analysis partitioned by gene only. In the parsimony analyses, the non-*Tangara* "core" tanager sequences were paraphyletic. The parsimony analyses varied in which species they identified as the sister taxon to *Tangara*. Trees from the equally weighted parsimony analyses did not agree on which of these species was the sister taxon to *Tangara*. Both the 5:1 and 50:1 analyses identified a clade containing *Neothraupis*, *Paroaria*, *Cissopis*, and *Schistochlamys* as the sister taxon. However, this relationship was not supported by bootstrap analyses (Table 2).

Based on plumage similarities, *Chlorochrysa* and *Tangara* have long been thought to be closely related; therefore, Innes (1979) suggested that species of *Chlorochrysa* should be placed within *Tangara*. However, none of our phylogenetic analyses identified *Chlorochrysa* as the sister taxon to *Tangara*; this indicates that plumage similarities may be the result of convergence. We recommend further sampling of species within the "core" tanager group to help clarify the closest living relative to *Tangara*. Nonetheless, results of this study and other recent studies confirm the monophyly of a "core" tanager group that includes *Tangara*. In addition, the monophyly of *Tangara*, to the exclusion of *Chlorochrysa*, is confirmed.

4.2. Comparisons with previous data sets—Tangara relationships

No previous phylogeny has investigated relationships within *Tangara*. However, linear-sequence classifications (e.g., Storer, 1970), have ordered taxa based on their presumed evolutionary relationships. The 13 species groups of Isler and Isler (1999) represent the most explicit phylogenetic statement to date concerning relationships of *Tangara*. These species groups were based on non-molecular characters, and show remarkable congruence to the results of this study. Ten of these groups were monophyletic in our study (Figs. 1 and 2, Table 2). For one of the remaining three groups (group 2) only one species, *T. palmeri* was sampled, so we can not comment on the monophyly of this group. Support is weak for the exact placement of *T. palmeri* in our trees, and its position differs between the two Bayesian trees. Inclusion of the only other member of its species group (*T. cabanisi* from southern Mexico and Guatemala) might help clarify the relationships of *T. palmeri* to other *Tangara*. The remaining two groups (group 9 and group 3) were not recovered as monophyletic in our analyses. For group 9, we sampled all three species and our results clearly show that they do not form a monophyletic group. *T. ruficervix* is found in one of the two main clades, whereas *T. labradorides* and *T. cyanotis* are found in the other. *T. labradorides* and *T. cyanotis* are paraphyletic relative to the other species of *Tangara*, and they show more plumage similarities to each other than they do to *T. ruficervix*. However, the exact position of any of these species is not strongly supported. The third species group for which monophyly was not recovered (group 3) contains three species (*T. chilensis*, *T. seledon*, and *T. fastuosa*). The phylogenetic relationship of these species to other *Tangara* agrees with their biogeographic distributions, but indicates that some plumage similarities are the result of convergence. These species have been linked based on similar plumage characters such as a greenish head, black on the back, and a colored rump. In our phylogenies (Figs. 1 and 2), *T. chilensis* is more closely related to members of species group 13 (*Tangara callophrys* and *T. velia*), and *T. seledon* and *T. fastuosa* form a sister clade to the clade containing members of group 4 (*T. desmaresti* and *T. cyanocephala*). *T. chilensis* shares some plumage similarities with members of 13 and also shares a similar Amazonian distribution. The monophyletic relationship of *T. seledon*, *T. fastuosa*, *T. desmaresti*, and *T. cyanocephala* is also supported by biogeographic similarities. All four species are found in the Atlantic Forest of eastern Brazil.

Other than the relationship between the clade containing *T. seledon* and *T. fastuosa* (group 3) and the clade containing *T. desmaresti* and *T. cyanocephala* (group 4), numerically adjacent species groups are not closely related to each other. In addition, species groups are scattered between the two clades, regardless of where they are in the arrangements. Thus, although some species were arranged in linear taxonomies in order of the presumed relationships, the arrangement of *Tangara* in linear classifications does not reflect their evolutionary relationships to one another beyond the species groups outlined by Isler and Isler (1999).

