

Phylogeography of a morphologically diverse Neotropical montane species, the Common Bush-Tanager (*Chlorospingus ophthalmicus*)

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Abstract

The Common Bush-Tanager (*Chlorospingus ophthalmicus*) is distributed in Neotropical cloud-forests from Mexico to Argentina and contains 25 subspecies divided into eight subspecies groups based on biogeography, eye coloration, presence of a postocular spot and chest band. All of Central America is occupied by a single subspecies group; whereas the Andes are believed to be occupied by seven additional subspecies groups. We used five mitochondrial genes to investigate the phylogeography and possible species limits of the *ophthalmicus* complex. A total of 14 monophyletic lineages were uncovered within the *ophthalmicus* complex, including three clades currently classified as separate species (*C. semifuscus*, *inornatus* and *tacarcunae*). Divergence estimates for these clades date between 0.8 and 5.2 million years ago (Ma). Contrary to expectations based on morphological diversity, phylogeographic structure was greatest in Mexico and Central America and weakest in the Andes. Morphological and genetic divergences were not significantly correlated and most morphologically defined subspecies groups were not supported. Our evidence suggests the *ophthalmicus* complex originated in Mexico ca. 6.0 Ma (million years ago) and spread south into the Andes ca. 4.7 Ma before the completion of the Isthmus of Panama. Three genetically divergent lineages of *ophthalmicus* that formed in the Andes possess a complex checkerboard distribution, with a single lineage represented by disjunct populations from Venezuela and the southern Andes, while intervening populations in Ecuador and Central Peru form two genetically and morphologically divergent lineages.

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1. Introduction

While the Neotropics possess more species than any other tropical region, the excess of species can be attributed in part to the rapid uplift of a complex system of highland regions extending from Mexico to Tierra del Fuego which promoted the formation of a distinctive highland fauna. Without this highland contingent, Neotropical species diversity would be comparable to other tropical regions

that lack extensive highlands, suggesting the importance of montane diversification in promoting the excess of Neotropical diversity. A recent review of Neotropical avian phylogenetic studies (Weir, 2006) suggested diversification rates in most highland genera have remained constant to the present while most lowland genera have experienced a decline in diversification rates through time. The contrast suggests ongoing speciation in the highland system may now contribute more to the buildup of biodiversity of the Neotropics than lowland faunas. However, only a handful of phylogeographic studies (Cadena et al., 2007; García-Moreno et al., 2004, 2006; Miller et al., 2007; Perez-Eman, 2005) have addressed population divergence within Neo-

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tropical highland avian species. These studies demonstrate that many widespread highland species have extensive phylogeographic structure suggesting that the speciation process is being initiated extensively in the highlands.

Here we examine the phylogeographic structure within one of the most widespread and polytypic highland species of Neotropical songbirds, the Common Bush-Tanager (*Chlorospingus ophthalmicus*). This species possesses approximately 25 subspecies (Dickinson, 2003; Isler and Isler, 1999) distributed in subtropical cloud-forest from Mexico to Argentina. These subspecies exhibit varying degrees of morphological divergence, with the most distinct-

tive in the Andes and the least distinctive in Central America. Isler and Isler (1999) divided *ophthalmicus* subspecies into eight groups based on geography, eye coloration (light or dark) and the presence or absence of a postocular spot and chest band (Fig. 5). This arrangement combined all Central American subspecies into a single group but recognized seven different groups in the Andes of South America (Fig. 1). Nevertheless, phylogenetic analysis of five *ophthalmicus* subspecies endemic to different highland regions in Mexico and Guatemala showed extensive genetic differentiation between subspecies in both mitochondrial DNA (García-Moreno et al., 2004) and allozymes (Peterson

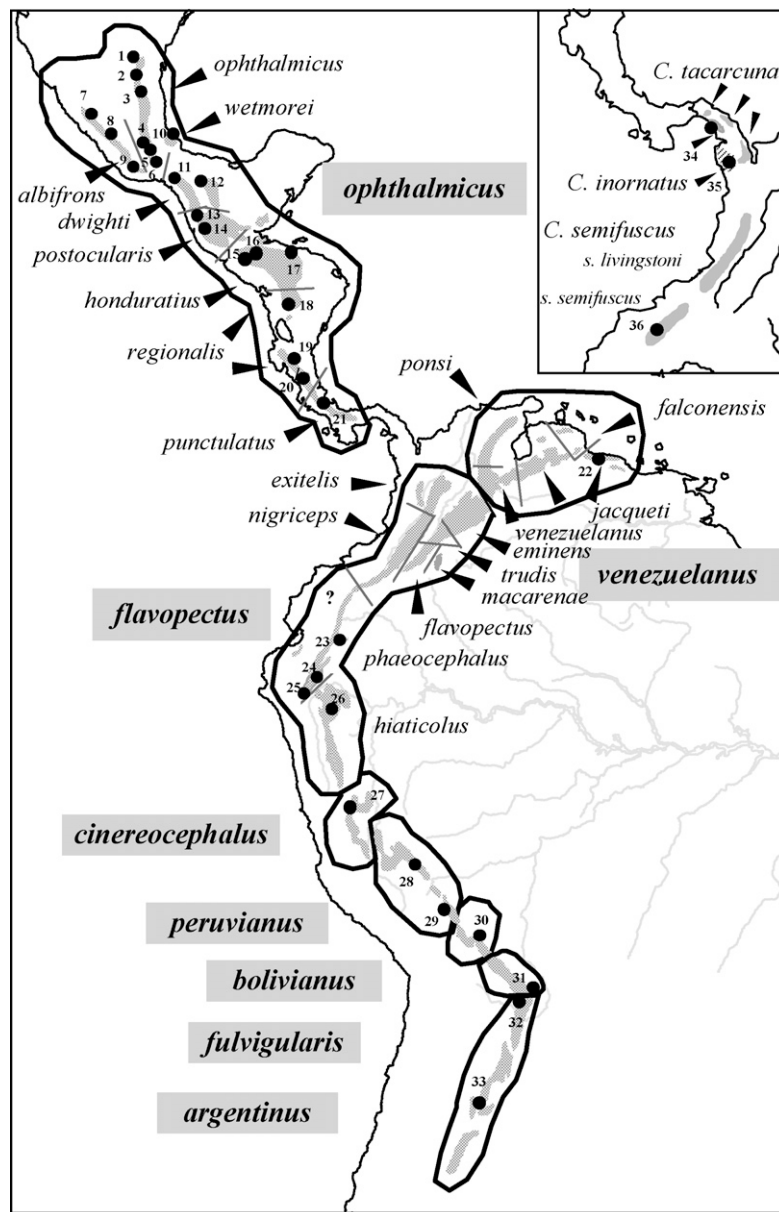


Fig. 1. Geographic distribution and sampling localities of the *Chlorospingus ophthalmicus* complex. Subspecies groups (names highlighted in gray) follow those defined by Isler and Isler (1999). Lines between populations delineate approximate subspecies boundaries. Boundaries are less well defined in Colombia and Venezuela. Inset panel shows distributions of three species (*semifuscus*, *inornatus* and *tacarcunae*) closely allied to *ophthalmicus*. The question mark shows the possible occurrence of *o. phaeocephalus* along the western Andes of north-western Ecuador. Numbered dots refer to sampling localities listed in Table 1.

et al., 1992). These subspecies examined comprise only part of a single subspecies group and much additional genetic variation may occur within the *ophthalmicus* complex. If morphological distinctiveness results from the gradual buildup of differences through time, then Andean subspecies which are the most distinctive morphologically should exhibit even deeper phylogenetic splits than exhibited within the Central American subspecies group.

Species boundaries of *ophthalmicus* are poorly understood. Three taxa currently classified as distinct species, *C. semifuscus*, *C. inornatus* and *C. tacarcunae*, have in the past been considered subspecies (Meyer de Schauensee, 1966) or allospecies (Sibley and Monore, 1990) of *ophthalmicus*. Alternatively, *C. tacarcunae* has been regarded as conspecific with *C. flavigularis* (Hellmayr, 1936), but both coexist in sympatry in central Panama (Blake, 1989) and hybridization has not been reported. In addition, *ophthalmicus punctulatus* from western Panama, *o. cinereocephalus* from central Peru, and the *o. flavopectus* group of subspecies (*flavopectus*, *trudis*, *exitelis*, *nigriceps*, *macarenae*, *phaeocephalus*, *hiaticolus*) from Colombia, Ecuador and northern Peru (Fig. 1), have in the past been considered species due to their morphological distinctiveness. All were considered conspecific with *ophthalmicus* by Zimmer (1947). Recently, Sánchez-González (2007) suggested several northern Mesoamerican *ophthalmicus* subspecies be elevated to species based on genetic and morphological considerations.

We investigated the phylogeographic structure within *C. ophthalmicus* by analyzing DNA sequences from five mitochondrial genes. Sequences were generated for all of the subspecies groups of *ophthalmicus* as defined by Isler and Isler (1999) and for *inornatus*, *tacarcunae*, and *semifuscus*. Phylogenetic analyses of these sequences were used to investigate the phylogeographic history of the *ophthalmicus* complex, the phylogenetic placement of *tacarcunae*, *semifuscus* and *inornatus*, and the concordance between morphological and phylogenetic divergence.

