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# MOLECULAR PHYLOGENETICS OF A CLADE OF LOWLAND TANAGERS: IMPLICATIONS FOR AVIAN PARTICIPATION IN THE GREAT AMERICAN INTERCHANGE

KEVIN J. BURNS[1] AND RACHEL A. RACICOT[2]

*Department of Biology, San Diego State University, 5500 Campanile Drive, San Diego, California 92182, USA*

ABSTRACT.—The importance of the formation of the Panamanian land bridge for mammalian diversification in the New World is well documented; however, studies investigating the role of this land bridge in avian diversification have only recently been reported. We used mitochondrial DNA data to reconstruct the phylogeny of a group of lowland tanagers (Thraupidae) that contains species distributed in both Central America and South America. Phylogenetic analyses identified a clade that includes all 26 species in the genera *Tachyphonus*, *Ramphocelus*, *Eucometis*, *Lanio*, *Trichothraupis*, *Coryphospingus*, and *Rhodospingus*. Three of these species (*Rhodospingus cruentus*, *Coryphospingus cucullatus*, and *C. pileatus*) have traditionally been classified with the finches (Emberizidae); here, we show that they are tanagers. The genus *Tachyphonus* is polyphyletic, with some species more closely related to species in the genus *Ramphocelus* than they are to other *Tachyphonus*. The ancestor of the entire clade was distributed in South America or was widespread there and in Central America. Reconstructing the biogeographic history of this group showed a dispersal bias from South America to Central America. Nine dispersal events were inferred, and eight of these involve dispersals from South America to Central America. Temporally, most dispersal events coincide with or postdate the final formation of the Panamanian isthmus. We also used our phylogeny to investigate plumage evolution. Two species are sexually monochromatic, and this condition was derived from sexual dichromatism through the evolution of more colorful female plumage. Although 11 species in the clade have crests, this feature evolved no more than twice within the group. *Received 4 October 2008, accepted 18 March 2009.*

Key words: biogeography, dispersal, Panamanian isthmus, plumage evolution, *Ramphocelus, Tachyphonus,* vicariance.

## Filogenética Molecular de un Clado de Tángaras de Tierras Bajas: Implicancias para la Participación de las Aves en el Gran Intercambio Americano

RESUMEN.—La importancia de la formación del puente terrestre de Panamá para la diversificación de los mamíferos en el Nuevo Mundo ha sido bien documentada. Sin embargo, sólo recientemente se han presentado estudios que investigan el rol de este puente de tierra en la diversificación de las aves. Empleamos datos de ADN mitocondrial para reconstruir la filogenia del grupo de las tángaras (Thraupidae) de tierras bajas que contiene especies distribuidas en Centro y Sur América. Los análisis filogenéticos identificaron un clado que incluye las 26 especies de los géneros *Tachyphonus*, *Ramphocelus*, *Eucometis*, *Lanio*, *Trichothraupis*, *Coryphospingus* y *Rhodospingus*. Tres de estas especies (*Rhodospingus cruentus*, *Coryphospingus cucullatus* y *C. pileatus*) han sido clasificadas tradicionalmente con los Emberizidae, pero aquí demostramos que son tángaras. El género *Tachyphonus* es polifilético, pues algunas especies están más cercanamente relacionadas con especies del género *Ramphocelus* que con otras del propio *Tachyphonus*. El ancestro de todo el clado estuvo distribuido en Sur América o estuvo ampliamente distribuido allí y en Centro América. La reconstrucción de la historia biogeográfica de este grupo mostró un sesgo de dispersión desde Sur América hacia Centro América: se infirieron nueve eventos de dispersión, ocho de los cuales fueron en esa dirección. Temporalmente, la mayoría de los eventos de dispersión coinciden con, o son posteriores a, la formación final del istmo de Panamá. También usamos nuestra filogenia para investigar la evolución del plumaje. Dos especies son sexualmente monocromáticas y esta condición se derivó a partir de una condición de dicromatismo sexual mediante la evolución de un plumaje más colorido en las hembras. Aunque 11 especies en el clado tienen crestas, esta característica no evolucionó más de dos veces en el grupo.

[1]E-mail: kburns@sciences.sdsu.edu
[2]Present address: Department of Geology and Geophysics, Kline Geology Lab, P.O. Box 208109, Yale University, New Haven, Connecticut 06520, USA.

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Of all zoogeographic realms, the Neotropics is the most species-rich, with about one-third of all avian species (Podulka et al. 2004). Several hypotheses have been invoked to explain the generation of this diversity: mountain building, long-term stability and accumulation of lineages, river barriers, marine incursions, the creation of refugia during climate fluctuations, and environmental gradients linked to adaptation (see reviews by Bates et al. 1998, Brumfield and Edwards 2007). Phylogeographic analyses and phylogenetic trees are the critical frameworks needed to address the roles these different processes have played in producing this rich pattern of biodiversity. When these analyses are placed into a temporal framework, they become an even more powerful mechanism for reconstructing speciation histories. In recent years, molecular phylogenies of birds in this region have proliferated, allowing new insight into the relative importance of these processes (Bates et al. 2008).

Some studies have focused on the evolution of taxa that occur in highland areas (e.g., García-Moreno et al. 2001, Navarro-Sigüenza et al. 2008), in lowland areas (e.g., Aleixo 2004, Ribas et al. 2005), or in both (e.g., Burns and Naoki 2004, Brumfield and Edwards 2007, Ribas et al. 2007). Here, using mitochondrial DNA (mtDNA) data, we reconstruct the phylogeny of a clade of lowland tanagers (Thraupidae). Initially, this study began as a phylogenetic analysis of species in the avian genus *Tachyphonus*. Over the course of the study, however, we discovered that this genus is not monophyletic. Thus, we expanded the study to include all species in the genera *Tachyphonus*, *Ramphocelus*, *Eucometis*, *Lanio*, *Trichothraupis*, *Coryphospingus*, and *Rhodospingus*. The 26 species in this group occur mostly at low elevations, though a few also are found at high elevations. Because they are distributed from southern Mexico to central Argentina, we were able to use our phylogeny to examine relationships across lowland Neotropical regions.

Tanagers are mainly a South American group and are often assumed to have South American origins (Yuri and Mindell 2002, Fjeldså and Rahbek 2006). However, many species occur in Central America, and the sister taxon to tanagers (Cardinalidae, the cardinals and grosbeaks; Klicka et al. 2007) is more speciose in Central America and North America than in South America. The extent to which lineages of tanagers radiated within Central America or the possibility of a Central American origin for some or all tanagers is largely unexplored because a comprehensive phylogeny of the group is lacking. The lowland tanagers we investigated provide an opportunity to explore the importance of Central America in generating patterns of tanager diversity. Five species are found only in Central America, 15 are found only in South America, and 6 are found in both regions. Given these distributions, we explored the dispersal and vicariance history of the group with respect to these two regions. One possibility is that the Central American species originated from a common ancestor that descended from a species that dispersed once to Central America from South America. Alternative hypotheses include a Central American origin of South American taxa, a more complicated scenario with multiple dispersals between Central America and South America, and a purely vicariant history of the group in which dispersal has not occurred. We explored these possibilities with respect to the timing of the final closure of the Panamanian isthmus 2.5–3.5 mya (Coates and Obando 1996).

Species in this lowland clade are some of the most conspicuous Neotropical birds, many acting as core species in mixed-species foraging flocks. Many species have plumage ornaments such as color patches, crests, and enlarged lower mandibles that are incorporated into their courtship displays (Moynihan 1962, 1966; Willis 1985; Isler and Isler 1999). Most have marked sexual dichromatism, males being more colorful than females. However, two species lack sexual dichromatism, both males and females having colorful plumage. The phylogeny presented here provides the opportunity to examine the evolution of these morphological features and their associated behaviors. We explore two of these here: sexual dichromatism and the presence of a colored patch of feathers on the head (i.e., a crest).

