Molecular phylogenetics of the hummingbird genus *Coeligena*

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ABSTRACT

Advances in the understanding of biological radiations along tropical mountains depend on the knowledge of phylogenetic relationships among species. Here we present a species-level molecular phylogeny based on a multilocus dataset for the Andean hummingbird genus *Coeligena*. We compare this phylogeny to previous hypotheses of evolutionary relationships and use it as a framework to understand patterns in the evolution of sexual dichromatism and in the biogeography of speciation within the Andes. Previous phylogenetic hypotheses based mostly on similarities in coloration conflicted with our molecular phylogeny, emphasizing the unreliability of color characters for phylogenetic inference. Two major clades, one monochromatic and the other dichromatic, were found in *Coeligena*. Closely related species were either allopatric or parapatric on opposite mountain slopes. No sister lineages replaced each other along an elevational gradient. Our results indicate the importance of geographic isolation for speciation in this group and the potential interaction between isolation and sexual selection to promote diversification.

1. Introduction

Hummingbirds represent one of the largest avian radiations and have proven an excellent system for studies of comparative biology (Altshuler et al., 2004a,b), biogeography (García-Moreno et al., 2006; McGuire et al., 2007), and macroecology (Rahbek and Graves, 2000). The success of these studies relies on hypotheses of the phylogenetic relationships among lineages in this group. Despite a good knowledge of higher-level relationships in the family (Bleiweiss, 2002; Bleiweiss et al., 1997; Gerwin and Zink, 1998; McGuire et al., 2007, 2009), few studies have addressed relationships at the species level (García-Moreno et al., 1999b, 2006; Gerwin and Zink, 1989). These studies have provided new insights into hummingbird systematics and predictions about the timing of speciation events (i.e., most speciation events may have occurred during the Pliocene and few during the Pleistocene, especially at higher elevations), the geography of speciation (i.e., most sister species are not sympatric), and the relationship between phenotypic and genetic divergence (i.e., weak phylogenetic signal in color traits). In this study, we present a multilocus phylogenetic hypothesis for the hummingbird genus *Coeligena*, compare this hypothesis to previous hypotheses, and discuss the phylogenetic distribution

of sexual dichromatism and the patterns of geographic overlap among closely related species.

Bleiweiss (1998a,b) and McGuire et al. (2007) showed that two clades of hummingbirds (Brilliants and Coquettes) comprising at least half the species in this family diversified along the Andes. Phylogeographic and phylogenetic patterns of Andean hummingbird groups can reveal the progression and geographic development of these diversification events (Donoghue and Moore, 2003; Richards et al., 2007; Wiens and Donoghue, 2004). To date, studies about the historical biogeography of hummingbirds have focused on the progressive colonization of the Andes from surrounding lowlands (Bleiweiss, 1998a; McGuire et al., 2007). Nonetheless, few studies have concentrated on radiations that occurred within the tropical Andes region. All species of *Coeligena* hummingbirds occur along humid montane forests and thus provide an ideal system to study tropical Andean radiations.

The genus *Coeligena* consists of 11 (Remsen et al., 2008) to 12 (Schuchmann, 1999) species distributed throughout the humid Andes from Venezuela to central Bolivia (Schuchmann, 1999; Fig. 1). Within that 3600 km latitudinal range, at least one species occurs in humid montane forests from timberline to its lower elevational limit. The genus contains more species than any other exclusively Andean genus of hummingbirds (except the genus *Eriocnemis*). Some species (*C. coeligena* and *C. torquata*) are found virtually throughout the Andes, whereas others are highly restricted (i.e., *C. prunellei*; Fig. 1). Some species inhabit mountain ranges with geological origins independent of the Andes, such as the Sierra

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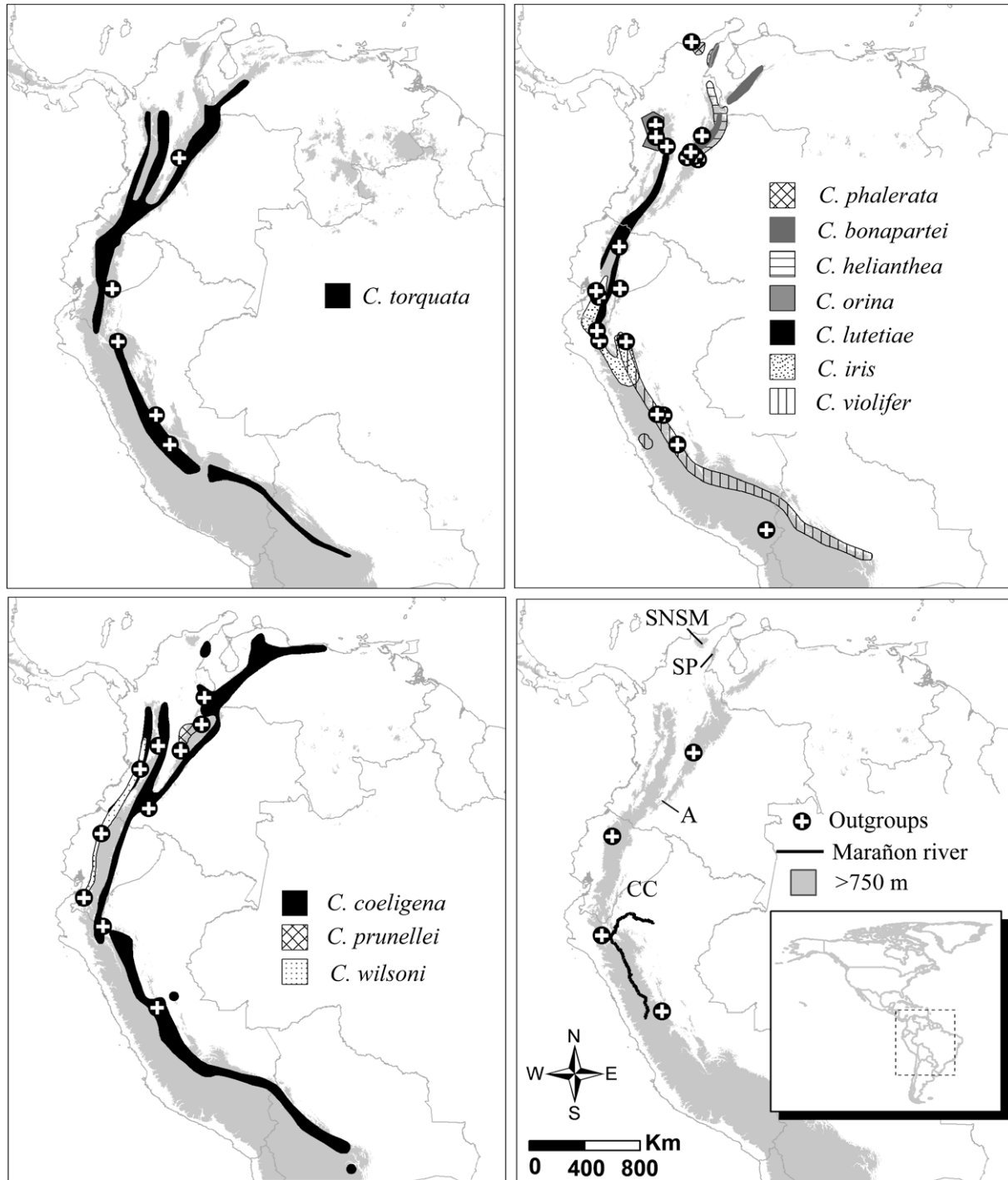*E-mail address:* juanluis@life.bio.sunysb.edu (J.L. Parra).

