

# Higher-level phylogeny and morphological evolution of tyrant flycatchers, cotingas, manakins, and their allies (Aves: Tyrannida)

Per G.P. Ericson<sup>a,\*</sup>, Dario Zuccon<sup>a,b</sup>, Jan I. Ohlson<sup>a,c</sup>, Ulf S. Johansson<sup>d</sup>,  
Herculano Alvarenga<sup>e</sup>, Richard O. Prum<sup>f</sup>

<sup>a</sup> Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden

<sup>b</sup> Dipartimento di Biologia Animale e dell' Uomo, Università di Torino, Via Accademia Albertina 17, 10123 Torino, Italy

<sup>c</sup> Department of Zoology, University of Stockholm, SE-106 91 Stockholm, Sweden

<sup>d</sup> DST/NRF Centre of Excellence at the Percy Fitzpatrick Institute, Evolutionary Genomics Group, Department of Botany and Zoology, University of Stellenbosch, Private Bag XI, Maitland 7602, South Africa

<sup>e</sup> Museu de História Natural de Taubaté, Rua Juvenal Dias de Carvalho, 111, Taubaté, SP, CEP 12070-640, Brazil

<sup>f</sup> Department of Ecology and Evolutionary Biology, and Peabody Museum of Natural History, Yale University, P.O. Box 208105, New Haven, CT 06520, USA

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## Abstract

Despite increased understanding of higher-level relationships in passerine birds in the last 15 years, the taxonomic boundaries and phylogenetic interrelationships of the major groups of the Tyrannida (including the cotingas, manakins, tityrines, and tyrant flycatchers) remain unclear. Here, we present an analysis of DNA sequence data obtained from two nuclear exons, three introns, and one mitochondrial gene for 26 genera of Tyrannida and 6 tracheophone outgroups. The analysis resulted in well-supported hypotheses about the earliest evolution within Tyrannida. The Cotingidae, Pipridae, Tityrinae (*sensu*) [Prum, R.O., Rice, N.H., Mobley, J.A., Dimmick, W.W., 2000. A preliminary phylogenetic hypothesis for the cotingas (Cotingidae) based on mitochondrial DNA. *Auk* 117, 236–241], Tyrannidae, and the tyrannid subfamilies Tyranninae and Pipromorphinae (*sensu*) [Sibley, C.G., Monroe, B. L. Jr., 1990. *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, CT] were all found to be reciprocally monophyletic (given the present taxon sampling). The Cotingidae and Pipridae form a clade that is the sister group to a well-supported clade including *Oxyruncus*, the Tityrinae, *Piprites*, and the Tyrannidae. *Oxyruncus* is the sister group to the Tityrinae, and *Piprites* is placed as the sister group to the Tyrannidae. The tyrannid subfamilies Tyranninae and Pipromorphinae are monophyletic sister taxa, but the relationships of *Platyrinchus mystaceus* to these two clades remains ambiguous. The presence of medial (= internal) cartilages in the syrinx is a synapomorphy for the *Oxyruncus*–Tityrinae–*Piprites*–Tyrannidae clade. Although morphological synapomorphies currently support the monophyly of both the Pipridae and the Cotingidae, convergences and/or reversals in morphological character states are common in Tyrannida. The relationship between *Oxyruncus* and the Tityrinae is congruent with additional syringeal synapomorphies and allozyme distance data. Accordingly, we propose the recognition the family Tityridae within the Tyrannida to include the genera *Schiffornis*, *Laniisoma*, *Laniocera*, *Iodopleura*, *Xenopsaris*, *Pachyramphus*, *Tityra*, and *Oxyruncus*.

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## 1. Introduction

The New World suboscines fall in two reciprocally monophyletic clades: the Tyrannida and Furnariida (Eric-

son et al., 2003). This division is unambiguously supported by molecular data (Sibley and Ahlquist, 1990; Irestedt et al., 2001; Johansson et al., 2002; Chesser, 2004), while morphological synapomorphies are known only for Furnariida (Ames, 1971). del Hoyo et al. (2003) list 558 species in the Tyrannida (their Tyranni) which are placed in three groups that long have been recognized by systematists as families

\* Corresponding author. Fax: +46 8 51954212.

E-mail address: [per.ericson@nrm.se](mailto:per.ericson@nrm.se) (P.G.P. Ericson).

(or subfamilies): the cotingas (Cotingidae; including plantcutters, *Phytotoma*, and sharpbill, *Oxyruncus*), the manakins (Pipridae) and the tyrant flycatchers (Tyrannidae; see Table 1 for taxonomic usage). Both molecular and morphological data support monophyly of the core-cotingas and core-manakins (Prum, 1990, 1992; Prum et al., 2000; Irestedt et al., 2001; Johansson et al., 2002; Chesser, 2004), but

the monophyly of tyrant flycatchers is less certain (McKittrick, 1985; Sibley and Ahlquist, 1985, 1990; Birdsley, 2002).

Nested among these three larger groups of taxa are several genera that have been considered taxonomically “problematic” and difficult to place in any of the main families of Tyrannida (Traylor, 1977; McKittrick, 1985; Prum and Lanyon, 1989; Prum, 1990). Among these are former cotinga genera

Table 1  
Species included in the study and their higher-level taxonomy

Species	Handbook of the Birds of the World (del Hoyo et al., 2003)—in brackets is given the corresponding name in Peters’ checklist (Traylor, 1979) if divergent	Distribution and Taxonomy of Birds of the World (Sibley and Monroe, 1990)	Ericson et al. (2003) used herein	Proposed taxonomy based on present result
Ingroup (least inclusive taxon)	Tyranni (Tyrannoidea)	Tyrannida	Tyrannida	Tyrannida
<i>Leptopogon amaurocephalus</i>	Tyrannidae: Elaeniinae	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae
<i>Todirostrum cinereum</i>	Tyrannidae: Elaeniinae	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae
<i>Corythopis delalandi</i>	Tyrannidae: Elaeniinae (Conopophagidae)	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae	Tyrannidae: Pipromorphinae
<i>Myiopagis viridicata</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Elaenia flavogaster</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Serpophaga subcristata</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Inezia inornata</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Stigmatura budytoides</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Platyrinchus mystaceus</i>	Tyrannidae: Elaeniinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Knipolegus striaticeps</i>	Tyrannidae: Fluvicolinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Fluvicola pica albiventer</i>	Tyrannidae: Fluvicolinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Gubernetes yetapa</i>	Tyrannidae: Fluvicolinae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Myiarchus tyrannulus</i>	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Tyrannus savana</i>	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae	Tyrannidae: Tyranninae
<i>Schiffornis virescens</i>	Pipridae	Tyrannidae: Tityrinae	Tityrinae <i>incertae sedis</i>	Tityridae
<i>Pachyramphus polychropterus</i>	Tyrannidae: Tityrinae	Tyrannidae: Tityrinae	Tityrinae <i>incertae sedis</i>	Tityridae
<i>Tityra cayana</i>	Tyrannidae: Tityrinae	Tyrannidae: Tityrinae	Tityrinae <i>incertae sedis</i>	Tityridae
<i>Iodopleura isabellae</i>	Cotingidae	Tyrannidae: Cotinginae	Tityrinae <i>incertae sedis</i>	Tityridae
<i>Oxyruncus cristatus</i>	Cotingidae (Oxyruncidae)	Tyrannidae: Cotinginae	Oxyruncidae	Tityridae
<i>Pyroderus scutatus</i>	Cotingidae	Tyrannidae: Cotinginae	Cotingidae	Cotingidae
<i>Rupicola peruviana</i>	Cotingidae	Tyrannidae: Cotinginae	Cotingidae	Cotingidae
<i>Phytotoma rutila</i>	Cotingidae (Phytotomidae)	Tyrannidae: Cotinginae	Cotingidae	Cotingidae
<i>Pipra fasciicauda</i>	Pipridae	Tyrannidae: Piprinae	Pipridae	Pipridae
<i>Chiroxiphia caudata</i>	Pipridae	Tyrannidae: Piprinae	Pipridae	Pipridae
<i>Manacus manacus</i>	Pipridae	Tyrannidae: Piprinae	Pipridae	Pipridae
<i>Piprites pileatus</i>	Pipridae	Tyrannidae: Piprinae	<i>incertae sedis</i>	<i>incertae sedis</i>
Outgroup	Furnarii (not recognized in Peters’ checklist)	Tyrannides	Furnariida	Furnariida
<i>Conopophaga aurita</i>	Conopophagidae	Conopophagidae	Conopophagidae	Conopophagidae
<i>Thamnophilus caeruleus</i>	Thamnophilidae (Formicariidae)	Thamnophilidae	Thamnophilidae	Thamnophilidae
<i>Furnarius cristatus</i>	Furnariidae	Furnariidae: Furnariinae	Furnariidae: Furnariinae	Furnariidae: Furnariinae
<i>Lepidocolaptes angustirostris</i>	Dendrocolaptidae	Furnariidae: Dendrocolaptinae	Furnariidae: Dendrocolaptinae	Furnariidae: Dendrocolaptinae
<i>Rhinocrypta lanceolata</i>	Rhinocryptidae	Rhinocryptidae	Rhinocryptidae	Rhinocryptidae

