



The Auk 124(1):134–154, 2007
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Printed in USA.

MOLECULAR PHYLOGENETICS OF THE TODY-TYRANT AND FLATBILL ASSEMBLAGE OF TYRANT FLYCATCHERS (TYRANNIDAE)

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ABSTRACT.—The tody-tyrant and flatbill assemblage, *sensu* Lanyon (1988a), includes 12 genera of tyrant flycatchers known variously as “tody-tyrants” and “flatbills.” Lanyon supported the monophyly of the group based on similar skull morphology and nest form, and built intergeneric relationships based on syringeal characters. However, these comparisons were made without a phylogenetic framework. A more recent study assessing relationships in the tyrant flycatchers using published morphological and behavioral data failed to recover monophyly of this assemblage (Birdsley 2002). Using DNA sequence data, we test for the monophyly of the tody-tyrant and flatbill assemblage and compare and contrast phylogenetic signals from mitochondrial and nuclear DNA character systems. We discuss our results in light of results from previous studies. We include representatives of the other major tyrant flycatcher assemblages for a total of 42 individuals (representing 27 tyrannid genera and 36 species). We sequenced 3,022 base pairs (bp) of three mitochondrial genes (ND2, ND3, and cytochrome *b*) and one nuclear intron (FIB5). Our results resolve many of the basal relationships of the tody-tyrant and flatbill phylogeny, but separate and combined analyses of data partitions are necessary to understand the nature of conflict among data sets. The tody-tyrants constitute a monophyletic clade, but the genera *Hemitriccus* and *Lophotriccus* are not monophyletic, and the limits of *Oncostoma*–*Lophotriccus* need to be revised with more complete sampling at the species level. The flatbills as defined by Lanyon are not monophyletic (*Onychorhynchus* and *Platyrrinchus* are not true flatbills). *Pseudotriccus* and *Corythopis* are sister taxa and, together with *Leptopogon* and *Mionectes*, are allied to the tody-tyrant and flatbill clade. Received 7 May 2005, accepted 12 January 2006.

Key words: β -fibrinogen intron 5, cytochrome *b*, flatbills, mitochondrial DNA, ND2, ND3, nuclear DNA, phylogeny, tody-tyrants, Tyrannidae, tyrant flycatchers.

Filogenia Molecular del Grupo de los Picochatos y Mosquiteros de la Familia Tyrannidae

RESUMEN.—El grupo de los picochatos y mosquiteros, *sensu* Lanyon (1988a), incluye a 12 géneros de la familia Tyrannidae conocidos en general como mosquiteros y picochatos. Lanyon apoyó la condición monofilética de este grupo basándose en la similitud de la morfología de sus cráneos y en la forma de sus nidos, y construyó relaciones inter-genéricas basándose en caracteres de la siringe. Sin embargo, estas comparaciones no fueron hechas en un contexto filogenético. Un estudio más reciente, que determinó las relaciones entre los Tyrannidae utilizando datos publicados de morfología y comportamiento, no consiguió detectar la condición monofilética de este grupo (Birdsley 2002). Utilizando datos de secuencia de ADN, probamos la condición monofilética del grupo de los picochatos y

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mosquiteros, y comparamos y contrastamos las señales filogenéticas de sistemas de caracteres de ADN mitocondrial y nuclear. Discutimos nuestros resultados a la luz de los resultados encontrados en estudios anteriores. Incluimos representantes de los principales grupos de Tyrannidae por un total de 42 individuos (representando a 27 géneros y 36 especies). Secuenciamos 3,022 pares de bases (pb) de tres genes de ADN mitocondrial (ND2, ND3, y citocromo *b*) y un intrón nuclear (*FIB5*). Nuestros resultados resuelven muchas de las relaciones basales de la filogenia de los picochatos y mosquiteros. Sin embargo, se requieren análisis por separado y en combinación para entender la naturaleza del conflicto entre los sets de caracteres. Los mosquiteros constituyen un grupo monofilético, pero los géneros *Hemitriccus* y *Lophotriccus* no son monofiléticos y los límites de *Oncostoma*–*Lophotriccus* deben ser revisados con un muestreo exhaustivo a nivel de especies. Los picochatos, como fueron definidos por Lanyon, no son un grupo monofilético (*Onychorhynchus* y *Platyrrinchus* no son picochatos verdaderos). *Pseudotriccus* y *Corythopsis* son grupos hermanos y junto con *Leptopogon* y *Mionectes* están aliados al clado de los picochatos y mosquiteros.

THE TYRANT FLYCATCHERS (Aves, Tyrannidae) are the largest, most diverse primarily Neotropical family of suboscine passerines. The family is divided into 104 genera and 429 species (Traylor 1977, 1979; Stotz et al. 1996; Fitzpatrick et al. 2004). The tyrant flycatchers display a plethora of morphological forms in a range of ecological niches, which in other continents are occupied by members of many different families (Fitzpatrick 1985). The great behavioral and ecological diversity observed in the tyrannids present rich opportunities for comparative tests of evolutionary and ecological hypotheses (Cicero and Johnson 2002). However, many aspects of their taxonomy remain controversial, and well-supported hypotheses of intrafamilial relationships are lacking (Birdsley 2002, Fitzpatrick et al. 2004).

A recent treatment of the Tyrannidae (Fitzpatrick et al. 2004) suggests that the family consists of four subfamilies: three “typical” (Elaeniinae, Fluvicolinae, Tyranninae) and one “atypical” (Tityrinae). Within the Elaeniinae, Lanyon (1988a) suggested an assemblage formed by 12 genera (and 70 species) of tyrannids known variously as “tody-tyrants,” “pygmy-tyrants,” and “flatbills.” Members of the tody-tyrant and flatbill assemblage are characterized by their relatively flat bills, which can vary from broad and swollen to narrower and spatulate in shape. Tody-tyrants (52 species) are small flycatchers, with proportionately long, somewhat flattened bills, short rictal bristles, and elongated tarsi. All forage by upward striking and build purse-like pendant nests. Flatbills (18 species) vary in size from small to medium;

they have broad, flat bills, moderate to heavy rictal bristles, and fairly short, slender tarsi. Like the tody-tyrants, they forage almost exclusively by upward striking, and several taxa build purse-like pendant nests—with the exception of spadebills (*Platyrrinchus* spp.), which build cup-shaped nests, and royal flycatchers (*Onychorhynchus* spp.), which build long pensile nests (Traylor and Fitzpatrick 1982).

Lanyon (1988a) supported the monophyly of this assemblage on the basis of skull morphology (characters included a poorly ossified nasal septum with no transverse trabecular plate, enlarged infraorbital fenestra, and supraorbital fenestra completely obliterated, or nearly so) and nest form (pendant nest, fully enclosed, with side entrance) and built intergeneric relationships based on syringeal characters (Fig. 1A). However, these comparisons were done without an explicit phylogenetic framework. Lanyon (1988a) defined two primary lineages within the group: one composed of *Todirostrum*, *Poecilotriccus*, *Hemitriccus* (including *Myiornis*), *Lophotriccus* (including *Atalotriccus*), and *Oncostoma*; and the other composed of *Rhynchocyclus*, *Tolmomyias*, *Onychorhynchus*, and *Platyrrinchus*. *Cnipodectes* did not fit into either of these lineages, and the limits of *Poecilotriccus* were enlarged by Lanyon to encompass the “*sylvia*” species group included in the genus *Todirostrum* by Fitzpatrick (1976) and Traylor (1977, 1979). Two genera, *Ramphotricon* and *Pseudotriccus*, previously considered to belong to the tody-tyrant and flatbill assemblage (von Berlepsch 1907, Hellmayr 1927, Traylor 1977, Traylor and Fitzpatrick 1982, for *Ramphotricon*;