2. Methods

2.1. Taxon sampling

We sampled *inornatus*, *tacarcunae*, *semifuscus* and 17 subspecies of *C. ophthalmicus* with at least one representative from each subspecies group. With the exception of the morphologically variable “*novicius*” from western Panama, a form now believed to be of mixed ancestry between *regionalis* and *punctulatus* (Olson, 1993), we follow the subspecies taxonomy of Isler and Isler (1999). All recognized subspecies from Central America and from the South American Andes south of the Colombian/Ecuadorian borders were included. In the Andes of Colombia and Venezuela, tissues were available only for *o. jacqueti*. Tissues were obtained from voucher museum specimens collected by the authors in Argentina, Mexico, Guatemala, Honduras, Nicaragua and Panama or from frozen-tissue collections

of the Museum of Natural Sciences, Louisiana State University, The Field Museum of Natural History, American Museum of Natural History, Marjorie Barrick Museum of Natural History, Smithsonian Tropical Research Institute, University of Kansas Natural History Museum and Academy of Natural Science, Philadelphia (Tables 1 and 2).

Our ongoing work (data not shown) on *Chlorospingus* systematics indicates that *C. pileatus* is the most closely related outgroup to a monophyletic clade that included *ophthalmicus*, *inornatus*, *tacarcunae* and *semifuscus*. Based on this analysis, we use *C. pileatus* as the outgroup for all the analyses presented here.

2.2. Laboratory protocols

Whole genomic DNA was isolated from muscle tissue using phenol chloroform extraction or commercially available extraction kits (Purgene). We sequenced the complete mitochondrial ATPase 6 and 8 coding region (842 base pairs [bp] including a 10 bp overlapping region between ATPase 8 and ATPase 6) for all samples using primers CO2QGL and CO3HMH (Hunt et al., 2001). We sequenced three additional mitochondrial genes for a subset of individuals representing the major lineages recovered in our analysis of the ATPase 6 and 8 dataset (Table 2). These genes were: the complete cytochrome *b* gene (cyt *b*: 1143 bp) using primers O-L14851 and O-H16065 (Weir and Schluter, 2007), the complete NADH dehydrogenase subunit 2 gene (ND2: 1041 bp) using primers L5215 (Hackett, 1996) and H6313 (Johnson and Sorenson, 1998), and a partial sequence of the cytochrome oxidase I gene (COI: 661 bp) using primers COI_f and COI_a (Palumbi, 1996). Amplification and sequencing of these genes followed standard protocols (i.e. Hunt et al., 2001).

2.3. Phylogenetic analysis

Sequences were edited and aligned in BioEdit (Hall, 1999). The 10 bp overlap between ATPase 6 and 8 was excluded following other authors (e.g. Lovette, 2004). A partition homogeneity test (Farris et al., 1995) conducted in PAUP* v.4.0b10 (Swofford, 2002) was used to determine if the phylogenetic signals in different gene regions in the five-gene dataset were compatible and thus could be combined in phylogenetic analysis (ATPase 6 and 8 were combined as a single gene region).

MrModeltest v2 (Nylander, 2004) was used to determine the likelihoods of nested models of sequence evolution for both our ATPase 6 and 8 and extended sequencing datasets. A hierarchical likelihood ratio test implemented in MrModeltest v2 (Nylander, 2004) was used to choose the model that best fit the data while minimizing the number of parameters to be estimated. The GTR- Γ model best fit the ATPase 6 and 8 dataset and the GTR- Γ -I model best fit the extended dataset.

Table 1
List of *Chlorospingus* samples used in this study

#	Taxon	Museum (tissue no.)	Locality	Accession no.
01	<i>ophthalmicus ophthalmicus</i>	MZFC 9715	Mexico: Querétaro, 7 km S of Tres Lagunas	AY609275 ^a
02	<i>ophthalmicus ophthalmicus</i>	MZFC BMM 085	Mexico: Hidalgo, 5 km E Tlanchinol	AY609276 ^a
02	<i>ophthalmicus ophthalmicus</i>	MZFC 10398	Mexico: Hidalgo, 5 km E Tlanchinol	AY609277 ^a
02	<i>ophthalmicus ophthalmicus</i>	FMNH 394061	Mexico: Hidalgo, 5 km E Tlanchinol	EU427577
02	<i>ophthalmicus ophthalmicus</i>	FMNH 394070	Mexico: Hidalgo, 5 km E Tlanchinol	EU427578
02	<i>ophthalmicus ophthalmicus</i>	FMNH 394066	Mexico: Hidalgo, 5 km E Tlanchinol	EU427579
03	<i>ophthalmicus ophthalmicus</i>	MZFC 11297	Mexico: Hidalgo, El Potrero, 5 km Tenango	AY609278 ^a
03	<i>ophthalmicus ophthalmicus</i>	MZFC 10981	Mexico: Hidalgo, El Potrero, 5 km Tenango	AY609279 ^a
04	<i>ophthalmicus ophthalmicus</i>	MZFC 12490	Mexico: Oaxaca, Sierra de Huautla	AY609281 ^a
04	<i>ophthalmicus ophthalmicus</i>	MZFC 11585	Mexico: Oaxaca, Sierra de Huautla	AY609282 ^a
05	<i>ophthalmicus ophthalmicus</i>	FMNH 346816	Mexico: Oaxaca, Nudo de Zempoaltepetl, 5 km below Totontepec	EU427580
05	<i>ophthalmicus ophthalmicus</i>	FMNH 393786	Mexico: Oaxaca, Totontepec, Cerro de Zempoaltepetl	EU427581
05	<i>ophthalmicus ophthalmicus</i>	FMNH 346811	Mexico: Oaxaca, Nudo de Zempoaltepetl, 5 km below Totontepec	EU427582
06	<i>ophthalmicus ophthalmicus</i>	MZFC MXJ 511	Mexico: Oaxaca, Cerro Zempoaltepetl, Totontepec	AY609283 ^a
07	<i>ophthalmicus albifrons</i>	MZFC MX1437	Mexico: Guerrero, El Iris, Sierra de Atoyac	AY609293 ^a
08	<i>ophthalmicus albifrons</i>	MBM dnb5526	Mexico: Guerrero, Carrizal de Bravo	EU427588
08	<i>ophthalmicus albifrons</i>	MBM dnb5527	Mexico: Guerrero, Carrizal de Bravo	EU427589
08	<i>ophthalmicus albifrons</i>	MBM dnb5545	Mexico: Guerrero, Carrizal de Bravo	EU427587
08	<i>ophthalmicus albifrons</i>	FMNH 393780	Mexico: Guerrero, Carrizal de Bravo	EU427590
09	<i>ophthalmicus albifrons</i>	MZFC 12810	Mexico, Oaxaca, Reyes Llano Grande	AY609294 ^a
09	<i>ophthalmicus albifrons</i>	MZFC 11579	Mexico, Oaxaca, Reyes Llano Grande	AY609295 ^a
10	<i>ophthalmicus wetmorei</i>	MZFC MX 1080	Mexico, Veracruz [Sierra de Los Tuxtlas], Volcan de Santa Marta	AY609284 ^a
10	<i>ophthalmicus wetmorei</i>	MZFC MX 1078	Mexico, Veracruz [Sierra de Los Tuxtlas], Volcan de Santa Marta	AY609285 ^a
10	<i>ophthalmicus wetmorei</i>	FMNH 393779	Mexico: Veracruz, El Bastonal	EU427584
10	<i>ophthalmicus wetmorei</i>	MBM 4952	Mexico: Veracruz, Sierra Santa Martha	EU427583
10	<i>ophthalmicus wetmorei</i>	MBM 4953	Mexico: Veracruz, Sierra Santa Martha	EU427585
10	<i>ophthalmicus wetmorei</i>	FMNH 393775	Mexico: Veracruz, El Bastonal (3 km SE)	EU427586
11	<i>ophthalmicus dwight</i>	MZFC 12084	Mexico, Oaxaca [Chimalapas] Chalchijapa, 20 km NE del campamento	AY609288 ^a
11	<i>ophthalmicus dwight</i>	LSUMZ B18090	Mexico, Oaxaca, Chimalapas	AY609290 ^a
11	<i>ophthalmicus dwighti</i>	LSUMZ B18089	Mexico, Oaxaca, Chimalapas	EU427591
12	<i>ophthalmicus dwight</i>	MZFC 9573	Mexico, Chiapas, 6 km NE de Pueblo Nuevo, camino Aurora-Ermita	AY609287 ^a
12	<i>ophthalmicus dwighti</i>	MZFC 9584	Mexico, Chiapas, 6 km NE de Pueblo Nuevo, camino Aurora-Ermita	AY609286 ^a
13	<i>ophthalmicus postocularis</i>	MZFC 8826	Mexico, Chiapas, Rio Mala, Volcan Tacana	AY609291 ^a
13	<i>ophthalmicus postocularis</i>	MZFC 8832	Mexico, Chiapas, Rio Mala, Volcan Tacana	AY609292 ^a
14	<i>ophthalmicus postocularis</i>	MBM dnb4454	Guatemala: Quezaltenango	EU427592
14	<i>ophthalmicus postocularis</i>	MBM gav2384	Guatemala: Quezaltenango	EU427594
14	<i>ophthalmicus postocularis</i>	MBM jk02-150	Guatemala: Quezaltenango	EU427593
15	<i>ophthalmicus honduratus</i>	UKNH 4895	El Salvador: Chalatenango, Cerro El Pital	EU427600
15	<i>ophthalmicus honduratus</i>	UKNH 5074	El Salvador: Chalatenango, Cerro El Pital	EU427599
16	<i>ophthalmicus honduratus</i>	MBM jk9974	Honduras: Copan, Copan Ruinas (15 km N)	EU427595
16	<i>ophthalmicus honduratus</i>	MBM gav1537	Honduras: Copan, Copan Ruinas (15 km N)	EU427596
17	<i>ophthalmicus honduratus</i>	MBM jk01243	Honduras: Atlantida, La Ceiba (9.7 km SW)	EU427597
17	<i>ophthalmicus honduratus</i>	MBM gav2026	Honduras: Atlantida, La Ceiba (9.7 km SW)	EU427598
18	<i>ophthalmicus regionalis</i>	MBM dab1325	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427602
18	<i>ophthalmicus regionalis</i>	MBM dab1291	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427601
18	<i>ophthalmicus regionalis</i>	MBM dab1331	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427603
19	<i>ophthalmicus regionalis</i>	LSUMZ B-16013	Costa Rica: Heredia, Virgen del Socorro (4 km SE)	EU427607
19	<i>ophthalmicus regionalis</i>	LSUMZ B-16018	Costa Rica: Heredia, Virgen del Socorro (4 km SE)	EU427606
20	<i>ophthalmicus regionalis</i>	FMNH 393087	Costa Rica: Cartago, Tres Rios, 4.5 km NE, near Finca Pizote	EU427604
20	<i>ophthalmicus regionalis</i>	FMNH 393086	Costa Rica: Cartago, Tres Rios, 4.5 km NE, near Finca Pizote	EU427605
21	<i>ophthalmicus punctulatus</i>	NMNH B-1490	Panama: Bocas del Toro, Los Planes (13 km N)	EU427608
21	<i>ophthalmicus punctulatus</i>	NMNH B-5274	Panama: Bocas del Toro, Los Planes (13 km N)	EU427615
21	<i>ophthalmicus punctulatus</i>	NMNH B-1491	Panama: Bocas del Toro, Los Planes (13 km N)	EU427613
21	<i>ophthalmicus punctulatus</i>	NMNH B-2020	Panama: Bocas del Toro, Los Planes (24 km N)	EU427611
21	<i>ophthalmicus punctulatus</i>	NMNH B-2019	Panama: Bocas del Toro, Los Planes (24 km N)	EU427614
21	<i>ophthalmicus punctulatus</i>	NMNH B-5403	Panama: Chiriqui, Lago Fortuna	EU427612
21	<i>ophthalmicus punctulatus</i>	LSUMZ B-28158	Panama: Chiriqui, Lago Fortuna (4 km S)	EU427609
21	<i>ophthalmicus punctulatus</i>	LSUMZ B-28177	Panama: Chiriqui, Lago Fortuna (4 km S)	EU427610
22	<i>ophthalmicus jaqueti</i>	AMNH GFB3143	Venezuela: Aragua, km 40 on El Junquito/Col. Tovvar Rd.	EU427616
23	<i>ophthalmicus phaeocephalus</i>	LSUMZ B6210	Ecuador: Morona-Santiago, Cordillera del Cutucu	EU427631
23	<i>ophthalmicus phaeocephalus</i>	LSUMZ B6242	Ecuador: Morona-Santiago, Cordillera del Cutucu	EU427632