*Tityra* and *Pachyramphus*, which Traylor (1977, 1979) placed in the Tityrinae as a new subfamily of tyrant flycatchers. Prum (1990) removed six genera from the manakins: *Schiffornis*, *Piprites*, *Sapayoa*, *Neopipo*, *Neopelma*, and *Tyrannneutes*. The *Neopelma* and *Tyrannneutes* clade was subsequently replaced in the manakins as the sister group to the rest of the manakins (Chesser, 2004; Hackett, S., pers. comm.). *Neopipo* was placed in an eclectic tyrannid clade lacking intrinsic syringeal muscles in a phylogenetic analysis of morphological characters (Mobley and Prum, 1995). *Sapayoa* has subsequently been recognized as a member of the “Old World” suboscines (Fjeldså et al., 2003; Chesser, 2004).

Prum and Lanyon (1989) proposed a monophyletic *Schiffornis* group composed of six genera of former piprids, cotingids, and tyrannids based on the presence of two synapomorphic characters in the syrinx. The *Schiffornis* group included *Schiffornis*, *Laniisoma*, *Laniocera*, *Iodopleura*, *Pachyramphus*, and *Xenopsaris*, but not *Tityra* (Prum and Lanyon, 1989). Neither Prum and Lanyon (1989) nor Prum (1990) established the phylogenetic position of the *Schiffornis* group. Later, Prum et al. (2000) added *Tityra* to the *Schiffornis* group based on a preliminary analysis of DNA data, and placed it as a subfamily of cotingids, again called the Tityrinae. (This usage will be used throughout this manuscript.) Various molecular data sets have supported not only the composition of the *Schiffornis* group plus *Tityra*, but also the interrelationships proposed by of Prum and Lanyon (1989; Prum et al., 2000; Johansson et al., 2002; Chesser, 2004). However, the systematic position of Tityrinae relative to other major groups of Tyrannida is unclear. The morphological data is ambiguous; while certain characters suggest an affinity between Tityrinae and the tyrant flycatchers (e.g., the presence of medial (= internal) syringeal cartilage and an intrinsic syringeal muscle with an oblique fiber direction), other instead are shared with the manakins and cotingas (e.g., the enlarged femoral artery and the insertion of the intrinsic muscle on the membrane between the A1 and B1 syringeal supporting elements). Prum et al. (2000) argued that the latter suite of characters in the tityrines are homologous with those in cotingas, suggesting that the Tityrinae is part of the cotinga radiation. This hypothesis also received weak support from their molecular data set, but not by others (Johansson et al., 2002; Chesser, 2004).

The analyses of DNA–DNA hybridization distance data unexpectedly suggested that a previously unrecognized group of genera constitutes the basal most branch within Tyrannida (Sibley and Ahlquist, 1990). The group, named Pipromorphinae, consisted of 54 species that in earlier classifications were nested within the tyrant flycatchers. This hypothesis suggested that the pipromorphines were sisters not only to all remaining tyrant flycatchers, but to all the rest of the Tyrannida. Although a pipromorphine clade has received support also in analyses of nucleotide sequence data (Johansson et al., 2002; Chesser, 2004) these studies provide no solid support for paraphyly of the traditional grouping of tyrant flycatchers (e.g., Tyrannidae *sensu* Traylor, 1979).

The difficulty with resolving the phylogenetic relationships among the major taxonomic clades of Tyrannida becomes particularly clear when investigating the complex and incongruent morphological variation within the clade. However, molecular data have also yielded conflicting results indicating that this difficulty may be explained by a more general phenomenon. It is conceivable that the group underwent an early, rapid cladogenesis leading to short internodes between the major clades. This would explain the difficulties of fully resolving the most ancient phylogenetic patterns of the group, regardless of the type of data. As a consequence, the precise taxonomic boundaries of major groups in Tyrannida and their phylogenetic relationships remain unclear.

In this paper, we address these issues with an analysis of DNA sequences obtained from representatives of all major clades of Tyrannida. The scope is similar to the study of Johansson et al. (2002), but we have augmented their data set with more taxa and three new genetic markers, all nuclear introns. The new markers are chosen because their evolutionary rates are intermediate between the comparatively slow *c-myc* and RAG-1 genes and relatively fast cytochrome *b* gene, which were used by Johansson et al. (2002). The phylogenetic signals in the six markers are compared to assess the robustness of the phylogenetic hypothesis based on the concatenated data set. We also evaluate the phylogenetic information in certain morphological characters that have been suggested to be especially useful for elucidating higher-level relationships in Tyrannida.

## 2. Materials and methods

### 2.1. Taxon sampling and outgroups

Blood or tissue samples were obtained from three species of the subfamily Pipromorphinae, 11 of Tyranninae, four of Tityrinae, three of Pipridae, and three of Cotingidae, in addition to *Oxyruncus cristatus*, and *Piprites pileatus*. At the genus level these species represent 13% of the genera of tyrant flycatchers, 17% of the manakins, and 10% of the cotingas (del Hoyo et al., 2003). As outgroups serve five representatives of Furnariida *sensu* Ericson et al. (2003), the sistergroup of Tyrannida (Sibley and Ahlquist, 1990; Irestedt et al., 2001). Sample information and GenBank Accession numbers are given in Table 2.

### 2.2. Extraction, amplification, and sequencing

Laboratory procedures for the extraction, PCR-amplification, and sequencing of *c-myc*, RAG-1, and cytochrome *b* follow protocols described by Ericson et al. (2000), Irestedt et al. (2001), and Johansson et al. (2002). Sequences from the myoglobin gene (intron 2), the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (intron 11), and the ornithine decarboxylase (ODC) gene (introns 6 and 7, along with the intercepting exon 7) were obtained according to methods described by Irestedt et al. (2002), Fjeldså et al. (2003), and Allen and Omland (2003), respectively.

Table 2  
Specimen data and GenBank Accession numbers for samples used in the study