4.3. Comparisons with previous data sets—species limits

Levels of sequence variation within species are affected by a variety of factors including time since the cessation of gene flow, different rates of sequence evolution, population size, and past history of bottlenecks.

Detailed phylogeographic studies involving numerous individuals across the range of a species are needed to attempt to tease apart these often conflicting factors. Despite this difficulty, because no previous genetic data have been reported for these birds, we wish to point out a few cases in which levels of sequence divergence indicate that species status of some *Tangara* warrant further investigation. Throughout this section, we wish to emphasize that levels of sequence divergence should not be used as a criterion for determining species status, and further studies involving multiple individuals will be needed in which species status is addressed in terms of either monophyly or reproductive isolation.

Within *Tangara*, some species show higher than expected levels of intraspecific sequence divergence. In contrast, some species are only weakly differentiated from their sister species. Levels of cyt *b* intraspecific sequence divergence in birds can vary widely, but most species typically show levels of variation below or near 1% (Avise and Walker, 1998; Ditchfield and Burns, 1998). Species showing levels of variation much greater than this usually represent species that have well-differentiated geographic units. Their species status is often later reassessed in light of degree of molecular divergence (e.g., Chesser, 1999; Zink et al., 1997). As expected, within *Tangara*, many species surveyed for more than one individual displayed less than 1.0% sequence divergence. These species include individuals from the same population (*T. cyanocephala*, *T. fastuosa*), adjacent island populations (*Tangara cucullata*), individuals from populations on the same slope of the Andes (*T. chilensis*, *T. ruficervix*), or relatively nearby populations in the Amazon basin (*T. velia*). Two species that were surveyed on both the east and west slope of the Andes (*Tangara cyanicollis* and *T. nigroviridis*) had slightly higher levels of divergence (1.8 and 1.0%); however, this amount of divergence is still typical of that observed among individuals of the same bird species.

Five species we studied showed higher than expected levels of divergence. We discuss the data for each species below, and suggest they be used as guidelines for future studies. For three of these species (*T. labradorides*, *Tangara arthus*, and *Tangara gyrola*), the Andes appear to act as a barrier, contributing to observed high levels of differentiation. For *T. labradorides*, two individuals were sequenced, one from the west slope of the Andes in Ecuador and one from the east slope in Peru. These individuals were from allopatric parts of the range and differed by 6.6% of their sequence. This is the highest level of intraspecific sequence divergence we observed, and is well above the level of divergence observed between some other species of *Tangara* whose status is not questioned. For example, *T. chilensis*, *T. callophrys*, and *T. velia* co-occur without interbreeding in parts of Amazonia, yet the average level of sequence divergence observed among these undisputed species is 3.9%. As in our sampling of *T. labradorides*, our two individuals of *T. arthus* were also from opposite sides of the Andes, and they also showed a relatively large amount of sequence divergence (3.6%). We sampled five individuals of *T. gyrola*, one of the most widespread and variable species of *Tangara*. Because of distinct morphological differences across the range of this species, three species may be involved (American Ornithologists' Union, 1998; Sibley and Monroe, 1990). All individuals we sampled were from one of these putative species (*Tangara gyroloides*, the Bay-and-blue Tanager). These five individuals were divided into two strongly supported, monophyletic groups, one that included two samples from Central America and one that included three samples from South America, on the eastern slope of the Andes. Levels of divergence between these two groups was high, averaging 4.6%. However, levels of variation within the two groups were much lower, being less than or just over 1% sequence divergence. Thus, populations from Central America seem to have been separated from those of the eastern Andes for a long time, with high levels of gene flow likely among populations within the two sampled regions. Two additional species also show high levels of intraspecific divergence. The range of *Tangara punctata* includes two allopatrically distributed portions, one in the Andes and one in Amazonia. We sampled one individual from each of these areas, and these two individuals differed by 6.0% sequence divergence. The range of *Tangara mexicana* is also separated into two allopatric areas, one in Amazonia and one in southeastern Brazil. The two individuals we sampled from these areas show high levels of divergence (4.1%), indicating a relatively long history of separation between individuals in these two areas.

