The Auk 119(4):984–995, 2002

BASAL PHYLOGENY OF THE TYRANNOIDEA BASED ON COMPARISONS OF CYTOCHROME *b* AND EXONS OF NUCLEAR c-*myc* AND RAG-1 GENES

ULF S. JOHANSSON,[1,2,4] MARTIN IRESTEDT,[1,2] THOMAS J. PARSONS,[3] AND PER G. P. ERICSON[1]

[1]*Department of Vertebrate Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden;*
[2]*Department of Zoology, University of Stockholm, SE-106 91 Stockholm, Sweden; and*
[3]*U.S. Armed Forces DNA Identification Laboratory, Armed Forces Institute for Pathology, 1413 Research Boulevard, Rockville, Maryland 20850, USA*

ABSTRACT.—The outlines of the phylogenetic relationships within the New World suboscine clade Tyrannoidea were investigated on the basis of nucleotide sequence data from two nuclear genes (c-*myc* and RAG-1) and one mitochondrial gene (cytochrome *b*), totaling over 2,400 bp. Representatives of the major tyrannoid lineages were sequenced, including *Pachyramphus*, *Schiffornis*, *Tityra*, and *Oxyruncus*. The data set with the three genes combined was analyzed under both the parsimony and maximum-likelihood criteria and under different character weighting schemes. The analyses resulted in similar topologies that differed only in poorly supported nodes. The three manakins (*Pipra*, *Manacus*, and *Chiroxiphia*) included in this study were found to be monophyletic, whereas *Schiffornis*—sometimes also considered to be a manakin—did not group with the manakins, but occurred with *Pachyramphus* and *Tityra* in the clade Tityrinae. The two clades Pipromorphinae and Tyranninae are also strongly supported in this analysis and appear as sister groups, thus supporting the monophyly of the tyrant flycatcher assemblage. *Phytotoma* was placed with the only cotingid species included in this analysis, whereas the position of *Oxyruncus* was unresolved.
Received 10 October 2000, accepted 6 May 2002.

RESUMEN.—Se investigó el perfil de las relaciones filogenéticas dentro del clado suboscino del Nuevo Mundo Tyrannoidea en base a datos de secuencias de nucleótidos de dos genes nucleares (*c-myc* y RAG-1) y un gen mitocondrial (citocromo *b*), con un total que sobrepasó las 2,400 pb. Se secuenciaron representantes de los principales linajes del clado Tyrannoidea, incluyendo *Pachyramphus*, *Schiffornis*, *Tityra*, y *Oxyruncus*. El conjunto de datos, con los tres genes combinados, fue analizado bajo los criterios de parsimonia y de máxima probabilidad y bajo diferentes esquemas de peso de los caracteres. Los análisis produjeron topologías similares que sólo difirieron en los nodos pobremente resueltos. Se encontró que los tres géneros *Pipra*, *Manacus*, y *Chiroxiphia* incluidos en este estudio fueron monofiléticos, mientras que *Schiffornis*, que a veces también se ha considerado como perteneciente a este grupo, no se agrupó con ellos, pero ocurrió con *Pachyramphus* y *Tityra* en el clado Tityrinae. Los dos clados Pipromorphinae y Tyranninae también fueron apoyados fuertemente por este análisis y aparecen como grupos hermanos, apoyando la monofilia del grupo de los cazamoscas tiránidos. *Phytotoma* se encontró con las únicas especies cotíngidas incluidas en este análisis, mientras que la posición de *Oxyruncus* fue irresoluta.

TYRANNOIDEA, ONE OF THE two major clades of New World suboscines, contains ~537 species (Sibley and Ahlquist 1990) and includes, for example, tyrant flycatchers, manakins, and cotingas. The vast majority of the tyrannoid species are confined to the Neotropics, but a few migratory species also occur in the Nearctic. Monophyly of this group has been proposed on the basis of myological data (Raikow 1987) and has further been corroborated by DNA–DNA hybridization (Sibley and Ahlquist 1990) and nuclear DNA sequence analyses (Irestedt et al. 2001).

The basal phylogenetic relationships within Tyrannoidea are less well understood. In many traditional classifications, the species included in that clade have been grouped into the five families Tyrannidae (tyrant flycatchers), Pipridae (manakins), Cotingidae (cotingas), Oxyruncidae (sharpbill), and Phytotomidae (plant-

[4] E-mail: ulf.johansson@nrm.se

cutters). The latter two families contain a single genus each that include one and three species, respectively. The phylogenetic positions of Oxyruncidae and Phytotomidae are uncertain, and they have variously been considered distinct families or included in any of the other families.

In addition to the taxa mentioned above, a few other enigmatic taxa have been difficult to place in any of the traditionally recognized families. Six of those taxa are *Iodopleura*, *Laniisoma*, *Laniocera*, *Xenopsaris*, *Schiffornis*, and *Pachyramphus*. Traditionally these genera have not been considered to be closely related, but have been allocated to different families. Prum and Lanyon (1989), however, suggested that these taxa actually constitute a monophyletic assemblage, referred to as the ''*Schiffornis*-group'', based on two syringeal characters. That group has also been recovered in a phylogenetic analysis based on 339 base pairs (bp) of the cytochrome-*b* gene (Prum et al. 2000), although this study further suggested that *Tityra* also is part of that clade, contrary to the result based on morphology. The clade containing the ''*Schiffornis*-group'' and *Tityra* has been referred to as Tityrinae (Prum et al. 2000). Certain morphological characters traditionally considered to be important in tyrannid classification suggest an affinity between Tityrinae and tyrant flycatchers (e.g. the possession of internal syringeal cartilage and an intrinsic syringeal muscle with an oblique fiber direction), whereas other characters 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 cotingids, suggesting that the Tityrinae is part of that radiation. Furthermore, their molecular data suggested a sister group relationship between the tityrines and the traditional cotingas.

Monophyly of the Tityrinae has also been supported by DNA–DNA hybridization data (Sibley and Ahlquist 1990), although *Pachyramphus*, *Tityra*, and *Schiffornis* were the only tityrinae genera included in that study. However, the DNA–DNA hybridization study did not support the cotingid affinity of Tityrinae, but grouped the tityrines with certain tyrant flycatchers (Fig. 1).