(continued on next page)

Table 1 (continued)

#	Taxon	Museum (tissue no.)	Locality	Accession no.
24	<i>ophthalmicus phaeocephalus</i>	ANSP 4841	Ecuador	EU427633
25	<i>ophthalmicus phaeocephalus</i>	LSUMZ B33884	Peru: Cajamarca	EU427630
26	<i>ophthalmicus hiaticolus</i>	ZMUC JGM6-160796	Peru, Dpt. Amazonas, Cordillera Colan,	AY609300 ^a
26	<i>ophthalmicus hiaticolus</i>	LSUMZ B5619	Peru: Amazonas, 30 km east of Florida	EU427629
27	<i>ophthalmicus cinereocephalus</i>	LSUMZ B8191	Peru: Dpt. Pasco, Playa Pampa (8 km NW of Cushi)	EU427626
27	<i>ophthalmicus cinereocephalus</i>	LSUMZ B7966	Peru: Dpt. Pasco, Playa Pampa (8 km NW of Cushi)	EU427628
27	<i>ophthalmicus cinereocephalus</i>	LSUMZ B1710	Peru: Pasco	EU427627
28	<i>ophthalmicus peruvianus</i>	FMNH 398409	Peru: Paucartambo, Suecia, km 138.5 on Cuzco-Shintuya Highway, Cosnipata Valley	EU427617
28	<i>ophthalmicus peruvianus</i>	FMNH 398412	Peru: Paucartambo, Suecia, km 138.5 on Cuzco-Shintuya Highway, Cosnipata Valley	EU427618
29	<i>ophthalmicus peruvianus</i>	LSUMZ B575	Peru: Puno	EU427619
30	<i>ophthalmicus bolivianus</i>	LSUMZ B-22831	Bolivia: La Paz, Cerro Asunta Pata	EU427623
31	<i>ophthalmicus fulvularis</i>	LSUMZ B31508	Bolivia: Santa Cruz, Florida (23 km E Samaipata)	EU427624
31	<i>ophthalmicus fulvularis</i>	AMNH CJV300	Bolivia: Santa Cruz, Parque National Amboro	EU427625
32	<i>ophthalmicus argentinus</i>	LSUMZ 39026	Bolivia: Cochabamba, Chapare, San Onofre (43 km W Villa Tunari)	EU427622
33	<i>ophthalmicus argentinus</i>	MBM gav666	Argentina: Tucuman, San Miguel de Tucuman (20 km N)	EU427620
33	<i>ophthalmicus argentinus</i>	MBM jag1918	Argentina: Tucuman, Tafi del Valle (20 km S, 6 km E)	EU427621
34	<i>tacarcunae</i>	MVUP 1977	Panama: Darien, Cerro Chucanti	EU427644
34	<i>tacarcunae</i>	MVUP 1078	Panama: Darien, Cerro Chucanti	EU427645
35	<i>inornatus</i>	LSUMZ B-1387	Panama: Darien, Cerro Pirre	EU427642
35	<i>inornatus</i>	LSUMZ B-1403	Panama: Darien, Cerro Pirre	EU427643
36	<i>semifuscus semifuscus</i>	ANSP 816	Ecuador	EU427639
36	<i>semifuscus semifuscus</i>	LSUMZ B6266	Ecuador: Pichincha	EU427641
36	<i>semifuscus semifuscus</i>	LSUMZ B34875	Ecuador: Pichincha	EU427640
37	<i>pileatus</i>	LSUMZ B-9957	Costa Rica: San Jose, La Georgina	EU427635
37	<i>pileatus</i>	LSUMZ B-9960	Costa Rica: San Jose, La Georgina	EU427634
38	<i>pileatus</i>	LSUMZ B-28243	Panama: Chiriqui, Boquete	EU427636
38	<i>pileatus</i>	LSUMZ B-28250	Panama: Chiriqui, Boquete	EU427637
39	<i>pileatus</i>	NMNH B-5503	Panama: Chiriqui, Cerro Hornito, Fortuna Reserva	EU427638

Genbank accession numbers for ATPase 6 and 8 are given. LSUMZ, Louisiana Museum of Natural History; AMNH, American Museum of Natural History; NMNH, National Museum of Natural History; MBM, Marjorie Barrick Museum of Natural History; FMNH, Field Museum of Natural History; STRI, Smithsonian Tropical Research Institute; MZFC, Museo de Zoología, Facultad de Ciencias; UKNH, University of Kansas Natural History; ZMUC, Zoological Museum, University of Copenhagen; ANSP, Academy of Natural Sciences, Philadelphia; MVUP, Museo de Vertebrados de la Universidad de Panamá.

^a Samples previously published in [García-Moreno et al. \(2004\)](#).

Bayesian analyses were carried out in a parallel processing version of MrBayes 3.1.2 ([Huelsenbeck and Ronquist, 2001](#)) using the appropriate model for each gene partition. Model parameters were estimated by the program. Both the ATPase 6 and 8 dataset and extended sequencing dataset were run for 20 million generations. To effectively sample the posterior distribution while minimizing autocorrelation between steps, trees were sampled only every 5000 generations after an initial “burnin” period of 2 million generations. Majority-rule consensus trees were constructed from the 3600 sampled trees. Equally weighted parsimony trees were also generated for the extended sequencing dataset using a heuristic search and the branching-and-bound option in PAUP* v.4.0b10 ([Swofford, 2002](#)).

We tested the validity of a global molecular clock for our extended sequencing dataset by comparing likelihoods with and without a clock assumption using a likelihood

ratio test ([Felsenstein, 1981](#); for details see [Weir and Schluter, 2004](#)). Likelihoods of both models were estimated in PAUP* v.4.0b10 ([Swofford, 2002](#)). Because a model with a global clock assumption was not rejected ($P = 0.15$), we used both PAUP* v.4.0b10 ([Swofford, 2002](#)) and BEAST v1.4.2 ([Drummond and Rambaut, 2006](#)) to obtain ultrametric estimates of branch length along the Bayesian tree topology. We ran BEAST with a yule prior for 50 million generations and sampled every 1000 generations after an initial “burnin” of 2 million generations.