## Methods

*Taxon sampling.*—Our data set included 61 individuals representing 44 species, including all 8 species in the genus *Tachyphonus* (Table 1; American Ornithologists' Union [AOU] 1998, Remsen et al. 2008). To guide our sampling, we used previous taxonomies and phylogenetic studies as well as an unpublished data set including DNA sequences of 91% of tanagers and finches included in Sibley and Monroe's (1990) Thraupini (K. J. Burns et al. unpubl. data). To address geographic variability, we included multiple individuals of several species. Previous generic-level phylogenetic studies that have included a representative of *Tachyphonus* have shown that this genus is closely related to *Eucometis*, *Lanio*, *Coryphospingus*, and *Ramphocelus* (Burns 1997; Burns et al. 2002, 2003; Yuri and Mindell 2002; Klicka et al. 2007). Thus, we included all the species in these genera. In addition, we included the monotypic genus *Rhodospingus* because of plumage similarities to *Coryphospingus* and because of the consistent placement of *Rhodospingus* near *Coryphospingus* in linear taxonomies (Paynter and Storer 1970, Sibley and Monroe 1990, Dickinson 2003). Likewise, we included representatives of all species in *Creurgops* and *Heterospingus* because these two species are placed near *Tachyphonus* in taxonomic arrangements (Paynter and Storer 1970, Sibley and Monroe 1990). Although *Habia*, *Chlorothraupis*, *Piranga*, and *Calochaetes* are often placed in this sequence as well, recent studies have shown that these species are not closely related to *Tachyphonus*. *Habia*, *Chlorothraupis*, and *Piranga* are grosbeaks (Klicka et al. 2007), and *Calochaetes* is a mountain tanager (Burns and Naoki 2004, R. E. Sedano and K. J. Burns unpubl. data). To help identify a monophyletic group that includes all species of *Tachyphonus* and their close relatives, we also included 14 additional species, representing each of the major lineages of tanagers (K. J. Burns et al. unpubl. data). The sister group to tanagers is Cardinalidae (cardinals and grosbeaks); thus, we used sequences of the Rose-breasted Grosbeak (*Pheucticus ludovicianus*) to root the entire tree.

*Character sampling.*—Cytochrome *b* (cyt *b*) and nicotinamide dehydrogenase subunit 2 (ND2) were used to infer relationships. These two mitochondrial markers were chosen because they have been successful in resolving relationships within other closely related species of tanagers (García-Moreno et al. 2001, Burns and Naoki 2004, Lijtmaer et al. 2004, Mauck and Burns 2009). DNA extractions were performed either with a 5% Chelex solution (Walsh et al. 1991) or using the QIAmp DNA MiniKit (Qiagen, Valencia, California). For ND2, only the first 330 base pairs were sequenced,

TABLE 1. Species names, GenBank numbers, voucher numbers,[a] and locality information.

| Species | GenBank numbers | Source (museum voucher number, collector, and locality) |
|---|---|---|
| *Pheucticus ludovicianus* | Cyt *b* (AF447373); ND2 (AF447298) | UMMZ 233649; Michigan |
| *Anisognathus somptuosus* | Cyt *b* (AY383090); ND2 (EU648011) | LSUMZ B566; T. Schulenberg; Peru: Dept. Puno, Abra de Maruncunca, 10 km SW San Juan del Oro |
| *Conirostrum speciosum* | Cyt *b* (AY190168); ND2 (FJ799835) | FMNH 334602; Bolivia: Santa Cruz, Chiquitos, San Jose-San Ignacio Rd., km 69 |
| *Coryphospingus cucullatus* | Cyt *b* (FJ799869) | FMNH 334587; Bolivia: Santa Cruz, Chiquitos, Purubi, 30 km S San Jose de Chiquitos |
| *C. cucullatus* | ND2 (AF447274) | UMMZ 235435, captive |
| *C. pileatus* | Cyt *b* (FJ799870); ND2 (FJ799836) | FMNH 392719; Brazil: Sergripe, Caninde do Sao Francisco, Curituba, Fazenda Brejo |
| *Creurgops dentata* | Cyt *b* (FJ799871); ND2 (FJ799837) | LSUMZ B580; T. Schulenberg; Peru: Puno, Abra de Maruncunca, 10 km SW San Juan del Oro |
| *C. verticalis* | Cyt *b* (FJ799872); ND2 (FJ799838) | LSUMZ B7974; K. Rosenberg; Peru: Pasco, Playa Pampa, 8 km NW Cushi on trail to Chaglla |
| *Cyanerpes cyaneus* | Cyt *b* (FJ799873); ND2 (FJ799839) | FMNH 427305; Brazil: Alagoas |
| *Dacnis venusta* | Cyt *b* (FJ799874); ND2 (FJ799840) | LSUMZ B26588; D. Dittman; Panama: Colon, 17 km by road NW Gamboa, Rio Agua Salud |
| *Diglossa albilatera* | Cyt *b* (EU647893); ND2 (EU647926) | AMNH DOT5023; Venezuela: Aragua, km 40 on El Junquito/Col. Tovar Rd. |
| *Eucometis penicillata* 1 | Cyt *b* (FJ799875); ND2 (FJ799841) | LSUMZ B6551; C. G. Schmitt; Bolivia: Santa Cruz, Rio Quizer |
| *E. penicillata* 2 | Cyt *b* (FJ799876); ND2 (FJ799842) | FMNH 334593; Bolivia: Santa Cruz, Chiquitos, San Jose-San Ignacio Rd. |
| *E. penicillata* 3 | Cyt *b* (EF529961); ND2 (EF529849) | MBM 14831; Panama: Colon |
| *Hemispingus atropileus* | Cyt *b* (AF006234) | LSUMZ B1889; S. Stoltz; Peru: Dept. Pasco, Cumbre de Ollon, ~12 km E Oxapampa |
| *H. atropileus* | ND2 (AF383135) | LSUMZ B14692; Peru: Dept. Pasco |
| *H. flavicollis* | Cyt *b* (AF006235); ND2 (EU647948) | LSUMZ B5102; S. Cardiff; Peru: Loreto, S Rio Amazonas, ~10 km SSW mouth of Rio Napo on E bank Quebrada Vainilla |
| *H. xanthopygius* | Cyt *b* (EU647915); ND2 (EU647949) | LSUMZ B2324; S. Lanyon; Panama: Darien, Cana on E slope Cerro Pirre |
| *Lanio aurantius* 1 | Cyt *b* (FJ799877); ND2 (FJ799843) | MBM 8738; Honduras: Depto. Atlantida, La Ceiba, 9.7 km SW Rio Quebrada |
| *L. aurantius* 2 | Cyt *b* (EF529962); ND2 (EF529850) | MBM 8966; Honduras: Atlantida |
| *L. fulvus* | Cyt *b* (EU647917); ND2 (EU647951) | LSUMZ B2694; S. Cardiff; Peru: Loreto, 1 km N Rio Napo, 157 km by river NNE Iquitos |
| *L. versicolor* | Cyt *b* (FJ799878); ND2 (FJ799844) | LSUMZ B1014; J. Remsen; Bolivia: La Paz, Rio Beni, ~20 km by river N Puerto Linares |
| *L. leucothorax* | Cyt *b* (FJ799879); ND2 (FJ799845) | STRI JTW572; Panama: Cocle, El Cope National Park |
| *Loxigilla portoricensis* | Cyt *b* (AF489886); ND2 (EU648044) | LSUMZ B-11351; P. Marra; Puerto Rico: Cabo Rojo, Boqueron, Penones de Melones, 1 km WNW intersection routes 301 and 303 |
| *Oryzoborus angolensis* | Cyt *b* (AF310055) | Ecuador: Santo Domingo; voucher not collected; see Sato et al. (2001) |
| *O. angolensis* | ND2 (FJ799846) | FMNH 433798; Peru: Madre de Dios, Moskitania, 13.4 km NNW Atalaya, l bank Alto Madre de Dios |
| *Poospiza cinerea* | Cyt *b* (FJ799880); ND2 (FJ799847) | USNM B05912; B. Schmidt; Argentina |
| *Ramphocelus bresilius* | Cyt *b* (U15724); ND2 (U15715) | AMNH; specimen from captivity; see Hackett (1996) |
| *R. carbo* | Cyt *b* (U15723); ND2 (U15714) | LSUMZ B4988; T. Davis; Peru: Loreto; S Río Amazonas, ~10 km SSW Río Napo on E bank Quebrada Vainilla |
| *Ramphocelus dimidiatus* 1 | Cyt *b* (FJ799881); ND2 (FJ799848) | LSUMZ B16559; G. Seutin; Panama: Panama Province, Old Gamboa Road-golf course, 4 km NW of Paraiso |
| *R. dimidiatus* 2 | Cyt *b* (EF529964); ND2 (EF529852) | MBM 14837; Panama: Colon |
| *Ramphocelus flammigerus* 1 | Cyt *b* (U15719); ND2 (U15710) | LSUMZ B12017; Ecuador: Prov. Esmeraldas; El Placer |
| *R. flammigerus* 2 | Cyt *b* (FJ799882); ND2 (FJ799849) | USNM B1238; S. Olson, T. Parsons; Panama: Punta Alegre, Peninsula Veliente |
| *R. melanogaster* | Cyt *b* (FJ799883); ND2 (FJ799850) | LSUMZ B44693; J. Mattos; Peru: Dept. San Martin; ~33 km NE Florida |
| *R. nigrogularis* | Cyt *b* (U15721); ND2 (U15712) | LSUMZ B2850; T. Davis; Peru: Dpto. Loreto; 1 km N Río Napo, 157 km by river NNE Iquitos |
| *R. costaricensis* 1 | Cyt *b* (U15722); ND2 (U15713) | LSUMZ B16134; J. O'Neill; Costa Rica: Prov. Puntarenas; Marenco Biological Station |
| *R. costaricensis* 2 | Cyt *b* (U15720); ND2 (U15711) | LSUMZ B16144; J. O'Neill; Costa Rica: Prov. Puntarenas; 2 km SE Dominical |
| *Ramphocelus passerinii* 1 | Cyt *b* (U15717); ND2 (U15726) | LSUMZ B16152; J. O'Neill; Costa Rica: Prov. Heredia; ~5 km by road S Puerto Viejo |

