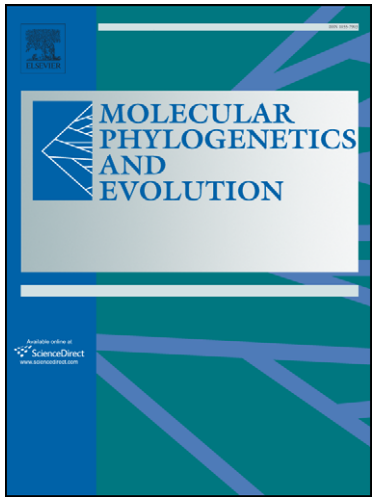

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Chlorospingus ophthalmicus Phylogeography

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.

Key Words: Emberizidae, *Chlorospingus ophthalmicus*, phylogeography, Neotropics, Andes, Central America, leap-frog patterns.

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 Neotropical 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 distinctive 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 post-ocular 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 *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 Monroe 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 et al. (2007) suggested several northern Mesoamerican *ophthalmicus* subspecies be elevated to species based on genetic and morphological considerations.

We investigated the phylogeographic structure within *Chlorospingus 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, 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 vouchered 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 and American Museum of Natural History (Tables 1 and 2). Also included were ATPase 6 & 8 sequences published in a previous study of Mexican *ophthalmicus* (García-Moreno et al 2004; Table 1).

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 & 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 & 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 COIf and COIa (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 & 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 & 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 & 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 & 8 dataset and the GTR-Γ-I model best fit the extended dataset.

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 & 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 branch-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-gamma-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 2008) for cyt *b*. To determine the validity of applying the cyt *b* molecular clock to our entire five-gene dataset, we compared model corrected (GTR-gamma-I model) genetic distances of cyt *b* with those of the remaining genes (ND2, ATPase 6 & 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 semsifuscus* = 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. fulvigularis* is polymorphic for eye color. Breast bands were scored

as present only if 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 posses multiple character states, *o. fulvigularis* was given both dark and pale eyes. However, separate analysis were run for pale and dark eyes in fulvigularis 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 & 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 & 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 & 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, fulvigularis* 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 & 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 & 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 analysis 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 & 8 dataset, in which all Central American and Mexican subspecies formed a monophyletic group. Constraining the topology to that uncovered in the ATPase 6 & 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 Figure 6. 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 or light for the polymorphic *o. fulvigularis* 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 likelihood ancestor state reconstruction, absence of a postocular spot received the strongest support but was not significant.

4. Discussion

Like other emberizids, *Chlorospingus 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. flavigularis* 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 to 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. honduriatus* all Mexican and Central American subspecies of *C. ophthalmicus* were reciprocally monophyletic for mitochondrial DNA haplotypes. Given that regionalis and honduriatus coalesce more than 3 Ma (Fig. 4), the placement of a single individual of *regionalis* within *honduriatus* is probably due to recent gene flow between these taxa rather than

299 incomplete lineage sorting (see below). In the Andes, only three reciprocally
300 monophyletic lineages of *ophthalmicus* were recovered: 1) *peruvianus, fulvigularis,*
301 *bolivianus*, *argentinus* and *jacqueti* (based on morphological similarity and
302 biogeographic proximity to *jacqueti,* unsampled subspecies of the *venezuelensis*
303 subspecies group probably belong to this lineage); 2) *phaeocephalus* and *hiaticolus*
304 (based on morphological similarity and biogeographic proximity to *phaeocephalus,*
305 unsampled subspecies of the *flavopectus* subspecies group probably belong to this
306 lineage) and 3) *cinereocephalus*.

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

319 Only two subspecies groups as defined by Isler and Isler (1999) correspond to
320 reciprocally monophyletic mitochondrial clades. These include *cinereocephalus*, which is
321 the sole member of its group, and the *flavopectus* group for which we sampled only two

322 subspecies, *o. phaeocephalus* and *o. hiaticolus*. Additional sampling of subspecies is
323 necessary to confirm the monophyly of this group. Although our sample sizes for Andean
324 subspecies were small, the Venezuelan group and the four groups from the southern
325 Andes defined by the Islers' shared mitochondrial haplotypes and they may not have had
326 time to form reciprocally monophyletic groups. Despite their unique combinations of
327 morphological characters, all appear closely related and recently diverged.

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

336 Phylogenetic studies are available for five other montane cloud forest inhabiting
337 species complexes believed to have originated in northern Middle America and spread
338 south through the Neotropics (Perez-Eman 2005, García-Moreno et al. 2006, Miller et al.
339 2007, Cadena et al. 2007; Fig. 6). Of these, *Myioborus miniatus* and *Baurremon*
340 *brunneinucha* show a highly congruent pattern to *Chlorospingus ophthalmicus* with
341 endemic clades in Mexico, the Middle American Plateau, Talamanca highlands, Darien
342 highlands and the Andes. Area cladograms between clades inhabiting these regions were
343 identical in *Chlorospingus* and *Myioborus* and suggest both may have experienced a
344 similar phylogeographic history, but were slightly different in *Buarremon* (Fig. 6).