Saturation plots revealed considerable saturation in uncorrected p -distances exceeding only 4% ([Fig. 2a](#)), thus we used the GTR- Γ -I model in all clock analysis. A large dataset of passerine molecular clocks strongly support an average molecular rate of 2% corrected sequence divergence per My^{-1} (clock calibrations used GTR- Γ model for corrected distances; [Weir and Schluter, in press](#)) for cyt *b*. To determine the validity of applying the cyt *b*

Table 2
Genbank accession numbers for the extended five-gene dataset

Species	Museum (tissue no.)	ATPase 6 and 8	cyt <i>b</i>	ND2	COI
<i>o. albifrons</i>	MBM ddb5526	EU427588	EU427662	EU427680	EU427647
<i>o. argentinus</i>	MBM jag1918	EU427621	EU427675	EU427692	EU427657
<i>o. bolivianus</i>	LSUMZ B-22831	EU427623	EU427673	EU427690	EU427656
<i>o. cinereocephalus</i>	LSUMZ B1710	EU427627	EU427672	EU427689	EU427655
<i>o. dwighti</i>	LSUMZ B18089	EU427591	EU427663	EU427681	EU427648
<i>o. fulvicularis</i>	AMNH CJV300	EU427625	EU427674	EU427691	
<i>o. honduratus</i>	UKNH 5074	EU427599	EU427667	EU427685	EU427651
<i>o. jaqueti</i>	AMNH GFB3143	EU427616	EU427670		
<i>o. punctulatus</i>	NMNH B-1490	EU427608	EU427669	EU427687	EU427653
<i>o. ophthalmicus</i>	FMNH 394061	EU427577	EU427665	EU427683	
<i>o. phaeocephalus</i>	LSUMZ B-6210	EU427631	EU427671	EU427688	EU427654
<i>o. postocularis</i>	MBM ddb4454	EU427592	EU427666	EU427684	EU427650
<i>o. regionalis</i>	MBM dab1325	EU427602	EU427668	EU427686	EU427652
<i>o. wetmorei</i>	FM 393775	EU427586	EU427664	EU427682	EU427649
<i>inornatus</i>	LSUMZ B-1387	EU427642	EU427676		EU427658
<i>pileatus</i>	LSUMZ B-9957	EU427635	EU427677	EU427693	EU427659
<i>semifuscus semifuscus</i>	LSUMZ B6266	EU427641	EU427678	EU427694	EU427660
<i>tacarcunae</i>	STRI PA-CPS2	EU427644	EU427679	EU427695	EU427661

molecular clock to our entire five-gene dataset, we compared model corrected (GTR- Γ -I model) genetic distances of cyt *b* with those of the remaining genes (ND2, ATPase 6 and 8, COI) combined (Fig. 2b). Model corrected genetic distances of the extended dataset (excluding cyt *b*) were closely correlated ($r = 0.8$) and had a 1:1 ratio to corrected distances for cyt *b* (Fig. 2b). Therefore, we used the five-gene dataset to construct ultrametric branch lengths and applied the traditional cyt *b* clock to date nodes.

2.4. Morphological analysis

Isler and Isler (1999) scored subspecies of *ophthalmicus* for presence or absence of a distinct yellow breast band, postocular spot and dark eye. These traits were used in combination with biogeographic data to group *ophthalmicus* subspecies into groups (Isler and Isler, 1999). We confirmed their scoring of postocular spot and breast band from adult male specimens in the American Museum of Natural History and the Field Museum of Natural History

and use their classifications here. Isler and Isler (1999) did not score other members of the *ophthalmicus* complex for plumage traits (*tacarcunae*, *inornatus*, *semifuscus* and *ophthalmicus punctulatus*). Plumage traits were scored by eye for these and for the nearest outgroup, *C. pileatus* by the lead author [(specimen voucher numbers from American Museum of Natural History; *punctulatus* = 246543, 187968, 246548, 246542, 246549, 246547, 246541, 246546, 246550, 246552, 187969; *tacarcunae* = 736366, 136368, 136362, 136364, 136637, 136363; *inornatus* = 233689; *semifuscus semifuscus* = 125208, 125207, 511417, 511418, 511414, 511420), (specimens from the Field Museum of Natural History; *pileatus* = 35397, 35387, 343715, 220207, 220205, 220204, 35390, 220200, 35388, 35403)] and eye color was taken from Isler and Isler (1999) and Ridgely and Gwynne (1989).

Morphological traits were scored as 0 (trait absent, dark eye absent) or 1 (trait or dark eye present). These plumage traits show almost no variability within subspecies with the exception that *o. fulvicularis* is polymorphic for eye color. Breast bands were scored as present only if

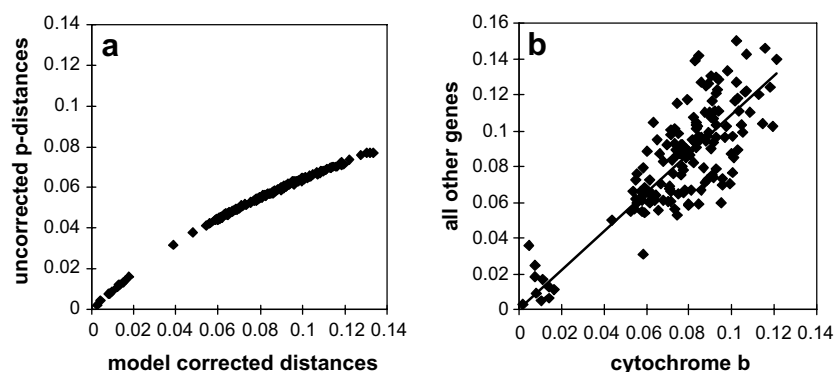


Fig. 2. (a) Saturation plot for the five-gene dataset; (b) relationship between maximum likelihood model corrected distances for cytochrome *b* only and the remaining four mitochondrial genes. The slope of the least squares regression line is 1.09, ($r^2 = 0.64$; $P < 0.0001$).

yellow coloration on the breast was distinct from the belly. *C. tacarcunae* has a yellowish breast and belly and so was scored as not possessing a breast band. *C. semifuscus* and *C. o. cinereocephalus* have slightly darker plumage on the breast but lack the bold yellow band seen in other subspecies of *ophthalmicus* and were scored as lacking bold chest bands. Isler and Isler (1999) likewise scored *o. cinereocephalus* as lacking a breast band. Postocular spots were scored as present if one or more white feathers occurred directly behind the eye. Parsimony and maximum likelihood (one parameter model with forward and backward transition rates equal) ancestor state reconstructions were performed for each morphological trait along the ultrametric five-gene phylogeny using Mesquite v1.12 (Maddison and Maddison, 2006). Clock-like branch lengths were used for maximum likelihood reconstructions. Because parsimony reconstructions in Mesquite v1.12 allow any given tree tip to possess multiple character states, *o. fulvicularis* was given both dark and pale eyes. However, separate analyses were run for pale and dark eyes in *fulvicularis* under maximum likelihood reconstructions because they allow only one character state for each tree tip.

3. Results

After deleting the 10 bp overlap, 832 bp of ATPase 6 and 8 were used for phylogenetic analysis; of these, 322 bp were variable. The partition homogeneity test showed no difference between genes ($P = 1.0$) and all gene regions were combined for phylogenetic analysis. The extended sequencing dataset, contained 3677 bp, 1029 of which were variable. Uncorrected distances for ingroup taxa in the extended sequencing dataset ranged from 0.5–8.1% for ATPase 6 and 8, 0.2–7.4% for *cyt b*, 0.2–10.3% for COI and 0.4–10.3% for ND2.

Evidence that our sequences are mitochondrial in origin are: (1) DNA was extracted from muscle tissue rich in mitochondria, (2) the absence of unexpected stop codons and insertions or deletions in protein coding genes and the ease at which sequences aligned with other published sequences from Genbank and (3) the similar tree topologies obtained from separate phylogenetic analysis of each of the protein coding genes (results not shown).

3.1. ATPase 6 and 8 analysis

All subspecies within *ophthalmicus* were reciprocally monophyletic with the following exceptions: (1) one individual of *regionalis* (from Nicaragua) grouped with *honduriatus*, but otherwise both subspecies were monophyletic, (2) *phaeocephalus* and *hiaticolus* together formed a monophyletic group but were not individually monophyletic and (3) *bolivianus*, *argentinus*, *jacqueti*, *fulvicularis* and *peruvianus* together formed a monophyletic group but those subspecies with multiple samples were not individually monophyletic. Relationships between many subspecies

groups of *ophthalmicus* were poorly resolved in the ATPase dataset.

3.2. Extended dataset analysis

The extended sequencing dataset included one sample from all species and subspecies in the ATPase 6 and 8 dataset except for *o. hiaticolus* which was phylogenetically nested within *o. phaeocephalus*; we included the latter to represent both subspecies. In contrast to the ATPase 6 and 8 analysis, most nodes received strong posterior and moderate to high bootstrap support in Bayesian and parsimony analysis of the extended dataset (Fig. 4).

Both Bayesian (Fig. 4) and parsimony (not shown) analyses uncovered similar tree topologies. These analyses confirm that *ophthalmicus* is not monophyletic with respect to *C. semifuscus*, *C. tacarcunae* and *C. inornatus*. In the *ophthalmicus* complex, three clades were strongly supported by both the Bayesian and parsimony analysis. The first clade comprised four subspecies of *ophthalmicus* from Mexico (MEX clade hereafter) and was basal to the remaining two clades. The second clade comprised four Central American subspecies of *ophthalmicus* from Guatemala to western Panama (CA clade) and the third, sister to the CA clade, comprised all Andean subspecies of *ophthalmicus* as well as the species *C. semifuscus*, *C. tacarcunae* and *C. inornatus* (SA clade). The monophyly of each of these clades was strongly supported. Relationships between taxa within these clades were fully resolved in all but the SA clade.

