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A molecular phylogeny of the cotingas (Aves: Cotingidae)

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Abstract

The phylogenetic relationships of members of Cotingidae were investigated using >2100 bp of sequence data from two nuclear introns (myoglobin intron 2 and G3PDH intron 11) and one protein-coding mitochondrial gene (cytochrome *b*). Strong support was found for a monophyletic clade including 23 traditional cotingid genera, corresponding to the Cotingidae *sensu* [Remsen, J.V. Jr., Jaramillo, A., Nores, M., Pacheco, J.F., Robbins, M.B., Schulenberg, T.S., Stiles, F.G., da Silva, J.M.C., Stotz, D.F., Zimmer, K.J., 2005. Version 2005-11-15. A classification of the bird species of South America. American Ornithologists' Union. <http://www.museum.lsu.edu/~Remsen/SACCBaseline.html>]. Neither *Oxyruncus* nor any of the genera in Tityrinae *sensu* [Prum, R.O, Lanyon, W.E., 1989. Monophyly and phylogeny of the Schiffornis group (Tyrannoidea). Condor 91, 444–461.] are members of Cotingidae. Within Cotingidae a polytomy of four well-supported clades was recovered: (1) the fruiteaters *Pipreola* and *Ampelioides*; (2) the *Ampelion* group, including *Phytotoma*; (3) *Rupicola* and *Phoenicircus*; and (4) the 'core cotingas' consisting of the remainder of the Cotingas (e.g. fruitcrows, *Cotinga*, *Procnias*, *Lipaugus*, and *Carpodectes*), with *Snowornis* in a basal position. The separation of *Snowornis* from *Lipaugus* [Prum, R.O, Lanyon, W.E., 1989. Monophyly and phylogeny of the Schiffornis group (Tyrannoidea). Condor 91, 444–461.] was strongly supported, as were the close relationships between *Gymnoderus* and *Conioptilon*, and between *Tijuca* and *Lipaugus*. However, basal relationships among 'core cotinga' clades were not resolved.

Keywords: Aves; Tyrannida; Cotingidae; Phylogeny; Systematics; Bayesian inference; Nuclear introns; Cytochrome *b*; Polygyny; Sexual dimorphism

1. Introduction

The cotingas (Cotingidae) are suboscine passerines distributed in lowland and montane humid forest of tropical Central and South America, with a few species in semi-arid and tree-line habitats. Most species are predominantly or exclusively frugivorous, and the family exhibits a wide range of variation in size, external morphology, and breeding ecology (see e.g. Snow, 1982, 2004). The majority of cotingas exhibit very pronounced sexual dimorphism that has evolved together with polygynous mating systems, in some cases with lekking behaviour. Many of the genera (e.g. *Cephalopterus*, *Perissocephalus*, *Pyroderus*, *Procnias*, *Phoenicircus*, and *Rupicola*) exhibit more or less extreme modifications in external morphology and vocalization. Some genera are composed of monogamous species, which are sexually monomorphic or nearly so (e.g. *Pipreola* and *Ampelion*). Most interestingly, there are a few genera of polygynous species that are sexually monomorphic and advertise using largely acoustic displays (e.g. *Lipaugus* and *Perissocephalus*). The sexually dimorphic, polygynous state has been hypothesized to be derived from the sexually monomorphic, monogamous conditions, (Snow, 1973), but there is no well resolved phylogenetic hypothesis to test various hypotheses about the evolution of sexual dimorphism and breeding system.

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The cotingas are members of the Tyrannida clade *sensu* Ericson et al. (2003) (=Tyrannoidea of Sibley and Ahlquist, 1985; Prum, 1990) of the New World suboscines. The compositional limits of the three traditional families (Tyrannidae, Cotingidae, and Pipridae) of the Tyrannida have been difficult to resolve (Prum, 1990). Several recent studies have supported the monophyly of a restricted cotingid clade, diagnosed by the possession of the derived insertion of the syringeal muscle musculus tracheolateralis on the lateral A1/B1 membrane, a piprid clade, and a tyrannid clade (Prum et al., 2000; Johansson et al., 2002; Chesser, 2004; Ericson et al., in press). Further, a number of studies have now supported the monophyly of an eclectic clade composed of the *Schiffornis* group of Prum and Lanyon (1989) plus *Tityra* (Sibley and Ahlquist, 1990; Prum et al., 2000; Johansson et al., 2002; Chesser, 2004; Ericson et al., in press). Prum et al. (2000) recommended that this clade, including former genera of cotingids, piprids, and tyrannids, be recognized as the Tityrinae, a subfamily of Cotingidae. Most recently, however, Ericson et al. (in press) strongly supported the placement of this clade as sister to the monophyletic tyrannids (plus *Piprites*), and recommended that it be recognized as Tityridae and that treatment is followed herein. Their data suggest the inclusion of *Oxyruncus* in this family, which is further supported by both syringeal synapomorphies (Prum and Lanyon, 1989) and allozyme distances (Lanyon, 1985).

The relationships within the Cotingidae are also poorly understood. Most cotingid genera are very distinctive in external appearance, to a degree that has made it difficult to draw any conclusions on their interrelationships and the several 'natural' groups that have been proposed in traditional classifications (e.g. Snow, 1973) are mostly based on rather scanty morphological and behavioural evidence that need to be evaluated phylogenetically with other types of data. A large proportion of the genera are monotypic (10 out of 24 in the classification of Prum and Lanyon (1989)) and most of the polytypic genera consist of a small number of taxa. Prum (1990) conducted a cladistic analysis of 12 morphological characters (e.g. internal syringeal cartilages, musculus obliquus ventralis, enlargement of the femoral artery and insertion of musculus tracheolateralis on the A1/B1 membrane of the syrinx) that supported a monophyletic Cotingidae, but did not resolve relationships among genera.

The only attempt to address the phylogeny within the cotingas with molecular sequence data is the preliminary study by Prum et al. (2000). This study included a short fragment of cytochrome *b* (ca. 350 bp) for 32 species in 26 genera, and parsimony bootstrap support was generally low. Only a few cotingas have been included in broader studies of passerine phylogeny (e.g. Johansson et al., 2002; Barker et al., 2004; Chesser, 2004; Ericson et al., in press).

The molecular study presented here includes representatives of the majority of cotingid genera (*sensu* Remsen et al., 2005), six tityrid genera (*sensu* Prum et al., 2000), and a sample of manakins, tyrannids, and tracheophone suboscines. We analyse sequence data from two nuclear introns (myoglobin intron-2 and G3PDH intron-11), and one mitochondrial protein-coding gene (cytochrome *b*) to investigate the relationships within the Cotingidae. The data address both the delimitation of a monophyletic Cotingidae and the phylogenetic relationships within the family. Our resulting phylogeny can provide a framework for investigating the evolution of morphology, breeding ecology, mating systems (e.g. lekking behaviour), sexual dimorphism, and other aspects of the biology of the Cotingidae.

2. Material and methods

2.1. Taxon sampling

Blood or tissue samples were obtained from representatives of 23 of the 24 genera of Cotingidae *sensu* Remsen et al. (2005) and 12 representatives from the other main lineages in Tyrannida (Johansson et al., 2002; Chesser, 2004). The only cotingid genus lacking was *Carpornis*, for which no tissue samples were available to us. *Phibalura*, which is usually included in Cotingidae (but not so in Remsen et al. (2005)) was unfortunately also unavailable to us. To evaluate the separation of *Snowornis* from *Lipaugus* (Prum et al., 2000; Prum, 2001), both members of *Snowornis* and two members of *Lipaugus sensu stricto* were included. The diverse genera *Cotinga* and *Pipreola* were represented by two species each. To evaluate the monophyly of Tityridae and their position in relation to Cotingidae, representatives of six of the seven proposed genera of Tityridae were included in the analysis. Two taxa from Furnariida, the sister group of Tyrannida (Ericson et al., 2003; Irestedt et al., 2002), were chosen as outgroup taxa. All the taxa, with sample identification and GenBank Accession Nos. are listed in Table 1.

