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MOLECULAR SYSTEMATICS AND EVOLUTION OF THE *CYANOCORAX* JAYS

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Abstract. Phylogenetic relationships were studied in the genus *Cyanocorax* (Aves: Corvidae) and related genera, *Psilorhinus* and *Calocitta*, a diverse group of New World jays distributed from the southern United States south to Argentina. Although the ecology and behavior of some species in the group have been studied extensively, lack of a molecular phylogeny has precluded rigorous interpretations in an evolutionary framework. Given the diverse combinations of plumage coloration, size, and morphology, the taxonomy of the group has been inconsistent and understanding of biogeographic patterns problematic. Moreover, plumage similarity between two geographically disjuct species, the Tufted Jay (*Cyanocorax dickeyi*) from western Mexico and the White-tailed Jay (*C. mystacalis*) from western Ecuador and Peru, has puzzled ornithologists for decades. Here, a phylogeny of all species in the three genera is presented, based on study of two mitochondrial and three nuclear genes. Phylogenetic trees revealed the non-monophyly of *Cyanocorax*, and the division of the whole assemblage in two groups: "Clade A" containing *Psilorhinus morio,* both species in *Calocitta, Cyanocorax violaceus*, *C. caeruleus*, *C. cristatellus*, and *C. cyanomelas,* and "Clade B" consisting of the remaining species in *Cyanocorax*. Relationships among species in Clade A were ambiguous and, in general, not well resolved. Within Clade B, analyses revealed the monophyly of the *"Cissilopha"* jays and showed no evidence for a sister relationship between *C. mystacalis* and *C. dickeyi*. The phylogenetic complexity of lineages in the group suggests several complications for the understanding biogeographic patterns, as well as for proposing a taxonomy that is consistent with morphological variation. Although multiple taxonomic arrangements are possible, recommendations are for recognizing only one genus, *Cyanocorax*, with *Psilorhinus* and *Calocitta* as synonyms.

Key words: *Cyanocorax, Psilorhinus, Calocitta,* Neotropics, New World Jays, Biogeography, Taxonomy.

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Resumen. Se estudiaron las relaciones filogenéticas en los géneros *Cyanocorax*, *Psilorhinus* y *Calocitta* (Aves: Corvidae), un grupo diverso de urracas del Nuevo Mundo cuyas especies se distribuyen desde el sur de los Estados Unidos hasta Argentina. Aunque la ecología y el comportamiento de algunas especies en el grupo han sido estudiadas extensamente, la falta de una filogenia molecular ha impedido la interpretación rigurosa de estos estudios en un marco evolutivo. Dadas las diversas combinaciones de coloración de plumaje, tamaño y morfología presentes en las especies del grupo, su taxonomía ha sido inconsistente y la interpretación de sus patrones biogeográficos ha sido problemática. Mas aún, la similitud de plumaje en especies que están geográficamente distantes, como *Cyanocorax dickeyi* del oeste de Mexico y *C. mystacalis* del oeste de Ecuador y Perú, ha sido difícil de interpretar. Se presenta una filogenia para todas las especies en los tres géneros, basada en el estudio de dos genes nucleares y dos genes mitocondriales. Los árboles filogenéticos mostraron la parafilia de *Cyanocorax* y la división de todas las especies en dos grupos: "Clado A" en el cual se encuentran *Psilorhinus morio,* ambas especies *Calocitta, Cyanocorax violaceus*, *C. caeruleus, C. cristatellus,* y *C. cyanomelas,* y "Clado B" en el cual se encuentran el resto de las especies de *Cyanocorax*. Las relaciones entre especies del Clado A fueron ambiguas y, en general, poco resueltas. En el Clado B, los análisis mostraron la monofilia de las especies en *"Cissilopha"*, pero no indicaron la monofilia de *C. mystacalis* + *C. dickeyi*. La complejidad filogenética de los linajes en el grupo sugiere varias complicaciones en el entendimiento de su biogeografía y taxonomía. Con base en los resultados filogenéticos se reconoce un solo género, *Cyanocorax*, con *Psilorhinus* y *Calocitta* como sinónimos.

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1. Introduction

Species in the genus *Cyanocorax* and allied genera *Psilorhinus* and *Calocitta*, constitute a morphologically, ecologically, and behaviorally diverse group of New World jays (NWJs), distributed from the extreme southern United States south to Argentina. Some species in the group have been studied extensively in terms of their social behavior (e.g., Crossin, 1967; Hardy, 1974; Raitt and Hardy, 1976, 1979; Langen, 1996; Williams and Hale, 2006), delayed soft-part color development (Hardy, 1973; Peterson, 1991), vocal repertoire (Hardy, 1961, 1979), and habitat preferences (e.g., Hardy, 1969; Raitt and Hardy, 1979; Amaral and Macedo, 2006). However, the lack of a robust hypothesis of relationships, as well as the paucity of detailed natural history studies for most South American species, has precluded rigorous interpretation of these characteristics in an evolutionary framework.

In addition to implications for understanding the evolution of the group, resolution of relationships among *Cyanocorax* species poses interesting systematic challenges. Owing to the diverse combinations of plumage coloration, size, and morphology found in *Cyanocorax* species, the genus has been described as "a pigeon-hole for a heterogeneous assemblage of jays" (Moore, 1935), apropos to the widely held perception that no characters support a coherent, natural group (e.g., Amadon, 1944; Goodwin, 1976). As a result, *Cyanocorax* has been subjected to several taxonomic overhauls involving large-scale splitting, lumping, and reallocation of taxa.

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1.1. Taxonomic History

Hellmayr (1934) presented the first comprehensive treatment of *Cyanocorax* and allied genera. He divided the current *Cyanocorax* into four genera: (1) *Xanthoura,* consisting solely of *X. yncas,* the only green-colored member of the group, distributed from southern Texas to northern Bolivia; (2) *Cissilopha,* consisting of four Mesoamerican jays—*C. beecheii, C. sanblasiana, C. yucatanica,* and *C. melanocyanea*—characterized by black heads and blue body plumage; (3) *Uroleuca,* represented by *U. cristatellus,* a distinctive species from central Brazil; and (4) *Cyanocorax,* containing one Mesoamerican–South American (*C. affinis*) and seven South American species (*C. caeruleus*, *C. violaceus*, *C. heilprini*, *C. cayanus*, *C. chrysops* [including *C. cyanopogon*], *C. cyanomelas,* and *C. mystacalis*). Also, he recognized two species in the (currently) monotypic genus *Psilorhinus* (*P. morio* and *P. mexicanus*), and one species of *Calocitta,* including the current *C. formosa* and *C. colliei*.

Based on subjective analysis of general morphology and plumage patterns, Amadon (1944) proposed a classification merging *Xanthoura* and *Uroleuca* into *Cyanocorax,* and placing *Cissilopha* into *Cyanocitta,* along with members of other NWJ genera (i.e., *Cyanocitta, Cyanolyca,* and *Aphelocoma*). He considered *Psilorhinus* and *Calocitta* as valid genera, and divided *Cyanocorax* into four "sections": (1) the "*Coronideus*" group, containing *C. caeruleus, C. cyanomelas,* and *C. violaceus*; (2) "*Uroleuca*"; (3) "*Xanthoura*"; and (4) a more restricted "*Cyanocorax*" including all other taxa, in addition to the newly described *C. dickeyi* (Moore, 1935).

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Blake and Vaurie (1962) divided the group in four genera: *Cissilopha* (sensu Helmayr, 1934), *Cyanocorax* (sensu Amadon, 1944), *Calocitta,* and *Psilorhinus.* Hardy (1969), in contrast, lumped all genera into a broad *Cyanocorax* with five subgenera: *Calocitta, Psilorhinus, Cissilopha, Uroleuca,* and *Cyanocorax* (including *Xanthoura*); also, he elevated *Cyanocorax cyanopogon* to a full species separate from *C. chrysops* (see also Meyer de Schauensee, 1966). Goodwin (1976) followed Blake and Vaurie (1962) in conferring generic status to *Cissilopha, Psilorhinus,* and *Calocitta.* Debates regarding the taxonomy of the group still continue. Whereas Monroe and Sibley (1993) and Madge and Burn (1994) recognized *Cyanocorax* (including *Xanthoura, Uroleuca,* and *Cissilopha*), *Calocitta,* and *Psilorhinus* (see also Bonaccorso and Peterson, 2007), others (AOU, 1983, 1998; Dickinson, 2003; Clements, 2007) recognized only *Cyanocorax* and *Calocitta*. In conclusion, the taxonomy of the group has been unstable, reflecting the lack of a robust hypothesis of relationships and insufficient morphological character variation on which to base a classification.