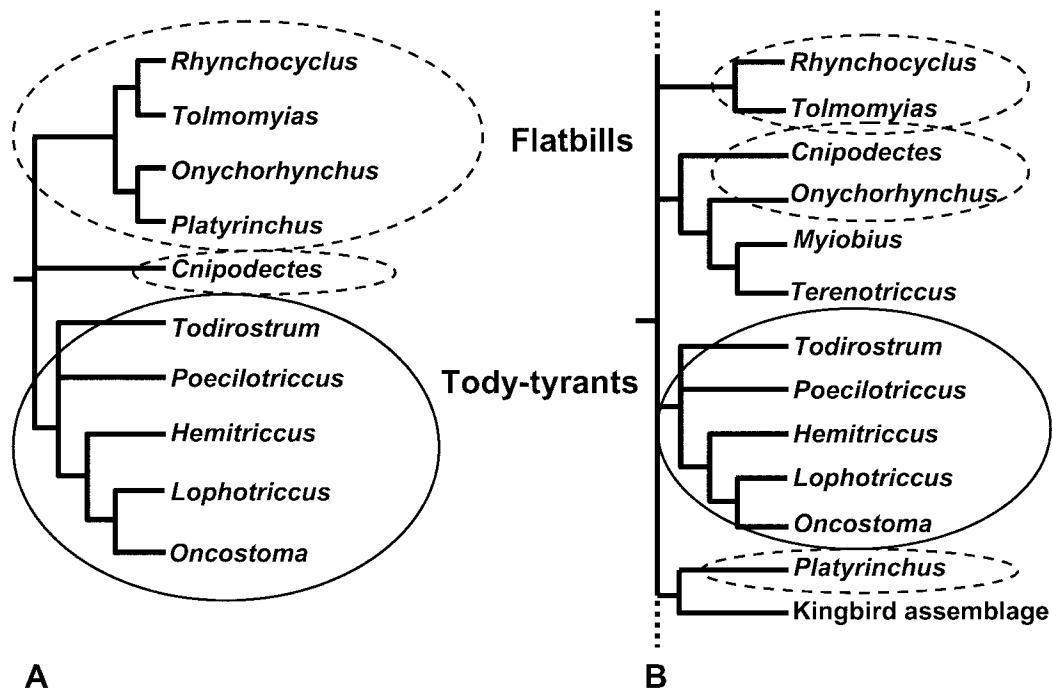


FIG. 1. Hypotheses of relationships for the tody-tyrant and flatbill assemblage: (A) Lanyon's (1988a) intergeneric relationships, based on syringeal characters; and (B) Birdsley's (2002) intergeneric relationships, based on published morphological and behavioral data.

Slater 1888, von Berlepsch 1907, Hellmayr 1927, Zimmer 1940, Meyer de Schauensee 1966, for *Pseudotriccus*), lacked the diagnostic characters for the tody-tyrant and flatbill assemblage and thus were considered to be outside this group (Lanyon 1985, 1988a). Although Lanyon (1988a) acknowledged previous uncertainties and controversies over the systematic positions of *Platyrinchus* and *Onychorhynchus*, including different nest types (Traylor 1977, Traylor and Fitzpatrick 1982) and cranial and syringeal morphology (Warter 1965, in Traylor 1977), he gave more weight to the nasal septum and the overall syringeal similarity in placing these genera within his tody-tyrant and flatbill assemblage.

Birdsley (2002), assessing the phylogenetic relationships of tyrant flycatchers using published morphological, nest, and behavioral data, did not recover Lanyon's tody-tyrant and flatbill assemblage as monophyletic, but his consensus tree (Birdsley 2002; Fig. 1B) recovered a clade formed by all tody-tyrants with the same topology of Lanyon's hypothesis. In Birdsley's phylogeny, *Platyrinchus* was closely related to the

Kingbird assemblage and *Cnipodectes* was sister to a clade formed by *Onychorhynchus*, *Myiobius*, and *Terenotriccus*, in which *Onychorhynchus* was the basal taxon.

We gathered mitochondrial and nuclear characters to examine the systematics of the tody-tyrant and flatbill assemblage. We included representatives of the other major flycatcher groups for a total of 42 individuals and two non-tyrannid outgroups. We discuss the implications of these data with the previous studies and compare phylogenetic signals from our mitochondrial and nuclear DNA character systems.

MATERIALS AND METHODS

TAXON SAMPLING AND DATA ACQUISITION

We sampled 42 flycatcher specimens, including representatives of all genera of the tody-tyrant and flatbill assemblage *sensu* Lanyon (1988a); representatives of other genera suggested to belong to this assemblage, with the

exception of the monotypic genus *Taeniotriccus* (Sclater 1888, von Berlepsch 1907, Hellmayr 1927, Zimmer 1940, Meyer de Schauensee 1966, Traylor 1977, Traylor and Fitzpatrick 1982); and representatives of the other major flycatcher groups, for a total of 27 tyrannid genera and 36 species (Appendix). Species names follow Sibley and Monroe (1990, 1993). Two non-tyrannid taxa, the Red-capped Manakin (Pipridae: *Pipra mentalis*) and the Gray Antbird (Thamnophilidae: *Cercomacra cinerascens*) (Irestedt et al. 2001, Chesser 2004), were used as outgroups for the analyses.

Total DNA was extracted from a small (~0.05 g, wet weight) portion of tissue using the Puregene Extraction kit (Gentra Systems, Minneapolis, Minnesota) following the manufacturer's directions. The final pellet was resuspended in 50 µL of Puregene DNA hydration solution. Polymerase chain reaction (PCR) primers designed for three mitochondrial gene regions, NADH dehydrogenase subunit 2 (ND2), NADH dehydrogenase subunit 3 (ND3), and cytochrome *b* (CYTB); and one nuclear intron, β-Fibrinogen intron 5 (FIB5), were used in the present study (Table 1).

The general PCR profile was as follows: an initial cycle of 3 min at 94°C, followed by 34

cycles of 45 s at 94°C, 30 s at 52°C, and 60 s at 72°C, with a final extension of 5 min at 72°C. For certain taxa and primer pairs, it was necessary to vary the annealing temperature and number of cycles to optimize PCR amplification. The PCR bands were visualized in 1% low-melting-point agar gels. The PCR bands were cut, and intact DNA was recovered using the GELase (Agarose Gel-Digesting) kit (Epicentre, Madison, Wisconsin). DNA sequencing was done using the ABI Big Dyes Cycle Sequencing kit, version 3 (Perkin Elmer, Foster City, California) for dye-terminator chemistry, following the manufacturer's instructions. Cycle sequencing reactions were precipitated in 75% solution of isopropanol following ABI protocol. Dried cycle-sequencing reactions were resuspended and electrophoresed on an ABI 3100 Genetic Analyzer (Perkin Elmer, Foster City, California). In most cases, both strands were sequenced to verify accuracy of the sequences. For ND2 and CYTB, however, there was only partial overlap in the middle of each gene, because each gene was amplified and sequenced using a single primer set (Table 1). All mitochondrial sequences were translated into amino acids to confirm that the reading frame corresponded to a functional mitochondrial gene. Additionally,

TABLE 1. Summary of molecular regions used in the present study.

Molecular region	Base pairs	Primers (reference) ^a
Mitochondrial DNA	2,437	
NADH dehydrogenase subunit 2 (ND2)	1,041	L5204: TAACTAAGCCGGCCCAT (designed by S. J. Hackett)-H6312: CTTATTTAAGGCTTTGAAGGCC (Cicero and Johnson 2001)
NADH dehydrogenase subunit 3 (ND3)	351	L10755: GACTTCCAATCTTTAAAATCTGG-H11151: GATTTGTTGAGCCGAAATCAAC (both primers from Chesser 1999)
Cytochrome <i>b</i> (CYTB)	1,045	L14990: CCATCCAACATCTCAGCATGAT GAAA-H16065: GGAGTCTTCAGTCTCTG GTTACAAGAC (both primers from Helm-Bychowski and Cracraft 1993)
Nuclear DNA	585 ^b	
β-Fibrinogen intron 5 (FIB5)	535–571	FIB5: CGCCATACAGAGTATACTGTGAC AT-FIB6: GCCATCCTGGCGATTCTGAA (both primers from Marini and Hackett 2002)

^a All primer sequences are listed 5' to 3'; numbers refer to the 3' base of the primer referenced to the mtDNA of chicken (*Gallus gallus domesticus*; Desjardins and Morais 1990); 'L' and 'H' refer to primers located on the light and heavy strands of the mitochondrial genome, respectively.

^b Total of aligned base pairs.

plots of overall pairwise genetic differences among mitochondrial genes and the nuclear intron were constructed to identify outliers that could represent nuclear copies in the mitochondrial data set (not shown). Where there was concern, DNA cloning was used to verify the mitochondrial nature of the sequences (Hillis et al. 1996), none of which gave results demonstrating the presence of nuclear copies. Saturation was assessed graphically using saturation plots of first-, second-, and third-position partitions, for both transitions and transversions (Hackett 1996).

SEQUENCE ALIGNMENT

Mitochondrial DNA sequences were aligned to the ND2, ND3, and CYTB sequences of chicken (*Gallus gallus domesticus*; Desjardins and Morais 1990) using SEQUENCHER, version 4.1 (Genecodes, Ann Arbor, Michigan) and checked by eye. β -Fibrinogen intron 5 sequences were aligned to each other and checked by eye to identify gap locations in the intron sequences and to find areas of ambiguous alignments (those regions characterized by the presence of gaps with uncertain positions, no matter which optimization criterion was used) in the nuclear data set. Final alignments of the nuclear sequences were done by eye (available from J.G.T.). Sequences were deposited in GeneBank (access numbers DQ294403–DQ294577).