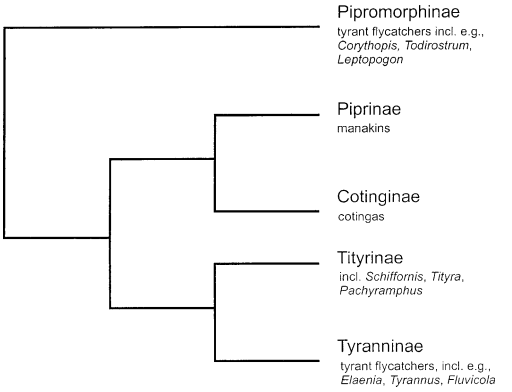

FIG. 1. A hypothesis of the phylogenetic relationships within Tyrannoidea based on DNA–DNA hybridization data (Sibley and Ahlquist 1990).

One of the more unexpected findings of the DNA–DNA hybridization study (Sibley and Ahlquist 1985, 1990) was that a group of genera traditionally regarded as tyrant flycatchers formed the sister group of all other tyrannoids, including manakins and cotingas (Fig. 1). That novel clade, named Pipromorphinae, included eight genera. Among those are *Mionectes*, *Todirostrum*, and *Leptopogon*.

In this study, we investigate the higher-level phylogenetic relationships within the Tyrannoidea on the basis of DNA sequence data from exons of two nuclear genes (c-*myc* and RAG-1) and one mitochondrial gene (cytochrome *b*), a total of 2,406 bp. The cytochrome-*b* gene has been widely used in avian phylogenetic studies, whereas the two nuclear genes only recently have received attention in this type of study (Groth and Barrowclough 1999, Ericson et al. 2000, Johansson et al. 2001, and Irestedt et al. 2001). Because these three genes have different properties and rates of base substitution (see e.g. Johansson et al. 2001 for a comparison of the two nuclear genes), they may be informative at different phylogenetic levels. In addition, congruence between different gene trees that presumably belong to different linkage groups would increase the probability that these trees actually reflect the evolutionary history of the group.

TABLE 1. Specimen and GenBank accession numbers for the samples used in the study. The taxonomy follows Traylor 1979. Acronyms: NRM = Swedish Museum of Natural History, ZMCU = Zoological Museum of the University of Copenhagen.

Family	Species	Sample no.	Origin	GenBank accession no.		
				c-*myc*	RAG-1	cyt *b*
Tyrannidae[a]	*Corythopsis delalandi*	NRM 937282	Paraguay	AF453779	AF453792	AF453805
Tyrannidae	*Myiopagis viridicata*	NRM 986779	Paraguay	AF453780	AF453793	AF453806
Tyrannidae	*Elaenia flavogaster*	NRM 966970	Paraguay	AF377279	AF453794	AF453807
Tyrannidae	*Leptopogon amaurocephalus*	NRM 937317	Paraguay	AF453781	AF453795	AF453808
Tyrannidae	*Todirostrum cinereum*	NRM 947036	Paraguay	AF453782	AF453796	AF453809
Tyrannidae	*Fluvicola pica albiventer*	NRM 956714	Paraguay	AF453783	AF453797	AF453810
Tyrannidae	*Gubernetes yetapa*	NRM 976700	Paraguay	AF295166	AF295188	AF453811
Tyrannidae	*Myiarchus tyrannulus*	NRM 937173	Paraguay	AF453784	AF453798	AF453812
Tyrannidae	*Tyrannus savana*	NRM 976722	Paraguay	AF295182	AF295203	AF453813
Tyrannidae	*Tityra cayana*	NRM 956584	Paraguay	AF295181	AF295202	AF453814
Tyrannidae	*Pachyramphus polychopterus*	NRM 967032	Paraguay	AF453785	AF453799	AF453815
Pipridae	*Schiffornis virescens*	NRM 937315	Paraguay	AF453786	AF453800	AF453816
Pipridae	*Pipra fasciicauda*	NRM 947271	Paraguay	AF295175	AF295196	AF453817
Pipridae	*Manacus manacus*		Trinidad	AF453787	AF453801	AF453818
Pipridae	*Chiroxiphia caudata*	NRM 956620	Paraguay	AF453788	AF453802	AF453819
Cotingidae	*Pyroderus scutatus*	NRM 967030	Paraguay	AF453789	AF453803	AF453820
Oxyruncidae	*Oxyruncus cristatus*	NRM 967078	Paraguay	AF453790	AF453804	AF453821
Phytotomidae	*Phytotoma rutila*	ZMCU S466	Bolivia	AF295173	AF295194	AF453822
Conopophagidae	*Conopophaga lineata*	NRM 956653	Paraguay	AF295163	AF295185	AY078173
Formicariidae	*Thamnophilus caerulescens*	NRM 967007	Paraguay	AF295180	AF295201	AY078176
Furnariidae	*Furnarius cristatus*	NRM 966772	Paraguay	AF295165	AF295187	AY064279
Dendrocolaptidae	*Lepidocolaptes angustirostris*	NRM 937184	Paraguay	AF295168	AF295190	AY078175
Rhinocryptidae	*Rhinocrypta lanceolata*	NRM 966793	Paraguay	AF295178	AF295199	AY078174

[a] Placed in Conopophagidae in Peters (1951:277), but shown by Ames et al. (1968) to belong to Tyrannidae.

METHODS

Taxon sampling and outgroups.—Eighteen species of tyrannoids were selected to represent the five traditionally recognized families (Traylor 1979), as well as the major lineages proposed by previous phylogenetic studies (W. E. Lanyon 1984, 1985, 1986, 1988a, b; Prum and Lanyon 1989; Prum 1990; Sibley and Ahlquist 1990). The trees were rooted according to the outgroup criterion (Farris 1972, Nixon and Carpenter 1993) with five representatives (*Thamnophilus*, *Conopophaga*, *Furnarius*, *Lepidocolaptes*, and *Rhinocrypta*) of the proposed sister group Furnarioidea (Sibley and Ahlquist 1990, Irestedt et al. 2001). Sample information of the included taxa and GenBank accession numbers are given in Table 1.