1.2. The origin of the Tufted Jay, Cyanocorax dickeyi

Within the *Cyanocorax* assemblage, morphologically similar species may have complex and discontinuous geographic distributions (Hardy, 1961; Goodwin, 1976). The most notorious example is the ~4000 km gap existing between two morphologically similar species, *C. dickeyi* and *C. mystacalis*. Whereas *C. dickeyi* inhabits a minute area in the Pacific slope of the Sierra Madre Occidental of Mexico (Moore, 1935; Crossin, 1967), *C. mystacalis* is endemic to southwestern Ecuador and northwestern Peru (Ridgely and Tudor, 1989). To explain this biogeographic pattern, Amadon (1944) hypothesized that *C. dickeyi* was a relict of a more widely distributed ancestor that was out-competed by other Mesoamerican jays (see also Moore, 1935).

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Others have proposed that a flock of *C. mystacalis* was storm-blown from the Pacific coast of South America (*in* Hardy, 1969), or that *C. dickeyi* was a descendent of *C. mystacalis* brought to Mexico by Native Americans (Haemig, 1979). Given the close morphological similarity between the two species (see Hope, 1989), molecular studies are necessary to uncover their phylogenetic affinities.

1.3. Phylogenetic Relationships

The first phylogenetic treatment of *Cyanocorax* jays (Hope, 1989) was based on discrete and meristic osteological characters of 12 of the 16 species in *Cyanocorax*, in addition to *Psilorhinus morio* and *Calocitta formosa*. Major patterns recovered included *"Cissilopha"* as monophyletic (in 2 out of 3 analyses) and *C. mystacalis* and *C. dickeyi* as sister species; *Psilorhinus* was placed as sister to *Calocitta* or closely related to *C. violaceus*, *C. caeruleus*, and *C. cyanomelas*. The only clade supported by a discrete, unreversed synapomorphy was *C. violaceus* + *C. caeruleus* + *C. cyanomelas* (i.e., Amadon's *"Coronideus"* group); relationships among other species were unstable and dependent on outgroup selection.

Molecular analyses of relationships among NWJs (Saunders and Edwards, 2000; Bonaccorso and Peterson, 2007) have supported the monophyly of *Cyanocorax* + *Psilorhinus* + *Calocitta*. However, because the most complete study considered only 6 of the 16 species of *Cyanocorax*, the question of the monophyly of the genus, as well as relationships among species, is unresolved. Herein, we expand the taxonomic sampling to include all species of *Cyanocorax*, in the context of other NWJ genera and a broad sampling of molecular markers, to provide a phylogenetic framework for understanding the evolution, systematics, and biogeography of the group.

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2. Methods

2.2. Taxon and Gene Sampling

We analyzed samples from 54 individuals, including all species in *Cyanocorax* and the allied genera *Psilorhinus* and *Calocitta*. For outgroup comparisons, we included representatives of all other NWJ genera (*Cyanocitta cristata*, *Aphelocoma coerulescens*, *Gymnorhinus cyanocephalus*, and *Cyanolyca viridicyanus*), which data were drawn from previous studies (Espinosa de los Monteros and Cracraft, 1997; Cicero and Johnson, 2001; Ericson et al., 2005; Bonaccorso and Peterson, 2007; Bonaccorso, 2009). Tissue samples for ingroup taxa were obtained through our sampling efforts (in Mexico, El Salvador, Guyana, Paraguay, and Argentina) via University of Kansas Natural History Museum general collecting expeditions, and from other museum collections in the U.S. (Table 1). Additionally, a subset of ingroup sequences was obtained from previous studies (Espinosa de los Monteros and Cracraft, 1997; Bonaccorso and Peterson, 2007; Bonaccorso, 2009).

The mitochondrial genes NADH dehydrogenase subunit 2 (ND2) and cytochrome *b* (cyt*b*) were analyzed as fast-evolving makers that could provide resolution at the tips of the tree. To obtain independent estimates of relationships, as well as information about deeper divergence, we sequenced three nuclear loci—Adenylate Kinase intron 5 (AK5), β-Fibrinogen intron 7 (βfb7), and TGFβ2.5—for representative individuals. Information on genes sequenced, GenBank accession numbers, catalog numbers, and associated locality data is summarized in Table 1.

List of tissue samples and GenBank accession numbers for sequences of species included in the present study. Acronyms: AMNH, American Museum of Natural History; ANSP, Academy of Natural Sciences; CUMZ, Cornell University Museum of Zoology; FMNH, Field Museum of Natural History; KUNHM, University of Kansas Natural History Museum; LSUMZ, Louisiana State University Museum of Zoology; MZFC, Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México; USMNH, National Museum of Natural History, Smithsonian Institution; YPM, Yale Peabody Museum. [a]Sequences from Esponosa de los Monteros and Cracraft (1997); [b]sequences from Bonaccorso and Peterson (2007);[c]sequences from Bonaccorso (2009).