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

352 Central America began as a North American peninsula. Collision of crustal plates
353 from the Pacific with plates in the Caribbean resulted in the gradual extension of this
354 peninsula southwards (Coates and Obando 1996). At ca. 3.1 Ma the peninsula finally
355 joined with South America forming a contiguous landbridge between these continents
356 (Coates and Obando 1996). The radiations of the five species complexes in Figure 6 were
357 underway in Mexico and northern Central America before the completion of the
358 landbridge. The interpretation of the area cladogram for the *ophthalmicus* complex that is
359 most consistent with the geological history of lower Central America is as follows. At ~
360 5.7 Ma *ophthalmicus* dispersed across the Isthmus of Tehuantepec from northern Mexico
361 into the Middle American plateau. At 4.7 (95% confidence interval, 4.2 to 5.3) Ma,
362 before the final completion of the landbridge, *ophthalmicus* dispersed from the Middle
363 American Plateau into the Andes.

364 Highland regions in the lower part of Central America from Nicaragua to
365 Colombia are believed to have formed no earlier than 4.5 Ma, sometime after the
366 subduction of the Cocos plate beneath the Caribbean plate and other key processes in the
367 formation of the Isthmus of Panama were initiated (Abratis and Worner 2001). If this

368 geological dating is correct, then endemic highland taxa probably did not occur in the
369 Talamanca or Darien highlands until some time after 4.5 Ma. The area cladograms
370 depicted in Figure 6 are consistent with taxa in these regions having colonized either
371 from a northern or southern route after the Talamanca and Darien highlands were uplifted
372 (Fig. 6). In *Chlorospingus ophthalmicus*, *Myioborus, Lampornis* and *Buarremon*, area
373 cladograms suggest Talamanca highland endemics colonized from the north. In *C.*
374 *ophthalmicus*, this occurred at approximately 3.2 Ma, at the final completion of the
375 Central American landbridge.

376 Darien highland endemics were phylogenetically embedded within Andean clades
377 in both the *Chlorospingus* and *Myioborus* complexes suggesting they colonized from
378 South America. *Myadestes* may have also colonized the Darien from the Andes though
379 other interpretations of its area cladogram (Fig. 6) are possible. In contrast, *Buarremon*
380 appears to have colonized the Darien from the north. In *Chlorospingus*, two separate
381 colonization events of the Darien highlands from South America probably resulted in the
382 two Darien endemics, *C. inornatus* and *C. tacarcunae.* These occurred at approximately
383 3.3 and 3.0 Ma, at the completion of the Central American landbridge. Alternatively, a
384 single colonization of the Darien followed by a back colonization into the Andes is
385 possible.

386 In the SA clade, Andean lineages of the *ophthalmicus* complex diverged between
387 2.4 and 3.5 Ma and form a phylogenetic leapfrog pattern (Figs 1,4,6). The lack of genetic
388 differentiation between populations from Venezuelan and southern Peru to Argentina
389 (Figs 3 and 4) is surprising given that intervening regions in Central Peru to northern
390 Colombia are occupied by the genetically and morphologically divergent lineages *C.*

391 *semifuscus*, *o. cinereocephalus* and the *o. flavopectus* subspecies group (represented in
392 Fig. 4 by *o. phaeocephalus*). Given the deep divergence events between these related
393 lineages, *semifuscus*, *cinereocephalus* and the *o. flavopectus* subspecies group have
394 probably occupied the Central Andes since shortly after the Andes were colonized (Fig.
395 4). A similar pattern was thought to occur in *Myioborus* redstarts with *M. brunniceps*
396 from the southern Andes similar morphologically to north Andean and Tepui taxa despite
397 intervening morphologically divergent species in the central Andes. However, molecular
398 phylogenetic analysis demonstrated that these *Myioborus* taxa are not closely related
399 (Perez-Eman 2005). We know of no other taxon that exhibits the genetic leapfrog pattern
400 observed in *Chlorospingus*. The lack of strong genetic differentiation between the
401 Venezuelan samples and south Andean subspecies suggests that a range expansion must
402 have occurred fairly recently within the last one million years of the Pleistocene. Whether
403 this involved a continuous expansion up the eastern edge of the Andes or a long distance
404 dispersal event is unknown.

405 Along the eastern edge of the Andes, major river valleys are believed to form
406 geographic barriers to dispersal in many Andean birds. Phylogenetic breaks in
407 *ophthalmicus* only correlated with one such river valley barrier. The Apurimac valley of
408 Peru separated the ranges of *o. cinereocephalus* and *o. peruvianus,* which last shared a
409 common ancestor ca. 3.5 Ma. Surprisingly, a phylogenetic break did not coincide with
410 the Marañon river valley of northern Peru, a barrier known to have caused such breaks in
411 other cloud-forest specialists (*Myadestes ralloides*, Miller et al. 2007; *Ochthoeca*
412 *cinnamomeiventris,* García-Moreno et al. 1998). Rather, a phylogenetic break occurs
413 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 Mexico, 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, 2002b), 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

437 clades but in the SA clade it is present only in the three southern most races *o.*
438 *argentinus, o. bolivianus* and *o. fulvigularis*. Maximum likelihood reconstructions gave
439 similar conclusions.