Extraction, amplification, and sequencing.—Laboratory procedures for the extraction, PCR-amplification, and sequencing of the two nuclear genes, RAG-1 and c-*myc*, follow protocols described by Ericson et al. (2000) and Irestedt et al. (2001). A similar protocol was also followed for the amplification and sequencing of the cytochrome-*b* gene. Initially, ~1,000 bp of this gene were amplified as a single fragment with either of the primer pairs L14841 (Kocher et al. 1989) and H15915 (Edwards and Wilson 1990) or L14841 together with Thr 1 (5′-TCT TTG GCT TAC AAG ACC AA-3′). The thermocycling conditions included an initial denaturation at 94°C for 5 min, followed by 40°C cycles of 94°C for 40 s, 49°C for 40 s, 72°C for 1 min, and completed with a final extension at 72°C for 5 min. The PCR products were cleaned with QIAquick™ PCR Purification Kit (Qiagen®, Valencia, California) following the protocol of the manufacturer. Sequencing reactions were carried out with Perkin Elmer Applied BioSystems PRISM terminator cycle sequencing kits with AmpliTaq FS polymerase and BigDye terminators, following the manufacturer's protocol. For the sequencing reactions, the following primers were used: L14841, P5L (5′-CCT TCC TCC ACG AAA CAG GCT CAA ACA ACC C-3′), H658 (5′-TCT TTG ATG GAG TAG TAG GGG TGG AAT GG-3′), and H15915 or Thr 1, with P5L and H658 as internal primers on the light and heavy strands, respectively. Sequencing products were run on a Perkin Elmer Applied BioSystems 377 automated fluorescent sequencing instrument. For each taxon, the multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SeqMan II™ (DNASTAR Inc., Madison, Wisconsin). At a few positions the nucle-

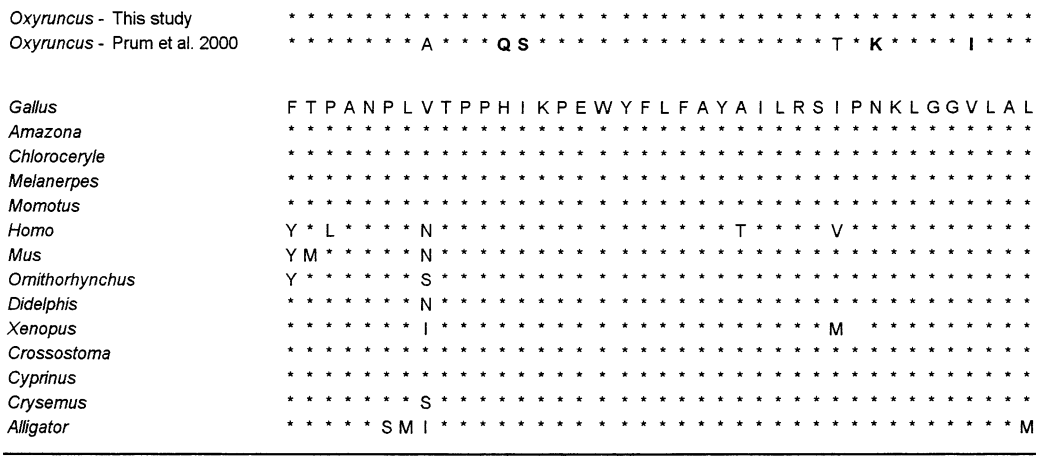

FIG. 2. Aligned amino acid sequences from a broad range of vertebrate taxa showing a highly conserved region of the cytochrome-*b* gene (position 15661 to 15777 relative the published *Gallus* sequence; Desjardin and Morais 1990). The alignment is based on sequences from five birds (*Gallus gallus*—GenBank accession number X52392; *Amazona ventralis*—U89178; *Chloroceryle americana*—U89183; *Melanerpes carolinus*—U89192; *Momotus mexicanus*—U89187), four mammals (*Homo sapiens*—J01415; *Mus musculus*—J01420; *Ornitorhynchus anatinus*—X83427; *Didelphis virginiana*—Z2957), one amphibian (*Xenopus laevis*—M10217), two fish (*Cyprinus carpio*—X61010; *Crossostoma lacustre*—M91245), one turtle (*Chrysemus picta*—AF069423), and one alligator (*Alligator mississippensis*—NC_001922). Nucleotides identical with the *Gallus* sequence are indicated with an asterisk. A comparison of the *Oxyruncus cristatus* sequences derived from this study with the published sequences by Prum et al. (2000) (GenBank accession number AF123631) shows that the *Oxyruncus* sequence from Prum et al. (2000) has acquired several amino acid substituions relative other vertebrates (unique changes shown in bold), indicating that this is likely to be a nuclear copy of the cytochrome-*b* gene.

otide could not be determined with certainty, and those 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™ (DNASTAR Inc., Madison, Wisconsin). No indels were observed and homologous positions were easily recognized.

Before the phylogenetic analysis, we compared our cytochrome-*b* sequences with previously published sequences deposited in GenBank. That revealed that our *Oxyruncus* sequence is different from that deposited in GenBank (accession number AF123631) by Prum et al. (2000). The two sequences differ in 83 out of 375 positions (22%), which is too many differences for conspecific cytochrome-*b* sequences. Because sample mix-up is a potential source of error in molecular studies, we sequenced a second individual of *Oxyruncus cristatus* (NRM 967091) collected at the same locality in Paraguay as the first individual. The two sequences were found to be identical (data not shown). Another likely source to the observed differences is that one of the sequences is a nuclear copy of the cytochrome-*b* gene (Arctander 1995, Quinn 1997, Sorenson and Quinn 1998). Amplifications of nuclear copies are more likely for certain types of source material, for example blood samples, which are relatively poor in mitochondria (Quinn 1997). Both our samples of *Oxyruncus* are extracted from muscle tissue. Nonfunctional nuclear copies of a mitochondrial protein-coding gene can possibly be detected by the unexpected presence of stop codons, indels, and mutations in regions conserved by structural constraints (Sorenson and Quinn 1998). To investigate the occurrence of unusual substitutions, we aligned the translated protein sequences of the cytochrome-*b* gene from a broad range of vertebrate taxa to identify highly conserved regions (Fig. 2). The result shows that in the region between position 15661 and 15777 in the published *Gallus* mitochondrial genome sequence (Desjardins and Morais 1990), our *Oxyruncus* sequence is identical with other vertebrates, whereas the *Oxyruncus*-sequence previously deposited in GenBank have acquired six amino acid substitutions. That suggests that the sequence analyzed in Prum et al. (2000) is likely to be of a nuclear origin.