2.2. Extraction, amplification, and sequencing

Genomic DNA was extracted from tissue or blood with the QIAamp® Mini Kit (QIAGEN®), following the instructions from the manufacturer. The entire myoglobin intron-2 (with 13 bp and 10 bp of the flanking exons 2 and 3, respectively), the entire G3PDH intron-11 and 999 bp of cytochrome *b* were amplified and sequenced using the primers listed in Table 2.

The thermocycling for myoglobin intron-2 and G3PDH intron-11 included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 40 s, 57–59 °C for 40 s and 72 °C for 1 min and a final extension at 72 °C for 8 min. Cytochrome *b* was amplified with a step down program with an initial denaturation at 95 °C for 5 min, followed by 4 cycles of 95 °C for 40 s, 55–52 °C for 1 min and 72 °C for 1 min; 4 cycles of 95 °C for 40 s, 52–50 °C for 1 min and 72 °C for 1 min and then 32 cycles of 95 °C for 40 s, 49–48 °C for 1 min and 72 °C for 1 min, and completed by a final extension of 72 °C for 8 min. The cytochrome *b* sequences were amplified as one fragment to reduce the risk of obtaining nuclear copies.

Table 1
Samples used in this study

Species	Origin	Sample Id.	Cytochrome *b*	Myoglobin	G3PDH
Cotingidae					
Ampelioides tschudii	Ecuador	ZMUC 127031	DQ470491	DQ470543	DQ470516
Ampelion rubrocristata	Peru	LSU B-7664	DQ470492	DQ470544	DQ470517
Carpodectes hopkei	Ecuador	ANSP 2381	DQ470493	DQ470545	DQ470518
Cephalopterus ornatus	Bolivia	LSU B-12300	DQ470494	DQ470547	DQ470519
Conioptilon mcilhennyi	Peru	KU B-1416	DQ470495	DQ470546	DQ470520
Cotinga cayana	Peru	LSU B-2653	DQ470496	DQ470548	DQ470521
Cotinga maynana	Peru	LSU B-4977	DQ470497	DQ470549	DQ470522
Doliornis sclateri	Peru	LSU B-3562	DQ470498	DQ470550	DQ470523
Gymnoderus foetidus	Bolivia	LSU B-9586	DQ470499	DQ470551	DQ470524
Haematoderus militaris	Guyana	KU B-1348	DQ470500	DQ470552	DQ470525
Lipaugus fuscocinereus	Ecuador	ANSP 5039	DQ470503	DQ470555	DQ470528
Lipaugus unirufus	Ecuador	ANSP 2399	DQ470505	DQ470556	DQ470529
Perissocephalus tricolor	Venezuela	AMNH uncat.	DQ470506	DQ470557	DQ470531
Phoenicircus nigricollis	Peru	LSU B-2898	DQ470507	DQ470558	DQ470530
Phytotoma rutila	Bolivia	ZMUC S466	AF453822[3]	AY338743[4]	AY336581[4]
Pipreola arcuata	Peru	LSU B-7654	DQ470508	DQ470559	DQ470532
Pipreola chlorolepidota	Peru	LSU B-5435	DQ470509	DQ470560	DQ470533
Porphyrolaema porphyrolaema	Peru	ANSP 3226	DQ470510	DQ470561	DQ470534
Procnias alba	Guyana	KU B-1244	DQ470511	DQ470562	DQ470535
Pyroderus scutatus	Paraguay	NRM 967030	AF453820[3]	AY065786[2]	AY336582[4]
Querula purpurata	Peru	LSU B-2785	DQ470512	DQ470563	DQ470536
Rupicola rupicola	Venezuela	LSU B-7575	DQ470513	DQ470564	DQ470537
Snowornis cryptolophus	Ecuador	ANSP 4445	DQ470502	DQ470565	DQ470538
Snowornis subalaris	Ecuador	ANSP 4884	DQ470504	DQ470566	DQ470539
Tijuca atra	Brazil	ZMUC 128821	DQ470514	DQ470567	DQ470540
Xipholena punicea	Captive	LSU B-20833	N/A	DQ470568	DQ470541
Zaratornis stresemanni	Peru	ZMUC 125021	DQ470515	DQ470569	DQ470542
Pipridae					
Chiroxiphia caudata	Paraguay	NRM 956620	AF453819[3]	DQ435516[6]	DQ435462[6]
Pipra fasciicauda	Paraguay	NRM 947271	AF453817[3]	AY065787[2]	AY336583[4]
Tityridae					
Iodopleura isabellae	Ecuador	ZMUC 125762	DQ435455[6]	DQ435519[6]	DQ435467[6]
Laniisoma elegans	Ecuador	ANSP 1558	DQ470501	DQ470553	DQ470526
Laniocera hypopyrra	Brazil	ZMUC 125879	N/A	DQ470554	DQ470527
Pachyramphus polychopterus	Paraguay	NRM 967032	AF453815[3]	AY338747[4]	AY336573[4]
Schiffornis virescens	Paraguay	NRM 937315	AF453816[3]	AY338741[4]	AY336574[4]
Tityra cayana	Paraguay	NRM 956584	AF453814[3]	AY338742[4]	AY336580[4]
Oxyruncus cristatus	Paraguay	NRM 967078	AF453821[3]	AY338745[4]	AY336572[4]
Tyrannidae					
Elaenia flavogaster	Paraguay	NRM 966970	AF453807[3]	AY064254[1]	DQ435464[6]
Fluvicola albiventer	Paraguay	NRM 956714	AF453810[3]	DQ435517[6]	DQ435465[6]
Leptopogon amaurocephalus	Paraguay	NRM 937317	AF453808[3]	DQ435520[6]	DQ435468[6]
Furnariidae					
Lepidocolaptes angustirostris	Paraguay	NRM 937184	AY078175[3]	AY065767[2]	AY336576[4]
Myrmoborus myotherinus	Brazil	ZMUC 125854	AY676961[5]	AY677008[5]	AY677058[5]

Classification follows Remsen et al. (2005), except in the treatment of Tityridae, which follows Ericson et al. (in press). Acronyms: AMNH, American Museum of Natural History, New York; ANSP, Academy of Natural Sciences, Philadelphia; KU, Natural History Museum, University of Kansas, Lawrence; LSU, Museum of Natural Science, Louisiana State University, Baton Rouge; NRM, Swedish Museum of Natural History, Stockholm; ZMUC, Zoological Museum of the University of Copenhagen. References: 1, Ericson et al. (2002); 2, Irestedt et al. (2002); 3, Johansson et al. (2002); 4, Fjeldså et al. (2003); 5, Irestedt et al. (2004); and 6, Ericson et al. (in press). N/A, not available.

2.3. Sequence assembly and alignment

Multiple sequence fragments obtained with the different sequencing primers for each gene and taxon were assembled to complete sequences with SeqMan II™ (DNASTAR). Apparent heterozygosities and other ambiguous positions were coded with the appropriate IUPAC codes. The sequences were aligned in MegAlign™ (DNASTAR), using the Clustal method and subsequently corrected by eye around gap regions. A low number of indels in the nuclear intron sequences made this procedure straightforward. All gaps inferred in the alignments were treated as missing data in the phylogenetic analyses. The alignments of the nuclear data sets have been deposited in EMBL under the Accession Nos. ALIGN_001006 (myoglobin, intron 2) and ALIGN_001007 (G3PDH, intron 11).