The relationships shown in the extended sequencing dataset are in conflict with the ATPase 6 and 8 dataset, in which all Central American and Mexican subspecies formed a monophyletic group. Constraining the topology to that uncovered in the ATPase 6 and 8 dataset (Fig. 3) did result in a significantly worse fit to the five-gene dataset ($P < 0.01$, Shimodaira–Hasegawa test with significance determined using a one-tailed bootstrap test of 1000 dataset permutations; Shimodaira and Hasegawa, 1999). While we are uncertain why these datasets differ in topology, we consider the relationships uncovered in the extended sequencing analysis to reflect the most accurate phylogenetic hypothesis for the genus.

Estimates of branch lengths under a global clock model were similar in PAUP and BEAST and only the results from the latter are reported here (Fig. 4).

3.3. Morphological analysis

Ancestor state reconstructions of morphological traits along the five-gene Bayesian phylogeny are presented in Fig. 5. Parsimony and maximum likelihood reconstructions were congruent for all traits at each node. Ancestor state probabilities are shown only at the ancestral node to the *ophthalmicus* complex. Treating eye color as dark

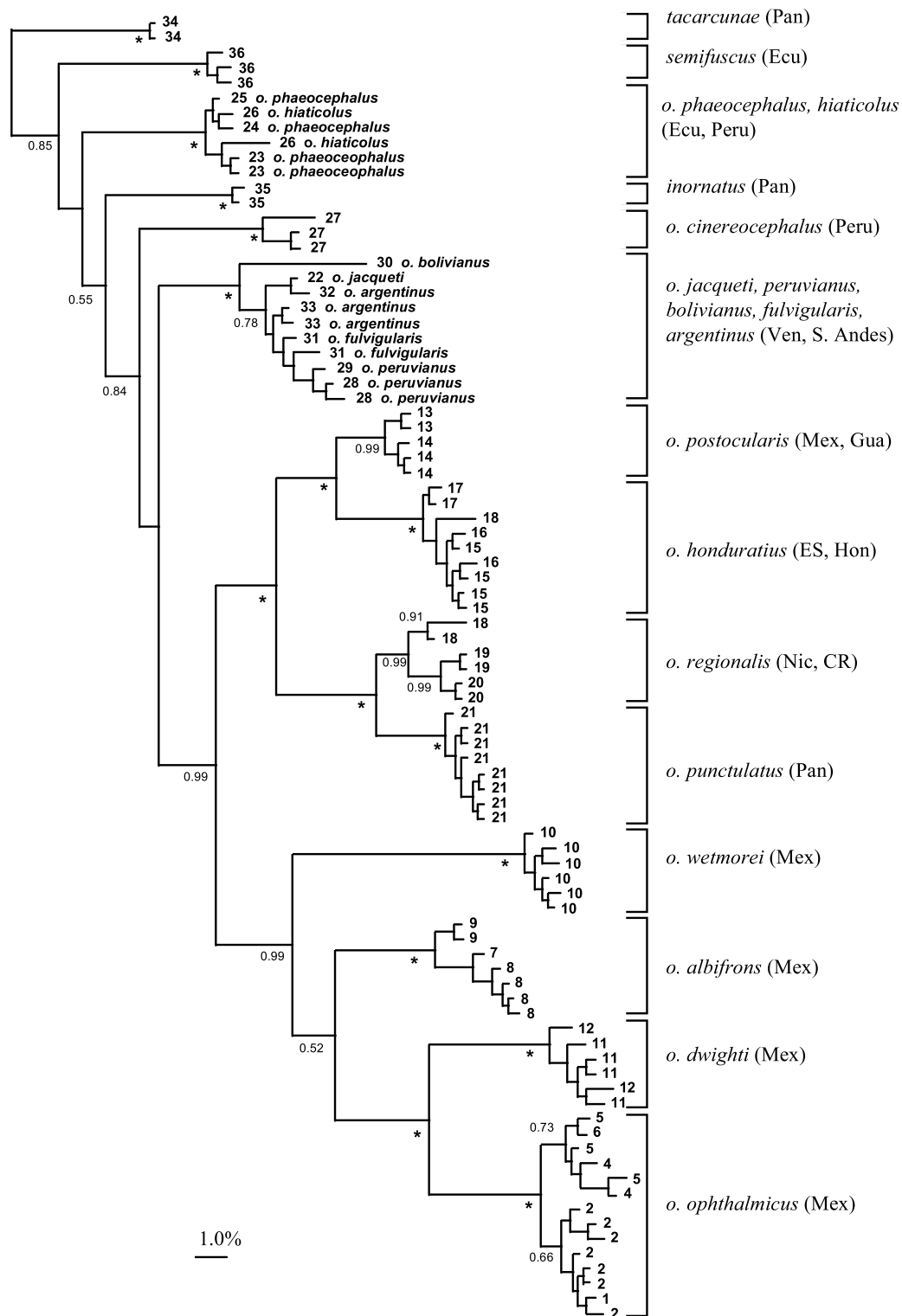


Fig. 3. Bayesian phylogeny of the *Chlorospingus ophthalmicus* complex for the ATPase 6 and 8 genes rooted to *C. pileatus* (not shown). Tip numbers refer to sampling localities in Table 1. Posterior probabilities are only shown for nodes connecting major lineages with support greater than 0.5 and for clarity are not shown within subspecies. Asterisks indicate a probability of 1.0. Country abbreviations as follows: Mexico (Mex), Guatemala (Gua), Honduras (Hon), El Salvador (ES), Nicaragua (Nic), Costa Rica (CR), Panama (Pan), Venezuela (Ven), Ecuador (Ecu).

or light for the polymorphic *o. fulvicularis* had no effect on parsimony reconstructions and resulted in only minor changes in character probabilities at nodes in the maximum

likelihood method. The ancestral lineage for the SA clade was reconstructed as uncertain in the parsimony ancestor state reconstruction for postocular spot. In the maximum

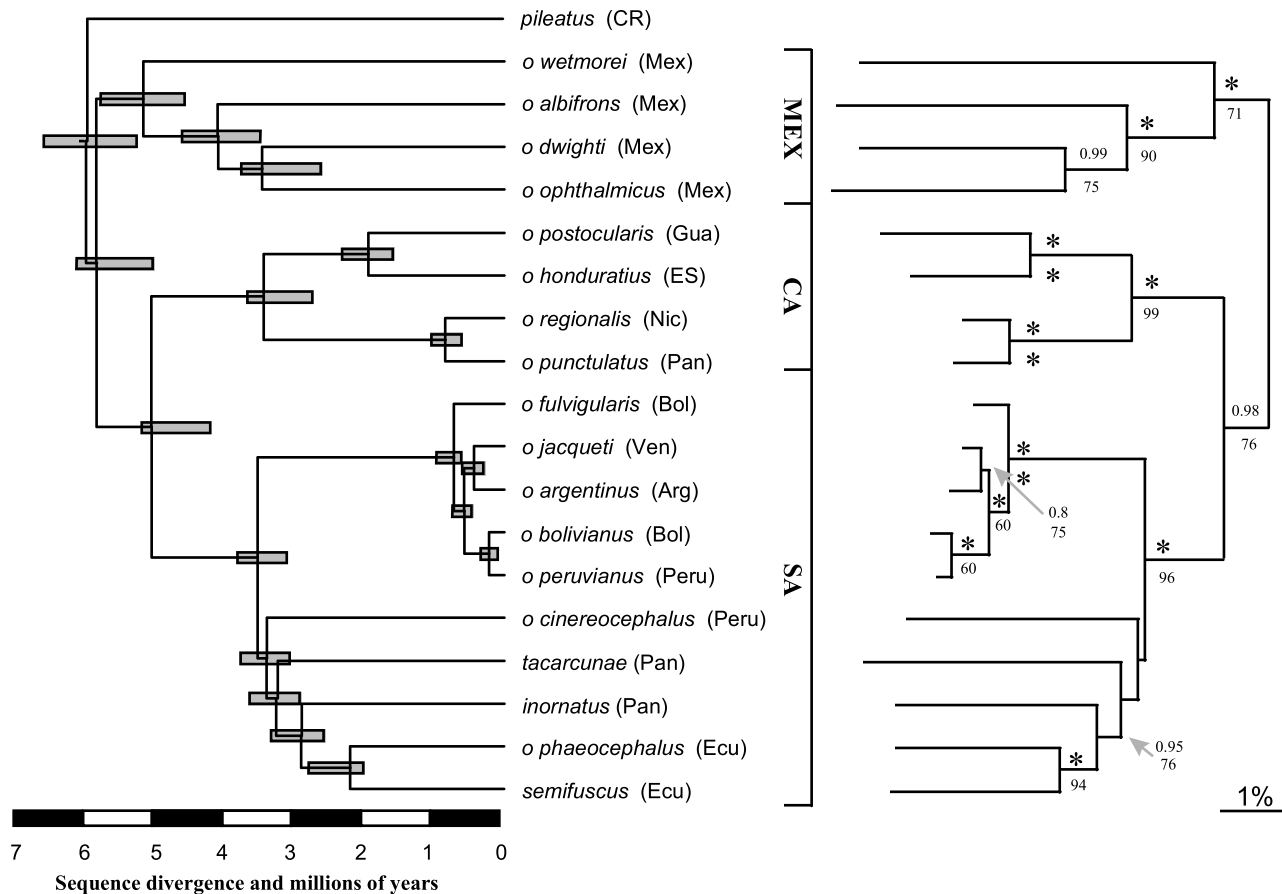


Fig. 4. Bayesian phylogeny of the *Chlorospingus ophthalmicus* complex for the five-gene dataset. (Right) Branch lengths without clock-like assumption and posterior probability shown above nodes as in Fig. 3 and parsimony bootstrap values (percentage of 1000 bootstrap replicates) shown below nodes. (Left) Branch lengths (and 95% confidence intervals) estimated with maximum likelihood under a global molecular clock. Scale bars show branch length in percent sequence divergence and in millions of years ago. Three main clades in the *ophthalmicus* complex are labeled MEX (Mexican), CA (Central American) and SA (South American). Country abbreviations as in Fig. 3 and as follows: Arg (Argentina), Bol (Bolivia).

likelihood ancestor state reconstruction, absence of a post-ocular spot received the strongest support but was not significant.