Species	Voucher No.	GenBank Accession numbers										
		c-myc		RAG-1		Cytochrome <i>b</i>		Myoglobin	G3PDH	ODC		
Ingroup												
<i>Leptopogon amaurocephalus</i>	NRM 937317	AF453781	2	AF453795	2	AF453808	2	DQ435520		DQ435468		DQ435487
<i>Todirostrum cinereum</i>	NRM 947036	AF453782	2	AF453796	2	AF453809	2	AY338740	5	AY336575	5	DQ435506
<i>Corythopis delalandi</i>	NRM 937282	AF453779	2	AF453792	2	AF453805	2	AY065788	3	DQ435463		DQ435479
<i>Myiopagis viridicatus</i>	NRM 986779	AF453780	2	AF453793	2	AF453806	2	DQ435522		DQ435470		DQ435490
<i>Elaenia flavogaster</i>	NRM 966970	AF377279	3	AF453794	2	AF453807	2	AY064254	4	DQ435464		DQ435480
<i>Serpophaga subcristata</i>	NRM 976683	DQ435452		DQ435514		DQ435460		DQ435527		DQ435475		DQ435502
<i>Inezia inornata</i>	NRM 947139	DQ435446		DQ435508		DQ435454		DQ435518		DQ435466		DQ435484
<i>Stigmatura budytoidea</i>	NRM 966804	DQ435453		DQ435515		DQ435461		DQ435528		DQ435476		DQ435503
<i>Platyrinchus mystaceus</i>	NRM 976724	DQ435450		DQ435512		DQ435458		DQ435525		DQ435473		DQ435497
<i>Knipolegus striaticeps</i>	NRM 966782	DQ435448		DQ435510		DQ435456		DQ435523		DQ435471		DQ435491
<i>Fluvicola pica albiventer</i>	NRM 956714	AF453783	2	AF453797	2	AF453810	2	DQ435517		DQ435465		DQ435481
<i>Gubernetes yetapa</i>	NRM 976700	AF295166	1	AF295188	1	AF453811	2	AY338739	5	AY336578	5	DQ435483
<i>Myiarchus tyrannulus</i>	NRM 937173	AF453784	2	AF453798	2	AF453812	2	DQ435521		DQ435469		DQ435489
<i>Tyrannus savana</i>	NRM 976722	AF295182	1	AF295203	1	AF453813	2	AY165826	6	AY336579	5	DQ435507
<i>Schiffornis virescens</i>	NRM 937315	AF453786	2	AF453800	2	AF453816	2	AY338741	5	AY336574	5	DQ435501
<i>Pachyrhamphus polychopterus</i>	NRM 967032	AF453785	2	AF453799	2	AF453815	2	AY338747	5	AY336573	5	DQ435493
<i>Tityra cayana</i>	NRM 956584	AF295181	1	AF295202	1	AF453814	2	AY338742	5	AY336580	5	DQ435505
<i>Iodopleura isabellae</i>	ZMUC 125762	DQ435447		DQ435509		DQ435455		DQ435519		DQ435467		DQ435485
<i>Oxyruncus cristatus</i>	NRM 967078	AF453790	2	AF453804	2	AF453821	2	AY338745	5	AY336572	5	DQ435492
<i>Pyroderus scutatus</i>	NRM 967030	AF453789	2	AF453803	2	AF453820	2	AY065786	3	AY336582	5	DQ435498
<i>Rupicola peruviana</i>	ZMUC 126003	DQ435451		DQ435513		DQ435459		AY338744	5	AY336584	5	DQ435500
<i>Phytotoma rutila</i>	ZMUC S466	AF295173	1	AF295194	1	AF453822	2	AY338743	5	AY336581	5	DQ435494
<i>Pipra fasciicauda</i>	NRM 947271	AF295175	1	AF295196	1	AF453817	2	AY065787	3	AY336583	5	DQ435495
<i>Chiroxiphia caudata</i>	NRM 956620	AF453788	2	AF453802	2	AF453819	2	DQ435516		DQ435462		DQ435477
<i>Manacus manacus</i>	L.S. uncat.	AF453787	2	AF453801	2	AF453818	2	AY338746	5	AY336571	5	DQ435488
<i>Piprites pileatus</i>	ZMUC 128817	DQ435449		DQ435511		DQ435457		DQ435524		DQ435472		DQ435496
Outgroup												
<i>Conopophaga aurita</i>	ZMUCS1245	AF295163	1	AF295185	1	AY078173	2	AY065784	3	AY336577	5	DQ435478
<i>Thamnophilus caerulescens</i>	NRM 967007	AF295180	1	AF295201	1	AY078176	2	AY065783	3	AY336587	5	DQ435504
<i>Furnarius cristatus</i>	NRM 966772	AF295165	1	AF295187	1	AY064279	4	AY064255	4	AY590066	7	DQ435482
<i>Lepidocolaptes angustirostris</i>	NRM 937184	AF295168	1	AF295190	1	AY078175	2	AY065767	3	AY336576	5	DQ435486
<i>Rhinocrypta lanceolata</i>	NRM 966793	AF295178	1	AF295199	1	AY078174	2	AY065775	3	DQ438953		DQ435499

Acronyms: NRM, Swedish Museum of Natural History; ZMUC, Zoological Museum of the University of Copenhagen; LS, Lisa Shorey. References: (1) Irestedt et al. (2001); (2) Johansson et al. (2002); (3) Irestedt et al. (2002); (4) Ericson et al. (2002); (5) Fjelds  et al. (2003); (6) Johansson and Ericson (2003); (7) Fjelds  et al. (2005).

For each taxon, the multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SeqMan II<sup>TM</sup> (DNASTAR Inc.). At a few positions where the nucleotide could not be determined with certainty, these were coded with the appropriate IUPAC code and treated as uncertainties in the phylogenetic analysis.

The sequences from the different species were aligned in MegAlign<sup>TM</sup> (DNASTAR Inc.). The alignment of the protein-coding genes was unproblematic as no insertions or deletions (indels) were observed. Also the myoglobin, G3PDH and ODC introns could easily be aligned by eye thanks to the low number of indels. All inserted gaps needed for the alignment of the sequences were treated as missing data in the analyses.

### 2.3. Phylogenetic analyses

The models for nucleotide substitutions were selected for each gene individually, prior to the MCMC, and using the Akaike Information Criterion (Akaike, 1973). The program

MrModeltest 2.2 (Nylander, 2002) in conjunction with PAUP\* (Swofford, 1998) was used to evaluate the fit of the data to different models for nucleotide substitutions. The models and parameter settings chosen for the individual genes were used also in the analysis of the combined data set. The posterior probabilities of trees and parameters were approximated with Markov chain Monte Carlo and Metropolis coupling using the program MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The chosen substitution models, base frequencies, and parameter settings used in the analysis (after reaching stability) are listed in Table 3.

For each gene region, we ran four analyses of 4 million generations each with trees sampled every 100 generation. After discarding the trees saved during the “burn-in phase” (as estimated graphically), the topologies of the 50% majority-rule consensus trees from the four runs were compared and in all cases found to be identical. The saved trees from each analysis were pooled and the posterior probabilities were based on a total of ca 150,000 trees. The analysis of the combined data set was conducted in the same manner



Table 3

Data characteristics and Bayes estimates of parameters for the six studied genetic markers

	<i>c-myc</i>	RAG-1	Cytochrome <i>b</i>	Myoglobin	G3PDH	ODC
Number of sites in alignment	477	930	999	774	370	733
Number of variable sites	69 (14%)	204 (22%)	480 (48%)	295 (38%)	211 (57%)	249 (34%)
Number of potentially informative sites	38 (8%)	80 (9%)	409 (41%)	122 (16%)	106 (29%)	67 (9%)
Base frequencies (%)						
A	34.6	31.4	33.6	30.5	22.0	28.1
C	24.0	19.4	39.0	20.5	18.9	17.3
G	23.0	24.2	8.0	20.8	30.0	20.6
T	18.4	24.9	19.3	28.3	29.1	34.0
Selected model for nucleotide evolution	GTR+I+G	GTR+I+G	GTR+I+G	GTR+G	GTR+G	GTR+G
Substitution model (rate matrix)						
A vs. C	0.98	1.00	1.20	1.01	0.98	1.16
A vs. G	8.89	4.28	15.93	4.67	4.36	3.12
A vs. T	0.22	0.75	4.48	0.52	0.63	0.58
C vs. G	0.57	0.73	1.07	1.59	1.93	0.97
C vs. T	13.40	6.76	69.72	4.81	5.57	2.53
Proportion of invariable sites ( <i>I</i> )	0.7071	0.4700	0.4595	0	0	0
Gamma distribution shape parameter	0.8046	3.3186	0.5583	0.9273	1.2380	1.0856

Substitution rate parameters given with the rate for G ↔ T set to 1. The models and parameter settings for the individual genes were used also in the analysis of the concatenated data set.

as for the individual genes. The 50% majority-rule consensus trees were identical also in these runs and the posterior probabilities are based on a total of 120,000 saved trees.