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

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

a complex history of character transition. The presence or absence of a white postocular spot was, however, partially concordant with phylogeography. Populations in Central America, Venezuela and the southern Andes all posses 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

C. 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 (Figures 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 *dwighti* and *postocularis* in Guatemala (Zimmer 1947), and *regionalis* and *punctulatus* in western Panama (Olson 1993). Our study found no genetic evidence of introgression between *dwighti* 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 analysis confirm gene flow between *dwighti* and

505 *postocularis*, it would suggest that lineages descended from the basal most split within
506 *ophthalmicus* have incomplete reproductive barriers despite approximately 5.7 million
507 years of evolutionary divergence. In the case of the more recently diverged *regionalis* and
508 *punctulatus*, our samples come from pure populations of *regionalis* and from a
509 population morphologically most similar to *punctulatus* but showing some signs of
510 morphological introgression with *regionalis* (Olson 1993). Mitochondrial haplotypes of
511 our *punctulatus*-like samples were genetically divergent from *regionalis* and showed no
512 signs of mixed ancestry, but analysis of multiple unlinked loci are needed for verification.
513 The only genetic evidence of gene flow uncovered by our study occurred between
514 *regionalis* and *honduriatus* which last shared a common ancestor about 3.2 Ma. We only
515 sampled three individuals of *regionalis* from Nicaragua, yet one of these individuals
516 possessed a haplotype belonging to the geographically proximate *honduriatus* (Figure 3).
517 Additional study of contact zones is necessary to assess the extent of gene flow and the
518 strength of reproductive barriers. In the absence of such information, we refrain from
519 making taxonomic recommendations under the biological species concept.

520 Three phylogenetic splits occurred within *ophthalmicus* subspecies. Populations
521 of the Mexican *o. ophthalmicus* from Hidalgo / Querétaro and Oaxaca / Veracruz formed
522 two genetically diverged, reciprocally monophyletic lineages. More than one taxon was
523 originally described from the range of *o. ophthalmicus* (i.e. *sumichrasti*; Ridgway 1901).
524 Likewise our samples of *o. albifrons* from Guerrero and Oaxaca formed two reciprocally
525 monophyletic groups. Populations of *albifrons* in Oaxaca are often considered a distinct
526 subspecies (*persimilis*; Phillips 1966), a conclusion supported by our findings. Although
527 sample sizes were low, Nicaraguan and Costa Rican populations of *o. regionalis* were

528 also genetically divergent from each other. Slight morphological differences have
529 previously been described between the Nicaraguan and Costa Rican populations (Zimmer
530 1947) and when combined with genetic data, suggest that two taxa may be involved.
531 However, these morphological differences could have resulted from introgression
532 between *regionalis* and *honduriatus* in Nicaragua as suggested by the placement of one
533 of our Nicaraguan samples of *regionalis* within *honduriatus*. Further molecular and
534 morphological analysis is necessary to determine the taxonomic status for each of these
535 populations.
536
537 **Conclusion**
538 The molecular phylogenetic hypothesis for the *ophthalmicus* complex suggests a
539 Mexican origin with subsequent colonization and diversification throughout the
540 Neotropical highland system, a conclusion reached by several other phyogeographic
541 studies of widespread Neotropical highland species (Perez-Eman 2005, García-Moreno et
542 al. 2006, Miller et al. 2007, Cadena et al 2007). Together, these studies suggest that a
543 North American cloud-forest fauna may have served as a source for many currently
544 widespread highland Neotropical taxa. The chain of isolated highland regions of Mexico
545 and Central America produced the most genetically distinctive but least morphologically
546 distinctive populations of *ophthalmicus*, while Andean populations were composed of
547 few genetically differentiated but multiple morphologically differentiated forms.
548 Morphological analysis of other widespread cloud-forest species complexes is necessary
549 to determine the generality of these patterns.

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Figures

Figure 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 (see text). Numbered dots refer to sampling localities listed in Table 1.

Figure 2

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

Figure 3

Bayesian phylogeny of the *Chlorospingus ophthalmicus* complex for the ATPase 6 & 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).

Figure 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 Figure 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 Figure 3 and as follows: Arg (Argentina), Bol (Bolivia).

Figure 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 fulvigularis* 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.

Figure 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), E) *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).

Table 1

List of *Chlorospingus* samples used in this study. Genbank Accession numbers for ATPase 6 & 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 Zoologia, 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

#	Taxon	Museum (Tissue Number)	Locality	Accession No
01	*ophthalmicus ophthalmicus*	MZFC 9715	Mexico: Querétaro, 7km S of Tres Lagunas	AY609275*
02	*ophthalmicus ophthalmicus*	MZFC BMM 085	Mexico: Hidalgo, 5km E Tlanchinol	AY609276*
02	*ophthalmicus ophthalmicus*	MZFC 10398	Mexico: Hidalgo, 5km E Tlanchinol	AY609277*
02	*ophthalmicus ophthalmicus*	FMNH 394061	Mexico: Hidalgo, 5km E Tlanchinol	EU427577
02	*ophthalmicus ophthalmicus*	FMNH 394070	Mexico: Hidalgo, 5km E Tlanchinol	EU427578
02	*ophthalmicus ophthalmicus*	FMNH 394066	Mexico: Hidalgo, 5km E Tlanchinol	EU427579
03	*ophthalmicus ophthalmicus*	MZFC 11297	Mexico: Hidalgo, El Potrero, 5km Tenango	AY609278*
03	*ophthalmicus ophthalmicus*	MZFC 10981	Mexico: Hidalgo, El Potrero, 5km Tenango	AY609279*
04	*ophthalmicus ophthalmicus*	MZFC 12490	Mexico: Oaxaca, Sierra de Huautla	AY609281*
04	*ophthalmicus ophthalmicus*	MZFC 11585	Mexico: Oaxaca, Sierra de Huautla	AY609282*
05	*ophthalmicus ophthalmicus*	FMNH 346816	Mexico: Oaxaca, Nudo de Zempoaltepetl, 5 km below Totontepec	EU427580
05	*ophthalmicus ophthalmicus*	FMNH 393786	Mexico: Oaxaca, Totontepec, Cerro de Zempoaltepetl	EU427581
05	*ophthalmicus ophthalmicus*	FMNH 346811	Mexico: Oaxaca, Nudo de Zempoaltepetl, 5 km below Totontepec	EU427582
06	*ophthalmicus ophthalmicus*	MZFC MXJ 511	Mexico: Oaxaca, Cerro Zempoaltepetl, Totontepec	AY609283*