PHYLOGENETIC ANALYSES

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods. The MP analyses were done using PAUP* (Swofford 2002). All searches were heuristic, with default options (keep best trees only, stepwise addition, swap on best starting trees only, hold one tree at each step, TBR branch-swapping, MULTREES, swap on best trees only), except that SETMAXTREES was set to 500, with an auto-increase by 100 for 100 random-addition sequence replicates. Phylogenies of the different molecular data sets were estimated using separate and combined analyses. For the mitochondrial genes, parsimony analyses were conducted using weighted characters using a step-matrix created with STMATRIX, version 2.2

(see Acknowledgments), which calculates the probabilities of reciprocal changes from one state to another and converts them to a cost of changes using the negative natural logarithm of the probability (Felsenstein 1981, Wheeler 1990). For the nuclear intron, regions of ambiguous alignment were incorporated in the phylogenetic analyses, following Lutzoni et al. (2000), as implemented in INAASE (see Acknowledgments). With this method, each ambiguous region is unequivocally coded as a new character, replacing its respective ambiguous region; then each coded character is subjected to a specific step matrix to account for the differential number of changes needed to transform one sequence into another (Lutzoni et al. 2000). The phylogenetic information of deletions present at regions of unambiguous alignment was preserved by creating additional binary characters that coded for the presence or absence of informative deletions in the data set. Support for nodes in the trees was estimated using bootstrapping (Felsenstein 1985). Bootstrap indices were generated in PAUP* from 100 bootstrap replicates. The contribution of each data partition to the overall node support of the simultaneous analysis was calculated using the "Partitioned Bremer Support Indices" option (Baker and DeSalle 1997, Baker et al. 1998) implemented in TREEROT, version 2 (Sorenson 1999). Bremer support indices (BPS) were generated in PAUP* from a command file generated by TREEROT using 100-replicate heuristic searches with random addition of taxa for each constraint statement.

For ML analyses, the Akaike information criterion (AIC; Akaike 1974, Hasegawa 1990) implemented in MODELTEST, version 3.5 (Posada and Crandall 1998) was used to find the model of DNA evolution that does not result in a significant increase in likelihood scores. Maximum-likelihood searches were performed in PAUP*, using the best model of evolution and parameters (base frequencies, matrix of substitution types, proportion of invariant sites, and shape of gamma distribution) suggested by MODELTEST. One hundred addition sequence replicates were performed using heuristic searches with TBR branch swapping, and performed with the neighbor-joining starting trees and other settings similar to those used in the MP runs. Bootstrap supports for the ML analyses were obtained using

PHYML (Guindon and Gascuel 2003). The PHYML program implements a hill-climbing algorithm that adjusts tree topology and branch length simultaneously, which permits analysis of large data sets in a comparatively short time (Guindon and Gascuel 2003). Parameters for each of the four genes and the combined data sets were estimated from the ML tree using PAUP*. Maximum-likelihood searches as implemented in PAUP* have the limitation of being restricted to a single model of evolution, which precludes exploring the effects of applying different models to each data partition. This may be particularly important when combining data from different molecular systems in which the models of molecular evolution may be quite different because of differences in the evolutionary process. Bayesian inference analysis, as implemented in MRBAYES, version 3.0b4 (Huelsenbeck and Ronquist 2001), allows the implementation of multiple model settings. Thus, BI analysis was used for tree searching and node support through estimation of Bayesian posterior probabilities (Rannala and Yang 1996, Yang and Rannala 1997). The Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling was performed, with four incrementally heated chains, starting from a random tree that was simultaneously run for 2 million generations, using the default priors as starting values for the model parameters. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of all trees retained after discarding those trees representing a "burn-in" period, which was determined graphically. Posterior probability values were considered statistically significant when $P \geq 0.95$. Every analysis was repeated twice (starting from different, randomly chosen trees) to check for potentially poor mixing of MCMCMC sampling. Independent analyses were considered to have converged if their log-likelihood values approached similar mean values. Finally, visual comparisons of the posterior probabilities of the independent runs were done to ensure congruence of the analyses. Bayesian runs were conducted using a four-partition model setting (each gene separated: ND2, ND3, CYTB, FIB5), allowing all parameters to be partitioned by a rate multiplier (i.e., using the unlink command in MRBAYES; Ronquist and Huelsenbeck 2003, Nylander et al. 2004).

CONGRUENCE AMONG DATA PARTITIONS

Differences in phylogenetic signal between data partitions (in terms of both genes and genetic system) were tested using the incongruence length difference (ILD) test (Farris et al. 1995a, b) implemented in PAUP* (the "partition homogeneity test"). The results of these tests were used to investigate conflicting phylogenetic signals in the different data partitions (but see Barker and Lutzoni 2002). We also tested for congruence between major data partitions (mitochondrial and nuclear) by inspecting bootstrap scores $\geq 70\%$ and posterior probabilities ≥ 0.95 resulting from the MP and ML-BI analyses, respectively (Mason-Gamer and Kellogg 1996). Nodes with bootstrap support $\geq 70\%$ and posterior probabilities ≥ 0.95 supporting two different phylogenetic relationships in two different partitions were considered incongruent.

RESULTS

SEQUENCING ALIGNMENT

The final mitochondrial DNA (mtDNA) alignment included a total of 2,437 base pairs (bp) (Table 1). Alignment of mtDNA sequences was straightforward because, as expected, no insertions or deletions were present. In contrast to mtDNA, the length of the nuclear FIB5 sequences varied from 535 bp in *Hemitriccus margaritaceiventer* to 571 bp in *Platyrinchus platyrhynchos*. The total length of the FIB5 alignment was 585 bp, and indel regions inferred from aligned sequences varied from 1 to 25 bp. Two regions of ambiguous alignment were identified, and their sizes were 43 and 4 bp, respectively. Because insertions and deletions (indels) were infrequent, the overall alignment did not appear to be compromised. Seven of these indels at regions of unambiguous alignment were coded as additional binary characters to preserve their phylogenetic information. Attempts to amplify the FIB5 for one of the two samples of *Cnipodectes subbrunneus* (ANSP 5887) failed; thus, nuclear sequences of this specimen were not included in the analyses. The final alignment of the combined mitochondrial and nuclear data sets included a total of 3,022 bp.

GENE PROPERTIES

The ND2 gene had the greatest proportion of variable and parsimony-informative sites, followed by ND3, CYTB, and FIB5 (Table 2). Bremer partition indices showed the contribution of each gene to the overall unweighted-tree topology (Table 2). According to those indices, ND2 contributed the most (52.5%), followed by CYTB (27.5%), FIB5 (11.3%), and ND3, which contributed the least (8.6%).

All the study markers, particularly FIB5, were relatively A+T rich, but the chi-square tests for base-composition heterogeneity across taxa did not show significant biases (Table 2). However, significant bias was found at third positions in the mtDNA. This significant effect was the result of base compositional biases at third positions of the ND2 and CYTB genes that deviated significantly from the stationary assumption (Table 3).

Comparison of pairwise divergence among the mitochondrial genes indicated that ND2 evolves ~1.2× faster than ND3 and ~1.4× faster than CYTB (not shown). The ND3 gene evolves ~1.2× faster than CYTB. The ND2, ND3, and CYTB genes exhibited elevated evolutionary rates, approximately 8×, 7×, and 6× faster than the nuclear intron, respectively. Overall, mitochondrial markers evolved ~7× faster than the nuclear intron (Fig. 2).

Mitochondrial genes also showed strong among-site rate heterogeneity, as indicated by low values of the alpha shape parameter, which contrasted with the higher alpha value of the nuclear intron (Table 2). Similarly, the proportions of invariant sites for the mitochondrial genes were higher than that for the intron. Despite the observed differences among the study markers, the ILD tests showed no statistically significant phylogenetic incongruence among all markers and genetic systems (all *P* > 0.05).

The ND2, ND3, and CYTB genes were characterized by a marked excess of transitions over transversions. Saturation plots showed evidence of multiple hits in third-position transition substitutions in all three mtDNA genes (not shown). By contrast, transitions and transversion substitutions at first and second positions, and third-position transversions, increased linearly with sequence divergence in all three mitochondrial genes, showing no signs of being saturated. Multiple hits in ND2, ND3, and CYTB were evident at levels of sequence

TABLE 2. Properties of three mitochondrial genes and one nuclear intron sequenced for 42 samples of tyrant flycatchers and two outgroup taxa (see Appendix).

