ARTICLE IN PRESS

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Molecular Phylogenetics and Evolution xxx (2007) xxx–xxx

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), with an emphasis on the elaeniine assemblage

Frank E. Rheindt [a,b,*], Janette A. Norman [a,b], Les Christidis [b,c]

[a] *Department of Genetics, University of Melbourne, Parkville Campus, Grattan Street, 3010 Melbourne, Vic., Australia*
[b] *Sciences Department, Museum Victoria, GPO Box 666, 3001 Melbourne, Vic., Australia*
[c] *Australian Museum, 6 College Street, 2010 Sydney, NSW, Australia*

Received 17 March 2007; revised 11 September 2007; accepted 14 September 2007

Abstract

The tyrant-flycatchers (Tyrannidae) are arguably the largest avian family in the Western Hemisphere with approximately 100 genera and 430 species. Although the composition of the family is largely settled, intergeneric relationships are poorly understood. Morphological and behavior-based classifications are in disagreement with DNA–DNA hybridization data, and both have recently been contradicted by DNA-sequence studies. However, previous DNA-sequence sampling has mostly focused on two out of the six traditional tribes. In this study, we have sampled mitochondrial and nuclear sequences of additional tyrannid genera from across the Tyrannidae, with particularly dense coverage of a third tribe (Elaeniini). Our data corroborate previous DNA-sequence studies that demonstrate a basal division of Tyrannidae into a pipromorphine group (recruited from two morphological tribes) and the core Tyrannidae. Furthermore, we identify a new assemblage that includes *Platyrinchus* and the enigmatic *Neopipo*, although the position of this lineage within the Tyrannidae remains *incertae sedis*. Within the core Tyrannidae, we find strong support for a monophyletic elaeniine assemblage, and discuss a number of strongly supported sub-clades and species-level arrangements that display varying levels of agreement with previous classifications. The elaeniine assemblage may be the sister group to all other core Tyrannidae, and it is in virtually complete congruence with a previous classificatory scheme based on syringeal morphology.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Tyrannidae; Elaeniini; *Elaenia* assemblage; Pipromorphinae; Bayesian inference; Phylogeny; Fib5; ND2; Morphology

1. Introduction

Comprising approximately 100 genera and 430 species, the tyrant flycatchers (Aves: Tyrannidae) are arguably the largest bird family in the Western Hemisphere (Fitzpatrick, 2004a). Confined to the New World, they constitute one of the four major bird radiations that make up the bulk of the Neotropics' unparalleled passerine diversity (the other three being furnariids, thamnophilid antbirds and nine-primaried oscines). Despite their spectacular diversification across the Neotropics, the Tyrannidae have received relatively little attention by phylogeneticists, and can be considered one of the last big pieces of *terra incognita* in avian family-level systematics. However, an increased understanding of phylogenetic relationships within the Tyrannidae would help us uncover the mechanisms that have led to such a great wealth of morphological and behavioral adaptations associated with this large avian radiation.

Though not exempt from the presence of odd taxa of problematic placement, the genus-level taxonomy within Tyrannidae and—to a lesser extent—its delineation towards other families have been relatively stable over the years (Fitzpatrick, 2004a). In contrast, the classification of tyrannid genera into subfamilies and tribes has been a task of substantial and on-going difficulty. Traylor's (1977, 1979) comprehensive revisions can be regarded as

* Corresponding author. Address: Department of Genetics, University of Melbourne, Parkville Campus, Grattan Street, 3010 Melbourne, Vic., Australia.
E-mail address: frankrheindt@yahoo.com.au (F.E. Rheindt).

1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2007.09.011

the first modern attempt at tyrannid classification. His treatment was expanded and improved upon by Lanyon (1986, 1988a, b), whose pioneering work synthesized previous morphological and behavioral data with skeletal traits, with a special emphasis on his own syringeal character data. Subsequently, Fitzpatrick (2004a) summarized these classifications in his family treatise (Fig. 1), but did not take into account information available from recent DNA-sequence studies.

In summary, Fitzpatrick's (2004a) treatment divides the Tyrannidae into three core subfamilies (Elaeniinae, Fluvicolinae, Tyranninae) plus the anomalous Tityrinae, which are sometimes raised to family level and placed as sister to the Tyrannidae (Fig. 1). Each of the core subfamilies is divided into two tribes. One of them, the Contopini, was newly erected by Fitzpatrick (2004b) to accommodate a number of genera that had variously been known as the "restricted *Empidonax* assemblage" (Birdsley, 2002) or "*Empidonax* group" (Lanyon, 1986) and that are distinct from other Fluvicolinae. Similarly, Lanyon (1988b) delineated his "*Elaenia* assemblage"—which is largely congruent with Fitzpatrick's (2004a) tribe Elaeniini—on the basis of an apomorphic configuration of the nasal septum, and he then offered a well-resolved morphology-based tree as a phylogenetic hypothesis for intergeneric relationships within this group (see different fonts in Fig. 1). As a result, Fitzpatrick (2004a) placed most of the remaining genera of the subfamily Elaeniinae in their own tribe Platyrinchini (Fig. 1), which is mainly made up of Lanyon's (1988a) "tody-tyrant and flatbill assemblage". The morphological studies of Traylor (1977, 1979) and Lanyon (1986, 1988a, 1988b) have been revisited using altered methodologies and aims (McKitrick, 1985; Birdsley, 2002); however, the resulting classifications were either not in great conflict with the previous works, or were poorly resolved.

Meanwhile, molecule-based methods applied to tyrannid systematics have yielded surprising results. Based on DNA–DNA hybridization, Sibley and Ahlquist (1985, 1990) placed a number of genera into tyrannid clades that largely coincide with Fitzpatrick's (2004a) scheme; however, they singled out certain genera (Fig. 1) into a new family Pipromorphidae (=Mionectidae) and placed it basal to all Tyranni, i.e., the group comprising the Tyrannidae and all their nearest neighbors, such as Tityridae, Cotingidae (cotingas) and Pipridae (manakins). This treatment renders the Tyrannidae polyphyletic. Although DNA-sequence studies have also identified a "pipromorphine" lineage, it was basal to other tyrannids, but not outside the family itself (Johansson et al., 2002; Ericson et al., 2003, 2006; Fjeldså et al., 2003; Chesser, 2004; Barker et al., 2004; Ohlson et al., 2007; Tello and Bates, 2007). This newly emerging clade Pipromorphinae includes as its core genera *Todirostrum*, *Hemitriccus*, *Mionectes*, *Leptopgon* and *Corythopis*, which have repeatedly come out in a highly supported lineage sister to all other tyrannids under study. Tello and Bates (2007) have added nine more genera to the pipromorphine assemblage, most of which have traditionally been considered part of the "tody-tyrant and flatbill assemblage" (Lanyon, 1988a) equivalent to Fitzpatrick's (2004a) Platyrinchini (Fig. 1).

Tello and Bates (2007) also uncovered an odd tyrannid clade consisting of three previously unsampled genera

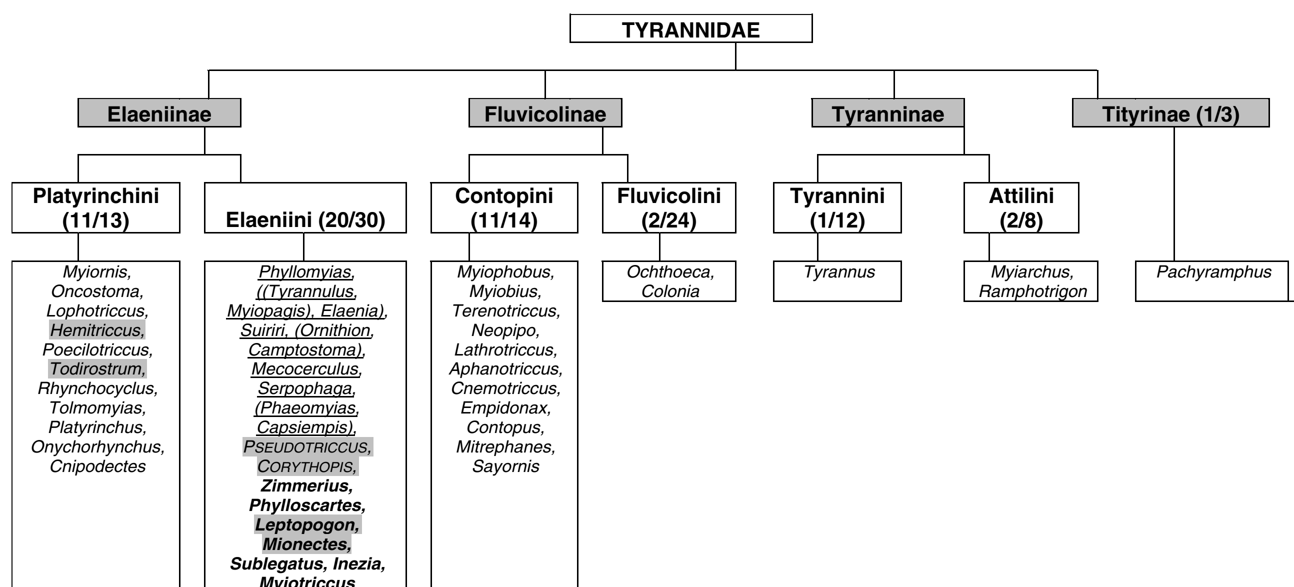

Fig. 1. Family classification as proposed by Fitzpatrick (2004a); the four gray boxes represent subfamilies, the six boxes below represent tribes; tribal names are accompanied by (number of genera sampled)/(total number of genera); tribal boxes connect to boxes containing all the constituent genera sampled in this study; within the Elaeniini, genera are individually marked to indicate their affinity to one out of three species groups as proposed by Lanyon (1988b): underlined "*Elaenia* group", small captials "*Pseudotriccus* group", bold "*Phylloscartes* group"; genera with a gray background were divided off into family Pipromorphidae by Sibley and Ahlquist (1985, 1990); however, these authors suggested that the majority of genera under tribe Platyrinchini should likewise be removed.