In addition to providing information on levels of intraspecific divergence, our data also provide information on interspecific sequence divergence that indicate some currently described species are only weakly differentiated from their closest living relative. That is, unusually low levels of sequence divergence were observed between some individuals from different species. Most notably, the individual we sequenced of *Tangara argyrofenges* and the individual of *Tangara heinei* only differed by 0.4% sequence divergence, an amount typically observed within species. This contrasts with the well-differentiated plumage of these two species (Graves and Weske, 1987; Isler and Isler, 1999). These two species differ in their back, belly, and wing coloration among other characters and are allopatrically distributed. Possible explanations for the lack of genetic divergence include rapid morphological evolution between the two taxa as well as mitochondrial introgression. A third species, *Tangara viridicollis*, is also only weakly differentiated (1.7%) from *T. argyrofenges* and *T. heinei*. These three species are allopatrically distributed and together with *T. phillipsi* (a species we were unable to sample) form a superspecies

complex (Graves and Weske, 1987; Isler and Isler, 1999). Low levels of sequence divergence were also observed among *Tangara cayana*, *Tangara vitriolina*, and *T. cucullata*. Sequence divergence averaged only 1.4% among these morphologically similar species. Based on other types of data, Isler and Isler (1999) indicated that *T. vitriolina* and *T. cayana* may be conspecific. Based on levels of sequence variation, we recommend further phylogenetic and field studies of these taxa as well as the *T. heinei*, *T. argyrofenges*, *T. phillipsi*, and *T. viridicollis* complex.

In contrast to these weakly differentiated species, our data show that some species whose status has been questioned are well differentiated genetically from their closest relative. For example, *T. fucosa* has been considered conspecific with *Tangara dowii* (Isler and Isler, 1999; Storer, 1970). However, Sibley and Monroe (1990) and the American Ornithologists' Union (1998) considered them separate species. The level of genetic variation we observed between these two species (6.9%) is well within the range of values observed among reproductively isolated and morphologically divergent species of *Tangara*.

Similarly, *Tangara nigrocincta* has been considered conspecific with *Tangara larvata* (Meyer de Schauensee, 1966, 1970), but as a separate species by others (American Ornithologists' Union, 1998; Eisenmann, 1957; Sibley and Monroe, 1990). Our data indicate that the two species are well-differentiated, with 4.4% pairwise sequence divergence. Moreover, *T. larvata* is actually more closely related to *T. cyanicollis* in our phylogenies (Figs. 1 and 2). These three species (*T. nigrocincta*, *T. larvata*, and *T. cyanicollis*) together form a superspecies complex (Isler and Isler, 1999; Storer, 1970).

Based on field observations and examining museum skins, Schulenberg and Binford (1985) described *Tangara meyerdeschauenseei* as a separate species. This species belongs to the species group 8 along with *T. vitriolina*, *T. cayana*, and *T. cucullata*. In contrast to the low levels of genetic variation observed among these three species (see above), *T. meyerdeschauenseei* is genetically well-differentiated from the other three species. Average pairwise sequence divergence between *T. meyerdeschauenseei* to the other three species is 3.8%, and *T. meyerdeschauenseei* is the sister taxon to the clade containing the other three species in our phylogenies (Figs. 1 and 2). Thus, the separate recognition that Schulenberg and Binford (1985) gave to *T. meyerdeschauenseei* based on other types of data corresponds to the molecular data of this study.