Gene properties.—The analyzed portion of the c-*myc* gene corresponds to the 477 bp (159 codons) long re-

gion between positions 756 and 1233 of exon 3 in the published *Gallus* sequence (Watson et al. 1983). Of the 477 bp, only 52 (11%) positions are variable, whereof 24 are uninformative. In addition, all but eight of those variable positions are at a third codon position. Within the ingroup taxa, the pairwise uncorrected sequence divergences range from 0.6% (*Fluvicola* and *Gubernetes*) to 2.6% (e.g. *Gubernetes* and *Pachyramphus*). Distances between the ingroup taxa and the furnaroid outgroups range between 1.9% (*Corythopis* and *Lepidocolaptes*) to 4.2% (*Phytotoma* and *Conopophaga*).

The analyzed portion of the single exon of the RAG-1 gene corresponds to the 930 bp (310 codons) between positions 1054 and 1983 in the chicken sequence (Carlson et al. 1991). Of those 930 bp, 176 characters were variable but only 65 were phylogenetically informative. The pairwise sequence divergence is low, ranging from 0.6% (*Fluvicola* and *Gubernetes*) to 3.7% (*Fluvicola* and *Phytotoma*). Between the ingroup and outgroup taxa the divergences range from 3.3% (*Chiroxiphia* and *Rhinocrypta*) to 5.7% (*Phytotoma* and *Thamnophilus*).

The analyzed portion of the cytochrome-*b* gene corresponds to the 999 basepairs (333 codons) between position 15037 and 16035 in the chicken mitochondrial genome sequence (Desjardins and Morais 1990). Of those, 531 bp (53%) are constant across taxa, 78 (8%) uninformative, and 390 (39%) phylogenetically informative. The pairwise sequence divergences within the ingroup range from 12.1% (*Manacus* and *Chiroxiphia*) to 20.5% (*Corythopis* and *Chiroxiphia*). The sequence divergences within the ingroup taxa are almost as high as those between the ingroup and outgroup taxa that range from 16.0% (*Pachyramphus* and *Rhinocrypta*) to 21.2% (*Phytotoma* and *Thamnophilus*).

Phylogenetic analyses.—The sequences from the three individual gene fragments were combined into a single matrix and analyzed with PAUP* 4.0b8 (Swofford 1998) under the parsimony and maximum-likelihood criteria. The three genes were also evaluated separately to compare the information provided by the individual genes. Minimum-length tree(s) were identified using heuristic searches with 500 random taxon additions and TBR branch swapping.

In the initial parsimony analysis of the combined data set, all characters were given equal weight. Saturation plots of the cytochrome-*b* gene have indicated that some partitions of the gene, especially transitions at third positions, may be saturated due to multiple substitutions when distantly related taxa are compared (Irwin et al. 1991). That saturation may obscure the phylogenetic signal, and in some studies the exclusion of these positions have improved the phylogenetic resolution and increased the bootstrap support (e.g. Groth 1998). However, those seemingly saturated positions may in fact contain phylogenetic information, and their inclusion may increase the number of supported nodes, especially when large data sets are analyzed (Källersjö et al. 1999). To evaluate the possibility of saturation in the cytochrome-*b* gene, the observed pairwise number of transitions and transversions at each codon position were plotted against the uncorrected (''p'') distances (Fig. 3). A nonlinear correlation for any of those partitions can be used as an indication of saturation (Moritz et al. 1987). Transitions at third positions in cytochrome *b* may be saturated (Fig. 3A), and those positions were thus excluded in an additional analysis of the combined data set. Also when analyzed separately, the cytochrome-*b* gene was evaluated with all positions equally weighted and with transitions at third positions excluded. Transitions were excluded by recoding all Cs to Ts and all As to Gs at third positions in MACCLADE (v. 3.0; Maddison and Maddison 1992). Saturation plots for the RAG-1 and c-*myc* genes (Groth and Barrowclough 1999, Irestedt et al. 2001, Johansson et al. 2001) have shown a linear correlation between the number of substitutions and sequence distances for even older divergences than are analyzed here. Consequently, all partitions of those genes are assigned equal weights in the parsimony analyses.

Support for individual clades was estimated by parsimony jackknifing (Farris et al. 1996) as implemented in XAC: Parsimony Jackknifer (Farris 1997) with 1,000 replicates, each with 10 random additions of taxa and branch swapping. Similar to a bootstrap analysis, parsimony jackknifing measures the nodal support by creating pseudoreplicates of the original data matrix. However, instead of randomly resampling all characters, each jackknifing pseudoreplicate excludes ~37% (e^{-1}) of the original characters. That deletion frequency is expected to yield support values comparable to those obtained by bootstrapping (Farris et al. 1996).

The model for the maximum-likelihood analysis was selected with the likelihood-ratio test implemented in MODELTEST 3.06 (Posada and Crandall 1998), which chooses the simplest model that cannot be rejected in favor of a more complex model. On the basis of the test of maximum-likelihood models, the general-time reversal (GTR) model with an estimate of invariable sites and a discrete (four rate categories) gamma distribution model of among site rate heterogeneity was selected for analysis in PAUP*. The search for the best-fit maximum-likelihood tree was performed in a stepwise procedure according to suggestions by J. Huelsenbeck (pers. comm.). In the first step, the gamma shape parameter, proportion of