Data partitions	Total sites (bp)	Variable sites	PI sites	CI	Tree length	MP trees	Summed PBS	PBS-		Base composition bias	π	α
								minimum steps	A+T (%)			
ND2	1,041	641 (61.6%)	579 (55.6%)	0.25	4,172	7	650 (52.5%)	0.59	57.5	NS	0.337	0.787
ND3	351	192 (54.7%)	167 (47.6%)	0.25	1,152	56	107 (8.6%)	0.34	56.5	NS	0.416	0.686
CYTB	1,045	506 (48.4%)	446 (42.7%)	0.24	3,238	11	341 (27.5%)	0.42	54.5	NS	0.467	0.608
All mtDNA	2,437	1,339 (54.9%)	1,192 (48.9%)	0.24	8,631	2	1,098 (88.7%)	0.49	56.2	NS	0.411	0.746
FIB5	585	285 (48.7%)	153 (26.2%)	0.67	430	970	140 (11.3%)	0.42	60.6	NS	0.000	2.103
All markers	3,010	1,624 (53.7%)	1,344 (44.5%)	0.26	9,047	4	1,238 (-)	0.48	57.0	NS	0.390	0.661

Abbreviations: PI = parsimony informative; CI = consistency index excluding uninformative sites; MP = maximum parsimony; PBS = partitioned Bremer support.

TABLE 3. Mean nucleotide ratios for three mtDNA genes and one nuclear intron.

	First						Second						Third						Overall						
	A	T	C	G	Bias ^a	A	T	C	G	Bias ^a	A	T	C	G	Bias ^a	A	T	C	G	Bias ^a	A	T	C	G	Bias ^a
ND2	0.354	0.215	0.276	0.155	NS	0.165	0.392	0.351	0.092	NS	0.375	0.225	0.340	0.061	S	0.298	0.277	0.322	0.103	NS	0.272	0.293	0.322	0.113	NS
ND3	0.246	0.255	0.312	0.188	NS	0.169	0.446	0.265	0.121	NS	0.402	0.179	0.389	0.030	NS	0.269	0.277	0.331	0.124	NS	0.282	0.280	0.326	0.113	NS
CYTb	0.261	0.238	0.288	0.214	NS	0.194	0.414	0.260	0.132	NS	0.351	0.180	0.444	0.026	S	0.301	0.306	0.171	0.223	NS	0.301	0.306	0.171	0.223	NS
mtDNA	0.298	0.230	0.286	0.185	NS	0.178	0.409	0.300	0.114	NS	0.368	0.199	0.392	0.041	S	0.301	0.306	0.171	0.223	NS	0.301	0.306	0.171	0.223	NS
FIB5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a S = significant differences, NS = nonsignificant differences.

divergence above 11%, 13%, and 16%, respectively (not shown). For all mitochondrial positions, saturation was reached at ~15% sequence divergence (Fig. 2). The FIB5 showed no evidence of multiple hits. Both transitions and transversions increase linearly with percentage of sequence divergence (not shown).

SEQUENCE VARIATION AT DIFFERENT TAXONOMIC LEVELS

Although not the focus of this study, mean values in conspecific comparisons for the different markers ranged from 2.6% to 3.2% for mtDNA (average of all three genes = 3.2%), and the mean was 0.5% for FIB5 (Table 4). Within flycatcher species, two samples of *Oncostoma cinereigulare* from the same site were identical in both mtDNA and FIB5 (0%), whereas samples of *Onychorhynchus coronatus* from different biogeographic regions were highly divergent (10.1% for mtDNA and 0.7% for FIB5).

Mean values in congeneric comparisons for the different markers ranged from 9.6% to 11.1% for mtDNA (average of all three genes = 10.3%), and the mean was 2.0% for FIB5. The most similar taxa were *Mionectes oleagineus* and *M. rufiventris* (3.7% for mtDNA, and 1.1% for FIB5), whereas *Ramphotrigon ruficauda* and *R. megalcephala* were the most divergent (14.2% for mtDNA and 2.2% for FIB5).

Among flycatcher genera, mean levels of sequence divergence for the different markers ranged from 16.0% to 22.2% for mtDNA (average of all three genes = 19.0%), and the mean was 6.1% for FIB5. The lowest level of divergence was between *Oncostoma* and *Lophotriccus* (4.8% for mtDNA, and 0.2% for FIB5), and the highest was between *Capsiempis* and *Onychorhynchus* (22.5% for mtDNA and 6.8% for FIB5).

Sequence divergence between the flycatcher taxa and the piprid outgroup ranged from 17.9% to 24.2% for mtDNA, and the mean was 6.7% for FIB5. Levels of divergence between the flycatcher taxa and the thamnophilid outgroup ranged from 19.5% to 26.0% for mtDNA, and the mean was 11.6% for FIB5.

PHYLOGENETIC ANALYSES: SEPARATE ANALYSES OF MAJOR DATA SETS (MTDNA AND NUCLEAR DNA)

Weighted parsimony analysis of the mtDNA data using the sepmatrix method resulted in

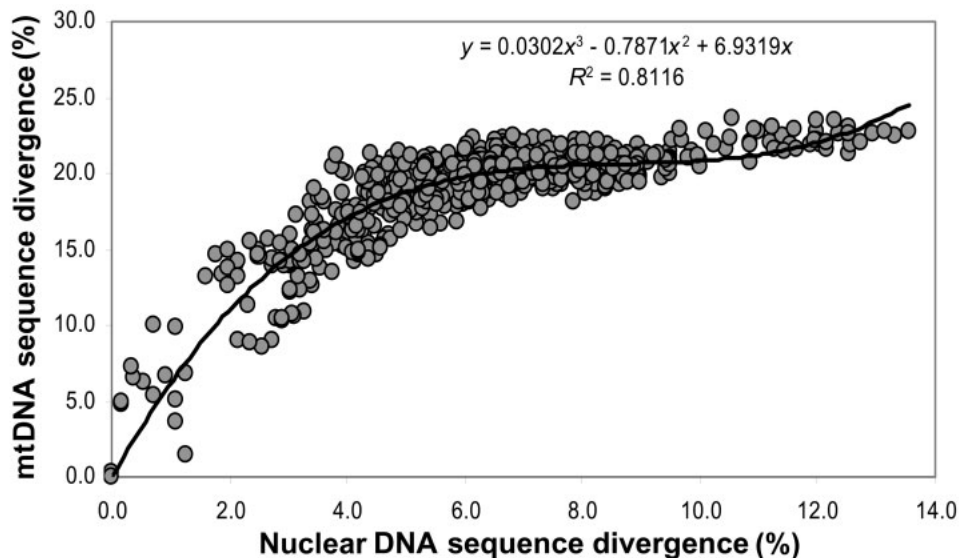


FIG. 2. Comparison of uncorrected percentage of sequence divergence between mtDNA and nuclear DNA. The slope is represented by its regression equation.

two most parsimonious trees (CI = 0.26 and 49.2% parsimony informative characters; Fig. 3A). The monophyly of the tody-tyrants was well supported by this tree, as well as the monophyly of the flatbills, with the exclusion of *Platyrinchus* and *Onychorhynchus*. Tody-tyrants and flatbills were sister groups. Relationships inside these major groups were also highly supported. Even though sampling of flycatcher species within genera was not comprehensive in many cases, all genera were monophyletic, except for *Hemitriccus* and *Lophotriccus*. Within the tody-tyrants, four clades were found: (I) one comprised *Oncostoma*, *Lophotriccus pileatus* (_2), *Hemitriccus minor* (_1) and *Atalotriccus*; (II) a second formed by *Hemitriccus zosterops* (_2); (III) a third formed by *Hemitriccus margaritaceiventer* (_3) and *Myiornis*; and (IV) a fourth comprising *Todirostrum* and *Poecilotriccus* (Fig. 3A). Clade I was sister to clade II, and this whole group was sister to clade III. Clade IV was basal to this whole group. Within the flatbills, *Rhynchocyclus* and *Tolmomyias* were sister taxa, and *Cnipodectes* was basal to this group. Close to the tody-tyrant and flatbill clade is a group including *Corythopsis*, *Pseudotriccus*, *Mionectes*, and *Leptopogon*. A sister relationship between *Pseudotriccus* and *Corythopsis* was strongly supported, but not the relationships of this clade with *Mionectes* and *Leptopogon*.

Platyrinchus was found to be at the base of the tody-tyrant, flatbill, and allies clade. This tody-tyrant, flatbill, and allies clade was related to a highly supported group formed by *Colonia*, *Elaenia*, *Capsiempis*, *Phyllomyias*, *Empidonax*, *Myiotriccus*, *Myiarchus*, *Ramphotrigon*, and *Tyrannus*. In this group, only a clade formed by *Myiarchus*, *Ramphotrigon*, and *Tyrannus* was highly supported. Finally, at the base of this major flycatcher clade was a well-supported group formed by *Onychorhynchus* that was related to a strongly supported clade formed by *Myiobius* and *Terenotriccus* (Fig. 3A).