#### 2.4. Morphological variation

The taxonomic distributions for selected morphological characters that are derived within Tyrannida (Table 4) were taken from Ames (1971), Prum and Lanyon (1989), and Prum (1990, 1992). The intrinsic syringeal muscle *M. obliquus ventralis* was reported by Ames (1971) to occur in most tyrant flycatchers (lacking only in a handful of the studied species including *Todirostrum cinereum*) (McKittrick, 1985). It has also been reported to occur in *Oxyruncus* and *Iodopleura*. However, the considerable variation in the morphology of the intrinsic muscles observed among taxa outside the tyrant flycatcher clade led Prum (1990, 1992) to consider that the condition in these groups to be non-homologous with *M. obliquus ventralis* of tyrant flycatchers. Herein we follow Prum (1990, 1992) when coding the variation in this character.

McKittrick (1985) suggested the possession of medial cartilages in the syrinx is a synapomorphy for a clade consisting of the tyrant flycatchers and a group of “problematic” members of Tyrannida (e.g., *Schiffornis*, *Pachyramphus*, *Tityra*, *Iodopleura*, *Piprites*, and *Oxyruncus*). Medial cartilages are absent in most piprids, most cotingids, and the outgroups. The coding of this character follows Ames (1971), Prum and Lanyon (1989), and Prum (1990) (Table 4).

Two other derived morphological characters that show taxonomic variation within Tyrannida are the enlargement of the femoral artery as the main blood supply to the thigh (in most other birds it is the ischiadic artery) (Prum, 1990),

and the insertion of *M. tracheolateralis* on the A1/B1 membrane of the syrinx (Ames, 1971; Prum, 1990). For most tyrant flycatchers the states for these characters are unknown or not explicitly stated. However, the derived states have never been observed in any of the species examined so far (cf. Prum and Lanyon, 1989; Prum, 1990; Prum et al., 2000). When coding the taxonomic distributions of character states we thus have assigned the primitive states for all tyrant flycatchers for which no detailed information is available (Table 4).

### 3. Results

#### 3.1. Sequence variation and alignments

Full sequences were obtained for all six genes in all taxa. After alignment the data sets had the following lengths and proportions of potentially informative sites: *c-myc*: 477 bp (8%); RAG-1: 930 bp (9%); cytochrome *b*: 999 bp (41%); myoglobin: 774 bp (16%); G3PDH: 370 bp (29%); and ODC: 733 bp (9%). After combining the six genes the final alignment consists of 4283 bp. The pairwise sequence distances (uncorrected *p* distances) among ingroup taxa varied considerably between genes: *c-myc* from 0.21% (*Gubernetes yetapa* vs. *Fluvicola pica*) to 4.19% (*Rupicola peruviana* vs. both *Iodopleura isabellae* and *Pachyramphus polychopterus*); in RAG-1 from 0.54% (both in *Stigmatura budytoidea* vs. *Elaenia flavogaster*, and in *Tyrannus savanna* vs. *Myiarchus tyrannulus*) to 3.67% (*Phytotoma rutila* vs. *Fluvicola pica*); in cytochrome *b* from 11.33% (*Pipra fasciicauda* vs. *Manacus manacus*) to 20.52% (*Corythopsis delalandi* vs. *Chiroxiphia caudata*); in myoglobin intron 2 from 0.97% (both in *Pipra fasciicauda* vs. *Manacus manacus*, and in *Tyrannus savanna* vs. *Myiarchus tyrannulus*) to 6.52%

Table 4  
Codings of morphological characters used in Fig. 3 a–d

Taxon	<i>M. obliquus ventralis</i> (following Prum, 1990)	Enlarged femoral artery	Internal cartilages	Insertion on A1/B1 membrane
<b>Ingroup</b>				
<i>Leptopogon</i>	+	—*	+	—*
<i>Todirostrum</i>	—	—*	+	—*
<i>Corythopsis</i>	+	—*	+	—*
<i>Myiopagis</i>	+ *	—*	+ *	—*
<i>Elaenia</i>	+	—*	+	—*
<i>Serpophaga</i>	+	—*	+	—*
<i>Inezia</i>	+	—*	+	—*
<i>Stigmatura</i>	+	—*	+	—*
<i>Platyrrhinus</i>	+	—*	+	—*
<i>Knipolegus</i>	+	—*	+	—*
<i>Fluvicola</i>	+	—*	+	—*
<i>Gubernetes</i>	+	—*	+	—*
<i>Myiarchus</i>	+	—*	+	—*
<i>Tyrannus</i>	+	—*	+	—*
<i>Schiffornis</i>	—	+	+	+
<i>Pachyrhamphus</i>	—	+	+	+
<i>Tityra</i>	—	+	+	+
<i>Iodopleura</i>	—	+	+	+
<i>Oxyruncus</i>	—	—	+	+
<i>Pyroderus</i>	—	+	—	+
<i>Rupicola</i>	—	—	—	+
<i>Phytotoma</i>	—	+	—	+
<i>Pipra</i>	—	+	—	—
<i>Chiroxiphia</i>	—	+	—	—
<i>Manacus</i>	—	+	—	—
<i>Piprites</i>	—	+	+	—
<b>Outgroup</b>				
<i>Conopophaga</i>	—	—	—	—
<i>Thamnophilus</i>	—	—	—	—
<i>Furnarius</i>	—	—	—	—
<i>Lepidocolaptes</i>	—	—	—	—
<i>Rhinocrypta</i>	—	—	—	—

A plus sign indicates presence of the structure in the taxon, and minus absence. An asterisk indicates that the character state is inferred from Prum (1990, Table 2).

(*Pyroderus scutatus* vs. *Platyrrhinus mystaceus*); in G3PDH intron 11 from 2.12% (*Gubernetes yetapa* vs. *Fluvicola pica*) to 13.27% (*Oxyruncus cristatus* vs. *Inezia inornata*); and in ODC introns 6–7 from 1.19% (*Stigmatura budyoides* vs. *Inezia inornata*) to 9.36% (*Rupicola peruviana* vs. *Piprites pileatus*). A summary of the gene properties (base frequencies, substitution rate matrices, and substitution model parameters) is shown in Table 3.

### 3.2. Phylogenetic analysis

The phylogenetic analysis of the combined data set resulted in a well-resolved tree in which most clades received high posterior probabilities (Fig. 1). At first glance, the individual gene trees differ considerably from this tree, especially at the deeper nodes (Figs. 2a–f). However, the differences are less dramatic if comparing only the well-supported clades in the gene trees, i.e., those receiving a 95% or higher posterior probability in the Bayesian analysis. Only six instances of a

highly supported alternative topology were observed (Table 5), and a single, highly supported node in the myoglobin gene tree (marked with an asterisk in Fig. 2d) is responsible for three of these six cases. At this node, the myoglobin data set supported a clade consisting of cotingas, manakins, and Tyranninae to the exclusion of *Oxyruncus* and *Platyrrhinus*, the Pipromorphinae, and Tityrinae (Fig. 2d). This topology was not recovered in any other gene tree.

The major results of the analysis are these:

1. The Tyrannida, the ingroup, was recovered as monophyletic with 100% posterior probability in the analyses of all data partitions (genes) as well as in the analysis of all these data combined.
2. The Cotingidae, Pipridae, Tityrinae, Tyranninae (excluding *Platyrrhinus*), and Pipromorphinae were all found to be reciprocally monophyletic (given the present taxon sampling) in the analysis of the combined data set. The cotinga clade (*Rupicola*, *Pyroderus*, and *Phytotoma*), the manakin clade (*Chiroxiphia*, *Manacus*, and *Pipra*), and the two clades with tyrant flycatchers (Tyranninae and Pipromorphinae) were recognized by all data partitions, except the more slowly evolving *c-myc* data set. Most analyses recovered these clades with 100% posterior probabilities (Table 5). Tityrinae was in turn recovered with a high posterior probability in most gene trees.
3. There is a low posterior probability (78%) in the combined data set for a clade of cotingas and manakins. This clade is recovered by the myoglobin data set only.
4. A monophyletic Tityrinae is recovered with 100% posterior probability in the combined tree, and overall high posterior probabilities in all gene trees except the *c-myc* tree. *Oxyruncus* is confidently placed as the sister to Tityrinae with 100% posterior probability in the combined tree, although this relationship is recovered only in the *c-myc* tree. Furthermore, the ODC data set supports an alternative hypothesis where *Oxyruncus* is sister to *Piprites pileatus* and the tyrant flycatchers (Fig. 2f).
5. A monophyletic Tyrannidae (Tyranninae plus Pipromorphinae of Sibley and Monroe, 1990) received 99% posterior probability in the combined tree and was also recovered in the cytochrome *b* and G3PDH trees, albeit with lower posterior probabilities (Figs. 2c and e). *Piprites pileatus* was identified as sister group to the tyrannids in the combined tree, as well as in the cytochrome *b* and G3PDH trees.
6. The phylogenetic position of *Platyrrhinus mystaceus* could not be confidently resolved. It groups with the Pipromorphinae in the myoglobin and G3PDH gene trees, albeit this relationship gets high posterior probability only in the myoglobin tree. In the ODC tree it associates with *Piprites pileatus*, although with posterior probabilities below 95%. In the combined tree the pipromorphine affinity of *Platyrrhinus mystaceus* receives only 74% posterior probability.
7. Within subfamily Tyranninae two major clades are recovered in the combined tree and RAG-1 and