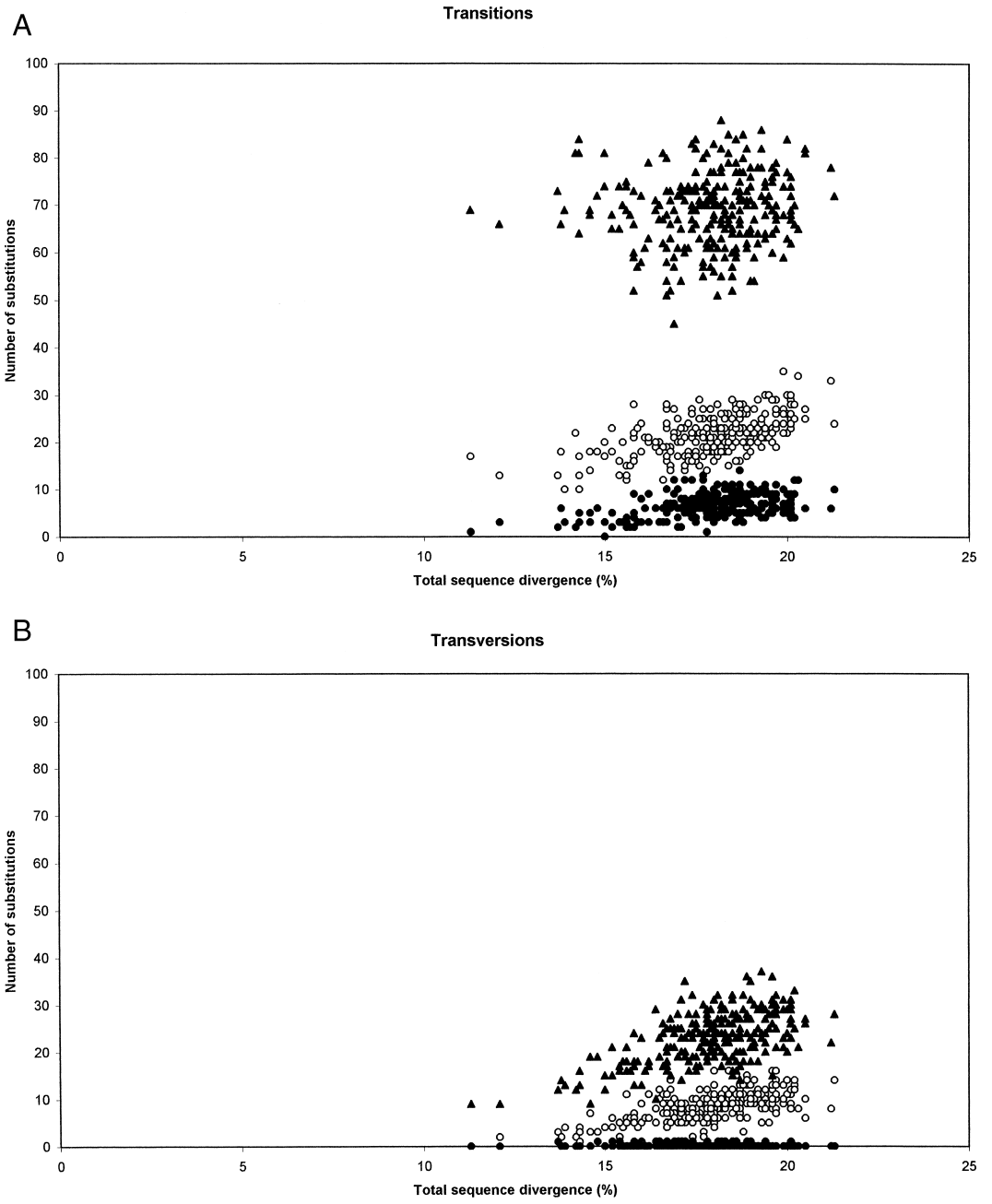

FIG. 3. Saturation plot for the cytochrome-*b* gene. Number of substitutions—(A) transitions, (B) transversion—for each pairwise comparison of taxa plotted against the pairwise uncorrected sequence divergence. Open circles indicate first positions, closed circles second positions, and triangles third positions.

invariable sites, and substitution rate parameters were estimated from a neighbor-joining tree. Those estimates were then used with the empirical base frequencies in a heuristic search with TBR branch-swapping. On the resulting tree, the gamma parameter, proportion of invariable sites, and substitution rate parameters were again estimated, and a new heuristic search with TBR branch-swapping was employed. A third estimation of the parameters was done on the basis of that tree, and those parameters

were then used in the final search for the best-fit tree. Nodal support for the maximum-likelihood tree was estimated with 200 bootstrap replicates.

RESULTS AND DISCUSSION

The strict consensus tree from the parsimony analysis of the combined data set with transitions at third positions in the cytochrome-*b* gene excluded is shown in Figure 4. That tree will be referred to as the ''-3TI'' tree below. In that tree, all but two of the recovered clades received jackknife support exceeding 94% (Fig. 4, Table 2). Those highly supported clades are also recovered in the analysis with all positions weighted equally (Table 2). Only the two weakly supported nodes in the -3TI analysis are not present in the equally weighted tree. First, the association of *Schiffornis* with *Pachyramphus* and *Tityra* is not recovered in the analysis of the equally weighted data set. Instead, *Schiffornis* is placed as sister to *Oxyruncus*, although that arrangement is unsupported by the jackknife analysis. The second difference is found within the clade consisting of *Leptopogon*, *Todirostrum*, and *Corythopis*. In the -3TI tree, *Corythopis* is placed together with *Leptopogon* with a 55% jackknife support, whereas in the equally weighted tree *Corythopis* is instead placed with *Todirostrum* with a 80% support and *Leptopogon* is basal of them.

The tree recovered by the maximum-likelihood analysis of the combined data set is identical to the -3TI tree, except that an additional clade including the taxa *Pyroderus*, *Phytotoma*, *Oxyruncus*, *Schiffornis*, *Pachyramphus*, and *Tityra* receives 74% bootstrap support (Fig. 5).

The number of supported nodes recovered by the jackknife analysis differ greatly between the different gene trees (Table 2). However, there are no conflicts between the supported nodes of one gene tree with the supported nodes of another gene tree. The c-*myc* tree is almost completely unresolved and only four ingroup clades are supported (Table 2). The RAG-1 gene tree is better resolved and support is obtained for some additional clades (Table 2). For instance, the Tyranninae (*sensu* Sibley and Ahlquist 1990) is recovered with an identical topology as that suggested by the combined analysis (Fig. 4). Also, the clade Pipromorphinae and the relationship of *Tityra* and *Pachyramphus* are supported in that gene tree. Although not supported by the jackknife analysis, *Schiffornis* groups with *Tityra* and *Pachyramphus* in the strict consensus tree (Table 2). All supported nodes in the RAG-1 gene tree are also present in the cytochrome-*b* gene tree, although not all of those nodes received a jackknife support above 50%. However, the jackknife support for those nodes increased considerably by the exclusion of the transitions at third positions (Table 2). The ''downweighted'' cytochrome-*b* data set thus contains phylogenetic signal very similar to that observed in the RAG-1 gene tree.