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

(*Onychorhynchus*, *Terenotriccus*, *Myiobius*) that came out at an even more basal position than the Pipromorphinae. Such a systematic treatment had never been suggested before, and indeed Tello and Bates's (2007) analyses left it open whether the non-tyrannid family Pipridae (manakins) is really more basal than this newly-identified flycatcher lineage. The fact that the three members of this strongly supported group are recruited from two different tribes (*sensu* Fitzpatrick, 2004a) highlights the strong disagreement between traditional morphology-based classifications and modern DNA sequence data. Furthermore, both Ericson et al. (2006) and Tello and Bates (2007) could not resolve the phylogenetic position of *Platyrinchus* within the Tyrannidae.

Outside of the new Pipromorphinae, sampling of tyrannid genera in DNA sequence studies has been limited. Cicero and Johnson (2002) clarified relationships among seven genera within the Contopini, however, assuming *a priori* that they constitute a monophyletic group. Ericson et al. (2006) incorporated 10 genera that came out as core tyrannids in their family-level sampling regime: A strongly supported fluvicoline group consisting of three genera (*Gubernetes*, *Knipolegus*, *Fluvicola*) emerged as sister to a *Myiarchus*-*Tyrannus* clade. This assemblage was placed as the sister group of a clade including five elaeniine genera (*Myiopagis*, *Serpophaga*, *Elaenia*, *Inezia*, *Stigmatura*). In Tello and Bates's (2007) work, the *Myiarchus*-*Tyrannus* clade was joined by *Ramphotrigon*, while two genera from the tribe Contopini (*Colonia*, *Empidonax*) and three elaeniines (*Capsiempis; Elaenia; Phyllomyias uropygialis*) grouped together as expected. The elaeniine *Myiotriccus*, however, variously emerged as sister to all core Tyrannidae.

Considering the large incongruences between the morphological and DNA-based classifications of the Tyrannidae, sequence data of more genera are required. To this end, we here provide DNA sequence data from 48 genera of Tyrannidae (Fig. 1; Appendix 1). We concentrated on, but did not limit ourselves to, the Elaeniini (*sensu* Fitzpatrick, 2004a; Fig. 1), bringing our genus coverage of that large tribe up to 67%. We also incorporate previously generated sequences (mainly Tello and Bates, 2007, and Cicero and Johnson, 2002; see Appendix 2) to cover 85% of the genera of Fitzpatrick's (2004a) now-invalidated Platyrinchini (most of which are in fact Pipromorphinae) and 79% of the genera of Contopini.

2. Materials and methods

2.1. Genetic and taxonomic sampling strategy

In this study, we analyzed the phylogenetics of New World flycatchers using one mitochondrial coding gene region, NADH dehydrogenase subunit 2 (ND2), and one nuclear intron, β-fibrinogen intron 5 (Fib5). For the ND2 dataset, we generated sequence data for 69 individuals spanning 44 species (Appendix 1), and supplemented them with another 46 Genbank sequences, mostly of complementary species (Appendix 2). The ingroup total for the ND2 dataset is 84 species. For the Fib5 dataset, we generated 61 sequences spanning 40 species (Appendix 1), which were complemented by 37 Genbank sequences (Appendix 2). Sampling for Fib5 amounted to 72 ingroup species. For the combined dataset, sequences were available for both gene regions in 93 individuals spanning 70 ingroup species.

All analyses were rooted using the Old World suboscine *Pitta*. Voucher information of the tissues used for sequence generation, as well as locality information and accession numbers of our samples and the additional Genbank samples are listed in the Appendix.

2.2. Extraction, sequence generation and alignment

Genomic DNA was extracted from frozen and ethanol-preserved tissue following standard extraction procedures as outlined in Gemmel and Akiyama (1996). For most samples, the complete ND2 gene (and partial sequence of the adjacent tRNA-Met) was amplified and sequenced in two overlapping fragments of approximately 370 and 750 base pairs (bp), using the primers L5215 with H5578 (Hackett, 1996) and FRND2.1 (5′-CAA TAG CAA TCT CAA TAA AAC TAG G-3′; this study) with H6315 (Kirchman et al., 2001), respectively. Additional sequences were amplified as a single fragment using L5215 and H6315. The Fib5 intron was amplified and sequenced using primers Fib5 and Fib6 (Driskell and Christidis, 2004). PCR conditions for both gene regions were similar to those described by Driskell and Christidis (2004). Amplified fragments were purified using either the GFX Gel Band and PCR purification Kit (Amersham Bioscience Corp., Piscataway, New Jersey) or the AMPure reagent (Agencourt Bioscience Corp., Beverly, Massachusetts). Purified PCR products were sequenced by Macrogen Corp., Inc. (Seoul, Korea) or on a MegaBACE 1000 capillary DNA sequencer utilising the methods described in Norman et al. (2007).

We aligned and edited sequences using the program SEQUENCHER v.4.1.4 (Gene Codes Corp., Ann Arbor, Michigan). All sequences were double-checked by eye. ND2 sequences were translated and checked for stop codons, anomalous substitution patterns and deviant base composition. Fib5 sequences contained numerous insertions and/or deletions (indels). Indels of ambiguous alignment were identified using the procedures outlined by Lutzoni et al. (2000).

2.3. Phylogenetic analysis

Maximum parsimony (MP) and Bayesian inference (BI) were employed in our phylogenetic analyses of both the combined data and the separate data partitions (nuclear and mitochondrial). We employed PAUP* (Swofford, 2002) for MP analyses, for testing partition homogeneity with an incongruence length difference (ILD) test, as well

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

as for estimating parameters for both data partitions. All PAUP* searches were heuristic, with default settings activated (keep best trees only, stepwise addition, swap on best starting trees only, simple addition sequence, TBR branch swapping, save multiple trees); however, SETMAXTREES was set to 200 with an auto-increase by 100. For PAUP* bootstrap analyses and the ILD test, we used 100 replicates, except for the bootstrap run for the Fib5 data partition, which was aborted after 12 days after reaching 20 replicates. The Fib5 partition contained a multitude of insertions and deletions (=indels); the phylogenetic information of those indels that could be unambiguously aligned was preserved in both MP and BI analyses by creating additional binary characters that coded for their presence or absence.

For MP analysis of the ND2 partition, we implemented a stepmatrix created with the program STMatrix 2.2 (François Lutzoni & Stefan Zoller, Department of Biology, Duke University; see also Miadlikowska et al., 2002), which calculates the probabilities of reciprocal changes from one state to another and converts them to a cost of change table using the negative natural logarithm of the probability. No such weighting scheme was used in MP analysis of the Fib5 partition in the absence of evidence for saturation (see Section 3). One 1–18 bp region within Fib5 could not be unambiguously aligned on account of the presence of indels. In MP analyses, this region was further analyzed using the program INAASE 2.3b (Lutzoni et al., 2000) and then removed. INAASE unequivocally codes ambiguous regions with a new character, which is then subjected to a specific stepmatrix to account for the differential number of changes (Lutzoni et al., 2000). Note that the number of these new INAASE characters exceeded MRBAYES program limitations (Ronquist et al., 2005), so this ambiguous region was removed and ignored in BI runs.

For BI analyses, we employed the Akaike information criterion (AIC; Akaike, 1974) as implemented in MODELTEST 3.06 (Posada and Crandall, 1998) in conjunction with PAUP* to evaluate the best fit among 56 different Maximum-Likelihood (ML) models. We then used the selected models for our BI searches, which were carried out using MRBAYES 3.1 (Ronquist and Huelsenbeck, 2003). However, as Bayesian searches attach only a restricted computational penalty to the estimation of parameters, we did not fix any of the model-specific parameters (such as the gamma shape parameter or the proportion of invariable sites) to the values given by MODELTEST 3.06, but we let MRBAYES 3.1 estimate these parameters instead (Ronquist et al., 2005). BI analyses employed Metropolis-coupled Markov chain Monte Carlo sampling with four incrementally heated chains running for 1 million generations with a sampling frequency of 100, and were repeated twice to ensure consistency between runs. In the combined analysis (Fib5 + ND2), we used different models for the data partitions as provided by MODELTEST 3.06. As branch support, posterior probabilities (PP) were derived from the 50% majority rule consensus of all trees retained after discarding the "burn-in", which constitutes those trees sampled before the BI runs had reached the optimal plateau areas of tree space. Burn-in was determined graphically following Ronquist et al. (2005).