Fig. 1. Geographic distribution of samples and species ranges (Ridgely et al., 2007). Unique localities are plotted with a white cross on top of their respective geographic range, except for the lower right panel where they represent outgroup localities. Sites mentioned in the text are shown as 'SNSM': Sierra Nevada de Santa Marta, 'SP': Sierra de Perijá, 'A': Andalucía, and 'CC': cordillera del Condor.

Nevada de Santa Marta in northern Colombia (Tschanz et al., 1974) and the Cordillera del Condor in Ecuador (Fig. 1). Although up to 3 species can be found locally sympatric, species replacement along an elevational gradient is the predominant pattern (and to a lesser extent along a latitudinal or longitudinal [i.e., on different slopes of the Andes] gradient; (Hilty, 2003; Hilty and Brown, 1986). Thus, the genus *Coeligena* provides an ideal model for the study of diversification patterns and processes in the tropical Andes region.

In general morphology, all *Coeligena* species are medium-sized (~6–8 g), have long (~28–34 mm total length) straight bills, and are sexually dimorphic in terms of bill length (Krabbe et al., 2005; Sánchez-Osés, 2003). In contrast to their morphological homogeneity, color patterns are heterogeneous. Three species are dull and sexually monochromatic, whereas the rest are highly dichromatic. Some species show little or no geographic variation in coloration, whereas others vary so strongly that species limits are controversial (see below).

There is no consensus about the phylogenetic relationships among sexually monochromatic and dichromatic species in this group. Previous studies of the evolution of sexual dichromatism

in passerine birds found that transitions from dichromatism to monochromatism were more frequent than the reverse (Price and Birch, 1996). This pattern follows the argument that the loss of ornamentation should be easier and faster than the gain in ornamentation (Badyaev and Hill, 2003; Burns, 1998). We predicted, therefore, that there should be no transition from mono- to dichromatism and that the monochromatic species should represent from a single to three terminal events representing losses of dichromatism.

The taxonomic history of *Coeligena* is complicated. The genus was first described by Lesson (1832) as *Ornysmia*, which included species currently known to be distant relatives of *Coeligena* (e.g., *Eugenes fulgens*). Subsequently, the genus went through a complicated shift of names until Peters (1945) established an arrangement that has been maintained since. However, taxonomic uncertainty remains about the taxonomic rank of certain taxa such as *Coeligena inca*, *C. eos*, and *C. orina*, the first generally regarded as a subspecies of *C. torquata*, and the last two as subspecies of *C. bonapartei* (Bleiweiss, 1988; Fjeldså and Krabbe, 1990; Krabbe et al., 2005; Schuchmann, 1999). Phylogenetic relationships among species in this group have been hypothesized mostly on the basis of similarities in coloration and distribution. Thus, it is important to determine the reliability of color traits to infer phylogenetic affinities. Fjeldså and Krabbe (1990), on the basis of specimens of intermediate phenotype, suggested that *C. bonapartei* and *C. helianthea* were recently derived sister species that could still hybridize (Fig. 2A). Schuchmann (1999) suggested (i) that *C. prunellei* and *C. wilsoni* formed a superspecies within a complex that also included *C. torquata* and *C. inca* (Fig. 2B), and (ii) that *C. lutetiae*, *C. violifer*, *C. helianthea*, and *C. bonapartei* (including *C. orina* and *C. eos*) formed a superspecies. Finally, Sánchez-Osés (2003), based on maximum parsimony analyses of plumage coloration, proposed another phylogenetic hypothesis for this group (Fig. 2C). Here we propose a phylogenetic hypothesis based on DNA sequence data from both mitochondrial and nuclear loci. We evaluate the fit of previously proposed hypotheses relative to this molecular phylogeny and discuss the implications of our results to the evolution of sexual dichromatism and the geography of speciation in this group.

2. Methods

2.1. Taxon sampling

A total of 36 individuals was used, from which tissue or blood samples were obtained through field collections and loans from various museums (Table 1). Whenever possible, we used two individuals per subspecies from geographically widespread samples within each subspecies' range (Fig. 1). Although all species in the genus were included in our analyses (Remsen et al., 2008), more than half of the designated subspecies (17 out of 33) were not included due to lack of tissue samples, which emphasizes the importance of continuous collecting efforts in the Andes (Cuervo et al., 2006). As outgroups, we used individuals from four species in the Brilliants clade (Bleiweiss et al., 1994), which have been proposed to be closely related to *Coeligena*, based on muscular anatomy (Zusi, 1982) or genetic data (McGuire et al., 2007).