Table 2
Sequences and references for primers used in this study

Primer name	Used for	Primer sequence (5′–3′)		Reference
Glyceraldehyde-3-phospho-dehydrogenase (G3PDH) intron 11				
G3P-13b	Amp	TCC ACC TTT GAT GCG GGT GCT GGC AT	Forward	Fjeldså et al. (2003)
G3P-14b	Amp, Seq	AAG TCC ACA ACA CGG TTG CTG TA	Reverse	Fjeldså et al. (2003)
G3PintL1	Amp, Seq	GAA CGA CCA TTT TGT CAA GCT GGT T	Forward	Fjeldså et al. (2003)
G3PintH1	Seq	AAG CTG TAT TCA TTC CAG GTA AG	Reverse	This study
G3PintH2	Seq	TCC ATA CTC GTT ATC ATA CCT G	Reverse	This study
Myoglobin intron 2				
Myo2	Amp, Seq	GCC ACC AAG CAC AAG ATC CC	Forward	Slade et al. (1993)
Myo3	Amp	CGG AAG AGC TCC AGG GCC TT	Reverse	Slade et al. (1993)
Myo3F	Amp, Seq	TTC AGC AAG GAC CTT GAT AAT GAC TT	Reverse	Heslewood et al. (1998)
MyoIntC	Seq	AGC CCT GGA GGA TCC ATT GG	Forward	Heslewood et al. (1998)
MyoIntL1	Seq	CTA TAT TAC ATA AGA CCT GTC A	Forward	Irestedt et al. (2002)
MyoIntNC	Seq	CCA ATG GAT CCT CCA GGG CT	Reverse	Heslewood et al. (1998)
MyoIntH1	Seq	TGA CAG GTC TTA TGT AAT ATA G	Reverse	Irestedt et al. (2002)
MyoIntH2	Seq	TCT AAA CTT GGA TAT TCA CAT	Reverse	Irestedt et al. (2002)
Cytochrome *b*				
L14841	Amp, Seq	AAC TGC AGT CAT CTC CGG TTT ACA AGA C	Forward	Kocher et al. (1989)
H15915	Amp, Seq	CCA TCC AAC ATC TCA GCA TGA TGA AA	Reverse	Edwards and Wilson (1990)
OscH1	Amp, Seq	AAT GGG TGT TCT ACT GGT TGG CT	Reverse	This study
Thr1	Amp, Seq	TCT TTG GCT TAC AAG ACC AA	Reverse	Johansson et al. (2002)
L401	Seq	CCA TGA GGC CAA ATA TCA TTC TGA GG	Forward	This study
L413	Seq	AAT ATC CTT CTG AGG AGC TAC AGT CAT	Forward	Ericson et al. (2005)
P5L	Seq	CCT TCC TCC ACG AAA CAG GCT CAA ACA ACC C	Forward	Irestedt et al. (2002)
P6L	Seq	CCA GAA AAC TTC ACA CCC GCC AAC CC	Forward	This study
H598	Seq	GTT GTT TGA GCC TGT TTC GTG TAG GAA	Reverse	Ericson et al. (2005)
H658	Seq	TCT TTG ATG GAG TAG TAG GGG TGG AAT GG	Reverse	Irestedt et al. (2002)
H859	Seq	GGG CTA GGA CTC CTC CTA GTT TGT T	Reverse	This study
OscH2	Seq	ATA GGA CTA GGA TGA TTG TGA AGT A	Reverse	This study
P10H	Seq	GGC CAA TGT ATG GGA TTG CTG AG	Reverse	This study

Amp, amplification; Seq, sequencing.

2.4. Controlling for quality of cytochrome b sequences

When amplifying mitochondrial genes there is a considerable risk of obtaining nuclear copies (Quinn, 1997; Sorenson and Quinn, 1998). As cytochrome *b* is a protein-coding gene, the occurrence of unexpected codon substitutions in conserved regions or stop codons anywhere in the sequence are strong indications that a nuclear copy has been amplified. Likewise, insertions or deletions anywhere in the sequence could also indicate this. Therefore we checked a conservative region corresponding to positions 15661–15777 (codon 209–247, positions 627–741 in our alignment) in the *Gallus* sequence of all our sequences for any anomalies against a broad range of vertebrate cytochrome *b* sequences, following the method described in Johansson et al. (2002). We also checked the entire cytochrome *b* alignment for stop codons, insertions, and deletions.

2.5. Phylogenetic analyses

Assessment of the homogeneity of the phylogenetic signal between the three genes was done with the ILD test (Farris et al., 1995,a) by running 1000 replicates of the partition homogeneity test in PAUP* 4.0b.10 (Swofford, 2002). Phylogenetic trees were estimated using Bayesian Inference as implemented in the program MrBayes 3.0b4 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) using the Markov chain Monte Carlo and Metropolis coupling. Prior to the analyses, nucleotide substitution models were selected for each gene separately, using the program MrModeltest (Posada and Crandall, 1998; Nylander, 2002) in conjunction with PAUP* 4.0b10 (Swofford, 2002). These models (Table 3) were used both in the analyses of the individual genes and in the combined analyses. For each data set three independent runs with four incrementally heated chains were run for $4^{\times}10^{6}$ generations and sampled every 100th generation. These were initiated from different randomly chosen starting trees, to decrease the risk of only reaching local optima in the tree space (Huelsenbeck et al., 2002). The 'prset unlink' option was implemented to allow parameters to vary independently between the three data partitions in the combined analyses. The burn-in phase was determined by plotting likelihood scores against generation number and trees saved during that phase were discarded. The 50% majority rule consensus trees from the four runs were compared to ensure that the individual runs had converged on the same target distribution (Huelsenbeck et al., 2002). The saved trees from each run were pooled and the posterior probabilities for each analysis were based on 108,000 saved trees.

A parsimony analysis on the combined dataset was performed in PAUP* 4.0b.10 (Swofford, 2002). The analysis was performed under the heuristic search option (with TBR-branch swapping), with all characters coded as

Table 3
Data characteristics and estimated substitution parameters for the three studied genetic markers and the combined data set

	G3PDH	Myoglobin	Cytochrome *b*	Combined
Number of sites in alignment	373	746	999	2118
Selected substitution model	GTR + G	GTR + G	GTR + G + I	N/A
Number of trees	108,000	108,000	108,000	108,000
Base frequencies (%)				
A	21.0935	29.4739	33.1082	
C	18.4727	20.6022	39.1256	
G	30.8091	21.5746	6.9793	
T	29.6247	28.3493	20.7869	
Substitution rates				
A–C	1.4025	1.0478	0.9485	
A–G	5.6013	4.3331	21.1270	
A–T	0.8615	0.3343	3.6716	
C–G	2.0525	1.3059	0.9898	
C–T	6.4977	4.6562	40.0736	
G–T	1	1	1	
Proportion of invariable sites (I)	N/A	N/A	0.4804	
Gamma distribution shape parameter (α)	1.4022	1.1944	0.7291	
−lnL	2827.06	3800.64	13807.11	20898.98

N/A, not applicable.

unordered, and with 10 random additions of taxa to reduce the risk of finding local optima only. Nodal supports were estimated by bootstrapping the data (200 replicates with 10 random additions of taxa).

Comparison of alternative topologies was done with the non-parametric SH test (Shimodaira and Hasagawa, 1999) as implemented in PAUP*4.0b.10 (Swofford, 2002). ML searches were performed to find the best tree under a specific constraint. The difference in likelihood value between the best tree from the constrained search and the best tree from an unconstrained search were compared using the RELL option with 1000 bootstrap replicates.