Branch support was considered significant at levels of PP = 1.0 and MP bootstrap > 80. Only incongruences among significantly supported branches between analyses were considered to be in conflict. Nodes of conflict between the two single data partitions were mapped onto the ND2 tree. Since the ILD test showed our two partitions to be significantly incongruent (see Section 3), we used PAUP* in conjunction with the program TREEROT v. 2 (Sorenson, 1999) for the combined analysis to evaluate partitioned branch support (PBS; Baker and DeSalle, 1997) for most of those branches identified as incongruent between data partitions. With this parsimony-based branch support parameter, we were able to investigate the relative contribution of either partition to selected nodes of the combined-analysis tree, independent of their contribution in single-partition analyses (Gatesy et al., 1999).

We utilized PAUP* in conjunction with the ML model specified by MODELTEST 3.06 for our ND2 partition to compute ML scores for the most likely tree under the enforcement of a molecular clock and under relaxed branch length assumptions. We then compared likelihood scores of both trees with a χ^2-test (df = 119) to see whether they differ significantly.

3. Results

3.1. Alignment, indels and genetic characterization

As expected for coding genes, no indels were detected in the ND2 and partial tRNA-Met sequences (hereafter referred to as ND2). Their alignment was straightforward and amounted to 1088 bp. As no anomalies were detected in the amino acid translation of these sequences, we rule out the possibility of amplifying nuclear pseudogenes. Sequence length of the Fib5 intron varied between 535 bp in *Hemitriccus margaritaceiventer* and 594 bp in *Elaenia ruficeps*. This length variation was mainly due to the presence of 23 parsimony-informative indels across the various taxa. Only one 1–14 bp region spanning a 9 bp indel (identified as indel no. 7 in Fig. 2) was identified as being of ambiguous alignment, and was consequently re-coded following the procedures outlined by Lutzoni et al. (2000) and excised. The final alignment of the combined dataset comprising both Fib5 and ND2 amounted to 1667 bp, plus 23 re-coded characters representing all parsimony-informative indels.

Of the 16 indels equalling or exceeding 3 bp in length, only one was in conflict with the Fib5 topology (see below), as it occurred in two phylogenetically distant individuals (*Myiotriccus* and *Terenotriccus*). In contrast, the remaining 15 indels were restricted to well-supported clades and could readily be mapped onto the Fib5 tree (Fig. 2). Note that

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

ARTICLE IN PRESS

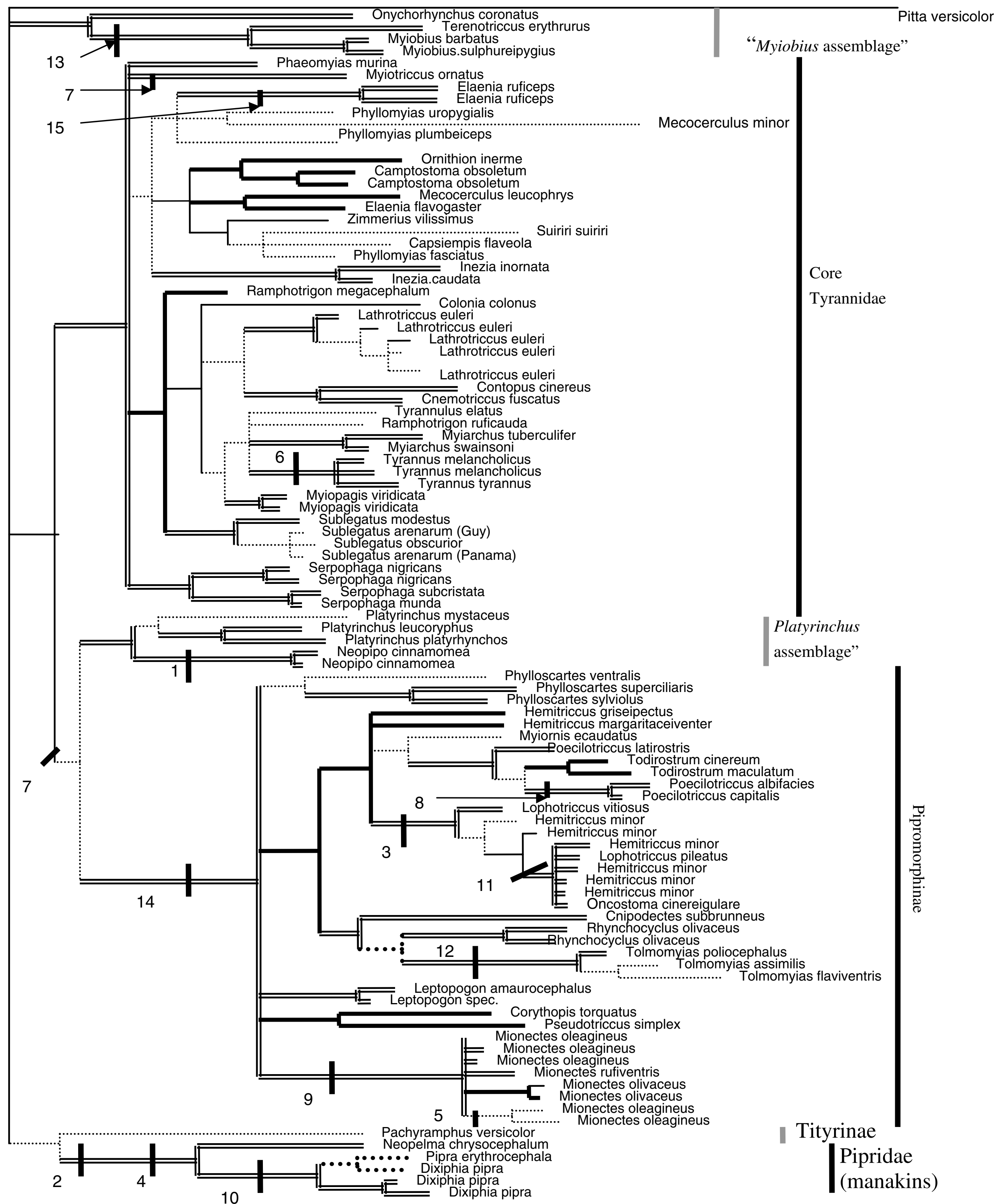

Fig. 2. Bayesian Inference tree of the Fib5 data partition; branch support indicated by branch thickness: thinly hatched branches, posterior probability (PP) < 95, Maximum parsimony bootstrap (BS) ⩽ 80; thin black branches, 0.95 ⩽ PP < 1.0, BS ⩽ 80; thickly hatched branches, PP < 95, BS > 80; thick black branches, PP = 1.0, BS ⩽ 80; thick hollow branches, PP = 1.0, BS > 80. Thick crossbars indicate the position of parsimony-informative indels: (1) 4 bp gap; (2) 7 bp gap; (3) 6 bp gap; (4) 6 bp gap (this may be homologous to an overlapping 8 bp gap in outgroup *Pitta* with respect to tyrannid ingroup); (5) 5 bp gap; (6) 4 bp gap; (7) 9 bp insertion; (8) 4 bp insertion; (9) 3 bp gap; (10) 3 bp gap; (11) 6–7 bp gap; (12) 9 bp gap; (13) 3 bp insertion; (14) 10 bp gap; and (15) 10 bp insertion.

one of them (a 9 bp insertion, no. 7 in Fig. 2) occurred at the base of all the Pipromorphinae and the *Platyrinchus*-*Neopipo* clade, but was additionally found in *Myiotriccus* (see Section 4).

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

Table 1 lists some properties and MP/ML parameters of each partition. Although the proportion of variable sites was roughly comparable between the two gene regions, Fib5 only exhibited about two thirds as many parsimony-informative sites as ND2 (Table 1). Comparisons of pairwise uncorrected sequence divergence between the two partitions (not shown) revealed that ND2 evolves about 4–7 times faster than Fib5. Chi-square tests of homogeneity of base frequencies across taxa did not show any evidence for base compositional bias, except for the third codon position in ND2 (Table 2). An ILD test revealed statistically significant incongruence between both data partitions ($P = 0.01$).

Plotting the number of pairwise substitutions (transitions and transversions) against uncorrected divergence did not reveal any deviation from linearity (=saturation) in Fib5 (not shown). However, it did show an abnormal transition/transversion ratio for a single individual (*Mecocerculus minor*; not shown), indicating that this species' phylogenetic position must be interpreted with caution. With respect to the ND2 partition, saturation was detected in third position transitions, starting at pairwise uncorrected divergences of 14% (not shown). In contrast, both types of substitution at first and second positions as well as third position transversions increased linearly with sequence divergence.

Pairwise uncorrected divergences were computed for all species combinations and for both partitions (not shown). Levels of maximum and minimum divergence were on average almost identical with those given by Tello and Bates (2007) for comparisons within species, within genera and between genera.

