Tyrant flycatchers coming out in the open: phylogeny and ecological radiation of Tyrannidae (Aves, Passeriformes)

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Tyrant flycatchers constitute a substantial component of the land bird fauna in all South American habitats. Past interpretations of the morphological and ecological evolution in the group have been hampered by the lack of a well-resolved hypothesis of their phylogenetic interrelationships. Here, we present a well-resolved phylogeny based on DNA sequences from three nuclear introns for 128 taxa. Our results confirm much of the overall picture of Tyrannidae relationships, and also identify several novel relationships. The genera *Onychorhynchus*, *Myiobius* and *Terenotriccus* are placed outside Tyrannidae and may be more closely related to Tityridae. Tyrannidae consists of two main lineages. An expanded pipromorphine clade includes flatbills, tody-tyrants and antpipits, and also *Phylloscartes* and *Pogonotriccus*. The spadebills, *Neopipo* and *Tachuris* are their closest relatives. The remainder of the tyrant flycatchers forms a well-supported clade, subdivided in two large subclades, which differ consistently in foraging behaviour, the perch-gleaning elaeniines and the sallying myiarchines, tyrannines and fluvicolines. A third clade is formed by the genera *Myiotriccus*, *Pyrrhomyias*, *Hirundinea* and three species currently placed in *Myiophobus*. Ancestral habitat reconstruction and divergence date estimation suggest that early divergence events in Tyrannida took place in a humid forest environment during the Oligocene. Large-scale diversification in open habitats is confined to the clade consisting of the elaeniines, myiarchines, tyrannines and fluvicolines. This radiation correlates in time to the expansion of semi-open and open habitats from the mid-Miocene (*c.* 15 Mya) onwards. The pipromorphine, elaeniine and myiarchine–tyrannine–fluvicoline clades each employ distinct foraging strategies (upward striking, perch-gleaning and sallying, respectively), but the degree of diversity in morphology and microhabitat exploitation is markedly different between these clades. While the pipromorphines and elaeniines each are remarkably homogenous in morphology and exploit a restricted range of microhabitats, the myiarchine–tyrannine–fluvicoline clade is more diverse in these respects. This greater ecological diversity, especially as manifested in their success in colonizing a wider spectrum of open habitats, appears to be connected to a greater adaptive flexibility of the search-and-sally foraging behaviour.

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Introduction

The South American avifauna is characterized by a high degree of continental endemism at high taxonomic levels. One of these radiations, the New World suboscines (Furnariida: ovenbirds, woodcreepers, antbirds, tapaculos and their allies; and Tyrannida: cotingas, manakins, tyrant flycatchers and their allies) makes up approximately two-thirds of the South American passerines. More than a third of the New World suboscines (*c.* 400 species in *c.* 100 genera) belong in Tyrannidae (tyrant flycatchers), one of the largest and most diverse of all bird families. Some well-known genera (e.g. *Myiarchus*, *Tyrannus* and *Pitangus*) include rather large and conspicuous species, reputed for their aggressive behaviour, but most members of the family are more anonymous, dull-coloured, small- to medium-sized insectivores. Tyrant flycatchers typically forage by different search-and-sally techniques or

by gleaning the foliage of trees and bushes like warblers, but many species also eat fruit and a few are specialized frugivores (Fitzpatrick 1980). They are distributed over the entire New World, but are most diverse in the Neotropical region, with a steep decline in diversity northward through Central America, with only five genera (*Tyrannus*, *Myiarchus*, *Contopus*, *Empidonax* and *Sayornis*) reaching temperate North America. In South America, the tyrant flycatchers have undergone an impressive morphological and ecological diversification. Here, they make up roughly one-third of the passerine bird communities in most habitats (Fitzpatrick *et al*. 2004) and, together with the furnariids, they are the predominant avian predators on small- to medium-sized arthropod prey. There is further a general behavioural distinction between these two groups: furnariids generally search for hidden prey in crevices and among debris and epiphytes, whereas tyrannids search for exposed prey.

While the phylogeny of Furnariida has been investigated in considerable detail (Irestedt *et al*. 2002; 2004a,b, 2006b), no comprehensive molecular phylogeny exists for the Tyrannida. Much of the effort spent on tyrannid systematics have been concentrated on the deeper relationships in the group, utilizing both morphology (e.g. McKitrick 1985; Prum & Lanyon 1989; Prum 1990), allozymes (S. M. Lanyon 1985), DNA–DNA hybridization data (Sibley & Ahlquist 1985, 1990) and DNA sequences (Irestedt *et al*. 2001; Johansson *et al*. 2002; Chesser 2004; Ericson *et al*. 2006). From these studies, it has emerged that Tyrannida consists of four main lineages: (i) Cotingidae (cotingas); (ii) Pipridae (manakins); (iii) Tyrannidae (tyrant flycatchers); and (iv) Tityridae (tityras, becards and mourners). Ericson *et al*. (2006) further demonstrated that Tyrannidae, Tityridae and the enigmatic *Piprites* (currently treated as *incerta sedis*; Remsen *et al*. 2007) form a monophyletic clade.

Genus-level relationships among the tyrant flycatchers have not yet been addressed with molecular data and Traylor's (1977, 1979) classification has essentially been the basis for linear classifications used in standard works since (e.g. Sibley & Monroe 1990; Dickinson 2003; Fitzpatrick *et al*. 2004; Remsen *et al*. 2007). Several studies have used morphological characters of the cranium and syrinx to clarify interrelationships among tyrant flycatchers and their relatives (Warter 1965; Ames 1971; Traylor 1977, 1979; W. E. Lanyon 1984, 1985, 1986, 1988a,b,c; Prum & Lanyon 1989; Mobley & Prum 1995; Birdsley 2002). In the most comprehensive of these studies, W. E. Lanyon used cranial characters to identify five main assemblages in Tyrannidae: flatbills and tody-tyrants (Lanyon 1988c); the *Elaenia* assemblage (Lanyon 1988a); the *Empidonax* assemblage (Lanyon 1986, 1988b); the myiarchine assemblage (W. E. Lanyon 1985); and the kingbirds and their allies (Lanyon 1984). Each of these assemblages was subdivided into smaller groups of genera, mainly based on syringeal characters. W. E. Lanyon (1984–1988c) did not analyse his data within a cladistic framework, but a subsequent cladistic analysis, extended with behavioural data from Fitzpatrick (1980), was performed by Birdsley (2002). He found support for a kingbird clade and for restricted myiarchine and *Empidonax* clades, which agreed with the findings of W. E. Lanyon, but found only weak support for his *Elaenia* and flatbill and tody-tyrant assemblages.

Several molecular studies have treated smaller groups of tyrannids: *Leptopogon* (Bates & Zink 1994), *Ochthoeca* (García-Moreno *et al*. 1998), *Anairetes* (Roy *et al*. 1999), *Muscisaxicola* (Chesser 2000), W. E. Lanyon's *Empidonax* group (Cicero & Johnson 2002), *Myiarchus* (Joseph *et al*. 2004) and the kingbird assemblage (Mobley 2002). These studies focus on evolutionary and biogeographical issues within these groups, and do not address phylogenetic relationships at deeper levels. The only larger groups of tyrant flycatchers that have been the target of molecular studies are the flatbill and tody-tyrant (Tello & Bates 2007) and *Elaenia* assemblages (Rheindt *et al*. 2008) of Lanyon (1988a and 1988c, respectively).

Due to the lack of a robust and well-resolved phylogenetic hypothesis for the tyrant flycatchers, the factors behind their ecological success and adaptive diversity are largely obscure. Fitzpatrick (1980, 1985) found that differences in foraging behaviour, and the morphological adaptations connected to them, correspond remarkably well with the taxonomic groupings outlined by Traylor (1977, 1979). However, his hypothesis on the evolution of foraging behaviours and ecological adaptations needs to be reinterpreted within a phylogenetic framework, and this is one aim of this paper. We use DNA sequence data from three nuclear introns to investigate the relationships between the majority of tyrant flycatcher genera. We use this phylogeny to investigate the evolution of the extant ecological diversity in Tyrannidae. We also calculate preliminary age estimates of certain divergence events among the tyrant flycatchers and their allies, and relate these to the climatic and ecological development in the Neotropics during the Tertiary.

Materials and methods

Taxon sampling and data acquisition

One hundred and three species, representing 83% of the genera in of Tyrannidae *sensu* Fitzpatrick *et al*. (2004) were sampled. Ten species of Tityridae (*sensu* Prum & Lanyon 1989; Ericson *et al*. 2006) were included, representing all genera except *Xenopsaris*. We also included *Piprites pileatus*, found to be the sister taxon to Tyrannidae by Ericson *et al*. (2006) and its supposed close relative *P. chloris*. We further included five representatives each of Cotingidae (*Ampelioides*, *Zaratornis*, *Rupicola*, *Pyroderus*, *Tijuca*) and Pipridae (*Tyranneutes*, *Neopelma*, *Chiroxiphia*, *Pipra*, *Lepidothrix*) and used two representatives of Furnariida (*Thamnophilus caerulescens* and *Furnarius cristatus*) as outgroups.

Three genera suspected to be non-monophyletic were more densely sampled. Members of *Myiophobus*, suggested by Lanyon (1986, 1988a) to belong to two different assemblages (the *Elaenia* and *Empidonax* assemblages), were especially densely sampled, with only *M. inornatus* lacking. *Mecocerculus*, suggested by Lanyon (1988a) to comprise of three distinctive groups, was represented by one species of each of these groups in order to assess their relationships (*Mecocerculus calopterus*, *M. poecilocercus* and *M. leucophrys*). Members of *Phyllomyias* were placed in five different genera (*Phyllomyias*, *Tyranniscus*, *Acrochordopus*, *Xanthomyias* and *Oreotriccus*) in earlier classifications (e.g. Meyer de Schauensee 1966). They were lumped under the name *Phyllomyias* by Traylor (1977), but Lanyon (1988b) did not recover them as a monophyletic group. To obtain an initial assessment of the relationships between these taxa we included two species from *Phyllomyias sensu stricto* (*fasciatus* and *griseiceps*) and one species each from *Tyranniscus* (*uropygialis*) and *Xanthomyias* (*virescens*). A few other genera (*Muscisaxicola*, *Ramphotrigon*, *Myiopagis*, *Ochthoeca*, *Hemitriccus*, *Pachyramphus*, *Tityra* and *Schiffornis*) were also represented by more than one species. GenBank accession numbers and sample identification for taxa used in this study are presented in Appendix 1. During the identification control of voucher specimens, we found that NRM 976683 referred to as *Serpophaga subcristata* in Ericson *et al.* (2006), in fact is a female *Myiopagis caniceps*. The taxonomy of this specimen has been corrected in the GenBank database.

Genomic DNA was extracted from muscle tissue or blood with the QIAamp® Mini Kit (QIAGEN®, Valencia, CA), following the protocol provided by the manufacturer. PCR-amplification and sequencing of myoglobin intron 2, glyceraldehyde-3-phosphate dehydrogenase intron 11 (G3PDH), ornithine decarboxylase introns 6 and 7 along with the intercepting exon 7 (ODC) were performed according to methods described by Irestedt *et al.* (2002), Fjeldså *et al.* (2003) and Allen & Omland (2003), respectively. DNA was obtained from footpads of museum skins for two taxa (*Capsiempis flaveola* and *Deltarhynchus flammulatus*), using the protocol described in Irestedt *et al.* (2006a). Primers used are listed in Table 1.

