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Historical biogeography and speciation in the Neotropical highlands: Molecular phylogenetics of the jay genus *Cyanolyca*

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ABSTRACT

Phylogenetic relationships were studied in the genus *Cyanolyca*, an assemblage of jays distributed from Mexico south to Bolivia. Given its fragmented distribution along the humid forests of the Neotropics, the genus *Cyanolyca* is a model group for exploring hypotheses on biogeography and speciation. Phylogenetic analyses were based on two mitochondrial and three nuclear loci; taxon sampling includes all species in the genus and most subspecies. Maximum parsimony, maximum likelihood, and Bayesian analyses produced trees that were congruent and highly robust at both terminal and deep nodes of the phylogeny. *Cyanolyca* comprises two major clades: one contains the Mesoamerican "dwarf" jays, and the other consists of two main groups—*C. cucullata* + *C. pulchra* and the "core" South American species. Prior hypotheses of relationships were explored statistically using Maximum Likelihood and Bayesian approaches. Dispersal-Vicariance analysis revealed the importance of the Northern Andes as a major center for biological diversification, and the effects of dispersal across the Panamanian Land Bridge in the composition of South American and Mesoamerican avifaunas. Phylogenetic patterns are highly congruent with an allopatric mode of speciation. Implications of these results are discussed in the context of the biogeography of Neotropical montane forests.

1. Introduction

Neotropical montane regions hold the world's highest diversity of birds, as well as that of many other organisms (Churchill et al., 1995; Stattersfield et al., 1998). These mountain chains stretch from Mexico south to Argentina and Chile, in a fragmented, complex mosaic of topographic units belonging to diverse geologic formations (Simpson, 1975; Ferrusquía-Villafranca, 1993; Coates and Obando, 1996; Gregory-Wodzicki, 2000). Regardless of its origins, the region sustains extensive tropical montane forests and numerous lineages that overlap broadly in areas with similar environmental conditions (Chapman, 1926; Hernández-Baños et al., 1995; Peterson et al., 1999).

Early distributional studies and recent empirical work suggest that Neotropical montane avifaunas are derived, at least partially, from lineages that have moved from lower to higher montane elevations (Chapman, 1926; Gerwin and Zink, 1989; Bates and Zink, 1994; García-Moreno et al., 1999a; Pérez-Emán, 2005; Brumfield and Edwards, 2007) and from lineages that have expanded their distributions via the Panama Land Bridge (Chapman, 1917; Haffer, 1974). Moreover, the complex topography and fragmented nature of Neotropical montane forests suggest that diversification in situ after initial biological interchange might play a decisive role in shaping distributions of largely overlapping lineages (Chapman, 1926; Remsen, 1984; Cracraft, 1985; Hernández-Baños et al., 1995; García-Moreno and Fjeldså, 2000).

Most models that attempt to explain geographic variation and speciation in situ depend on the following: effects of deep river valleys as barriers to gene flow and consequent evolution of distinctive geographic forms (Chapman, 1926; Vuilleumier, 1969; Remsen, 1984; Cracraft, 1985; García-Moreno and Fjeldså, 2000); the linearity of the Andes, which results in elongate geographical ranges and reduces potential contact and gene flow among parapatric forms (Remsen, 1984; Graves, 1985, 1988) and the effects of Pleistocene glaciations on the cyclic fragmentation, isolation, and reconnection of montane forests (Hooghiemstra et al., 2000) and their avifaunas (Vuilleumier, 1969; Haffer, 1974; Hackett, 1995). Clearly, these propositions are not mutually exclusive, and could operate across various temporal, spatial, and taxonomic scales.

Cyanolyca jays are model organisms for testing hypotheses of diversification across the Neotropical montane forests. Being a relatively small assemblage that represents one of the two New World jay (NWJ) lineages that reached South America, these jays are sedentary and inhabit humid montane forests from Mexico south to Bolivia (Fig. 1). Most are allopatric, and their ranges are highly subdivided, creating (putatively) isolated and morphologically distinct populations (Hellmayr, 1934). Current taxonomic

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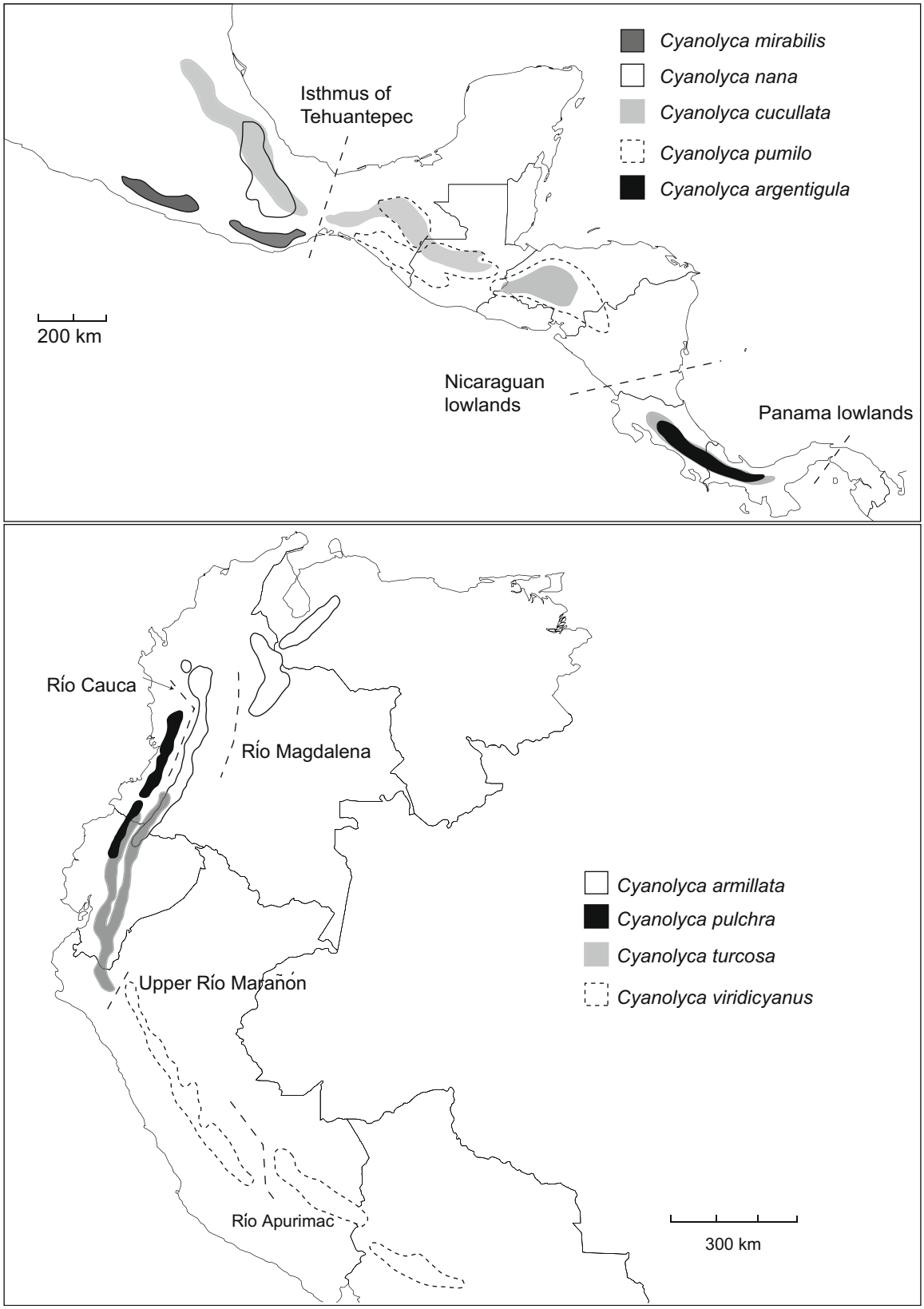

Fig. 1. Maps of Mesoamerica and northern South America showing the distribution of the nine species of *Cyanolyca*.

treatments (e.g., Sibley and Monroe, 1990; Madge and Burn, 1994; Dickinson, 2003) recognize nine species: *Cyanolyca mirabilis*, *C. nana*, *C. pumilo*, *C. argentigula*, *C. pulchra*, *C. cucullata*, *C. armillata*, *C. turcosa*, and *C. viridicyanus*. The first four species, the so-called "dwarf jays," are allopatric and have been recognized as full species since early revisions (e.g., Hellmayr, 1934; Blake and Vaurie,

1962; Goodwin, 1976). The taxonomic status of the remaining species is more problematic. Hellmayr (1934) treated the Mesoamerican *C. cucullata* and the South American *C. pulchra* as conspecific. Blake and Vaurie (1962) considered *C. turcosa* and *C. armillata* as subspecies of *C. viridicyanus*, whereas others (Hellmayr, 1934; Fjeldså and Krabbe, 1990) recognized *C. turcosa*, but lumped *C. armillata* and *C. viridicyanus* into a single species. Other authors have recognized all five as full species, based on discrete plumage differences (Ridgely and Tudor, 1989), vocalizations (Goodwin, 1976), and geographic ranges (Zimmer, 1953).

The only hypothesis of relationships within *Cyanolyca* is that of Goodwin (1976), who proposed that dwarf jays arose from a single ancestor, with *C. mirabilis* and *C. argentigula* as sister species. In his arrangement, *C. cucullata* and the South American forms are closely related, with *C. cucullata* and *C. pulchra* as sister species. Also, he suggested that *C. viridicyanus* and *C. armillata* may form a superspecies, sister to *C. turcosa*. These propositions were based on subjective interpretation of overall plumage similarity, and therefore they are subject to the observation that plumage characters seem to be extremely labile among birds (Hackett and Rosenberg, 1990; Burns, 1998; Omland and Lanyon, 2000; Weibel and Moore, 2005).