Table 1
Genetic properties and parameters of phylogenetic inference for the two data partitions and for the combined dataset

Data partition	Fib5	ND2	Combined
Total sites (bp)	627	1088	1715
Variable sites (bp)	390 (62.2%)	708 (65.1%)	1098 (64.0%)
PI sites (bp)	248 (39.6%)	658 (60.5%)	906 (52.8%)
Length of best MP trees (steps)	847	8895	12885.37
CI of best MP trees	0.656	0.148	0.206
Number of best MP trees	744700	35	2
Best ML model	TVM + G	GTR + I + G	—
AIC of best ML model	11263.816	65738.688	—

Abbreviations: PI, parsimony-informative; MP, maximum parsimony; CI, consistency index; PBS, partitioned branch support; ML, maximum likelihood; AIC, akaike information criterion.

Table 2
Base composition of all three mitochondrial codon positions and of the nuclear intron; parameters of χ^2-test for base compositional homogeneity

	ND2 1st position	ND2 2nd position	ND2 3rd position	ND2 overall	Fib5
A	35.6%	16.4%	37.8%	29.9%	30.3%
C	27.7%	35.0%	32.8%	31.8%	16.9%
T	21.7%	39.3%	5.8%	28.2%	30.8%
G	15.0%	9.3%	23.6%	10.0%	22.0%
χ^2 (df)	114.35 (360)	35.71 (360)	627.11 (360)	285.46 (360)	40.44 (297)
Homogeneity	HS	HS	NS	HS	HS

Abbreviations: df, degrees of freedom; HS, highly significant; NS, non-significant.

3.2. Phylogenetic results

The heuristic MP search on the Fib5 dataset (incl. the recoded indel information) found 744,700 most-parsimonious trees (see Table 1 for details). A restricted bootstrap run (see Section 2) provided high branch support for a number of nodes (Fig. 2). A BI analysis provided a tree (Fig. 2) that was in complete agreement with all the strongly supported branches of the MP analysis. While the BI tree exhibited strong branch support at a substantially higher number of nodes, there were two nodes that were strongly supported by MP but only weakly so by BI (see Fig. 2).

Our weighted MP analyses of the ND2 data partition using the step matrix approach yielded 35 most-parsimonious trees (see Table 1 for tree scores). A MP bootstrap run produced high branch support especially for terminal clades in the tree, while many deeper nodes remained unresolved (Fig. 3). The BI tree for ND2 (depicted in Fig. 3) was considerably better resolved than the Fib5 topology, including high branch support on some of the deeper nodes. More importantly, BI and MP analyses were entirely congruent. While the BI tree exhibited almost twice as many highly supported branches, the MP tree displayed better support in two minor instances: one involving internal relationships within *Mionectes oleagineus*; and a second in providing 100% bootstrap for a monophyletic *Phyllomyias griseiceps*, which is rendered paraphyletic with respect to *P. fasciatus* by BI, albeit with no support (not shown in Fig. 3). Enforcing a molecular clock on the ND2 data resulted in a ML score that was significantly lower than the one resulting from relaxed conditions ($P \approx 0$).

The trees produced by each of the two data partitions were not entirely congruent, although conflict between significantly supported branches was confined to only four regions within the tyrannid ingroup (Fig. 3). This partial incongruence may have been due to significant non-homogeneity of the two partitions (as shown by the ILD test, see above), and exacerbated by non-symmetrical sampling, with considerably more samples for the ND2 dataset. Within the Pipromorphinae, conflict among significantly supported branches between the two partitions was restricted to the node comprising *Lophotriccus pileatus*, *Oncostoma*, and the five samples of *Hemitriccus minor*. Like Tello and Bates (2007), we also noted an equivocal placement of *Cnipodectes*, either as a sister group to the flatbill clade (as indicated by Fib5; Fig. 2) or to the tody-tyrant clade (as indicated by ND2; Fig. 3). However, the latter arrangement received no significant support and

ARTICLE IN PRESS

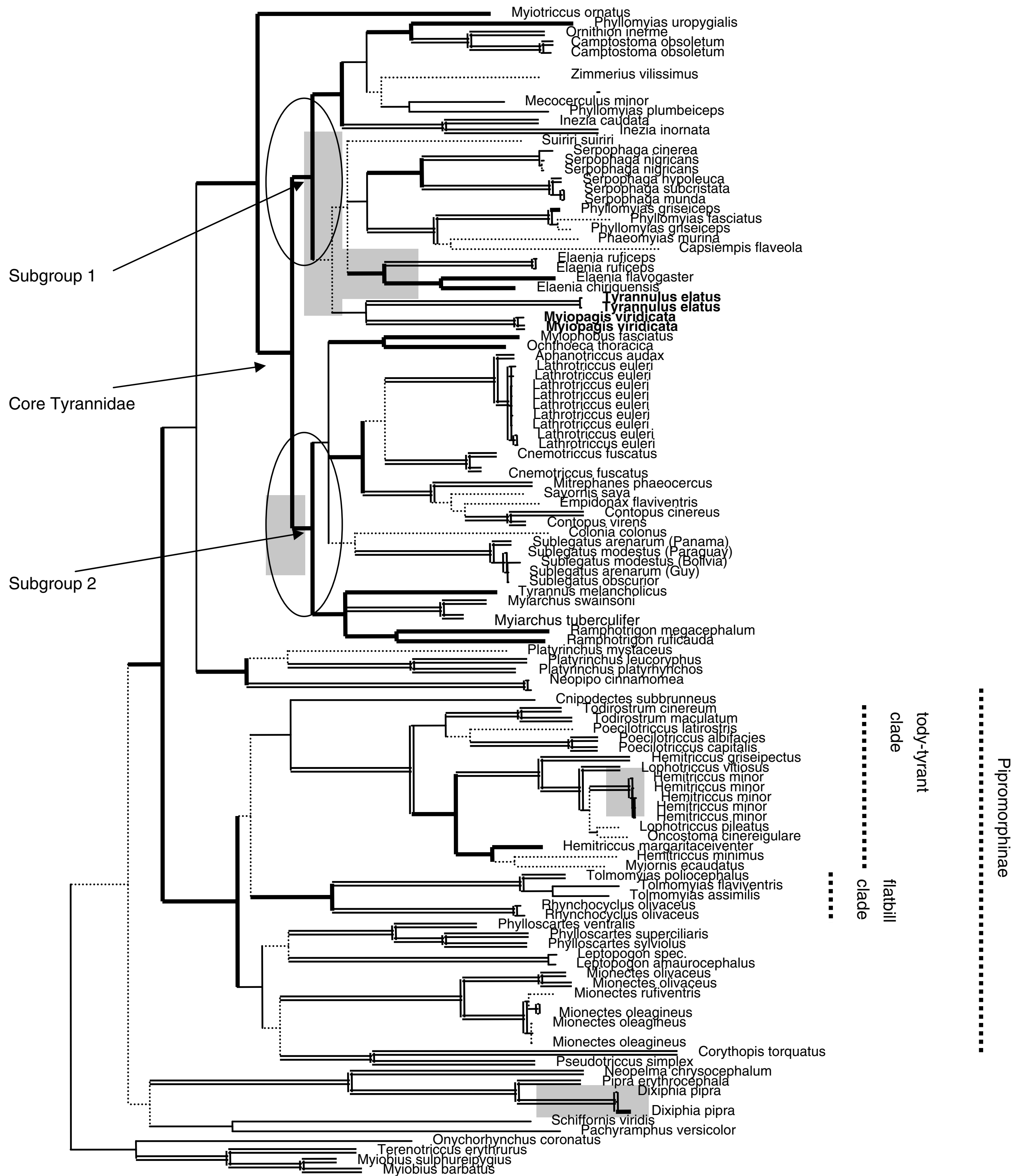

Fig. 3. Bayesian Inference tree of the ND2 data partition; branch support indicated by branch thickness, see legend Fig. 2. Gray background indicates regions of conflict among significantly supported nodes of ND2 and Fib5 data partition. Within the core Tyrannidae, two strongly supported subgroups are labeled; species that fall into the opposite subgroup in the Fib5 data partition are printed in bold (see Section 4).

was therefore not interpreted as incongruent. Within the core Tyrannidae, the ND2 sequences revealed the presence of two well-supported subgroups (subgroups 1 and 2; Fig. 3), each of which contained a number of significantly-supported and unsupported clades. Though Fib5 results were much less resolved, they supported or failed to contradict an identical placement of most members of these two ND2 subgroups. However, Fib5 analyses did associate four samples (belonging to the two genera *Myiopagis* and *Tyrannulus*, printed bold in Fig. 3) with core mem-