(*Continued*)

Table 1. Continued.

| Species | GenBank numbers | Source (museum voucher number, collector, and locality) |
|---|---|---|
| *R. passerinii* 2 | Cyt *b* (EF529965); ND2 (EF529853) | MBM 8627; Honduras: Atlantida |
| *R. sanguinolentus* | Cyt *b* (U15718); ND2 (U15709) | Mexico: Veracruz; Sierra de Santa Martha, El Bastanol; see Hackett (1996) |
| *Rhodospingus cruentus* | Cyt *b* (FJ799884); ND2 (FJ799851) | LSUMZ B5184, D. Dittman; Peru: Lambayeque Department, Las Pampas; 885 km on Pan-American Hwy, 11 km on road from Olmos |
| *Sporophila nigricollis* | Cyt *b* (AF310053) | Ecuador: Santo Domingo; voucher not collected; see Sato et al. (2001) |
| *S. nigricollis* | ND2 (FJ799852) | FMNH 427217; Brazil: Alagoas, Ibateouara, Envenho Ceimba, Usina Serra Grande |
| *Stephanophorus diadematus* | Cyt *b* (EU647992); ND2 (EU648053) | AMNH DOT 9915; Argentina: Buenos Aires, Partido Escobar |
| *Tachyphonus coronatus* 1 | Cyt *b* (FJ799885); ND2 (FJ799853) | AMNH DOT 2452, Argentina: Misiones, Departamento San Ignacio, near border Parque Prov. Urugua-i, ~1 km W park headquarters, Ruta Prov. 19 |
| *T. coronatus* 2 | Cyt *b* (FJ799886); ND2 (FJ799854) | FMNH Tissue 5944; Brazil: São Paulo, Boroceia National Park |
| *T. coronatus* 3 | Cyt *b* (FJ799887); ND2 (FJ799855) | MVZ FC20068; Paraguay: Depto. Itapua, El Tirol, 19.5 km NNE (by road) Encarnacion |
| *T. cristatus* 1 | Cyt *b* (FJ799888); ND2 (FJ799856) | LSUMZ B9548; J. Remsen; Bolivia: Pando, Nicholas Suarez, 12 km by road S of Cojiba, 8 km W on road to Mucden |
| *T. cristatus* 2 | Cyt *b* (FJ799889); ND2 (FJ799857) | LSUMZ B2693; T. Davis; Peru: Loreto, 1 km N Rio Napo, 157 km by river NNE Iquitos |
| *T. delatrii* 1 | Cyt *b* (FJ799890); ND2 (FJ799858) | LSUMZ B11710; F. Gill; Ecuador: Esmeraldas, El Placer, ~670 m |
| *T. delatrii* 2 | Cyt *b* (EF529966); ND2 (EF529854) | MBM 15562; Panama: Cocle |
| *T. luctuosus* 1 | Cyt *b* (FJ799891); ND2 (FJ799859) | LSUMZ B2279; S. Lanyon; Panama: Darien, Cana on E slope Cerro Pirre |
| *T. luctuosus* 2 | Cyt *b* (FJ799892); ND2 (FJ799860) | MVZ FC21573; Peru: Depto. Madre de Dios, Albergue, Rio Madre de Dios, 12 km E Puerto Maldonado |
| *T. luctuosus* 3 | Cyt *b* (EF529967); ND2 (EF529855) | MBM 8846; Honduras: Atlantida |
| *T. phoenicius* | Cyt *b* (FJ799893); ND2 (FJ799861) | AMNH DOT 4797; Venezuela, Bolivar, Cerro Guanay |
| *T. rufiventer* 1 | Cyt *b* (FJ799894); ND2 (FJ799862) | LSUMZ B22777; S. Cardiff; Bolivia: La Paz, B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata |
| *T. rufiventer* 2 | Cyt *b* (FJ799895); ND2 (FJ799863) | LSUMZ B3629; M. Robbins; Peru: Loreto, S bank Rio Maranon, along Rio Samiria, Est. Biol. Pithecia, Base Tacsha Cocha |
| *T. rufus* 1 | Cyt *b* (FJ799896); ND2 (FJ799864) | LSUMZ B6668; D. Schmitt; Bolivia: Santa Cruz, Rio Tucavaca |
| *T. rufus* 2 | Cyt *b* (FJ799897); ND2 (FJ799865) | UWBM 54452; Argentina: Provincia de Corrientes, Corrientes, 45 km S, Manuel Derqui |
| *T. rufus* 3 | Cyt *b* (FJ799898); ND2 (FJ799866) | FMNH NKK959; Tobago: Northside Co., Main Ridge Forest Reserve, Center Hill Trail, 11°16′N, −60°37′W |
| *T. surinamus* | Cyt *b* (EU647923); ND2 (EU647959) | LSUMZ B4795; T. Davis; Peru: Loreto, S Rio Amazonas, ~10 km SSW Rio Napo |
| *Tangara gyrola* | Cyt *b* (AY383131); ND2 (EU648071) | LSUMZ B22850; E. Cardiff; Bolivia: Dept. La Paz, Prov. B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata |
| *Tiaris fuliginosa* | Cyt *b* (AF489900); ND2 (EU648107) | LSUMZ B-12612; D. Schmitt; Bolivia: Departamento Santa Cruz, Velasco; 50 km ESE Florida, Arroyo del Encanto |
| *Trichothraupis melanops* 1 | Cyt *b* (FJ799899); ND2 (FJ799867) | AMNH DOT 2464; Argentina: Misiones, Departamento San Ignacio, ~20 km SE San Ignacio |
| *T. melanops* 2 | Cyt *b* (FJ799900); ND2 (FJ799868) | FMNH Tissue 5934; Brazil: São Paulo, Boroceia National Park Rondonia |

[a] AMNH = American Museum of Natural History; FMNH = Field Museum of Natural History; LSUMZ = Louisiana State University Museum of Natural Science Collection of Genetic Resources; MBM = University of Nevada Las Vegas, Barrick Museum of Natural History; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; STRI = Smithsonian Tropical Research Institute; UMMZ = University of Michigan Museum of Zoology; USNM = National Museum of Natural History; UWBM = University of Washington Burke Museum.

and these were amplified using primers L5215 and H5578 (Hackett 1996). The entire cyt-*b* gene (1,143 base pairs) was amplified using primer pairs L14851 with H15297, L15206 with H15710, and L15656 with H16058 (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, <1 s at 43–50°C, 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). The final product of polymerase chain reaction was purified using either a GeneClean Kit (Bio101) or Exonuclease I and Shrimp Alkaline phosphatase. The product was then cycle sequenced (96°C for 1 min, 96°C for 30 s, 50°C for 15 s, 60°C for 4 m—28 cycles) using Big Dye terminator reaction mix (Applied Biosystems, Foster City, California). Samples were cleaned with a Sephadex bead column before being sequenced on either an ABI 377 or ABI 3100 DNA sequencer (Applied Biosystems). SEQUENCHER (Gene Codes, Ann Arbor, Michigan) 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 included sequencing both heavy and light strands, using overlapping fragments of cyt *b*, checking for amino-acid translation without stop codons or gaps, and comparing levels of sequence divergence separately for the three cyt-*b* fragments as well as the ND2 fragment. Table 1 provides GenBank numbers of new sequences as well as those we took from previous studies (Hacket 1996; Burns 1997; Sato et al. 2001; Burns et al. 2002, 2003; Lovette and Bermingham 2002; Yuri and Mindell 2002; Burns and Naoki 2004; Klicka et al. 2007; Mauck and Burns 2009; R. E. Sedano and K. J. Burns unpubl. data).