2.6. Evolution of polygyny

Data on breeding systems were taken from standard sources (e.g. Snow, 1982, 2004). These were analysed using parsimony algorithm in MacClade 4.0 (Maddison and Maddison, 2000) (Fig. 3). The characters were optimised in the tree under the assumption that reversals are more common than convergences in this data set. The biological implications of the observed distributions are commented on below.

3. Results

3.1. Sequence variation and characteristics

The ILD tests did not recover any significant incongruence between gene partitions ($p = 0.99$), thus allowing them to be analysed as a combined data set. Nevertheless, we also analysed the individual genes to investigate topological differences between them.

Concatenation of the data sets for all three gene regions yielded a final alignment of 2118 bp. The alignment length, base frequencies, and substitution rates for each gene are given in Table 3. Full sequences for all genes were obtained for all 41 taxa with the exception of *Xipholena punicea* and *Laniocera hypopyrrha*, for which no cytochrome *b* sequences could be obtained. These two taxa are thus only included in the analyses of the nuclear introns. For myoglobin, the fragment length ranged between 679 bp (*Lepidocolaptes angustirostris*) and 727 bp (*Rupicola rupicola* and *Phoenicircus nigricollis*). The uncorrected pairwise distances ranged between 0.3% (between *Pyroderus scutatus* and *Cephalopterus ornatus*) and 5.7% (between *Ampelioides tschudii* and *Cephalopterus ornatus*) within Cotingidae, up to 7.2% (between *Ampelioides tschudii* and *Fluvicola albiventer*) within Tyrannida and up to 10.4% (between *Conioptilon mcilhennyi* and *Lepidocolaptes angustirostris*) when the outgroup taxa were included. For G3PDH the fragment length ranged between 326 bp (*Tijuca atra*) and 347 bp (*Phoenicircus nigricollis*). The uncorrected pairwise distances ranged between 0.9% (between *Xipholena punicea* and *Carpodectes hopkei*) and 8.6% (between *Porphyrolaema porphyrolaema* and *Pipreola chlorolepidota*) within the Cotingidae, up to 13.8% (between *Zaratornis stresemanni* and *Laniocera hypopyrrha*) within Tyrannida and up to 19.2% (between *Laniocera hypopyrrha* and *Lepidocolaptes angustirostris*) when the outgroup taxa were included. We obtained 999 bp of the cytochrome *b* gene corresponding to positions 15037–16035 in the chicken mitochondrial genome sequence (Desjardins and Morais, 1990). The uncorrected pairwise distances ranged between 4.7% (between *Perissocephalus tricolor* and *Cephalopterus ornatus*) and 20.2% (between *Snowornis subalaris* and *Zaratornis stresemanni* and *S. subalaris* and *Pipreola chlorolepidota*) within Cotingidae, up to 21.0% (between *Porphyrolaema porphyrolaema* and *Oxyruncus cristatus*, and between *Snowornis subalaris* and *Schiffornis virescens*) within

Tyrannida and up to 21.1% (between *S. subalaris* and *Myrmoborus myotherinus*) when the outgroup taxa were included.

No stop or unexpected codons were found in any of the cytochrome *b* sequences. However, a one-codon deletion was found in the sequence of *Phoenicircus nigricollis*, at position 562–564 (codon 188, corresponding to positions 15598–15600 in the chicken mitochondrial genome sequence (Desjardins and Morais, 1990). However, the sequence did not exhibit any other anomalies that might suggest a nuclear origin and the systematic position of *Phoenicircus* in the cytochrome *b* analyses was identical to those in the analyses of the nuclear introns. Codon deletion in the avian mtDNA has only been reported once: *Melospiza georgiana* and *M. lincolnii* lacking one codon in the Atp8 gene compared to their sister species *M. melodia* (Carson and Spicer, 2003).

3.2. Insertions and deletions

Several indel events were observed in the two nuclear data sets. Most of these were autapomorphic, but 10 were phylogenetically informative (Fig. 2). Two indel events are synapomorphic for the entire Cotingidae clade: one deletion of 4 bp in the myoglobin sequence (at position 61–64 in the alignment) and one insertion in the G3PDH sequence of 2 bp at position 344–345 in the alignment (TG in all taxa except *Porphyrolaema porphyrolaema* which has CR). Three indel events in the myoglobin and five in the G3PDH are synapomorphic for smaller clades. In the myoglobin alignment, a deletion of 6 bp at position 515–520 is shared by *Pachyramphus polychopterus*, *Tityra cayana* and *Iodopleura isabellae*; an insertion of 2 bp (GT) at position 696–697 is shared by *Phytotoma rutila*, *Ampelion rubrocristata* and *Doliornis sclateri* and an 11-bp insertion (CGAATGGCATG) at the position 200–210 is shared by *Phoenicircus nigricollis* and *Rupicola rupicola*. In the G3PDH alignment the two *Cotinga* species share a 2-bp deletion at position 23–24, a 4-bp insertion at position 125–128 and a 3-bp deletion at position 131–133; the fruiteaters (*Pipreola* and *Ampelioides*) share a 2-bp deletion at position 138–139 and *Pachyramphus polychopterus* and *Tityra cayana* share a 4-bp deletion at position 207–210.

With only a single exception, all indels support clades that are supported by the Bayesian analysis of the combined data with 1.00 posterior probability (Fig. 2). The *Phytoma–Ampelion–Doliornis* clade, which is diagnosed by a 2-bp insertion, is supported as monophyletic in the Bayesian analysis of the combined data with a posterior probability of only 0.78 (Fig. 2).

3.3. Phylogenetic results

In the Bayesian analyses, similar, well-resolved topologies were found in the separate cytochrome *b* (Fig. 1A) and myoglobin (Fig. 1B) analyses, whereas the G3PDH analysis (Fig. 1C) yielded a rather poorly resolved tree. The 50% consensus tree from the Bayesian analysis of the concatenated data set showed generally good resolution and high posterior probabilities (Fig. 2). However, the basal branching order in Tyrannida was not resolved, consisting of a polytomy of tyrannids, cotingids, piprids, tityrids ,and *Oxyruncus*. Each clade received 1.00 posterior probability. There was a strong support for a monophyletic Cotingidae