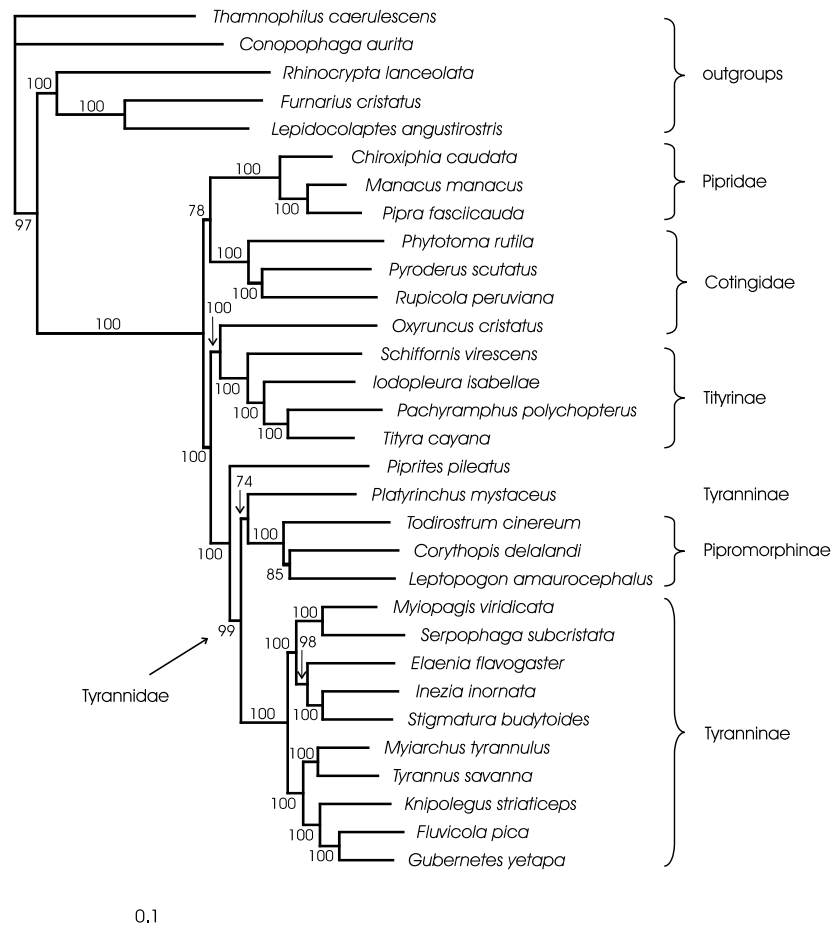


Fig. 1. Majority-rule consensus tree obtained from the Bayesian analysis of the combined data set. Numbers indicate posterior clade probabilities for individual nodes.

G3PDH gene trees. One clade includes *Elaenia*, *Inezia*, *Myiopagis*, *Serpophaga*, and *Stigmatura*, for which the internal relationships differ between the analyses, however. The other clade consists of a *Fluvicola*, *Gubernates*, and *Knipolegus* lineage as sister to *Myiarchus* and *Tyrannus*. This large clade receives 95% posterior probability or larger in all analyses, except in those of the cytochrome *b* and ODC data sets. The ODC data set suggests a highly supported, alternative arrangement within Tyranninae (Fig. 2f).

8. Tityrinae, *Oxyruncus*, *Piprites*, Pipromorphinae, and Tyranninae form a well-supported clade in the combined tree. This clade is recovered with 96% in the ODC tree but not in any of the other gene trees (Fig. 2). An alternative topology receives high support in the myoglobin data set (Fig. 2d).

### 3.3. Taxonomic distribution of morphological characters

Four morphological characters were parsimoniously mapped onto the phylogenetic tree obtained in the Bayesian analysis of the combined data set (Figs. 3a–d). The biological implications of the observed distributions are commented on below.

## 4. Discussion

### 4.1. Phylogenetic signal and congruence between gene trees

Although the gene trees in Fig. 2 exhibit several differences in their topologies, the overall pattern is that the six data partitions contain similar phylogenetic signals. This is evident when comparing only those nodes for which high posterior probabilities were obtained in the analyses (Table 5). For the other nodes there is simply too little data to yield statistical support. Non-supported relationships are not necessarily wrong, but it cannot be ruled out whether they are recovered for purely stochastic reasons. For example, the low number of well-supported nodes in the *c-myc* tree (Fig. 2a) is most likely a consequence of the low rate of mutations in this gene in combination with the rather short sequence length. As a consequence, the number of phylogenetically informative characters in this data set is low. Instances of homoplasy caused by, e.g., multiple changes at the same nucleotide position may influence phylogenetic reconstruction based on this data set considerably more than in a data set obtained from longer sequences of a more variable locus.

However, a short internode in a tree may not have arisen by chance; it could also reflect a rapid cladogenesis. Such a