4. Discussion

Like other emberizids, *C. ophthalmicus* is comprised of numerous morphologically distinct subspecies. However, unlike many temperate sparrows and other passerines whose subspecies rarely exhibit genetic differentiation in mitochondrial DNA (Zink, 2004), many subspecies of *ophthalmicus* were highly differentiated genetically and formed reciprocally monophyletic groups. This result was paralleled by phylogeographic analysis of two other widespread Neotropical highland species of sparrow (*Buarremon brunneinucha* and *B. torquatus*; Cadena et al., 2007). Phillimore and Owens (2006) recently estimated that approximately half of all avian subspecies distributed south of the northern hemisphere temperate and arctic zones were phylogenetically distinct. This average is exceeded by *ophthalmicus* with 60% (9 of 15 sampled subspecies) of its subspecies distinct. This high degree of

genetic differentiation highlights an extended history of diversification.

4.1. Phylogenetics and phylogeography

The phylogenetic evidence confirmed Zimmer's (1947) hypothesis that the species *C. semifuscus* and *C. inornatus* were derived from within a paraphyletic *ophthalmicus* (Fig. 4). Together with the *o. flavopectus* subspecies group, these species formed a well-supported, monophyletic subclade within the SA clade. In addition, *C. tacarcunae* was also nested within the SA clade of *ophthalmicus*, and was not closely related to *C. fulvularis* as previously suggested (Hellmayr, 1936). The *ophthalmicus* complex, expanded to include these species, forms a monophyletic group that is sister to *C. pileatus*.

The *C. ophthalmicus* complex is composed of at least 14 monophyletic and moderately to deeply diverged lineages. These lineages date between ~0.8 and 5.2 Ma. In addition to *semifuscus*, *inornatus* and *tacarcunae*, 11 genetically divergent lineages occurred within *ophthalmicus* as currently defined. With the exception of *o. honduratus* all

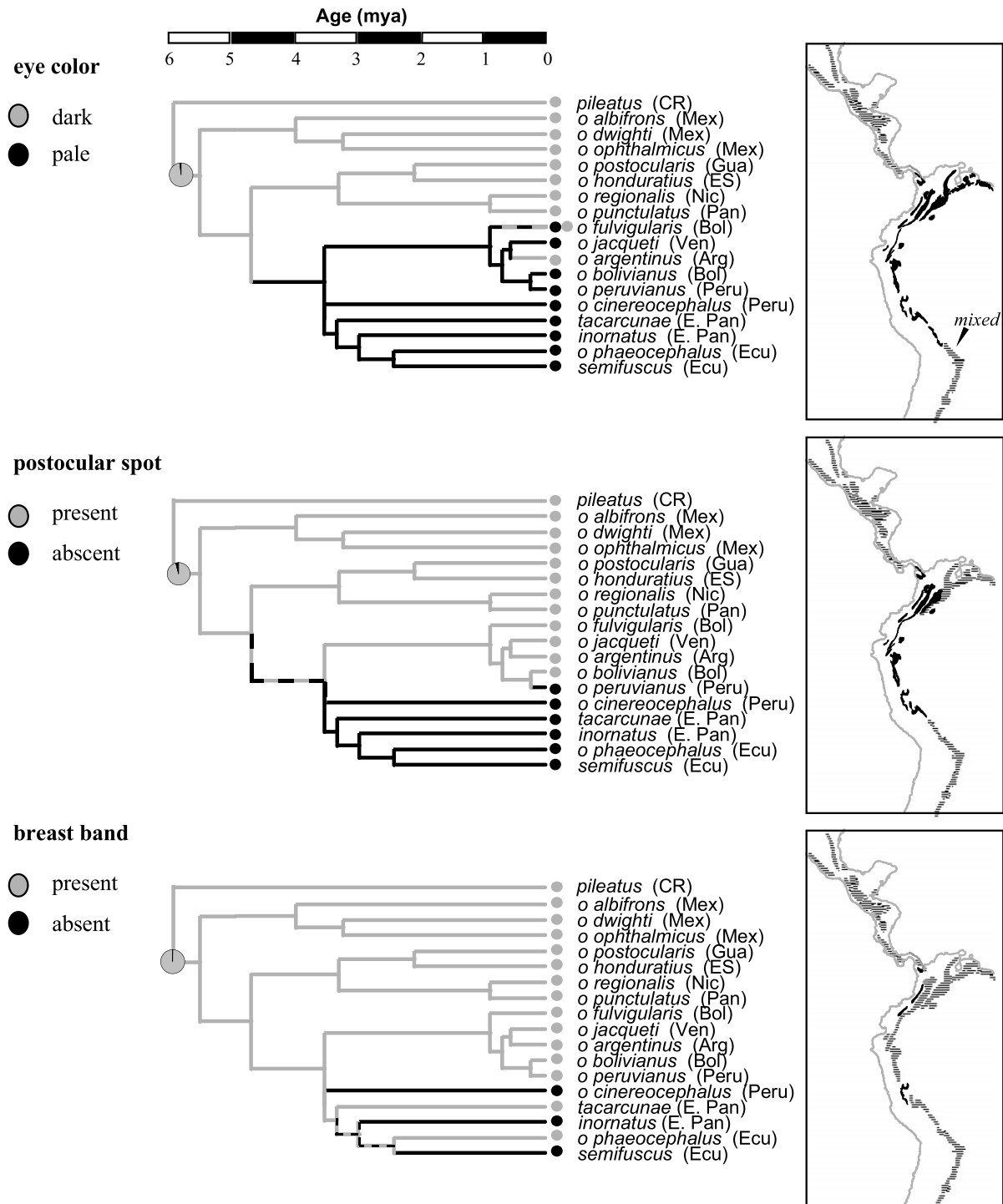


Fig. 5. Ancestor state reconstructions of morphological traits along the five-gene phylogeny for the *Chlorospingus ophthalmicus* complex. Character states for each taxon are shown at tree tips. The most parsimonious reconstruction is plotted using different shading along tree branches. Pie diagrams show maximum likelihood support for character states in the ancestral *ophthalmicus*. *Ophthalmicus fulvularis* is polymorphic for eye color. Treating eye color as dark or pale did not change reconstructions. Geographic distribution of character states are shown on maps. Presence of trait shown by gray (hatching) and absence of trait shown by black on trees and maps.

Mexican and Central American subspecies of *C. ophthalmicus* were reciprocally monophyletic for mitochondrial DNA haplotypes. Given that *regionalis* and *honduratus* coalesce more than 3 Ma (Fig. 4), the placement of a single individual of *regionalis* within *honduratus* is probably due

to recent gene flow between these taxa rather than incomplete lineage sorting (see below). In the Andes, only three reciprocally monophyletic lineages of *ophthalmicus* were recovered: (1) *peruvianus*, *fulvularis*, *bolivianus*, *argentinus* and *jacqueti* (based on morphological similarity and bio-