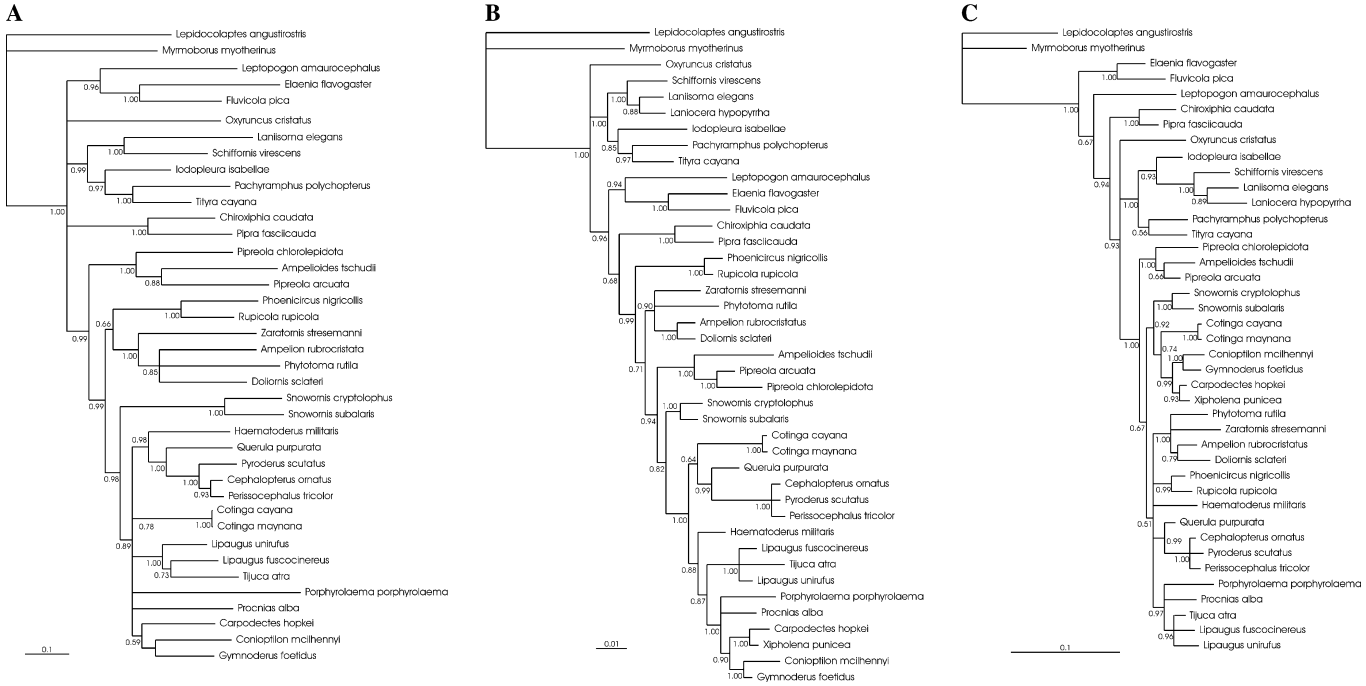

Fig. 1. 50% Majority rule consensus trees obtained from the Bayesian analysis of the individual genes: (A) cytochrome *b*, (B) myoglobin intron-2, and (C) G3PDH intron-11. Numbers indicate posterior probability for each node.

clade *sensu* Remsen et al. (2005), i.e. including *Phytotoma*, *Rupicola*, *Phoenicircus*, *Ampelioides*, *Pipreola*, *Lipaugus*, and *Snowornis* but excluding all the tityrid genera and *Oxyruncus*. *Oxyruncus* associated with the tityrids, but with low posterior probability (66%).

The basal branching order in the cotingid clade was not resolved, but formed a polytomy of four well-supported clades: (1) the fruiteaters *Pipreola* and *Ampelioides*; (2) the *Ampelion* group; (3) *Rupicola* and *Phoenicircus*; and (4) a large clade including the remainder of the Cotingidae *sensu* Remsen et al. (2005), hereafter referred to as the 'core cotingas' (see Fig. 2). All four clades received 1.00 posterior probability values. The fruiteater clade was the sister group of the other cotingids in the combined analysis, but with only 0.81 posterior probability. This topology was found in both the cytochrome-*b* and G3PDH trees, although it received strong support only in the cytochrome-*b* tree (Fig. 1A). In the myoglobin tree, the fruiteaters were placed together with the 'core cotingas' (Fig. 1B), with near-significant support (0.94).

In the fruiteater clade, the *Ampelioides tschudii* is sister to a monophyletic *Pipreola*, represented by *chlorolepidota* and *arcuata*, two highly differentiated species within the genus (Fig. 2). In the *Ampelion* clade, there is an indication that *Zaratornis stresemanni* is basal to *Phytotoma rutila*, *Doliornis sclateri*, and *Ampelion rubrocristata* (Fig. 2). The posterior probability for this topology is too low to be statistically significant (0.78), but a shared inser-

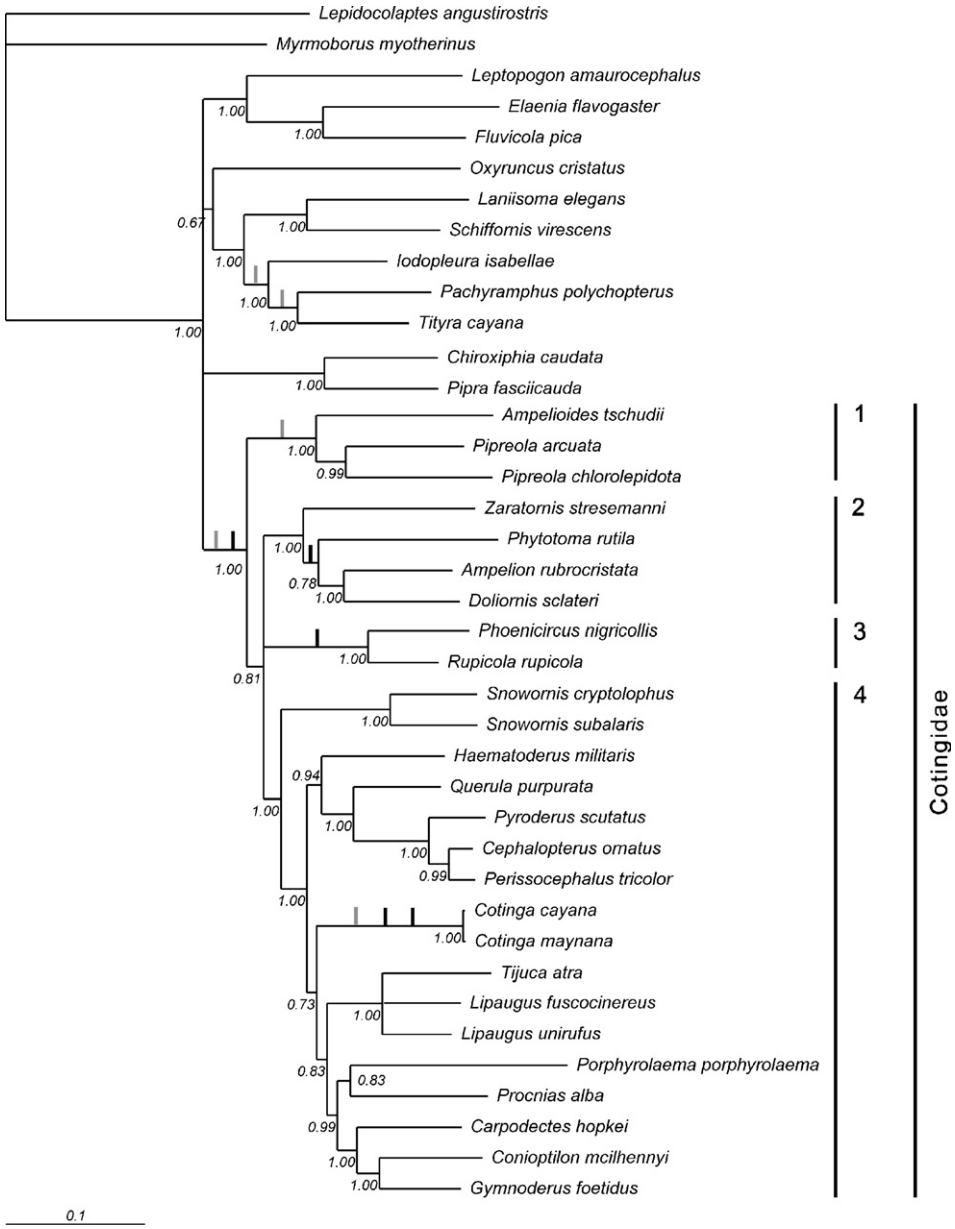

Fig. 2. 50% Majority rule consensus tree obtained from the Bayesian analysis of the combined data set. Numbers indicate posterior probability for each node. Numbered clades: (1) fruiteaters, (2) the *Ampelion* clade, (3) the *Rupicola* clade, and (4) the 'core cotingas'. Synapomorphic insertions and deletions are marked with black and grey bars, respectively.