*Phylogenetic analyses*.—Phylogenetic analyses were performed using maximum-likelihood (ML) and Bayesian inference methods. The ML analyses were run using GARLI, version 0.951 (Zwickl 2006), applying the GTR+I+Γ model of evolution. Multiple analyses were run, and each run resulted in the same tree topology. The data set was also bootstrapped for 1,000 replicates to assess support for each node. The resulting trees from the bootstrapped analysis were used to construct a 50% majority-rule consensus tree in PAUP*, version 4.0b10 (Swofford 2002).

Bayesian analyses were implemented in MRBAYES, version 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003), with the data partitioned by codon and gene region. Models of evolution for each codon position for both cyt-*b* and ND2 data were inferred using MRMODELTEST, version 2.2 (Nylander 2004) and Akaike's information criterion. The GTR+I+Γ model was inferred for each of these six partitions. Two independent runs were implemented. One run for 6 million generations each was sampled every 1,000 generations, and another run for 3 million generations was sampled every 500 generations. The log likelihood values from each run were plotted against number of generations using TRACER, version 1.4 (Rambaut and Drummond 2007), to determine the point at which stationarity was reached. For both runs, stationarity was reached well before 500,000 generations, and we chose a burn-in of 1 million generations for each. Results of each analysis were compared, and the same topology and similar posterior probabilities were recovered. Thus, all of the post-burn-in trees for both analyses were imported into PAUP*, and a consensus tree was constructed using 50% majority rule, including all compatible groupings. Posterior probabilities (PP) of 0.95 and higher were considered strongly supported.

*Biogeographic analyses*.—To examine patterns of dispersal and vicariance between Central America and South America, we used DISPERSAL-VICARIANCE ANALYSIS, version 1.1 (DIVA; Ronquist 1997). DIVA uses distributions of extant species together with phylogeny to reconstruct ancestral distributions. We coded each extant species as occurring in Central America, South America, or both regions using relevant references (Ridgely and Tudor 1989, Parker et al. 1996, Isler and Isler 1999). We follow DaCosta and Klicka (2008) in considering Central America as the area north of the Panamanian state of Darien.

For each branch on which a dispersal could have occurred, we calculated an upper and a lower bound to the dispersal date. The earliest possible date for the dispersal is considered the age of origin of a particular branch, and the latest date is the point at which that branch diversifies into other species. Thus, we estimated the minimum and maximum age of each internode in which DIVA identified a dispersal event. Some extant species are found in both Central America and South America, but the most recent common ancestor they share with another living species was found in only one of these regions. Thus, in each case, there has been a recent expansion (dispersal) into either Central America or South America. The dispersal must have occurred no earlier than the split between these species and their closest living relatives. In most cases, we included at least one population in both Central America and South America, and the divergence dates of these two populations were used to establish a date of minimum age of dispersal. However, we note that detailed intraspecific studies are desirable for each of these species to further clarify the timing of the dispersal.

To estimate dates of dispersal events and their associated error, we used BEAST, version 1.4.7 (Drummond and Rambaut 2007). For birds, a variety of mtDNA sequence-divergence rates have been estimated, but the most widely used calibration is 2% sequence divergence $Ma^{-1}$, first estimated from restriction fragment length polymorphism data in geese. Subsequently, this rate has continued to be identified in a variety of bird studies. In a recent study, Weir and Schluter (2008) confirmed the generality of this rate for cyt *b* in avian divergences more recent than 12 mya, within the range of diversification of the clade we studied. Using cyt *b* and fossil and biogeographic evidence, Weir and Schluter (2008) examined 90 lineages and found 74 of these (many of which were tanagers) to correspond to a rate of 2.1% $Ma^{-1}$. Thus, we employed this rate (2.1% = 0.0105 substitutions $lineage^{-1}$ $Ma^{-1}$) when examining the divergence times within our lowland tanager clade. Using only our cyt-*b* data and a strict molecular clock, we ran BEAST analyses for 20 million generations with data partitioned by codon and gene and employing the GTR+I+Γ model of nucleotide substitution. We used TRACER to explore our results and to calculate mean ages of each node and the 95% highest posterior density interval (HPD) associated with each node. Before the BEAST analyses were run, rate homogeneity was confirmed by comparing likelihood scores with and without a molecular clock enforced.

*Plumage evolution*.—The presence of sexual dichromatism and the presence of a crest were mapped onto the phylogenies. The evolution of these two plumage characters was optimized using pasimony as implemented in MACCLADE, version 4.08 (Maddison and Maddison 2005).

## RESULTS

*Phylogenetic analyses*.—Both ML and Bayesian analyses (Figs. 1 and 2) identified a large clade of lowland tanagers that includes all species in the genera *Tachyphonus*, *Ramphocelus*, *Trichothraupis*, *Eucometis*, *Lanio*, *Coryphospingus*, and *Rhodospingus*. Support for the monophyly of this clade was high (1.0 PP, 69% bootstrap). However, the placement of this clade in relation to other tanagers was not strongly supported in any analyses. The first divergence within this clade identifies two strongly supported clades (clades A and B), each of which contains several species of *Tachyphonus* as well as other genera. Clade A contains the silverbeaks (genus *Ramphocelus*) and three species of *Tachyphonus*. Clade B includes the shrike-tanagers (genus *Lanio*) and a variety of species with crests, including additional species of *Tachyphonus*, Neotropical finches in the genera *Rhodospingus* and *Coryphospingus*, and the