To date, neither the relationships among species, nor the validation of current species as independent historical entities (sensu Simpson, 1961; Wiley, 1978) have been approached in a phylogenetic context. A higher-level phylogenetic analysis of the mitochondrial Control Region (CR) revealed high sequence divergence among *Cyanolyca* species compared to divergences in related genera (Saunders and Edwards, 2000), which may indicate that speciation in *Cyanolyca* occurred deep in history, but without major morphological change. Therefore, assessments of relationships based on overall morphological similarity may not reflect the complexity and evolutionary history of lineages in the group.

Herein, I study the phylogenetic relationships among *Cyanolyca* jays based on the analysis of two mitochondrial and three nuclear loci. Based on the results, I test previous hypotheses of relationships among currently recognized species, and identify potential independent lineages that might represent different evolutionary species. Finally, I reconstruct the ancestral distributional areas for *Cyanolyca*, and discuss speciation scenarios in the context of the biogeography of Neotropical montane avifaunas.

2. Materials and methods

2.1. Taxon and gene sampling

I analyzed 40 individuals of *Cyanolyca*, including at least one representative of each species and representatives of all subspecies except *C. armillata armillata* (from the eastern Andes of Colombia and extreme west of the Venezuelan Andes). For geographically widespread or polytypic taxa (e.g., *C. turcosa, C. cucullata, C. armillata,* and *C. viridicyanus*), sampling spanned geographic populations to encompass genetic variation among the extremes of their distributions. Tissue samples were obtained from ornithological collections in the US and Mexico, as well as from my collecting efforts in Mexico, Ecuador, and Venezuela (Table 1).

DNA sequences used for outgroup comparisons were obtained from previous studies (Cicero and Johnson, 2001; Ericson et al., 2005; Bonaccorso and Peterson, 2007), and included species representing all New World jay genera (*Aphelocoma*, *Cyanocitta*, *Gymnorhinus*, *Calocitta*, and *Cyanocorax*) and more distantly related corvid genera (*Dendrocitta* and *Perisoreus*). A novel CR sequence for *Dendrocitta formosae* and sequences of the Transforming Growth Factor β-2 intron 5 (TGFβ2.5) for all outgroup taxa were generated to complete the outgroup dataset (GenBank Accession Nos. FJ598301 to FJ598308 and FJ618563).

I obtained sequences of CR for the full ingroup dataset and sequences of the NADH dehydrogenase subunit 2 (ND2) for 38 individuals. Sequences of representative individuals were obtained for the nuclear genes Adenylate Kinase intron 5 (AK5), β-Fibrinogen intron 7 (βfb7), and TGFβ2.5 (Table 1). For combined mitochondrial and mitochondrial/nuclear analyses, sequences of *Perisoreus canadensis* were concatenated with the published CR sequence of *Perisoreus infaustus*. Rooting the tree with this technically chimeric sequence is justified because the *P. infaustus* sequence is probably more closely related to *P. canadensis* than to any other sequence in the combined dataset (Saunders and Edwards, 2000).

2.2. DNA amplification, sequencing, and aligning

Genomic DNA was extracted from frozen tissue with the DNeasyTissue extraction kit (Qiagen Inc.) or a modified salt precipitation method (M. Fujita, unpubl.). DNA of *Cyanolyca nana* was obtained from a museum skin sample (KU 106856) courtesy of R. Fleischer in the laboratories of the National Museum of Natural History and National Zoological Park, using established protocols (Fleischer et al., 2000, 2001). PCR amplification was completed using the following primer pairs: L5216 and H6313 (Sorenson et al., 1999) for ND2; JCR13 (Saunders and Edwards, 2000) and H1248 (Tarr, 1995) for CR; AK5b+ and AK6c− (Shapiro and Dumbacher, 2001) for AK5; FIB-B17U and FIB-B17L (Prychitko and Moore, 1997) for βfb7; and TGFb2.5F and TGFb2.6R for TGFβ2.5 (Sorenson et al., 2004). To amplify DNA extracted from the museum skin of *Cyanolyca nana*, I designed a set of internal primers that were used in combination with published primers (Appendix 1). In order to avoid cross contamination with other samples, amplification was carried out in a separate lab facility, using proper controls and fresh reagents.

Mitochondrial genes were amplified using a standard PCR protocol (Bonaccorso and Peterson, 2007), whereas nuclear genes were amplified using a touchdown protocol (i.e., an initial denaturation of 94 °C/3 min; 5 cycles of 94 °C/30 s, 60 °C/30 s, 72 °C/40 s; 5 cycles of 94 °C/30 s, 56 °C/30 s, 72 °C/40; 35 cycles of 96 °C/30 s, 52 °C/30 s, 72 °C/40 s; and a final extension of 72 °C/10 min; R. Moyle, pers. comm.). When multiple bands persisted, target bands were purified using the QIAquick Gel Extraction Kit (Quiagen, Inc.). Single PCR products were treated with ExoSAP-IT (Affymetrix) to degrade unincorporated primers and dNTP's. Cycle sequencing was completed with the corresponding PCR primers and BigDye Terminator 3.1 chemistry (Applied Biosystems). Sequencing reaction products were purified with CleanSEQ magnetic beads (Agencourt) and resolved on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Data from heavy and light strands were assembled to obtain a consensus sequence for each sample, using Sequencher 4.1 (Gene Codes Corporation, 2000). Nucleotide sequences were aligned in CLUSTAL X using default settings (Thompson et al., 1997). MacClade ver. 4.0 (Maddison and Maddison, 2000) was used to adjust alignments by eye and to translate nucleotide sequences into amino acids to verify absence of stop codons.

2.3. Phylogenetic analyses

Best-fit models of molecular evolution were selected in Modeltest v.3.7 (Posada and Crandall, 2001) under the Akaike Information Criterion (AIC) for each gene and codon position (i.e., ND2) and for mitochondrial and mitochondrial/nuclear datasets. General models estimated in Modeltest were used in further maximum likelihood (ML) and Bayesian analyses. Model parameter values estimated for ND2 were used for obtaining pairwise ML-corrected distances in PAUP* v.4.0b10 (Swofford, 2002).

Phylogenetic inferences were conducted using maximum parsimony (MP) and ML for all genes. Comparisons of individual gene

Table 1
List of tissue samples and GenBank accession numbers for sequences of *Cyanolyca* included in the present study. Acronyms: AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History; LSUMZ, Louisiana State University Museum of Natural Science; QCAZ, Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito; EBGR, Museo Estación Biológica Rancho Grande, Venezuela; CVULA, Colección de Vertebrados, Universidad de los Andes, Venezuela; NMNH, National Museum of Natural History, Smithsonian Institution; ANSP, Academy of Natural Sciences; MZFC, Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México; KUNHM, University of Kansas Natural History Museum.