For each taxon, the multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SeqMan II™ (DNASTAR Inc., Madison, WI). Positions where the nucleotide could not be determined with certainty were coded with the appropriate IUB codes and treated as uncertainties in the phylogenetic analysis.

Alignment and phylogenetic analyses

The sequences from the different species were aligned in MegAlign™ (DNASTAR Inc.), using the Clustal V algorithm and subsequently checked by eye. Indel events did not pose any problems in homology assessment and all inferred gaps in the alignments were treated as missing data in the analyses.

Phylogenetic trees were estimated using parsimony jackknifing (PJ) (Farris *et al.* 1996) and Bayesian inference (Huelsenbeck & Ronquist 2001). The PJ analysis was implemented in *XAC: Parsimony Jackknifer* (Farris 1997). The analysis was performed with 1000 replicates, each with 10 random additions of taxa and branch swapping. Selection

Table 1 Sequences and references for primers used in this study. A, amplification; S, sequencing.

Primer name	Use	Primer sequence (5′–3′)	Reference
Glyceraldehyde-3-phosphodehydrogenase (G3PDH) intron 11			
G3P-13b	A	TCC ACC TTT GAT GCG GGT GCT GGC AT	Fjeldså *et al.* (2003)
G3P-14b	A, S	AAG TCC ACA ACA CGG TTG CTG TA	Fjeldså *et al.* (2003)
G3PintL1	S	GAA CGA CCA TTT TGT CAA GCT GGT T	Fjeldså *et al.* (2003)
Myoglobin intron 2			
Myo2	A, S	GCC ACC AAG CAC AAG ATC CC	Slade *et al.* (1993)
Myo3	A	CGG AAG AGC TCC AGG GCC TT	Heslewood *et al.* (1998)
Myo3F	A, S	TTC AGC AAG GAC CTT GAT AAT GAC TT	Heslewood *et al.* (1998)
MyoIntC	S	AGC CCT GGA GGA TCC ATT GG	Irestedt *et al.* (2002)
MyoIntNC	S	CCA ATG GAT CCT CCA GGG CT	Irestedt *et al.* (2002)
Myo309L	A, S	CAT AAG ACC TGT CAG TGG CTG GA	Irestedt *et al.* (2006a)
MyoNso030L	A, S	ATC TGG AGG TAT GGA AAA GGG CA	Irestedt *et al.* (2006a)
MyoSub149L	A, S	GTA CAG GCA GCA GGA GGC ACA GA	This study
MyoSub218H	A, S	GCA TGT GGT GTT TGG AAT GGG AA	Irestedt *et al.* (2006a)
MyoTyr345H	A, S	CCT CTA GGG CTT GCT CTA AAA TTG TA	This study
Ornithine decarboxylase (ODC) introns 6–7 (with exon 7)			
OD6	A, S	GAC TCC AAA GCA GTT TGT CGT CTC AGT GT	Allen & Omland (2003)
OD8R	A, S	TCT TCA GAG CCA GGG AAG CCA CCA CCA AT	Allen & Omland (2003)
ODintF	A, S	ATG CCC GCT GTG TGT TTG	Irestedt *et al.* (2006a)
ODintF2	A, S	CAC TTA AGA CTA GCA GGC TTC TTC TGG A	Irestedt *et al.* (2006a)
ODintR2	S	CTT ACT CCC ATA TCA AAC ACA CA	This study
ODintR3	A, S	CAA ACA CAC AGC GGG CAT CAG A	Irestedt *et al.* (2006a)
ODintR4	A, S	CAT ATT GAA GCC AAG TTC AGC CTA	Irestedt *et al.* (2006a)

of the best-fitting substitution model for each of the gene partitions for the Bayesian analyses were performed in the program MrModeltest (Posada & Crandall 1998; Nylander 2002) in conjunction with paup* 4.0b10 (Swofford 2002). Phylogenetic analyses using Bayesian inference were implemented in the program MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using the Markov chain Monte Carlo and Metropolis coupling (MC3). The Akaike information criterion (AIC, Akaike 1973), as applied in MrModeltest, recommended using GTR + Γ for all three genes. The preferred models (Table 2) were used both in the analyses of the individual genes and in the combined analyses. In the combined analyses we allowed all parameters, except branch length and topology to vary independently between partitions by using the commands *unlink* and *prset ratepr = variable*. For each data set, two independent analyses were run with four incrementally heated MC3 chains for 5×10^6 generations with trees sampled every 100th generation. Trees saved before the run had reached stationarity (the 'burn-in' phase, as estimated by plotting –log likelihood values against generation number) were discarded and posterior probabilities were estimated from the remaining generations. The 50% majority rule consensus trees from the individual runs were compared to ensure that the individual runs had converged on the same target distribution (Huelsenbeck *et al*. 2002).

A genetically distant outgroup might potentially affect topology and support values by introducing a rooting problem in the ingroup (Wheeler 1990; Smith 1994). The branch leading to Tyrannida turned out to be very long, therefore, we tested the sensitivity to outgroup choice by deleting the Furnariids and restricting the outgroup to one representative each from Cotingidae and Pipridae.

Divergence time estimations

We used PATHd8 (Britton *et al*. 2006) to obtain a linearized tree from which the dates of the phylogenetic splits could be calculated. In the absence of suitable fossils, we adopted a different strategy to obtain a fixed calibration point for the analysis. This was done by combining previously published estimates of the age of the oldest split in Passeriformes and an estimate of the substitution rate of the myoglobin intron 2, a gene segment that evolves relatively slowly and appears to have a rather constant substitution rate in the passerine tree. An estimate of the average percent sequence distance in the myoglobin intron 2 in passerines was calculated by comparing sequence divergences between 39 species of oscines and 41 species of suboscines (most sequences available on GenBank). Each of these 1599 pairwise comparisons provides an estimate of the true sequence divergence between oscines and suboscines since they split from their common ancestor. The average of these divergences was 11.56%. Two independent estimates of the age of the oscine–suboscine split are available (Ericson *et al*. 2002; Barker *et al*. 2004). Both are based on the assumption that *Acanthisitta* on New Zealand diverged from the rest of the Passeriformes when New Zealand became isolated from Gondwanaland, dating the oscine–suboscine split to 71 or 77 Mya, respectively. Calculating the substitution rate for myoglobin intron 2 from these two dates yields two estimates of the average mutation rate in myoglobin intron 2 in passerines: 0.1628 and 0.1501% Ma^{-1}. We used

Table 2 Data characteristics and Bayes estimated of parameters for the three studied genetic markers. Number of sites in alignments is given both for the total alignments and for alignments with long autapomorphic insertions omitted (see the section 'Sequence characteristics' under Results for details). The number of variable and parsimony informative sites is calculated from the alignment without the long autapomorphic insertions. Substitution rates are calculated with the G–T rate set to 1.

	G3PDH	Myoglobin	ODC
Number of sites in alignment			
Total alignment	712	825	2925
Long autapomorphic insertions omitted	396	825	761
Sequence length variation	318–646	691–753	547–1885
	median = 340	median = 720	median = 675
Number of variable sites	294 (74%)	445 (54%)	461 (61%)
Number of parsimony informative sites	208 (53%)	205 (25%)	282 (37%)
Selected substitution model	GTR + Γ	GTR + Γ	GTR + Γ
Base frequencies (%)			
A	0.23	0.30	0.29
C	0.20	0.21	0.22
G	0.29	0.21	0.21
T	0.28	0.28	0.28
Substitution rate			
r(AC)	1.32	1.14	0.85
r(AG)	5.39	5.30	4.16
r(AT)	0.95	0.52	0.69
r(CG)	1.86	1.50	0.99
r(CT)	7.04	5.68	2.51
r(GT)	1	1	1
Γ shape parameter (α)	1.378	0.936	1.096

the mean of these estimates: 0.156% Ma^{-1} and calculated the date for the split between the Furnariida and Tyrannida to 62.18 Mya. This age was used as a fixed calibration point in our PATHd8 analysis of the combined Bayesian tree.

PATHd8 is a nonparametric method, which smoothes substitution rates sequentially by taking averages over path lengths from an internode to all its descending terminals (Britton *et al*. 2007). The smoothing is thereby done between sister groups, as opposed to most other methods, where rate smoothing is done between mother and daughter lineages. This has the effect of better preserving the pattern of heterogeneous branch lengths in the phylogram. Another property of the method is that zero or near-zero branch lengths collapse, which seems reasonable, considering that these branch lengths probably represent short time or uncertainties in the phylogeny (or both). However, PATHd8 performs best when multiple internal calibration points can be used. With few calibration points available, as in the present study, deviating branch lengths can have a strong impact on the chronogram (e.g. by exaggerating the length of an internode leading to a clade with short branches).

Evolution of foraging strategies and ecological adaptations

From data gathered from general sources (e.g. Ridgely & Tudor 1994; Ridgely & Greenfield 2001; Hilty 2003; Fitzpatrick *et al*. 2004), we defined four broad habitat categories as input data for the terminal taxa (Fig. 2): (i) humid forest interior, including dense thickets at edge zones; (ii) humid forest canopy (although adjacent edge zones are occasionally visited); (iii) semi-open habitats, including both humid to deciduous forest borders and woodlands and dry forest, such as chaco and thorn forest; and (iv) open grass-dominated habitats, such as marshes, open cerrado, dry scrub, as well as austral and montane grassland and scrub. We made no distinction between low- and highland types of any habitat types. In most cases, the habitat range of the represented genera is homogenous enough not to cause any severe ambiguity in coding. However, some genera (e.g. *Elaenia*, *Ochthoeca*, *Knipolegus*) inhabit a wider range of habitats than that covered by our taxon sampling. As we cannot make any a priori assumption about their ancestral habitat, these were coded according to the overall habitat range in the genus. The evolution of ecological adaptations in these clades must be studied with a more comprehensive taxon sampling. Ancestral states for habitat categories were optimized on the Bayesian combined tree, in the Mesquite program package, version 1.12 (Maddison & Maddison 2006). Characters were treated as unordered and were optimized under a ML criterion, using the Markov *k*-state one-parameter model (Lewis 2001). A proportional likelihood value of 70 or above was considered a reliable estimate of the ancestral state.

Data on foraging behaviour were taken chiefly from Fitzpatrick (1980, 1985), updated with information from the same sources as the habitat data. With some minor modifications, character coding followed Birdsley (2002). These were mapped on the Bayesian combined tree, and we also performed a ML-based analysis in Mesquite, using the same settings as for the habitat analysis. Several of the categories of foraging behaviour designated by Fitzpatrick (1980, 1985) are variants of the sallying strategy. This strategy is characterized by relatively long search time from a vantage point, scanning the surroundings for prey, which are caught by an approach flight, either to adjacent surfaces or to the air. This is different from the more active foraging strategies upward striking and perch gleaning, where search time are much shorter. We also performed a simplified analysis, where all foraging behaviours based on sallying were coded as one character state.