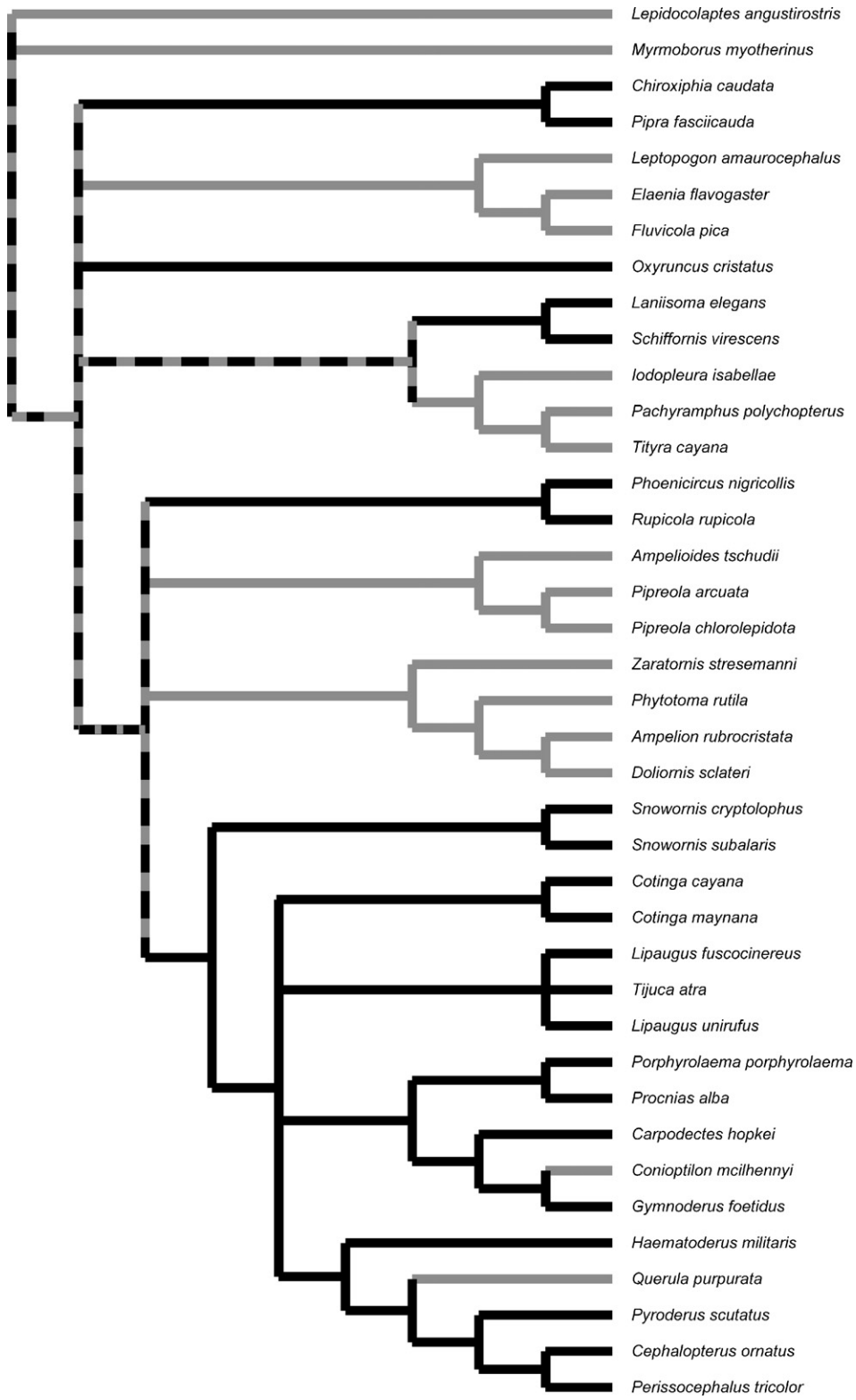

Fig. 3. Evolution of polygyny in Cotingidae. Grey lines, monogamy; black lines, polygyny; and dashed lines, equivocal state.

tion of two base pairs in the myoglobin sequences of *Phytotoma*, *Ampelion*, and *Doliornis* suggest that these may be closer to each other than to *Zaratornis*. Within the 'core cotingas' the position of *Snowornis* as sister to the remaining genera was supported with a posterior probability of 1.00, but the next basal lineages were poorly resolved in all analyses (posterior probability <0.85) (Fig. 2). A fruitcrow clade (*Querula*, *Pyroderus*, *Cephalopterus*, and *Perissocephalus*), a piha clade (*Lipaugus*, and *Tijuca*) and a clade consisting of *Porphyrolaema*, *Procnias*, *Carpodectes*,

Conioptilon, and *Gymnoderus* (here-after referred to as the 'canopy cotingas') each received strong support (posterior probability values 0.99–1.00). The monophyly of the genus *Cotinga* was supported by a sister group relationship between *C. cayana* and *C. maynana*; however, the position of *Cotinga* within the 'core cotinga' clade was unresolved.

Within the 'canopy cotinga' clade, a sister group relationship between *Conioptilon* and *Gymnoderus* was recovered with 1.00 posterior probability, as was a sister group relationship between *Xipholena* and *Carpodectes* in the nuclear analyses (we did not obtain any cytochrome *b* sequence for *Xipholena*). A sister group relationship between these two pairs of genera was also strongly supported. A placement of *Porphyrolaema* and *Procnias* basal to that clade also received high posterior probability (0.99).

The nuclear gene trees were inconclusive regarding the position of *Haematoderus* to other fruitcrows, but in the cytochrome *b* analysis it was placed basally in the fruitcrow clade with good support (0.98 posterior probability, Fig. 1A). In the combined analysis, the support was slightly weaker (0.94). Otherwise the internal nodes in the fruitcrow clade were well supported.

In the combined analyses, there was an indication of the fruiteater clade being basal to the remainder of the cotingas (Fig. 2), but the posterior probability was low, however, in effect giving a polytomy of four clades. This is a consequence of the two conflicting placements of the fruiteater clade in the myoglobin and cytochrome *b* trees, respectively, which both received high posterior probability values. (compare Fig. 1A and B). In the myoglobin tree, the fruiteater clade was placed together with the 'core cotingas', with a posterior probability value of 0.94 (Fig. 1B). In the cytochrome *b* tree, the fruiteater clade was basal to all other cotingas with a posterior probability of 0.99 (Fig. 1A). This incongruence affects basal topology and support values in the cotingid clade in the combined analysis. One clade that is not congruent with results of the myoglobin or cytochrome *b* analysis were recovered with high posterior probability (0.97) in the G3PDH tree (Fig. 1C): *Porphyrolaema* and *Procnias* were placed in a clade together with *Lipaugus* and *Tijuca*.

The parsimony analysis of the combined dataset resulted in one most parsimonious tree (not shown) (tree length = 4374, ci = 0.3320, ri = 0.3766). It showed the same main clades in Tyrannida (Tyrannidae, Tityridae, Oxyruncidae, Pipridae, and Cotingidae) and within Cotingidae (fruiteaters, the *Ampelion* clade, *Rupicola-Phoenicircus*, and the 'core cotingas') as the combined Bayesian analysis, except that *Snowornis* did not group with the other 'core cotingas'. Bootstrap support for the 'core cotinga' and *Ampelion* clades were low (0.61) and basal relationships in both the 'core cotingas', Cotingidae, and Tyrannida were unresolved. Well-supported clades at all levels were congruent with those found in the Bayesian analysis.