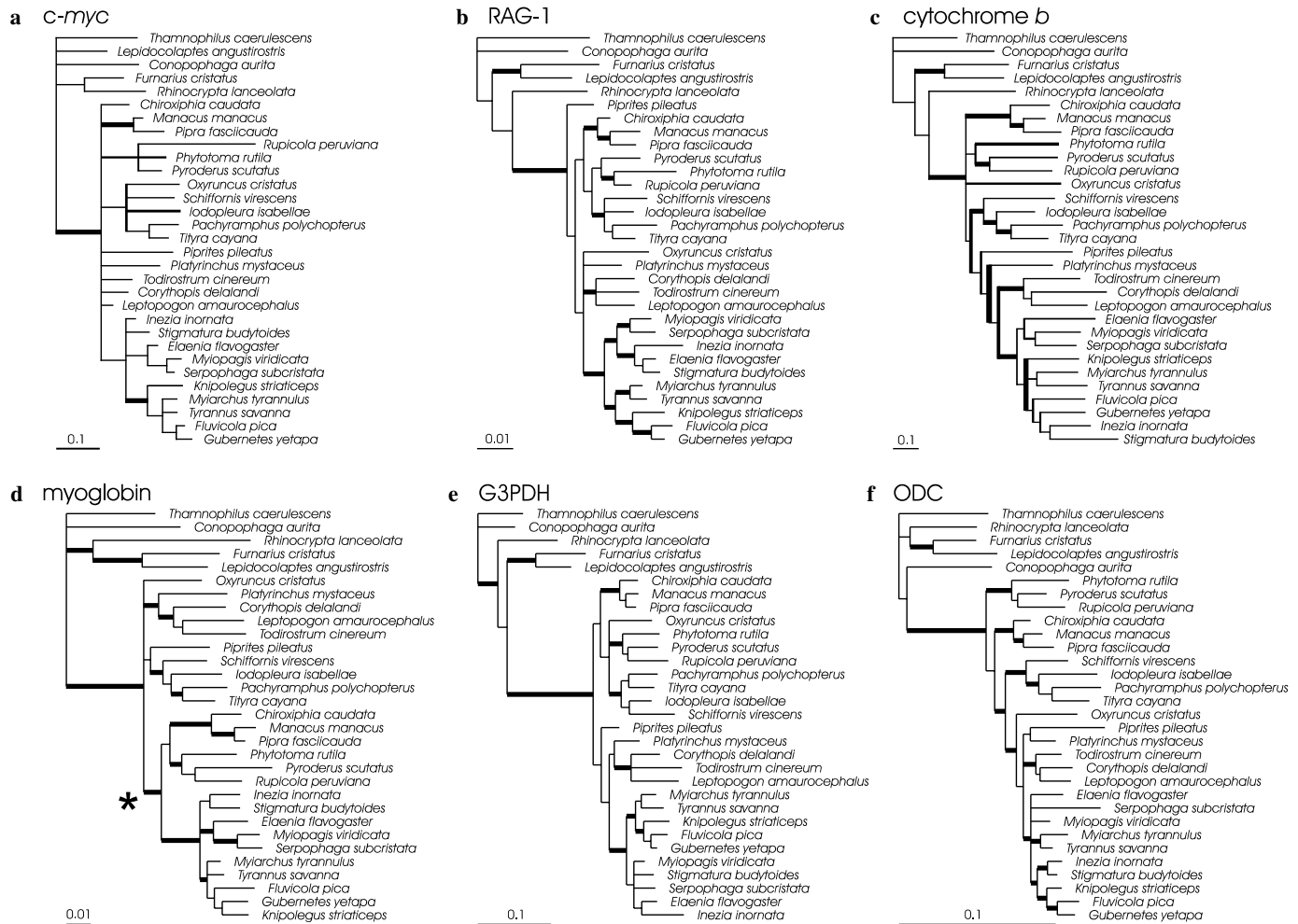


Fig. 2. Majority-rule consensus trees obtained from Bayesian analyses of individual genes: (a) *c-myc* exon 3, (b) *RAG-1*, (c) *cytochrome b*, (d) *myoglobin* intron 2, (e) *G3PDH* intron 11, (f) *ODC* introns 6 and 7. Thick branches indicate clades that received a 95% or higher posterior probability in the Bayesian analysis. Most of the highly supported clades in the gene tree are either present in other trees as well, or at least not contradicted by highly supported, alternative topologies (cf. Table 5). An exception is the well supported clade of *Pipridae*, *Cotingidae* and *Tyrannidae* in the *myoglobin* tree (marked with an asterisk)—a phylogenetic arrangement that has not been recovered in any other gene tree.

node would yield poor support in data sets where only one or few nucleotide substitutions had occurred between speciation events. In this case, the true evolutionary relationships may be revealed through observed topological congruence between different gene trees. For example, in the present analysis the monophyly of a clade including the tyrant flycatchers (*Tyrannidae*) plus *Piprites pileatus* was recovered by three of the six data sets, but posterior probabilities below 95% in all of them (Table 5). The clade was not recovered at all in the poorly resolved trees based on the slowly evolving *c-myc* and *RAG-1* genes, and the *myoglobin* data set supported an alternative topological arrangement. Despite this, the analysis of the combined data set yielded a 100% support for the *Piprites*–*Tyrannidae* clade.

#### 4.2. Higher-level relationships within *Tyrannidae*

The phylogenetic hypothesis based on the combined data set provides additional support for the monophyly of the

main clades within the *Tyrannidae*, but also give more resolution to interrelationships of these clades than previous DNA sequence data (Johansson et al., 2002; Chesser, 2004). The addition of three nuclear introns and more taxa considerably increased the resolution of more ancient relationships in the combined tree and the number of well-supported nodes compared to the results of Johansson et al. (2002). The clades corresponding to the *Cotingidae*, *Pipridae*, *Tityrinae*, *Tyrannidae*, and *Pipromorphinae* were also recognized in their analysis, even though fewer taxa were included, but the interrelationships between them were left largely unresolved (Johansson et al., 2002, Figs. 4 and 5). Chesser's (2004) analysis left the higher-level relationships within *Tyrannidae* almost unresolved, except for an 87% bootstrap support in the maximum-likelihood analysis for *Tyrannidae* together with *Pipromorphinae*, and an 84% support for *Tityrinae* with *Pipridae*.

The analysis of the combined data set suggests an early division of *Tyrannidae* into two or three major evolutionary lineages. The first leads to the tyrant flycatchers, *tityrines*,



Table 5

Posterior probabilities for selected clades recovered in the phylogenetic tree based on the concatenated data set compared to corresponding numbers for the individual gene trees

Clade present in the concatenated data set	Combined tree	Individual gene trees					
		<i>c-myc</i>	RAG-1	<i>cyt b</i>	Myoglobin	G3PDH	ODC
Monophyletic Pipridae	100%	Not recovered	100%	100%	100%	100%	100%
Monophyletic Cotingidae	100%	Yes, <95%	Yes, <95%	Yes, <95%	95%	98%	100%
Monophyletic clade with Cotingidae and Pipridae	Yes, <95%	Not recovered	Not recovered	Not recovered	Yes, <95%	Not recovered	Not recovered
Monophyletic Tityrinae	100%	Yes, <95%	99%	98%	99%	100%	100%
Monophyletic Tityrinae and <i>Oxyruncus</i>	100%	Yes, <95%	Not recovered	Not recovered	Not recovered	Not recovered	Alternative topology supported with >94%
Monophyletic Pipromorphinae	100%	Not recovered	100%	100%	99%	100%	100%
Monophyletic clade with Pipromorphinae and <i>Platyrinchus</i>	Yes, <95%	Not recovered	Not recovered	Not recovered	98%	Yes, <95%	Not recovered
Monophyletic Tyranninae	100%	Yes, <95%	100%	100%	100%	100%	99%
Monophyletic clade with <i>Elaenia</i> , <i>Inezia</i> , <i>Stigmatura</i> , <i>Myiopagis</i> , and <i>Serpophaga</i>	100%	Not recovered	99%	Not recovered	Not recovered	Yes, <95%	Alternative topology supported with >94%
Monophyletic clade with <i>Knipolegus</i> , <i>Fluvicola</i> , <i>Gubernetes</i> , <i>Myiarchus</i> , and <i>Tyrannus</i>	100%	99%	95%	Not recovered	95%	98%	Alternative topology supported with >94%
Monophyletic Tyrannidae (Tyranninae and Pipromorphinae, including <i>Platyrinchus</i> )	99%	Not recovered	Not recovered	Yes, <95%	Alternative topology supported with >94%	Yes, <95%	Not recovered
Monophyletic clade with <i>Piprites</i> and Tyrannidae	100%	Not recovered	Not recovered	Yes, <95%	Alternative topology supported with >94%	Yes, <95%	Yes, <95%
Monophyletic clade with Tityrinae, <i>Oxyruncus</i> , <i>Piprites</i> , and Tyrannidae	100%	Not recovered	Not recovered	Not recovered	Alternative topology supported with >94%	Not recovered	96%

*Oxyruncus*, and *Piprites*. Although this clade is not recovered with strong support in any of the individual gene trees, it is supported by the presence of internal cartilages in the syrinx of these taxa. A strongly supported, alternative arrangement of the major groups of Tyrannida in the gene trees is found only in the myoglobin tree. As mentioned above, the myoglobin data set does not support monophyly of the tyrant flycatchers. Instead, the subfamily Tyranninae groups with the cotingas and manakins, while the relative positions of Pipromorphinae, Tityrinae, and *Oxyruncus* are left unresolved.

The other major lineage of Tyrannida includes the cotingas and manakins. The phylogenetic tree based on the combined data set suggests monophyly of a cotinga–manakin clade, but the support is below 95%. Furthermore, only the myoglobin tree groups the cotingas and manakins together. Evidently, the current data set cannot unambiguously resolve the branching patterns between the basal radiations in Tyrannida, further supporting the prediction that the group underwent a rapid, early cladogenesis.