07	*ophthalmicus albifrons*	MZFC MX1437	Mexico, Guerrero, El Iris, Sierra de Atoyac	AY609293*
08	*ophthalmicus albifrons*	MBM dhb5526	Mexico: Guerrero, Carrizal de Bravo	EU427588
08	*ophthalmicus albifrons*	MBM dhb5527	Mexico: Guerrero, Carrizal de Bravo	EU427589
08	*ophthalmicus albifrons*	MBM dhb5545	Mexico: Guerrero, Carrizal de Bravo	EU427587
08	*ophthalmicus albifrons*	FMNH 393780	Mexico: Guerrero, Carrizal de Bravo	EU427590
09	*ophthalmicus albifrons*	MZFC 12810	Mexico, Oaxaca, Reyes Llano Grande	AY609294*
09	*ophthalmicus albifrons*	MZFC 11579	Mexico, Oaxaca, Reyes Llano Grande	AY609295*
10	*ophthalmicus wetmorei*	MZFC MX 1080	Mexico, Veracruz [Sierra de Los Tuxtlas], Volcan de Santa Marta	AY609284*
10	*ophthalmicus wetmorei*	MZFC MX 1078	Mexico, Veracruz [Sierra de Los Tuxtlas], Volcan de Santa Marta	AY609285*
10	*ophthalmicus wetmorei*	FMNH 393779	Mexico: Veracruz, El Bastonal	EU427584
10	*ophthalmicus wetmorei*	MBM 4952	Mexico: Veracruz, Sierra Santa Martha	EU427583
10	*ophthalmicus wetmorei*	MBM 4953	Mexico: Veracruz, Sierra Santa Martha	EU427585
10	*ophthalmicus wetmorei*	FMNH 393775	Mexico: Veracruz, El Bastonal (3 km SE)	EU427586
11	*ophthalmicus dwight*	MZFC 12084	Mexico, Oaxaca [Chimalapas] Chalchijapa, 20km NE del campamento	AY609288*
11	*ophthalmicus dwight*	LSUMZ B18090	Mexico, Oaxaca, Chimalapas	AY609290*
11	*ophthalmicus dwighti*	LSUMZ B18089	Mexico, Oaxaca, Chimalapas	EU427591
12	*ophthalmicus dwight*	MZFC 9573	Mexico, Chiapas, 6km NE de Pueblo Nuevo, camino Aurora-Ermita	AY609287*
12	*ophthalmicus dwighti*	MZFC 9584	Mexico, Chiapas, 6km NE de Pueblo Nuevo, camino Aurora-Ermita	AY609286*
13	*ophthalmicus postocularis*	MZFC 8826	Mexico, Chiapas, Rio Mala, Volcan Tacana	AY609291*
13	*ophthalmicus postocularis*	MZFC 8832	Mexico, Chiapas, Rio Mala, Volcan Tacana	AY609292*
14	*ophthalmicus postocularis*	MBM dhb4454	Guatemala: Quezaltenango	EU427592
14	*ophthalmicus postocularis*	MBM gav2384	Guatemala: Quezaltenango	EU427594
14	*ophthalmicus postocularis*	MBM jk02-150	Guatemala: Quezaltenango	EU427593
15	*ophthalmicus honduratus*	UKNH 4895	El Salvador: Chalatenango, Cerro El Pital	EU427600
15	*ophthalmicus honduratius*	UKNH 5074	El Salvador: Chalatenango, Cerro El Pital	EU427599
16	*ophthalmicus honduratius*	MBM jk9974	Honduras: Copan, Copan Ruinas (15 km N)	EU427595
16	*ophthalmicus honduratius*	MBM gav1537	Honduras: Copan, Copan Ruinas (15 km N)	EU427596
17	*ophthalmicus honduratius*	MBM jk01243	Honduras: Atlantida, La Ceiba (9.7 km SW)	EU427597
17	*ophthalmicus honduratius*	MBM gav2026	Honduras: Atlantida, La Ceiba (9.7 km SW)	EU427598