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

ARTICLE IN PRESS

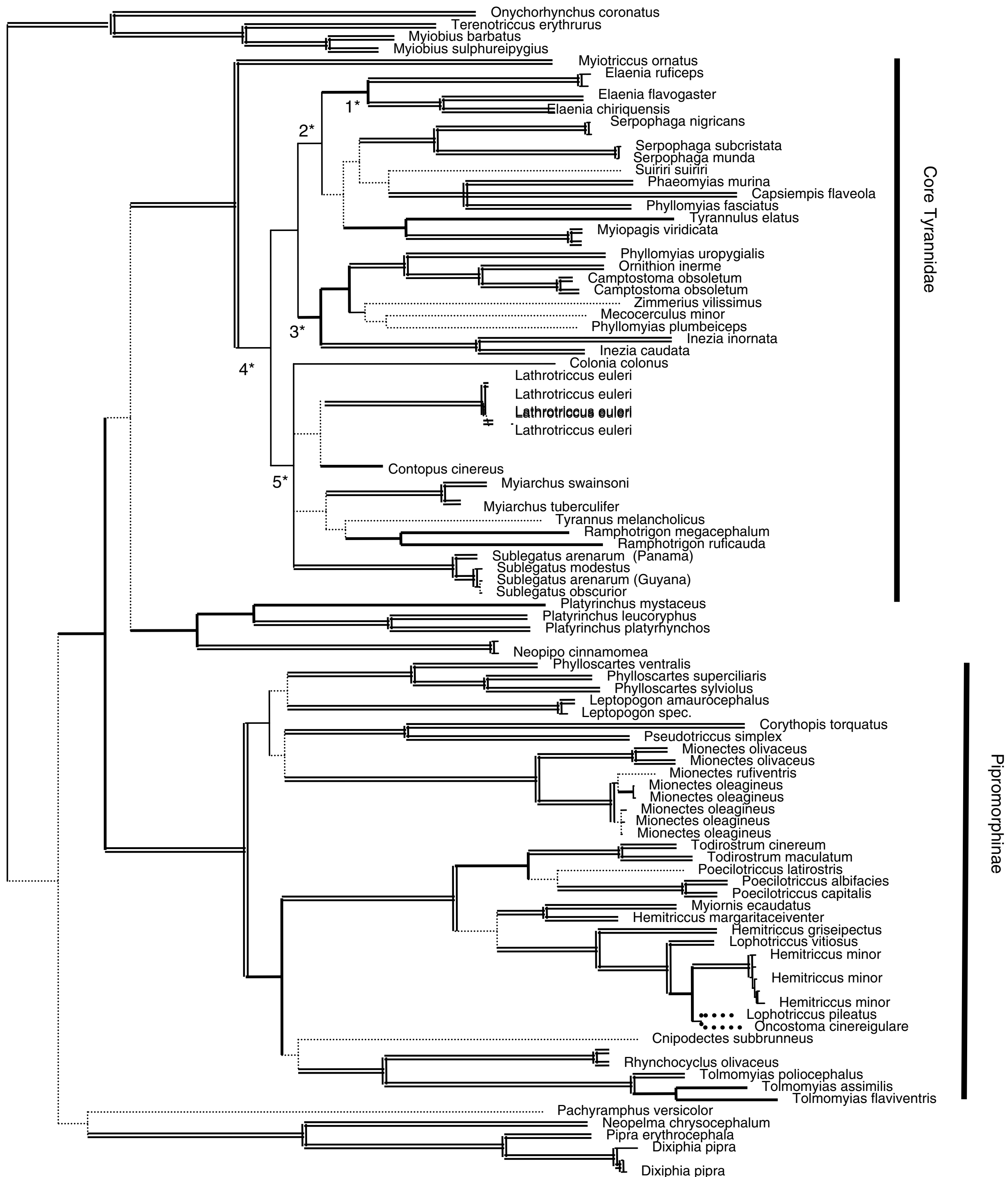

Fig. 4. Bayesian Inference tree of the combined dataset; branch support indicated by branch thickness, see legend Fig. 2; five nodes for which partitioned branch support is presented in Table 3 are labeled with asterisks.

bers of the opposite subgroup. This clade of two genera accounted for both basal regions of conflict between the two data partitions within the core Tyrannidae (Fig. 3).

Our MP analyses of the combined dataset, in which stepmatrix weighting was only applied to the ND2 partition, yielded two most-parsimonious trees (see Table 1 for tree scores). As in the separate-partition analyses, bootstrap support was high for many clades (Fig. 4). Even more resolution was attained in BI analyses (BI tree depicted in Fig. 4), which additionally delivered high posterior probabilities to many clades unsupported by MP. Again, BI and MP analyses were in complete agreement.

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

ARTICLE IN PRESS

Table 3
Partitioned branch support values of five tree nodes for both data partitions and summed

	ND2	Fib5	Total
Node 1	7.4	4	11.4
Node 2	5.6	0	5.6
Node 3	1.5	2	3.5
Node 4	10.3	1	11.3
Node 5	1	1	2

Node number refers to node labels in Fig. 4; for ease of interpretation, zero values have a gray background (negative values missing).

Within the core Tyrannidae, we calculated partitioned branch support (PBS) for all nodes that displayed significant conflict between the two data partitions, and additionally for two key nodes that were not in significant conflict between partitions, but that had only marginally significant support (as measured in PP or MP bootstrap). Table 3 lists the PBS for all five of these nodes, which can be found in the combined-analysis tree topology in Fig. 4. All of these five controversial nodes were supported by ND2, but not necessarily by Fib5, in the single-partition analyses. However, in the combined analysis, four out of these five nodes received additional PBS backing by Fib5, indicating that the Fib5 partition contains hidden branch support for these arrangements that is not evident in single-partition analyses but unfolds in the combined analysis. The remaining node (node 2; Fig. 4) received no additional support from the partitioned analysis, probably because Fib5 strongly supports an arrangement in which *Tyrannulus* and *Myiopagis* are associated with the core tyrannid subgroup 2 (Fig. 3) rather than subgroup 1.

4. Discussion

4.1. Phylogenetic utility of the genetic markers

In the single-partition analyses, both markers converged on tree topologies that were largely reconcilable with each other (Figs. 2 and 3). There were only four significantly supported tyrannid branches (three within the core Tyrannidae) that were in conflict between the two data partitions (Fig. 3). In order to test the significance of these conflicts, we calculated partitioned support for three incongruent core-tyrannid nodes, plus for an additional two key nodes within the core Tyrannidae that were generally poorly supported, in order to gauge individual support for these nodes by either of the two partitions, and to evaluate whether their presence in the combined tree was only backed by one partition but not by the other (Table 3). Of the five nodes thus assessed, four exhibited support by both partitions in the combined analysis although they had partially lacked Fib5 support in the single-partition analysis, which indicates that the Fib5 dataset contains hidden branch support for these nodes that only unfolds in the combined analysis (Gatesy et al., 1999). Only one node (node 2; Fig. 4) remained unsupported by Fib5, mainly because it contains two species (the *Tyrannulus*-*Myiopagis* clade) that Fib5 placed in another lineage with high support.

Considering the extensive congruence between Fib5 and ND2 results, and the demonstration that their areas of conflict in single-partition analyses were mostly invalidated by hidden branch support that unfolds in combined analyses, we based our phylogenetic inferences on strongly supported clades in the combined topology (Fig. 4), except in the case of node 2 (Fig. 4), the only such node that was shown to be supported by ND2 but not by Fib5.

4.2. Phylogenetic relationships

4.2.1. The ‘‘*Myiobius* assemblage’’

Recently, Tello and Bates (2007) were the first to reveal a clade (henceforth the ‘‘*Myiobius* assemblage’’) comprising three genera whose DNA had previously been unsampled (*Terenotriccus*, *Myiobius*, *Onychorhynchus*) and which are deeply divergent from all other tyrannid flycatchers. Indeed, Tello and Bates (2007) could not rule out the possibility that manakins (Pipridae) rather than the ‘‘*Myiobius* assemblage’’ are in fact the sister group of all other Tyrannidae (they lacked Tityridae as an outgroup). The basal tyrannid sampling regime in the present study is slightly enhanced with regard to Tello and Bates’s (2007) analysis (with one additional *Myiobius* species and 1–2 representatives of the Tityridae—*Pachyramphus* and *Schiffornis*). Our results corroborate recognition of the ‘‘*Myiobius* assemblage’’ and its deep divergence from all other Tyrannidae, and we were equally unable to distinguish between any branching order among the Tyrannidae, Tityridae, Pipridae and the ‘‘*Myiobius* assemblage’’ (Fig. 4).

The members of the ‘‘*Myiobius* assemblage’’ are recruited from two of Fitzpatrick’s (2004a) ‘‘morphological tribes’’, namely Contopini and Platyrinchini. *Terenotriccus* and *Myiobius* have always been considered problematic, to the extent that Fitzpatrick (2004b) placed them *incertae sedis* within the tribe Contopini. He emphasized their unusual pensile nests as a character that may unite them with a number of other tyrannid genera of equal nesting habit, e.g., *Onychorhynchus* or *Cnipodectes*. Although his prediction was correct with regard to *Onychorhynchus*, pensile nest builders are also found within the Pipromorphinae, suggesting that this trait may have evolved more than once in tyrannid evolution.

All the members of the ‘‘*Myiobius* assemblage’’ lack an intrinsic syringeal Musculus obliquus ventralis, a character that has been used to define the tyrant-flycatchers in general and that is absent in tityrids and manakins (Fitzpatrick, 2004a). However, this muscle is also missing in a small number of odd tyrannids that are firmly embedded (see below) within the Pipromorphinae and core Tyrannidae (e.g., *Zimmerius*, *Todirostrum*, *Tolmomyias*) and in genera that have yet to be analyzed by DNA sequence data (e.g., *Pyrrhomyias*, *Hirundinea*).