Results

Sequence characteristics

With a few exceptions detailed below, we obtained sequences for the entire target regions from the three genetic markers for all taxa. Total alignment length, proportion of variable and parsimony informative sites, base composition, substitution patterns are given in Table 2. G3PDH sequences varied in length between 318 bp (*Terenotriccus erythrurus*) and 345 bp (*Sirystes sibilator* and *Rupicola peruvianus*). Due to an autapomorphic insertion, the G3PDH sequence of *Myiophobus phoenicomitra* was 646 bp long. For *Alectrurus risora* and *Piprites chloris*, we were unable to get a good reading for the last 10 bp of G3PDH. For myoglobin, we were unable to sequence the first 31 bp of *Deltarhynchus flammulatus*, and the last 30 bp for *Phyllomyias griseiceps*, *Anairetes alpinus* and *Satrapa icterophrys*. With the exception of these, sequence length varied between 691 bp (*Furnarius cristatus*) and 753 bp (*Corythopis delalandei*) with all but a few taxa ranging between 712 and 727 bp. For ODC, we obtained slightly truncated sequences for *Capsiempis flaveola* and *Deltarhynchus flammulatus*, due to the use of specific primers designed for amplification from study skins. For the same reason, these two sequences lack a small portion of exon 7. Due to autapomorphic insertions, a small number of taxa had strongly deviating sequence lengths (*Phyllomyias uropygialis*, 1885 bp; *Piprites chloris*, 1296 bp, *Myiophobus flavicans* and *M. roraimae*, 993 bp) or deletions (*Furnarius cristatus*, 558 bp; *Myiobius villosus*, 547 bp; *Cnipodectes subbrunneus*, 621 bp). Apart from these outliers, sequence lengths ranged between 648 bp (*Thamnophilus caerulescens*) and 702 bp (both *Schiffornis* species and *Laniisoma elegans*).

Phylogenetic results

Comparisons between analyses. The combined tree with the three genes treated as separate and independently evolving partitions (Fig. 1) exhibited good resolution and generally well-supported relationships. Using only one cotingid and


Thamnophilus caerulescens
Furnarius cristatus
Ampelioides tschudii
Zaratornis stresemanni
Rupicola peruvianus
Pyroderus scutatus
Tijuca atra
Cotingidae
Tyranneutes stolzmanni
Neopelma pallescens
Chiroxiphia caudata
Lepidothrix coronata
Pipra fasciicauda
Pipridae
Tyrannida
B
Onychorhynchus occidentalis
Myiobius villosus
Terenotriccus erythrurus
Oxyruncus cristatus
Schiffornis turdinus
Schiffornis virescens
Laniisoma elegans
Laniocera hypopyrrha
Iodopleura isabellae
Tityra cayana
Tityra inquisitor
Pachyramphus validus
Pachyramphus castaneus
Pachyramphus polychopterus
Tityridae
A
Piprites chloris
Piprites pileatus
Platyrinchus mystaceus
Neopipo cinnamomea
Tachuris rubrigastra
Pseudotriccus ruficeps
Corythopis delalandi
Phylloscartes ventralis
Pogonotriccus poecilotis
Mionectes rufiventris
Leptopogon amaurocephalus
Rhynchocyclus fulvipectus
Tolmomyias flaviventris
Cnipodectes subbrunneus
Poecilotriccus ruficeps
Todirostrum cinereum
Hemitriccus diops*
Lophotriccus pileatus
Hemitriccus margaritaceiventer*
Myiornis auricularis
Pipromorphines
Tyrannidae
E-3
Mecocerculus leucophrys*
Polystictus pectoralis
Anairetes alpinus
Anairetes parulus
Culicivora caudacuta
Pseudocolopteryx acutipennis
Serpophaga munda
E-1
Elaenia flavogaster
Myiopagis caniceps
E
Myiopagis viridicata
Suiriri suiriri
Phaeomyias murina
Capsiempis flaveola
Phyllomyias fasciatus*
Phyllomyias griseiceps*
Elaeniines
Zimmerius chrysops
Stigmatura budytoides
Inezia inornata
Euscarthmus meloryphus
E-2
Ornithion brunneicapillus
Phyllomyias uropygialis*
Camptostoma obsoletum
Phyllomyias virescens*
C
Mecocerculus calopterus*
Mecocerculus poecilocercus*
G
Myiotriccus ornatus
Hirundinea ferruginea
Pyrrhomyias cinnamomeus
Myiophobus pulcher*
Myiophobus lintoni*
Myiophobus ochraceiventris*
Myiarchus tyrannulus
Sirystes sibilator
Casiornis rufus
Rhytipterna simplex
Myiarchines
Attila spadiceus
Attila torridus
Legatus leucophaius
Machetornis rixosus
Pitangus sulphuratus
Myiozetetes similis
Megarynchus pitangua
Myiodynastes maculatus
Empidonomus varius
Tyrannus savana
D
Tyrannines
Ramphotrigon megacephalum*
Deltarhynchus flammulatus
Ramphotrigon ruficauda*
Muscigralla brevicauda
Ochthoeca cinnamomeiventris*
F-1
Tumbezia salvini
Ochthoeca diadema*
Myiophobus phoenicomitra*
Myiophobus flavicans*
Myiophobus roraimae*
F
F-2
Colonia colonus
Arundinicola leucocephala
Fluvicola albiventer
Pyrocephalus rubinus
Alectrurus risora
Gubernetes yetapa
Sublegatus modestus
Myiophobus cryptoxanthus*
Myiophobus fasciatus*
F-4
Lathrotriccus euleri
Cnemotriccus fuscatus
Sayornis nigricans
Mitrephanes phaeocercus
Empidonax alnorum
Contopus sordidulus
Knipolegus striaticeps
Hymenops perspicillatus
Satrapa icterophrys
F-3
Muscisaxicola alpinus
Muscisaxicola maculirostris
Lessonia rufa
Neoxolmis rufiventris
Myiotheretes fumigatus
Agriornis andicola
Xolmis irupero
Polioxolmis rufipennis
Cnemarchus erythropygius
Fluvicolines
0.1

Table 3 Posterior probabilities for selected clades recovered in the phylogenetic tree based on the concatenated data set compared to corresponding values for the gene trees. N.R., not recovered; S.A.T., supported alternative topology (posterior probability of 95 or above). Nodes with support values in parentheses are affected by the alternative placement of an individual taxon, but are otherwise similar to the combined tree. These cases are explained at the bottom of the table.

	Combined	G3PDH	Myoglobin	ODC
Tyrannida	**100**	**97**	**100**	**100**
Cotingidae	**100**	**97**	**95**	89
Pipridae	**100**	**100**	**100**	**100**
Clade A	**100**	S.A.T.	89	**100**
Clade B, Oxyruncus and Tityridae	93	N.R.	N.R.	S.A.T.
Clade B	**100**	89	91	**100**
Tityridae	**100**	**100**	**100**	**100**
Tyrannidae and *Piprites*	**100**	N.R.	79	**97**
Tyrannidae	**99**	66	N.R.	N.R.
Pipromorphines, *Platyrinchus*, *Neopipo* and *Tachuris*	**100**	85	89	N.R.
Platyrinchus and *Neopipo*	**100**	66	**100**	56
Pipromorphines	**100**	N.R.	**100**	**100**
Flatbills and tody-tyrants	**100**	N.R.	88	N.R.
Clade C	**100**	**100**	**100**	**100**
Elaeniines	**100**	N.R.	N.R.	N.R.
Clade E-1	**100**	N.R.	(95)*	S.A.T.
Clade E-2	**99**	(96)†	S.A.T.*	N.R.
Clade E-3	**100**	**100**	83	(91)‡
Clade D and clade G	81	92	N.R.	N.R.
Clade D	**100**	N.R.	**97**	**96**
Clade G	**100**	N.R.	**100**	N.R.
Fluvicolines, *Muscigralla*, *Ramphotrigon* and *Deltarhynchus*	**97**	N.R.	N.R.	**100**
Fluvicolines	**100**	N.R.	N.R.	N.R.
Clade F-1	**100**	S.A.T.	**100**	(85)§
Clade F-2	**98**	61	N.R.	N.R.¶
Clades F-3 and F-4	**100**	83	N.R.	N.R.
Clade F-3	**100**	N.R.	N.R.	(91)¶
Clade F-4	**100**	71	N.R.	N.R.§
Clade F-4 excluding *Myiophobus* s.s.	**100**	**98**	N.R.	N.R.
Myiarchines	**100**	N.R.	N.R.	91
Tyrannines	**100**	66	N.R.	N.R.
Myiarchines, tyrannines and Attila	66	N.R.	N.R.	**95**

*Clade El-1 recovered, but 'invaded' by *Euscarthmus* (in clade El-2 in the combined tree).

†For clade El-2 except *Stigmatura* and *Zimmerius*.

‡For clade El-3 except *Mecocerculus leucophrys*.

§Clade Fl-1 recovered, but 'invaded' by *Myiophobus* s.s. (in clade Fl-4 in the combined tree).

¶Clade Fl-3 recovered, but 'invaded' by *Colonia* (in clade Fl-2 in the combined tree).

one piprid as outgroup did not alter topology or support values. Separate analyses of the elaeniine and tyrannine clades under a three-partition model yielded the same topologies and support values as when included in the complete data set. The three gene trees (not shown) were poorly resolved in the basal regions of the large elaeniine/tyrannine radiation (clade C), despite having slightly different substitution rates. However, differences between well-supported nodes in the individual gene trees and the combined tree were few (Table 3). The G3PDH data set places clade B, *Oxyruncus* and Tityridae with Cotingidae and Pipridae instead of in clade A, and *Ochthoeca cinnamomeiventris* and *Tumbezia salvini* as the sister

Fig. 1 Fifty percent consensus tree of Tyrannida from the Bayesian analyses of the combined data set (myoglobin intron 2, G3PDH intron 11 and ODC introns 6–7). Nodes with a posterior probability of 95 or above are in bold type. The basal branch leading to Tyrannida has been shortened. Major taxonomic groupings (in brackets) and numbered clades are referred to in the text. Nodes with posterior probabilities < 95 are drawn in thinner lines with posterior probability values given below. Species with placements radically different from previous hypotheses are underlined and species representing non-monophyletic genera are marked with an asterisk (*).

group to clade F-3 + F-4 instead of as part of clade F-1. In the ODC tree, *Elaenia* and *Myiopagis* were placed outside clade E-1, and the rest of that clade was placed as the sister to clade E-3. Some well-supported clades in the individual trees were recovered with low posterior probabilities in the combined tree, for example, support in the ODC tree for the myiarchines and the tyrannines forming a clade together with *Attila* (posterior probability = 0.95 vs. posterior probability = 0.66 in the combined tree) and support in the G3PDH tree for *Pseudotriccus* + *Corythopis* and *Mionectes* + *Leptopogon* being sister groups (posterior probability = 0.96 vs. unresolved in the combined tree). A placement of *Oxyruncus* and clade B as the sister to Tyrannidae + *Piprites* was supported in ODC (posterior probability = 0.99), but the combined tree had a posterior probability = 0.93 for a clade consisting of clade B, Tityridae and *Oxyruncus*. The PJ analysis resulted in a tree (not shown) that did not conflict with the combined Bayesian tree, although resolution and support levels were generally lower in the intermediate regions of the tree, e.g. at the base of clade C.