18	*ophthalmicus regionalis*	MBM dab1325	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427602
18	*ophthalmicus regionalis*	MBM dab1291	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427601
18	*ophthalmicus regionalis*	MBM dab1331	Nicaragua: Matagalpa, Matagalpa (10 km N)	EU427603
19	*ophthalmicus regionalis*	LSUMZ B-16013	Costa Rica: Heredia, Virgen del Socorro (4 km SE)	EU427607
19	*ophthalmicus regionalis*	LSUMZ B-16018	Costa Rica: Heredia, Virgen del Socorro (4 km SE)	EU427606
20	*ophthalmicus regionalis*	FMNH 393087	Costa Rica: Cartago, Tres Rios, 4.5 km NE, near Finca Pizote	EU427604
20	*ophthalmicus regionalis*	FMNH 393086	Costa Rica: Cartago, Tres Rios, 4.5 km NE, near Finca Pizote	EU427605
21	*ophthalmicus punctulatus*	NMNH B-1490	Panama: Bocas del Toro, Los Planes (13 km N)	EU427608
21	*ophthalmicus punctulatus*	NMNH B-5274	Panama: Bocas del Toro, Los Planes (13 km N)	EU427615
21	*ophthalmicus punctulatus*	NMNH B-1491	Panama: Bocas del Toro, Los Planes (13 km N)	EU427613
21	*ophthalmicus punctulatus*	NMNH B-2020	Panama: Bocas del Toro, Los Planes (24km N)	EU427611
21	*ophthalmicus punctulatus*	NMNH B-2019	Panama: Bocas del Toro, Los Planes (24km N)	EU427614
21	*ophthalmicus punctulatus*	NMNH B-5403	Panama: Chiriqui, Lago Fortuna	EU427612
21	*ophthalmicus punctulatus*	LSUMZ B-28158	Panama: Chiriqui, Lago Fortuna (4 km S)	EU427609
21	*ophthalmicus punctulatus*	LSUMZ B-28177	Panama: Chiriqui, Lago Fortuna (4 km S)	EU427610
22	*ophthalmicus jaqueti*	AMNH GFB3143	Venezuela: Aragua, km 40 on El Junquito/Col. Tovvar Rd.	EU427616
23	*ophthalmicus phaeocephalus*	LSUMZ B6210	Ecuador: Morona-Santiago, Cordillera del Cutucu	EU427631
23	*ophthalmicus phaeocephalus*	LSUMZ B6242	Ecuador: Morona-Santiago, Cordillera del Cutucu	EU427632
24	*ophthalmicus phaeocephalus*	ANSP 4841	Ecuador	EU427633
25	*ophthalmicus phaeocephalus*	LSUMZ B33884	Peru: Cajamarca	EU427630
26	*ophthalmicus hiaticolus*	ZMUC JGM6-160796	Peru, Dpt. Amazonas, Cordillera Colan,	AY609300*
26	*ophthalmicus hiaticolus*	LSUMZ B5619	Peru: Amazonas, 30 km east of Florida	EU427629
27	*ophthalmicus cinereocephalus*	LSUMZ B8191	Peru: Dpt. Pasco, Playa Pampa (8 km NW of Cushi)	EU427626
27	*ophthalmicus cinereocephalus*	LSUMZ B7966	Peru: Dpt. Pasco, Playa Pampa (8 km NW of Cushi)	EU427628
27	*ophthalmicus cinereocephalus*	LSUMZ B1710	Peru: Pasco	EU427627
28	*ophthalmicus peruvianus*	FMNH 398409	Peru: Paucartambo, Suecia, km 138.5 on Cuzco-Shintuya Highway, Cosnipata Valley	EU427617
28	*ophthalmicus peruvianus*	FMNH 398412	Peru: Paucartambo, Suecia, km 138.5 on Cuzco-Shintuya Highway, Cosnipata Valley	EU427618
29	*ophthalmicus peruvianus*	LSUMZ B575	Peru: Puno	EU427619
30	*ophthalmicus bolivianus*	LSUMZ B-22831	Bolivia: La Paz, Cerro Asunta Pata	EU427623

31	*ophthalmicus fulvigularis*	LSUMZ B31508	Bolivia: Santa Cruz, Florida (23 km E Samaipata)	EU427624
31	*ophthalmicus fulvigularis*	AMNH CJV300	Bolivia: Santa Cruz, Parque National Amboro	EU427625
32	*ophthalmicus argentinus*	LSUMZ 39026	Bolivia: Cochabama, Chapare, San Onofre (43 km W Villa Tunari)	EU427622
33	*ophthalmicus argentinus*	MBM gav666	Argentina: Tucuman, San Miguel de Tucuman (20 km N)	EU427620
33	*ophthalmicus argentinus*	MBM jag1918	Argentina: Tucuman, Tafi del Valle (20 km S, 6 km E)	EU427621
34	*tacarcunae*	MVUP 1977	Panama: Darien, Cerro Chucanti	EU427644
34	*tacarcunae*	MVUP 1078	Panama: Darien, Cerro Chucanti	EU427645
35	*inornatus*	LSUMZ B-1387	Panama: Darien, Cerro Pirre	EU427642
35	*inornatus*	LSUMZ B-1403	Panama: Darien, Cerro Pirre	EU427643
36	*semifuscus semifuscus*	ANSP 816	Ecuador	EU427639
36	*semifuscus semifuscus*	LSUMZ B6266	Ecuador: Pichincha	EU427641
36	*semifuscus semifuscus*	LSUMZ B34875	Ecuador: Pichincha	EU427640
37	*pileatus*	LSUMZ B-9957	Costa Rica: San Jose, La Georgina	EU427635
37	*pileatus*	LSUMZ B-9960	Costa Rica: San Jose, La Georgina	EU427634
38	*pileatus*	LSUMZ B-28243	Panama: Chiriqui, Boquete	EU427636
38	*pileatus*	LSUMZ B-28250	Panama: Chiriqui, Boquete	EU427637
39	*pileatus*	NMNH B-5503	Panama: Chiriqui, Cerro Hornito, Fortuna Reserva	EU427638