Sample	Collection	Voucher	Locality	GenBank Accession Numbers				
				ND2	CR	AK5	βfb7	TGF
C. viridicyanus viridicyanus 1	AMNH	CJV 29	Bolivia, La Paz: Piara, near Pelechuco	FJ598146	FJ598182	FJ598220	FJ598246	FJ598272
C.v. viridicyanus 2	AMNH	CBF MH 35	Bolivia, La Paz: Piara, near Pelechuco	DQ912606*	FJ598183	DQ912622*	DQ912643*	FJ598273
C. v. viridicyanus 3	LSUMZ	B1268	Bolivia, La Paz: Ca 1km S Chuspipata	FJ598147	AF218933**	FJ598221	FJ598247	FJ598274
C. v. viridicyanus 4	LSUMZ	B22738	Bolivia, La Paz: Saavedra, 12 km E Charazani	FJ598148	FJ598184	FJ598222	FJ598248	FJ598275
C. v. cyanolaema 5	FMNH	430148	Cuzco Peru: Paucartambo, Pillahuata	FJ598149	FJ598185	FJ598223	FJ598249	FJ598276
C. v. jolyaea 6	LSUMZ	B3501	Peru, Huanuco: ca. 14 km W Panao	FJ598150	FJ598186	FJ598224	FJ598250	FJ598277
C. v. jolyaea 7	LSUMZ	B8249	Peru, Pasco: Millpo	FJ598151	FJ598187	FJ598225	FJ598251	FJ598278
C. v. jolyaea 8	LSUMZ	B8412	Peru, Pasco: Millpo	FJ598152	FJ598188	FJ598226	FJ598252	_
C. v. jolyaea 9	LSUMZ	B43820	Peru, San Martin: Ca 22km ENE Florida	FJ598153	FJ598189	FJ598227	_	FJ598279
C. v. jolyaea 10	LSUMZ	B44528	Peru, San Martin: Ca 22km ENE Florida	FJ598154	FJ598190	FJ598228	FJ598253	FJ598280
C. armillata quindiuna 1	QCAZ	2955	Ecuador, Napo: Oyacachi	FJ598155	FJ598191	FJ598229	FJ598254	FJ598281
C. a. quindiuna 2	QCAZ	2956	Ecuador, Napo: Oyacachi	FJ598156	FJ598192	FJ598230	FJ598255	FJ598282
C. a. quindiuna 3	QCAZ	2957	Ecuador, Napo: Oyacachi	FJ598157	FJ598193	_	_	_
C. a. meridana 4	EBRG	12238	Venezuela, Mérida: La Mucuy	FJ598158	FJ598193	FJ598231	FJ598256	FJ598283
C. a. meridana 5	CVULA	563	Venezuela, Mérida: La Mucuy	FJ598159	FJ598195	FJ598232	FJ598257	FJ598284
C. a. meridana 6	CVULA	564	Venezuela, Mérida: La Mucuy	FJ598160	FJ598196	FJ598233	FJ598258	FJ598285
C. turcosa 1	NMNH	B03153	Ecuador, Sucumbíos: Cocha Seca	_	FJ598197	_	_	_
C. turcosa 2	NMNH	B03154	Ecuador, Sucumbíos: Cocha Seca	_	FJ598198	_	_	_
C. turcosa 3	ANSP	512	Ecuador, Carchi: Between Maldonado and Tulcán	FJ598161	FJ598199	_	_	_
C. turcosa 4	QCAZ	2958	Ecuador, Pichincha: Palmeras, 35 km NW Quito	FJ598162	FJ598200	FJ598234	FJ598259	FJ598286
C. turcosa 5	LSUMZ	B7770	Ecuador, Pichincha: SW side Cerro Pichincha	FJ598163	FJ598201	_	_	_
C. turcosa 6	LSUMZ	B7784	Ecuador, Pichincha: SW side Cerro Pichincha	FJ598164	FJ598202	_	_	_
C. turcosa 7	ANSP	4055	Ecuador, Loja: 7 km SE Saraguro	FJ598165	FJ598203	_	_	_
C. turcosa 8	ANSP	5046	Ecuador, Zamora-Chinchipe: 6 km NW San Andres	FJ598166	FJ598204	_	_	_
C. turcosa 9	LSUMZ	B31759	Peru, Cajamarca: Quebrada Lanchal	FJ598167	FJ598205	FJ598235	FJ598260	FJ598287
C. turcosa 10	LSUMZ	B31823	Peru, Cajamarca: Quebrada Lanchal	FJ598168	FJ598206	FJ598236	FJ598261	FJ598288
C. turcosa 11	LSUMZ	B31834	Peru, Cajamarca: Quebrada Lanchal	FJ598169	FJ598207	FJ598237	FJ598262	FJ598289
C. cucullata cucullata 1	NMNH	B05557	Panama, Chiriquí: Los Planes	FJ598170	FJ598208	FJ598238	FJ598263	FJ598290
C. c. cucullata 2	LSUMZ	B26406	Panama, Chiriquí: Cordillera Central	FJ598171	FJ598209	_	_	_
C. c. cucullata 3	LSUMZ	B26418	Panama, Chiriquí: Cordillera Central	FJ598172	FJ598210	FJ598239	FJ598264	FJ598291
C. c. mitrata 4	FMNH	394011	Mexico, Hidalgo: 5 km E Tlanchinol	FJ598173	FJ598211	FJ598240	FJ598265	FJ598292
C. c. mitrata 5	FMNH	343730	Mexico, Hidalgo: 5 km E Tlanchinol	FJ598174	FJ598212	FJ598241	FJ598266	FJ598293
C. c. mitrata 6	MZFC	11233	Mexico, Oaxaca: San Martín Caballero	FJ598175	FJ598212	_	FJ598267	FJ598294
C. pulchra	QCAZ	3000	Ecuador, Pichincha: Palmeras, 35 km NW Quito	FJ598176	FJ598214	FJ598242	FJ598268	FJ598295
C. nana	KUNHM	106856	Oaxaca, Mexico: Totontepec	FJ598177	FJ598215	_	_	_
C. mirabilis	FMNH	343601	Mexico, Guerrero: El Iris, Sierra de Atoyac	DQ912606*	AF218934**	DQ912623*	DQ912644*	FJ598296
C. pumilo	MZFC	B19493	Mexico, Chiapas: 5 km N Coapilla	FJ598178	FJ598216	FJ598243	FJ598269	FJ598297
C. argentigula 1	LSUMZ	B19770	Costa Rica, San José: Villa Mills	FJ598179	FJ598217	FJ598244	FJ598270	FJ598298
C. argentigula 2	LSUMZ	B19790	Costa Rica, San José: Villa Mills	FJ598180	FJ598218	FJ598245	_	FJ598299
C. argentigula 3	LSUMZ	B19819	Costa Rica, San José: Villa Mills	FJ598181	FJ598219	_	FJ598271	FJ598300

Note: Accession numbers will be added upon acceptance of the paper.
* Sequences from Bonaccorso and Peterson (2007).
** Sequences from Saunders and Edwards (2000).

trees and their non-parametric bootstrap support were used as a gross measure of phylogenetic congruence among datasets (Bull et al., 1993; de Queiroz et al., 1995; Wiens, 1998); i.e., whenever conflicting topologies were highly supported, potential for gene incongruence was taken in account. For each gene, base frequencies were examined for nucleotide bias among taxa, using the χ^2 test of homogeneity in PAUP. Evolutionary rate heterogeneity among lineages was tested using a likelihood-ratio test (Felsenstein, 1981) by comparing the likelihood scores of the ML trees with and without the molecular clock enforced.

Combined mitochondrial and mitochondrial/nuclear trees were obtained under MP, ML, and Bayesian analyses. The mitochondrial dataset consisted of all CR and ND2 sequences. Combined mitochondrial/nuclear analyses were based on: (1) all available sequences (69% of taxa fully sampled); and (2) a pruned dataset, in which every individual in the tree was represented by all loci, with the exception of that of *C. nana*. Parsimony analyses were performed with gaps coded as missing data and heterozygous positions treated as polymorphisms. Trees were obtained through heuristic searches in PAUP using 10,000 stepwise random additions (TBR branch-swapping). Clade support was estimated via heuristic searches using 1000 bootstrap pseudoreplicates (Felsenstein, 1985), with each pseudoreplicate consisting of 10 stepwise random additions.

Maximum likelihood trees were estimated using GARLI (Genetic Algorithm for Rapid Likelihood Inference, ver. 0.951; Zwickl, 2006), which provides considerable advantages over PAUP in terms of computational efficiency. It uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously (Zwickl, 2006). GARLI analyses for individual genes and the combined datasets were conducted using the general model specified by Modeltest but with parameter values estimated from the data. Individual solutions were selected after 10,000 generations with no significant improvement in likelihood, with the significant topological improvement level set at 0.01; then, the final solution was selected when the total improvement in likelihood score was lower than 0.05, compared to the last solution obtained. Default values were used for other GARLI settings, as per recommendations of the developer (Zwickl, 2006). For each dataset, at least 20 independent analyses were run to assure that they produced consistent likelihood scores. Bootstrap support was assessed via 100 and 1000 pseudoreplicates for the individual gene and the combined datasets, respectively, and bootstrap searches were performed under the same settings used during tree search.

Bayesian analyses were performed in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), implementing a partition by gene and codon position (ND2), and assigning to each partition its best-fit model "family"; thus, the combined mitochondrial analysis consisted of four partitions (three codon positions for ND2, and CR), whereas the combined mitochondrial/nuclear analyses consisted of seven (three codon positions for ND2, and one for CR, AK5, βfb7, and TGFβ2.5, respectively). In all cases, parameters were unlinked between partitions, except topology and branch lengths, and rate variation (prset ratepr = variable) was invoked. Analyses consisted of five independent runs of 10×10^6 generations and 10 Markov chains (temperature = 0.20), with trees sampled every 1000 generations. Stationarity was assessed by plotting −ln L per generation in Tracer 1.3 (Rambaut and Drummond, 2004), and plotting posterior probabilities of clades as a function of generation number using AWTY (Wilgenbusch et al., 2004). Comparison of performance of multiple runs allowed selection of those runs that converged to high likelihood values and reflected stability in the posterior probabilities of clades. All five runs fulfilled these conditions and reached stationarity after 1×10^6 generations. Of the 10,000 trees resulting per run, the first 2000 were discarded as "burn in." Then, the remaining trees were combined to calculate the posterior probabilities in a 50% majority-rule consensus tree.

2.4. Hypothesis testing

Once the "best" phylogenetic hypothesis was recovered, the topology obtained was tested against those representing previous hypotheses of relationships. Statistical comparisons were conducted on the topology resulting from the mitochondrial/nuclear pruned analyses, using three different methods: the Shimodaira–Hasegawa test of topology (SH test; Shimodaira and Hasegawa, 1999; Goldman et al., 2000); the likelihood-ratio test of monophyly (Huelsenbeck et al., 1996a) based on parametric bootstrapping (Efron, 1985; Huelsenbeck et al., 1996b; Goldman et al., 2000); and evaluation of Bayesian posterior probabilities of alternative tree topologies (Huelsenbeck and Rannala, 2004). For the SH test, the ML tree was compared with a set of trees including ML trees under the null hypotheses, and trees under other possible realizations of the null; in doing this, I avoided breaking up monophyletic groups that were compatible with both the null hypotheses and the ML tree (Buckley et al., 2001). Trees were compared in PAUP, running 1000 bootstrap pseudoreplicates under RELL optimization.

The parametric bootstrap test of monophyly (Huelsenbeck et al., 1996a) compares the likelihood between the best ML topology (T_1) and that showing the monophyly of the group of interest (T_0). Significance of likelihood difference (δ) is assessed by comparing observed differences with a null distribution obtained by means of Monte Carlo simulation (Efron, 1985; Felsenstein, 1988; Huelsenbeck et al., 1996b; Goldman et al., 2000). A total of 100 simulated matrices were obtained using Batch Architect (Maddison and Maddison, 2004a) in Mesquite 1.05 (Maddison and Maddison, 2004b); all ML tree searches were performed in GARLI with the same settings described before. The Bayesian approach consisted of taking the post burning-in trees from the posterior probability distribution and filtering all trees compatible with the null hypothesis in PAUP. In this case, the percent of the trees retained indicates the posterior probability that the hypothesis is correct (conditional on the model, data, prior probabilities, and convergence of the MCMC; Huelsenbeck and Rannala, 2004).