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

Considering that both our partitions and both modes of analysis consistently placed the "*Myiobius* assemblage" basal to the manakins, cotingas and tyrant-flycatchers (albeit with no support; Figs. 2 and 3), it is highly likely that this group may deserve recognition at the familial level. Further generic sampling is required to ascertain the exact composition of this assemblage and its relation to other major lineages.

4.2.2. Pipromorphinae

We found support for a monophyletic Tyrannidae (excluding the "*Myiobius* assemblage") that comprises the Pipromorphinae and the remaining Tyrannidae (henceforth the core Tyrannidae; Fig. 4). This result is consistent with other DNA sequence studies (Johansson et al., 2002; Ericson et al., 2003, 2006; Fjeldså et al., 2003; Chesser, 2004; Barker et al., 2004; Ohlson et al., 2007; Tello and Bates, 2007) that contradict Sibley and Ahlquist's (1985, 1990) proposal to remove pipromorphine genera from the tyrant-flycatchers. Our studies confirm Tello and Bates's (2007) arrangement of the Pipromorphinae into Lanyon's (1988a) "tody-tyrant and flatbill assemblage" (minus *Onychorhynchus* and *Platyrinchus*) and a clade comprising *Leptopogon*, *Mionectes*, *Corythopis* and *Pseudotriccus*; moreover, we add the large genus *Phylloscartes* to the Pipromorphinae (Fig. 4), although its exact internal placement requires further sampling. Monophyly of the pipromorphines as here defined was strongly supported in all analyses (Fig. 4) and confirmed by a shared 10 bp indel in the Fib5 partition with respect to the outgroup and other tyrant-flycatchers (Fig. 2).

4.2.3. The "Platyrinchus assemblage"

Tello and Bates (2007) could not determine the affinities of the spadebills (*Platyrinchus*)—traditionally considered a typical member of the pipromorphine "flatbill assemblage" (Lanyon, 1988a; Fitzpatrick, 2004a)—with respect to the Pipromorphinae and the core Tyrannidae (Fig. 4), although Fib5 branch support for their association with the Pipromorphinae was moderately high in that study. Our increased taxon sampling demonstrates that *Platyrinchus* forms a highly supported clade (here termed the "*Platyrinchus* assemblage") with the enigmatic *Neopipo* (Fig. 4), a genus previously considered a manakin until placed with the atypical cliff flycatcher (*Hirundinea*) and cinnamon flycatcher (*Pyrrhomyias*) by Mobley and Prum's (1995) on morphological grounds. When Fitzpatrick (2004b) erected the tribe Contopini for—amongst others—*Hirundinea*, *Pyrrhomyias* and *Neopipo*, he left the latter genus *incertae sedis* and suspected it may some day be proved to be affiliated with a group of atypical pensile nest builders, such as *Terenotriccus*, *Onychorhynchus* and *Myiobius*. However, all three of these are now shown to be members of the "*Myiobius* assemblage" possibly outside of the true tyrant-flycatchers (see above). Mobley and Prum's (1995) prediction about *Neopipo*'s affinity to *Hirundinea* and *Pyrrhomyias* equally needs further testing, as no DNA sequences of the latter two have hitherto been investigated.

Although our analyses produced a very high support for the monophyly of the "*Platyrinchus* assemblage", neither of our data partitions yielded convincing branch support for its association with either the core Tyrannidae or the Pipromorphinae (Figs. 2 and 3). However, we did detect a characteristic 9 bp insertion that linked the "*Platyrinchus* assemblage" with the Pipromorphinae, in accordance with Tello and Bates's (2007) Fib5 bootstrap. This 9 bp insertion was also present in *Myiotriccus*, the most basal member of the core Tyrannidae (see below), so it may be a tyrannid apomorphy that was secondarily lost by the core Tyrannidae (minus *Myiotriccus*). *Platyrinchus* and *Neopipo* also lack the 10 bp indel present in the Pipromorphinae (see above).

The "*Platyrinchus* assemblage" may best be treated as a subfamily of its own. However, more genera need to be sampled before naming such a subfamily. Moreover, note that the name "Platyrinchinae" may not be available, because Fitzpatrick (2004a) used the subordinate name Platyrinchini to denote an unnatural tribe including *Platyrinchus* and an additional 12 genera, none of which have emerged as members of the "*Platyrinchus* assemblage".

4.2.4. The core Tyrannidae

Our analyses provide strong support for the placement of all other flycatchers inside a monophyletic group that we here label the "core Tyrannidae" (Fig. 4). One of its members, the ornate flycatcher (*Myiotriccus ornatus*), an unusually colored monospecific genus formerly placed in Lanyon's (1988b) "*Elaenia* assemblage" (=Fitzpatrick's (2004a) Elaeniini), repeatedly shifted position in Tello and Bates's (2007) study depending on analysis, but emerged as the sole basal member of the core Tyrannidae with near-significant branch support in our study (Fig. 4). Additionally, a characteristic 9 bp insertion in the Fib5 partition present in the Pipromorphinae, the "*Platyrinchus* assemblage" and *Myiotriccus*, but not in the remainder of the core Tyrannidae, lends additional support to placing *Myiotriccus* basal to all other core tyrannids. PBS values show that this basal arrangement of *Myiotriccus* is mainly supported by ND2, but Fib5 does not contradict it despite lesser support (node 4; Table 3).

4.2.4.1. The elaeniine assemblage. In the context of our sampling regime, the core Tyrannidae were subdivided into two major groups that roughly correspond with Fitzpatrick's (2004a) Elaeniini (subgroup 1; Fig. 3) and various members of the Fluvicolinae and Tyranninae (subgroup 2; Fig. 3). However, a clade of two genera (the *Myiopagis-Tyrannulus* clade; printed bold in Fig. 3) is placed into different subgroups by each of the two partitions, respectively. This clade's traditional affiliation has been with subgroup 1 (Elaeniini), where Lanyon's (1988b) placed it on account of apomorphic configurations in syringeal morphology. This is also where ND2 places it (Fig. 3), and

where it consequently emerges on the combined tree (Fig. 4). Considering that Lanyon's (1988b) use of the syrinx as a taxonomic marker is congruent with our findings in all 11 other elaeniine genera sampled, we suggest that the association of the *Myiopagis*-*Tyrannulus* clade with subgroup 1 (Fig. 3) is correct. Subgroup 1 (henceforth the elaeniine assemblage) is completely identical with Lanyon's (1988b) "*Elaenia* group", nested within his "*Elaenia* assemblage", as far as our sampling permits us to conclude, and it may thus be one of the few tyrannid tribes whose taxonomic circumscription on morphological grounds is not contradicted by DNA sequences.

This elaeniine assemblage is further subdivided into two lineages (node 2 and 3; Fig. 4). However, the first lineage (node 2) lacks support by Fib5 in both single-partition and combined analyses (see node 2, Table 3). This conflict is probably due to the mixed phylogenetic signal of the *Myiopagis*-*Tyrannulus* clade (see above), but may be exacerbated by the uncertain placement of *Suiriri* between data partitions (Figs. 2 and 3). While we are thus not strongly confident about the correctness of node 2, our data are highly supportive of some of the clades contained within this node. For instance, there is strong signal for a monophyletic *Serpophaga* (for which ND2 species coverage was 100%, Fig. 3). Additionally, there is high support in both partitions for a clade comprising *Phaeomyias*, *Capsiempis* and *Phyllomyias s. str.* (Figs. 2–4). The close affinity between *Phaeomyias* and *Capsiempis* was predicted by Lanyon (1988b) on morphological grounds. Equally, it has long been suspected that *Phyllomyias* may eventually turn out to be polyphyletic, which is supported by our data placing the two true members of *Phyllomyias* (*P. griseiceps* and *P. fasciatus*) here, while *P. uropygialis* and *P. plumbeiceps* are phylogenetically distant (see below). Finally, monophyly of the four samples of the genus *Elaenia* is strongly supported by ND2 (Fig. 3). Though the Fib5 partition initially contradicted this arrangement (Fig. 2), it produced considerable positive hidden branch support for this clade in the combined analysis (node 1; Table 3).

The second lineage (node 3; Fig. 4) within the elaeniine assemblage receives near-significant support from both partitions in the combined analysis (node 3; Table 3), and additionally there is no conflict between significantly supported branches in single-partition analyses. There is a number of clades within this lineage that are highly supported, such as a strongly monophyletic *Inezia* (two species sampled), and the *Ornithion*-*Camptostoma* clade (as correctly predicted by Lanyon (1988b)), which is joined by *Phyllomyias uropygialis* with high posterior probability. The latter has long been suspected to be unrelated to *Phyllomyias s. str.* (see above) and was therefore split off into *Tyranniscus* along with two or more other species (see Fitzpatrick, 2004a), a treatment that seems to be borne out by our data. Another *Phyllomyias* species (*P. plumbeiceps*) also falls near the *Ornithion*-*Camptostoma* clade, though not as a sister species to *P. uropygialis*. *P. plumbeiceps* has variously been considered a member of *Phyllomyias*, or *Tyranniscus* (along with *P. uropygialis* and others), or of a genus of its own (*Oreotriccus*; Fitzpatrick, 2004a). While it seems to be safe to conclude that *P. plumbeiceps* needs to be removed from *Phyllomyias*, we would like to await denser taxon sampling to decide whether it deserves its own genus or should be assigned to *Tyranniscus*.