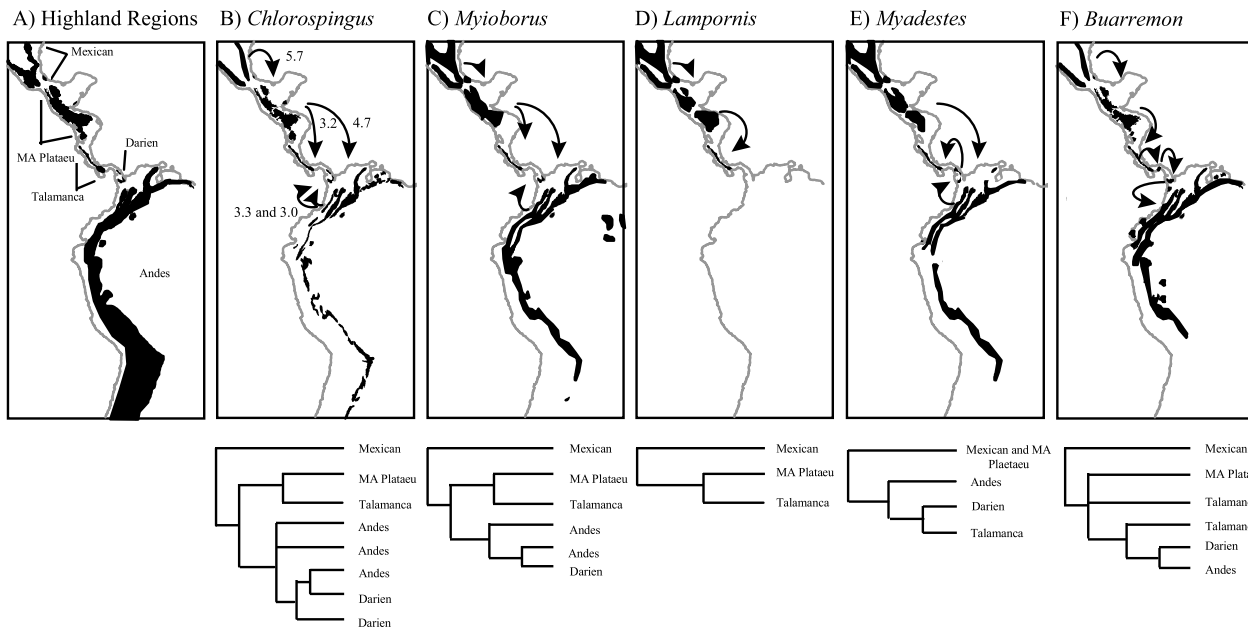


Fig. 6. Comparative phylogeography of five species complexes with an inferred north to south colonization route. (A) Neotropical highland regions, (B) *Chlorospingus ophthalmicus* complex, (C) *Myioborus miniatus* complex (Perez-Eman, 2005), (D) *Lampornis* (García-Moreno et al., 2006) and (E) *Myadestes* (Miller et al., 2007), (F) *Buarremon brunneinucha* complex (Cadena et al., 2007). Branch lengths do not represent time or genetic distance. Arrows on maps depict one possible interpretation of the phylogeographic history for each area cladogram below. Other interpretations are possible but conflict with the known geological history of the Central American landbridge (see text).

geographic proximity to *jacqueti*, unsampled subspecies of the *venezuelensis* subspecies group probably belong to this lineage); (2) *phaeocephalus* and *hiaticolus* (based on morphological similarity and biogeographic proximity to *phaeocephalus*, unsampled subspecies of the *flavopectus* subspecies group probably belong to this lineage) and (3) *cinereocephalus*.

Relationships between these genetic lineages do not conform well to the morphologically and biogeographically based subspecies groups defined by Isler and Isler (1999). The Islers arranged all Mexican and Central American subspecies of *ophthalmicus* into a single subspecies group. The phylogeny generated using only the 862 base pairs of ATPase 6 and 8 agrees with this arrangement. However, in the five-gene analysis, the MEX and CA clades were not sister to each other. Rather, the CA clade was sister to the SA clade containing all Andean subspecies of *ophthalmicus* and the species *semifuscus*, *inornatus* and *tacarcunae*. The MEX clade was sister to these. We are uncertain why topologies differ between the ATPase 6 and 8 versus five-gene datasets, but given the much larger number of base pairs in the five-gene dataset, and better levels of support at nodes, we consider its topology to most closely represent the evolutionary history of this group.

Only two subspecies groups as defined by Isler and Isler (1999) correspond to reciprocally monophyletic mitochondrial clades. These include *cinereocephalus*, which is the sole member of its group, and the *flavopectus* group for which we sampled only two subspecies, *o. phaeocephalus* and *o. hiaticolus*. Additional sampling of subspecies is necessary to confirm the monophyly of this group. Although

our sample sizes for Andean subspecies were small, the Venezuelan group and the four groups from the southern Andes defined by the Islers' shared mitochondrial haplotypes and they may not have had time to form reciprocally monophyletic groups. Despite their unique combinations of morphological characters, all appear closely related and recently diverged.

In a recent phylogenetic investigation of *ophthalmicus* subspecies from Mexico and northern Central America, García-Moreno et al. (2004) suggested that *ophthalmicus* originated in South America and spread northwards into Central America and finally Mexico. Our five-gene dataset rejects this hypothesis (Fig. 4). The sister relationship between the SA and CA clades with the MEX clade basal suggests that Andean forms were derived from a northern ancestor. Moreover, the sister to the entire *ophthalmicus* complex, *C. pileatus*, is also endemic to Central America lending further support for an origin outside of South America.

Phylogenetic studies are available for five other montane cloud-forest inhabiting species complexes believed to have originated in northern Middle America and spread south through the Neotropics (Perez-Eman, 2005; García-Moreno et al., 2006; Miller et al., 2007; Cadena et al., 2007; Fig. 6). Of these, *Myioborus miniatus* and *Buarremon brunneinucha* show a highly congruent pattern to *C. ophthalmicus* with endemic clades in Mexico, the Middle American Plateau, Talamanca highlands, Darien highlands and the Andes. Area cladograms between clades inhabiting these regions were identical in *Chlorospingus* and *Myioborus* and suggest both may have experienced a similar

phylogeographic history, but were slightly different in *Buarremon* (Fig. 6). *Lampornis* lacks clades in the Darien and Andes but otherwise has an identical area cladogram to *Chlorospingus*. *Myadestes* has a similar pattern, but has two species distributed widely throughout Mexico and the Middle American Plateau rather than lineages endemic to each of these regions, and has a slightly different area cladogram. In all cases, the basal-most divergence events within the Neotropics occurs between Mexican/Middle American Plateau lineages and all southern lineages. These cladograms are consistent with a northern origin and southward colonization route.

Central America began as a North American peninsula. Collision of crustal plates from the Pacific with plates in the Caribbean resulted in the gradual extension of this peninsula southwards (Coates and Obando, 1996). At ca. 3.1 Ma the peninsula finally joined with South America forming a contiguous landbridge between these continents (Coates and Obando, 1996). The radiations of the five species complexes in Fig. 6 were underway in Mexico and northern Central America before the completion of the landbridge. The interpretation of the area cladogram for the *ophthalmicus* complex that is most consistent with the geological history of lower Central America is as follows. At ~5.7 Ma *ophthalmicus* dispersed across the Isthmus of Tehuantepec from northern Mexico into the Middle American plateau. At 4.7 (95% confidence interval, 4.2–5.3) Ma, before the final completion of the landbridge, *ophthalmicus* dispersed from the Middle American Plateau into the Andes.

Highland regions in the lower part of Central America from Nicaragua to Colombia are believed to have formed no earlier than 4.5 Ma, sometime after the subduction of the Cocos plate beneath the Caribbean plate and other key processes in the formation of the Isthmus of Panama were initiated (Abratis and Worner, 2001). If this geological dating is correct, then endemic highland taxa probably did not occur in the Talamancan or Darien highlands until some time after 4.5 Ma. The area cladograms depicted in Fig. 6 are consistent with taxa in these regions having colonized either from a northern or southern route after the Talamancan and Darien highlands were uplifted (Fig. 6). In *C. ophthalmicus*, *Myioborus*, *Lampornis* and *Buarremon*, area cladograms suggest Talamancan highland endemics colonized from the north. In *C. ophthalmicus*, this occurred at approximately 3.2 Ma, at the final completion of the Central American landbridge.

Darien highland endemics were phylogenetically embedded within Andean clades in both the *Chlorospingus* and *Myioborus* complexes suggesting they colonized from South America. *Myadestes* may have also colonized the Darien from the Andes though other interpretations of its area cladogram (Fig. 6) are possible. In contrast, *Buarremon* appears to have colonized the Darien from the north. In *Chlorospingus*, two separate colonization events of the Darien highlands from South America probably resulted in the two Darien endemics, *C. inornatus* and *C.*

tacarcunae. These occurred at approximately 3.3 and 3.0 Ma, at the completion of the Central American landbridge. Alternatively, a single colonization of the Darien followed by a back colonization into the Andes is possible.

In the SA clade, Andean lineages of the *ophthalmicus* complex diverged between 2.4 and 3.5 Ma and form a phylogenetic leap-frog pattern (Figs. 1, 4 and 6). The lack of genetic differentiation between populations from Venezuelan and southern Peru to Argentina (Figs. 3 and 4) is surprising given that intervening regions in Central Peru to northern Colombia are occupied by the genetically and morphologically divergent lineages *C. semifuscus*, *o. cinereocephalus* and the *o. flavopectus* subspecies group (represented in Fig. 4 by *o. phaeocephalus*). Given the deep divergence events between these related lineages, *semifuscus*, *cinereocephalus* and the *o. flavopectus* subspecies group have probably occupied the Central Andes since shortly after the Andes were colonized (Fig. 4). A similar pattern was thought to occur in *Myioborus* redstarts with *M. bruniceps* from the southern Andes similar morphologically to north Andean and Tepui taxa despite intervening morphologically divergent species in the central Andes. However, molecular phylogenetic analysis demonstrated that these *Myioborus* taxa are not closely related (Perez-Eman, 2005). We know of no other taxon that exhibits the genetic leap-frog pattern observed in *Chlorospingus*. The lack of strong genetic differentiation between the Venezuelan samples and south Andean subspecies suggests that a range expansion must have occurred fairly recently within the last one million years of the Pleistocene. Whether this involved a continuous expansion up the eastern edge of the Andes or a long distance dispersal event is unknown.