Deep lineages in Tyrannida. The consensus tree from the Bayesian analysis of the combined three-partition data set is shown in Fig. 1. We recovered three well-supported main clades in Tyrannida: Cotingidae (Cotingas), Pipridae (Manakins) and a large clade consisting of Tityridae, *Oxyruncus*, *Piprites* and all members of Tyrannidae (clade A). Tityridae was shown to be composed of two well-supported and ecologically distinctive clades: one containing the mourners (*Schiffornis*, *Laniocera* and *Laniisoma*) and the other the purpletufts, tityras and becards (*Iodopleura*, *Tityra* and *Pachyramphus*).

Onychorhynchus, *Myiobius* and *Terenotriccus*, which traditionally have been included in Tyrannidae, formed a strongly supported clade (clade B) outside of Tyrannidae. In the combined tree, clade B, Tityridae and *Oxyruncus* formed a monophyletic clade, but support for this was not very strong (posterior probability = 0.93).

The two species of *Piprites* came out together with strong support and were placed as the sister group to a well-supported clade encompassing the majority of genera traditionally assigned to Tyrannidae, as was found by Ericson *et al*. (2006). The Tyrannidae, in turn, was divided into two well-defined clades. The first of these consists of *Platyrinchus*, *Neopipo*, *Tachuris* and a large clade corresponding to an expanded 'flatbill and tody-tyrant assemblage' (the pipromorphines). The other (clade C) contains most of the genera of Fitzpatrick *et al*.'s (2004) Elaeniini, Fluvicolinae and Tyranninae. This is in accordance with the subdivisions found in earlier studies based on DNA sequence data, based on smaller taxon samples (Johansson *et al*. 2002; Chesser 2004; Ericson *et al*. 2006). However, several genera in Tyrannidae were found to have relationships different from what has previously been suggested, these genera are underlined in Fig. 1.

The pipromorphine clade contains not only the typical flatbills and tody-tyrants of Lanyon (1988c), but also *Phylloscartes* and *Pogonotriccus*. Within this clade, the tody-tyrants (*Todirostrum*, *Poecilotriccus*, *Hemitriccus*, *Lophotriccus* and *Myiornis*) group together with strong support, with *Cnipodectes* as its sister taxon, with the flatbills (*Rhynchocyclus* and *Tolmomyias*) outside of these. The rest of the pipromorphines consists of three pairs of relatively fine-billed genera (*Phylloscartes* + *Pogonotriccus*, *Leptopogon* + *Mionectes* and *Pseudotriccus* + *Corythopis*), which traditionally have been placed among the elaeniines (Traylor 1977; Lanyon 1988b; Fitzpatrick *et al*. 2004). Interrelationships between these three pairs of genera were not resolved, neither was there strong support for them forming a monophyletic clade (posterior probability of 0.91 in the combined tree). Three genera of debated affinities, *Platyrinchus*, *Neopipo* and *Tachuris*, grouped together with the pipromorphines with strong support. The interrelationships between these taxa were unresolved, while *Platyrinchus* and *Neopipo* were strongly supported as sister taxa.

Relationships among 'true tyrant flycatchers'. Clade C consists of three subclades, but the relationships between these were not fully resolved. The first of the two larger clades (D) encompasses the majority of genera in Tyranninae and Fluvicolinae of Fitzpatrick *et al*. (2004). The second of the larger clades (clade E) contains the traditional elaeniine genera except for the ones that we place in the pipromorphine clade (see above). An unexpected outcome was that *Myiotriccus*, *Pyrrhomyias*, *Hirundinea* and three species traditionally placed in *Myiophobus* (*M. pulcher*, *M. lintoni* and *M. ochraceiventris*) formed a well-supported clade (clade G) alongside with clades D and E. They may be the sister group to clade D, but the posterior probability value was too low (0.81) to draw any definite conclusions. The three '*Myiophobus*' species formed a clade that is sister to *Pyrrhomyias* and *Hirundinea*, with *Myiotriccus* in a basal position.

Our elaeniine clade corresponds to the *Elaenia* group of Lanyon (1988a), with the addition of *Zimmerius*, *Stigmatura*, *Euscarthmus* and *Inezia*, which he placed in other groups in the *Elaenia* assemblage. It further includes *Culicivora*, which was unavailable to W. E. Lanyon, and *Phyllomyias fasciatus* and *griseiceps*, which he treated as *incertae sedis* due to their aberrant syrinx morphology. The elaeniine clade and its three subclades were all well-supported in the combined Bayesian analysis, although the deeper relationships were poorly resolved. *Elaenia*, *Myiopagis*, *Suiriri*, *Phaeomyias*, *Capsiempis*, *Phyllomyias fasciatus* and *P. griseiceps*, formed clade E-1. Clade E-2 contains two well-supported subclades, one consisting of *Stigmatura*, *Euscarthmus* and *Inezia*, the other of *Ornithion*, *Camptostoma*, two species currently placed in *Mecocerculus* (*M. calopterus* and *M. poecilocercus*) and two species currently placed in *Phyllomyias* (*P. uropygialis* and *P. virescens*), with *Zimmerius* in an unresolved

position. Finally, clade E-3 contains *Serpophaga*, *Anairetes*, *Polystictus*, *Culicivora*, *Pseudocolopteryx* and *Mecocerculus leucophrys*.

Clade D includes most of the genera placed in the traditional groupings Tyranninae and Fluvicolinae (e.g. Traylor 1977, 1979; Fitzpatrick *et al*. 2004) and consists of three well-supported clades corresponding to the traditional groupings of myiarchine, tyrannine and fluvicoline genera (W. E. Lanyon 1984, 1985, 1986). Clade D further includes the genera *Machetornis*, *Muscigralla* and *Colonia*, which Lanyon (1984, 1986) treated as *incertae sedis*, and *Sublegatus*, which previously has been associated with the elaeniines. Interrelationships between the three clades were not resolved, and the genera *Attila*, *Ramphotrigon*, *Deltarhynchus* and *Muscigralla* occupy positions outside these clades. Contrary to what has been suggested from syringeal and cranial morphology (Lanyon 1984) *Ramphotrigon* and *Deltarhynchus* showed a closer relationship with fluvicolines than with the myiarchines, whereas *Attila* was loosely associated with the myiarchine and kingbird clades. *Muscigralla* was also placed in an unresolved position at the root of the clade formed by *Ramphotrigon*, *Deltarhynchus* and the fluvicolines.

Within the fluvicoline clade (F), four subclades were strongly supported, but their interrelationships were not fully resolved. The two *Ochthoeca* species, *Tumbezia salvini* and three species currently placed in *Myiophobus* (*M. phoenicomitra*, *M. flavicans* and *M. roraimae*) constitute clade F-1, with *O. diadema* in an unresolved position. Another well-supported clade consists of *Colonia*, *Fluvicola*, *Pyrocephalus*, *Arundinicola*, *Alectrurus* and *Gubernetes* (clade F-2). There was further strong support for a clade F-3 (*Knipolegus*, *Hymenops*, *Satrapa*, *Muscisaxicola*, *Lessonia*, *Neoxolmis*, *Myiotheretes*, *Agriornis*, *Xolmis*, *Cnemarchus* and *Polioxolmis*) and of a clade F-4 consisting of 'typical' flycatcher-like genera with the highest diversity in Central and North America. This clade was well resolved, with *Myiophobus sensu stricto* (*M. fasciatus* and *M. cryptoxanthus*) in a basal position, and with *Cnemotriccus* and *Lathrotriccus* as the sister group to a clade containing the mainly Central and North American genera *Contopus*, *Empidonax*, *Mitrephanes* and *Sayornis*. A sister relationship between clades F-3 and F-4 was also strongly supported. Finally, *Sublegatus* was found to belong to Fluvicolini, but its exact position was not resolved.

Both the myiarchine clade (*Myiarchus*, *Sirystes*, *Casiornis* and *Rhytipterna*) and tyrannine clade (*Legatus*, *Pitangus*, *Machetornis*, *Myiozetetes*, *Megarynchus*, *Myiodynastes*, *Empidonomus* and *Tyrannus*) were strongly supported. Relationships recovered within these two clades were mostly uncontroversial, except that we found *Machetornis* to be member of the tyrannines, despite the aberrant syringeal morphology reported by Lanyon (1984).

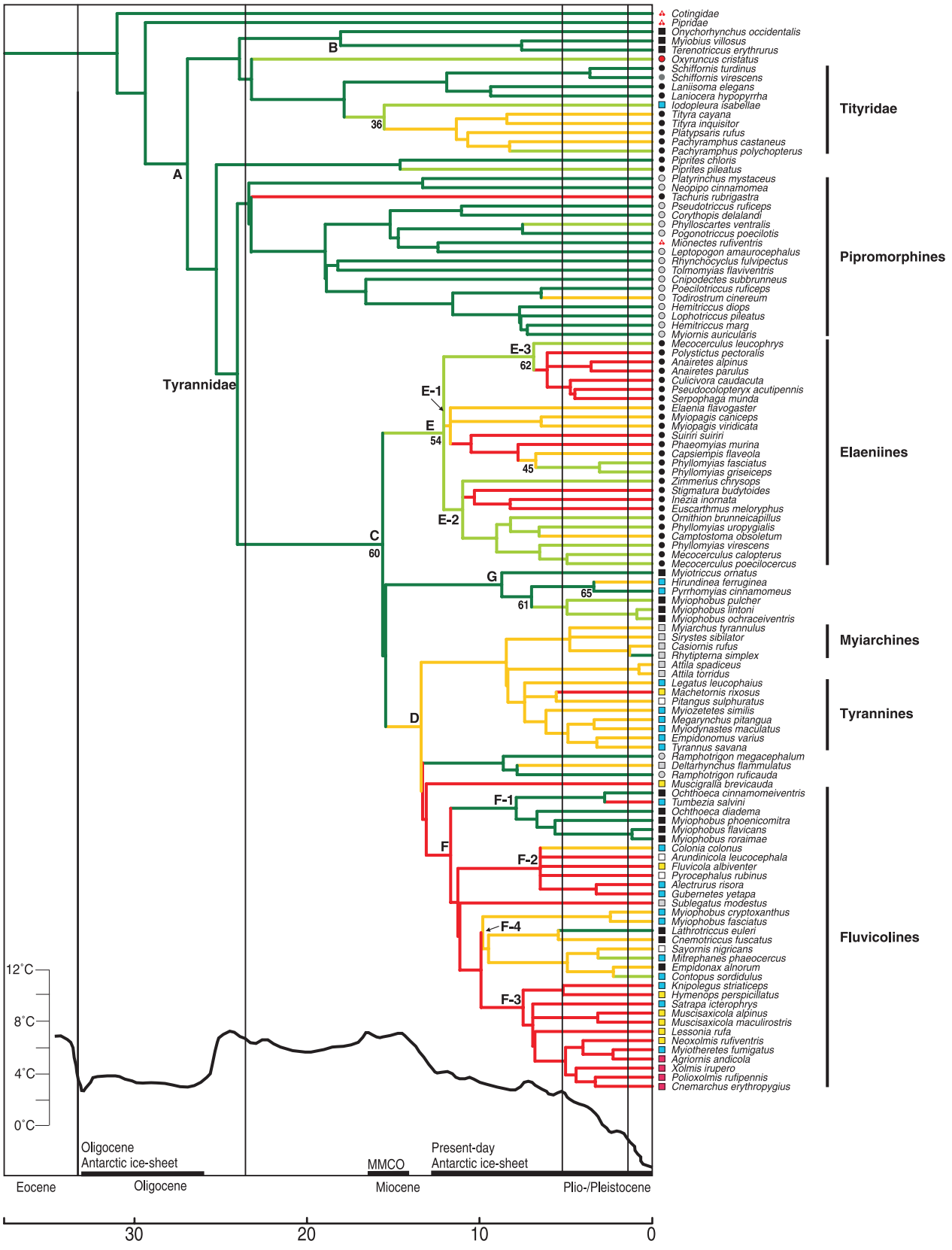
Divergence age and evolution of ecological adaptations