4.2.4.2. The fluvicoline/tyrannine assemblage. Subgroup 2 (Fig. 3) within the core Tyrannidae consists of a number of genera that have traditionally been grouped with the subfamilies Tyranninae and Fluvicolinae (Fitzpatrick, 2004a). Note, however, that this subgroup also contains a monophyletic *Sublegatus* (100% species coverage), though with marginal support and in a basal position (Fig. 4). *Sublegatus* has previously been placed with the Elaeniini (Fitzpatrick, 2004a). Partitioned branch support for subgroup 2 is marginal for both data partitions (node 5; Table 3), which is at least partially caused by the mixed partition-specific signal of the *Tyrannulus*-*Myiopagis* clade (see above). Our taxon sampling for this huge assemblage is tiny; thus, phylogenetic inferences are beyond the scope of this study and must await the completion of investigations with a denser coverage of genera.

4.2.5. Species-level relationships

Though not the focus of this study, our sampling uncovered several species-level arrangements that warrant further investigation.

Our addition of *Mionectes olivaceus* to Tello and Bates's (2007) sampling of *Mionectes* corroborated the monophyly of the genus (Fig. 4). However, our two *olivaceus* samples originate from different subspecies groups (Fitzpatrick, 2004a), one cis-Andean (east-slope in Peru) and one trans-Andean (Panama). They are separated by 5.3% uncorrected sequence divergence in mitochondrial ND2, which is considerably larger than comparisons between the congeneric and poorly differentiated *M. oleagineus* and *M. rufiventris* (3.5–3.7%). Different members of *Mionectes* would benefit from inquiries into phylogenetics and vocalizations to determine whether current taxonomy correctly reflects biological species diversity.

Within the tody-tyrants, *Hemitriccus minimus* emerged as part of Tello and Bates's (2007) clade III (along with *H. margaritaceiventer* and *Myiornis*), far from the vicinity of *H. minor* (Fig. 3), of which it had formerly been considered a weakly defined race (Fitzpatrick, 2004a). Uncorrected mitochondrial divergences supported the distinct status of *H. minimus*, with a 15–15.4% difference between *H. minimus* and *H. minor* and 11.4% between *H. minimus* and *H. margaritaceiventer*. Within *H. minor* itself, sampling of six individuals from large swathes of its Amazonian range revealed little mitochondrial differentiation (0.2–0.5% ND2 uncorrected p), suggesting that the highly fragmented distribution of this elusive species may be a sampling artefact. However, this assertion requires confirmation from broader sampling and a population-genetic approach.

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

ARTICLE IN PRESS

The three members of *Sublegatus* have formerly been united into a single species and then split on account of minor differences in plumage and major ones in vocalizations (Fitzpatrick, 2004a). Our coverage of all three species provides strong support for a basal position of the isolated Central American nominate *S. arenarum* with respect to all other taxa (Figs. 3 and 4). Indeed, there is limited differentiation in the ND2 sequences among *S. obscurior*, *S. modestus* and Guianan *S. arenarum* (0–1.6%), whereas their divergence towards nominate *S. arenarum* is higher at 3.2–4.2%. Additionally, within the four poorly-differentiated South American samples, mitochondrial divergence is largest between Bolivian *S. modestus* and the remainder (1.2–1.6%), while divergence between the other two species (*S. obscurior* and Guianan *S. arenarum*) and between them and a second sample of *S. modestus* from Paraguay is virtually zero (0–0.3%). Sampling locations suggest that misidentification is an unlikely factor (Appendix). However, since little is known about the austral migration routes of *S. modestus* to the north of South America, it may often be confused with *S. obscurior* or even *S. arenarum* during the non-breeding season. Moreover, Fitzpatrick (2004a) reports on a hitherto undescribed species of *Sublegatus* from Guyana, to which our Guianan sample may actually belong, and which would explain the paraphyly of *S. arenarum* in our tree. Only additional sampling of more representatives of each taxon, coupled with a rigorous vocal and plumage assessment, will be able to determine the number of species in *Sublegatus*.

Another genus of which we have attained 100% coverage and which yielded surprising arrangements despite strong support for its monophyly is *Serpophaga* (Figs. 3 and 4). The five species of *Serpophaga* were divided into two sub-clades of high branch support (Fig. 4) and of comparatively great depth (15.1–16.7 % uncorrected ND2 divergence): one comprising the two streamside inhabitants *S. nigricans* and *S. cinerea*, and the other consisting of the Amazonian river-island specialist *S. hypoleuca* and the two generalists *S. subcristata* and *S. munda*. However, within both *Serpophaga* sub-clades, species exhibited low mitochondrial differentiation (1.6–1.7% uncorrected ND2 divergence between *S. nigricans* and *S. cinerea*, 0.2% between *S. munda* and *S. subcristata*, and 2.3–2.4% between *S. hypoleuca* and the two generalists). Note that *S. nigricans* and *S. cinerea* are allopatric and have been considered closely related in the past (Fitzpatrick, 2004a), although they do show pronounced plumage differences and have never been lumped. *S. munda* and *S. subcristata*, which are near-identical in ND2 sequence, were formerly considered one species, but do differ substantially in belly color and are reported to be parapatric with limited hybridization (Fitzpatrick, 2004a). Undoubtedly the genus *Serpophaga* requires a more in-depth study to reveal whether incorrect species boundaries were drawn in the past, or whether identical mitochondrial genomes between species are the result of past hybridization events and subsequent mitochondrial introgression. Unfortunately, we lack nuclear sequences for 2 of the 5 species in this study to resolve this issue further.

Myiobius sulphureipygius and *M. barbatus* were formerly considered two subspecies groups (a trans-Andean and a cis-Andean one, respectively) of a single species (Fitzpatrick, 2004a). ND2 comparisons between them were at the level of 7.6% uncorrected divergence. While taxonomic decisions must not rely on isolated divergence values that are not put into a comparative context with closely related taxa, this value does considerably exceed the level of divergence between distinct biological sister species in the present study and in Tello and Bates's (2007) investigation, and therefore lends additional support for splitting these two allopatric forms.

Finally, within our sampling of *Phyllomyias s. str.*, the two sister taxa *P. griseiceps* and *P. fasciatus* showed 6.5–6.8% mitochondrial divergence as observed in many other congeneric tyrannid species. However, within the monotypic *P. griseiceps*, two Ecuadorian samples from west and east of the Andes, respectively, exhibited 2.4% divergence. Again, such an isolated divergence value does not permit any taxonomic conclusions, but it does constitute a comparatively high level of mitochondrial differentiation between two samples from the same subspecies that are in relative geographical proximity (though separated by the Andes). Additional sampling from all over the range of *P. griseiceps* is required to uncover hidden racial or species diversity in this species.

4. Conclusions

Past workers on anatomical, behavioral and plumage characters have been in some disagreement about tyrannid classification into subfamilies and tribes (Traylor, 1977, 1979; McKitrick, 1985; Lanyon, 1986, 1988a, 1988b; Birdsley, 2002). Sibley and Ahlquist's (1985, 1990) research using DNA–DNA hybridization exacerbated this disagreement. Most importantly, however, modern DNA-sequence based studies have—on many counts—shown key morphological characters to be homoplasious, while at the same time disagreeing with results from DNA–DNA hybridization (Johansson et al., 2002; Cicero and Johnson, 2002; Ericson et al., 2003, 2006; Fjeldså et al., 2003; Chesser, 2004; Barker et al., 2004; Ohlson et al., 2007; Tello and Bates, 2007). Nevertheless, most of these DNA-sequence studies have lacked a thorough taxon sampling within the Tyrannidae, with the exception of Cicero and Johnson (2002), who covered well Lanyon's (1986) "*Empidonax* assemblage", and Tello and Bates (2007), who unravelled relationships within the Pipromorphinae.

This study is one further step in elucidating the systematic relationships of the myriad genera and species in the confusing radiation that is the Tyrannidae by adding taxon coverage especially to the elaeniine subgroup (Lanyon, 1988b). Our results corroborated recent findings of a tyran-

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011

ARTICLE IN PRESS

nid division into Pipromorphinae and core Tyrannidae, with a small "*Myiobius* assemblage" falling outside of the Tyrannidae, and a newly defined "*Platyrinchus* assemblage" falling somewere in between the two big tyrannid groups. Our analyses also added new taxa to some of these confirmed lineages. Within the core Tyrannidae, where resolution has so far been lacking, our data hint at a monophyletic core tyrannid clade that we term the elaeniine assemblage, which is identical to Lanyon's (1988b) "*Elaenia* group" and may be the sister group of all other core tyrannids. Within this elaeniine assemblage, there is support for the monophyly of a number of genera (*Elaenia*, *Serpophaga*, *Inezia*) as far as taxon sampling permits, and for the close relationship between *Capsiempis*/*Phaeomyias*/*Phyllomyias s. str.*, *Camptostoma*/*Ornithion*/*Tyranniscus* and *Tyrannulus*/*Myiopagis*, respectively. Many of these relationships are in congruence with Lanyon's (1988b) taxonomic recommendations based on morphological characters. Therefore, while a number of recent DNA studies into tyrannid systematics have been at odds with morphological arrangements (e.g., as concerns the Pipromorphinae, or the "*Platyrinchus*" assemblage), our data on the elaeniine assemblage show good concordance with past morphological analyses. However, it is still too early to make inferences about broad-scale patterns in the evolutionary history of morphological traits in Tyrannidae, and such studies must await a fuller resolution of major tyrannid subgroups, particularly of the Fluvicolinae and Tyranninae.