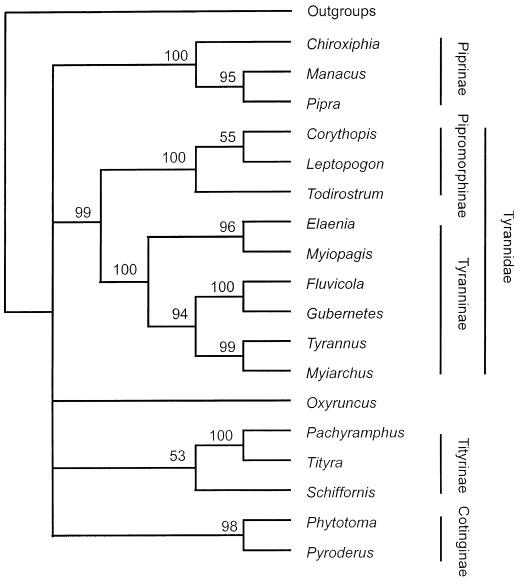

FIG. 4. Strict consensus tree of five most parsimonious trees (1,353 steps) obtained from the analysis of the three genes (c-*myc*, RAG-1, and cytochrome *b*) combined, with transitions in the third positions of the cytochrome-*b* gene excluded. Parsimony jackknife support for the clades are indicated above the branches.

Sibley and Ahlquist (1990) divided the Tyrannoidea (their Tyrannida) into five main lineages: Pipromorphinae, Cotinginae, Piprinae, Tityrinae, and Tyranninae. All those clades are recovered with jackknife support by the present study. However, *Oxyruncus* did not group unambiguously with any of those clades (Fig. 4, Table 2). Although the interrelationships between most of those clades are left un-

TABLE 2. Jackknife support values obtained for the clades of tyrannoids in phylogenetic analyses of various partitions of the dataset, using different optimality criteria.

	Combined	Combined (cyt *b* 3rd position transitions excluded)	Maximum likelihood (GTR + I + G)	RAG-1	c-*myc*	cyt *b*	cyt *b* (3rd position transitions excluded)
Monophyly of Cotingidae (sensu Prum 2000)	X	X	74	X	X	X	X
Monophyly of Pipridae (sensu Prum 1990)	100	100	100	77	X	99	100
Manacus together with *Pipra*	96	95	100	87	70	88	57
Monophyly of Tyrannidae (excluding *Pachyramphus* and *Tityra*)	96	99	99	X	X	92	97
Monophyly of Pipromorphinae	99	100	100	73	X	98	96
Corythopis together with *Leptopogon*	X[a]	55	64	X	X	X[b]	57
Monophyly Tyranninae	100	100	100	86	X	82	75
Myiopagis together with *Elaenia*	92	96	98	62	X	<50%	84
Fluvicola together with *Gubernetes*	97	100	100	85	58	60	87
Tyrannus together with *Myiarchus*	92	99	99	85	X	<50%	94
Monophyly of *Fluvicola, Gubernetes, Tyrannus, Myiarchus*	85	94	98	86	85	<50%	54
Phytotoma together with *Pyroderus*	77	98	91	62	59	<50%	82
Monophyly of Tityrinae	X	53	71	<50%	X	X	X
Tityra together with *Pachyramphus*	95	100	100	56	X	92	99

"X" indicates that the clade is not recovered in the strict consensus tree. "<50%" indicates that the clade is recovered in the strict consensus tree but not supported by the jackknife analysis.

[a] *Corythopis* is associated with *Todirostrum* with 80% jackknife support.

[b] *Corythopis* is associated with *Todirostrum* with 81% jackknife support.

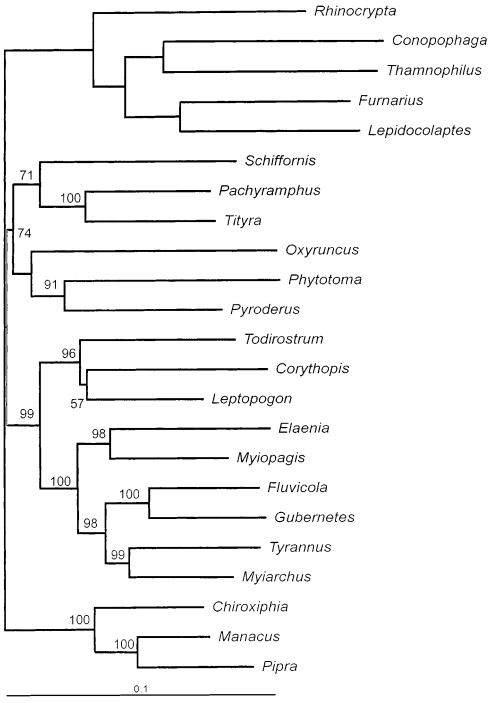

FIG. 5. Maximum-likelihood tree calculated with the general time reversible (GTR) model with estimates of invariable sites and among site rate heterogeneity. Log-likelihood score for this tree is −13,999.25 and the bootstrap support for the recovered clades are indicated at nodes.

resolved by the present analysis, one association is strongly supported and differs from the phylogenetic hypothesis on the basis of DNA–DNA hybridization data. Contrary to the results of the DNA–DNA hybridization study (Fig. 1), our data support the monophyly of the entire tyrant flycatcher assemblage and places the clades Pipromorphinae and Tyranninae as sister groups (Fig. 4). The precise phylogenetic delimitations of Pipromorphinae and Tyranninae are uncertain, but besides the taxa included herein (*Corythopis*, *Todirostrum*, and *Leptopogon*), the Pipromorphinae may also include *Mionectes*, *Pseudotriccus*, *Hemitriccus* (including *Idioptilon*), *Poecilotriccus*, and *Taeniotriccus* (Sibley and Ahlquist 1990). Lanyon (1988a) recognized an assemblage within the tyrant flycatchers consisting of 32 genera (named the *Elaenia*-assemblage), which included, for example, *Corythopis*, *Leptopogon*, *Elaenia*, and *Myiopagis*. Monophyly of that group was suggested by a hypothesized derived state of the nasal septum. Our data do not support the monophyly of that assemblage, and *Corythopis* and *Leptopogon* are placed together with *Todirostrum* in Pipromorphinae, whereas *Elaenia* and *Myiopagis* group with *Fluvicola*, *Gubernetes*, *Myiarchus*, and *Tyrannus* in Tyranninae.