Differences in statistical power between the SH and the parametric bootstrapping test are expected because knowledge on the underlying distribution is available in the second approach, but not to the first (Goldman et al., 2000). However, the cost of this power is an increased reliance on the evolutionary model. In the same way, Bayesian posterior probability values are highly dependent on the adequacy of the evolutionary model (Larget and Simon, 1999; Huelsenbeck and Bollback, 2001; Shimodaira, 2001). Therefore, to assure the adequacy of the evolutionary model selected by Modeltest, I performed an absolute goodness-of-fit test (Goldman, 1993). Under this approach, goodness-of-fit can be assessed by evaluating the difference between the multinomial (unconstrained) likelihood and the likelihood under the ML model being examined; the null hypothesis is that the difference calculated from the empirical data is not greater than it would be expected by chance were there is a perfect fit between model and data (Sullivan et al., 2000). The null distribution was generated in Mesquite, via simulation of 100 matrices under the best-fit model, with parameters estimated from the original data. ML trees were estimated in GARLI, and multinomial and ML values were obtained by scoring the trees in PAUP.

2.5. Ancestral area reconstruction

To explore implications of the phylogeny for the historical biogeography of the *Cyanolyca* jays, I optimized the areas occupied by

each species onto the final tree topology using Dispersal-Vicariance analysis (DIVA; Ronquist, 1996, 1997). Dispersal-Vicariance analysis is a simple biogeographic model based on a three-dimensional step matrix that takes into account vicariance, dispersal, and extinction. Speciation is assumed to subdivide the ranges of widespread species into vicariant components and the optimal ancestral distributions are those that minimize the number of implied dispersal and extinction events (Ronquist, 1997).

A simple scheme attempted to capture the most important biogeographic patterns in the genus, with species assigned to three main areas as character states: (1) Mesoamerica (= from Mexico to Panama; *C. nana*, *C. mirabilis*, *C. pumilo*, *C. argentigula*, and *C. cucullata*); (2) Northern Andes (= from Venezuela south to the Huancabamba Deflexion in Peru; *C. pulchra*, *C. armillata*, and *C. turcosa*); and (3) Central Andes (= from the Huancabamba Deflexion south to Bolivia; *C. viridicyanus*). To explore all possible DIVA optimizations, I ran two analyses: one in which the maximum number of ancestral areas was set to 3 (= number of total areas) and one in which this parameter was set to 2 areas. Biogeographic optimizations were conducted based on ingroup taxa only because, although the sister relationship of *Cyanolyca* jays with all other NWJ species is strongly supported (Zusi, 1987; Espinosa de los Monteros and Cracraft, 1997; Saunders and Edwards, 2000; Bonaccorso and Peterson, 2007), relationships among the remaining NWJ species are not completely resolved.

3. Results

3.1. Sequence attributes

The ND2 sequence fragments, including those for *Cyanolyca nana,* showed no patterns suggesting amplification of nuclear pseudogenes (Zhang and Hewitt, 1996; Sorenson and Quinn, 1998). CR and the nuclear introns showed indels of variable size, but were aligned easily after minor adjustments. A 78 bp indel found in the AK5 sequence of *Dendrocitta formosae* was excluded from all analyses. Variable/parsimony-informative sites within gene fragments were distributed as follows: 459/379 out of 1014 for ND2; 338/269 out of 653 for CR; 96/28 out of 607 for AK5; 111/45 out of 871 for βfb7; and 67/32 out of 600 for TGFβ2.5. Primer pairs used for amplifying *C. nana* produced three overlapping fragments of CR (608 bp; 648 aligned positions) and ND2 (397 bp). According to the AIC, Modeltest selected the GTR + Γ + I model for CR; TrN + Γ + I for ND2 (K81uf + Γ + I for the first, TrN + I for the second, and TIM + Γ for the third codon); HKY + I for AK5; GTR + Γ for βfb7; and TrN + Γ for TGFβ2.5; parameter values estimated based on ML trees are listed in Table 2. Nucleotide composition bias across lineages was non-significant for all datasets ($P = 0.999$). Evolutionary rate heterogeneity was detected for both mitochondrial genes ($P < 0.001$), even when outgroup taxa where excluded from analyses, whereas, for the nuclear genes, the assumption of clock-like evolution was not rejected ($P > 0.05$).

3.2. Phylogeny

Given that phylogenetic trees based on ML analysis of ND2 and CR produced the same general topology, I present the results of the combined mitochondrial analyses, which show high MP and ML bootstrap support, and high Bayesian posterior probabilities for all major relationships (Fig. 2). According to these analyses *Cyanolyca* consists of two major clades, each with several geographically defined subclades. The first clade consists of Mesoamerican dwarf jays, with *C. pumilo* and *C. argentigula* as sisters and reciprocally monophyletic with respect to *C. nana* + *C. mirabilis*. The second clade is divided into two main groups—one composed of *C. cucullata* + *C. pulchra* and the other containing the "core" South American species, with *C. armillata* as sister of *C. turcosa* + *C. viridicyanus*. Parsimony analysis of the mitochondrial dataset produced 1153 equally parsimonious trees, which were highly homoplastic (consistency index [CI] = 0.47, rescaled consistency index [RC] = 0.37); despite the high level of homoplasy, the MP majority-rule consensus tree recovered the same major groupings as the ML trees.

Parsimony and ML trees of the nuclear loci were in general agreement with the mitochondrial tree (Fig. 3). Analyses of TGFβ2.5 resulted in trees showing major relationships obtained in mitochondrial analyses with few exceptions. The other nuclear genes were more limited in their resolution. AK5 recovered the monophyly of *Cyanolyca* and the sister relationships between *C. pulchra* and *C. cucullata*, *C. viridicyanus* and *C. turcosa*, and *C. argentigula* and *C. pumilo* (ML only). βfb7 failed to depict the sister relationship between *C. pulchra* and *C. cucullata*, and showed *C. viridicyanus* as paraphyletic with respect to *C. turcosa*, a pattern observed in all nuclear gene trees. Topological discordances across loci were observed only within the dwarf jay clade. The mitochondrial dataset and TGFβ2.5 supported the sister relationship between *C. pumilo* and *C. argentigula*, βfb7 supported *C. pumilo* + *C. mirabilis*, and AK5 was ambiguous (*C. pumilo* + *C. argentigula* in ML, and *C. argentigula* + *C. mirabilis* in MP; both having less than 50% bootstrap support). Sources of these topological disagreements are difficult to determine given that single samples were available for *C. pumilo* and *C. mirabilis*, and no nuclear sequence was available for *C. nana*.

Independently of sampling limitations, mitochondrial and nuclear gene trees may disagree under different circumstances. Mitochondrial DNA tends to be effectively haploid and, thus, has a larger probability of tracking the species tree (Birky et al., 1989; Moore, 1995; Palumbi et al., 2001). Furthermore, congruence between the mitochondrial and the TGF2.5 trees, provides further support for suggesting that they may represent the true topology, whereas the topology of the βfb7 tree may result from other phenomena such as hybridization, retention of nuclear polymorphisms (deep coalescence), or gene paralogy. The short genetic distance between *C. pumilo* and *C. mirabilis* inferred from the βfb7 tree, compared with the long distance between these two taxa inferred from other loci, favors a hypothesis of hybridization and argue against

Table 2
Summary of model parameters and tree scores estimated from maximum likelihood analyses. Values were estimated in PAUP based on the GARLI ML trees.

Gene	Base frequencies				Rate matrix					α	Pinv	−ln likelihood
	A	C	G	T	AC	AG	AT	CG	CT			
CR	0.345	0.228	0.103	0.324	4.463	17.154	2.058	0.703	13.753	1.089	0.343	5118.106
ND2	0.333	0.346	0.095	0.226	0.768	17.930	0.914	0.665	13.431	1.452	0.478	7142.754
Combined mt	0.333	0.296	0.101	0.269	1.604	16.754	1.522	0.792	12.323	1.234	0.420	12,269.423
AK5	0.219	0.287	0.312	0.182	Kappa = 6.206					—	0.401	1489.069
βfb7	0.320	0.174	0.191	0.316	0.875	5.681	0.353	2.093	3.302	0.701	—	1863.282
TGFβ2.5	0.239	0.237	0.214	0.310	1.338	7.985	1.228	1.417	3.884	0.449	—	1378.646
Total evidence	0.304	0.259	0.169	0.269	2.171	11.075	1.738	0.845	14.614	0.455	0.466	17,560.402