2.2. DNA amplification and sequencing

Genomic DNA was obtained from tissue using the DNAeasy Blood & Tissue Extraction kit (Qiagen) or the guanidine–thyocinate extraction protocol. The GenElute Blood Genomic DNA kit (Sigma–Aldrich) was used to extract DNA from blood. Two mitochondrial protein-coding genes and three nuclear introns were amplified and sequenced for all individuals. The mitochondrial genes were
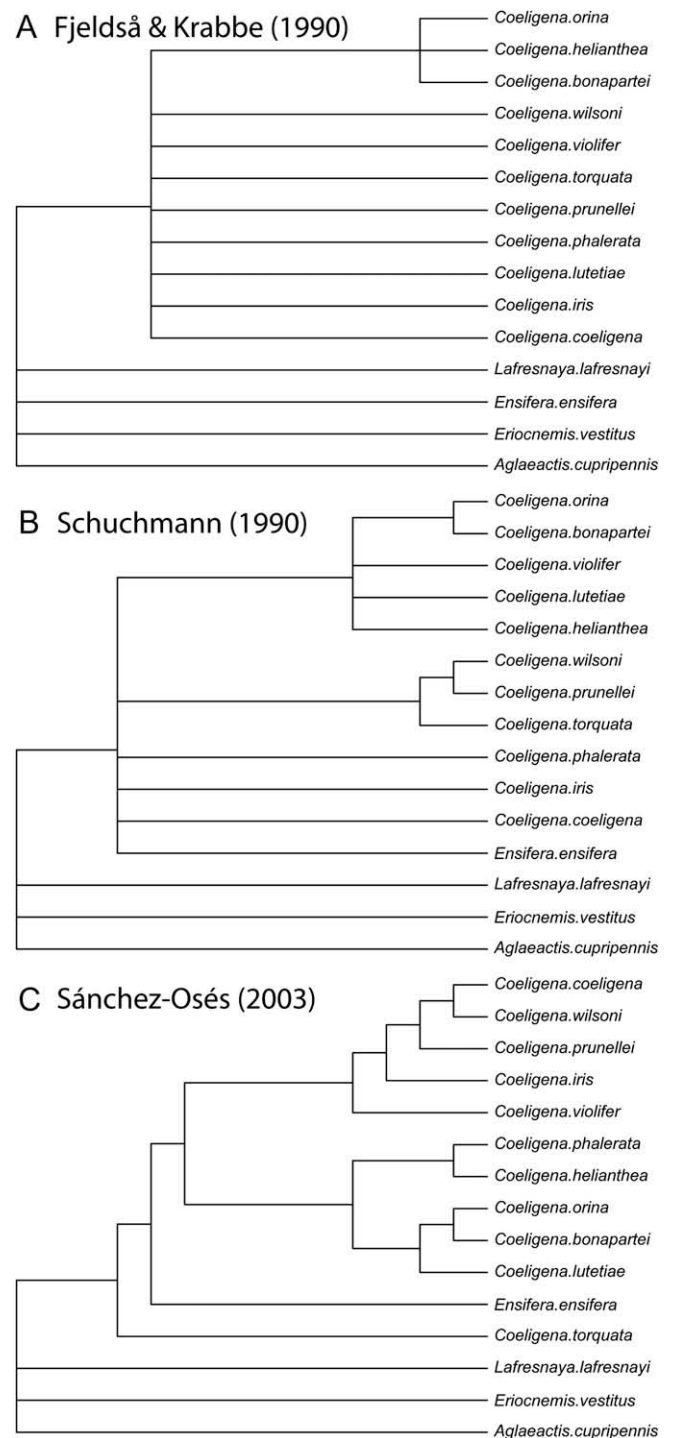

Fig. 2. Phylogenetic hypotheses for species in the genus *Coeligena* proposed by various authors in the past, mostly on the basis of general plumage coloration patterns and distribution. See text for complete references.

the nicotinamide dehydrogenase subunits 2 (ND2) and 4 (ND4), and the nuclear loci were the first intron of the adenylate kinase gene (AK1), the 7th intron of the Beta-fibrinogen gene (Bfib7; Prychitko and Moore, 1997), and the ornithine decarboxylase introns 6 and 7 (ODC; Friesen et al., 1999). The ND2, ND4, AK1, and Bfib7 genes were amplified and sequenced using the primers and protocols described in McGuire et al. (2007), whereas the ODC introns were amplified using either the original chicken-based primers, or a pair of newly developed primers ODC2-F (5′-GCGTGCAAAAGA-ACTTGACC-3′) and ODC2-R (5′-AGCCACCACCAATAT CAAGC-3′).

Table 1
Locality and species information for the 36 tissue samples used.

No.	Museum[a]	Catalogue No.[b]	Species	Subspecies	Country	Department	Latitude	Longitude
1	LSUMZ	B.6304	*Aglaeactis cupripennis*	NA	Ecuador	Pichincha	−0.10667	−78.63022
2	IAvH	4188	*Coeligena bonapartei*	*bonapartei*	Colombia	Boyaca	5.84750	−73.46278
3	IAvH	JLPV.74	*Coeligena bonapartei*	*bonapartei*	Colombia	Cundinamarca	4.92898	−74.11205
4	IAvH	752	*Coeligena coeligena*	*columbiana*	Colombia	Caqueta	1.34861	−76.10306
5	IAvH	1869	*Coeligena coeligena*	*columbiana*	Colombia	Norte de Santander	7.56889	−72.96833
6	IAvH	4500	*Coeligena coeligena*	*ferruginea*	Colombia	Risaralda	4.86500	−75.55500
7	IAvH	JLPV.33	*Coeligena coeligena*	*ferruginea*	Colombia	Valle del Cauca	3.56498	−76.58560
8	LSUMZ	B.8015	*Coeligena coeligena*	*obscura*	Peru	Pasco	−9.85000	−75.61667
9	LSUMZ	B.33476	*Coeligena coeligena*	*obscura*	Peru	Cajamarca	−5.28333	−78.66333
10	IAvH	2504	*Coeligena helianthea*	*helianthea*	Colombia	Meta	4.49389	−73.69250
11	IAvH	2569	*Coeligena helianthea*	*helianthea*	Colombia	Cundinamarca	4.70361	−73.85111
12	LSUMZ	B.31851	*Coeligena iris*	*flagrans*	Peru	Cajamarca	−5.68667	−79.25000
13	LSUMZ	B.31972	*Coeligena iris*	*flagrans*	Peru	Cajamarca	−5.68667	−79.25000
14	UCLA	01N8410	*Coeligena iris*	*hesperus*	Ecuador	Azuay	−3.21667	−79.26667
15	UCLA	01N8752	*Coeligena iris*	*hesperus*	Ecuador	Azuay	−2.88708	−79.42700
16	IAvH	1639	*Coeligena lutetiae*	NA	Colombia	Caldas	5.24833	−75.44028
17	UCLA	00N5086	*Coeligena lutetiae*	NA	Ecuador	Napo	−0.37292	−78.13656
18	LSUMZ	B.340	*Coeligena lutetiae*	NA	Peru	Cajamarca	−5.11667	−79.38333
19	IAvH	5169	*Coeligena orina*	NA	Colombia	Antioquia	6.45000	−76.08334
20	IAvH	PCPR.1	*Coeligena orina*	NA	Colombia	Antioquia	5.77175	−76.05389
21	IAvH	485	*Coeligena phalerata*	NA	Colombia	Magdalena	11.11333	−74.05278
22	IAvH	JLPV.42	*Coeligena prunellei*	NA	Colombia	Cundinamarca	4.61170	−74.31513
23	IAvH	JLPV.64	*Coeligena prunellei*	NA	Colombia	Santander	6.07395	−73.12926
24	LSUMZ	B.8019	*Coeligena torquata*	*insectivora*	Peru	Pasco	−9.85000	−75.61667
25	MVZ	ccw.1115	*Coeligena torquata*	*insectivora*	Peru	Junin	−11.51078	−74.84241
26	LSUMZ	B.44126	*Coeligena torquata*	*margaretae*	Peru	San Martin	−5.72306	−77.75027
27	IAvH	JLPV.38	*Coeligena torquata*	*torquata*	Colombia	Cundinamarca	4.60695	−74.30578
28	LSUMZ	B.6229	*Coeligena torquata*	*torquata*	Ecuador	Morona-Santiago	−2.75000	−78.08330
29	LSUMZ	B.1258	*Coeligena violifer*	*dichroura*	Peru	La Paz	−16.31667	−69.85000
30	LSUMZ	B.3504	*Coeligena violifer*	*dichroura*	Peru	Huanuco	−9.81667	−76.00000
31	LSUMZ	B.44151	*Coeligena violifer*	*dichroura*	Peru	San Martin	−5.72306	−77.75027
32	LSUMZ	B.7870	*Coeligena wilsoni*	NA	Ecuador	El Oro	−3.66740	−79.72800
33	LSUMZ	B.12064	*Coeligena wilsoni*	NA	Ecuador	Pichincha	−0.04806	−78.77000
34	LSUMZ	B.8224	*Ensifera ensifera*	NA	Peru	Pasco	−9.93838	−75.83728
35	IAvH	JLPV.75	*Eriocnemis vestitus*	NA	Colombia	Cundinamarca	4.60072	−74.06521
36	LSUMZ	B.32771	*Lafresnaya lafresnayi*	NA	Peru	Cajamarca	−5.66667	−79.20333