Figure 2 summarizes the age estimates for divergence events in clade A. Although it should be borne in mind that these estimates are highly tentative, some general remarks can be made. The initial divergences in Tyrannida, between Cotingidae, Pipridae and clade A took place in the Oligocene (*c*. 30 Mya). The main lineages in clade A diverged during a relatively short period in the second half of the Oligocene and at the beginning of the Miocene (*c*. 25 Mya), giving rise to the ancestors of clade B, Tityridae, *Piprites* and Tyrannidae. The extant lineages in clade C (clades D, E and G) did not diverge until the Middle Miocene (at *c*. 15 or 10 Mya after the split from their sister clade). Soon after this, extensive radiation took place in clades D and E, with most of their extant generic diversity in existence by the beginning of Pliocene.

The analysis of habitat characteristics in Tyrannida reveals some striking patterns (colouring of branches in Fig. 2). The ancestral habitat for most nodes could be reconstructed with a proportional likelihood value of 70 or above. The nodes with more ambiguous reconstructions are denoted in Fig. 2 by the values for the most likely ancestral state. The deeper divergence events all involve lineages largely restricted to the interior of humid forest, while clade C is dominated by taxa that have proliferated in a wide spectrum of semi-open and open (i.e. light intensive) habitat types, from humid forest canopy and borders to barren grass steppes. Adaptation to light intensive habitats (mostly forest canopy) has also occurred independently in some clades outside of clade C: *Oxyruncus*, *Iodopleura*, *Tityra*, *Pachyramphus*, *Piprites pileatus*, *Tachuris* and a few pipromorphine taxa.

In clade C, the only subclades with a large proportion of humid forest interior forms are clades G and F-1, which are both restricted to the Andes. However, clade F-1 contains taxa that exploit habitat types ranging from humid montane forest undergrowth to dry and scrubby habitats. *Ramphotrigon* and *Attila*, which occupy unresolved basal positions in clade D, are also mostly tied to tropical forest biomes, although usually favouring bamboo thickets (*Ramphotrigon*) or forest edges (*Attila*). Roughly half of the elaeniines are tied to humid forest canopy and borders. Optimization of the ancestral habitats for the basal divergences in clades C, the elaeniines and D had a higher degree of uncertainty, due to several instances of short basal branches and unresolved nodes. It was also influenced by the habitat category of *Muscigralla*, a single terminal taxon with long-branch length.

The three subclades in the elaeniine clade exhibit slightly different trends in habitat preferences and distribution patterns. Clade E-1 is distributed over a wide spectrum of lowland habitats, with a majority in canopy and edge situations and in open wooded habitats. Clade E-2 inhabit mostly mesic to humid forest canopy and borders, except for *Stigmatura*, *Inezia* and *Euscarthmus*, which are mainly found in dry scrub. Except for the basal *Mecocerculus leucophrys*, which is mainly found in humid montane forest borders, genera in clade E-3 inhabit scrubby and often dry habitats. This clade includes the only


Cotingidae
Pipridae
Onychorhynchus occidentalis
Myiobius villosus
Terenotriccus erythrurus
Oxyruncus cristatus
Schiffornis turdinus
Schiffornis virescens
Laniisoma elegans
Laniocera hypopyrrha
Iodopleura isabellae
Tityra cayana
Tityra inquisitor
Platypsaris rufus
Pachyramphus castaneus
Pachyramphus polychopterus
Pipites chloris
Pipites pileatus
Platyrinchus mystaceus
Neopipo cinnamomea
Tachuris rubrigastra
Pseudotriccus ruficeps
Corythopis delalandi
Phylloscartes ventralis
Pogonotriccus poecilotis
Mionectes rufiventris
Leptopogon amaurocephalus
Rhynchocyclus fulvipectus
Tolmomyias flaviventris
Cnipodectes subbrunneus
Poecilotriccus ruficeps
Todirostrum cinereum
Hemitriccus diops
Lophotriccus pileatus
Hemitriccus marg
Myiornis auricularis
Mecocerculus leucophrys
Polystictus pectoralis
Anairetes alpinus
Anairetes parulus
Culicivora caudacuta
Pseudocolopteryx acutipennis
Serpophaga munda
Elaenia flavogaster
Myiopagis caniceps
Myiopagis viridicata
Suiriri suiriri
Phaeomyias murina
Capsiempis flaveola
Phyllomyias fasciatus
Phyllomyias griseiceps
Zimmerius chrysops
Stigmatura budytoides
Inezia inornata
Euscarthmus meloryphus
Ornithion brunneicapillus
Phyllomyias uropygialis
Camptostoma obsoletum
Phyllomyias virescens
Mecocerculus calopterus
Mecocerculus poecilocercus
Myiotriccus ornatus
Hirundinea ferruginea
Pyrrhomyias cinnamomeus
Myiophobus pulcher
Myiophobus lintoni
Myiophobus ochraceiventris
Myiarchus tyrannulus
Sirystes sibilator
Casiornis rufus
Rhytipterna simplex
Attila spadiceus
Attila torridus
Legatus leucophaius
Machetornis rixosus
Pitangus sulphuratus
Myiozetetes similis
Megarynchus pitangua
Myiodynastes maculatus
Empidonomus varius
Tyrannus savana
Ramphotrigon megacephalum
Deltarhynchus flammulatus
Ramphotrigon ruficauda
Muscigralla brevicauda
Ochthoeca cinnamomeiventris
Tumbezia salvini
Ochthoeca diadema
Myiophobus phoenicomitra
Myiophobus flavicans
Myiophobus roraimae
Colonia colonus
Arundinicola leucocephala
Fluvicola albiventer
Pyrocephalus rubinus
Alectrurus risora
Gubernetes yetapa
Sublegatus modestus
Myiophobus cryptoxanthus
Myiophobus fasciatus
Lathrotriccus euleri
Cnemotriccus fuscatus
Sayornis nigricans
Mitrephanes phaeocercus
Empidonax alnorum
Contopus sordidulus
Knipolegus striaticeps
Hymenops perspicillatus
Satrapa icterophrys
Muscisaxicola alpinus
Muscisaxicola maculirostris
Lessonia rufa
Neoxolmis rufiventris
Myiotheretes fumigatus
Agriornis andicola
Xolmis irupero
Polioxolmis rufipennis
Cnemarchus erythropygius
Tityridae
Pipromorphines
Elaeniines
Myiarchines
Tyrannines
Fluvicolines
A
B
C
D
E
E-1
E-2
E-3
F
F-1
F-2
F-3
F-4
G
Tyrannidae
36
62
54
45
60
61
65
12°C
8°C
4°C
0°C
Oligocene
Antarctic ice-sheet
MMCO
Present-day
Antarctic ice-sheet
Eocene
Oligocene
Miocene
Plio-/Pleistocene
30
20
10
0

elaeniine genera specialized on grass-dominated habitats (*Polystictus*, *Culicivora* and *Pseudocolopteryx*). Members of the small clade F-2 are widely distributed in wetland and savanna habitats of South America with one species (*Pyrocephalus rubinus*) extending up into southern North America. Clade F-3 contains scrub- and ground-living genera of mostly austral and high Andean regions. Members of clade F-4 have a wide distribution in New World wooded habitats. The myiarchines and tyrannines occupy a wide range of wooded habitats, almost invariably confined to canopy, border and semi-open niches.

When mapping foraging behaviour on the combined tree (coloured symbols in Fig. 2), a strong phylogenetic pattern in the distribution of these characters was apparent, especially in Tyrannidae. Upward striking is entirely restricted to the pipromorphines, *Platyrinchus* and *Neopipo*; perch-gleaning to the elaeniines and the various sallying-based strategies to the myiarchines, tyrannines, fluvicolines and clade G. The optimization of foraging behaviour weakly favoured perch-gleaning as ancestral in clade A. Foraging behaviour that can be categorized as sallying-based has also evolved in clade B, but differs somewhat from that typical for most members of clade D.

Discussion

Phylogenetic results

Basal relationships in Tyrannida. Basal relationships in Tyrannida have generally been poorly resolved by morphological characters (e.g. Warter 1965; Ames 1971; McKitrick 1985; Prum 1990). These studies have notably failed in placing taxa that appear (from our data) to represent independent deep branches (e.g. clades B, G, *Piprites*, *Muscigralla*, *Tachuris*). Within Tyrannidae, there is some overall agreement between the results of our phylogenetic analysis and the assemblages of tyrant flycatchers defined by W. E. Lanyon (1984–1988c), but it is notable that very few of his groupings could be recovered as monophyletic. Those all involve closely related pairs of genera or small and uncontroversial clades. Along the same line, especially the syringeal characters used by W. E. Lanyon turn out to be highly homoplastic. This is also evident from Birdsley's (2002) cladistic re-analysis of W. E. Lanyon's data, which neither did succeed in clarifying deeper relationships in Tyrannidae, nor recover much phylogenetic structure among elaeniine genera.

The deeper relationships that we recovered in Tyrannida are in accordance with the findings of Ericson *et al.* (2006), with Tityridae, *Oxyruncus*, *Piprites* and Tyrannidae forming the well-supported monophyletic clade A. However, their well-supported sister group relationship between *Oxyruncus* and Tityridae was not recovered in our analyses. This can be attributed to the addition of *Onychorhynchus*, *Myiobius* and *Terenotriccus*, which turned out to form a distinct clade outside of Tyrannidae (clade B). When these were excluded from the analysis, *Oxyruncus* shifted back to a well-supported position as the sister to Tityridae. This underscores the importance of dense taxon sampling in species-rich groups with poorly known interrelationships (e.g. Omland *et al.* 1999). Several of our findings, both at basal and terminal levels, prompt a reappraisal of the taxonomy and classification of Tyrannidae, and this will be dealt with in a separate article.