4. Discussion

4.1. Basal relationships in Tyrannida

Consistent with previous studies on molecular sequence data (Johansson et al., 2002; Chesser, 2004; Ericson et al., in press), our data support four main clades within Tyrannida, corresponding to Tyrannidae, Pipridae, Cotingidae, and Tityridae (including *Oxyruncus*). Our data do not support any confident conclusions about the sister group of Cotingidae. Ericson et al. (in press) placed Pipridae as the sister group to Cotingidae, but with non-significant support.

Some previous studies (Sibley et al., 1984; Sibley and Ahlquist, 1985, 1990; Prum et al., 2000) have suggested that *Oxyruncus* is embedded in Cotingidae, whereas previous molecular sequence studies (Johansson et al., 2002; Chesser, 2004), have placed *Oxyruncus* in an unresolved position alongside the four main clades in a basal polytomy. In the combined tree (Fig. 1) it shows a weakly supported association with the tityrids. This relationship receives stronger support in a recent study by Ericson et al. (in press) and has also been suggested from studies of allozyme data (Lanyon, 1985). Interestingly, Prum and Lanyon (1989) documented several syringeal features that are shared between *Oxyruncus* and members of the tityrids, including horseshoe-shaped medial syringeal. A relationship of *Oxyruncus* with *Tityra* and *Pachyramphus*.

4.2. A monophyletic cotingid clade

A well-supported cotingid clade is recovered in all three individual gene trees, with the same constituent taxa and largely the same topology in both of the well-resolved trees (myoglobin and cytochrome *b*). This clade is further supported by two synapomorphic indels in the nuclear introns (Fig. 2) and one syringeal character: the insertion of the musculus tracheolateralis between the A1 and B1 segments of the syrinx (Prum, 1990). *Procnias* and *Lipaugus* have independently derived intrinsic syringeal muscles that insert on the A1/B1 membrane. The monophyletic Cotingidae also includes *Rupicola*, *Phoenicircus*, *Snowornis*, *Ampelioides*, and *Pipreola* which all lack an enlarged femoral artery. The derived presence of the enlarged femoral artery has been proposed as a synapomorphy of a clade including cotingas, manakins, and tityrids, and secondarily lost in the aforementioned genera (Prum, 1990).

4.3. Interrelationships in Cotingidae

Many of the phylogenetic relationships among cotingid supported by this study have been suggested previously. There is good overall concordance with the results of Prum et al. (2000) in the taxonomic composition of the four main clades: (1) fruiteaters; (2) the *Ampelion* clade; (3) *Rupicola* and *Phoenicircus*; and (4) the 'core cotingas', a large clade consisting of the genera *Snowornis*, *Haematoderus*, *Querula*, *Pyroderus*, *Cephalopterus*, *Perissocephalus*, *Cotinga*, *Lipaugus*, *Procnias*, *Porphyrolaema*, *Xipholena*, *Carpodectes*,

Gymnoderus, and *Conioptilon*. In the phylogeny of Prum et al. (2000) the *Ampelion* group (their Phytotominae) occupied a basal position, fruiteaters (there including *Oxyruncus*) and *Rupicola* + *Phoenicircus* formed one clade (their Rupicolinae) that was the sister group to the 'core cotingas', whereas in our result the relationships between these four clades are unresolved. Prum et al. (2000) presented a parsimony analysis of a short sequence of cytochrome *b*; the hypothesis had generally low bootstrap values and was sensitive to different weighting strategies.

The mainly Andean fruiteaters form a well-supported clade in all individual analyses, and they further share a 2-bp deletion in the G3PDH intron-11 sequence (Fig. 2). The close relationship of *Pipreola* and *Ampelioides* is expected from morphological data (Prum, 1990) and in accordance with traditional classifications (e.g. Snow, 1973). A sister group relationship between the fruiteaters and the *Rupicola* + *Phoenicircus* clade was recovered by Prum et al. (2000), when their data were equally weighted. However, when transversions were weighted three times more than transitions, basal branching in Cotingidae collapsed into a polytomy of four clades, consistent with our results.

A relationship between *Rupicola* and *Phoenicircus* has been suggested on morphological grounds (Sclater, 1888; Hellmayr, 1929) and this was supported by allozyme data (Lanyon, 1985), but was entirely unanticipated by Snow (1973, 1982). Instead Snow (1973) suggested a relationship between *Phoenicircus* and the piprids based on outer toe fusion, or syndactyly, colouration, and the suspected possession of a communal courtship display. *Phoenicircus* performs an elaborate communal courtship display, resembling those of both *Rupicola* and several piprids (Trail and Donahue, 1991). In this study *Rupicola* and *Phoenicircus* are unambiguously recovered as sister taxa without any indications of a relationship with Pipridae. Their close relationship is further corroborated by a unique shared insertion of 11-bp in the myoglobin intron-2 sequence (Fig. 2).

This study corroborates the close relationship of *Phytotoma* to the high Andean cotingas in the genera *Zaratornis*, *Ampelion*, and *Doliornis* that has been suggested by several studies (Küchler, 1936; Lanyon, 1985; Lanyon and Lanyon, 1989; Prum, 1990; Johansson et al., 2002; Chesser, 2004). However, there is no compelling evidence that *Ampelion*, *Doliornis*, and *Zaratornis* form a clade to the exclusion of *Phytotoma*. Posterior probability values are too low to be conclusive, but an insertion of 2 bp in the myoglobin intron-2 sequence shared by *Phytotoma*, *Ampelion*, and *Doliornis* to the exclusion of *Zaratornis* (Fig. 2) may indicate that *Zaratornis* is the basalmost taxon in this clade. This was also indicated by allozyme data (Lanyon and Lanyon, 1989). Further, Lowery and O'Neill (1966) state that the skull anatomy of *Zaratornis* differs markedly from that of *Ampelion*, but they do not give any details.

Except for the position of the Andean *Snowornis* as the sister group to a predominantly lowland humid forest inhabiting clade of fruitcrows, pihas, and 'canopy cotingas', there is little basal resolution in the phylogeny of the 'core cotinga' clade. This part of the tree is poorly resolved in all three individual gene trees, possibly reflecting a rapid diversification.

The separation of *Snowornis* from *Lipaugus* was first suggested by Snow (1982) who suggested that the species *cryptolophus* and *subalaris* were only distantly related to other *Lipaugus*. This was confirmed by both morphology and DNA sequence data (Prum, 1990; Prum et al., 2000) and *cryptolophus* and *subalaris* have subsequently been separated as the genus *Snowornis* (Prum, 2001). The separation of *Snowornis* from *Lipaugus* is well supported here, but the relationships of the two genera to other cotingas differ between the current study and that of Prum et al. (2000). Prum et al. (2000) place *Snowornis* nested among genera that in our study are members of the well-supported 'canopy cotinga' clade while *Lipaugus* is placed as the sister to all 'core cotingas' except *Procnias* and *Cotinga*. The support for their topology is generally low (decay indexes of one in most cases). Our results also show low resolution between 'core cotinga' clades. It is clear, however that *Snowornis* is the sister to the remainder of the clade and that *Lipaugus* is placed among other genera with polygynous breeding systems, elaborate male courtship display and generally strong sexual dimorphism.

A close relationship between *Lipaugus* and *Tijuca* has been suggested earlier from external morphology, behaviour, and feather protein data (Snow, 1982, pp.108–109). This hypothesis is strongly supported here, but reciprocal monophyly of *Lipaugus* and *Tijuca* cannot be established without a better taxon sampling. The syrinx of *Tijuca* has not been described, so it cannot be established whether it exhibits the syringeal synapomorphies of *Lipaugus* (Prum, 2001).