4.4. Biogeography

Based on knowledge of tanager phylogeny at the time, Burns (1997) suggested a Caribbean origin for all tanagers. However, recent studies (Klein et al., in press; Yuri and Mindell, 2002) have shown that the basal taxa driving this interpretation are not tanagers, and the relationships among many genera are still ambiguous (Figs. 1 and 2; Burns et al., 2002, 2003). A full species-level phylogeny of all tanagers and related finches (Burns, in prep.) is needed in order to determine with any confidence the geographic origin of the group and the distributional history of species leading up to *Tangara*, including ancestral distributions within the clade of core tanagers. However, by analyzing geographic distributions among *Tangara* species, the current study clearly shows that the immediate ancestor of the genus *Tangara* is Northern Andean in origin. In addition, the identification of older nodes in the *Tangara* phylogeny as Andean indicates that many of the oldest speciation events within *Tangara* occurred within the Andes as well. Other relationships indicate that speciation has continued to occur within the Andes to recent times (e.g., *T. arthus*, *Tangara icterocephala*, and *Tangara florida*). However, many recent speciation events also occurred in areas outside the Andes. The presence of species of *Tangara* outside of the Andes is the result of subsequent dispersal and recent speciation within these other areas, including lowland regions (Fig. 3). At least for *Tangara*, this contrasts with the general idea that montane areas in the Neotropics are the site of more recent speciation events than lowland areas (Bates and Zink, 1994; Fjeldså, 1994; Roy et al., 1997). Few molecular phylogenetic studies of South American birds have included several highland and lowland species (e.g., Bates and Zink, 1994; Garcia-Moreno et al., 1999; Voelker, 1999). Our results agree with Voelker's (1999) study of *Anthus*, a cosmopolitan group that occurs in the Andes as well as other parts of South America. Within the South American species of *Anthus*, the Northern Andes was identified as part of the ancestral area, and subsequent speciation involved dispersal events out of the Andes into lowland areas of South America. Although the *Anthus* results agree with our results for *Tangara*, more phylogenetic studies are needed to determine if directionality between highland and lowland areas can be generalized across all birds.

The omission of some species from our study raises the possibility that our interpretations may change with the inclusion of the additional taxa. However, we feel this is unlikely given our results and the distributions of most of these missing taxa. Two of the six missing species (*T. phillipsi* and *T. rufigenis*) include the Andes in their distribution. For the remaining species (*T. cabanisi*, *T. cyanoventris*, *T. peruviana*, and *T. preciosa*), they would likely need to be basal to the rest of *Tangara* in order to outweigh the current reconstructions of biogeography. This possibility is unlikely given their firm placement within the species groups (2, 4, and 8) of Isler and Isler (1999) and given the general reliability of Isler and Isler's groupings.

The identity of the Andes as an important area for early and continued *Tangara* speciation agrees with the relative timing of geologic events in the area. Although using a molecular clock requires many assumptions (Hillis et al., 1996), it provides a rough framework for formulating preliminary biogeographic hypotheses and estimating relative divergence times. For bird mtDNA, a number of studies have converged on a rate of roughly 2% sequence divergence per million years (Shields and Wilson, 1987; Tarr and Fleischer, 1993; see references cited in Klicka and Zink, 1997). Assuming this rate applies for *Tangara*, the genus diverged from other members of the core tanager clade no earlier than 6.5 million years ago (range of average uncorrected sequence divergence = 9.4–12.9%). Within *Tangara*, species began diverging from each other as early as 6 million years ago, with most splits occurring between 3.5 and 5.5 million years ago (average uncorrected sequence divergence = 8.8%, range = 0.4–12.1%). Thus, most speciation events in *Tangara* occurred during the late Miocene and through the Pliocene. This was a time of continued uplift in the Andes when a variety of factors such as habitat changes, fragmentation, climatic cycles, and tectonic activity could have provided opportunities for isolation and subsequent speciation (Clapperton, 1993; Hooghiemstra and Van der Hammen, 1998; Potts and Behrensmeyer, 1992).