Weighted parsimony analysis of the nuclear DNA data, using the INAASE method (excluding the ambiguous regions), plus the addition of binary characters, representing unambiguous indels, resulted in 430 most parsimonious trees (CI = 0.73 and 27.6% proportion of parsimony-informative characters; Fig. 3B). Overall, the mitochondrial and nuclear MP trees had similar topologies (Fig. 3). The main differences between these two trees were at the topologic level at which they resolve nodes. In the mtDNA tree, the highly supported nodes resolved relationships toward the tips of the tree, where the nuclear tree did not have strong resolution (Fig. 3A). The nuclear tree exhibited increased support at the base of the tree compared with the mitochondrial tree (Fig. 3B).

TABLE 4. Uncorrected percentage of sequence divergence (p) at different taxonomic levels for three mitochondrial genes and one nuclear intron (mean \pm SE, range in parentheses).

Comparison	n^a	ND2	ND3	CYTB	All mtDNA	FIB5
Intraspecific	6, 5	2.61 \pm 1.00 (0.10–5.86)	2.85 \pm 1.65 (0.00–10.83)	3.21 \pm 1.59 (0.00–9.86)	3.23 \pm 1.58 (0.04–10.05)	0.53 \pm 0.24 (0.00–1.25)
Interspecific–intrageneric	10, 10	11.11 \pm 1.44 (3.65–16.14)	10.11 \pm 1.40 (3.42–16.52)	9.55 \pm 1.03 (3.25–13.49)	10.30 \pm 1.19 (3.65–14.16)	1.99 \pm 0.35 (0.35–3.41)
Intergeneric–intrafamilial	845, 805	22.19 \pm 0.11 (5.09–27.19)	18.31 \pm 0.10 (4.56–24.79)	16.02 \pm 0.08 (4.50–19.71)	18.98 \pm 0.09 (4.84–22.53)	6.09 \pm 0.06 (0.18–10.02)
Interfamilial						
Tyrannidae vs. Pipridae outgroup	42, 41	24.23 \pm 0.16 (21.52–26.32)	21.69 \pm 0.26 (17.09–24.79)	17.92 \pm 0.11 (16.75–19.33)	21.16 \pm 0.09 (19.94–22.36)	6.73 \pm 0.14 (4.80–8.59)
Tyrannidae vs. Thamnophilid outgroup	42, 41	25.98 \pm 0.19 (22.96–28.82)	19.49 \pm 0.20 (16.52–22.79)	19.54 \pm 0.11 (17.80–21.05)	22.28 \pm 0.10 (20.72–23.68)	11.62 \pm 0.15 (9.53–13.54)
Piprid outgroup vs. Thamnophilid outgroup	1, 1	24.59	22.79	20.86	22.73	10.11

^a Sample sizes for the pairwise comparisons, mitochondrial and nuclear, respectively. Nuclear data exclude *Cnipodectes subbrunneus_2* (see Appendix) because attempts to amplify the complete intron failed.

This could be observed at three different places in the nuclear topology: (1) the node joining *Cnipodectes* with the *Rhynchocyclus–Tolmomyias* clade; (2) the node joining the “true” flatbills to the tody-tyrants; and (3) the node joining *Platyrinchus* to the tody-tyrant, flatbill, and allies clade. Overall, there was no evidence of statistically significant phylogenetic incongruence among the two major data sets (ILD test, $P > 0.05$). This is further supported by the lack of conflictive nodes (i.e., those supporting two different phylogenetic relationships in the two major data partitions; Fig. 3).

PHYLOGENETIC ANALYSES: COMBINED ANALYSES OF MAJOR DATA SETS (MTDNA AND NUCLEAR DNA)

Maximum parsimony.—The weighted MP analysis (stepmatrix for mtDNA and INAASE method, plus addition of binary indel characters for nuclear DNA) of the combined mtDNA and nuclear DNA data resulted in one most-parsimonious tree, 45.0% of the characters were parsimony-informative, and 63.4% of its nodes have >70% bootstrap support (Fig. 4A). With the exception of three poorly supported nodes outside the tody-tyrant, flatbill, and allies clade, the topology of this tree was similar to that of the mtDNA tree. Three nodes strongly supported by the nuclear DNA data did not get high support in the combined data set (Fig. 5). Nodes with little support from either mtDNA or nuclear DNA did not get strong support in the combined analyses, with the exception of one node, which was outside of the tody-tyrants and flatbills.

Maximum likelihood–Bayesian inference.—The ML and BI analyses resulted in identical trees that were similar to the MP combined tree, except for the position of *Platyrinchus* (Fig. 4B). As seen in the separate analyses, *Platyrinchus* lies at the base of a major clade including the tody-tyrants, the flatbills, and allies in the MP tree (Fig. 3), though the bootstrap support was <50%. Conversely, the ML–BI tree placed *Platyrinchus* outside the previous clade, at the base of a clade that included several other flycatchers (Fig. 4B). The posterior probability and ML bootstrap support of this node were also low (0.53 and 64, respectively), which indicates low confidence for the placement of this taxon. The ML–BI tree recovered *Mionectes* as sister to *Leptopogon* (though with low node

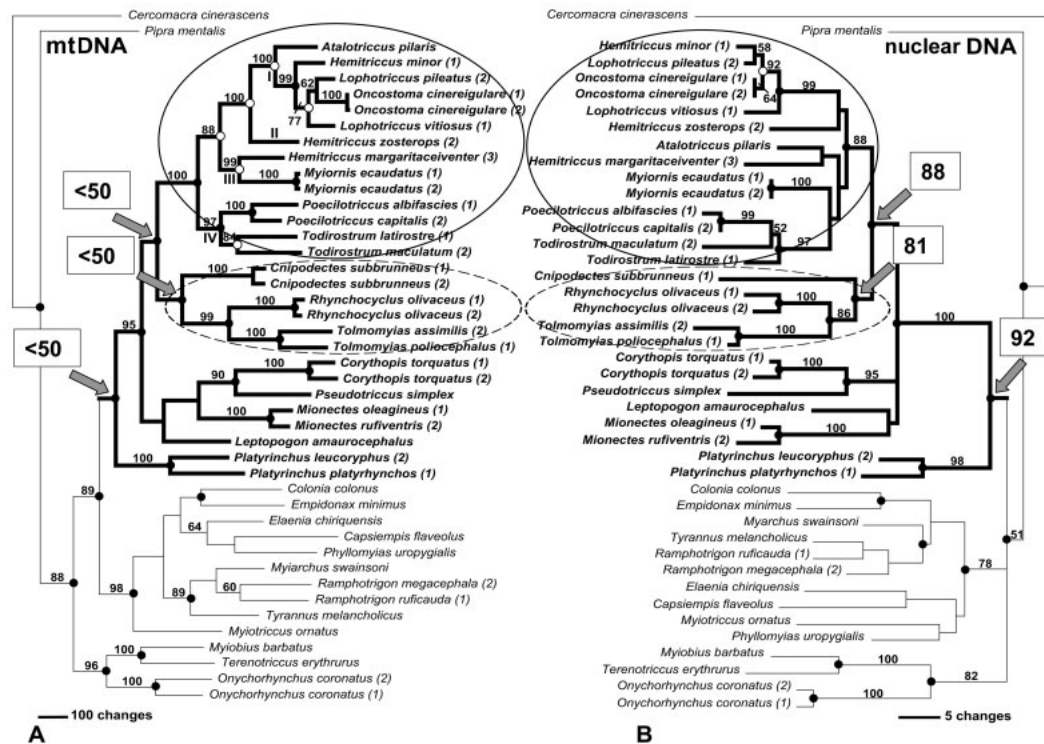


FIG. 3. Separated phylogenetic analyses of the tody-tyrant and flatbill assemblage of tyrant flycatchers. (A) One of the two most-parsimonious trees from a weighted analysis of mtDNA sequences (2,437 bp from ND2, ND3, and CYTB genes). (B) One of 430 most-parsimonious trees from a weighted analysis of nuclear DNA sequences (585 aligned bp from FIB5). In both trees, numbers above the branches represent bootstrap values. Black circles represent nodes that are found in both trees, white circles represent nodes found only in one tree. Arrows indicate selected nodes with low support in the mitochondrial tree and high support in the nuclear tree.

support), and this clade was found to be sister to the *Pseudotriccus*–*Corythopsis* clade. This tree also found strong support for a clade formed by *Colonia*, *Empidonax*, *Myiarchus*, *Ramphotrigon*, and *Tyrannus*, which was previously recovered by the MP tree with low support. However, neither the ML nor BI trees recovered the basal node joining all tyrannid taxa (Fig. 4B).

DISCUSSION

We consider the topology of the combined data (based on MP and ML–BI analyses) to be the best estimate for this group, because of the nuclear results and the lack of apparent conflict with the combined data set. Therefore, we base our taxonomic conclusions on this topology (Fig. 5).