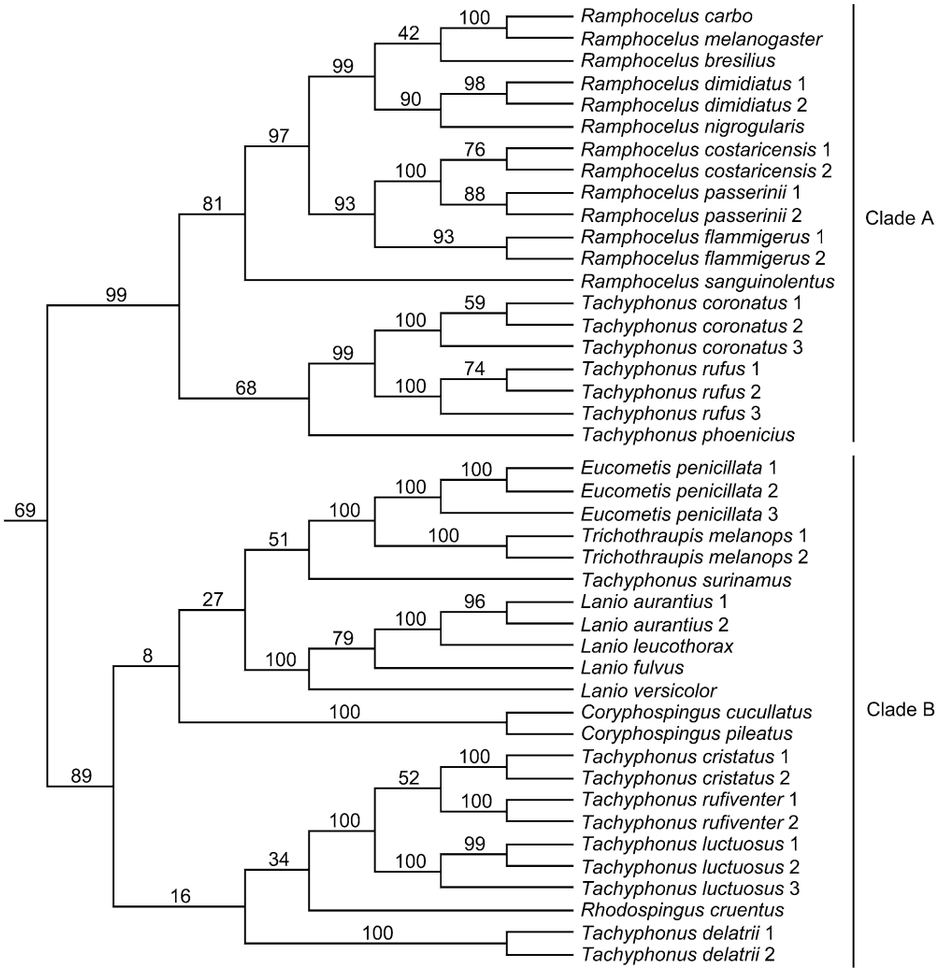


FIG. 1. Maximum-likelihood bootstrap consensus tree. Numbers on nodes indicate the percentage of 1,000 bootstrap replicates in which that clade was retained. Clades A and B are discussed in the text.

army-ant-following tanagers *Eucometis* and *Trichothraupis*. The ML and Bayesian topologies were very similar, with similar levels of support for each node. Only a few nodes differed between the two analyses, and none of these nodes shows strongly supported conflict. The two trees differ mainly in the relative positions of *Tachyphonus surinamus*, *Coryphospingus*, *Tachyphonus delatrii*, and *Rhodospingus* (Figs. 1 and 2). In both sets of analyses, all species for which more than one individual was sampled were found to be monophyletic, with strong support.

*Biogeography.*—The reconstructed ancestral distributions show that species found in the same region are not always each other's closest relative. Thus, there is not a single origin to all Central American or all South American taxa, and there have been multiple dispersals throughout the history of the group. Using either the Bayesian or the ML tree, DIVA produced three equally optimal reconstructions (ML tree shown; Fig. 3). The earliest member of the clade either was found in South America only or was widespread there and in Central America. None of the reconstructions identified a Central American ancestor to the clade. For each reconstruction, nine dispersal events were identified, but the reconstructions differed on which branches some of these dispersal events occurred (Fig. 3 and Table 2). For six branches (branches 1, 2, 3, 4, 5, and 11), all three reconstructions showed dispersal events. For another five branches (branches 6, 7, 8, 9, and 10), some reconstructions showed dispersal whereas others did not. All three reconstructions showed a dispersal bias from South America to Central America. Of the nine dispersal events of each reconstruction, only one involves a dispersal from Central America to South America. The rest involve dispersal from South America to Central America. The one dispersal to South America occurred on either branch 7 or branch 8. Thus, the dispersal to South America happened within the *Ramphocelus* clade. In some reconstructions, it involves the ancestor of all *Ramphocelus* except *R. sanguinolentus*. In other reconstructions, it occurred later and involves the speciation of *R. flammigerus*, *R. costaricensis*, and *R. passerinii*. Given that all other dispersals involve movement

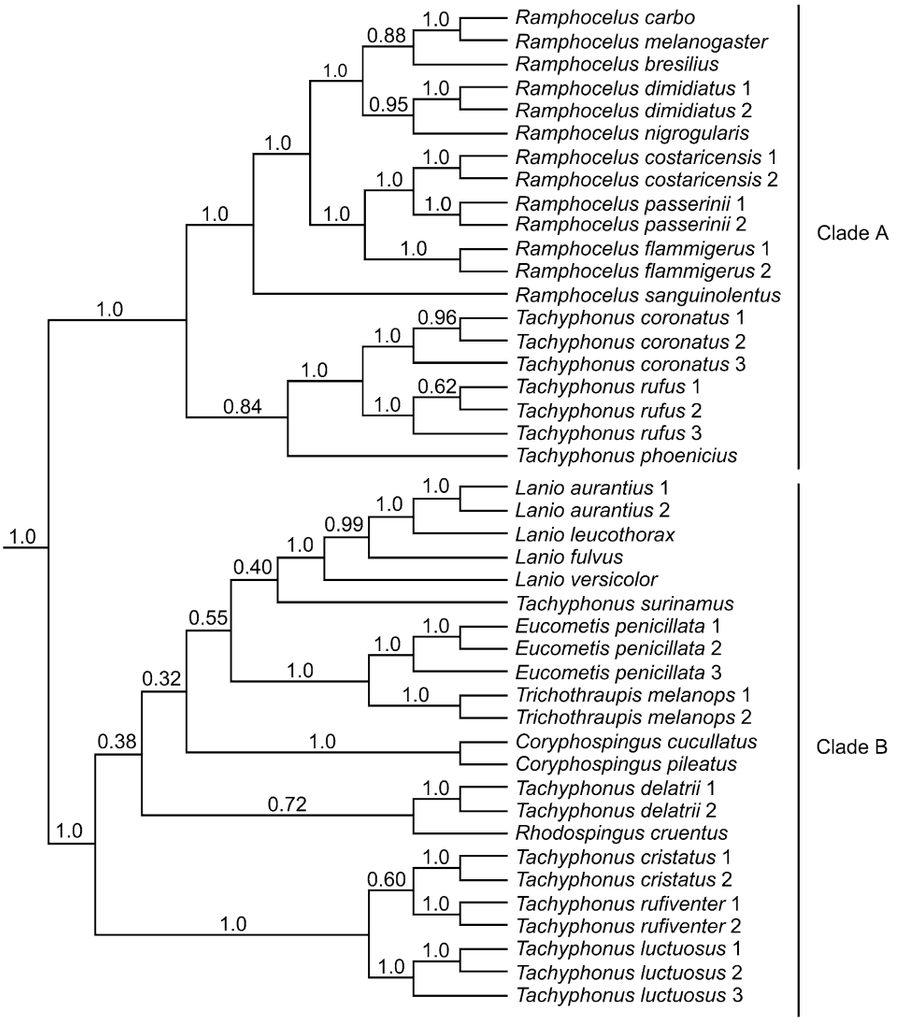


FIG. 2. Majority-rule consensus tree of the 18,000 trees that resulted from the Bayesian analysis. Numbers on nodes indicate the posterior probabilities of a particular clade. Clades A and B are discussed in the text.

from South America to Central America, the Central American distributions of most species within this clade are the result of multiple relatively recent dispersals to the region. Although there were several dispersals to Central America, not all speciation events in Central America involved dispersal to the region. Examples of speciation events occurring solely within Central America include *Lanio aurantius* and *L. leucothorax* as well as *Ramphocelus costaricensis* and *R. passerinii*.

A likelihood ratio test supported the hypothesis of rate homogeneity ($\chi^2 = 77.65$, df = 59, $P > 0.05$). Thus, assuming a molecular clock of 2.1% $Ma^{-1}$ (Weir and Schluter 2008), we used BEAST to estimate the dates of hypothesized dispersal events and compared them with the timing of the final closure of the Panamanian isthmus at 2.5–3.5 mya (Coates and Obando 1996). Given the large 95% HPD associated with each date (Table 2), specifying the exact timing of dispersal is difficult. However, of the 11 possible dispersal events inferred on the three different DIVA reconstructions, at least six of these (branches 1, 3, 5, 6, 7, and 11) likely coincided with or followed the final formation of the Panamanian isthmus (Table 2 and Fig. 3). One of the remaining dispersal events (branch 2) has mean maximum and minimum ages slightly older than the final formation of the isthmus (4.2–4.6 mya), but, given the 95% HPD, this dispersal may have occurred after isthmus formation. Another dispersal event (branch 4) has too broad a range of dates for us to infer with confidence whether dispersal corresponds with isthmus formation (1.0–8.8 mya). The remaining three dispersal events (8, 9, and 10) are older than the isthmus; however, not all of these dispersal events appear on each of the three reconstructions. Thus, for any given DIVA reconstruction, most but not all dispersal events likely correspond with or postdate the final formation of the isthmus.