This temporal framework for *Tangara* speciation corresponds well with that of other groups of co-distributed Andean birds for which molecular data are available. García-Moreno and Fjeldså (2000) reviewed data from 18 different groups of Andean birds and concluded that diversification has been continuous from the upper Miocene into the Pleistocene. Within this time frame, different groups diversified earlier than others, but the most intensive speciation occurred in the upper Miocene, Pliocene, and mid-Pleistocene. Late Pleistocene glacial cycles have been hypothesized as an important mechanism for generating current levels of avian species diversity (e.g., Haffer, 1969, 1974; Rand, 1948). However, García-Moreno and Fjeldså (2000) found few pairings of Andean species that correspond to the late Pleistocene. For *Tangara*, only the split between *T. argyrofenges* and *T. heinei* corresponds to the period of dramatic climatic cycling in the late Pleistocene (less than 250,000 years ago). If the temporal period is extended to consider the last 800,000 years when Pleistocene glacial cycles were also extreme (García-Moreno and Fjeldså, 2000; Haffer, 1974), then the split among *T. cayana*, *T. vitriolina*, and *T. cucullata* corresponds to this period as well. However, the majority of splits among species of *Tangara* occurred well before the onset of the Pleistocene. The universality of the late Pleistocene speciation model has been challenged for Temperate zone, North American birds (Klicka and Zink, 1999; but see Avise and Walker, 1998). Our study and other recent molecular studies of birds (García-Moreno and Fjeldså, 2000) indicate that a late Pleistocene model may not apply universally in montane regions of the Neotropics as well (but see Chesser, 2000; Garcia-Moreno et al., 1999). We agree with Bates (2001) that more pattern-based phylogenetic studies are needed to help infer the processes that have generated biodiversity in the New World tropics.

4.5. Speciation within Tangara

Although many species of *Tangara* presently occur sympatrically and others have elevationally parapatric distributions, these distributions appear to be the result of more recent events as speciation within *Tangara* likely occurred in allopatry. Many sister species of *Tangara* identified in our phylogeny have allopatric distributions (although they may occur in the same zoogeographic region). For example, of the 11 sister species pairs identified in our codon and gene partitioned Bayesian analysis (Fig. 2), five have completely allopatric distributions (*T. heinei–T. argyrofenges*, *T. vitriolina–T. cucullata*, *T. dowii–T. fucosa*, *T. inornata–T. mexicana*, and *T. seledon–T. fastuosa*). Two of the remaining six have sympatric distributions (*T. callophrys–T. velia*, *T. desmaresti–T. cyanocephala*), and four are at least partly parapatric (*T. larvata–T. cyanicollis*, *T. punctata–Tangara xanthogastra*, *T. icterocephala–T. florida*, and *T. lavinia–T. gyrola*). The sympatric and parapatric pairs are separated by a larger amount of average pairwise sequence divergence compared to the allopatric pairs (4.5% versus 3.4%). Thus, they may have been separated for a longer period of time than the allopatric sister pairs. This longer time frame could have allowed subsequent dispersal and secondary contact in these sister species. The allopatric nature of speciation within *Tangara* is also supported by the distribution of subspecies within the group. None of the 108 currently recognized subspecies of *Tangara* are found elevationally parapatric, and all subspecies in the Andes are found latitudinally allopatric separated by dry valleys or found in the eastern and western sides of the Andes separated by the tree-less Andean ridge (Isler and Isler, 1999). In addition, hybridization between two *Tangara* species is only known for a few species. Therefore, most, if not all, *Tangara* in the Andes speciated allopatrically along a north–south axis, and the elevationally parapatric distributions are probably the result of secondary contact after the establishment of reproductive isolation. This pattern of speciation is consistent with that described by García-Moreno and Fjeldså (2000) for other Andean birds, whereby species are initially isolated into relictual areas and subsequent sympatry is the result of later dispersal following the evolution of adaptations in isolation. We know nothing about how reproductive isolation was established in the *Tangara* or how they

recognize conspecific individuals. Rapid diversification and reproductive isolation may have been achieved by extremely diverse plumage colors or their simple, but species-specific songs. As a result, sexual selection may have played a central role in producing numerous ecologically similar species (Price et al., 2000). In this scenario, a fine segregation in arthropod foraging adaptations (Naoki, 2003; Naoki and Burns, in prep.) could have later facilitated coexistence of ecologically similar species by decreasing competition.

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