[a] Acronyms for Institutions: LSUMZ: Louisianna State University Museum of Natural Science; IAvH: Instituto Alexander von Humboldt; UCLA: Center for Tropical Research, University of California, Los Angeles; MVZ: Museum of Vertebrate Zoology, University of California, Berkeley.

[b] For tissue samples that have not been catalogued, the collector number was provided. Acronyms for collectors: JLPV: Juan Parra; PCPR: Paulo Pulgarin; and ccw: Christopher Witt.

PCR and sequencing protocols for ND2, ND4, AK1, and Bfib7 follow McGuire et al. (2007). For ODC, initial amplifications were conducted in a 12.5 µl solution containing 1 mM PCR Buffer, 1.5 mM of MgCl₂, 0.25 mM of each dNTP, 0.25 mM of each primer, and 0.02 mM of Taq (Roche). Amplification conditions were as following: 94 °C for 3 min for denaturation, 35 cycles of 94, 57, and 72 °C, each for 30 s to anneal and extend, and a final extension step of 72 °C for 5 min. After cleaning amplified products with ExoSAP-IT (USB), each strand was cycle sequenced using the ABI Big Dye Terminator v. 3.1 Cycle Sequencing kit. Sequencing products were cleaned using Sephadex columns and finally processed in an ABI 377 Automated Sequencer (Applied Biosystems). All sequence data are deposited in Genbank (Accession Nos. FJ903501-FJ903680).

To check for the potential amplification of pseudogenes in mitochondrial protein-coding genes, especially in blood extracted samples (Sorenson and Fleischer, 1996; Sorenson and Quinn, 1998), we looked for stop codons or frame shifts in the sequences obtained, and performed long PCRs for both ND2 (~4.5 kb, primers L2258 and H6681; Sorenson et al., 1999) and ND4 (~4 kb, primers L8928 and H13022) from which we sequenced the fragment of interest to determine whether the sequences obtained in this way were the same as the ones obtained when amplifying the fragment directly.

2.3. Phylogenetic analyses and statistical tests

The character set used for the analysis contains 1013 base pairs (bp) of the ND2 coding region; 666 bp of the ND4 coding region;

400 bp of AK1 intron; 895 bp of Bfib7 intron, and 94 bp of coding plus 457 bp of introns 6 and 7 of ODC, for a total of 3525 base pairs. Gaps were excluded from the analyses and polymorphic sites in introns were coded with their respective ambiguity code.

We conducted maximum parsimony, maximum likelihood and Bayesian phylogenetic analyses using PAUP v.4b10 (Swofford, 1991), RaxML v.7.0 (Stamatakis, 2006b), and MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), respectively. Using the maximum parsimony criterion we performed a 500 bootstrap-heuristic search on the mtDNA alone, nDNA alone, and concatenated datasets using tree bisection reconnection and a stepwise starting tree with random addition and 10 replications. All characters were given equal weight and treated as unordered.

Prior to running full maximum likelihood analyses, we assessed the compatibility of 10 different partitions (each codon position for each mitochondrial protein-coding gene, Ak1, Bfib7, ODC intron, and ODC exon) using the incongruence length difference test (Farris et al., 1995) as implemented in PAUP v.4b10 (partition homogeneity test) with 1000 replicates. We also evaluated the effect of different partitioning schemes when using the concatenated dataset (Brown and Lemmon, 2007) by performing 100 bootstrapped runs using the fast bootstrap algorithm in RaxML under the GTR-CAT approximation (Stamatakis, 2006a), followed by a search for the best tree under the general time reversible (GTR) model of evolution using 4 discrete rate categories. The choice of the GTR model over simplified versions of it has been discussed by Brown and Lemmon (2007). We compared trees obtained using different par-

Table 2
Overall levels of variation and empirical base frequencies for each partition used in the phylogenetic analyses. Tree length obtained from RAxML analyses represents the average number of expected substitutions per site. 'Var' refers to variable and 'PI' refers to parsimony informative sites.