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Species	#	Museum and tissue number	Locality Information	ND2	cyt*b*	AK5	βfib7	TGFβ2.5
Cyanocorax cayanus	1	KUNHM 5817	Guyana: E. Barima River	DQ912614[b]	DQ912599[b]	DQ912631[b]	DQ912650[b]	GU144911
	2	KUNHM 5819	Guyana: E. Barima River	GU144811	GU144854			
	3	AMNH ROP 252	Venezuela: Bolivar, 40 km E Tumeremo	GU144812	GU144855			
	4	LSUMZ 25552	Brazil: Amapa	GU144813	GU144856			
	5	FMNH 391652	Brazil: Amapa	GU144814	GU144857			
C. cyanomelas	6	KUNHM 134	Paraguay: Concepción, San Luís	GU144815	GU144858	GU144898	GU144904	GU144912
	7	KUNHM 3171	Paraguay: Alto Paraguay, Estancia Triunfo	GU144816	GU144859			
	8	FMNH 324102	Peru: Madre de Dios, Hacienda Amazonia	GU144817	GU144860			
	9	FMNH 334725	Bolivia: Santa Cruz, Chiquitos	GU144818	GU144861			
	10	AMNH 2279	Bolivia: Santa Cruz, Velazco, near El Tuna	GU144819	GU144862			
C. chrysops	13	KUNHM 171	Paraguay: Concepción, San Luís	DQ912609[b]	U77334[a]	DQ912626[b]	DQ912646[b]	FJ59830[b]
	14	KUNHM 3667	Paraguay: Itapua, San Rafael N.P., San Pedro Mi	GU144820	GU144863			
	15	AMNH 2249	Bolivia: Santa Cruz, Comunidad Karapari	GU144821	GU144864			
	16	CUMZ 52152	Argentina: Jujuy	GU144822	GU144865			
	17	LSUMZ 18785	Bolivia: Santa Cruz	GU144823	GU144866			
C. affinis	18	LSUMZ 28539	Panama: Colón	GU144824	GU144867			
	19	LSUMZ 28602	Panama: Colón	GU144825	GU144868			
	20	USMNH BO 1960	Panama: Bocas del Toro, 4 km W Chriquí Grande	GU144826	GU144869	GU144899	GU144905	GU144913
	21	USMNH BO 1980	Panama: Bocas del Toro, 4 km W Chriquí Grande	GU144827	GU144870			
C. mystacalis	22	ASNP 1813	Ecuador: Loja, SE Celica, along Río Catamayo	GU144828	GU144871			
	24	ASNP 4153	Ecuador: Loja, 4 km N of Zapotillo	GU144829	GU144872			
	25	ASNP 4596	Ecuador: Loja, 10 km E Mangahurcu	GU144830	GU144873			
	26	LSUMZ 5160	Peru: Lambayeque	GU144831	GU144874	GU1448900	GU144906	GU144914
C. heilprini		LSUMZ 48619	Brazil: Amazonas, Rio Uaupés, Jauareté	GU144832	GU144875			
C. cyanopogon	27	FMNH 392998	Brazil: Alagoas, Pirañas, Fazenda Mecejana	GU144833	GU144876			
	28	FMNH 392999	Brazil: Alagoas, Pirañas, Fazenda Mecejana	GU144834	GU144877	GU144901	GU144907	GU144915
C. caeruleus		YPM 80868	Brasil: Sao Paulo, Riberao Onca Parda	GU144835	GU144878			
C. violaceus	29	FMNH 324104	Peru: Madre de Dios, Hacienda Amazonia	GU144836	GU144879	GU144902	GU144908	GU144916
	30	FMNH 398598	Peru: Madre de Dios, 2.75 km E Shintuya	—	GU144880			
	31	ANSP 5697	Ecuador: Sucumbíos, Ca. 14 km N Tigre Playa	GU144837	GU144881			
	32	ANSP 5940	Ecuador: Sucumbíos, Ca. 20 km NE Lumbaqui	GU144838	GU144882			
C. cristatellus	33	LSUMZ 13888	Bolivia: Santa Cruz	GU144839	GU144883	GU144903	GU144909	GU144917
	33b	LSUMZ 13889	Bolivia: Santa Cruz	GU144840	GU144884			
	33c	LSUMZ 13915	Bolivia: Santa Cruz	GU144841	GU144885			
C. dickeyi	33s	MZFC 15315	Mexico: Sinaloa	DQ912611[b]	DQ912596[b]	DQ912628[b]	DQ912647[b]	GU144918
	34s	MZFC 15666	Mexico: Sinaloa	GU144842	GU144886			
	40s	MZFC 15316	Mexico: Sinaloa	GU144843	GU144887			
C. yncas	35	LSUMZ 30899	USA: Texas	GU144844	GU144888			
	36	LSUMZ 43650	Peru: San Martín	GU144845	GU144889			
	37	LSUMZ 6114	Ecuador: Morona-Santiago	GU144846	GU144890			
	39	MZFC 15927	Mexico: San Luís Potosí, San Nicolás de los Montes	GU144847	GU144891			
	40	MZFC 15722	Mexico: Querétaro, Laguna de la Cruz	DQ912610[b]	DQ912595[b]	DQ912627[b]	GU144910	GU144919
C. yucatanicus	41	MZFC 14340	Mexico: Campeche, 9 km W Tenabo	GU144848	GU144892			
	42	MZFC B1661	Mexico: Yucatán	DQ912613[b]	DQ912598[b]	DQ912630[b]	DQ912649[b]	GU144920
C. sanblasianus	1	KUNHM 106859	Mexico: Nayarit, 9 mi E Las Varas	GU144849	GU144893			
	2	KUNHM 106860	Mexico: Guerrero, El Arenal	GU144850	GU144894			
C. beecheii	1	KUNHM 101842	Mexico: Nayarit	GU144851	GU144895			
	2	MZFC 20513	Mexico: Sinaloa	GU144852	GU144896			
C. melanocyaneus	43	KUNHM 7657	El Salvador: San Vicente	DQ912612[b]	DQ912597[b]	DQ912629[b]	DQ912648[b]	GU144921
	44	KUNHM 4998	El Salvador: Morazán	GU144853	GU144897			
Psilorhinus morio	45	KUNHM B1896	Mexico: Campeche	DQ912607[b]	DQ912593[b]	DQ912624[b]	DQ912645[b]	FJ598304[c]
	46	KUNHM B2169	Mexico: Campeche	DQ912608[b]	DQ912594[b]			
Calocitta formosa	47	KUNHM 9352	El Salvador: Usulután	DQ912602[b]	U77336[a]	DQ912620[b]	DQ912639[b]	FJ598302[c]
C. colliei	48	FMNH 343602	Mexico: Sinaloa	DQ912603[b]	DQ912591[b]			

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2.2. DNA Amplification and Sequencing

Genomic DNA was extracted from frozen tissue using salt precipitation protocols (M. Fujita, unpubl.). Amplification was carried out using the following primers: L5143 or L5216, and H6313 (Sorenson et al., 1999) for ND2; L14990 (Kocher et al., 1989) and H16065 (Tim Birt, unpubl.) for cyt*b*; FIB-B17U and FIB-B17L (Prychitko and Moore, 1997) for βfb7; AK5b+ and AK6c- (Shapiro and Dumbacher, 2001) for AK5; and TGFb2.5F and TGFb2.6R for TGFb2.5 (Sorenson et al., 2004). DNA extracts from *Cyanocorax sanblasianus, C. beecheii* (sample 1, Table 1), *C. heilprini,* and *C. caeruleus* were obtained from museum skin samples in a dedicated ancient DNA laboratory (Fleischer et al., 2000, 2001); DNA extracts from an additional degraded tissue sample of *C. beecheii* (sample 2, Table 1) were obtained using the DNeasyTissue extraction kit (Qiagen Inc.). Amplification from skin-extracted DNA was carried out using several internal primers (Appendix I), which allowed amplifying and sequencing of short DNA fragments (150–350 bp). In all ancient DNA work, independent laboratory facilities were used to separate pre- and post-PCR manipulations, and special care (e.g., multiple controls, use of fresh lab supplies) was taken to reduce risk of contamination.

We used a standard PCR protocol (Bonaccorso and Peterson, 2007) for mitochondrial genes, and a touchdown protocol for nuclear genes (i.e., an initial denaturation of 94˚C/3min; 5 cycles of 94˚C/30 sec, 60˚C/30 sec, 72˚C/40 sec; 5 cycles of 94˚C/30 sec, 56˚C/30 sec, 72˚C/40; 35 cycles of 96˚C/30 sec, 52˚C/30 sec, 72˚C/40 sec; and a final extension of 72˚C/10 min; R. Moyle, pers. comm.). Amplification 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

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resolved in 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 Corp., 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.

2.3. Sequence Aligning and Phylogenetic Analyses

Best-fit models of molecular evolution were selected in MODELTEST v.3.7 (Posada and Crandall, 1998, 2001) under the Akaike Information Criterion (AIC) for each gene and codon position (i.e., ND2, cyt*b*), and for combined datasets (see below). Model parameters estimated from MODELTEST were used in subsequent model-based analyses.

Individual gene trees were estimated using maximum parsimony (MP), and maximum likelihood (ML) analyses. Tree topology and clade support were used as gross measures of congruence in phylogenetic signal (Bull et al., 1993; de Queiroz et al., 1995; Wiens, 1998); "strong" incongruence was identified by the presence of conflicting nodes showing $\geq$ 70% non-parametric bootstrap support. To explore potential sources of conflict among genes, sequence data were tested for stationarity in base frequencies (χ^2 test of homogeneity in PAUP* v.4.0b10 [Swofford, 2002]) and rate homogeneity among lineages, via a likelihood ratio test (Felsenstein, 1981), by comparing the likelihood scores of the ML trees with and without the molecular clock enforced. After preliminary examination of individual gene trees, we performed combined analyses to amplify potentially congruent signals, but questioned weakly supported nodes that reflected conflicting signals (Wiens, 1998). Combined mitochondrial and mitochondrial + nuclear trees were estimated, using MP, ML, and Bayesian analyses. Mitochondrial analyses

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were performed over all available samples; individual nuclear-gene and mitochondrial + nuclear analyses included one sample for each species, with exception of *Cyanocorax heilprini, C. sanblasianus, C. beecheii, C. caeruleus,* and *Calocitta colliei.*

Parsimony analyses were performed treating gaps as missing data, and heterozygous positions (in nuclear genes) as polymorphisms. Trees were obtained through heuristic searches in PAUP with 10,000 stepwise random additions (TBR branch-swapping). Clade support was estimated via heuristic searches with 1000 bootstrap pseudoreplicates (Felsenstein, 1985), each pseudoreplicate consisting of 100 stepwise random additions.

Maximum likelihood trees were estimated using GARLI (Genetic Algorithm for Rapid Likelihood Inference, ver. 0.951; Zwickl, 2006), which estimates tree topology, branch lengths, and model parameters that maximize the –ln likelihood, in a simultaneous approach. Analyses were conducted specifying the model "family" obtained by MODELTEST, but allowing the program to estimate parameter values from the data. For each dataset, ≥10 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; bootstrap searches were performed under the same settings used in tree search.