In some earlier classifications, *Tityra* and *Pachyramphus* were placed in the Cotingidae, but were subsequently removed to the Tyrannidae by Traylor (1977, 1979). Our data support a close relationship between those taxa, but not their inclusion in the Tyrannidae. The parsimony analyses of the combined data leave the basal relationships unresolved, whereas the maximum-likelihood analysis weakly supports a clade consisting of the cotingas, *Phytotoma*, *Oxyruncus*, and Tityrinae (Fig. 5). That arrangement is consistent with the Cotingidae *sensu* Prum et al. (2000). Both the -3TI and the maximum-likelihood trees indicate the monophyly of Tityrinae, and the close relationship of *Pachyramphus* and *Tityra* exclusive of *Schiffornis* (Figs. 4 and 5). That topology is identical to that indicated by DNA–DNA hybridization data (Sibley and Ahlquist 1990). Allozyme distance data (S. M. Lanyon 1985) also indicate a close relationship of *Pachyramphus* and *Tityra*, although in that analysis *Schiffornis* was not placed near those two. However, a different scenario of relationship has been suggested based on morphology. Prum and Lanyon (1989) found that *Pachyramphus* and *Schiffornis* share two syringeal synapomorphies (the insertion of the intrinsic muscle on the AI/B1 membrane and a unique configuration of the tracheobronchial junction) with four other taxa and included them in their *Schiffornis* group. Those two characters were not found in *Tityra* which thus was not included in that clade.

The three manakins (Pipridae *sensu* Prum 1990) included in this study are monophyletic. *Schiffornis*, which sometimes has been included in the Pipridae (e.g. Traylor 1979), does not group with the manakins in our analyses (Figs. 4 and 5).

The phylogenetic position of *Oxyruncus* is not conclusively resolved in the present study. In the -3TI analysis of the cytochrome-*b* gene, *Oxyruncus* is placed with *Schiffornis* with weak jackknife support (63%), and in the maximum-

likelihood tree it is placed in a clade with Tityrinae (which includes *Schiffornis*), *Pyroderus*, and *Phytotoma*. *Oxyruncus* possesses an intrinsic muscle that has been considered homologous with the *M. obliquus ventralis* found in the Tyrannidae (Ames 1971, McKitrick 1985), although that homology has been questioned (Prum and Lanyon 1989, Prum 1990). Allozyme distance data (S. M. Lanyon 1985) suggests that *Oxyruncus* 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 the Tyrannidae, whereas most cotingids possess an enlarged femoral artery (Prum 1990). Based on cytochrome-*b* sequence data, Prum et al. (2000) placed *Oxyruncus* within the Cotingidae in a clade of aberrant cotingas that lack the enlarged femoral artery. However, that study was flawed by the apparent use of a nuclear copy of cytochrome *b*.

In all present analyses, *Phytotoma* groups with the single cotingid species (*sensu* Traylor 1979) included in our comparisons, and that association receives jackknife support in all but one of the gene trees (cytochrome *b*—equally weighted) (Table 2). A cotingid affinity of the genus *Phytotoma* has often been suggested, but that taxon is nevertheless placed in a separate family in most classifications. *Phytotoma* shares an enlarged femoral with many cotingid and piprid taxa and also possesses the insertion of *M. tracheolateralis* on the A1/B1 membrane similar to the Cotingidae (*sensu* Prum et al. 2000). A close relationship between *Phytotoma* and the cotingas was also indicated by allozyme distance data (S. M. Lanyon 1985), and Lanyon and Lanyon (1989) placed it next to the cotingid genus *Ampelion* on the basis of allozyme data, as well as synapomorphies in the syrinx. Our data are consistent with a cotingid affinity of *Phytotoma*, although its precise relationship with that clade can only be resolved with the inclusion of additional cotingids.

Acknowledgments

Most of the samples used in this study have been collected during fieldwork in Paraguay in a collaboration between Swedish Museum of Natural History and Museo Nacional de Historia Natural del Paraguay, San Lorenzo. The Direccion de Parques Nacional y Vida Silvestre issued the necessary collecting and export permits in Paraguay. The other samples have kindly been put to our disposal by the Zoological Museum, Copenhagen (J. Fjeldså and J. Garcia-Moreno), and L. Shorey. We are thankful to M. Arvidsson and P. Eldenäs who have been of invaluable help in the laboratory. Thanks also to S. Farris and M. Källersjö who ran the jackknife analysis for us; and, finally, thanks to J. Fjeldså, M. Källersjö, and two anonymous reviewers for comments on earlier drafts of this manuscript. The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the U.S. Department of Defense or the U.S. Department of the Army.

Literature Cited

Ames, P. L. 1971. The morphology of the syrinx in passerine birds. Peabody Museum of Natural History, Yale University, Bulletin, no. 37.

Ames, P. L., M. A. Heimerdinger, and S. L. Warter. 1968. The anatomy and systematic position of the antpipits *Conopophaga* and *Corythopis*. Postilla 114:1–31.

Arctander, P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. Proceedings of the Royal Society of London, Series B 262:13–19.

Carlson, L. M., M. A. Oettinger, D. G. Schatz, E. L. Masteller, E. A. Hurley, W. T. McCormack, D. Baltimore, and C. B. Thompson. 1991. Selective expression of RAG-2 in chicken *b* cells undergoing immunoglobulin gene conversion. Cell 64:201–208.