Onychorhynchus, *Myiobius* and *Terenotriccus* have often been regarded as atypical tyrannids with unclear relationships. They all have broad, flat bills and unusually long rictal bristles, specializations for capturing flying insects in dense forest undergrowth. They also share some ecological traits, as they inhabit humid forest understorey and build long, enclosed, pendant nests. They were proposed to be related to each other by Ames (1971), based on their possession of two double A elements in the syrinx and the absence of intrinsic syringeal muscles, although Ames also included *Pyrrhomyias* and possibly also *Piprites* in this group. This treatment was followed by Traylor (1977, 1979), who placed the three genera in Fluvicolinae. In contrast, Lanyon (1988c) placed *Onychorhynchus* in the Flatbill/Tody-tyrant assemblage, and *Myiobius* and *Terenotriccus* in a separate group in the *Empidonax* assemblage together with some *Myiophobus* species (Lanyon 1988b). In his re-analysis of W. E. Lanyon's data, Birdsley (2002) found a close relationship between *Onychorhynchus*, *Myiobius* and *Terenotriccus*, but suggested a relationship with *Cnipodectes*. Here, they are demonstrated to constitute an independent, old lineage in Tyrannida, possibly more closely

Fig. 2 Chronogram estimated in PATHd8 summarizing the ecological adaptations over time in Tyrannida. The furnariid outgroup taxa have been excluded and the cotingid and piprid clades condensed to one edge each. Major taxonomic groupings and clade labels as in Fig. 1. The black curve at bottom of the figure shows ocean temperature as estimated from deep-sea oxygen isotope record (δO^{18}) (adapted from Zachos *et al.* 2001). The approximate temporal extent of the Oligocene Antarctic ice-sheet, the mid-Miocene Climatic Optimum (MMCO) and the present-day Antarctic ice-sheet are shown as thicker black bars at the bottom of the figure. Branch colours refer to broad habitat categories used in the ancestral habitat reconstruction performed in MESQUITE: dark green, humid forest interior; light green, humid forest canopy; yellow, semi-open habitat generalist, including humid to mesic forest edge, woodlands and dry forest; red, open habitats, including marshlands, open cerrado, austral and montane grassland and scrub. All nodes have a likelihood value of 70 or higher except those marked with numbers. Main foraging strategy of each genus is shown as a coloured box in front of the species name: cherry, fruit; red circle, probing and prying; grey circle, upward striking; black circle, perch gleaning; square boxes, foraging strategies based on sallying techniques: black, enclosed perch-hawk; blue, aerial sallying; grey, outward hover-gleaning; white, near ground generalists; red, pounce (perch-to-ground); yellow, ground foraging.

related to Tityridae and *Oxyruncus*. Tello & Bates (2007) and Rheindt *et al*. (in press) also found *Onychorhynchus*, *Myiobius* and *Terenotriccus* to group outside Tyrannidae, although the taxon sampling in these studies did not allow them to resolve their systematic position.

The traditional placement of *Piprites* in Pipridae has long been questioned (e.g. Snow 1975). S. M. Lanyon (1985) and Prum (1990) presented evidence that *Piprites* was not a manakin, but they could not come up with any supported conclusion as to its real affinities. Ericson *et al*. (2006) found *P. pileatus* to be the sister taxon to Tyrannidae, and this is corroborated in this study, with *P. chloris* firmly placed as its sister taxon. Thus, the similar syringeal morphology (e.g. lack of intrinsic syringeal musculature) found in *Piprites* and members of our clade B (Ames 1971) can be interpreted as plesiomorphic. The presence of the intrinsic syringeal *Musculus obliquus ventralis* (and of intrinsic syringeal musculature in general) was suggested as a possible synapomorphy for Tyrannidae in Ericson *et al*. (2006). Intrinsic syringeal musculature is absent in cotingas and manakins (Prum 1990), and in a number of genera in clade A (*Onychorhynchus*, *Myiobius*, *Terenotriccus*, *Piprites*, *Neopipo*, *Todirostrum*, *Zimmerius*, *Pyrrhomyias*, *Hirundinea* and *Machetornis*). Similar structures are present in Tityridae, although these were hypothesized to be of an independent origin (Prum 1990). The absence in the above-mentioned Tyrannidae genera was interpreted by Lanyon (1984, 1986, 1988a,c) as independent losses, and not treated as a character state at all by Birdsley (2002). The most reasonable interpretation of our results is that intrinsic syringeal musculature evolved in the ancestor of Tyrannidae, as suggested by Ericson *et al*. (2006). It was subsequently independently lost in *Neopipo*, *Todirostrum*, *Zimmerius*, *Pyrrhomyias*, *Hirundinea* and *Machetornis*, as these are all embedded in clades that possess intrinsic syringeal musculature.

Tody-tyrants, flatbills and allies. The clade containing the flatbills and tody-tyrants turns out to be a more diverse group than previously understood. Compared to Lanyon's (1988c) flatbill and tody-tyrant assemblage and Fitzpatrick *et al*.'s (2004) Platyrinchini it does not include *Onychorhynchus* (see previous section). On the other hand, it includes the aberrant *Neopipo* and *Tachuris* and, in accordance with Rheindt *et al*. (in press), a number of fine-billed genera (*Pseudotriccus*, *Corythopis*, *Leptopogon*, *Mionectes*, *Phylloscartes* and *Pogonotriccus*) that traditionally have been associated with various elaeniine genera (Traylor 1977; Lanyon 1988a; Fitzpatrick *et al*. 2004). A relationship between *Phylloscartes*, *Pogonotriccus*, *Leptopogon* and *Mionectes* is in accordance with Lanyon (1988a) results, although he placed them in the *Elaenia* assemblage together with the elaeniine genus *Zimmerius*. Lanyon's (1988a) also considered *Corythopis* and *Pseudotriccus* to be closely related, in agreement with Sibley & Ahlquist (1985, 1990), but kept them in the *Elaenia* assemblage. Tello & Bates (2007) did not include *Phylloscartes* or *Pogonotriccus*, but found a close relationship between *Corythopis* and *Pseudotriccus* and also found some support for a relationship between them and *Mionectes* and *Leptopogon*, as well as for these four genera being the sister group to the Tody-tyrants. Most members of *Phylloscartes*, *Pogonotriccus* and *Leptopogon* share a number of plumage characters, for example, a grizzled facial pattern and a dark crescent on the ear coverts. They also share the peculiar 'single-wing flicking' behaviour and forage mainly by upward striking (Fitzpatrick 1980), the latter being unique for the pipromorphines, *Platyrinchus* and *Neopipo*.

Within the pipromorphines, there was strong support for a sister relationship between *Rhynchocyclus* and *Tolmomyias*, and for the monophyly of the mostly thicket-specialized tody-tyrants (*Todirostrum*, *Poecilotriccus*, *Lophotriccus*, *Hemitriccus* and *Myiornis*). This is in accordance with the results of Tello & Bates (2007) and Rheindt *et al*. (in press) and our studies all strongly indicate that current generic limits in this clade need to be re-evaluated. However, these cannot be clarified without a much denser taxon sampling. The exact position of *Cnipodectes* is less clear, however. Lanyon (1988c) placed in an unresolved position in the Flatbill and Tody-tyrant assemblage, where it also comes out in the study by Rheindt *et al*. (in press) It was placed as the sister to *Rhynchocyclus* and *Tolmomyias* by Tello & Bates (2007), whereas our data favour a placement as the sister taxon to the Tody-tyrants.

Contrary to Ericson *et al*. (2006), Tello & Bates (2007) and Rheindt *et al*. (in press), we found support for *Platyrinchus* having their closest affinities with the tody-tyrants, flatbills and allies, although they are separated from these by a long internode. One of the most unexpected findings in our study, which was also found by Rheindt *et al*. (in press), was that the poorly known *Neopipo* consistently comes out as the sister taxon to *Platyrinchus*. *Neopipo* was traditionally placed in Pipridae, until Prum (1990) showed that it lacks the syringeal and hind limb artery traits that united the Cotingidae and Pipridae. Based on morphological characters, both Mobley & Prum (1995) and Birdsley (2002) found it to belong with the *Myiophobus* group in the *Empidonax* assemblage of Lanyon (1986, 1988a), that is, *Myiobius*, *Terenotriccus*, *Pyrrhomyias*, *Hirundinea* and some *Myiophobus* species. We found these genera to belong in widely different parts of the tree, but no support for a close relationship of *Neopipo* to any of them. To ascertain that this was not the result of sample mix-up, we sequenced G3PDH for a second specimen of *Neopipo cinnamomea* (sample identification: USNM B11430). The two sequences were identical and only one of them was used in the analysis. *Neopipo* possesses the complete, ossified bronchial A elements that Lanyon (1988a) used to define the *Myiophobus* group in his *Empidonax* assemblage (Mobley & Prum 1995). However,

this is also present in several other tyrant flycatchers, including some species of *Platyrinchus* (Lanyon 1988c). More importantly, in the light of our results, *Neopipo* shares the upward strike foraging strategy typical of *Platyrinchus* and the pipromorphines (Fitzpatrick *et al*. 2004, p. 353).

Although *Tachuris* was placed in an unresolved, basal position, its affinities with the pipromorphines and *Platyrinchus* are as unexpected as for *Neopipo*. A relationship to *Pseudocolopteryx* was suggested for *Tachuris* by Traylor (1977), arguably because they inhabit similar habitats and show some vague similarities in shape and colour pattern. Indeed, from morphology and behaviour, there is little to suggest an affinity with the pipromorphines, and especially in habitat selection and foraging behaviour, *Tachuris* is set apart from other pipromorphines. The largely un-ossified nasal capsule of *Tachuris* (Birdsley 2002) is, however, in concordance with this placement, as this appears to be plesiomorphic in Tyrannida and clade C in general is characterized by a higher degree of cranial ossification (W. E. Lanyon 1984–1988c). Further, *Platyrinchus* and *Tachuris* build very similar neat, tiny cup-shaped nests (see Fitzpatrick *et al*. 2004, pp. 224–225), which differ strikingly from the enclosed nests of the pipromorphines. The nest of *Neopipo* is unfortunately unknown, but judging from its affinities it might be expected to have a neat, cup-shaped nest.

Elaeniines, tyrannines, fluvicolines and allies. The bulk of clade C is made up of two large clades roughly corresponding to the Elaeniini and Fluvicolinae + Tyranninae of Fitzpatrick *et al*. (2004). It further contains a small clade of chiefly Andean humid forest forms (clade G), which all have been subject to various degrees of taxonomic confusion. In external appearance, they are similar to many typical fluvicoline genera (e.g. *Mitrephanes* and *Cnemotriccus*) and they were treated as such by Traylor (1977). Lanyon (1986, 1988a), on the other hand, divided them between the *Elaenia* (*Myiophobus lintoni*, *M. ochraceiventris* and *Myiotriccus*) and *Empidonax* assemblages (*Pyrrhomyias*, *Hirundinea* and provisionally also *Myiophobus pulcher*, of which he lacked samples). One of the two cranial morphologies used by Lanyon (1986) to define his *Empidonax* assemblage (a forked posterior end of trabecular plate in the nasal septum) is absent in all of these taxa. *Pyrrhomyias* and *Hirundinea* were included in the *Empidonax* assemblage, although they lack this latter trait (Lanyon 1986, p. 55; 1988c). A close relationship between *Pyrrhomyias* and *Hirundinea* has been suggested earlier, based on behaviour, external appearance and syringeal morphology. The placement of *Myiophobus pulcher*, *M. lintoni* and *M. ochraceiventris* far away from their traditional congeners confirms earlier suspicions of non-monophyly of *Myiophobus*. However, our subdivision of this genus differs in several respects from that proposed by Lanyon (1986, 1988a). He proposed that the closest relatives to *Myiophobus lintoni* and *M. ochraceiventris* (and *Myiotriccus*) were *Myiophobus phoenicomitra* and *M. roraimae*, but our data place them with *Ochthoeca* (see below). A sister relationship with clade D is indicated by our data, and would also be in accordance with behaviour and morphology, but as this only receives a posterior probability of 0.81 this question remains open. Rheindt *et al*. (in press) placed *Myiotriccus* as the sister to all other 'core tyrannids', a results that is not contradicted by our results.