Bayesian analyses of the combined datasets were performed in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), implementing partitions by gene and by codon position (ND2 and cyt*b*), and assigning to each partition its best-fit model family of nucleotide substitution. All parameters were unlinked between partitions (except topology and branch lengths), and rate variation (prset ratepr = variable) was invoked. Analyses consisted of four independent runs of 5 $\times 10^6$ generations and 10 Markov chains (temperature = 0.20), with trees sampled every 1000 generations. Stationarity was assessed by plotting -lnL per generation in Tracer 1.3 (Rambaut

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and Drummond, 2004) and plotting posterior probabilities of clades as a function of number of generations in AWTY (Wilgenbusch et al., 2004). Comparison of performance of multiple runs allowed selection of those runs that converged to the highest likelihood values and reflected stability in the posterior probabilities of clades. All four runs fulfilled these conditions and reached stationarity after 500,000 generations. From the 5000 resulting trees per run, the first 1000 were discarded as "burn in." The remaining 16,000 trees were combined to calculate posterior probabilities in a 50% majority-rule consensus tree.

2.4. Hypothesis Testing

Statistical comparison between the ML tree and a ML tree resulting from enforcing the monophyly of the genus *Cyanocorax* and the monophyly of *C. mystacalis* + *C. dickeyi* was conducted via the likelihood-ratio test of monophyly (Huelsenbeck et al., 1996a). This test 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 it to a null distribution obtained via parametric bootstrapping (Effron, 1985; Felsenstein, 1988; Huelsenbeck et al., 1996b); in short, replicated datasets are created by simulation under the model and model parameter values estimated from the original data optimized over the null topology. Then, two different ML searches are conducted for each simulated dataset to estimate the likelihood of (1) the ML tree and (2) the ML tree with the null topology enforced. Calculation of likelihood difference between trees produced by (1) and (2) provides the null distribution for assessing whether δ deviates from random expectations. Given that two independent tests were performed (monophyly of *Cyanocorax* and monophyly of *C. mystacalis* +

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C. dickeyi), significance of δ-values was assessed after adjusting the *P* level using a Bonferroni correction (Sokal and Rohlf, 1995).

To avoid potential problems related to missing data (e.g., distortion of branch lengths), parametric bootstrapping was implemented over the mitochondrial + nuclear, pruned dataset. A total of 100 matrices was simulated for each test (i.e., monophyly of *Cyanocorax* and of *C. mystacalis* + *C. dickeyi*) with Batch Architect (Maddison and Maddison, 2004a) in Mesquite 1.05 (Maddison and Maddison, 2004b). We used GARLI for conducting all ML searches (i.e., ML trees and ML "constrained" trees, on real and simulated datasets).

3. Results

3.1. Sequence Attributes and Model Selection

For all well-preserved tissues (except *Cyanocorax violaceus* sample 30 and *C. cyanomelas* sample 10), we obtained 1002 bp of ND2 and 999 bp of cyt*b*. Use of internal primers allowed amplification and sequencing of samples for which preserved tissues were not available. Species and numbers of base-pairs sequenced from such samples are as follows: *C. heilprini* (1002 bp for ND2, 486 bp for cyt*b*), *C. beecheii* 1 (675 bp for ND2, 411 bp for cyt*b*), *C. beecheii* 2 (676 bp for ND2, 979 bp for cyt*b*) *C. caeruleus* (960 bp for ND2, 486 bp for cyt*b*), *C. sanblasianus* 1 (1002 bp for ND2, 411 bp for cyt*b*) and *C. sanblasianus* 2 (867 bp for ND2, 411 bp for cyt*b*). Maximum Likelihood-corrected pair-wise distances based on cyt*b* sequences are provided in Appendix II. Among nuclear introns, apart from specific length variation observed in outgroup sequences, indels were limited to 1–16 base pairs, allowing unambiguous alignment in all cases.

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Variable/parsimony-informative positions across genes were distributed as follows: 457/375 out of 1002 for ND2, 394/311 out of 999 for cyt*b*, 77/15 out of 518 for AK5, 99/33 out of 856 for βfb7, and 53/18 out of 564 for TGFβ2.5. According to the AIC, MODELTEST selected models nested within the GTR + I + Γ model "family" for all but one data partition (HKY + Γ was selected for cyt*b* second-codon positions), and for the individual mitochondrial genes and combined mitochondrial and mitochondrial + nuclear datasets. The best-fit model families for the nuclear genes were HKY + Γ for AK5, and GTR + Γ for βfb7 and TGFβ2.5. Model parameter values estimated via ML analysis of individual genes, are summarized in Table 2. Nucleotide composition bias across lineages, considering codon positions (mitochondrial genes) and complete gene fragments, was non-significant in all cases ($P > 0.05$), and the assumption of a molecular-clock mode of evolution was not rejected for any loci ($P > 0.05$).

Table 2. Summary of nucleotide substitution models, model parameters, and tree scores estimated from maximum likelihood trees.

	Base frequencies				Rate matrix							
Gene	A	C	G	T	AC	AG	AT	CG	CT	α	Pinv	-ln l
ND2	0.330	0.348	0.087	0.236	0.880	23.122	0.669	0.657	11.500	1.146	0.466	-8645.9573
cyt*b*	0.309	0.368	0.120	0.204	0.953	10.132	1.082	0.257	15.627	1.482	0.553	-7973.7999
Mitochondrial	0.321	0.346	0.102	0.231	0.900	17.619	0.547	0.372	12.417	0.208	0.011	-13124.2970
AK5	0.217	0.303	0.306	0.175	Transition/transversion ratio = 3.460					0.426	N/A	-1170.3705
βfb7	0.322	0.176	0.186	0.316	1.567	0.785	2.982	7.160	0.654	0.475	N/A	-1886.9103
TGFβ2.5	0.243	0.244	0.211	0.302	0.779	7.706	0.633	1.142	4.440	0.375	N/A	-1176.6071
Mitochondrial + nuclear (total evidence)	0.299	0.291	0.164	0.247	1.387	12.679	0.732	0.429	15.877	0.331	0.490	-16981.317
Mitochondrial + nuclear (pruned)	0.296	0.286	0.166	0.251	1.711	12.358	0.919	0.592	18.617	0.311	0.481	-14682.317

3.2. Phylogenetic Analyses

Individual MP and ML analyses of ND2 and cyt*b* revealed general congruence in phylogenetic signal (not shown). Although specific details of relationships differ among genes and among analyses, no strong conflicts in topology were encountered; thus, we combined both genes in a single mitochondrial dataset. Figure 1 shows the Bayesian tree of the mitochondrial dataset, including Bayesian posterior probabilities and ML bootstrap support. The topology of

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the MP 50% majority rule consensus tree based on the mitochondrial dataset (24 equally parsimonious trees, 2306 steps; consistency index = 0.439, rescaled consistency index = 0.350; not shown) was in general agreement with those of ML and BA, unless otherwise specified.