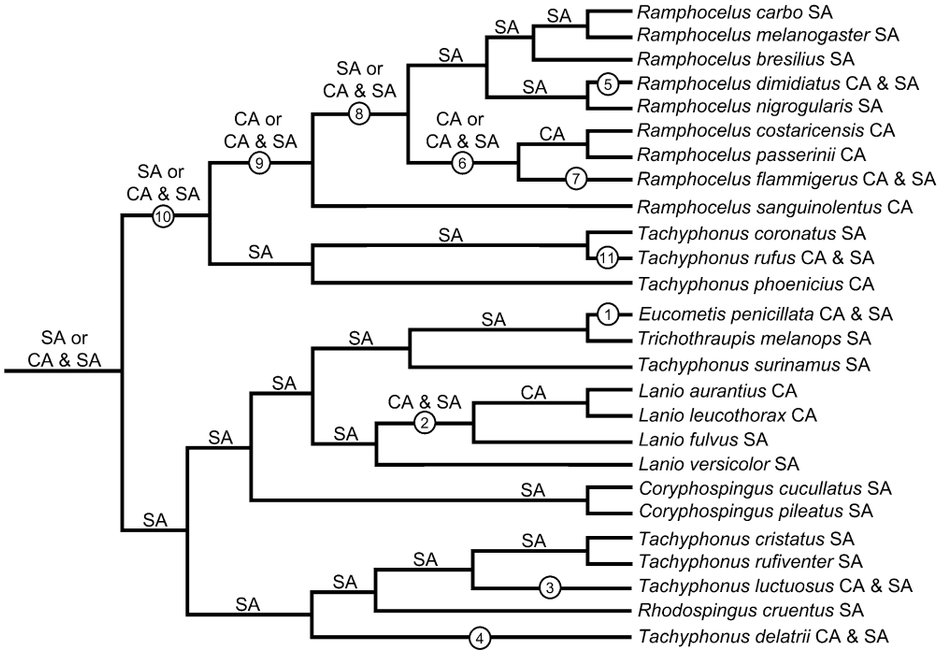


FIG. 3. Dispersal-vicariance analysis using the maximum-likelihood tree. Letters correspond to an ancestral area reconstructed for that node (CA = Central America, SA = South America, CA & SA = both regions). Areas separated by "or" represent equally optimal reconstructions. Numbers on branches correspond to Table 2 and indicate branches on which a dispersal event occurred.

## DISCUSSION

The phylogenies of the present study identified a novel clade of morphologically diverse tanagers. Although the species had not been suspected of forming a clade, some previous molecular studies with only generic-level sampling (Burns 1997, Yuri and Mindell 2002, Burns et al. 2003) have suggested that some of these species are related. In addition, the monophyly of all species in this group has been confirmed in a larger study that is in progress, involving multiple nuclear and mitochondrial genes and most species of tanagers (K. J. Burns et al. unpubl. data). Although this clade is diverse, members of this group have several features in common. All species except one, *R. melanogaster* (altitudinal range = 600–1,700 m), occur at lowland elevations. Only three other species are also found above 1,500 m (*Trichothraupis melanopsis*, *Coryphospingus cucullata*, and *R. dimidiatus*), but none of these occurs above 1,700 m (Parker et al. 1996). Many species in the clade act as core species in mixed-species flocks (Isler and Isler 1999). Isler and Isler (1999) noted similarities in foraging behavior among *Trichothraupis*, *Eucometis*, and some *Tachyphonus*, and similarities in behavior and plumage between *Eucometis penicillata* and *Trichothraupis melanopsis* led Willis (1985) to

TABLE 2. Timing and direction of dispersal events between Central America and South America based on DIVA reconstruction using the maximum-likelihood tree. Branch numbers match those in Figure 3.

| Branch | Maximum age[a] | Minimum age[a] | Direction of dispersal |
|---|---|---|---|
| 1 | 4.2 (3.4–5.2) | 2.6 (2.0–3.4) | To Central America |
| 2 | 4.6 (3.6–5.5) | 4.2 (3.2–5.2) | To Central America |
| 3 | 5.6 (4.6–6.6) | 3.1 (2.3–3.9) | To Central America |
| 4 | 8.8 (7.7–9.9) | 1.0 (0.6–1.4) | To Central America |
| 5 | 0.9 (0.5–1.2) | Undetermined | To Central America |
| 6 | 3.7 (3.1–4.4) | 2.7 (2.1–3.3) | To Central America or no dispersal |
| 7 | 2.7 (2.1–3.3) | 1.3 (0.8–1.8) | To South America or no dispersal |
| 8 | 5.2 (4.3–6.1) | 3.7 (3.1–4.4) | To South America or no dispersal |
| 9 | 6.2 (5.3–7.1) | 5.2 (4.3–6.1) | To Central America or no dispersal |
| 10 | 9.0 (7.9–10.1) | 6.2 (5.3–7.1) | To Central America or no dispersal |
| 11 | 3.7 (2.9–4.6) | Undetermined | To Central America |

[a]Age is in millions of years before present, and ranges are 95% highest posterior density intervals.

argue that these two species are congeneric. Most species are strongly sexually dichromatic, males typically having more melanin (black) and carotenoid (red, yellow, orange) patches than females. In several species, these patches form boldly contrasting patterns. Other plumage patterns shared among some species include white patches on either side of the wing (e.g., *Tachyphonus*, *Trichothraupis*, and *Rhodospingus*). Many species also have crests on their heads that are exposed or raised during display. Species in the genus *Ramphocelus* have an enlarged lower mandible that is silver in color (Isler and Isler 1999). Similarly, some *Tachyphonus* have differently colored upper and lower mandibles (e.g., *T. surinamus*) or a lower mandible with a blue-gray base (e.g., *T. rufus*, *T. luctuosus*; Ridgely and Gwynne 1989, Restall et al. 2006). The presence of these plumage and bill ornaments and their associated displays (Moynihan 1962, 1966; Willis 1985; Isler and Isler 1999) suggest that sexual selection is strong in this group. Our phylogeny's identification of these species as members of a single monophyletic group should facilitate the future study of these behaviors and plumages.

*Systematic implications*.—Although most of the species in this clade are tanagers, three species have traditionally been placed with New World finches and sparrows (Emberizidae). Using DNA–DNA hybridization, Bledsoe (1988) was the first to show that some Neotropical emberizids are actually more closely aligned to traditional tanagers. Subsequent DNA–DNA hybridization studies (Sibley and Ahlquist 1990) and, more recently, several DNA sequencing studies (e.g., Lougheed et al. 2000; Burns et al. 2002, 2003) also have shown that many of these species are more closely aligned to tanagers. However, several workers have been reluctant to move these Neotropical finches to the tanagers, awaiting more comprehensive analyses. The present study demonstrates that the three species in the genera *Rhodospingus* and *Coryphospingus* are clearly more closely related to tanagers and, thus, should be included within the Thraupidae. In the case of *Rhodospingus*, Paynter (1971) earlier argued that *Rhodospingus cruentus* was a tanager and identified characters that this species shares with species of *Coryphospingus*, *Tachyphonus*, and *Trichothraupis*. In addition to DNA characters, the red and black plumage, relatively thin bill shape, and presence of a crest in *Rhodospingus* and *Coryphospingus* support their placement within this clade of lowland tanagers.

Perhaps our most surprising finding is the lack of monophyly of the genus *Tachyphonus*. Three species belong to clade A with *Ramphocelus*, and the other five belong to clade B. The five *Tachyphonus* spp. found within clade B do not form a monophyletic group; however, support values for other nodes in this clade do not provide enough evidence to eliminate the possibility of monophyly for these five *Tachyphonus*. Within clade A, the three species of *Tachyphonus* show strong support for monophyly, and the species are the sister group to *Ramphocelus*. Although the nonmonophyly of *Tachyphonus* may seem surprising, these three species share several features with *Ramphocelus* that support their placement. All these species lack the obvious crests of the other *Tachyphonus*. Although *T. coronatus* has red feathers on its head like some of the crested *Tachyphonus*, these feathers are usually concealed, unlike the crests of the species of *Tachyphonus* of clade B. There also are similarities in habitat preference between species of *Ramphocelus* and the three *Tachyphonus* of clade A. Unlike other *Tachyphonus*, these three species typically occur in second-growth, semi-open, and edge habitats (Ridgely and Tudor 1989, Isler and Isler 1999), where most *Ramphocelus* spp. are also common. In addition, *R. bresilius* and *T. coronatus* have hybridized in captivity (Sick 1993), which suggests a close relationship among these species.