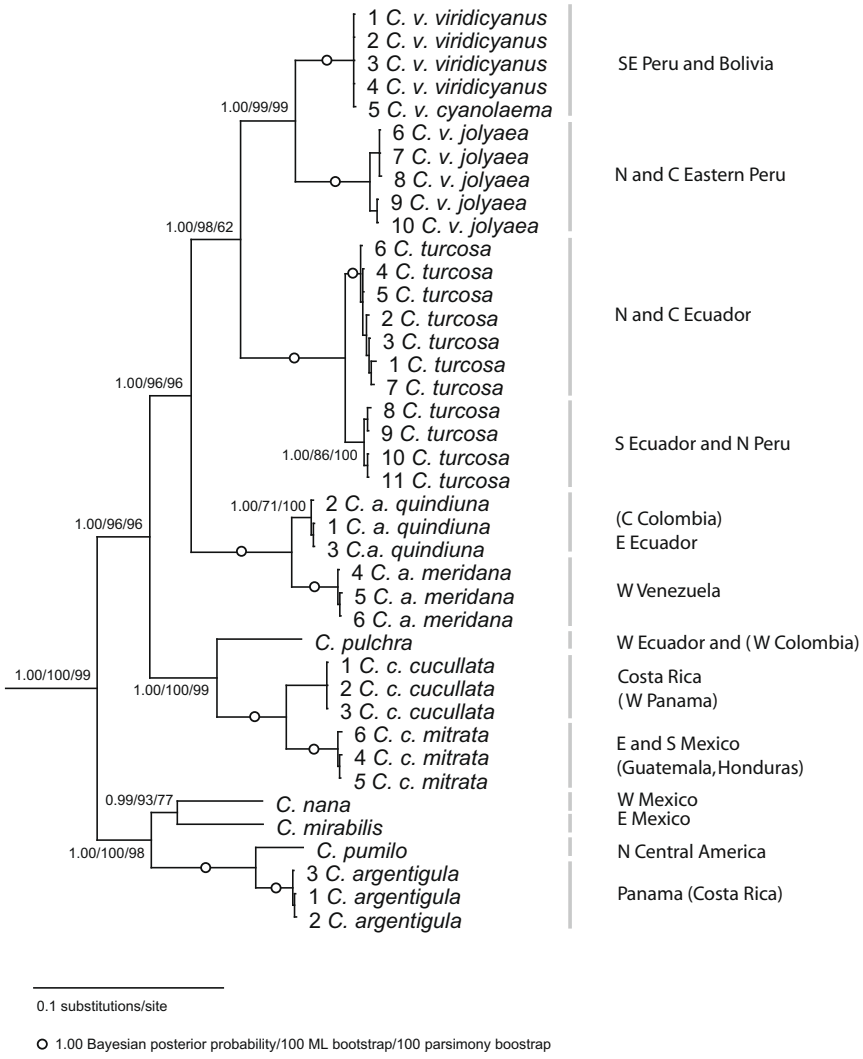

Fig. 2. Bayesian 50% majority-rule consensus tree estimated from the combined analysis of the mitochondrial Control Region and ND2. Bayesian posterior probabilities and maximum likelihood and maximum parsimony bootstrap values are indicated whenever nodes were recovered with less than 1.00 posterior probability or 100% bootstrap support.

retention of ancestral polymorphisms (Holder et al., 2001). Moreover, the low population sizes of both *C. pumilo* (Harris and Pimm, 2008) and *C. mirabilis* (Birdlife International, 2000) decrease the probabilities of deep coalescence scenarios. In addition, the observation that some loci (e.g., βfb7), but not others, may "leak" as result of a small scale hybridization event (Coyne and Orr, 2004) reinforces the plausibility of gene flow between these species in the recent past. However, representative sample sizes and sequences for more nuclear loci are needed to test any of these hypotheses.

Given that topological conflicts were limited to a terminal branch, I combined all loci in further analyses. Parsimony analyses of the total evidence and pruned datasets resulted in the same topology observed in the combined mitochondrial analyses; however, the total evidence dataset produced 13,021 equally parsimonious trees (CI = 0.5228, RC = 0.4112) whereas the pruned dataset generated only 12 (CI = 0.5227, RC = 0.3717). In both cases, disagreements among equally parsimonious trees were caused by alternate arrangements among outgroup sequences or within subspecies. The high number of trees produced in the total evidence analysis is not surprising since, frequently, a higher amount of trees is generated when incomplete datasets are analyzed (Huelsenbeck, 1991; Wiens and Reeder, 1995; Wilkinson, 1995).

Maximum likelihood analyses of the total evidence and pruned datasets generated exactly the same topology. For both datasets, independent ML-GARLI runs produced highly consistent likelihood scores, and all ML trees showed the same relationships as the MP and the Bayesian 50% majority-rule consensus trees. Also, nodal support was equally high across analyses, showing no trend towards a decrease or increase attributable to incorporation of incomplete data. Together, similarity of both topology and nodal support suggest that the number of characters available for the incompletely sampled taxa included in the total evidence analyses is sufficient for obtaining the level of accuracy of the complete sampling (pruned) analyses, an idea suggested by recent simulation studies (Wiens, 2003). Given the consistency of results obtained under different sampling strategies and optimization criteria, I present the Bayesian 50% majority-rule consensus tree resulting from the pruned dataset, indicating the nodal support recovered from Bayesian, ML, and MP analyses (Fig. 4A).

3.3. Geographic structure within Cyanolyca species

On finer scales, individuals in polytypic species segregated clearly into geographic groups (Fig. 2). Within *Cyanolyca cucullata*, samples from the Sierra Madre Oriental of Mexico (*C. c. mitrata*) are

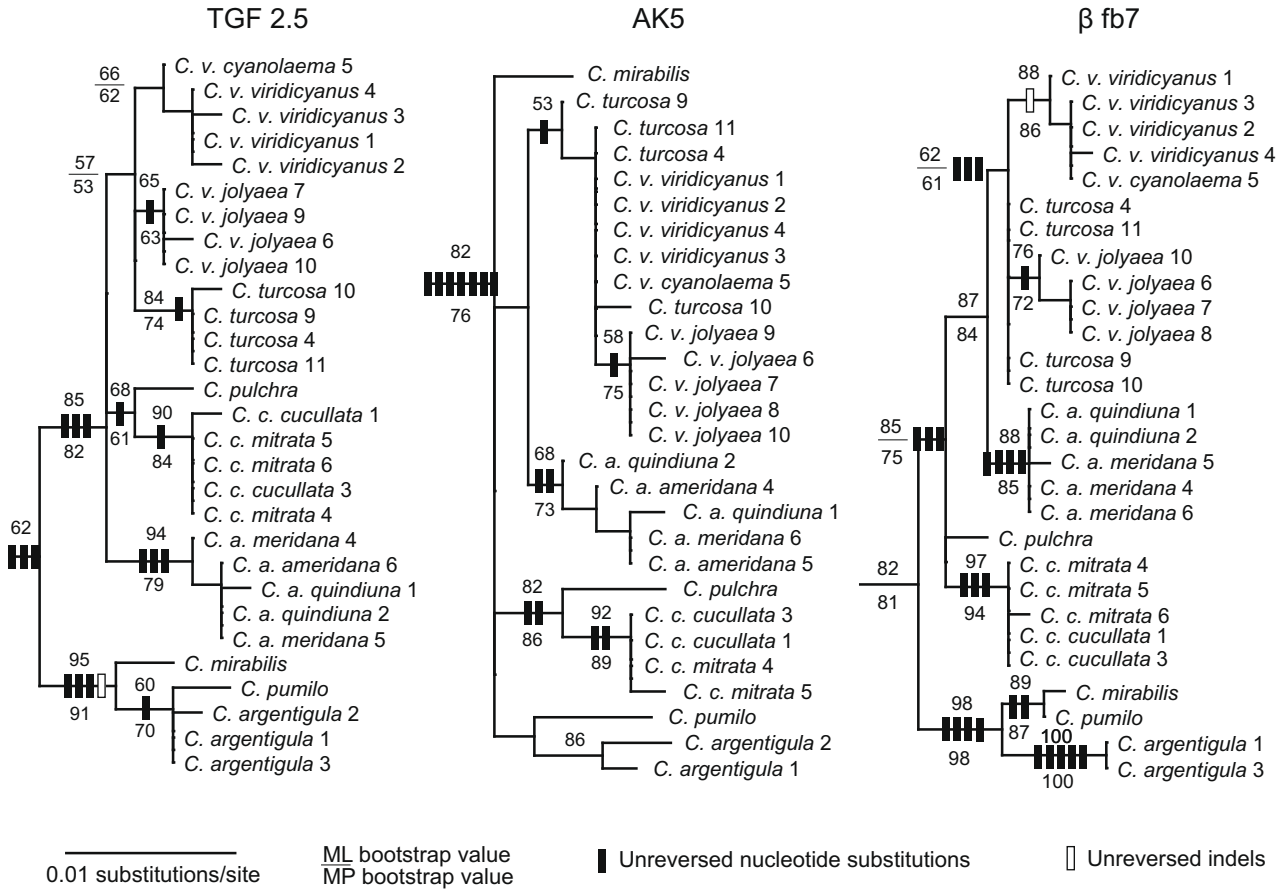

Fig. 3. Maximum likelihood trees estimated for the nuclear introns TGF 2.5, AK5, and βfb7. Values on nodes indicate maximum likelihood (above) and maximum parsimony (below) bootstrap proportions.

distinct from those of the southern Central American highlands of Costa Rica (*C. c. cucullata*). In South America, individuals of *C. armillata* from the Andes of Venezuela (*C. a. meridana*) are distinct from those of the eastern Andes of Ecuador (*C. a. quindiuna*), whereas samples of *C. viridicyanus* split in two groups, one corresponding to populations from the eastern Andes of Peru (*C. v. jolyaea*), and the other corresponding to those from the southern extreme of the eastern Andes of Peru (*C. v. cyanolaema*) and the Andes of Bolivia (*C. v. viridicyanus*). Interestingly, the tree shows an additional separation between individuals of *C. turcosa* from most of Ecuador versus those from southern tip of Ecuador and northern Peru, a distinction not obvious on morphological grounds.