Our elaeniine clade includes all the taxa that Lanyon (1988a) united in his *Elaenia* group, based on the possession of a syringeal drum made up by fused tracheal elements. However, four genera that lack this character also fell within the elaeniine clade (*Zimmerius*, *Stigmatura*, *Inezia* and *Euscarthmus*). Although taxonomic sampling differs, similar results were also found by Rheindt *et al*. (in press). Our results suggest a rapid initial divergence in the elaeniine clade, as many of the basal nodes are short and unresolved (although the clades themselves are strongly supported). It is also clear that generic limits as currently defined do not reflect the true relationships, and as was suggested by Lanyon (1988a) and Rheindt *et al*. (in press), neither *Mecocerculus* nor *Phyllomyias* are monophyletic. *Mecocerculus leucophrys* is not close to any of its traditional congeners, but is instead the sister taxon of a number of genera which mainly inhabit dry scrub and grassland (clade E-3). *Phyllomyias fasciatus* and *P. griseiceps*, which Lanyon (1988a) considered aberrant enough to be excluded from the *Elaenia* assemblage altogether, are closely related to *Capsiempis* and *Phaeomyias* in clade E-1. The other sampled species in *Phyllomyias* (*uropygialis* and *virescens*) and *Mecocerculus* (*calopterus* and *poecilocercus*) belong to a clade with *Ornithion* and *Camptostoma* (clade E-2). A more comprehensive taxon sampling and additional molecular markers are clearly needed to clarify both the early evolutionary history and generic limits in the elaeniine clade.

The fluvicolines, myiarchines and tyrannines all constitute strongly supported clades, but their interrelationships are not resolved. The genera *Attila*, *Ramphotrigon*, *Deltarhynchus* and *Muscigralla* occupy positions outside these three 'core' groups in the poorly resolved basal part of clade D. *Muscigralla* was treated as *incerta sedis* within Tyrannidae by Lanyon (1986), due to several peculiarities in its syringeal morphology. Traylor (1977) suggested on vague grounds that it might be 'an early offshoot of the fluvicoline stock'. This is not contradicted by our results, although it is obviously an isolated taxon with highly derived morphology.

Ramphotrigon and *Deltarhynchus* share a number of morphological and ecological traits with the myiarchines, e.g. nesting in tree cavities. Due to marked differences in syringeal morphology, W. E. Lanyon (1985) proposed that *Ramphotrigon* should be removed from the vicinity of the flatbills *Tolmomyias* and *Rhynchocyclus* to a position near *Myiarchus* and *Deltarhynchus*. Birdsley (2002) likewise

recovered a relationship between the *Myiarchus* assemblage and *Deltarhynchus* and *Ramphotrigon*. *Deltarhynchus* is reminiscent of *Myiarchus* in general appearance and they were suggested to be close relatives by W. E. Lanyon (1982, 1985). *Attila* was associated with the myiarchines and tyrannines by W. E. Lanyon (1985), albeit with some doubts, and this uncertainty is borne out in our phylogeny as well, where *Attila* is placed with very weak support together with the myiarchines and tyrannines. Our result supports the suggestion of W. E. Lanyon (1985) of a close relationship between *Ramphotrigon* and *Deltarhynchus*, but we did not find clear evidence for a close relationship with *Myiarchus*. Instead, they are basal to the fluvicolines, with good support in the combined tree. However, the results of Tello & Bates (2007) and Rheindt *et al*. (in press), using different molecular markers, strongly indicate that at least *Ramphotrigon* belongs with the myiarchines and tyrannines. This is also suggested by certain unique features of the syrinx, nesting behaviour, and in the case of *Deltarhynchus*, also by external appearance. The position of these two genera must be explored further. Besides that, the myiarchine and tyrannine clades agree well with previously suggested hypotheses (Lanyon 1984; Birdsley 2002), with the only notable difference that *Machetornis* is embedded among the tyrannines as well, somewhat unexpectedly as the sister to *Pitangus*. A kingbird affinity has been suggested previously for *Machetornis*, based on general morphology and behaviour, but this was contradicted by its aberrant cranial and syringeal morphology (Lanyon 1984).

Our delimitation of the fluvicolines generally agrees well with earlier classifications (Traylor 1977; Fitzpatrick *et al*. 2004), but there were several differences in the internal relationships compared to the *Empidonax* assemblage of Lanyon (1986) and Birdsley (2002). Except for the relatively clear-cut *Empidonax* group, none of W. E. Lanyon's groups is recovered by our data. Contrary to its traditional placement in Elaeniini (e.g. Fitzpatrick *et al*. 2004), but in agreement with Rheindt *et al*. (in press), we find *Sublegatus* embedded among the fluvicolines, although its exact position is unclear. Lanyon (1988a) placed it in the *Phylloscartes* group of his *Elaenia* assemblage, a group that is demonstrated here to be composed of genera of widely different affinities.

Basal relationships among the fluvicolines were not well resolved. There was some indication that clade F-1 may be the sister group to the remainder of the genera, but the support was not particularly strong (posterior probability of 0.93). A basal position of clade F-1 in the fluvicoline radiation is nevertheless an intriguing suggestion, as this clade contains the only fluvicoline taxa confined to humid forest interior. Furthermore, the taxonomic composition of this clade is novel, as a close affinity of any *Myiophobus* species to *Ochthoeca* has not been suggested previously. The three *Myiophobus* species included here (*M. phoenicomitra*, *M. flavicans* and *M. roraimae*) were suggested by Fitzpatrick *et al*. (2004) to be closely related (and also including *M. inornatus*), although Lanyon (1986, 1988a) placed them in different assemblages. The unresolved position of *Ochthoeca diadema* and the sister relationship between *Ochthoeca cinnamomeiventris* and *Tumbezia salvini* indicates that *Ochthoeca* as currently delimited may not be monophyletic. Lanyon (1986) recommended subsuming *Tumbezia* in *Ochthoeca* and proposed the genus *Silvicultrix* for the species *diadema*, *frontalis* (including *jelskii*) and *pulchella*, but this was refuted by García-Moreno *et al*. (1998). More comprehensive taxon sampling is needed (also including the purported relative *Coloramphus*) to clarify both generic limits and adaptive history in this clade.

Although well supported, clade F-2 shows very little internal resolution, which is surprising given the morphological distinctiveness of its members. This group was not recovered by Lanyon (1986); he found a close relationship between *Fluvicola*, *Arundinicola* and *Alectrurus* although he placed them in a group together with *Ochthoeca*. *Pyrocephalus*, which he suggested to be close to *Lessonia*, *Knipolegus* and *Hymenops*, is also placed here, as is *Gubernetes*, which he associated with genera in our clade F-3. Furthermore, *Colonia*, which Lanyon (1986) placed as *incerta sedis*, comes out in this clade in our analyses. It was placed together with *Sublegatus* outside the restricted *Empidonax* clade by Birdsley (2002).

The monophyly of W. E. Lanyon's *Empidonax* group is strongly supported by our data and was also recovered by Cicero & Johnson (2002), but we further demonstrate that *Myiophobus sensu stricto* is their sister group, together forming our clade F-4. Our results are also in accordance with their hypothesis on relationships within the clade and like in earlier studies (Lanyon & Lanyon 1986; Cicero & Johnson 2002), we found a close relationship between *Lathrotriccus* and *Cnemotriccus*. Cicero & Johnson (2002) hypothesized that the *trans*-isthmian genera in the *Empidonax* group had their origins in South America. This is indeed expected, and our results further suggest mesic to deciduous lowland forest as a possible ancestral habitat. This is supported by the fact that both their successive sister groups (*Lathrotriccus*, *Cnemotriccus* and *Myiophobus sensu stricto*) are widely distributed over such habitats. However, this suggestion must be substantiated by analyses of a comprehensive taxon sample, especially including Neotropical representatives of *Contopus*, which hitherto never have been included in any molecular studies.

Most of the tyrannid genera characteristic of austral and 'high Andean' regions (e.g. *Muscisaxicola*, *Agriornis* and *Myiotheretes*) have generally been assumed to be close relatives. Here, they cluster together in clade F-3, which is placed as the sister group to clade F-4. In fact, the relationships depicted in the Adams consensus tree in Birdsley (2002, fig. 6), obtained with behavioural and ecological characters, are remarkably similar to our results, including a basal position of *Satrapa*.

This genus has sometimes been associated to *Tumbezia*, mainly based on plumage characteristics (Traylor 1977). It was shown by Lanyon (1986) to be related to the genera in our clades F-3 and F-4, although he could not determine its closest relative. Lanyon (1986) separated *Knipolegus*, *Hymenops* and *Lessonia* in the *Knipolegus* group together with *Pyrocephalus*. This group was not defined by any anatomical characters but only by 'marked sexual dimorphism'. Similar to the situation in clade F-4, the basal genera in the clade, *Knipolegus* and *Hymenops*, are distributed mostly in lowland habitats.

Ecological radiation in Tyrannidae

Temporal scale of diversification. The understanding of the extensive ecological radiation in Tyrannida has been limited by the difficulties in resolving the interrelationships between major lineages. Traylor (1977) suggested that fluvicolines, myiarchines and tyrannines radiated and diversified in response to the spread of non-forest habitats, although he presumed the fluvicolines to be an old lineage, due to the 'high degree of phenetic variability' in the group.

With a well-resolved phylogeny at hand, we can draw the conclusion that the ancestral Tyrannida inhabited the interior of humid forest. The earliest divergence events in the group gave rise to two clades of humid forest frugivores (Cotingidae and Pipridae), and a clade of predominantly insectivorous forms (clade A). Today, the Cotingidae and Pipridae are comparatively species-poor (65 and 51 species, respectively), whereas extensive diversification has taken place within clade A (441 species).

With the caveats given above (subsection Divergence time estimation under Materials and methods section), our chronogram (Fig. 2) presents a tentative estimate of divergence times among tyrannid lineages. The early divergences in clade A are estimated to be of Oligocene age (*c.* 25 Mya) and involved clades largely restricted to humid forest, strongly suggesting that much of the early diversification in clade A likewise took place in a humid forest environment. The existence of several small clades of ancient rainforest tyrannids (*Oxyruncus*, *Onychorhynchus*, *Myiobius*, *Terenotriccus*, *Piprites*, *Neopipo*) suggests a high persistence of lineages in this environment but few opportunities for speciation. Apart from a number of clades that today still are restricted to humid forest interior, the early period of divergences also gave rise to clade C, which encompasses slightly more than 50% of the extant Tyrannida species (*c.* 300 species in 75 genera), the majority of which are adapted to habitats other than humid forest interior. The diversification in clade C is correlated with the expansion of open habitats during late Neogene climatic cooling and aridification (Fig. 2). This overall pattern is in accordance with what is known about the large-scale Tertiary climatic and palaeoecological development globally and in South America (e.g. Burnham & Graham 1999; Zachos *et al.* 2001; MacFadden 2006). According to our PATHd8 estimate, clade C diverged from its sister lineage at *c.* 25 Mya, near the onset of the late Oligocene warming episode. However, the extensive radiation leading to its extant diversity did not initiate until *c.* 10 Mya later. This delay in diversification corresponds to a period of generally warm and humid climate from the late Oligocene to the mid-Miocene climatic optimum (Zachos *et al.* 2001). Apparently, a major adaptive shift coupled to the colonization of open habitats took place along this branch, but it is impossible to be more precise about timing of the adaptive change.