	Base pairs	Var.Sites	% Var.Sites	Var.Sites-Gaps	PI sites	Empirical base frequencies				Tree length
						A	C	G	T	
ND2pos1	337	88	26	88	60	0.35	0.27	0.16	0.21	0.18
ND2pos2	337	34	10	34	22	0.17	0.36	0.09	0.38	0.02
ND2pos3	338	282	83	282	225	0.40	0.39	0.06	0.15	3.19
ND4pos1	222	44	20	44	31	0.34	0.33	0.16	0.18	0.11
ND4pos2	223	12	5	12	6	0.16	0.31	0.14	0.40	0.04
ND4pos3	222	182	82	182	157	0.39	0.42	0.04	0.15	2.04
AK1	400	68	17	46	22	0.20	0.31	0.28	0.21	0.06
Bfib	895	204	23	120	48	0.32	0.19	0.17	0.31	0.13
ODCexon	94	6	6	6	1	0.18	0.17	0.29	0.36	0.00
ODCintron	457	91	20	59	27	0.27	0.17	0.19	0.37	0.10

tition schemes with one another for best fit of the data on the basis of the Akaike Information Criterion (AIC, Burnham and Anderson, 2002). After identifying the partition scheme that maximized the likelihood, we performed a thorough ML analysis using an exhaustive algorithm under that partition in RaxML v.7.0 with the GTR+GAMMA model and 4 rate categories with 1000 non-parametric bootstrap replicates. Bayesian phylogenetic inference was performed on the partition scheme that maximized the likelihood with the GTR+I+Gamma model for each partition and unlinked parameter optimization. Two independent runs with four chains each were run for 20 million generations and assessed for convergence by examining stationarity in log likelihood scores as by examining the correlation of split frequencies between runs (Nylander et al., 2008).

To assess statistically the conflict between previous phylogenetic hypotheses relative to the obtained molecular phylogeny, we employed the Shimodaira Hasegawa test (Shimodaira, 2001, 2002) using 1000 bootstraps and RELL optimization for results from concatenated not partitioned datasets in PAUP v.4b10, and results from concatenated and partitioned datasets in RAxML v7.0.

3. Results

3.1. Variation among partitions and partition schemes

Mitochondrial genes exhibited more nucleotide variation than nuclear introns, and in coding regions, third site positions were more variable relative to first and second site positions (Table 2). Transition and transversion rates were variable among partitions, which justifies the independent estimation of parameters for each gene and gene region. Topologies obtained based on analyses of sequences from each partition exhibited no significant conflict among each other (ILD test, $P = 0.42$). Topologies estimated using mitochondrial genes had more resolution than topologies estimated using only nuclear genes (Fig. 3). Therefore, we concatenated the independent loci used in this study to infer the species phylogeny. Nevertheless, we acknowledge that lack of resolution from the nuclear introns does not imply that the topology recovered from the mitochondrial genes is indicative of the species tree (Edwards et al., 2007).

Increasing the number of partitions of the concatenated dataset significantly improved the fit to the data ($L_{nopartitions} = -13927.9$, $L_{5partitions} = -13181$, $L_{10partitions} = -12378.7$), but the topology obtained did not change depending on the partition scheme chosen. All partitions schemes were able to produce a completely resolved tree, in which case the AIC test reduces to comparing the likelihoods among topologies because the number of parameters is the same (Felsenstein, 2004). Nonetheless, branch lengths were greatly affected by choice of partition scheme (i.e., ML tree length

using no partition: 0.38 expected substitutions per site; ML tree length using 10 partitions = 0.17).

3.2. Phylogenetic results

Plots of the convergence of likelihood scores from independent Bayesian runs indicated that chains reached stationarity after six million generations. Thus, we conservatively discarded the first eight million generations as burnin. The topology estimated from the Bayesian partitioned analyses was identical to the topology estimated from ML and MP analyses, except for the lack of support for the sister relationship between *C. prunellei* and *C. wilsoni* in MP (Figs. 3 and 4). The topology obtained strongly supports *Coeligena* as monophyletic in relation to the four outgroups used (Fig. 4). All species were recovered as monophyletic groups with high support, except *C. bonapartei* and *C. helianthea*. Two clades were recovered within *Coeligena*, one with 3 species, including *C. coeligena* at the base and *C. wilsoni* and *C. prunellei* as sister taxa. The other clade was composed of eight species, including *C. torquata* at the base, and following in a pectinate fashion *C. violifer*, *C. iris*, *C. phalerata*, *C. orina*, *C. lutetiae*, and finally, *C. helianthea* and *C. bonapartei*, which appear as indistinguishable with the genes used in this study. Average maximum likelihood pairwise distance under the GTR+I+Gamma model for the concatenated dataset was 0.08 ± 0.03. Average distance within the three-species clade was 0.078 ± 0.03 and within the eight-species clade was 0.04 ± 0.03.

Two of the three previous phylogenetic hypotheses were rejected on the basis of SH tests (Table 3). The hypothesis of Schuchmann (1999) differs from our gene-based phylogeny in the placement of *C. torquata* in a clade with *C. wilsoni* and *C. prunellei*; in the placement of *C. orina* as a subspecies of *C. bonapartei*; and the suggestion that *Ensifera ensifera* might belong in the genus *Coeligena*. The hypothesis of Sánchez-Osés (2003) has several conflicts with the gene-based phylogeny. In particular, the placement of *C. violifer* and *C. iris* in the same clade as *C. coeligena*, *C. wilsoni*, and *C. prunellei*, and the sister relationship of *C. phalerata* and *C. helianthea* were refuted by our analyses. The hypothesis of Fjeldså and Krabbe (1990) concerning close relationship between *C. bonapartei* and *C. helianthea* was not rejected with the analyzed molecular data, although the designation of *C. orina* as a subspecies of *C. bonapartei* was not supported.