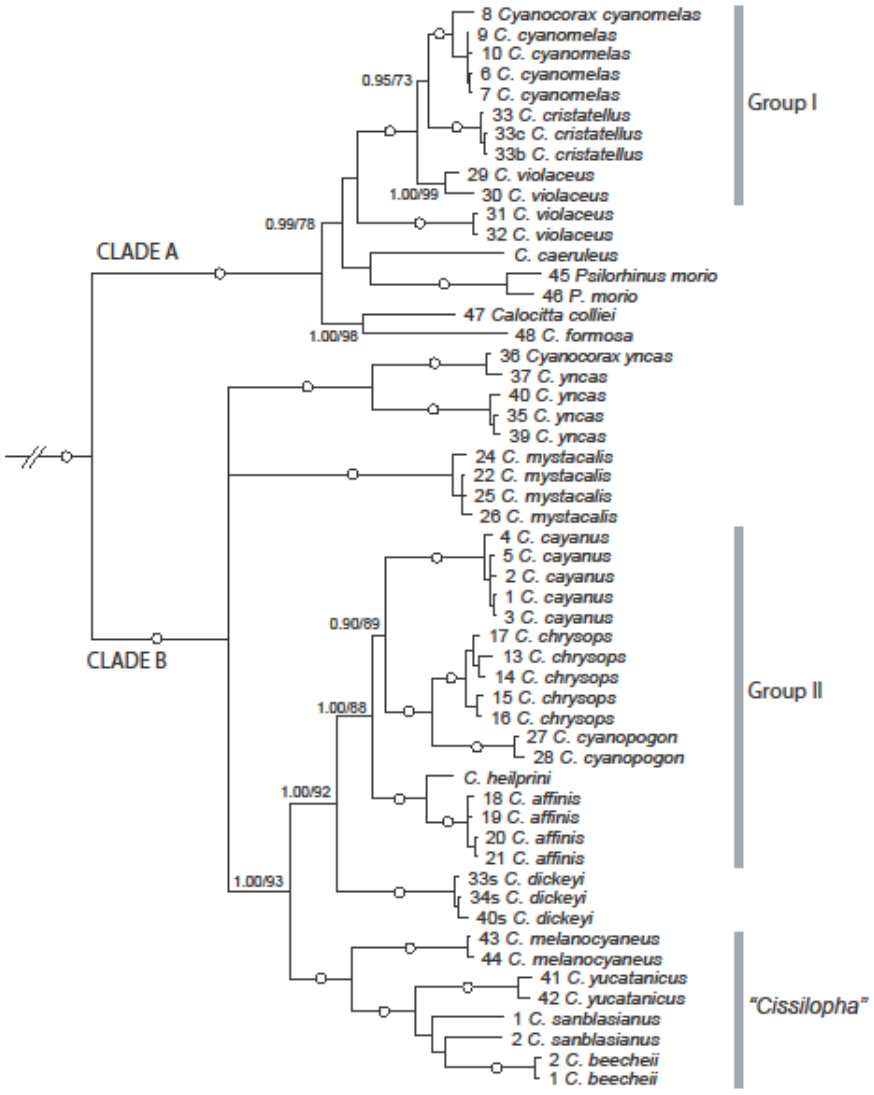

FIG. 1

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Fig. 1. Bayesian 50% majority rule consensus tree estimated from the mitochondrial dataset (ND2 and cyt*b*; 2001 bp). Bayesian posterior probabilities/maximum likelihood bootstrap values (below branches) are indicated whenever nodes were recovered with less than 1.00 posterior probability or 100% bootstrap support.

Analyses of the mitochondrial dataset (Fig. 1) recovered *Cyanocorax* + *Psilorhinus* + *Calocitta* as monophyletic, and show a further division into two main clades: Clade A, containing *Calocitta, Psilorhinus, Cyanocorax violaceus, C. caeruleus, C. cristatellus,* and *C. cyanomelas*; and Clade B, including the remaining species of *Cyanocorax*. Within Clade A, *Calocitta* was sister to a group containing all other species (0.99 Bayesian posterior probabilities, 78% ML bootstrap support, Fig. 1), but this relationship was not recovered by the MP 50% majority rule consensus tree (not shown). All other lineages in Clade A are represented by a virtual polytomy among *Psilorhinus morio*, *C. caeruleus*, *C. violaceus* from Ecuador, and a strongly supported clade ("Group I") formed by *C. violaceus* from Peru + (*C. cristatellus* + *C. cyanomelas*), which renders *C. violaceus* paraphyletic. Nonetheless, different, weakly supported topologies were obtained from individual-gene (ND2 and cyt*b*) trees, with the positions of *Psilorhinus, Cyanocorax caeruleus,* and *C. violaceus* from Ecuador being unstable across analyses. These discrepancies reflected in low support for nodes within Clade A, except for that uniting Group I (1.00 Bayesian posterior probabilities, 100% ML and MP bootstrap support). Relationships within Clade B are represented by another polytomy among *C. yncas, C. mystacalis,* and a well-supported clade containing "*Cissilopha*" jays + all other *Cyanocorax* species. Within "*Cissilopha*," *C. sanblasianus* was recovered as paraphyletic with respect to *C. beecheii*, although the node uniting both species showed low support. The sister clade of "*Cissilopha"* is *C. dickeyi* + "Group II", whereas in "Group II", *C. affinis* + *C. heilprini* is sister to *C. cayanus* (*C. chrysops* + *C. cyanopogon*). Relationships within Clade B were consistent among MP, ML, and Bayesian analyses of the mitochondrial dataset, with two exceptions. Maximum Likelihood and MP analyses showed *C. mystacalis* and *C. yncas* as monophyletic and

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sister to all other species (56% ML and 60% MP bootstrap support), and MP did not recover the node of *C. sanblasianus* + *C. beecheii.*

In general, individual analyses of the three nuclear introns were less informative regarding relationships within *Cyanocorax* (Fig. 2). AK5 recovered Clade A with high bootstrap support. βfb7 supported the monophyly of the *Cyanocorax*, *Calocitta*, *Psilorhinus* assemblage, the monophyly of species in Clade A, and a sister relationship between *Psilorhinus* and *Calocitta*; other relationships disagree with those resulting from the mitochondrial analyses, but showed low bootstrap support. TGFβ2.5 provided the most information, supporting the monophyly of the whole assemblage, Clades A and B, and *Calocitta* + *Psilorhinus*.

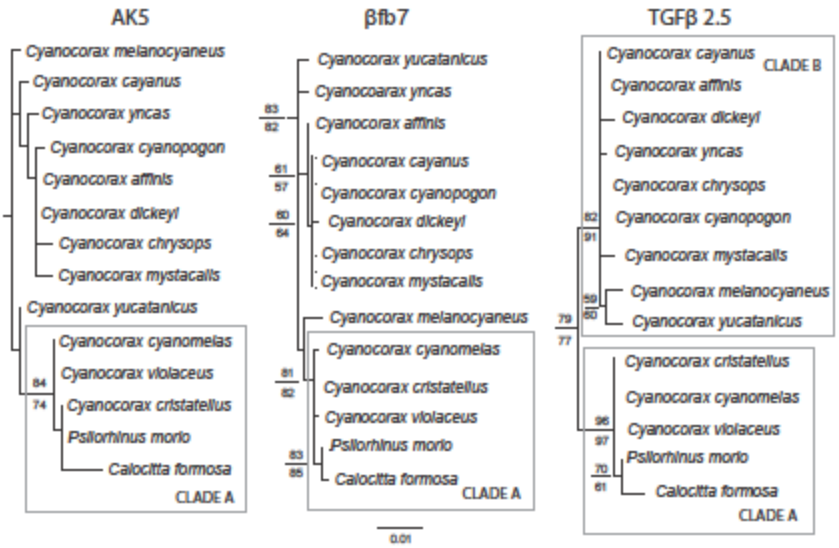

FIG. 2

Fig 2. Maximum likelihood (ML) trees estimated for the individual nuclear genes (AK5, βfib7, and TGFβ2.5). Values on nodes indicate maximum likelihood (above) and maximum parsimony (below) bootstrap support.

Analyses of the combined mitochondrial + nuclear dataset produced similar topologies to those obtained from the mitochondrial dataset. However, the position of *C. mystacalis*, *C. yncas*,

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Psilorhinus, and *Calocitta* remained unstable or conflicting between ML and Bayesian analyses (Fig. 3). The MP analysis of the mitochondrial + nuclear dataset produced one most parsimonious tree (1974 steps, CI = 0.5329, RC = 0.2879; not shown), which resulted in the same general topology of the Bayesian and ML trees, with two exceptions: it reconstructed the same relationships as the Bayesian tree with respect to the position of *C. yncas* and *C. mystacalis*, and the same relationships as the ML tree with respect to *Psilorhinus* and *Calocitta* (MP bootstrap supports < 50% in both cases).