THE TODY-TYRANT AND FLATBILL ASSEMBLAGE

Our molecular results support a major group of tyrant flycatchers composed of two clades, the tody-tyrants (*Oncostoma*, *Lophotriccus*, *Hemitriccus*, *Atalotriccus*, *Myiornis*, *Todirostrum*, and *Poecilotriccus*) and what we call the “true” flatbills (*Cnipodectes*, *Tolmomyias*, and *Rhynchocyclus*). Although the tody-tyrant clade agrees with previous hypotheses (Traylor 1977, Lanyon 1988a, Birdsley 2002), the true flatbill clade, as uncovered by the present study, has not been previously suggested. In our results, the spadebills and royal flycatchers, *Platyrinchus* and *Onychorhynchus*, previously suggested by Lanyon (1988a) to belong to this group fall outside this clade. Thus, these results suggest that the characters (nasal septum poorly ossified [both

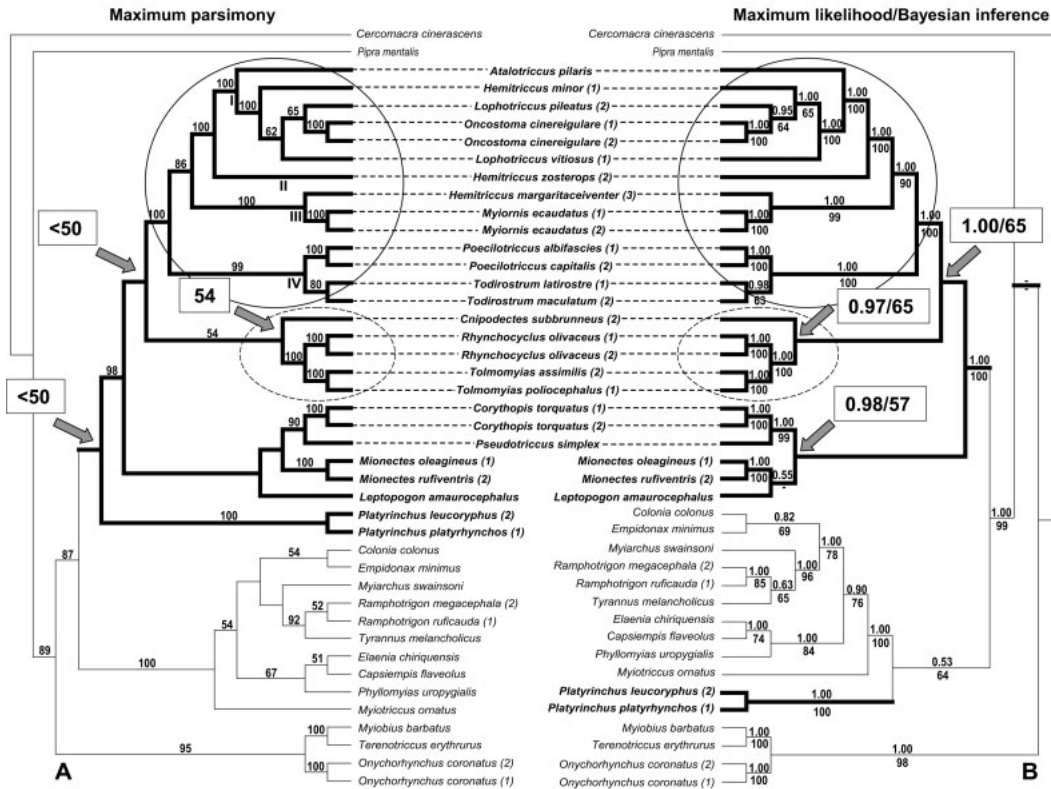


FIG. 4. Combined phylogenetic analyses of the tody-tyrant and flatbill assemblage of tyrant flycatchers. (A) The most-parsimonious tree from the weighted analysis of the combined mitochondrial and nuclear data (3,010 bp from ND2, ND3, CYTB, and FIB5 genes). Numbers above the branches represent bootstrap values. (B) Maximum-likelihood tree (-lnL = 39,458.98, GTR+Γ+I model). Numbers above the branches represent posterior probabilities values from the Bayesian analysis using a complex model partition setting containing four partitions (ND2, ND3, CYTB, and FIB5). Numbers below the branches represent bootstrap values from the maximum-likelihood analysis. Arrows indicate nodes with low bootstrap support in the maximum-parsimony tree and high posterior probability support in the maximum-likelihood and Bayesian inference tree.

taxa] and long pendant nest in *Onychorhynchus*) used by Lanyon (1988a) to support the inclusion of these two genera in his tody-tyrant and flatbill assemblage are convergent.

THE TODY-TYRANT CLADE

The tody-tyrants were grouped in three, perhaps four, clades, with two genera being non-monophyletic: clade I, comprising *Oncostoma*, *Lophotriccus_2* (*pileatus*), *Lophotriccus_1* (*vittosus*), *Hemitriccus_1* (*minor*), and *Atalotriccus*; clade II, formed by *Hemitriccus_2* (*zosterops*); clade III, comprising *Hemitriccus_3* (*margaritaceiventer*) and *Myiornis*; and clade IV, comprising *Todirostrum*

and *Poecilotriccus*. Clade I was sister to clade II, and this whole group was sister to clade III. Clade IV was basal to this whole group (Fig. 5).

Within clade I, *Oncostoma* was embedded within *Lophotriccus*, *Hemitriccus_1* (*minor*) was sister to the *Lophotriccus*-*Oncostoma* clade, and *Atalotriccus* came at the base of clade I. The generic status of *Oncostoma* has been questioned previously (Fitzpatrick 1976, Traylor and Fitzpatrick 1982). It has been suggested that *Oncostoma* is closer to the *Idioptilon*-*Hemitriccus* group of Fitzpatrick (1976) than to either *Lophotriccus* or *Atalotriccus* (Hellmayr 1927). However, Fitzpatrick (1976) did not include *Lophotriccus* and *Atalotriccus* in his systematic

than to the rest of *Hemitriccus*, a proposal supported by our molecular results.

A close relationship between *Myiornis* and *Hemitriccus* was suggested by Lanyon (1988a) on the basis of similar syringeal morphology and lack of significant differentiation in external morphology. This led him to suggest that they were congeners. However, he also pointed out that this may be a reflection of the limited number of variable characters in the syrinx at this lower taxonomic level. Our results support his latter suggestion. *Myiornis* was found to be the sister to "*Hemitriccus*" *margaritaceiventer*, and the two were ~11% divergent from each other in their mtDNA.

Todirostrum and *Poecilotriccus* (*sensu* Traylor 1977) formed a monophyletic clade at the base of the tody-tyrants (Fig. 5). A sister relationship between these two genera has not been directly proposed. However, the species *P. capitalis* (_2) was formerly in *Todirostrum* (Fitzpatrick 1976), until Traylor (1977) placed it in *Poecilotriccus*. Furthermore, Lanyon (1988a) found differences in syringeal morphology between the two *Todirostrum* species groups (*cinereum* and *sylvia*), and syringeal similarities between the *T. sylvia* group and *Poecilotriccus* that led him to suggest merging these two lineages in *Poecilotriccus*. This arrangement has been adopted by Fitzpatrick et al. (2004). Samples of *Todirostrum* used for this study included one member of each *Todirostrum* group: *T. latirostre* (_1) from the *sylvia* group, and *T. maculatum* (_2) from the *cinereum* group. Because *Todirostrum* was monophyletic in our results, our study does not support Lanyon's merging of *Poecilotriccus* and the *T. sylvia* group. However, the amount of genetic differentiation between the two *Todirostrum* samples was as high as that found for *Todirostrum* vs. *Poecilotriccus* (up to 12.7% and 14.5%, respectively). Thus, the *sylvia* group may represent a distinct genus.

The status of another member of the group, the monotypic genus *Taeniotriccus*, further exemplifies generic issues in this group. *Taeniotriccus andrei* was lumped in *Poecilotriccus* on the basis of syringeal similarities (Lanyon 1988a) and previously recognized shared plumage similarities, including the presence of sexual dimorphism (Traylor 1977). However, overall external morphology and vocalizations suggest that this taxon should stay in its own genus, *Taeniotriccus* (Fitzpatrick et al. 2004, Zimmer and Whittaker 2004). A more extensive assessment

of relationships in the tody-tyrants is needed to delimit the boundaries of these genera.

THE FLATBILL CLADE

The flatbills consist of three genera: *Tolmomyias*, *Rhynchocyclus*, and *Cnipodectes*. The genera *Platyrrinchus* and *Onychorhynchus*, previously suggested to be placed in this group (Lanyon 1988a), were found to be outside (Fig. 4). With the current sampling, the position of *Platyrrinchus* is uncertain (see above), and *Onychorhynchus* was found to be closer to *Myiobius* and *Terenotriccus* from the Fluvicolinae (Traylor 1977, Fitzpatrick et al. 2004). This later relationship has been suggested on the bases of similarities of the syrinx (two or more double, complete, and medially ossified A elements; Ames 1971), external morphology (extreme development of the rectal bristles extending beyond the tip of the broad, flat bills; Traylor 1977), and nest type (pendent, purse-shaped nest; Traylor 1977, Traylor and Fitzpatrick 1982).