Along the eastern edge of the Andes, major river valleys are believed to form geographic barriers to dispersal in many Andean birds. Phylogenetic breaks in *ophthalmicus* only correlated with one such river valley barrier. The Apurimac valley of Peru separated the ranges of *o. cinereocephalus* and *o. peruvianus*, which last shared a common ancestor ca. 3.5 Ma. Surprisingly, a phylogenetic break did not coincide with the Marañon river valley of northern Peru, a barrier known to have caused such breaks in other cloud-forest specialists (*Myadestes ralloides*, Miller et al., 2007; *Ochthoeca cinnamomeiventris*, García-Moreno et al., 1998). Rather, a phylogenetic break occurs between *C. o. hiaticolus* and *C. o. cinereocephalus*. These subspecies are distributed north and south of the Rio Apurimac valley region in Huanuco. A similar phylogenetic break occurs in this region in *Ochthoeca frontalis* (García-Moreno et al., 1998).

4.2. Morphological evolution

All subspecies from Central America and Mexico were phylogenetically distinctive despite their morphological uniformity in eye color, presence of breast band and postocular spot (Figs. 4 and 5). The deepest phylogenetic split within *ophthalmicus* occurred in Central America and Mex-

ico, separating morphologically similar subspecies into the deeply diverged MEX and CA clades (Fig. 4). By contrast, only one Andean subspecies was phylogenetically distinctive (*o. cinereocephalus*) despite the greater morphological variability of Andean subspecies. The lack of genetic differentiation between five of the seven morphologically defined subspecies groups in the Andes (Isler and Isler, 1999) suggests rapid race formation following a recent range expansion in the mid to late Pleistocene. The rapid formation of boldly patterned races is reported in high latitude sparrows (Mila et al., 2007a,b; Fry and Zink, 1998; Klicka et al., 1999) and a number of other species (e.g. Odeen and Bjorklund, 2003; Pavlova et al., 2005; Zink et al., 2002a,b), but has not been reported at this scale in a Neotropical species. In Andean *ophthalmicus*, the origin of many subspecies may be related to intense climatic cycles of the late Pleistocene in the Andes that are thought to have promoted rapid divergence of some avian groups (Weir, 2006).

Ancestor state reconstructions based on parsimony (Fig. 5) suggest the immediate common ancestor to the *ophthalmicus* clade possessed a dark eye, postocular spot, and breast band. This ancestral morphotype is retained in all subspecies of the MEX and CA clades but in the SA clade it is present only in the southern most race *o. argentinus* and in some individuals of *o. fulvicularis*. Maximum likelihood reconstructions gave similar conclusions.

Each of these characters exhibits leap-frog patterns in which populations sharing a character state are geographically bisected by populations exhibiting an alternative state (Fig. 5). Such leap-frog patterns characterize more than 20% of Andean species complexes and are thought to play an important role in diversification and speciation (Remsen, 1984). Whether geographically separated populations exhibiting similar color patterns are more closely related to each other than to intervening populations (and thus represent both morphological and phylogenetic leap-frog patterns) has not previously been tested.

In *C. ophthalmicus* most leap-frog patterns arose from either multiple transitions to a single state along the phylogeny (absence of breast band and postocular spot) or from back transitions to the ancestral character state (eye color; Fig. 5). Eye color is dark in Central America (excluding eastern Panama) and the southern Andes with intervening populations possessing light colored eyes. A transition to light colored eyes occurred in the ancestor of the SA clade, and then switched back to dark colored eyes in populations of the southern Andes. The absence of a breast band occurs in three populations: the Darien highlands of Panama, the western slope of the Andes of southern Colombia and Ecuador and in Central Peru with intervening populations possessing breast bands (Fig. 5). Although uncertainty exists in the reconstruction of breast bands, it is probable that breast bands were lost at least twice. These results suggest that some leap-frog patterns are not mirrored by concordant phylogenetic patterns but resulted from a complex history of character transition. The pres-

ence or absence of a white postocular spot was, however, partially concordant with phylogeography. Populations in Central America, Venezuela and the southern Andes all possess a postocular spot while intervening populations from eastern Panama to Central Peru do not. The loss of this spot by populations in the Central Andes dates to about 3.5 Ma. Venezuelan and southern Andean subspecies are not genetically differentiated, which suggests a recent dispersal event of birds with postocular spots around the Central Andean populations that lack them. This represents the only Andean case we are aware of where two geographically disjunct populations, that share a morphological trait, are more closely related to each other than to the intervening populations that lack the trait. The absence of a postocular spot in *o. peruvianus* might represent a second loss of this character state. Alternatively, it may have resulted from past introgression with Andean forms to the north.

None of these morphological traits unambiguously characterize the major clades (MEX, CA or SA clades) of the *ophthalmicus* complex. No traits characterize the separation of the MEX and CA clades and only pale eye coloration characterizes most, but not all members of the SA clade. Instead, the multiple transitions between character states at different points along the phylogeny have resulted in a geographic patch-work of morphological traits which bears little resemblance to phylogeny or geography and helps explain the high subspecies diversity in this species. Analysis of other plumage traits (crown color, throat color) that vary between populations is needed.

4.3. Taxonomic considerations

Chlorospingus ophthalmicus as currently defined is paraphyletic with respect to three taxa currently recognized as species: *C. semifuscus*, *C. inornatus* and *C. tacarcunae*. Though all three of these latter taxa have been considered conspecific within *ophthalmicus* (Meyer de Schauensee, 1966), each possesses a number of unique plumage features and on these grounds are generally afforded species status (Remsen et al., 2007; American Ornithologists' Union, 1998). In addition, *semifuscus* possesses a unique social system in which males form singing assemblages resembling leks (Bohorquez and Stiles, 2002). The exact role of these singing assemblages is uncertain and it is not apparent whether they would render this taxon reproductively isolated from other *ophthalmicus* taxa.

Under the phylogenetic or evolutionary species concepts *semifuscus*, *inornatus* and *tacarcunae* along with the 11 additional genetically divergent lineages in the *ophthalmicus* complex (Figs. 3 and 4) appear to represent separate species. Under the biological species concept, species boundaries are difficult to judge in this complex, as most forms are completely allopatric. In several cases where genetically divergent subspecies come in geographic contact, morphologically intermediate populations are reported to occur, suggesting gene flow.

Morphological evidence of gene flow is best documented by populations which are morphologically and geographically intermediate between *dwrighti* and *postocularis* in Guatemala (Zimmer, 1947), and *regionalis* and *punctulatus* in western Panama (Olson, 1993). Our study found no genetic evidence of introgression between *dwrighti* and *postocularis* but we lacked samples from populations believed to be of mixed origin. Further analysis is necessary to confirm the mixed ancestry of morphologically intermediate populations. If such analyses confirm gene flow between *dwrighti* and *postocularis*, it would suggest that lineages descended from the basal-most split within *ophthalmicus* have incomplete reproductive barriers despite approximately 5.7 million years of evolutionary divergence. In the case of the more recently diverged *regionalis* and *punctulatus*, our samples come from pure populations of *regionalis* and from a population morphologically most similar to *punctulatus* but showing some signs of morphological introgression with *regionalis* (Olson, 1993). Mitochondrial haplotypes of our *punctulatus*-like samples were genetically divergent from *regionalis* and showed no signs of mixed ancestry, but analysis of multiple unlinked loci are needed for verification. The only genetic evidence of gene flow uncovered by our study occurred between *regionalis* and *honduriatus* which last shared a common ancestor about 3.2 Ma. We only sampled three individuals of *regionalis* from Nicaragua, yet one of these individuals possessed a haplotype belonging to the geographically proximate *honduriatus* (Fig. 3). Additional study of contact zones is necessary to assess the extent of gene flow and the strength of reproductive barriers. In the absence of such information, we refrain from making taxonomic recommendations under the biological species concept.

Three phylogenetic splits occurred within *ophthalmicus* subspecies. Populations of the Mexican *o. ophthalmicus* from Hidalgo/Querétaro and Oaxaca/Veracruz formed two genetically diverged, reciprocally monophyletic lineages. More than one taxon was originally described from the range of *o. ophthalmicus* (i.e. *sumichrasti*; Ridgway, 1902). Likewise our samples of *o. albifrons* from Guerrero and Oaxaca formed two reciprocally monophyletic groups. Populations of *albifrons* in Oaxaca are often considered a distinct subspecies (*persimilis*; Phillips, 1966), a conclusion supported by our findings. Although sample sizes were low, Nicaraguan and Costa Rican populations of *o. regionalis* were also genetically divergent from each other. Slight morphological differences have previously been described between the Nicaraguan and Costa Rican populations (Zimmer, 1947) and when combined with genetic data, suggest that two taxa may be involved. However, these morphological differences could have resulted from introgression between *regionalis* and *honduriatus* in Nicaragua as suggested by the placement of one of our Nicaraguan samples of *regionalis* within *honduriatus*. Further molecular and morphological analysis is necessary to determine the taxonomic status for each of these populations.

5. Conclusion

The molecular phylogenetic hypothesis for the *ophthalmicus* complex suggests a Mexican origin with subsequent colonization and diversification throughout the Neotropical highland system, a conclusion reached by several other phylogeographic studies of widespread Neotropical highland species (Perez-Eman, 2005; García-Moreno et al., 2006; Miller et al., 2007; Cadena et al., 2007). Together, these studies suggest that a North American cloud-forest fauna may have served as a source for many currently widespread highland Neotropical taxa. The chain of isolated highland regions of Mexico and Central America produced the most genetically distinctive but least morphologically distinctive populations of *ophthalmicus*, while Andean populations were composed of few genetically differentiated but multiple morphologically differentiated forms. Morphological analysis of other widespread cloud-forest species complexes is necessary to determine the generality of these patterns.

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