3.3. Sexual dichromatism

The two clades recovered within *Coeligena* separate the dichromatic from the monochromatic species (Fig. 4). The dichromatic clade has almost three times (three vs. eight) the number of species of the monochromatic clade, indicating potential differences in speciation or extinction rates (but see below). As stated previously, the average pairwise distance among sexually dichromatic species
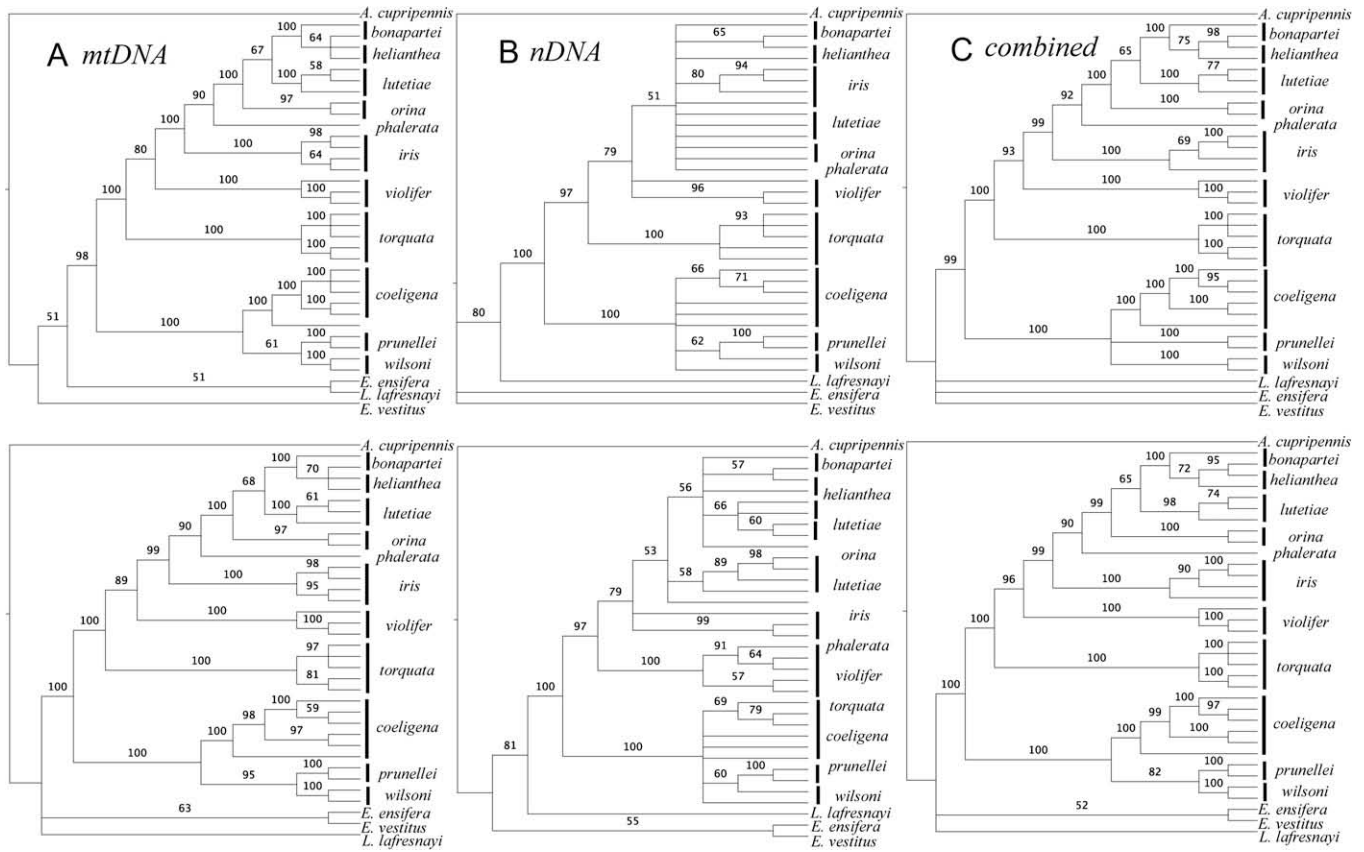
Fig. 3. Majority rule consensus trees for maximum parsimony (upper row) and maximum likelihood (lower row) analyses based on the mitochondrial DNA dataset (A), nuclear dataset (B), and both combined (C). Node values refer to bootstrap support from 100 pseudoreplicates. Vertical bars indicate 2 or more tips that belong to the same species.
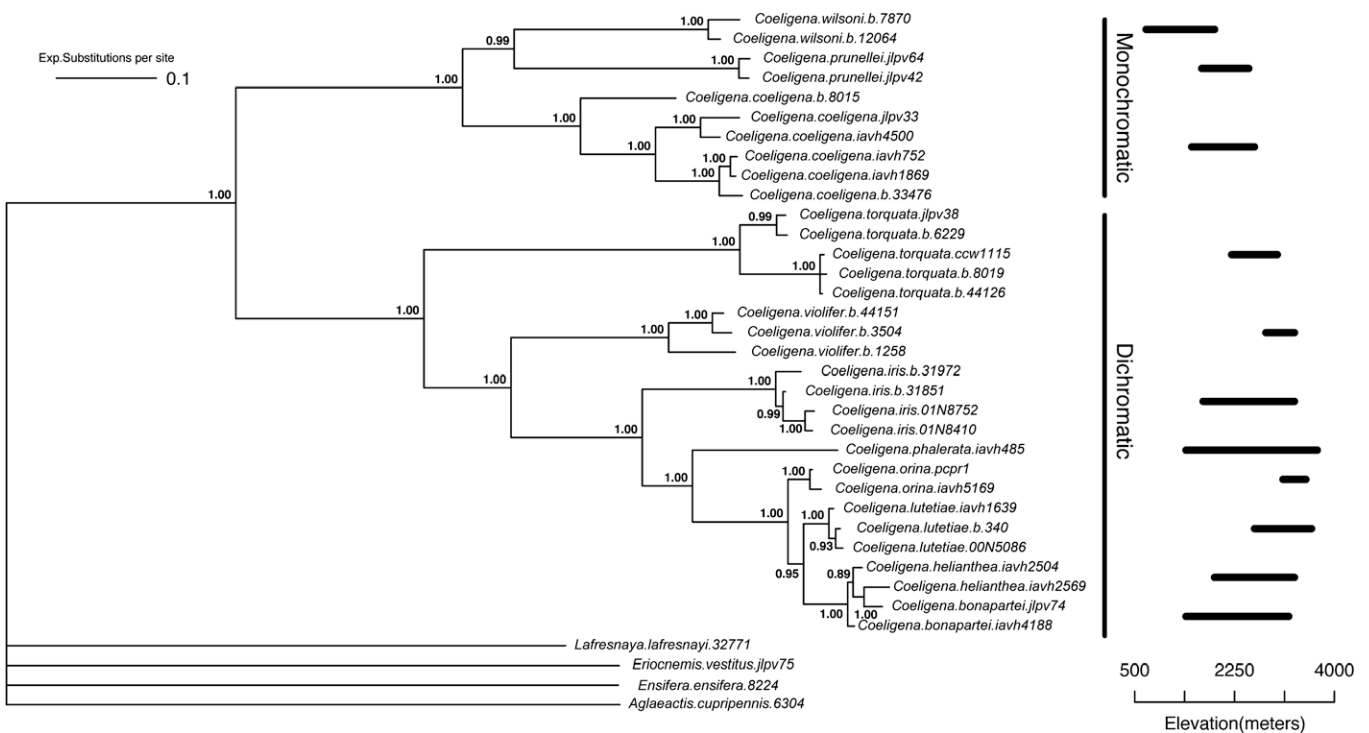

Fig. 4. Bayesian tree with posterior probabilities from a complete partitioned analyses using GTR+I+Gamma as a substitution model for each partition. Monochromatic and dichromatic clades are highlighted as well as elevational ranges for each species.