Tolmomyias and *Rhynchocyclus* resemble each other in external and syringeal morphology, as well as nest structure, and their relationship has been recognized previously (Hellmayr 1927, Traylor 1977, Lanyon 1988a). Warter (1965) and Traylor (1977) suggested a close relationship of these genera to *Cnipodectes* based on cranial morphology (type I nasal septum) and overall external shape. Lanyon's (1988a) coding of cranial morphology agreed with that from previous studies, but he could not find syringeal characters to support a close relationship of *Rhynchocyclus* and *Tolmomyias* with *Cnipodectes* (Fig. 1A).

GENERA ALLIED TO THE TODY-TYRANT AND FLATBILL CLADE

We found four genera allied to the tody-tyrant and flatbill clade: *Pseudotriccus*, *Corythopsis*, *Mionectes*, and *Leptopogon* (Fig. 5). Among these genera, *Pseudotriccus* was found to be sister to the antpipits, *Corythopsis*. *Corythopsis* may have the most checkered systematic history in the tyrannids, having formerly been part of the Conopophagidae and not a tyrant flycatcher at all until the work of Ames et al. (1968). Cranial and syringeal morphology support the *Pseudotriccus*-*Corythopsis* clade (Lanyon 1988b). Also, the two genera are characterized by the presence of long tarsi with almost

obsolete scutes (tarsi covered by several long, continuous platelike scales [scutes], with no overlapping scales), which give them a smooth booted effect (Lanyon 1988b). These two genera also share similar feeding habits, being ground (*Corythopsis*) or near-to-ground (*Pseudotriccus*) foragers that use upward strikes to snatch insects from the undersides of leaves (Traylor and Fitzpatrick 1982). Lanyon (1988b) placed the *Pseudotriccus*–*Corythopsis* clade in a group that included his *Stigmatura* group (*Stigmatura* and *Pseudelaenia*) and *Euscarthmus*, but this relationship was based on a single cranial character. Our results do not support this relationship.

A relationship between *Mionectes* and *Leptopogon* was recovered by both the nuclear MP and the combined ML–BI trees, but support for this node was low (Fig. 4). This relationship has been previously suggested on the basis of similarity of syringeal morphology (Lanyon 1988b, Birdsley 2002), DNA–DNA hybridization (Sibley and Ahlquist 1985, 1990), and nuclear and mitochondrial DNA sequences (Chesser 2004). A sister relationship between this clade and the *Pseudotriccus*–*Corythopsis* clade was highly supported by the ML–BI tree (Fig. 4B). The latter result is consistent with results of other molecular studies (Sibley and Ahlquist 1985, Chesser 2004).

Both *Mionectes* and *Leptopogon* build pensile nests, purse- and globe-shaped, respectively (Traylor and Fitzpatrick 1982). Pensile nests are a character shared with all members of the tody-tyrant and flatbill clade whose nests have been described; with the exception of *Corythopsis*, which has a ground oven-shaped nest (Oniki and Willis 1980), and *Pseudotriccus*, which has a globular nonpensile nest (H. Greeney et al. unpubl. data). Outside the tody-tyrant, flatbill, and allies clade, pensile nests are found only in the *Onychorhynchus*–*Terenotriccus*–*Myiobius* clade (Traylor and Fitzpatrick 1982), where it seems to have evolved independently. As has been found for other avian lineages (Sheldon and Winkler 1999, Zyskowski and Prum 1999), these results suggest that the distribution of nest types in this tyrannid group track well their phylogenetic history.

PHYLOGENETIC PROPERTIES OF THE STUDY MARKERS

Mitochondrial genes and the nuclear intron exhibit important differences that influence their

ability to better resolve different regions of the flycatcher phylogeny (Table 2). Although the combined mitochondrial data set was 4× larger than the nuclear one and evolved 7× faster, differences in the percentage of variable sites were not as marked (55% and 49%, respectively). The FIB5 sequences were more A–T rich than the mitochondrial sequences, which is characteristic of markers under low or no selective constraints (Li and Graur 1991, Li 1997). The estimated alpha value (2.10) for the FIB5 was much higher than that for the combined mitochondrial data set (0.75), which suggests a lower among-site rate heterogeneity in the nuclear intron (Yang 1996, Lin and Danforth 2004). The alpha value of the nuclear intron was higher than previously reported values for the β -Fibrinogen intron 7 (Weibel and Moore 2002); the alpha value of the combined mitochondrial genes also was higher than an mtDNA value previously reported by the same authors (0.75 vs. 0.19). It has been suggested that high alpha values are correlated with low levels of homoplasy and, therefore, are indicative of high phylogenetic information content (Yang 1996, Weibel and Moore 2002, Lin and Danforth 2004). This is supported by the relative frequencies of substitutions for each nucleotide class reconstructed in the mitochondrial and nuclear trees according to the MP criterion (not shown). The changes are more homogeneously distributed for the nuclear positions than for the third-position transitions of the mitochondrial data. A similar pattern of changes was found for mitochondrial first and second positions (not shown). This also was observed in the saturation curves for the mitochondrial genes, which exhibited saturation at 3% nuclear divergence (Fig. 2). Nonetheless, the mitochondrial tree did not differ topologically from the nuclear tree at basal nodes (defined as either within or near the base of the tody-tyrant, flatbill, and allies clade), except that support was low at those nodes (Fig. 3A, B).

Trees from the MP, ML, and BI analyses of the combined data were very similar in topology (Fig. 4). In general, nodes with high bootstrap support ($\geq 70\%$) in the MP tree exhibited high posterior probabilities in the ML–BI tree (≥ 0.95) (Fig. 4B). However, the ML–BI tree showed stronger support for those basal nodes that received low support in the MP tree (Fig. 4A), with the exception of the position of *Platyrrinchus*. In the ML–BI tree, *Platyrrinchus* was placed sister to a major clade that included several flycatchers

from Sibley and Ahlquist's (1990) Tyranninae. Nuclear data alone strongly supported inclusion of *Platyrinchus* sister to the tody-tyrant, flatbill, and allies clade (Fig. 3B), in contrast to the results of the combined ML–BI tree. This may illustrate the effect of a relatively long branch separating *Platyrinchus* from the tody-tyrant, flatbill, and allies clade (Fig. 3B), which most likely is attributable to the limited taxon sampling beyond the study group, or to failure of the mitochondrial data to resolve this ancient node because of saturation; this may also explain why the ML–BI tree did not recover the Tyrannidae as monophyletic (Fig. 4B). In any case, placement of *Platyrinchus* outside the flatbills is consistent with other characters, including the nasal septum (type 3 instead of type 1) and nest type (cup-shaped instead of pendant; Traylor 1977). These differences led Traylor (1977) to place this genus at the end of the Elaeniinae in his linear classification of the Tyrannidae.

CONCLUSIONS

A combination of mitochondrial and nuclear data helped to resolve the backbone of the tody-tyrant and flatbill phylogeny, but separate and combined analyses of data partitions are necessary for understanding the nature of conflict among data sets. The tody-tyrants constitute a monophyletic clade, and the flatbills as defined by Lanyon (1988a) do not constitute a natural group (*Onychorhynchus* and *Platyrinchus* are not true flatbills). The tody-tyrants and the true flatbills were found to be sister clades. *Hemitriccus* is polyphyletic and *Lophotriccus* is paraphyletic, and the limits of *Oncostoma*–*Lophotriccus* need to be revised with more complete sampling at the species level. *Pseudotriccus* and *Corythopsis* are sister taxa and, together with *Leptopogon* and *Mionectes*, are allied to the tody-tyrants and flatbills. With current sampling, the genus *Platyrinchus* falls outside this group, a placement that is consistent with other divergent characteristics, such as the cup-shaped nest built by *Platyrinchus* species, as compared with the pendant nest built by the tody tyrants, flatbills, and allies. *Onychorhynchus* was found to be closer to *Myiobius* and *Terentotriccus* from the Fluvicolinae, which is consistent to previously recognized similarities in morphology and nest type (Ames 1971, Traylor 1977, Traylor and Fitzpatrick 1982). But the ML–BI tree failed

to recover the Tyrannidae as monophyletic. Greater resolution of these basal nodes will require the addition of more nuclear characters and the inclusion of more tyrannid taxa.