Population structure translated into relatively high sequence divergence in ND2 (ML-corrected pair-wise distances; Appendix 2). Sequence divergence between geographically segregated groups, represented as number of substitutions per site, can be summarized as follows: *C. turcosa* of northern Ecuador vs. *C. turcosa* of southern Ecuador and northern Peru, 0.013–0.015; *C. armillata meridana* vs. *C. a. quindiuna*, 0.029–0.03; *C. cucullata cucullata* vs. *C. c. mitrata*, 0.048–0.05; and *C. viridicyanus jolyaea* vs. *C. v. cyanolaema* and *C.v. viridicyanus* 0.08–0.084. Consistent with this pattern of divergence, nuclear synapomorphies in the form of nucleotide substitutions or indels support some of these groups (Fig. 3).

3.4. Hypothesis testing

The final ML tree topology did not include some of the relationships proposed by Goodwin (1976) and others (Hellmayr, 1934; Fjeldså and Krabbe, 1990), being these the sister-group relationship of (1) *Cyanolyca argentigula* and *C. mirabilis* and (2) *C. viridicyanus* and *C. armillata*. Trees depicting these null hypotheses were generated under both independent and joint scenarios, and a set of compatible trees was built by incorporating relationships supported by Goodwin (1976) and the ML tree: i.e., monophyly of *Cyanolyca*, monophyly of the dwarf jays, and ((*C. pulchra* + *C. cucullata*) South American jays). Combinations of these constraints produced 27 topologies that were compared with the "best" (ML pruned) tree, using the SH test. This analysis rejected topologies containing *Cyanolyca argentigula* + *C. mirabilis* ($P < 0.01$) as equally good explanations of the data, but was unable to reject topologies containing *C. viridicyanus* + *C. armillata*.

Because the SH test tends to be highly conservative (Strimmer and Rambaut, 2001; Buckley, 2002; Shimodaira, 2002), further efforts focused on exploring the *C. viridicyanus* + *C. armillata* topology (Goodwin, 1976) under the parametric bootstrapping and evaluation of Bayesian posterior probabilities. According to the parametric bootstrapping test, the *Cyanolyca viridicyanus* + *C. turcosa* topology recovered herein, had a statistically better likelihood than the *C. viridicyanus* + *C. armillata* topology ($\delta = 17.1$, $P < 0.01$). From the Bayesian standpoint, no tree from the posterior probability distribution showed the *C. viridicyanus* + *C. armillata* arrangement, implying that the Bayesian posterior probability of this topology is close to zero.

The goodness-of-fit test could not reject the null hypothesis of perfect fit between model and data. Although the difference between the multinomial likelihood and the likelihood under the ML model for the real data was relatively high ($\delta = 4045.484$), it fell

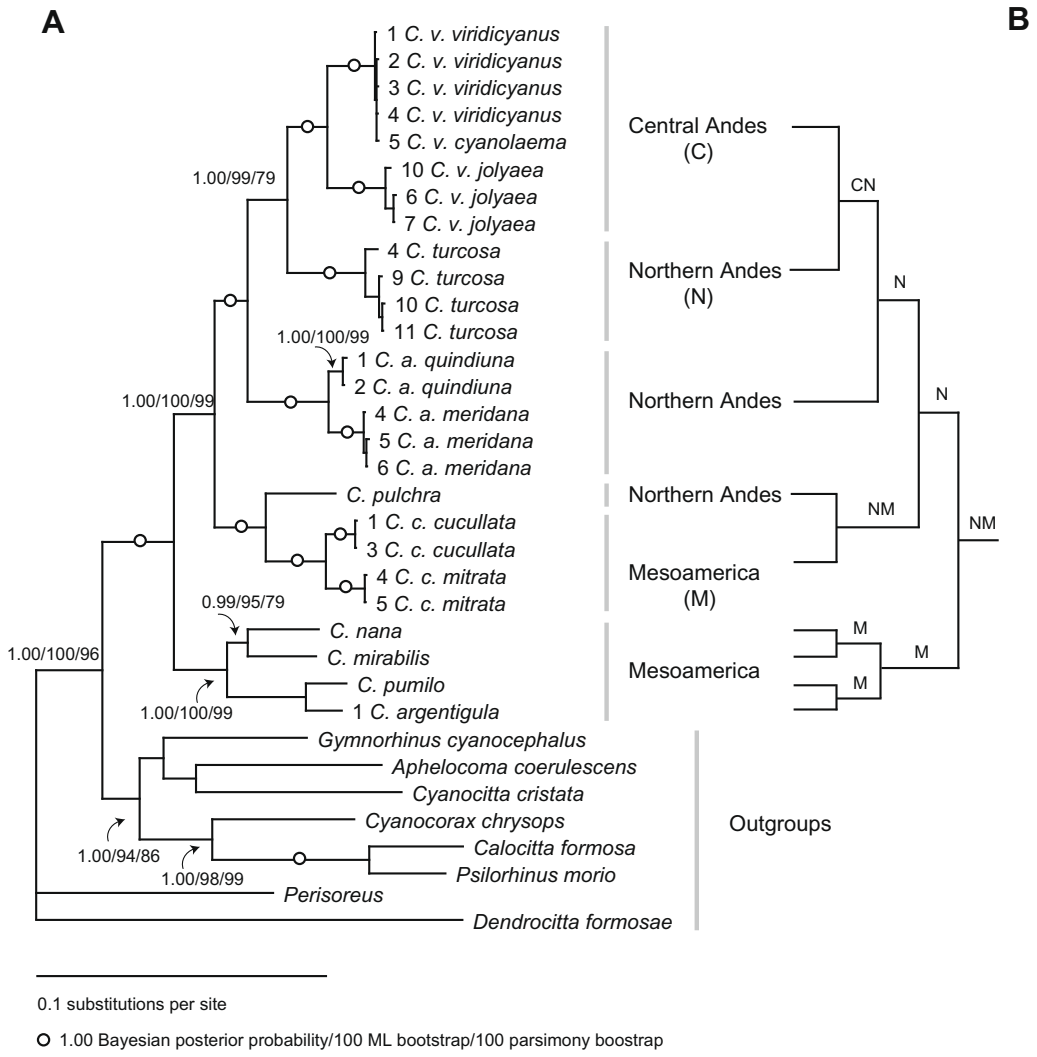

Fig. 4. (A) Bayesian 50% majority-rule consensus tree estimated from the combined, pruned analysis (Control Region, ND2, TGF 2.5, AK5, and βfb7). Bayesian posterior probabilities and maximum likelihood and maximum parsimony bootstrap values are indicated whenever nodes were recovered with less than 1.00 posterior probability or 100% bootstrap support. (B) Optimization of ancestral areas onto the phylogeny by means of Dispersal-Vicariance analysis; N = Northern Andes, C = Central Andes, M = Mesoamerica.

within the 95% of the null distribution. Therefore, it can be assumed that the chosen model encompasses the major features of the distribution of the data, supporting the validity of the parametric bootstrapping test and the evaluation of Bayesian posterior probabilities.

3.5. Ancestral area reconstruction

Optimization of geographic areas onto the tree provided simple, analytical means for exploring the biogeography of *Cyanolyca*. Dispersal-Vicariance analyses produced the same solution regardless of the maximum number of ancestral areas (either 2 or 3), with optimizations requiring 2 dispersal events (Fig. 4B). These reconstructions imply an ancestor distributed in Mesoamerica and the Northern Andes, and a vicariant event between the ancestor of the dwarf jays (in Mesoamerica) and the ancestor of the remaining species (in the Northern Andes). Then, dispersal of the ancestor of *C. pulchra* + *C. cucullata* into Mesoamerica was followed by a vicariant event that separated what is now *C. cucullata* (in Mesoamerica) from *C. pulchra* (in the Northern Andes). The ancestral area of the core South American clade is the Northern Andes, with the ancestor of *C. viridicyanus* + *C. turcosa* dispersing into the Central Andes, and a vicariant event separating both species, *C. turcosa* (in the Northern Andes) and *C. viridicyanus* (in the Central Andes).

4. Discussion

4.1. Phylogeny of ***Cyanolyca*** jays

The inferred hypothesis of relationships for the *Cyanolyca* jays is highly robust and is in general agreement with previous ideas (i.e., Goodwin, 1976). The robustness of the combined phylogenetic tree seems to be a product of having genes that (1) support nodes at different branching levels and (2) overall, are congruent with one another. Although these results are conditional on the data at hand, it seems that it would take large amounts of contrary evidence to recover alternate topological rearrangements among taxa.

Monophyly of *Cyanolyca* has not been seriously questioned, particularly since Zusi's (1987) work. All NWJ species share a synapomorphy involving modifications in the lower jaw (i.e., the "buttress complex"; Zusi, 1987); in all *Cyanolyca* examined by Zusi (1987) this complex shows a shared state. However, having

complete sampling at the species-level and a robust hypothesis of relationships allows inferences about evolutionary processes in the group.

Common ancestry of the dwarf jays is consistent with their smaller size and allopatric distributions across the highlands of Mesoamerica. Although *Cyanolyca argentigula* and *C. mirabilis* share plumage similarities, the *C. argentigula* + *C. pumilo* and *C. mirabilis* + *C. nana* relationships are more congruent with their geographic distributions. *Cyanolyca pumilo* inhabits montane forests between the Isthmus of Tehuantepec and the lowlands of Nicaragua, and it is replaced by *C. argentigula* in the mountains of Costa Rica and western Panama. Genetically and morphologically divergent populations and sister species have been documented for other bird lineages in these two regions (e.g., *Lampornis* hummingbirds, García-Moreno et al., 2006; *Aulacorhynchus* toucanets, Puebla-Olivares et al., 2008; *Chlorospingus* bush-tanagers, Bonaccorso et al., 2008). Similarly, *C. nana* inhabits patchy areas in the mountains of eastern Mexico (Veracruz and Oaxaca), whereas *C. mirabilis* inhabits the Sierra Madre del Sur (Guerrero and Oaxaca) in western Mexico. As a whole, dwarf jays form two reciprocally monophyletic groups distributed east and west of the Isthmus of Tehuantepec, another important geographic barrier for montane forest specialists (Sullivan et al., 2000; García-Moreno et al., 2004, 2006; Pérez-Emán, 2005; Cadena et al., 2007).