Table 3
Results from Shimodaira Hasegawa tests among tree topologies. Previously proposed hypotheses (see Fig. 2) were used to find a ML tree under those constraints, and those trees were compared with the ML tree (best). Maximum likelihood values (L) are reported for each tree and the difference in log likelihoods (dif ln L) between the proposed tree and the best tree is used to assess statistical significance of the test. Tests were run in PAUP for a concatenated dataset under a single model of evolution, and in RAxML for a partitioned dataset under mixed models of evolution for each partition.

	PAUP			RAxML			
	−ln L	dif ln L	Significance	−ln L	dif ln L	SD	Significance
Schuchmann	14080.5321	164.40243	0	−12986.4201	−153.158931	25.385049	Yes
Oses	14245.27953	329.14986	0	−13016.84466	−183.583497	29.255859	Yes
Krabbe	13916.12968	0	1	−12839.20734	−5.946175	4.855185	No
Best	13916.12968	–	–	−12833.26117	–	–	–

was shorter than the average pairwise distance among sexually monochromatic species. Two of the dichromatic species (*C. bonapartei* and *C. helianthea*), despite their marked phenotypic differences, were not distinguishable based on the analyzed molecular data.

3.4. Biogeographic context of speciation

The two most basal species in the dichromatic and monochromatic clades had the most widespread geographic ranges, whereas the more derived species had restricted ranges and were concentrated in the northern Andes. The sister species in the monochromatic clade (*C. wilsoni* and *C. prunellei*) are allopatric and highly divergent (0.05 ± 0.004 corrected sequence divergence). *Coeligena wilsoni* and *C. prunellei* are both restricted to the western slope of the Western and Eastern Andes of Colombia, respectively. *Coeligena coeligena* replaces *C. wilsoni* at higher elevations, but is not present on the western slope of the Eastern Andes of Colombia, where it is replaced by *C. prunellei*. The sister species in the dichromatic clade (*C. bonapartei* and *C. helianthea*) are parapatric, occupying opposite slopes on the Eastern Andes of Colombia, and the lack of detected genetic differences between them suggests that they have probably diverged very recently (although not possible yet to rule out recent introgression). These two species differ dramatically in coloration; for example, Sánchez-Osés (2003) scored them as differing in at least six characters including the color of the lower back, upper and under tail coverts. Some of the remaining dichromatic species replace each other along a latitudinal gradient, especially at the Marañon River depression (*C. violifer* and *C. lutetiae*), whereas the others are found within discrete topographic units, such as *C. phalerata* in the Sierra Nevada de Santa Marta, *C. lutetiae* in the Central Andes of Colombia and Ecuador, *C. orina* in the Western Andes of Colombia, and *C. bonapartei* and *C. helianthea* in the Eastern Andes of Colombia. Species that replace each other along the elevational gradient (*C. coeligena* – *C. torquata*, *C. wilsoni* – *C. coeligena*, *C. wilsoni* – *C. torquata*, *C. torquata* – *C. violifer*, *C. torquata* – *C. lutetiae*) are not each other closest relatives.

4. Discussion

We present a multilocus phylogenetic hypothesis for hummingbird species in the genus *Coeligena* with new insights into their systematic relationships. Using this phylogenetic hypothesis as a framework, we analyzed patterns in the evolution of sexual dichromatism and the geography of speciation in this group. Four independent molecular markers were used that provide different levels of resolution but were not in conflict with each other. A partitioned-mixed model phylogenetic analysis resulted in a fully resolved tree, with sexually dichromatic species forming a monophyletic group, sister to a clade of monochromatic species. Closely related species did not replace each other along an elevational gradient, but were either parapatric occupying opposite

slopes, or allopatric inhabiting different chains of the Andes, as has been shown in other Andean birds (Burns and Naoki, 2004; Cadena et al., 2007; García-Moreno and Fjeldså, 2000; Roy et al., 1997). Thus, we found no evidence for parapatric speciation in this clade despite widespread parapatry in the genus along broad elevational gradients.

The molecular data obtained resolved most species relationships in this group with a high confidence level (90–100% bootstrap support or >95% posterior probability). A pair of sexually dichromatic species that differ strongly in coloration (*C. bonapartei* and *C. helianthea*) could not be separated using the molecular markers employed in this study. This supports previous suggestions of recent hybridization between these species (Fjeldså and Krabbe, 1990), but is also consistent with a recent speciation event. *Coeligena bonapartei* and *C. helianthea* are easily distinguished based on plumage coloration, and to a lesser extent on body size and bill length, and they are usually not sympatric (Hilty and Brown, 1986). These two species occupy opposite slopes of the Eastern Andes in Colombia and meet along the Sabana de Bogotá (Stiles et al., 2000). They are the only example within *Coeligena* of currently parapatric sister species and should be a good system for further detailed studies of the efficacy of phenotypic differentiation in reproductive isolation. The taxonomic validity of *C. orina* as a distinct species (Krabbe et al., 2005) is supported by our results. Recent discoveries based on freshly collected specimens of *C. orina*, which allowed a more rigorous assessment of the status of this species, also support its distinctness and possible relation to other species such as *C. lutetiae* (Krabbe et al., 2005). Contrary to previous suggestions that *C. orina* should be considered a race of *C. bonapartei* (Schuchmann, 1999), we found no evidence to support a sister relationship between the two. *Coeligena orina*, *C. lutetiae*, *C. bonapartei,* and *C. helianthea* formed a monophyletic group of mostly allopatric species. Although the close relationship among these species was expected (Schuchmann, 1999), their specific relationships had not been possible to elucidate until now. *Coeligena torquata*, a wide-ranging dichromatic species proposed to form a species group with the monochromatic *C. prunellei* and *C. wilsoni* (Bleiweiss, 1988; Schuchmann, 1999) based on plumage coloration similarities, is found to be phylogenetically distant and basal within the dichromatic clade. The phylogenetic hypothesis proposed by Sánchez-Osés (2003) based on color characters was rejected by the gene-based phylogeny, which reflects the weak phylogenetic signal of color traits in this group as observed in many other birds (Allen and Omland, 2003; Burns and Naoki, 2004; Endler et al., 2005; García-Moreno et al., 2006; Gerwin and Zink, 1989; Omland and Lanyon, 2000; Price et al., 2007). Although our analysis included all taxa currently ranked as species (Remsen et al., 2008), the future addition of individuals representing subspecies in geographic extremes such as Venezuela, Bolivia, the Sierra de Perijá in Colombia, and the southern part of the Andes in Peru, will be essential to provide a complete perspective on this Andean radiation.