ACKNOWLEDGMENTS

We thank the following museums and their curatorial and collection management staff for making tissue samples available for this study: Field Museum (S. Hackett, D. Willard, T. Gnoske); Academy of Natural Sciences of Philadelphia (L. Joseph, N. Rice); Louisiana State University Museum of Natural Science, Baton Rouge (F. Sheldon, R. Brumfield, D. Dittmann); and University of Michigan Museum of Zoology, Ann Arbor (R. Payne, D. Mindell, J. Hinshaw). Thanks to J. Perez for checking the identification of a specimen from the ornithological collection at the Fundación La Salle in Venezuela. DNA sequencing was carried out at the Field Museum's Pritzker Lab for Molecular Systematics and Evolution, operated with support from the Pritzker Foundation. We thank K. Feldheim, J. Hunt, and R. Bowie from the Pritzker Lab for advice during sequencing work and E. Sackett for help with lab work. Help with data analyses was provided by S. Hackett, L. Olson, A. Miller, F. Lutzoni, and C.-P. Lin. This research was supported by a National Science Foundation grant (Doctoral Dissertation Enhancement Grant, INT-0135532) awarded to J.M.B. and J.G.T. An early stage of the manuscript was improved by comments of S. Hackett, M. Ashley, D. Stotz, D. Nyberg, and S. Williams. M. Cohn-Haft made comments to the final version of the manuscript. The programs STMATRIX and INAASE are available as free downloads at www.lutzonilab.net/pages/download.shtml#Step.

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Associate Editor: K. P. Johnson

APPENDIX. Collection data and voucher information for tissue samples used in this study.

Species	Voucher number ^a	Collector	Collection locality
<i>Cercomacra cinerascens</i>	FMNH 389251	D. F. Stotz	Brazil: Roraima: Fazenda Santa Cecilia, east bank of Rio Branco, across from Boa Vista.
<i>Pipra mentalis</i>	LSUMNS B-18078	A. T. Peterson	Mexico.
<i>Elaenia chiriquensis</i>	FMNH 391472	J. M. Cardoso da Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
<i>Empidonax minimus</i>	FMNH 434044	B. J. O'Shea	El Salvador: Ahuachapan: Municipio San Pedro Puxtla, Cantón Concepción.
<i>Tyrannus melancholicus</i>	FMNH 390003	A. T. Peterson	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana.
<i>Myiarchus swainsoni</i>	FMNH 391516	J. M. Cardoso da Silva	Brazil: Amapá.
<i>Colonia colonus</i>	FMNH 323355	J. W. Fitzpatrick	Perú: Madre de Dios: Hacienda Amazonia.
<i>Ramphotrigon ruficauda</i>	FMNH 391180	J. M. Bates	Bolivia: La Paz: T.C.O Campamento Araona, "Palmasola," Rio Manupari.
<i>Ramphotrigon megacephala</i>	FMNH 389988	D. E. Willard	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana.
<i>Onychorhynchus coronatus</i>	FMNH 391185	J. M. Bates	Bolivia: La Paz: T.C.O Campamento Araona, "Palmasola," Rio Manupari.
<i>Onychorhynchus coronatus</i>	FMNH 389231	D. F. Stotz	Brazil: Sao Paulo: Boraceia Biological Station, Salesopolis.
<i>Phylomyias uropygialis</i>	FMNH 430002	T. Pequeño S.	Perú: Cuzco: Paucartambo: Pillahuata.
<i>Myiorticus ornatus</i>	FMNH 433614	T. Pequeño S.	Perú: Cuzco: Paucartambo: Consuelo, 15.9 km southwest of Pilcopata
<i>Terenotriccus erythrurus</i>	FMNH 391186	J. G. Tello	Bolivia: El Beni: Hacienda Los Angeles, 10 km east of Riberalta.
<i>Capsiempis flaveolus</i>	FMNH 391485	J. M. Cardoso da Silva	Brazil: Amapá.
<i>Myiobius barbatus</i>	FMNH 389975	A. T. Peterson	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana.
<i>Platyrinchus platyrhynchos</i>	FMNH 391509	J. M. Cardoso da Silva	Brazil: Amapá.
<i>Platyrinchus leucorhynchus</i>	FMNH 395438	D. F. Stotz	Brazil: Sao Paulo: Boraceia.
<i>Mionectes olegineus</i>	FMNH 391170	J. G. Tello	Bolivia: El Beni: Hacienda Los Angeles, 10 km east of Riberalta.
<i>Mionectes rufiventris</i>	FMNH 395447	D. F. Stotz	Brazil: Sao Paulo: Boraceia.
<i>Leptopogon amaurocephalus</i>	LSUMNS B-14499	J. M. Bates	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km southeast of Catarata Arco Iris.
<i>Corythopsis torquatus</i>	FMNH 389960	A. T. Peterson	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana.
<i>Corythopsis torquatus</i>	FMNH 391486	J. M. Cardoso da Silva	Brazil: Amapá: Amapá, Fazenda Itapoa.
<i>Pseudotriccus simplex</i>	FMNH 430018	T. Pequeño S.	Perú: Cuzco: Paucartambo: San Pedro.
<i>Cnipodectes subbrunneus</i>	FMNH 395582	Unknown	Brazil: Acre: Reserva Extravista Alto Jurua, Rio Tejo.
<i>Cnipodectes subbrunneus</i>	ANSP 5887	A. P. Capparella	Ecuador: Sucumbios: ~20 km northeast of Lumbaqui.
<i>Tolmomyias poliocephalus</i>	FMNH 391187	J. G. Tello	Bolivia: El Beni: Hacienda Los Angeles, 10 km east of Riberalta.
<i>Tolmomyias assimilis</i>	FMNH 389997	J. W. Fitzpatrick	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana.

APPENDIX. Continued.

Species	Voucher number ^a	Collector	Collection locality
<i>Rhynchocyclus olivaceus</i>	FMNH 391502	J. M. Cardoso da Silva	Brazil: Para: Serra dos Carajás.
<i>Rhynchocyclus olivaceus</i>	FMNH 427086	S. A. Roda	Brazil: Alagoas: Ibateouara, Engenho Coimbra, Usina Serra Grande.
<i>Lophotriccus vittosus</i>	FMNH 391488	J. M. Cardoso da Silva	Brazil: Amapá: Amapá, Fazenda Itapoa.
<i>Lophotriccus pileatus</i>	FMNH 430020	T. Pequeño S.	Perú: Cuzco: Paucartambo: San Pedro.
<i>Atalotriccus pilaris</i>	ANSP 5780	T. D. Pederson	Panama: Veraguas: Cascajilloso.
<i>Oncostoma cinereigulare</i>	FMNH 434038	B. J. O'Shea	El Salvador: Ahuachapan: Municipio San Francisco Menend: Parque Nacional El Imposible, Sector La Fincona.
<i>Oncostoma cinereigulare</i>	FMNH 434039	B. J. O'Shea	El Salvador: Ahuachapan: Municipio San Francisco Menend: Parque Nacional El Imposible, Sector La Fincona.
<i>Hemitriccus minor</i>	FMNH 391494	J. M. Cardoso da Silva	Parque Nacional El Imposible, Sector La Fincona.
<i>Hemitriccus zosterops</i>	FMNH 391178	J. M. Bates	Brazil: Para: Serra dos Carajás.
<i>Hemitriccus margaritaceiventer</i>	UMMZ 600	P. Lau	Bolivia: La Paz: T.C.O Campamento Araona, "Palmasola," Rio Manupari.
<i>Poecilotriccus albifascies</i>	FMNH 433657	T. Pequeño S.	Venezuela: Sucre: Pueblo Mochima.
<i>Poecilotriccus capitalis</i>	FMNH 334374	D. F. Stotz	Perú: Madre De Dios: Moskitania, 13.4 km north-northwest of Atalaya, left bank of Alto Madre de Dios.
<i>Todirostrum latirostre</i>	FMNH 433653	B. J. O'Shea	Brazil: Rondônia: Sitio Amaro, 30 km southwest of Cachoeira Nazare on Jaru Road.
<i>Todirostrum maculatum</i>	FMNH 391498	J. M. Cardoso da Silva	Perú: Madre De Dios: Moskitania, 13.4 km north-northwest of Atalaya, left bank of Alto Madre de Dios.
<i>Myiornis ecaudatus</i>	FMNH 389978	S. M. Lanyon	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
<i>Myiornis ecaudatus</i>	FMNH 391177	J. G. Tello	Brazil: Rondônia: Cachoeira Nazare, west bank of Rio Jiparana. Bolivia: El Beni: Hacienda Los Angeles, 10 km east of Riberalta.

^a Museum abbreviations: FMNH = Field Museum of Natural History; LSUMNS = Louisiana State University Museum of Natural Science; ANSP = Academy of Natural Sciences of Philadelphia; UMMZ = University of Michigan Museum of Zoology.