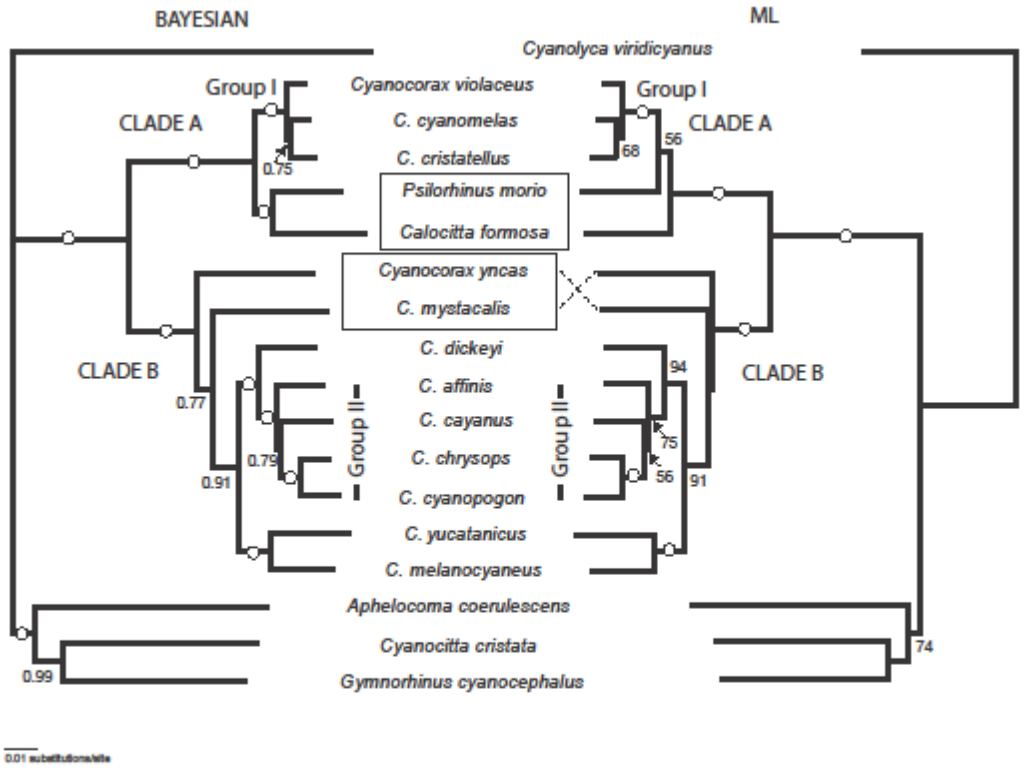

FIG. 3

Fig. 3. Bayesian 50% majority rule consensus tree (left) and maximum likelihood tree estimated from the total evidence dataset (ND2, cyt*b*, AK5, βfb7, and TGFβ2.5; 4077 bp). Bayesian posterior probabilities and maximum likelihood bootstrap values are indicated in each case.

3.3. Hypothesis Testing

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According to the parametric bootstrapping tests, the null hypotheses of monophyly of *Cyanocorax* and of that of a sister relationship between *C. mystacalis* and *C. dickeyi* were rejected with high significance. In testing the monophyly of *Cyanocorax*, the difference between the ML tree and the constraint ML tree drawn from the real dataset (–ln likelihood = 15669.408 vs. 15729.410; $\delta = 60.002$ ln units) was significantly different from δ values obtained by simulation ($P < 0.05$). Therefore, *Calocitta* and *Psilorhinus* must be considered integral components of the *Cyanocorax* radiation. Also, the difference between the ML tree and the constraint ML tree enforcing the monophyly of *C. mystacalis* + *C. dickeyi*, was significant (–ln likelihood = 15669.408 vs. 15688.984; $\delta = 19.576$ ln units; $P < 0.05$); then, according to this analysis, they should not be considered as sister species.

4. Discussion

4.1. Phylogeny of Cyanocorax

Phylogenetic trees based on mitochondrial and nuclear genes, as well as on different analyses, produced a basic structure for species-level relationships within *Cyanocorax* and allied genera (Figs. 1–3). The most important result is the paraphyly of *Cyanocorax* with respect to *Psilorhinus* and *Calocitta*; this hypothesis is supported statistically by the parametric bootstrapping test, rejecting the monophyly of the current concept of *Cyanocorax*. A second major result is the division of all species into two well-supported groups, Clades A and B: Clade A containing *Psilorhinus*, *Calocitta*, *Cyanocorax caeruleus*, *C. violaceus*, *C. cyanomelas*, *C. cristatellus*, and *C. violaceus*; and Clade B containing the "*Cissilopha*" jays and the remaining species of *Cyanocorax*.

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Phylogenetic Relationships within Clade A.—Relationships within this clade were particularly unstable with respect to the position of *Psilorhinus morio*. This species was placed as sister to *C. caeruleus* based on combined mitochondrial analyses, but as sister to *Calocitta* in analyses of independent nuclear loci (βfb7 and TGFβ2.5, Fig. 2) and the Bayesian analysis of the combined mitochondrial + nuclear dataset (Fig. 3). Similar difficulties were encountered by Hope (1989), whose analyses showed *Psilorhinus* as sister to *Calocitta*, or to *C. violaceus* + *C. caeruleus* + *C. cyanomelas*, depending on outgroup selection.

Ambiguity and uncertainty on the positions of *Calocitta, Psilorhinus, Cyanocorax caeruleus*, and *C. violaceus* from Ecuador contrast with the high support obtained for Group I (*C. cyanomelas, C. cristatellus*, and *C. violaceus* from Peru) in all analyses. Common ancestry of *C. cyanomelas, C. violaceus*, and *C. caeruleus* was expected based on plumage similarity (i.e., the "*Coronideus*"group; Amadon, 1944), as well as two osteological synapomorphies (one unreversed, another present also independently in *Cyanolyca*; Hope, 1989). However, in our phylogeny, *C. cristatellus* (not *C. caeruleus*) is closely related to *C. cyanomelas* and *C. violaceus* from Peru. Because *C. cristatellus* was not analyzed by Hope (1989), it is not possible to assess whether its molecular affinities with *C. violaceus* and *C. cyanomelas* coincide with osteological synapomorphies. In any case, the phylogenetic positions of both *C. caeruleus* and *C. violaceus* from Ecuador are in conflict with previous assessments of relationships based on morphology.

With regard to the paraphyly of *C. violaceus*, examination of study skins from Ecuador did not reveal marked differences from those from Peru, other than slight color variation (N. Rice, pers. comm.). Given the relatively long and well-supported branch leading to Group I (*C. violaceus* from Peru + *C. cyanomelas* + *C. cristatellus*), it is improbable that this structure is caused by stochastic phylogenetic error (Funk and Omland, 2003). Moreover, it does not seem

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plausible that this structure is the result of amplification of nuclear pseudogenes, because our independently obtained sequences of ND2 and cyt*b* provide the same signal, and it is unlikely that sequences obtained in both cases come from nuclear pseudogenes. Alternate explanations include that samples from Ecuador (1) represent a cryptic species north of the Amazon River or (2) carry ancestrally polymorphic mitochondrial haplotypes. It seems clear at this point that answering either question may require extensive sampling of populations of *C. violaceus* and closely related species across their ranges, as well as incorporation of faster-evolving nuclear markers.

Phylogenetic Relationships within Clade B.—In general, phylogenetic structure within Clade B was stable across analyses, although the positions of *Cyanocorax mystacalis* and *C. yncas* were ambiguous. Regardless of its position in the tree, *C. yncas* sequences separated into distinct groups corresponding the disjunct North and South American portions of the range of the species (Fig. 4). Differences in plumage, habitat preferences (e.g., Meyer de Shauensee, 1966; Goodwin, 1976), social behavior (Alvarez, 1975; Gayou, 1986), and vocalizations suggest that these populations might represent distinct species (Ridgely and Greenfield, 2001; Hilty, 2003): *C. yncas* (from northern Colombia and Venezuela to northern Bolivia) and *C. luxuosus* (from Texas to northern Honduras). Further analysis of populations from across the range of the species, particularly in Central America and northern South America, will be crucial in assessing their validity as independent evolutionary lineages (Wiley, 1978).