The present analysis confidently groups Tyranninae with Pipromorphinae, thus recovering a monophyletic Tyrannidae. With the exception of the ambiguous placement of *Platyrinchus*, our data is also congruent with Sibley and Ahlquist (1985, 1990) about the composition of the Pipromorphinae and the Tyranninae. Our findings, however, contradict Sibley and Ahlquist (1985, 1990) who suggested that Pipromorphinae was the most basal branch within Tyrannida. Non-monophyly of Tyrannidae was unexpected when first suggested in 1985, but was not disregarded off hand. Partly this was due to the novelty of using DNA data, partly to the incomplete understanding of higher-level relationships among suboscines at the time (Lanyon, 1985). McKittrick (1985) was the first to investigate the monophyly of Tyrannidae within a cladistic framework using nine morphological characters extracted from the works of Ridgway (1907), Warter (1965), Ames (1971), and W.E. Lanyon (unpublished observations). McKittrick (1985) could not corroborate monophyly of

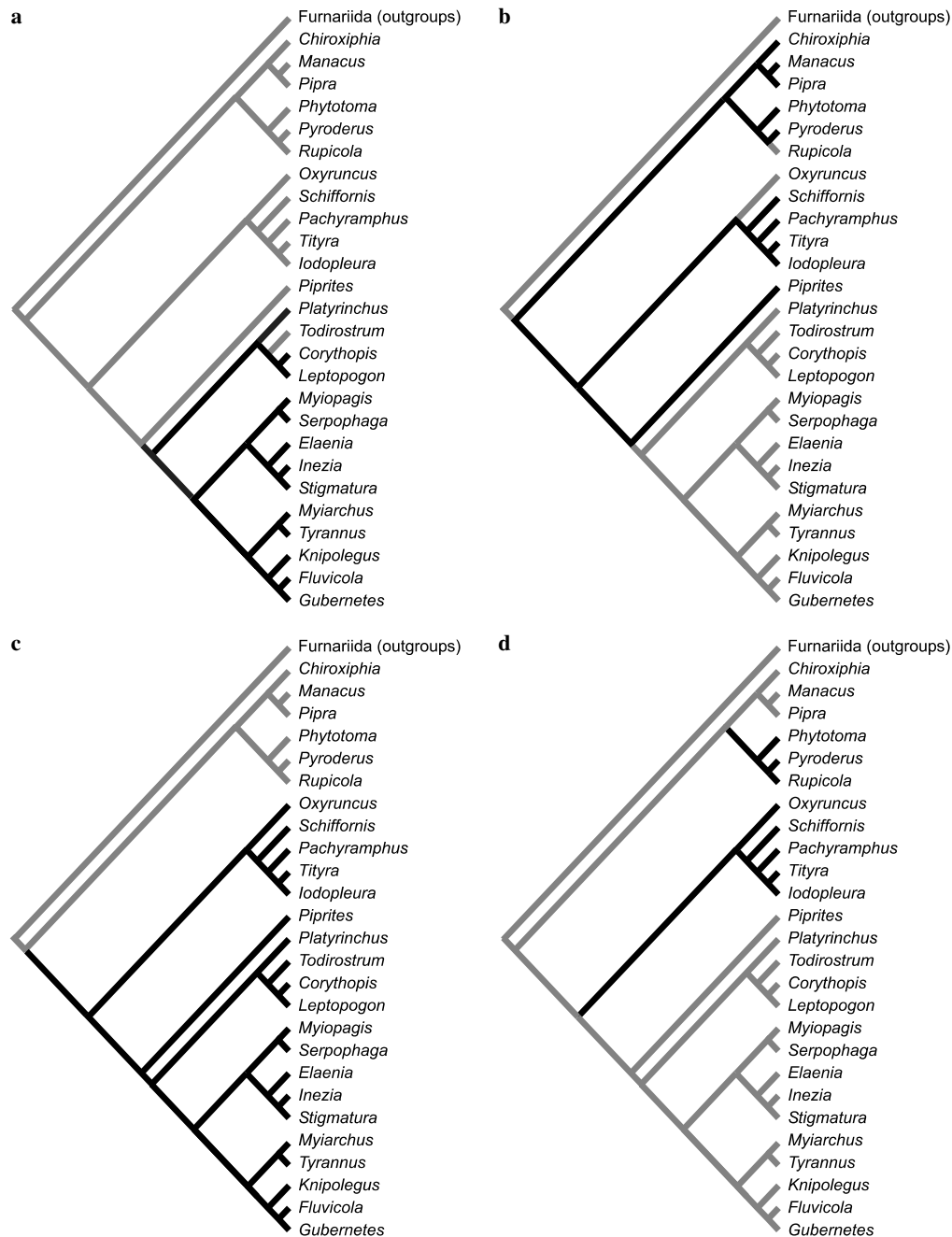


Fig. 3. Morphological character states mapped onto the phylogenetic tree based on the combined data set. (a) intrinsic syringeal muscle *M. obliquus ventralis*; (b) enlargement of the femoral artery as the main blood supply to the thigh; (c) medial (= internal) syringeal cartilages; (d) insertion of *M. tracheolateralis* on the A1/B1 membrane of the syrinx. Branches in black indicate presence of the derived state in the taxon (Table 4).

Tyrannidae by a cladistic analysis of these and other characters. In a re-analysis of available morphological characters, Birdsley (2002) also came to the conclusion that monophyly of Tyrannidae is ambiguous. In his analysis, an equally parsimonious result was that a group of flatbill and tody-tyrant genera are more distantly related to the other tyrant flycatchers than are cotingas and manakins (Birdsley, 2002, p. 726). However, the monophyly of the tyrannid clade as identified in this analysis was proposed by Prum (1990) based on the shared possession of the intrinsic syringeal muscle *M. obliquus ventralis* (see

below). Even though the taxon sampling within the Tyranninae is modest, the combined tree also successfully groups the 10 genera sampled into three clades that are congruent with the traditional subfamilies of Traylor (1977, 1979): Elaeniinae (the *Myiopagis* through *Stigmatura* clade), Fluvicolinae (*Fluvicola*, *Gubernetes*, and *Knipolegus*), and Tyranninae (*Tyrannus* and *Myiarchus*) (Fig. 1).

The identification of a monophyletic Tityrinae is congruent with previous molecular analyses (Prum et al., 2000; Johansson et al., 2002; Chesser, 2004), but this eclectic clade

was originally proposed on the basis of morphological synapomorphies to exclude *Tityra* (Prum and Lanyon, 1989). These well-supported phylogenetic results imply: (1) that syringeal morphology can be highly phylogenetically informative in suboscine passerines (i.e., sufficient to identify a clade of genera distributed among three families), and (2) that the syringeal morphology of *Tityra* has been secondarily radically simplified. The syringeal morphology of *Tityra* is typical of a generalized cotinga, and, except for some very rudimentary medial cartilages, *Tityra* entirely lacks the anatomical synapomorphies of the group (Prum and Lanyon, 1989; Prum, 1990).

The sharpbill, *Oxyruncus cristatus*, is a problematic tyrannoid that has proven exceedingly difficult to place systematically. Allozyme distance data (Lanyon, 1985) suggested that it is related to *Pachyramphus*, *Tityra*, and *Piprites*, whereas DNA–DNA hybridization data place it among the cotingids (Sibley et al., 1984; Sibley and Ahlquist, 1985, 1990). The main hindlimb artery in *Oxyruncus* is the ischiadic as in Tyrannidae, whereas most cotingids, manakins, and tityrines possess an enlarged femoral artery (Prum, 1990). Based on cytochrome *b* sequence data, Prum et al. (2000) placed *Oxyruncus* within Cotingidae in a clade of aberrant cotingas that lack the enlarged femoral artery. However, that conclusion was flawed by the apparent use of a nuclear copy of cytochrome *b* for *Oxyruncus*. In this analysis, *Oxyruncus* groups confidently as the sister group to the Tityrinae. This position is supported independently by Lanyon's (1985) allozyme data and by three derived syringeal characters Prum and Lanyon (1989; see below): presence of derived horse-shoe shaped medial cartilages, the insertion of oblique ventral intrinsic syringeal muscles on the A1/B1 membrane, and the insertion of the *M. tracheolateralis* on the ventral ends of the A1 elements (shared with *Pachyramphus* and *Xenopsaris* and reversed in other members).