Desjardin, P., and R. Morais. 1990. Sequence and gene organization of the chicken mitochondrial genome: A novel gene order in higher vertebrates. Journal of Molecular Biology 212:599–634.

Edwards, S. V., and A. C. Wilson. 1990. Phylogenetically informative length polymorphism and sequence variability in mitochondrial DNA of Australian songbirds (*Pomatostomus*). Genetics 126:695–711.

Ericson, P. G. P., U. S. Johansson, and T. J. Parsons. 2000. Major divisions of oscines revealed by insertions in the nuclear gene c-*myc*: A novel gene in avian phylogenetics. Auk 117:1077–1086.

Farris, J. S. 1972. Inferring phylogenetic trees from distance matrices. American Naturalist 106:645–668.

Farris, J. S. 1997. XAC: Parsimony Jackknifer. Molekylärsystematiska laboratoriet. Naturhistoriska riksmuseet. Stockholm, Sweden.

FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12:99–124.

GROTH, J. G. 1998. Molecular phylogenetics of finches and sparrows: Consequences of character state removal in cytochrome *b* sequences. Auk 19:377–390.

GROTH, J. G., AND G. F. BARROWCLOUGH. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Molecular Phylogenetics and Evolution 12:115–123.

IRESTEDT, M., U. S. JOHANSSON, T. J. PARSONS, AND P. G. P. ERICSON. 2001. Phylogeny of major lineages of suboscines (Passeriformes) analyzed by nuclear DNA sequence data. Journal of Avian Biology 32:15–25.

IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

JOHANSSON, U. S., T. J. PARSONS, M. IRESTEDT, AND P. G. P. ERICSON. 2001. Clades within the ''higher land birds'', evaluated by nuclear DNA sequences. Journal of Zoological Systematics and Evolutionary Research 39:37–51.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Science USA 86: 6196–6200.

KÄLLERSJÖ, M., V. A. ALBERT, AND J. S. FARRIS. 1999. Homoplasy increases phylogenetic structure. Cladistics 15:91–93.

LANYON, S. M. 1985. Molecular perspectives on higher-level relationships in the Tyrannoidea (Aves). Systematic Zoology 34:404–418.

LANYON, S. M., AND W. E. LANYON. 1989. The systematic position of the Plantcutters, *Phytotoma*. Auk 196:422–432.

LANYON, W. E. 1984. A phylogeny of the kingbirds and their allies. American Museum Novitates, no. 2797.

LANYON, W. E. 1985. A phylogeny of the myiarchine flycatchers. Ornithological Monographs 36:361–380.

LANYON, W. E. 1986. A phylogeny of the thirty-three genera of the *Empidonax* assemblage of tyrant flycatchers. American Museum Novitates, no. 2846.

LANYON, W. E. 1988a. A phylogeny of the thirty-two genera in the *Elaenia* assemblage of tyrant flycatchers. American Museum Novitates, no. 2914.

LANYON, W. E. 1988b. A phylogeny of the flatbill and tody-tyrant assemblage of tyrant flycatchers. American Museum Novitates, no. 2923.

MADDISON, W. P., AND D. R. MADDISON. 1992. MACCLADE, version 3.0. Sinauer Associates, Sunderland, Massachusetts.

MCKITRICK, M. C. 1985. Monophyly of the Tyrannidae (Aves): Comparision of morphology and DNA. Systematic Biology 34:35–45.

MORITZ, C., T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and sytematics. Annual Review of Ecology and Sytematics 18: 269–292.

NIXON, K. C., AND J. M. CARPENTER. 1993. On outgroups. Cladistics 9:413–426.

PETERS, J. L. 1951. Check-list of Birds of the World, vol. 7. Museum of Comparative Zoology, Cambridge, Massachusetts.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

PRUM, R. O. 1990. A test of the monophyly of the manakins (Pipridae) and of the cotingas (Cotingidae) based on morphology. Occasional Papers of the Museum of Zoology, University of Michigan, no. 723.

PRUM, R. O., AND W. E. LANYON. 1989. Monophyly of the *Schiffornis* group (Tyrannoidea). Condor 91: 444–461.

PRUM, R. O., N. H. RICE, J. A. MOBLEY, AND W. W. DIMMICK. 2000. A preliminary phylogenetic hypothesis for the cotingas (Cotingidae) based on mitochondrial DNA. Auk 117:236–241.

QUINN, T. W. 1997. Molecular evolution of the mitochondrial genome. Pages 3–28 *in* Avian Molecular Evolution and Systematics (D. P. Mindell, Ed.). Academic Press, New York.

RAIKOW, R. J. 1987. Hindlimb myology and evolution of the Old World suboscine passerine birds (Acanthisittidae, Pittidae, Philepittidae, Eurylaimidae). Ornithological Monographs, no. 41.

SIBLEY, C. G., AND J. E. AHLQUIST. 1985. Phylogeny and classification of New World suboscine Passerine birds (Passeriformes:Oligomyodi:Tyrannides). Ornithological Monographs 36:396–428.

SIBLEY, C. G., AND J. E. AHLQUIST. 1990. Phylogeny and Classification of Birds. Yale University Press, New Haven, Connecticut.

SIBLEY, C. G., S. M. LANYON, AND J. E. AHLQUIST. 1984. The relationship of the Sharpbill (*Oxyruncus cristatus*). Condor 86:48–52.

SORENSON, M. D., AND T. W. QUINN. 1998. Numts: A challenge for avian systematics and population biology. Auk 115:214–221.

SWOFFORD, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

TRAYLOR, M. A., JR. 1977. A classification of the tyrant flycatchers (Tyrannidae). Bulletin of the Museum of Comparative Zoology 148:129–184.

TRAYLOR, M. A., JR. 1979. Check-list of the Birds of the World, vol. 8. Museum of Comparative Zoology, Cambridge, Massachusetts.

WATSON, D. K., E. P. REDDY, P. H. DUESBERG, AND T. S. PAPAS. 1983. Nucleotide sequence analysis of the chicken c-*myc* gene reveals homologous and unique coding regions by comparision with the transforming gene of avain myelocytomatosis virus MC29, *delta gag-myc*. Proceedings of the National Academy of Science USA 80:2146–2150.

Associate Editor: F. Sheldon