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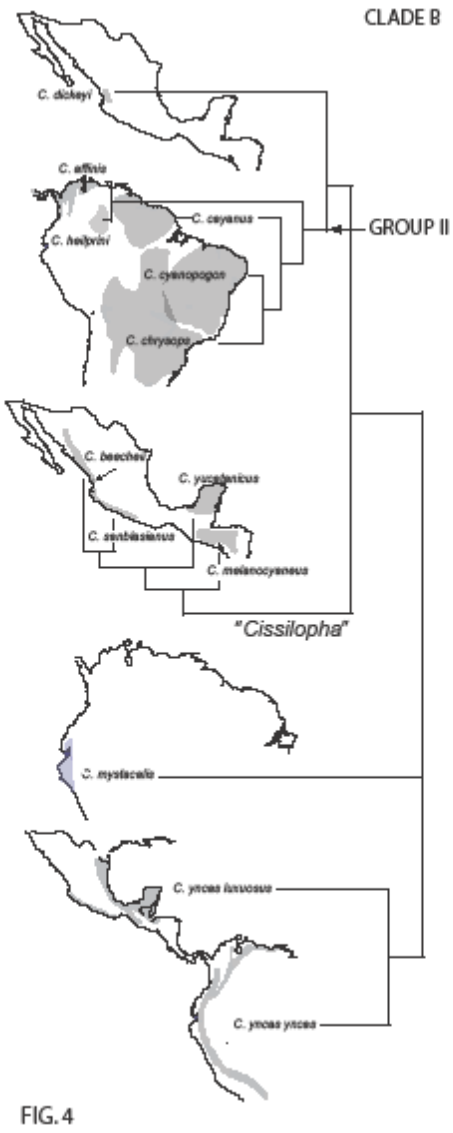

Fig. 4. Geographic distribution and phylogenetic relationships among species in Clade B (tree topology from Fig. 1). Darker shades (and arrow in the case of *Cyanocorax beecheii* and *C. sanblasianus*) indicate areas of range overlap.

Among other results, the monophyly of the *"Cissilopha"* jays is consistent with their shared plumage characters, as well as their geographic distribution in Mexico and Central America, fitting previous hypothesis of relationships (e.g., Amadon, 1944; Goodwin, 1976; Hardy, 1969; Hope, 1989). Although paraphyly of *C. sanblasianus* with respect to *C. beecheii* is

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unexpected given their differences in plumage and size, the short internodal branches conducting to *C. sanblasianus* 1 + (*C. sanblasianus* 2 + *C. beecheii*) and *C. sanblasianus* 2 + *C. beecheii*, might indicate that speciation in this group occurred in a relatively short period of time, and not enough synapomorphies accumulated in the mitochondrial genes analyzed herein. This same scenario might also explain the low support for the *C. sanblasianus* + *C. beecheii* clade. Although cases of species paraphyly were once seen as exceptions rather than rules, in recent years the number of studies showing this phenomenon has been directly related to deep scrutiny of sister-species relationships (Johnson and Cicero, 2004), and to analyses including multiple samples per species (Funk and Omland, 2003; for several examples among Neotropical birds see Pérez-Emán, 2005; Weir et al., 2008; DaCosta and Klicka, 2008).

An alternate explanation would be introgression in the area of overlap between *Cyanocorax beecheii* and *C. sanblasianus* in western Nayarit (Fig. 4). Although examination of the specimens involved did not reveal any deviations from their expected morphology, hybridization and introgression could have occurred in the history of these species. Occasional reports of hybrids between NWJ species (e.g., *Psilorhinus* × *Calocitta* from western Chiapas, Mexico; Pitelka et al., 1956) suggest that hybridization occurs in the wild. Given that only mitochondrial sequences were available for these taxa, discarding hybridization/introgresion is not possible. Additionally, judging by the relatively low the degree of differentiation observed among our nuclear introns, adding sequences from these loci may prove non-informative.

Most relationships among the South American jays in Group II resulted as expected. The sister relationship of *Cyanocorax chrysops* and *C. cyanopogon* is consequent with their early placement in a single species (Hellmayr, 1934). Also, a close relationship between *C. chrysops* + *C. cyanopogon* with *C. cayanus* is consistent with their similar morphology and geographic

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distribution north (*C. cayanus*) and south (*C. chrysops* + *C. cyanopogon*) of the Amazon River (Fig. 4). More interesting from the morphological standpoint is the sister relationship between *C. heilprini* and *C. affinis*, given that *Cyanocorax heilprini* differs from all other species in having a dark venter. Finally, the position of *C. dickeyi* as sister to Group II and the statistical significance of the parametric bootstrapping test, contradict previous hypothesis regarding its close relationship with *C. mystacalis* (Haemig, 1979; Hope, 1989). This result supports the hypothesis of retention of plesiomorphic plumage characters in *C. mystacalis* or homoplasy.

4.2. Biogeography

The phylogenetic relationships reconstructed herein illustrate why past attempts to understand the biogeography of *Cyanocorax* and allied genera have been unfruitful and puzzling (e.g., Hardy, 1961; Hardy, 1969; Goodwin, 1976). The general pattern that emerges is one in which relatively recent radiations (e.g., Group II and the "*Cissilopha*" jays) fit general expectations based on biogeography. However, farther back in evolutionary time, traces of ancestral biogeographic history seem to be lost from the phylogenetic record. Two clear cases of discontinuous geographic distributions are recovered by the phylogeny: (1) *Calocitta* and *Psilorhinus*, two species from Mesoamerica, being most closely related to species in the Amazon Basin and central South America (Fig. 5); and (2) *C. dickeyi* from a minute area of northwestern Mexico, as sister to a clade of mostly South American jays (Fig. 4).

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Fig. 5. Geographic distribution of species in Clade A. Darker shades indicated areas of range overlap.

Repeated instances of discontinuous distributions across the phylogeny could be explained by two different processes—i.e., long-distance dispersal or local extinction of widely distributed ancestors. Curiously, some species in this group are known to stray from their customary distributional areas (e.g., *C. yncas* [Hilty, 2003]), occasionally by hundreds of kilometers (*C. sanblasianus* in Arizona [Phillips, 1950]; "*Cissilopha*" sp. in Texas, [J. Eitniear, pers. comm.]). Also among NWJs, the Blue Jay, *Cyanocitta cristata*, is at least partially migratory in the northern portion of its range (Pitelka, 1946), a pattern repeated in several species of *Corvus* (Madge and Burn, 1994). As demonstrated for orioles (Kondo and Omland, 2007), New World thrushes (Outlaw et al., 2003), and Old World warblers (Helbig, 2003), migratory behavior may evolve independently across the phylogeny of a group. Nonetheless, the partial or relatively short-distance nature of migrations among corvids makes the possibility of long-distance migration less plausible than in other lineages.

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On the other hand, local extinction of a broadly distributed ancestor has been invoked to explain the peculiar distribution of *Cyanocorax dickeyi* (Amadon, 1944). In fact, extinction seems to be the most plausible explanation for broad distributional gaps observed in non-migratory avian taxa, for which reasonable geographic sampling has been accomplished: e.g., the azure-winged magpie (*Cyanopica cyanus*) disjunct distribution in the Iberian peninsula and Asia, (Fok et al., 2002); wrentits, *Chamaea fasciata*, from western North America, most closely related to the Old World genus *Sylvia* (Barhoum and Burns, 2002); and New World and Asian piculets, *Picumnus*, most closely related to each other (Benz et al., 2006). Among *Cyanocorax* species, the geographic gap between Mesoamerican and South American populations of *C. yncas* (Fig. 4), could represent the early stage of a geographic gap produced by local extinction. Given a high potential for extinction, the exercise of reconstructing ancestral areas would be misleading in this case, and could produce simply erroneous results.

4.3. Taxonomic Implications

Phylogenetic analysis of species in *Cyanocorax* and allied genera revealed that *Cyanocorax* is paraphyletic. This result is well supported by individual-gene and combined analyses, and by the statistical rejection of the null hypothesis of monophyly of the genus. Thus, if phylogenetic relationships are to be reflected in systematic classification, and if taxa are to be natural groups, the genus *Cyanocorax* must be redefined. From the relationships recovered for the overall assemblage, two different approaches could be taken.