A single transition event was found to explain the existence of monochromatic species in this group. Although this poses an

interesting line of comparison between the mono- and dichromatic clades, it represents one event (Badyaev and Hill, 2003) and is confounded by factors that covary between the clades. For example, if we consider sexual dichromatism as an indicator of sexual selection (Hamilton and Zuk, 1982; Nadeau et al., 2007), we expect the dichromatic clade to have more species than the monochromatic clade (Kaneshiro and Boake, 1987; Kirkpatrick and Ravigne, 2002; Lande, 1981; Panhuis et al., 2001). Although this prediction is fulfilled, the pattern could also arise by the occupation of higher elevations in the sexually dichromatic clade, which are more fragmented and thus provide more opportunities for speciation by geographic isolation (Graves, 1985). In fact, the topology and branch lengths of the *Coeligena* phylogeny are consistent with the predictions of the oldest species at the lowest elevations and youngest at highest elevations (e.g., Bates and Zink, 1994). The roles of ecological and sexual isolation are difficult to disentangle when sister lineages are in geographic isolation (Price, 1998), which is most often the case in *Coeligena*. On the other hand, the restriction of gene flow due to geographic isolation can facilitate the operation of sexual selection (Kirkpatrick and Ravigne, 2002; Ödeen and Florin, 2002) and thus, it might be difficult to separate the two.

Based on our phylogenetic hypothesis and what is known about elevational ranges in these species (Hilty, 2003; Hilty and Brown, 1986; Krabbe et al., 2005; Renjifo et al., 2002; Schuchmann, 1999), we found no evidence for the replacement of sister species along an elevational gradient, which is consistent with other studies in tropical mountains (Arctander and Fjeldså, 1994; Moritz et al., 2000; Patton and Smith, 1992; Roy et al., 1997). On the contrary, we found examples of either replacement of congeners across opposite slopes and many examples of closely related species in allopatry. Although the idea of divergence in allopatry has been long-supported in birds (Mayr, 1949), divergence on opposite mountain slopes has not received much attention (Brumfield and Edwards, 2007; Chaves et al., 2007). The occurrence of different environments on opposite slopes (Chapman, 1917) and the climate fluctuations experienced during the Quaternary (Hooghiemstra and Van der Hammen, 2004) might also catalyze population divergence both in response to geographic isolation and natural selection (Vuilleumier, 1969). In addition to isolation in different mountain slopes, the history of uplift of the Andes also provides a scenario promoting periods of isolation with subsequent connections. For example, the high elevations (>1000 m) present in Mérida (Venezuela) and Perijá (Colombia and Venezuela) 10 million years ago (Mya) were not present in the Eastern Andes in Colombia until 2–5 Mya (Hoorn et al., 1995). Thus, what currently looks like a nearly continuous mountain chain actually consisted of patches that were much more isolated in the past, providing opportunities for divergence in isolation and eventual reconnection of these populations.

One of the most studied patterns of diversification in the Andes has been the colonization of highlands by lowland ancestors (Bates and Zink, 1994; Brumfield and Edwards, 2007; García-Moreno et al., 1999b; McGuire et al., 2007; Ribas et al., 2007). This hypothesis implies that speciation events are linked to colonization of highlands. An alternative hypothesis is that speciation events occur at high elevations along mountain ridges (García-Moreno et al., 1999b) followed by subsequent dispersal into the lowlands, and current elevation ranges are the result of interspecific competition (Cadena, 2007). Our results could be interpreted under either biogeographic model. The basal lineages within the mono- and dichromatic clades (*C. coeligena* and *C. torquata*) have widespread ranges and inhabit elevations up to 2600 and 2800 m, respectively. In the dichromatic clade, *Coeligena torquata* is sister to a group of species that inhabit elevations reaching above 3000 m, a pattern similar to the one occurring in the hummingbird genus *Metallura* (García-Moreno et al., 1999b). This is consistent with a model of

colonization and speciation at higher elevations. Nevertheless, it is difficult to rule out the alternative model of allopatric speciation followed by subsequent spread and establishment of current elevational ranges through competitive displacement (cf. Cadena, 2007; Cadena et al., 2007). The fact that closely related species are often in allopatry and that species replace each other along an elevation gradient is consistent with the latter. The biogeographic distribution of species in the monochromatic clade can also be interpreted under both models. *C. coeligena* is sister to a clade of two species, *C. prunellei,* which inhabits a similar elevation range but is isolated from other species in this clade, and *C. wilsoni,* which inhabits a lower elevational range but is replaced at higher elevations either by *C. coeligena* in Colombia (Hilty and Brown, 1986) or *C. torquata* in Ecuador (J. Chaves and J. Freile, pers. comm.). Although the more recent highlands were probably colonized progressively from the lowlands, it is important to differentiate if this colonization lead to further speciation, or if it simply allowed for the coexistence of more species.

Recent phylogenetic and phylogeographic studies involving Andean taxa have revealed a complex variety of responses in terms of biogeography and timing of events among lineages to a single history (Cadena et al., 2007; García-Moreno et al., 1998, 1999a,b; García-Moreno and Fjeldså, 1999; Hall, 2005; Koscinski et al., 2008; Navarro-Siguenza et al., 2008; Puebla-Olivares et al., 2008; Ribas et al., 2007). Knowledge about the phylogenetic relationships of Andean species and their population structure will continue to inform biogeographic and evolutionary hypotheses (i.e., Remsen, 1984), which can then be tested with additional data. Our results emphasize the potential importance of opposite mountain slopes, historical isolation, and sexual selection as promoters of diversification in this group.

Acknowledgments

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