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

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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 poco 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 clade 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 cotingidas incluidas en este análisis, mientras que la posición de Oxyruncus fue irresoluto.

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-


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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).

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, To- dirostrum, 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.
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; Prum and Lanyon 1989; Prum 1990; Sibley and Ahlquist 1990). The trees were rooted according to the outgroup criterion (Farris 1972, Nixon and Irestedt et al. 2001). A similar protocol was 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, 5SL (5'-CCT TCC ACC AAA CAG GCT CAA ACA ACC C-3'), H658 (5’TCT TTG GAG TAG TAG GGG TGG GAT GG-3'), and H15915 or Thr 1, with 5SL 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; Desjardins 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 mississippiensis*—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 substitutions relative other vertebrates (unique changes shown in bold), indicating that this is likely to be a nuclear copy of the cytochrome-\(b\) gene.

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-
region between positions 756 and 1233 of exon 3 in the published Callus 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 furnarioid 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 Jacknifer (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
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

Fig. 3. Saturation plot for the cytochrome-β 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.
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, Todiostrotrum, and Corythopsis. In the -3TI tree, Corythopsis is placed together with Leptopogon with a 55% jackknife support, whereas in the equally weighted tree Corythopsis is instead placed with Todiostrotrum 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 Pipromorphini 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.

Sibley and Ahlquist (1990) divided the Tyrannoidea (their Tyrannida) into five main lineages: Pipromorphinae, Cotinginae, Piprininae, 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.

<table>
<thead>
<tr>
<th>Clade Description</th>
<th>Combined</th>
<th>Combined (cyt b 3rd position transitions excluded)</th>
<th>Maximum likelihood (GTR + I + G)</th>
<th>RAG-1</th>
<th>c-myc</th>
<th>cyt b (3rd position transitions excluded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monophyly of Cotingidae (sensu Prum 2000)</td>
<td>X</td>
<td>X</td>
<td>74</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Monophyly of Pipridae (sensu Prum 1990)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>77</td>
<td>X</td>
<td>99.00</td>
</tr>
<tr>
<td>Manacus together with Pipra</td>
<td>0.96</td>
<td>0.95</td>
<td>1.00</td>
<td>0.87</td>
<td>0.87</td>
<td>0.99</td>
</tr>
<tr>
<td>Monophyly of Tyrannidae (excluding Pachyramphus and Tityra)</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>X</td>
<td>X</td>
<td>0.99</td>
</tr>
<tr>
<td>Monophyly of Pipromorphinae</td>
<td>0.99</td>
<td>0.99</td>
<td>0.73</td>
<td>X</td>
<td>X</td>
<td>0.98</td>
</tr>
<tr>
<td>Corythopis together with Leptopogon</td>
<td>X</td>
<td>0.55</td>
<td>0.64</td>
<td>X</td>
<td>X</td>
<td>0.97</td>
</tr>
<tr>
<td>Monophyly Tyranninae</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.86</td>
<td>0.86</td>
<td>0.97</td>
</tr>
<tr>
<td>Myiopagis together with Elaenia</td>
<td>0.92</td>
<td>0.96</td>
<td>0.98</td>
<td>0.62</td>
<td>X</td>
<td>&lt;0.50%</td>
</tr>
<tr>
<td>Phylia together with Gubernates</td>
<td>0.97</td>
<td>1.00</td>
<td>0.85</td>
<td>0.58</td>
<td>0.58</td>
<td>0.87</td>
</tr>
<tr>
<td>Tyrannus together with Myiarchus</td>
<td>0.92</td>
<td>0.99</td>
<td>0.99</td>
<td>0.85</td>
<td>X</td>
<td>&lt;0.50%</td>
</tr>
<tr>
<td>Monophyly of Phylia, Gubernates, Tyrannus, Myiarchus</td>
<td>0.85</td>
<td>0.94</td>
<td>0.98</td>
<td>0.86</td>
<td>0.86</td>
<td>0.94</td>
</tr>
<tr>
<td>Phytotoma together with Pyroderus</td>
<td>0.77</td>
<td>0.98</td>
<td>0.91</td>
<td>0.62</td>
<td>0.62</td>
<td>&lt;0.50%</td>
</tr>
<tr>
<td>Monophyly of Tityridae</td>
<td>X</td>
<td>0.53</td>
<td>0.71</td>
<td>&lt;0.50%</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Tityra together with Pachyramphus</td>
<td>0.95</td>
<td>1.00</td>
<td>1.00</td>
<td>0.96</td>
<td>X</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*“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.
* Corythopis is associated with Toxorrostus with 80% jackknife support.
* Corythopis is associated with Toxorrostus with 81% jackknife support.
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 Taniotriccus (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 Tityr
inae (which includes Schifformis), Pyroderus, and Phytotoma. Oxyruncus possesses an intrisin
c 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 cotingas (Sibley et al. 1984; Sibley and Ahlquist 1985, 1990). The main hindlimb artery in Oxyruncus is the ischiadic as in the Tyrannidae, whereas most cotingas 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 an enlarged femoral artery (Prum 1990).

ACKNOWLEDGMENTS

Most of the samples used in this study have been collected during fieldwork in Paraguay in a collabora-
tion between Swedish Museum of Natural History and Museo Nacional de Historia Natural del Paraguay, San Lorenzo. The Direccióon 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. García-Moreno), and L. Shorey. We are thankful to M. Arvidsson and P. Eldén 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.

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Associate Editor: E. Sheldon