The close relationship between *C. pulchra* and *C. cucullata* is consistent with their distributions being separated by the Panamanian and Colombian lowlands, as well as their inhabiting lower elevations than all other species in the genus (Ridgely and Tudor, 1989; Stiles and Skutch, 1989; Howell and Webb, 2004). Geographic separation of this clade from the main northern Andean lineage might have been caused by the formation of the Río Cauca Valley in western Colombia, whereas sympatry of *C. pulchra* with *C. turcosa* in western Ecuador might have resulted from post-speciation range expansions.

The core South American taxa form a monophyletic group, with *Cyanolyca armillata* sister to *C. turcosa* + *C. viridicyanus* (*contra* Hellmayr, 1934; Meyer de Schauensee, 1966; Goodwin, 1976; Fjeldså and Krabbe, 1990; parametric bootstrap, $P < 0.01$). This relationship is intriguing considering that no geographic break is evident between these two clades (i.e., *C. armillata* vs. *C. turcosa* + *C. viridicyanus*). In fact, the eastern Andes of Ecuador and the central Andes of Colombia—where *C. armillata* and *C. turcosa* overlap—are considered part of the same geomorphological unit (Simpson, 1975). Therefore, details of the original barrier separating these two clades, as well as the potential causes of cladogenesis, remain obscure. In the present, local sympatry between *C. armillata* and *C. turcosa* might be facilitated by differences in habitat preferences, since *C. armillata* seems to occupy less disturbed habitats (Ridgely and Tudor, 1989; Ridgely and Greenfield, 2001).

Finally, the close relationship between *C. turcosa* (Andes of Ecuador) and *C. viridicyanus* (Andes of Bolivia and Peru) is consistent with their ranges being adjacent to and separated by the Río Marañon Valley in northern Peru, considered one of the most prominent geographic barriers in the Andes (e.g., Vuilleumier, 1969; Parker et al., 1985). Genetic differentiation between sister taxa on both sides of this valley has been documented for *Leptopogon* flycatchers (Bates and Zink, 1994) and *Myadestes ralloides* (Miller et al., 2007).

4.2. Geographic structure and species limits

Sampling of widely distributed species provided a preliminary assessment of molecular differentiation of populations, as well as identification of potential independent lineages within species. Among the least expected results was the segregation of individuals of *Cyanolyca turcosa* into two genetically divergent groups. *Cyanolyca turcosa* is distributed mainly along the two Andean cordilleras of Ecuador, which are separated by a dry valley; however, the two cordilleras meet in southern Ecuador, as do some bird populations distributed along them (Ridgely and Greenfield, 2001). Sequence divergence in *C. turcosa* seems to indicate an abrupt disruption of gene flow between populations along the Andes of Ecuador, and those restricted to the southern tip of the Andes of Ecuador and northern Peru. This genetic disruption coincides with a geographic break in southern Ecuador around the Río Zamora Valley (Fig. 5). Morphological differentiation of other birds across this valley has been documented by Krabbe (2008), who proposed it as the most important geographic break of the eastern Ecuadorian Andes. Further and denser sampling at the population level is needed to assess the effectiveness of Río Zamora Valley as a barrier to gene flow, not only for *C. turcosa* but also for other lineages.

Samples of *Cyanolyca armillata* from the Andes of Venezuela (*C. a. meridana*) and the eastern Andes of Ecuador (*C. a. quindiuna*, also in the central Andes of Colombia), segregated into two lineages. Unfortunately, individuals from the geographically intermediate populations along the eastern Andes of Colombia (*C. a. armillata*) were not available for inclusion in this study. Still, the relatively low genetic differentiation between individuals from these extreme populations is congruent with their slight variation in coloration and size. Analysis of samples from the eastern and central Andes of Colombia, as well as careful documentation of morphological variation, is crucial in assessing potential geographic structure in this species.

Morphological differences and nucleotide-based synapomorphies separating allopatric populations of *Cyanolyca cucullata* south and north of the lowlands of Nicaragua, suggest that they represent independent evolutionary lineages (sensu Simpson, 1961; Wiley, 1978). From the point of view of the Phylogenetic Species Concept, study of museum skins shows that the criterion of diagnosability (Cracraft, 1983) is met for populations on both sides of the geographic break; *C. c. mitrata* has a well-defined white line on the crown that extends to the loral region, whereas in *C. c. cucullata*, the line is lacking, but a light, whitish shadow on the crown is present. Further population-level studies, including more samples across the intervening populations of northern Central America [*C. c. guatemalae* and *C. c. hondurensis* of Pitelka (1951)], which correspond morphologically to *C. c. mitrata*, are needed to confirm the reciprocal monophyly of *C. c. cucullata* and *C. c. mitrata*.

Finally, the most dramatic molecular differences were observed between populations of *Cyanolyca viridicyanus* north (*C. v. jolyaea*) and south (*C. v. cyanolaema* and *C. v. viridicyanus*) of the Río Apurimac valley, one of the most important biogeographic boundaries in the humid Andes (Vuilleumier, 1969; Haffer, 1974; Remsen and Brumfield, 1996), which defines the limits of several avian taxa (Ridgely and Tudor, 1989). Accordingly, this genetic differentiation coincides with discrete morphological differences: specimens from south of the Río Apurimac have a well-defined white line at the crown, whereas those from the north show no line but a whitish area on the crown, have a narrower white band on the throat, and are darker in overall plumage (Ridgely and Tudor, 1989; Madge and Burn, 1994; this study). Morphological, mitochondrial, and nuclear characters support the reciprocal monophyly (McKitrick and Zink, 1988; Zink and McKitrick, 1995) and diagnosability (Cracraft, 1983) of two historical and evolutionarily independent entities. On these grounds, I propose to recognize *Cyanolyca viridicyanus* (Lafresnaye and d'Orbigny, 1838) and *Cyanolyca jolyaea* (Bonaparte, 1852) as two different species.

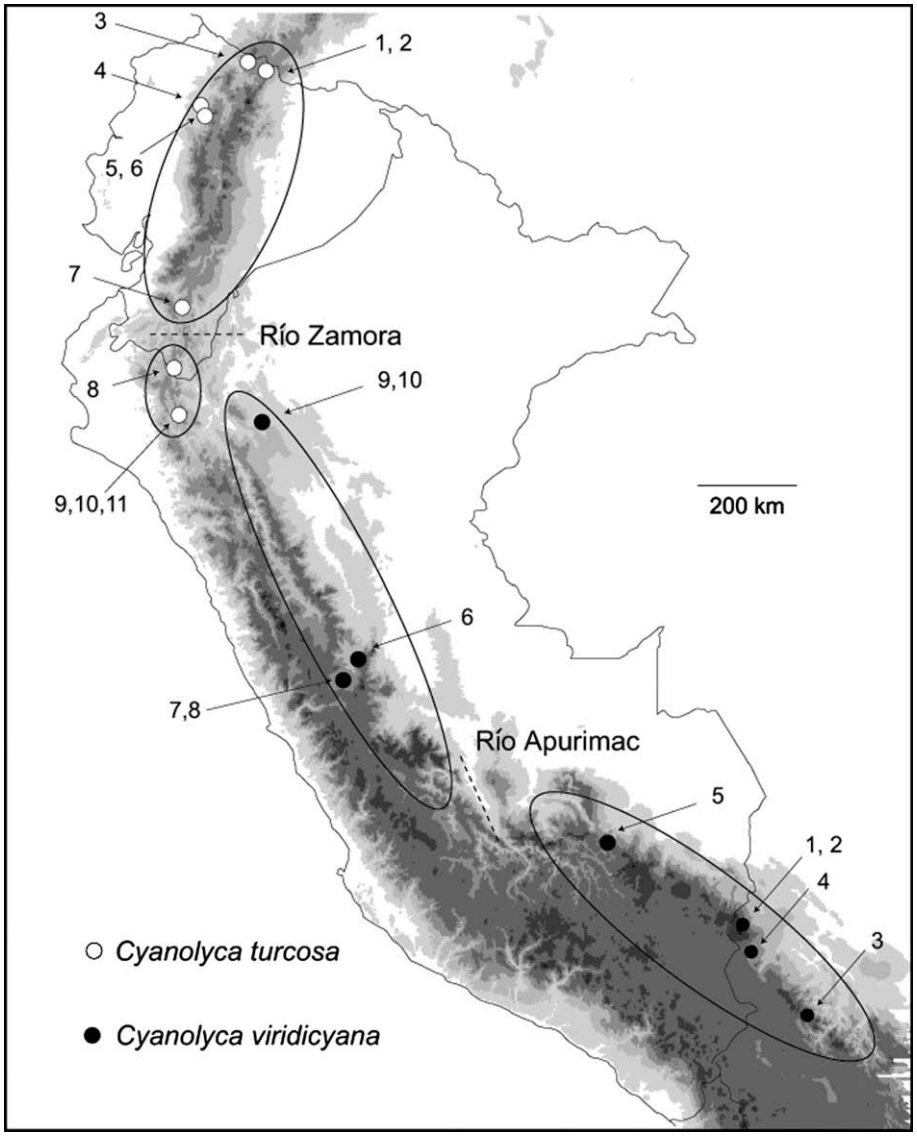

Fig. 5. Sampling localities in Ecuador and Peru for *Cyanolyca turcosa* and *C. viridicyanus*. Ovals indicate different geographic groups identified in the phylogenetic analyses of the Control Region and ND2.