* samples previously published in Garcia-Moreno et al. 2004

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

Species	Museum (Tissue Number)	ATPase 6&8	Cyt B	ND2	COI
o. albifrons	MBM dhb5526	EU427588	EU427662	EU427680	EU427647
o. argentinus	MBM jag1918	EU427621	EU427675	EU427692	EU427657
o. bolivianus	LSUMZ B-22831	EU427623	EU427673	EU427690	EU427656
o. cinereocephalus	LSUMZ B1710	EU427627	EU427672	EU427689	EU427655
o. dwighti	LSUMZ B18089	EU427591	EU427663	EU427681	EU427648
o. fulvigularis	AMNH CJV300	EU427625	EU427674	EU427691	
o. honduratius	UKNH 5074	EU427599	EU427667	EU427685	EU427651
o. jaqueti	AMNH GFB3143	EU427616	EU427670		
o. punctulatus	NMNH B-1490	EU427608	EU427669	EU427687	EU427653
o. ophthalmicus	FMNH 394061	EU427577	EU427665	EU427683	
o. phaeoceophalus	LSUMZ B-6210	EU427631	EU427671	EU427688	EU427654

o. postocularis	MBM dhb4454	EU427592	EU427666	EU427684	EU427650
o. regionalis	MBM dab1325	EU427602	EU427668	EU427686	EU427652
o. wetmorei	FM 393775	EU427586	EU427664	EU427682	EU427649
inornatus	LSUMZ B-1387	EU427642	EU427676		EU427658
pileatus	LSUMZ B-9957	EU427635	EU427677	EU427693	EU427659
semifuscus semifuscus	LSUMZ B6266	EU427641	EU427678	EU427694	EU427660
tacarcunae	STRI PA-CPS2	EU427644	EU427679	EU427695	EU427661