Traditionally, *Piprites* has been treated as an odd manakin. Although Prum (1990) removed it from the piprids, he was unable to identify a well-supported hypothesis for its phylogenetic relationships to the major tyrannoid clades. Herein, we present evidence that *Piprites*, represented by the species *pileatus*, is an ancient lineage of the Tyrannida, and that it is the sister group to the tyrant flycatchers. The relationship to the tyrannids is in agreement with Ames (1971) who placed *Piprites* with the tyrant flycatchers based on syringeal morphology. However, the only derived syringeal character shared by *Piprites* and the tyrannids is the presence of medial syringeal cartilages (Prum, 1990). DNA of *Piprites*, which was not available for DNA–DNA hybridizations in the 1980s, was first analyzed by Prum et al. (2000) who included *Piprites chloris* in a study of Cotingidae. The only conclusion supported was that *Piprites* is not a cotinga or a manakin (no tyrant flycatcher was included in the study).

#### 4.3. Morphological variation

As hypothesized by Prum (1990), the presence of the intrinsic syringeal muscle *M. obliquus ventralis* is optimized

as a synapomorphy of the Tyrannidae in the combined tree (Fig. 3a). This optimization also indicates a loss of *M. obliquus ventralis* in *Todirostrum*, however, the overwhelming majority of the tyrant flycatchers possess this syringeal muscle. It has been secondarily lost in *Neopipo*, *Hirundinea*, *Pyrrhomyias*, *Myiobius*, *Terenotriccus*, *Zimmerius*, *Machetornis*, *Todirostrum*, *Poecilotriccus*, and *Onychorhynchus* (Ames, 1971; McKittrick, 1985; Prum, 1990; Mobley and Prum, 1995; Birdsley, 2002). Its loss may define restricted clades within the Tyrannidae (Mobley and Prum, 1995). Ames (1971) reported the presence of *M. obliquus ventralis* in a few taxa outside the tyrant flycatcher clade (e.g., *Oxyruncus*, *Iodopleura*, *Laniisoma*, and *Laniocera*), but Prum (1990) regarded the intrinsic muscles in *Oxyruncus* and the *Schiffornis* groups as sufficiently different to be non-homologous with *M. obliquus ventralis* of the tyrant flycatcher clade. A non-homologous *M. obliquus ventralis* has also convergently evolved in *Sapayoa aenigma* (Prum, 1990; Fjeldså et al., 2003).

Among Tyrannida, the femoral artery is enlarged in all known piprids and cotingids except the cotingid genera *Snowornis*, *Pipreola*, *Ampelioides*, *Carpornis*, *Rupicola*, and *Phoenicircus* (Prum and Lanyon, 1989; Prum, 1990). Enlargement of the femoral artery, instead of the ischiadic, is a condition that can be optimized in the combined tree as a synapomorphy for the entire Tyrannida with reversals in *Rupicola*, *Oxyruncus*, and the tyrannids (Fig. 3b). However, it is equally parsimonious to optimize this character as convergently evolved in the cotingid–piprid clade (subsequently lost in *Rupicola*), in the Tityrinae, and in *Piprites*. Both optimizations require four steps.

Medial syringeal cartilages are present in all tyrant flycatchers and tityrines included herein as well as in *Oxyruncus* and *Piprites pileatus*, and their presence may thus be optimized as a synapomorphy for this clade in the combined tree (Fig. 3c). In addition to the taxa included here, medial syringeal cartilages are present in the tityrine genera *Xenopsaris*, *Laniisoma*, and *Laniocera* (Ames, 1971; Prum and Lanyon, 1989; Prum, 1990), which is congruent with the optimization suggested in the present analysis. Medial syringeal cartilages are also present in at least two species of the cotingid genus *Lipaugus* (*L. unirufus* and *L. vociferans*), and in the piprid genera *Neopelma* and *Tyrannetes* (Prum and Lanyon, 1989; Prum, 1990), but these are independently derived (Chesser, 2004; Ohlson et al., in press). Birdsley (2002) subdivided the medial syringeal cartilage character complex into eleven independent characters in search for synapomorphies for the tyrant flycatcher clade. He found no such character. Instead it seems as the character complex as a whole may provide morphological support for a taxonomically more inclusive clade consisting not only of tyrant flycatchers, but also the tityrines and other more or less “problematic” members of Tyrannida—an idea first suggested by McKittrick (1985).

The insertion of *M. tracheolateralis* on the A1/B1 lateral membrane of the syrinx is a derived condition present in Cotingidae. In *Oxyruncus* and the Tityrinae, the intrinsic

syringeal muscles also insert on the A1/B1 lateral membrane. Prum (1990) and Prum et al. (2000) hypothesized that these intrinsic muscles were derived from the cotingid condition. The combined tree suggests that the insertion of syringeal muscles on the A1/B1 lateral membrane evolved independently in the cotingas and in the ancestor of *Oxyruncus* and the Tityrinae (Fig. 3d). Alternatively but less parsimoniously, if the two states of muscle insertion were homologous, then the derived insertion would be a synapomorphy of the Tyrannida, and later lost in the ancestor of the manakins, and in the ancestor of the *Piprites*—tyrant flycatchers clade (Fig. 3d).

Almost all of the additional morphological characters that have been suggested to be phylogenetically informative have a complicated taxonomic distribution within Tyrannida. This problem has long been realized and is the most important reason why the higher-level systematics of this group were so difficult to resolve in the pre-DNA era, even after the application of phylogenetic methods. The present study, which targets four of the phylogenetically most promising characters, also bears evidence of this. Although all four characters apparently may be optimized as synapomorphies for subgroups of Tyrannida, there are most often taxa nested within these groups that lack the derived morphology. This calls for ad hoc explanations like secondary losses (reversals) or convergent evolution of character states. However, the systematic relationships within Tyrannida still are far from being fully understood. Only when we have solid phylogenetic hypotheses for at least the higher-level relationships is it possible to reconstruct the history of morphological evolution of this clade.

#### 4.4. Taxonomic implications

When Prum and Lanyon (1989) first suggested that six genera from all three major families of the Tyrannida—*Schiffornis*, *Laniisoma*, *Laniocera*, *Iodopleura*, *Pachyramphus* and *Xenopsaris*—formed the monophyletic *Schiffornis* group, this hypothesis was rather unexpected. However, molecular studies (Prum et al., 2000; Johansson et al., 2002; Chesser, 2004; this study; Ohlson et al., in press) have consistently recovered members of the *Schiffornis* group plus *Tityra* as a clade and a deep branch within Tyrannida. Furthermore, this study does not support the conclusion of Prum et al. (2000) that this clade can be recognized as a subfamily Tityrinae in an expanded and monophyletic Cotingidae. Rather, this clade has closer relationships to the tyrannids than to the cotingids and piprids. Therefore, we propose the recognition of the Tityrinae of Prum et al. (2000)—including *Tityra* and the six genera in the *Schiffornis* group—as a family level taxon; Tityridae. This treatment puts more emphasis on the great morphological and ecological diversity in Tyrannida than does an inclusion of all members of the group in one single family.

This study also supports the placement of *Oxyruncus* as the sister to the rest of the Tityridae. This phylogenetic relationship is congruent with a number of derived syringeal

characters for the group identified by Prum and Lanyon (1989), including the horse-shoe shaped medial cartilages and the insertion of the tracheolateralis on the ventral ends of the A1 elements. Furthermore, a similar placement was suggested by allozyme data (Lanyon, 1985). In recognition of the congruence between three data sets of remarkably different nature (i.e., DNA sequences, allozyme distances, and derived syringeal morphology characters), we recommend that *Oxyruncus* be placed within the family Tityridae as its basal member.

We prefer the name Tityridae over Oxyruncidae for the more inclusive clade that includes both *Tityra* and *Oxyruncus* even though, strictly speaking, Oxyruncidae Ridgway, 1906 (1831) has priority over Tityridae G. R. Gray, 140 (1832–33) (see Bock, 1994). The name Tityridae should be conserved because it has been consistently recognized to apply to a diverse taxon including, since Traylor (1977, 1979), *Tityra* and *Pachyramphus*, and since Prum (2001), *Tityra* and the *Schiffornis* group genera. In contrast, Oxyruncidae has always been used to refer to a family that includes only the monotypic genus *Oxyruncus*.

From our phylogeny, an argument could be also made for the inclusion of *Piprites* in the Tyrannidae, but we await a more comprehensive study and evidence of congruence with other data sets before any taxonomical proposals are made for this genus. Hence, we tentatively place *Piprites* as a genus *incertae sedis* within the Tyrannida.

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