The first option involves maintaining the genus *Cyanocorax* specifically for species in Clade B—*C. chrysops* Boie 1826 is the type species (Hellmayr, 1934)—and naming species in Clade A with the next available name, which would be *Psilorhinus* Ruppell 1838 (Hellmayr,

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1934); this alternative involves changing the genus of six species (*Cyanocorax cyanomelas*, *C. cristatellus*, *C. violaceus*, *C. caeruleus*, *Calocitta formosa*, *C. colliei*). The second approach is lumping all taxa into a broader concept of *Cyanocorax*, given its taxonomic priority; in this case, only three species would change genus (*Calocitta formosa*, *C. colliei*, and *Psilorhinus morio* [according to some classifications]). Because most recent classifications (AOU, 1983, 1998; Dickinson, 2003; Clements, 2007) already submerge *Psilorhinus morio* into *Cyanocorax*, placing species of *Calocitta* within *Cyanocorax* would be a minimal change in taxonomy.

Based on the phylogenetic results, the monophyletic group including *Cyanocorax*, *Calocitta*, and *Psilorhinus*, is highly diverse from morphological (e.g., Moore, 1935; Amadon, 1944; Hardy, 1969; Hope, 1989), behavioral (Alvarez, 1975; Gayou, 1986; Peterson, 1991), and ecological (e.g., *C. heilprini*) points of view. Although a previous study (Bonaccorso and Peterson, 2007) discussed the uniqueness of *Psilorhinus morio* in terms of its possession of a "furcular pouch" (Sutton and Gilberg, 1942), reflecting the actual morphological variation within the overall assemblage would require recognizing a multiplicity of genera: *Cyanocorax* (Clade B), *Calocitta* and *Psilorhinus* (*if* reciprocally monophyletic and sister to all other species in Clade A), and *Uroleuca* Bonaparte 1859 (including *Cyanocorax cristatellus*, *C. violaceus*, *C. cyanomelas*, *C. caeruleus*), *if* all other species in Clade A formed a monophyletic group.

On the contrary, the merging *Psilorhinus* and *Calocitta* into *Cyanocorax* meets most of the criteria recently proposed for naming monophyletic taxa (Guayasamin et al., 2009), these being: 1) significant statistical support and congruence among phylogenetic estimation methods; 2) congruence (or no conflict) among genetic markers; and 3) traditional use of names, minimizing the number of taxonomic changes (also in ICZN, 1999). A fourth and final criterion, based on morphological and/or behavioral distinctiveness, cannot be applied since, to our

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knowledge, no unreversed morphological/behavioral synapomorphies unite the group (other than the overly general "large, crowlike body form," which is unique among NWJs).

5. Conclusions

Analyses of phylogenetic relationships among *Cyanocorax, Psilorhinus,* and *Calocitta,* revealed consistent patterns indicating overall monophyly of the group, but paraphyly of the current *Cyanocorax*. Also, division of ingroup taxa into two reciprocally monophyletic groups, Clades A and B, is highly supported. Within Clade A, a robust node indicates the monophyly of *C. violaceus* from Peru + *C. cristatellus* + *C. cyanomelas* (Group I). Based on previous morphological studies (Hope, 1989), it is possible that this clade includes also *C. caeruleus;* however, the ambiguous phylogenetic position of *C. caeruleus* and *Psilorhinus morio,* and the paraphyly of *C. violaceus* prevent solid conclusions on relationships within this clade. Within Clade B, ambiguous relationships were limited to resolving the positions of *C. yncas* and *C. mystacalis.*

Further resolution of problematic, weakly supported relationships associated with inconsistencies between mitochondrial and nuclear genes (e.g., *Psilorhinus* and *Calocitta*), as well as those involving relatively short internodal branches (e.g., the positions of *C. mystacalis* and *C. yncas*, and *C. sanblasianus* and *C. beecheii*), seems difficult at this point. In the case of relationships involving short branches, theoretical models indicate that when the time of shared ancestry is short compared to that of independent ancestry (e.g., short internodal branches leading to long terminal branches), the number of informative characters will be so small that the probabilities of recovering a bifurcating pattern is low (Lanyon, 1988). Moreover, signals of common ancestry (i.e., synapomorphies) are most likely to be overwritten by homoplasy

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(McCracken and Sorenson, 2005). Thus, resolving these relationships might require considerably greater numbers of mitochondrial and fast-evolving nuclear characters.

The phylogenetic complexity of lineages in the current *Cyanocorax* suggests several complications for the taxonomy of the group. Although multiple taxonomic arrangements are possible, our recommendations are for recognizing a single inclusive genus, *Cyanocorax*, including species currently assigned to *Psilorhinus* and *Calocitta*. This proposition maximizes stability of a systematic classification consistent with phylogeny.

Genealogical patterns recovered in this study highlight the importance of analyzing multiple samples per taxa, particularly as concerns detection of species paraphyly, cryptic species, and ancestral polymorphisms. Still, extensive population-level, multi-locus analyses will be necessary to understand the origin of these patterns. Finally, these results emphasize the importance of collecting and preserving proper voucher specimens when trying to detect coupled genetic and morphological change, as well as traces of hybridization.

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Appendix I. Internal primers used to amplify sequences from museum study skin samples.

Gene	Primers
ND2	**L155** CAT CGA AGC AGC YAC TAA RTA YTT CC
	L350 AGG TCA TGC AAG GCT CAT CTC TCA
	L449 TGA ATA GGA CTA AAY CAA ACA C
	L493 GGA GGA TGA ATA GGA CTA AAC
	L664 ATG AAA GTY CTA AAA CTA TCA ACR C
	L835 GCA ATA ATC ATY TCA CTT CTG TC
	H200 GAG GCA GCT TGT ACY ARG
	H416 ATT GGT GGG AAT TTY ATY ACY GTG G
	H574 TAG CTA TTC AGC CCA GGT GAG CAA
	H727 TTG TGC TAA GTG AAG GTG
	H903 TGT TGC RCA GTA TGC TAG GCG AAG
cyt*b*	**L691** CTA GGA TTY GCA CTA ATR CTA RTC CTA C
	L939 ACG CTC AAT AAC CTT CCG TC
	H998 AGG TCT GCA ACT AGK GTT CAR AAT AG
	cyt*b*2.RC and **cyt*b*2.wow** (Dumbacher et al. 2003)

Appendix II. Mitochondrial (cyt*b*) maximum likelihood-corrected pair-wise distances (substitutions per site) among *Cyanocorax* species. Distances for species for which DNA was extracted from skins or degraded samples (*C. heilprini*, *C. caeruleus*, *C. beecheii*, *C. sanblasianus*) are not included, given considerable amounts of missing data.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Cyanocorax cayanus* 1																
2	*C. cyanomelas* 6	0.20															
3	*C. chrysops* 13	0.08	0.19														
4	*C. affinis* 20	0.07	0.19	0.08													
5	*C. mystacalis* 26	0.12	0.21	0.14	0.13												
6	*C. cyanopogon* 28	0.07	0.20	0.06	0.08	0.13											
7	*C. violaceus* 29	0.19	0.03	0.20	0.20	0.20	0.20										
8	*C. violaceus* 31	0.19	0.07	0.21	0.20	0.20	0.21	0.07									
9	*C. cristatellus* 33	0.20	0.03	0.22	0.21	0.20	0.22	0.03	0.07								
10	*C. dickeyi* 33s	0.08	0.19	0.09	0.07	0.13	0.09	0.20	0.21	0.21							
11	*C. yncas* 36	0.17	0.25	0.19	0.15	0.17	0.19	0.24	0.24	0.25	0.16						
12	*C. yncas* 40	0.19	0.23	0.19	0.17	0.18	0.19	0.23	0.24	0.25	0.16	0.08					
13	*C. yucatanicus* 42	0.11	0.23	0.12	0.12	0.15	0.13	0.23	0.22	0.23	0.11	0.17	0.18				
14	*C. melanocyaneus* 43	0.11	0.23	0.11	0.12	0.14	0.12	0.23	0.24	0.24	0.11	0.17	0.15	0.09			
15	*P. silorhinus* 45	0.23	0.10	0.24	0.21	0.23	0.25	0.09	0.12	0.10	0.20	0.28	0.30	0.23	0.26		
16	*C. formosa*	0.25	0.10	0.27	0.26	0.26	0.25	0.10	0.11	0.10	0.27	0.31	0.31	0.30	0.28	0.14	
17	*C. colliei*	0.20	0.08	0.22	0.22	0.22	0.23	0.07	0.09	0.07	0.23	0.28	0.27	0.25	0.24	0.11	0.09

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