Given these similarities and the strong support values for the placement of *Tachyphonus* spp. into two separate clades (Figs. 1 and 2), it is unlikely that any further data would result in an exclusive, monophyletic group containing all *Tachyphonus* spp. Thus, *Tachyphonus* can be added to the growing list of avian genera that have been found to be nonmonophyletic in taxon-intensive phylogenies. Because *Tachyphonus* is not monophyletic, the taxonomy of the group needs to be revised. We see two reasonable alternatives. Given the strong support for all the species in this lowland group, all the genera included in the present study (all species in Figs. 1 and 2) could be placed within a single genus. In this case, all species would be placed within *Ramphocelus*, which has taxonomic priority because it was the first genus in this clade to be described. Alternatively, clades A and B could each be given separate generic names. Clade A would be *Ramphocelus*. Because the type species for *Tachyphonus* (*T. rufus*) belongs to clade A, the name *Tachyphonus* is no longer available for species in clade B. Thus, all the species in clade B would be placed within *Lanio*, the oldest remaining generic name in clade B.

Retaining the current usage of the generic names *Lanio*, *Eucometis*, *Trichothraupis*, *Coryphospingus*, and *Rhodospingus* is problematic because it would require the naming of three new genera (a monotypic genus for *T. delatrii*, a monotypic genus for *T. surinamus*, and a new genus name for the clade containing *T. luctuosus*, *T. cristatus*, and *T. rufiventer*). *Eucometis*, *Trichothraupis*, and *Rhodospingus* are all monotypic genera that were likely named as unique genera because their relationships to other species were unclear. Results of the present study, however, identify their close relatives, and classifications should reflect this information. Thus, we advocate either a single genus for the whole group (*Ramphocelus*) or two genera to define the two main clades (*Ramphocelus* and *Lanio*).

Although some molecular phylogenies have previously addressed generic-level relationships, Hackett's (1996) study of *Ramphocelus* is the only previous phylogenetic work involving species-level relationships of the taxa included in the present study. She included all but two species of *Ramphocelus* in her phylogeny. Here, we include the additional two species (*R. dimidiatus* and *R. melanogaster*). The phylogenies agree for the species in common between the present study and Hackett's (1996) study. However, the biogeographic scenario presented by Hackett (1996) becomes more complicated with the addition of the two missing species.

Levels of sequence divergence alone, especially when only a few individuals have been studied, cannot be used as the sole criterion for determining species status (Moritz and Cicero 2004). Nevertheless, given that no genetic data have previously been published for many of these species, we highlight cases in which our DNA data indicate that further investigation is needed. Most of the species we investigated show typical levels of cyt-*b* intraspecific divergence (Avise and Walker 1998, Ditchfield and Burns 1998). For three species, low levels of sequence divergence were found among individuals from the same or geographically nearby

populations (*Trichothraupis*, 0.9%; *L. aurantius*, 0.5%; *R. dimidiatus*, 0.4%). For another three species, our sampling occurred at distant parts of the range; nevertheless, low levels of sequence divergence were again recorded (*Tachyphonus rufus*, 0.82%; *T. rufiventer*, 0.6%; *T. coronatus*, 0.8%).

By contrast, some species showed surprisingly high levels of intraspecific sequence divergence. *Tachyphonus cristatus* is the most variable species in the genus, with 10 recognized subspecies (Dickinson 2003). We sampled two individuals, one from Peru and one from Bolivia (see Table 1 for specific localities). These two individuals showed 2.5% sequence divergence, despite the fact that they were relatively close geographically. Even higher levels of sequence divergence were recorded among another variable species, *T. luctuosus*. For this species, we included three individuals. An individual from Peru and one from southern Panama (Darien Province) differed from each other by 2.5%, and an individual from Honduras showed an average 4.8% sequence divergence from these two. This level of sequence divergence is higher than that found in many recognized avian species and genera. Similar levels of sequence divergence were observed between Central American and South American populations of *E. penicillata*. Two individuals adjacent to each other in Bolivia were similar in sequence (0.35%), but these two individuals were 4.8% divergent from an individual *E. penicillata* from Panama. Thus, both *E. penicillata* and *T. luctuosus* likely consist of multiple independent evolutionary units that perhaps represent different species. Moderately high levels of divergence were also found in two other species, with sampling in both Central America and South America. Two individual *T. delatrii* were included. One of these was from Central America and another from South America; however, these two individuals showed less sequence divergence (1.8%) than the species described above that also occur in these two regions. Likewise, two *R. flammigerus* (both in the *icteronotus* subspecies group), one from Panama and one from Ecuador, showed 2.2% sequence divergence.

Our data also can be used where full species status has been questioned. For example, the two *Coryphospingus* spp. hybridize in Brazil (Sick 1993), and Paynter and Storer (1970) suggested that the two might be conspecific. However, these two species show 5.9% sequence divergence, reflecting a long history of isolation. *Ramphocelus bresilius*, *R. carbo*, *R. dimidiatus*, and *R. melanogaster* form a superspecies (Paynter and Storer 1970), with hybridization recorded between *R. carbo* and *R. bresilius* (Sibley and Monroe 1990, Sick 1993) and between *R. carbo* and *R. melanogaster* (Paynter and Storer 1970). Although levels of divergence between *R. carbo* and *R. melanogaster* are somewhat low (1.1%), levels of divergence among all the other species in this group are similar to expected values for well-differentiated species (2.4–4.0%). *Ramphocelus passerinii*, *R. costaricensis*, and *R. flammigerus* also form a superspecies (Paynter and Storer 1970). *Ramphocelus flammigerus* shows 4.7% sequence divergence from *R. passerinii* and *R. costaricensis*, which indicates isolation of *R. flammigerus* from the other two species. Although considered two species by the AOU (1998), *R. passerinii* and *R. costaricensis* are considered one species in some taxonomies (Sibley and Monroe 1990, Dickinson 2003). Hackett (1996) argued for species status of these two taxa on the basis of DNA differences, plumage differences, allozyme data, and lack of recorded hybridization. We included the three individuals from Hackett (1996) as well as an additional representative of *R. passerinii* reported by Klicka et al. (2007), so *R. passerinii* and *R. costaricensis* are each represented by two individuals in our study. Both species are monophyletic, and levels of divergence within the two species (0.1%, 0.6%) are much less than levels of divergence between them (1.8%). Thus, although the interspecific divergence is lower than that observed between most species in the present study, our analyses are consistent with Hackett's (1996) argument for full species status for these two diagnosable taxa. Within *Lanio*, *L. fulvus* and *L. versicolor* are considered one superspecies (Isler and Isler 1999) and *L. aurantius* and *L. leucothorax* are considered another superspecies (Sibley and Monroe 1990). *Lanio fulvus* and *L. versicolor* replace each other on either side of the Amazon and are well differentiated (7.0%). However, considering them part of the same superspecies to the exclusion of other *Lanio* spp. is not appropriate, given that they are not each other's closest living relative. *Lanio fulvus* is more closely related to *L. aurantius* and *L. leucothorax* than to *L. versicolor*. In contrast to the large DNA divergence observed between *L. fulvus* and *L. versicolor*, *L. aurantius* and *L. leucothorax* are only 0.9% divergent with each other, a value similar to that observed within species of this group. *Lanio aurantius* and *L. leucothorax* are allopatrically distributed in Central America, and their distributions approach each other in Honduras. The two species differ mainly in throat color, and some populations of *L. leucothorax* also have a black rump, belly, and undertail coverts. Our sample of *L. leucothorax* is from Panama, at the farthest point in the distribution of *L. leucothorax*. Thus, it is unlikely that the similarity in genetic sequence is attributable to a recent hybridization event. These two species warrant further study, because they likely represent a recent speciation event coupled with rapid plumage divergence.