4.3. Biogeography

Biogeographic reconstructions emphasize the importance of the Northern Andes as a major center for biological diversification (Gentry, 1982; Fjeldså, 1994; Duellman, 1999), and suggest, once again, that periodic establishment and disruption of gene flow across the Panamanian Land Bridge has been important in affecting the composition of South American and Mesoamerican avifaunas (e.g., DaCosta and Klicka, 2008). Four other montane forest lineages that span their distributions from Mexico to the southern Andes—*Myioburus*: Pérez-Emán (2005); *Myadestes*: Miller et al. (2007); *Buarremon*: Cadena et al. (2007); and *Chlorospingus*: Weir et al. (2008)—are hypothesized to have originated in northern Middle America and spread south through the Neotropics (Weir et al., 2008). *Cyanolyca* jays show a similar pattern of radiation from Mesoamerica south to the Andes; this observation is supported by the fact that most lineages in its sister group—all other NWJs—are restricted to Mesoamerica and North America. Even more, *Cyanolyca* jays appear to have radiated from the Northern Andes back into the highlands of Mesoamerica up to Mexico. This second "wave" of radiation, represented by *C. cucullata*, may have coincided with those of lineages of South American origin that are though to have invaded Mesoamerica in the recent past (e.g., *Aulacorhynchus prasinus*; Puebla-Olivares et al., 2008).

Timing two independent dispersals across the Isthmus of Panama is not trivial, because it involves placing the phylogeny of *Cyanolyca* in an absolute-time framework. Unfortunately, fossils available are not useful because they are too young (i.e., Late Pleistocene: Brodkorb, 1957; Weigel, 1967; Holman, 1959), ambiguous as per their identification as NWJs, or dated imprecisely (Brodkorb, 1972). Given the widespread evolutionary rate heterogeneity detected for several lineages in the group (i.e., NWJs as a whole [Bonaccorso and Peterson, 2007]; *Aphelocoma* jays [Peterson, 1992; McCormack et al., 2008]; *Cyanocorax* jays [Bonaccorso et al., unpubl.]; and *Cyanolyca* jays [this study]), using standard estimates of evolutionary rates drawn from other avian lineages (e.g., Fleischer et al., 1998; Arbogast et al., 2006) would be both arbitrary and misleading. However, high phylopatry of species in *Cyanolyca*,

their deep genetic differentiation across geographic barriers, and their absence from lower montane areas (e.g., the Darien highlands) support the idea that dispersal into South America occurred only after the completion of the Panama Land Bridge (3.1 Mya; Coates and Obando, 1996) in times when montane forests were relatively continuous along the region.

Biogeography of *Cyanolyca* also reveals interesting ecological patterns. Populations of *C. cucullata* are marginally sympatric with three of the dwarf jays (Fig. 1), but occupy lower elevations (Stiles and Skutch, 1989; Howell and Webb, 2004). This altitudinal segregation, which seems to facilitate marginal sympatry, is observed also between two species that co-occur in the Northern Andes (*C. turcosa* and *C. pulchra*). Detailed analyses on ecology and niche preferences are necessary to corroborate these patterns, as well as their potential association with competition avoidance mechanisms.

4.4. Speciation in the Cyanolyca jays

Phylogenetic analysis of *Cyanolyca* species provides evidence for discussing modes of speciation. Observed patterns of relationship do not support models involving recent divergence along altitudinal gradients, because species that replace each other in altitude are not each other's closest relatives. On the other hand, marked geographic differentiation provides support for hypothesis based on the effect of the linearity of the Andes in limiting contact among parapatric forms (especially in *Cyanolyca viridicyanus*), and the disruptive effect of geographic barriers in promoting speciation, not only in the Andes, but along the full range of the genus. With only one exception (i.e., the clade formed by *C. armillata* and *C. turcosa* + *C. viridicyanus*), diverging lineages (sister species, as well as sister clades) are distributed on either side of potentially effective barriers to gene flow (Fig. 1). In the case of *Cyanolyca armillata* vs. *C. turcosa* + *C. viridicyanus*, original species distributions are obscured by likely subsequent range expansions and by the fact that no current barriers separate these two groups.

Although allopatry is widely accepted as the most important cause of speciation among birds (Mayr, 1942, 1963; Chesser and Zink, 1994; Barraclough et al., 1998), empirical evidence regarding Andean montane forest birds is still limited (e.g., Bates and Zink, 1994; Pérez-Emán, 2005; Miller et al., 2007; Weir et al., 2008). In most studies, adjacent, allopatric species and populations have turned out to be more genetically similar than originally predicted (e.g., *Cranioleuca* Spinetails: García-Moreno et al., 1999b; *Buarremon bruneinucha* complex: Cadena et al., 2007; *Aulacorhynchus prasinus* complex: Puebla-Olivares et al., 2008; *Chlorospingus opthtalmicus* complex: Weir et al., 2008). Clearly, levels of divergence between allopatric species and populations are expected to vary according to several factors (e.g., evolutionary rates, time since isolation, dispersal abilities, and demography of populations [Mindell et al., 1990; Barraclough et al., 1998; Barraclough and Savolainen, 2001; Knowles and Maddison, 2002]). From the high levels of differentiation observed in *Cyanolyca* (Appendix 2), it is likely that evolutionary rates are relatively high, or that geographic isolation occurred earlier (or has been more effective) than in other lineages studied to date. Considering the wide spectrum of sequence divergence recorded among different populations, development of studies for estimating coalescent-based divergence times seems promising in linking geological events with molecular and morphological evolution, as well as in assessing the potential effects of Pleistocene climatic fluctuations on population structure.

Finally, it is important to notice that, although major genetic differentiation and speciation in *Cyanolyca* seems to be promoted by the isolating effects of unsuitable habitat and dry river valleys, current climates and barriers do not represent the conditions that have prevailed for the last 2 million years. For example, barriers that appear major now may have been easier to cross during glacial periods, and alternatively, areas with no current barriers may have sustained unsuitable habitats that could have promoted isolation and speciation.

In summary, study of *Cyanolyca* jays has provided a unique perspective on phenomena responsible for the unparalleled biodiversity of the Neotropical mountain avifaunas. It has also pointed out the value of analyzing both inter- and intra-specific divergence in understanding distributional patterns and in discovering the potential for answering broader questions in evolutionary biology. Collaborative efforts directed towards amassing complete sampling for many other taxa across the Neotropical mountains, especially in the Andes, are critical in assembling comparative studies to elucidate shared biogeographic patterns of the avifauna of this complex region.

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Appendix 1

Primer pairs used to amplify sequences of Cyanolyca nana. Primers for which sequences are provided were designed especially for this study.

Gene	Primer pair
ND2	L5740 (TGAATAGGACTAAAYCAAACAC) and H5968 (TGGTGCTAAGTGAGGTG) L5937 (TGCATGAACAAAAGCACCTTC) and H6148 (TAATTGTTGCGCAGTATGCG) L6076 (GCAACARTCATCTCRCTCC) and H6313 (Sorenson et al., 1999)
CR	JCR13 and JCR16 (Saunders and Edwards, 2000) CRL171 (GGACATATTTATTTTCCTTTCG) and CRH349 (GAAAAGTTAAGTGTATACATATG) JCR19 and H1248 (Saunders and Edwards, 2000)

Appendix 2

ND2 maximum likelihood-corrected pair-wise distances (substitutions per site) within (diagonal) and among taxa in *Cyanolyca*. Distances for *C. nana* are not included, given that the considerable amount of missing data for this species could inflate distance values.

		1	2	3	4	5	6	7	8	9	10	11	12
1	*C. viridicyanus viridicyanus*	0											
2	*C. v. cyanolaema*	0.001	—										
3	*C. v. jolyaea*	0.080–0.083	0.080–0.083	0.001–0.010									
4	*C. armillata quindiuna*	0.149–0.121	0.147–1.149	0.150–0.165	0–0.001								
5	*C. a. meridana*	0.145–0.158	0.143–0.156	0.159–0.177	0.03	0							
6	*C. turcosa*	0.116–0.124	0.111–0.122	0.117–0.134	0.164–0.172	0.170–0.191	0–0.017						
7	*C. cucullata cucullata*	0.18	0.183	0.190–0.195	0.219–0.221	0.229–0.235	0.180–0.188	0					
8	*C. c. mitrata*	0.178–0.182	0.176–0.180	0.201–0.211	0.211–0.217	0.231–0.239	0.177–0.196	0.048–0.05	0–0.003				
9	*C. pulchra*	0.181	0.179	0.190–0.195	0.186–0.189	0.209	0.170–0.186	0.120	0.138–0.142	—			
10	*C. mirabilis*	0.239	0.236	0.258–0.263	0.258–0.260	0.249–0.271	0.236–0.244	0.197	0.194–0.204	0.204	—		
11	*C. pumilo*	0.257	0.254	0.272–0.285	0.262–0.265	0.254–0.275	0.261–0.266	0.234	0.229–0.241	0.218	0.140	—	
12	*C. argentigula*	0.255–0.260	0.253–0.257	0.288–0.295	0.277–0.282	0.272–0.299	0.251–0.268	0.238–0.242	0.219–0.235	0.230	0.132	0.051	0–0.001

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