The traditional fruitcrows (*Gymnoderus*, *Haematoderus*, *Querula*, *Cephalopterus*, *Perissocephalus*, and *Pyroderus*) have been hypothesized to constitute a natural group because of their large size, wide gapes, and powerful bills (e.g. Snow, 1973). Prum et al. (2000) recovered a monophyletic clade of fruitcrows, including *Haematoderus*, but excluding *Gymnoderus*. A similar fruitcrow clade of is recovered in all our analyses with strong support for monophyly of *Querula*, *Pyroderus*, *Cephalopterus*, and *Perissocephalus*, but the basal position of *Haematoderus* within the fruitcrows is unambiguously supported only in the cytochrome *b* analysis, and only weakly so in the combined analysis. Our study is congruent with Prum et al. (2000) in the hypothesized interrelationships among the fruitcrows and, the placement of *Gymnoderus* outside the fruitcrow clade.

The canopy living genera *Cotinga*, *Xipholena*, *Carpodectes*, and *Porphyrolaema* have commonly been assumed to form a closely related group, based on similar behaviour and similar type of sexual dimorphism (conspicuously coloured males and dull females). They were not recovered as a monophyletic clade in Prum et al. (2000) or in this study. *Xipholena*, *Carpodectes*, and *Porphyrolaema* form a well-

supported clade together with *Conioptilon*, *Gymnoderus*, and *Procnias*, whereas the position of *Cotinga* is unresolved within the 'core cotingas'. The SH test did however not reject a topology where *Cotinga* is part of the 'canopy cotinga' clade ($\delta L = 1.97$, $p = 0.27$).

A close relationship between *Carpodectes* and *Xipholena* has been suggested in earlier studies (e.g. Snow, 1973; Prum et al., 2000) and is confirmed by both the myoglobin and G3PDH data sets of this study. Prum et al. (2000) found that *Xipholena* and *Carpodectes* are sister taxa and further that *Gymnoderus* is the sister taxon to them. This clade is recovered in our analyses, and also includes *Conioptilon* as the sister taxon to *Gymnoderus*. The placement of *Conioptilon* is not unexpected, although it differs substantially from that in Prum et al. (2000). *Conioptilon* exhibits similarities to *Gymnoderus*, *Carpodectes*, and *Xipholena* in both cranial morphology and by the possession of extensive patches of powder down (Lowery and O'Neill, 1966). Thus the four cotingid genera with powder down form a well-supported monophyletic group. Within Tyrannida, powder down also occurs to some extent in the tityrids *Tityra* and *Iodopleura* (Lowery and O'Neill, 1966).

The affinities of *Procnias* to other cotingas have been enigmatic, mainly due to its highly derived syrinx (Ames, 1971; Snow, 1973; Prum, 1990). In the combined analysis, *Procnias* is placed among in the 'canopy cotinga' clade.In general appearance and type of sexual dimorphism *Procnias* is quite similar to the 'canopy cotingas'. The display behaviour of *Procnias* males is also quite similar to that of *Carpodectes* and *Xipholena*, although with a much stronger vocal component. It is probable that the development of a large and highly muscularized syrinx is connected to these extraordinary vocal capacities.

4.4. Implications for morphological character evolution

Prum (1990) analysed the morphological characters used to in the traditional classification of families in the Tyrannida, and concluded from a cladistic analysis that seven of them are phylogenetically informative (see also Prum et al., 2000). Because of homoplasy in the evolution of two of these characters—medial (or internal) syringeal cartilages and the enlarged femoral artery—the phylogeny was not resolved, however. Piprids, tityrids, and cotingids were associated in all phylogenetic reconstructions because of their shared derived femoral artery. The molecular data presented here indicate that the cotingid genera lacking the derived femoral artery—*Pipreola*, *Ampelioides*, *Rupicola*, *Phoenicircus*, and *Snowornis*—have secondarily lost this state. Based on the combined molecular phylogenetic hypothesis (Fig. 2), the most parsimonious optimisation of this character would require multiple losses and derivations of the enlarged femoral artery in the cotingas— e.g. independent losses in the fruiteaters, *Rupicola–Phoenicircus*, and *Snowornis*; or a loss in the ancestor of cotingas with subsequent gains in the *Ampelion* clade (clade 3) and the core cotingas excluding *Snowornis*. Further, the medial syringeal cartilages of *Lipaugus* are independently derived and the presence of medial syringeal cartilages in *Tityra* is congruent with the other members of the newly constituted tityrids.

4.5. The evolution of polygyny, lekking, and plumage sexual dimorphism

Breeding ecology is poorly known for several genera of Cotingidae, but in the majority of species no pairs bonds are formed and males perform various forms of ritualised courtship display, either solitary or in communal lekking arenas (e.g. Snow, 1982, 2004). The present phylogenetic hypothesis provides some insights into the evolution of cotinga breeding systems and sexual dimorphism.

The preferred hypothesis for the ancestral breeding system in cotingas depends upon the sister group of the family and the resolution of the basal relationships within the cotingas (Fig. 3). If the manakins are the sister group to the cotingas, which receives weak support in the analysis of Ericson et al. (in press), polygyny is more likely to be ancestral to the cotinga family. However, the fruiteater clade, which may be basal within the family, consists entirely of monogamous species. The *Ampelion* clade also consists entirely of monogamous species, whereas the *Rupicola* clade and the 'core Cotingas' consist of largely polygynous species. In the combined phylogenetic hypothesis, the *Ampelion* clade has unresolved relationships to the polygynous *Rupicola* clade and to the 'core Cotingas', but it is phylogenetically bracketed by largely polygynous clades in each of the separate data sets.

Regardless of whether monogamy is ancestral within cotingas, reversals from polygyny to social monogamy have evolved in *Conioptilon* (Tobias, 2003), and to monogamy with helpers at the nest in *Querula* (Snow, 1982, 2004). Given the tree topologies supported by the various genes, monogamy is also likely to have evolved secondarily from polygyny in the *Ampelion* clade. Whether monogamy in the fruiteater clade is ancestral or derived remains ambiguous.

The polygynous breeding system has evolved further into complex communal leks in the *Rupicola* clade and in the *Pyroderus–Cephalopterus–Perissocephalus* clade (Snow, 1982, 2004) (Fig. 3). Thus, the behavioural similarities previously identified among the species within each of these clades are homologous (Snow, 1982, 2004; Trail and Donahue, 1991).

Using either the manakins or the tityrids as the sister group to the cotingas, it appears that sexual dimorphism in plumage colouration is likely to be the primitive state in Cotingidae. Not surprisingly, sexual dimorphism is secondarily reduced or lost in the species of the monogamous *Ampelion* clade and in the monogamous *Conioptilon*. More interestingly, there is a derived loss in sexual plumage dimorphism in the lekking pihas (*Lipaugus*), and a derived reduction culminating in a loss of sexual plumage dimorphism in the lekking fruitcrows (*Pyroderus*, *Cephalopterus*, and *Perissocephalus*) (Fig. 3). Although the fruitcrows are

highly sexually dimorphic in size, the loss of sexual dichromatism with increasing sexual selection indicates that female preferences have transferred to other aspects of male phenotype. In both instances, elaborate vocal advertisements—explosive whistles in *Lipaugus* and booming calls in fruitcrows—appear to have evolved coincidentally with the reduction of plumage ornamentation. While further analyses are required to verify these patterns in more detail, current data indicate that female preferences in these lineages have evolved to switch from plesiomorphic plumage traits to novel vocal and behavioural traits, resulting in a decrease in plumage dichromatism.

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