Fig. 1

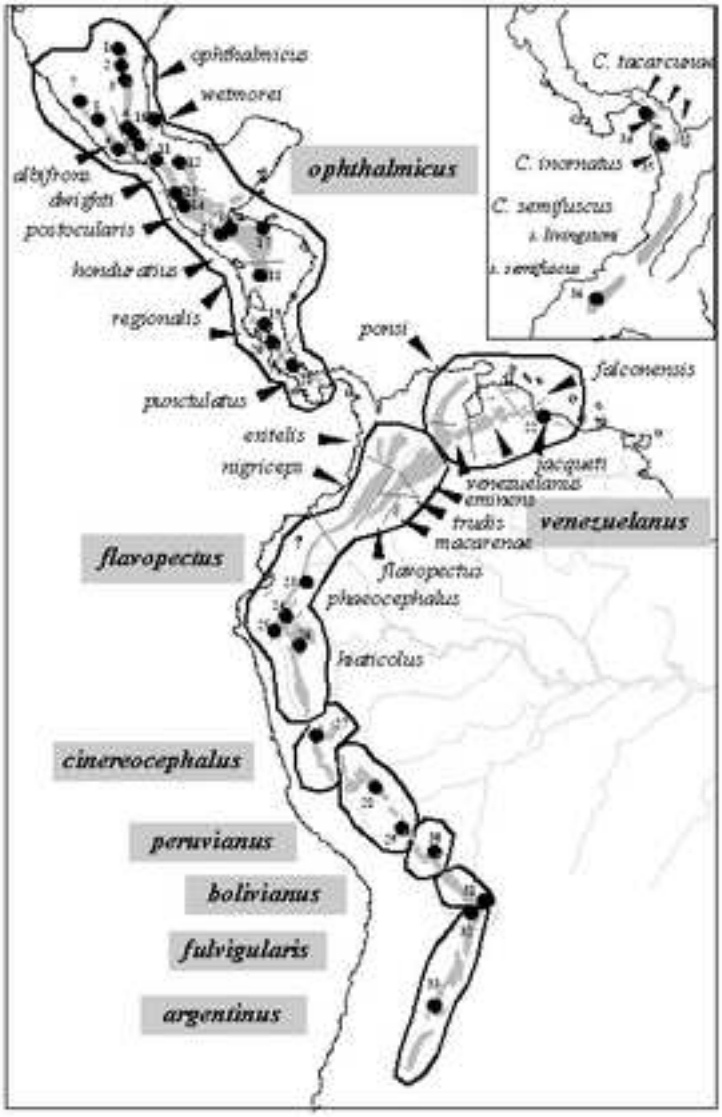

Fig. 2

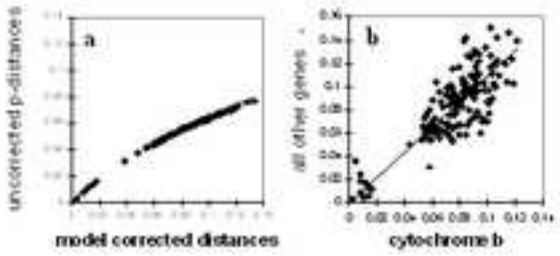

Figure 3