*Plumage evolution*.—The phylogeny presented here provides an opportunity to examine the evolution of plumage characters. Most species within this group are sexually dichromatic, with males having more elaborate plumage than females. As a result, tracing the evolution of sexual dichromatism onto the tree reveals that the ancestor of these species was sexually dichromatic. Although strong sexual dichromatism is a trademark of this group, two species within the clade are not obviously sexually dichromatic (Fig. 4). In *E. penicillata*, both males and females are similar and have bright yellow plumage that is likely carotenoid-based. In *R. sanguinolentus*, both males and females have the same bold red and black pattern. In both of these cases, the loss of sexual dichromatism in the group is most parsimoniously explained by a gain in elaborate female plumage. Thus, the species are sexually monomorphic not because males have become less colorful, but because females have increased in colorfulness. This finding agrees with the general pattern described for the evolution of sexual dichromatism in tanagers, whereby female plumage changes have happened more frequently during the evolutionary history of the group than changes in male plumage (Burns 1998). There are several potential explanations for why female plumage changes are driving the patterns of evolution of sexual dichromatism. Sexual selection on male plumage likely maintains sexual dimorphism in most lineages. However, in a few lineages, selection pressures on female plumage have changed. This could be the result of sexual selection acting on female plumage in terms of female–female competition or male choice (Amundsen 2000). Alternatively, natural selection for cryptic female plumage could be relaxed in the

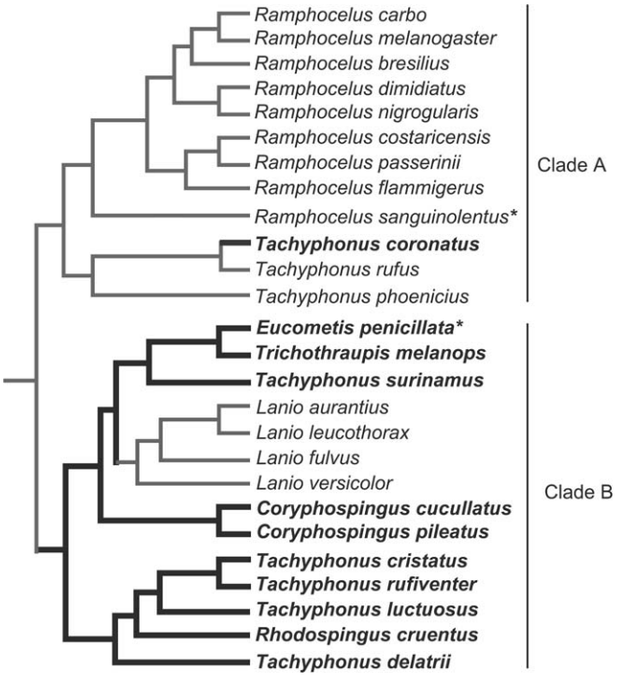


FIG. 4. Presence of a plumage crest mapped onto the maximum-likelihood tree using parsimony. Thick branches and species in bold indicate the presence of a crest. The crest evolved twice, once within *Tachyphonus coronatus* and once in the ancestor of clade B. The same results are obtained with the Bayesian tree. Species that are sexually monomorphic are indicated with an asterisk.

two monochromatic species. Further behavioral research on species in this group would help distinguish between the relative roles these two factors may have played. The present study adds to the growing number of studies (e.g., Hofmann et al. 2008) that clarify the relative influence of male and female plumage in driving patterns of dichromatism by evaluating male and female plumage separately in a phylogenetic context.

Another plumage feature of many of the species in this group is the presence of a crest, a distinctive patch of colored feathers on the head that can be raised and lowered. Crests are found in many species of birds and are present in several tanagers. In addition to being found in some of the species we investigated, crests also occur in the following tanagers: *Creurgops verticalis*, *Gubernatrix cristata*, some species of the genus *Paroaria*, both species of *Lophospingus*, and *Charitospiza eucosma*. We included 11 species of crested tanagers in the present study (Fig. 4). Ten of these species are found in clade B, and the other (*T. coronatus*) is in clade A. However, the crest of the latter species is different from those of the crested species in clade B in that, in *T. coronatus*, the crest is visible only in display. Regardless of whether *T. coronatus* is scored as crested, crest evolution has been limited in the group. When mapped onto the phylogeny (Fig. 4), crests evolved only twice and were lost only once in the group. In addition to these crested tanagers, some of the other crested tanagers are also closely related. *Gubernatrix cristata*, the genus *Paroaria*, and the genus *Lophospingus* are found within the same clade of mountain tanagers and relatives (R. E. Sedano and K. J. Burns unpubl. data). Thus, when placed in a phylogenetic context, crest evolution appears to be fairly conservative within tanagers.

*Biogeographic history.*—Multiple geological events probably contributed to the present-day pattern of species distributions seen in this group. In our DIVA analysis, we characterized distributions broadly into two general areas, Central America and South America. Thus, DIVA was not able to identify more fine-scale examples of vicariance and dispersal. However, allopatric distributions of most terminal sister taxa at least suggest that vicariance plays an important role in the diversification of the group. In many cases, important barriers separate distributions of related species. For example, *R. passerinii* and *R. costaricensis* are separated by Central American highlands, and *L. versicolor* is separated from its sister lineage by the Amazon River. *Tachyphonus rufiventer* is separated from its sister species, *T. cristata*, in the north by the Amazon River, and in the east, the border of these two species corresponds roughly to the Fitzcarrald arch, a structural uplift arch of Amazonia. Late Pleistocene glacial cycles have been hypothesized to be an important mechanism for generating Neotropical bird diversity via isolation into refugia (Haffer 1969, 1974), but none of the speciation events in our phylogeny corresponds to the period of dramatic climatic cycling in the late Pleistocene (<250,000 years ago). However, three speciation events reconstructed in the present study (*L. aurantius–L. leucothorax*, *R. dimidiatus–R. nigrogularis*, and *R. costaricensis–R. passerinii*) likely correspond to the past 800,000 years, when Pleistocene glacial cycles were also extreme. The considerable diversity within some of the recognized species could have been influenced by climatic oscillations during the late Pleistocene. Nonetheless, most speciation events are older than the late Pleistocene; thus, either climatic cycling earlier in the Cenozoic (Haffer 1997) or other events likely influenced speciation within the group.

The species we studied diversified mostly in South America, with subsequent dispersals into Central America. Furthermore, the group dispersed into Central America not once, but several times. This suggests that the formation of the Panamanian isthmus may have facilitated movement of several contemporaneous lineages north. The importance of the formation of the Panamanian isthmus in mammal diversification is well documented and is often described as the "Great American Interchange" (Stehli and Webb 1985). The interchange of bird species between these two areas is less well known, mainly because of the lack of fossil evidence (Mayr 1964, Vuilleumier 1985). Furthermore, birds are often assumed to have been unaffected by such a barrier because of their ability to fly. However, tropical birds can be very sedentary and have more limited dispersal than is often appreciated (Moore et al. 2008). With the advent of molecular data and complete phylogenies of avian clades, the relevance of the formation of the isthmus in avian diversification patterns is just now coming to light. In particular, four recent avian studies focused on this question and included comprehensive sampling of their respective groups (warblers in the genus *Myioborus*, Pérez-Emán 2005; wrens in the genus *Campylorhynchus*, Barker 2007; the genus *Trogon*, DaCosta and Klicka 2008; and *Chlorospingus ophthalmicus*, Weir et al. 2008). Two of these studies (Barker 2007, DaCosta and Klicka 2008) showed that most dispersal events occurred after final

isthmus formation. In one of the studies (Pérez-Emán 2005), the timing of dispersal occurred just before or during final closure. However, Weir et al. (2008) showed dispersal occurring before the final formation. All four of these cases revealed that diversification began north of the Panamanian isthmus and was followed by later dispersal into South America. By contrast, in the present study we identified the opposite pattern, namely that repeated invasions into Central America led to greater diversity in these lowland tanagers. Most of these invasions occurred during or after isthmus formation. In another recent tanager study (R. E. Sedano and K. J. Burns unpubl. data), dispersal into Central America after isthmus formation was identified in both highland and lowland species of mountain tanagers and species in the genus *Tangara*. In addition, dispersal from South America to Central America was identified in flowerpiercers in the genus *Diglossa* (Hackett 1995, Mauck and Burns 2009). Future studies of avian taxa are needed to contribute to our growing knowledge of the role birds played in the Great American Interchange.

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