Acknowledgments

For the provision of tissue samples, we are greatly indebted to the following people at the following institutions: Donna Dittmann, Van Remsen and Robb Brumfield (Louisiana State University Museum of Natural Sciences, Baton Rouge, Louisiana), Nate Rice and Leo Joseph (Academy of Natural Sciences of Philadelphia), Mark Robbins and A. Townsend Peterson (University of Kansas Natural History Museum, Lawrence, Kansas), David Willard and Shannon Hackett (The Field Museum of Natural History, Chicago), Paul Sweet (American Museum of Natural History), Christopher Huddleston, Gary Graves and James Dean (Smithsonian National Museum of Natural History, Washington, DC), Cristina Miyaki (Laboratório de Genética e Evolução Molecular de Aves, São Paulo), José Álvarez Alonso (Iquitos, Peru), and Armando Valdés-Velásquez (Universidad Cayetano-Heredia, Lima, Peru). For assistance with some of the labwork, Gustavo Cabanne deserves warm thanks. We thank the Instituto Nacional de Recursos Naturales (INRENA) of Peru for granting collection permits for fieldwork. This work was undertaken at the Population and Evolutionary Genetics Unit, Museum Victoria, which was established with the generous support of the Ian Potter Foundation and Amersham Biosciences (now GE Healthcare).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.09.011.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE T. Automat. Contr. 19, 716–723.

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Syst. Biol. 46, 654–673.

Barker, F.K., Cibois, A., Schikler, P., Feinstein, J., Cracraft, J., 2004. Phylogeny and diversification of the largest avian radiation. PNAS 101, 11040–11045.

Birdsley, J.S., 2002. Phylogeny of the tyrant flycatchers (Tyrannidae) based on morphology and behavior. Auk 119, 715–734.

Chesser, R.T., 2004. Molecular systematics of the New World suboscine birds. Mol. Phylogen. Evol 32, 11–24.

Cicero, C., Johnson, N.K., 2002. Phylogeny and character evolution in the *Empidonax* group of tyrant flycatchers (Aves: Tyrannidae): a test of W. E. Lanyon's hypothesis using mtDNA sequences. Mol. Phylogen. Evol. 22, 289–302.

Driskell, A.C., Christidis, L., 2004. Phylogeny and evolution of the Australo-Papuan honeyeaters (Passeriformes, Meliphagidae). Mol. Phylogen. Evol. 31, 943–960.

Ericson, P.G.P., Irestedt, M., Johansson, U.S., 2003. Evolution, biogeography, and patterns of diversification in passerine birds. J. Avian Biol. 34, 3–15.

Ericson, P.G.P., Zuccon, D., Ohlson, J.I., Johansson, U.S., Alvarenga, H., Prum, R.O., 2006. Higher-level phylogeny and morphological evolution of tyrant flycatchers, cotingas, manakins, and their allies (Aves: Tyrannida). Mol. Phylogen. Evol. 40, 471–483.

Fitzpatrick, J.W., 2004a. Family Tyrannidae (Tyrant-Flycatchers). In: del Hoyo, J., Elliott, A., Christie, D.A. (Eds.), Handbook of the Birds of the World, Cotingas to Pipits and Wagtails, vol. 9. Lynx Edicions, Barcelona, pp. 170–462.

Fitzpatrick, J.W., 2004b. Description of a new tribe of fluvicoline flycatchers. In: del Hoyo, J., Elliott, A., Christie, D.A. (Eds.), Handbook of the Birds of the World, Cotingas to Pipits and Wagtails, vol. 9. Lynx Edicions, Barcelona, pp. 25–27.

Fjeldså, J., Zuccon, D., Irestedt, M., Johansson, U.S., Ericson, G.P.G., 2003. Sapayoa aenigma: a New World representative of the 'Old World suboscines'. Proc. R. Soc. Lond. B 270 (Suppl. 2), S238–S241.

Gatesy, J., O'Grady, P., Baker, R.H., 1999. Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. Cladistics 15, 271–313.

Gemmel, N., Akiyama, S., 1996. An efficient method for extraction of DNA from vertebrate tissues. Trends Genet. 12, 338–339.

Hackett, S.J., 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Mol. Phylogen. Evol. 5, 368–382.

Johansson, U.S., Irestedt, M., Parsons, T.J., Ericson, P.G.P., 2002. Basal phylogeny of the Tyrannoidea based on comparisons of cytochrome *b* and exons of nuclear c-*myc* and RAG-1 genes. Auk 119, 984–995.

Kirchman, J.J., Hackett, S.J., Goodman, S.M., Bates, J.M., 2001. Phylogeny and systematics of the ground rollers (Brachypteraciidae) of Madagascar. Auk 118, 849–863.

Lanyon, W.E., 1986. A phylogeny of the thirty-three genera in the *Empidonax* assemblage of tyrant flycatchers. Amer. Mus. Novit. 2846, 1–29.

Lanyon, W.E., 1988a. A phylogeny of the flatbill and tody-tyrant assemblage of tyrant flycatchers. Am. Mus. Novit. 2923, 1–41.

Lanyon, W.E., 1988b. A phylogeny of the thirty-two genera in the *Elaenia* assemblage of tyrant flycatchers. Am. Mus. Novit. 2914, 1–57.

Lutzoni, F., Wagner, P., Reeb, V., Zoller, S., 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. 49, 628–651.

McKitrick, M.C., 1985. Monophyly of the Tyrannidae (Aves): comparison of morphology and DNA. Syst. Zool. 34, 35–45.

Miadlikowska, J., McCune, B., Lutzoni, F., 2002. *Pseudocyphellaria perpetua*, a new lichen from Western North America. The Bryologist 105, 1–10.

Mobley, J.A., Prum, R.O., 1995. Phylogenetic relationships of the Cinnamon Tyrant, *Neopipo cinnamomea*, to the tyrant flycatchers (Tyrannidae). Condor 97, 650–662.

Norman, J.A., Rheindt, F.E., Rowe, D.L., Christidis, L., 2007. Speciation dynamics in the Australo-Papuan *Meliphaga* honeyeaters. Mol. Phylogen. Evol. 42, 80–91.

Ohlson, J.I., Prum, R.O., Ericson, P.G.P., 2007. A molecular phylogeny of the cotingas (Aves: Cotingidae). Mol. Phylogen. Evol. 42, 25–37.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Ronquist, F., Huelsenbeck, J.P., van der Mark, P., 2005. MrBayes 3.1 Manual. Online manual available at <http://mrbayes.csit.fsu.edu/manual.php>.

Sibley, C.G., Ahlquist, J.E., 1985. Phylogeny and classification of New World suboscine passerine birds (Passeriformes: Oligomyodi: Tyrannides). In: Foster, M.S. (Ed.), Neotropical ornithology. Ornithol. Monogr. 36, 396–428.

Sibley, C.G., Ahlquist, J.E., 1990. Phylogeny and classification of birds. Yale University Press, London.

Sorenson, M.D., 1999. TreeRot, version 2. Boston University, Boston, Massachusetts.

Swofford, D.L., 2002. PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Tello, J.G., Bates, J.M., 2007. Molecular phylogenetics of the tody-tyrant and flatbill assemblage of tyrant flycatchers (Tyrannidae). Auk 124, 134–154.

Traylor, M.A., 1977. A classification of the tyrant flycatchers (Tyrannidae). Bull. Mus. Comp. Zool. 148, 128–184.

Traylor, M.A., 1979. Check-list of Birds of the World: A Continuation of the Work of James L. Peters. vol. 8. Museum of Comparative Zoology, Harvard University Press, Cambridge, Massachusetts.

Further reading

Fuchs, J., Bowie, R.C., Fjeldså, J., Pasquet, E., 2004. Phylogenetic relationships of the African bush-shrikes and helmet-shrikes (Passeriformes: Malaconotidae). Mol. Phylogenet. Evol. 33, 428–439.

Marini, M.A., Hackett, S.J., 2002. A multifaceted approach to the characterization of an intergeneric hybrid manakin (Pipridae) from Brazil. Auk 119, 1114–1120.

Moyle, R.G., Chesser, R.T., Prum, R.O., Schikler, P., Cracraft, J., 2006. Phylogeny and evolutionary history of Old World suboscine birds (Aves: Eurylaimides). American Museum Novitates 3544, 1–22.

Slack, K.E., Delsuc, F., McLenachan, P.A., Arnason, U., Penny, D., 2007. Resolving the root of the avian mitogenomic tree by breaking up long branches. Mol. Phylogen. Evol. 42, 1–13.

Please cite this article in press as: Rheindt, F.E. et al., Phylogenetic relationships of tyrant-flycatchers (Aves: Tyrannidae), ..., Mol. Phylogenet. Evol. (2007), doi:10.1016/j.ympev.2007.09.011