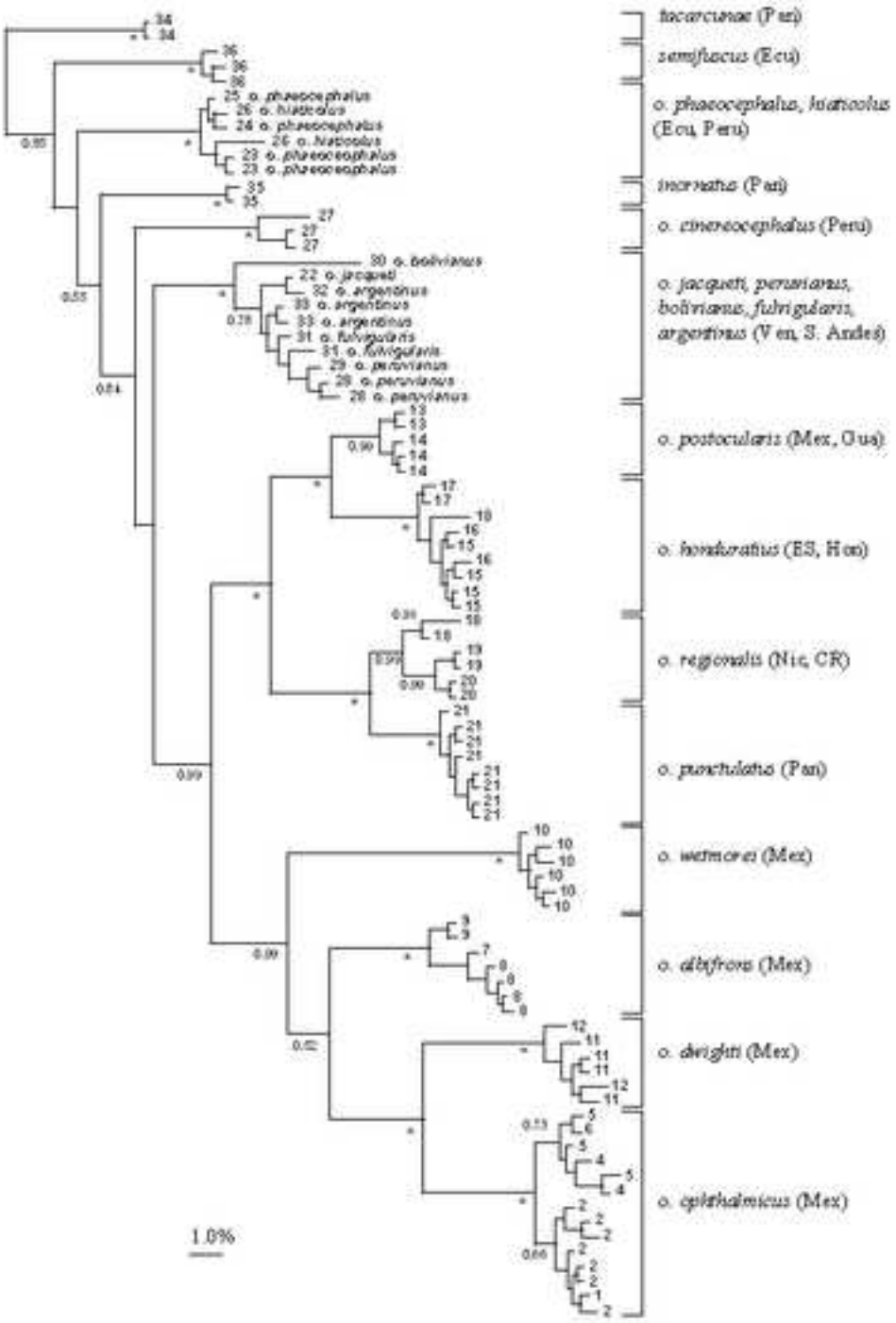

Fig. 4

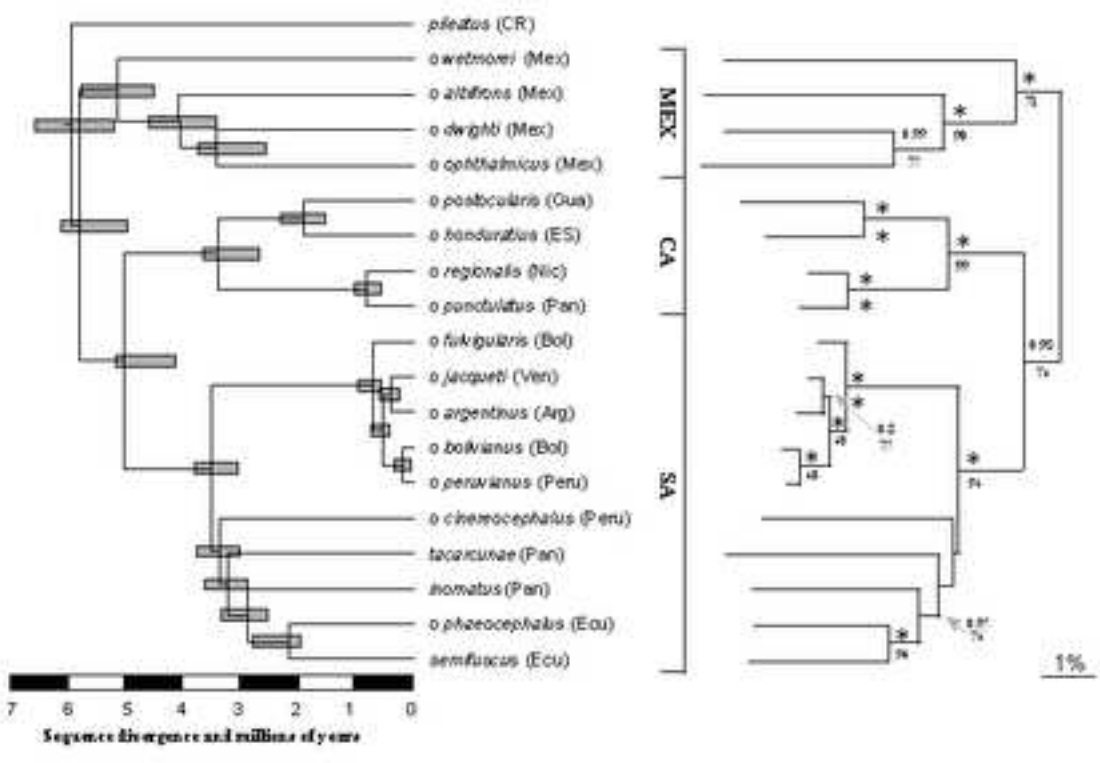

Fig. 5

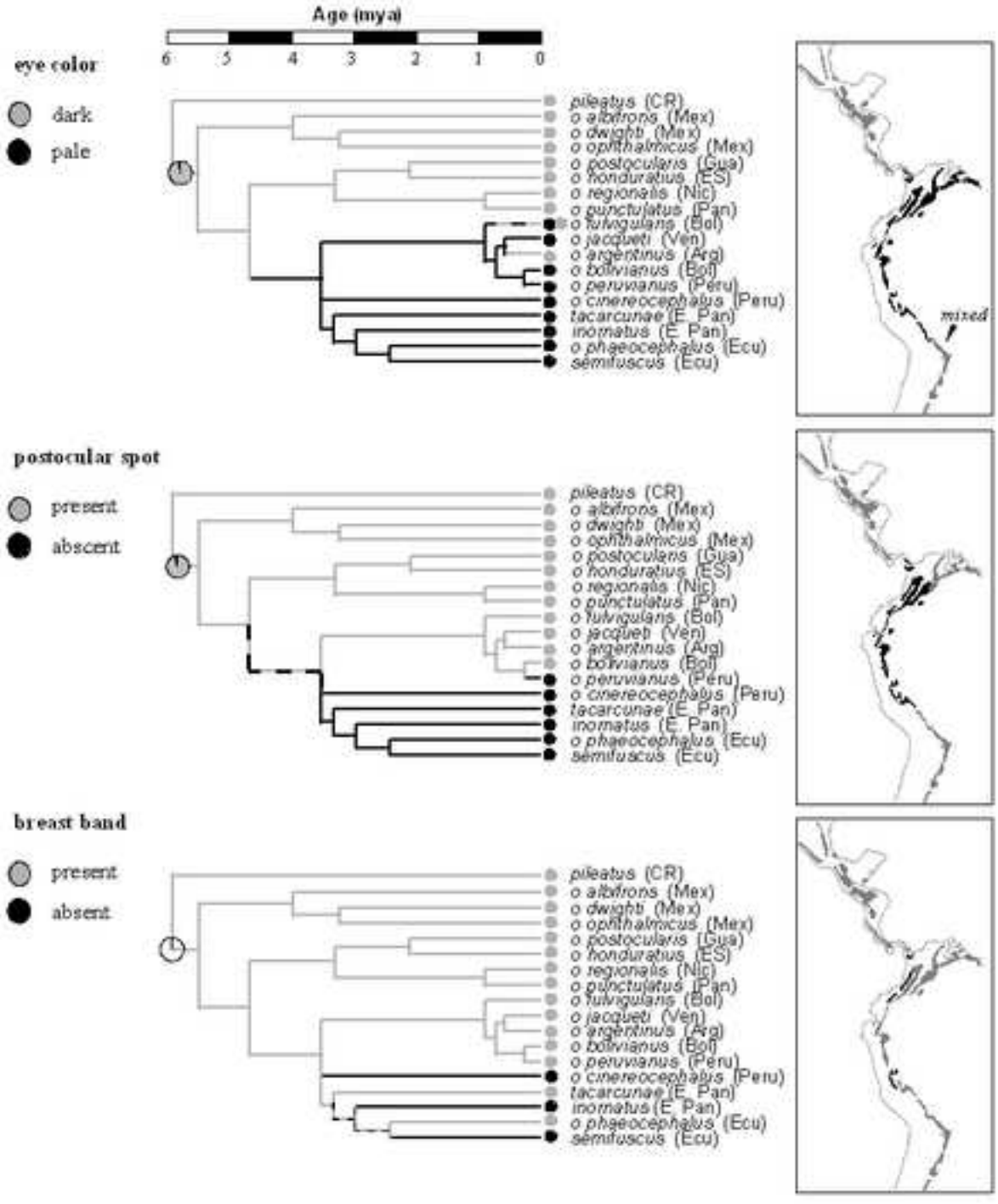

Figure 6