Following the mid-Miocene climatic optimum (*c.* 15 Mya) South America experienced another trend of cooling and aridification (Zachos *et al.* 2001, fig. 2) with the concomitant expansion of grass-dominated and other open ecosystems (e.g. Jacobs *et al.* 1999). An increase of C4 grasses, a strong indicator of arid tropical grasslands, is evident at *c.* 9–5 Mya (Jacobs *et al.* 1999). Coincident with this climatic trend, substantial diversification of open-habitat lineages occurred in clade C. The densely packed nodes after the mid-Miocene Climatic Optimum (Fig. 2) indicate a rapid diversification throughout the subsequent periods of global cooling, as they colonized a variety of emerging open habitats.

Although our divergence time estimates are tentative, there is a good correlation between the appearance of open habitat forms and known environmental changes during the Miocene. First, the substantial diversification of open habitat taxa in clade C lies almost entirely within the time frame of the cooling and drying climatic trend following the mid-Miocene Climatic Optimum (see Fig. 2). Second, the emergence of clades restricted to grass-dominated habitats (clades E-3, F-2 and F-3), show a rather strong correlation with the earliest evidence for an increase of C4 grasses in South America (*c.* 9 Mya) (Jacobs *et al.* 1999).

Foraging behaviour and colonization of open habitats. Fitzpatrick (1980, 1985) found Traylor's (1977, 1979) major lineages in Tyrannidae to be remarkably homogenous in foraging behaviour, showing a strong correlation between the diversification of foraging behaviour and adaptive success in different habitats. This pattern is even more obvious when viewed in the light of our phylogenetic hypothesis (Fig. 2), which also allows some further interpretations of the evolution of foraging behaviours. The deeper lineages in clade A are inhabitants of forest understorey and are characterized by close range foraging techniques with short search times, chiefly upward striking and short sallies. Of these lineages, only the pipromorphines exhibit a large diversity, with almost 100 extant species. This clade is strikingly conservative in ecology and behaviour and its substantial radiation took place without any notable modifications of the ancestral foraging behaviour. With few exceptions, the pipromorphines are tied to humid

forest interior with on average large leaf size, or to dense thickets. As observed by Fitzpatrick (1980, 1985) their predominant foraging strategy, upward striking, is remarkably stereotyped, and it is further retained in the few members of *Phylloscartes*, *Tolmomyias* and *Todirostrum* that have colonized canopy and edge habitats with more finely divided foliage.

Along a few long branches, more drastic changes have taken place, often coupled with marked habitat shifts or ecological adaptations that set them apart from their closest relatives: *Iodopleura* (aerial sallier in canopy), *Oxyruncus* (prober/pryer in epiphytes), *Tachuris* (perch-gleaner in marshes) and *Muscigralla* (ground gleaner in desert scrub). Long branches do not lend themselves easily to meaningful reconstruction of character changes, as there is no way to estimate where on the branch the change took place. This makes it impossible to estimate the age of these specializations; we can only say that they are not necessarily as old as the branches themselves.

Colonization of 'forest exterior' and of open habitats that emerged in the mid- to late Miocene occurred independently in several different subclades in clade A. Apart from in clade C, it also took place in *Tachuris* and some species in the pipromorphine genera *Tolmomyias*, *Phylloscartes* and *Todirostrum*, and in *Tityra* and several species of *Pachyramphus* in Tityridae. However, it apparently resulted in a large-scale diversification only in clade C, which today encompasses the overwhelming majority of small insectivorous birds in South American semi-open and open habitats. Some of the basal clades in clade C are tied to humid forest; clades G and F-1, which are both restricted to the Andes (except the cliff specialist *Hirundinea*) and *Attila* and *Ramphotrigon*, which chiefly inhabit lowland humid forest edge. This may suggest that taxic diversification in clade C started before open habitats became widespread and that the initial diversification took place in a humid forest environment. The colonization of open habitat by the ancestors of elaeniines, myiarchines, tyrannines and fluvicolines would thus be independent events (see Strömberg 2005, for a similar hypothesis for grasses in North America). However, a humid forest interior origin for clade C is only weakly favoured as the ancestral state in the MESQUITE optimization. To clarify this, better resolution is needed for the initial divergence events in that clade. This requires resolving the positions of clade G, *Ramphotrigon*, *Attila* and *Muscigralla*, which at present are not satisfactorily established. The ancestral state reconstructions for both clade D and the fluvicolines are heavily affected by the specialized *Muscigralla*, which lives in arid scrub. When the branch leading to *Muscigralla* is coded as such, this is also favoured as the ancestral habitat for both of them. However, if *Muscigralla* is excluded, that is, if its character state is treated as an uninformative autapomorphy, or assumed to have occurred somewhere along the branch and not at the point of divergence, the ancestral state for the fluvicolines becomes ambiguous with almost equal likelihood for humid forest interior, mesic woodland and open habitat.

Both the elaeniines and clade D are ecologically very successful in terms of generic and species-level diversity. Interestingly, they show very consistent differences in principal foraging behaviour. Like the pipromorphines and allies, the elaeniines are remarkably homogenous in both foraging behaviour and habitat preference. Although they occur in a broad range of habitats from humid forest canopy to dense arid scrub, they are remarkably narrow in microhabitat use. With few exceptions, members of the elaeniines inhabit leafy microhabitats with high light intensity and a complex and finely divided foliage, where they forage actively by perch-gleaning and upward hover-gleaning, with several species accompanying mixed feeding flocks. This foraging behaviour has not undergone much modification during their history of diversification.

In contrast to the marked homogeneity regarding habitat and foraging behaviour in the pipromorphines and allies and elaeniines, the pattern of ecological diversification has taken a strikingly different course in clade D, where an ancestral sallying technique has evolved into a diverse array of foraging behaviours linked to the colonization of semi-open to open habitats. The four principal fluvicoline clades exhibit distinct ecological characteristics, when habitat and foraging behaviour are taken together. Several species in clade F-1 inhabit shady interior of humid Andean forest, a life zone that is otherwise rarely occupied by the fluvicolines. A previous study (García-Moreno *et al.* 1998) indicated that adaptation to the interior of humid forest is the derived state in *Ochthoeca*. This conclusion needs to be re-evaluated with more data and the use of model-based phylogenetic methods.

Members of clade F-2 are mostly distributed in wetland and savanna habitats of South America with one species (*Py. rubinus*) extending up into southern North America. Clades F-3 and F-4 reach their greatest diversity outside the humid lowlands of South America. The morphologically diverse clade F-3 radiated extensively in tree-less habitats in the Austral and high Andean regions. In clade F-4, three genera with similar morphology (*Contopus*, *Empidonax* and *Sayornis*), but different foraging strata (Cicero & Johnson 2002), have spread and diversified in forested habitats in Central and North America. The basal members of these two clades occupy various bushy or wooded South American habitats, mainly in the lowlands: *Knipolegus* in clade F-3 and *Myiophobus sensu stricto*, *Cnemotriccus* and *Lathrotriccus* (and likely also *Aphanotriccus*, see Cicero & Johnson 2002) in clade F-4.

The myiarchines and tyrannines are predominantly birds of forest borders and woodland, and include two genera (*Myiarchus* and *Tyrannus*) that have extended into North America. Myiarchines occur in a broad range of habitats (Joseph *et al.* 2004), but without exception forage by 'outward

hover-gleaning' (Fitzpatrick 1980), searching from a more or less exposed perch after sedentary prey in adjacent foliage. Tyrannines exhibit a broader range of foraging behaviour; mostly variants of aerial hawking from exposed perches, with only one genus (*Machetornis*) adapted to a terrestrial life style. They are often adaptable generalists, reaching a culmen in the extremely versatile and opportunistic *Pitangus sulphuratus*. Neotropical forms are most diverse in mesic to humid woodland and forest edge habitats in lowlands, where their opportunities may now have been favoured by anthropogenic habitat modifications.

Given the plasticity of traits connected with foraging behaviour, it is interesting to note that the elaeniines and clade D differ markedly in adaptive success in different habitats and in the degree of morphological and ecological diversity. This might be due to a greater adaptive versatility inherent in the sallying technique. The predominant foraging strategy of the elaeniines is perch-gleaning, which is an active close-range approach to stationary, hidden prey. This strategy is successful in microhabitats with dense foliage, but may be less amenable to adaptations to non-wooded habitats. Most elaeniines that have colonized non-forest habitats are restricted to microhabitats with dense foliage (e.g. *Phaeomyias*, *Camptostoma* and *Anairetes*), while an adaptation to grassland has only occurred in a few genera in clade E-3 (*Culicivora*, *Polystictus* and *Pseudocolopteryx*). Both the pipromorphines and allies and the elaeniines are characterized by a more mobile foraging behaviour, with generally short search times. The typical sallying strategy of clade D is based on longer search times, often from a more or less exposed vantage point, and medium- to long-range approach to the prey, and this may be more easily modified for adaptation to strictly non-wooded habitats, for example, the perch-to-ground strategy of *Xolmis* and other genera (Fitzpatrick 1980). This may explain the success of the fluvicolines in colonizing open habitats, such as grasslands, where elaeniines are poorly represented.

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Corrigendum

After this paper had been accepted, it was discovered that two of the samples used in this study had been misidentified. First, the sample identified as *Myiophobus roraimae* (ZMUC 126 361) has been shown to be a *Myiophobus flavicans*. Second, the sample identified as a *Contopus sordidulus* (ZMUC 126 771), is from an *Empidonax* species, most probably *E. alnorum*. Neither of these errors have any important consequences for the main conclusions of the paper.

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Appendix 1 List of samples used this study, with collecting locality and GenBank accession numbers. All samples except those marked with an asterisk (*) are from vouchered specimens. Taxa without physical vouchers are all documented with photographs of the individual sampled. Institutional acronyms: NRM, Swedish Museum of Natural History; ZMUC, Zoological Museum, University of Copenhagen; USNM, National Museum of Natural History, Smithsonian Institution.

Taxon	Family	Sample ID	Provenance	G3P	Myoglobin	ODC
*Agriornis andicola**	Tyrannidae	ZMUC 125 678	Azuay, Ecuador	EU231738	EU231836	EU231939
Alectrurus risora	Tyrannidae	NRM 947 227	Presidente Hayes, Paraguay	EU231722	EU231820	EU231923
Anairetes alpinus	Tyrannidae	ZMUC 125 189	Cuzco, Peru	EU231677	EU231775	EU231878
Anairetes parulus	Tyrannidae	ZMUC 125 198	Apurímac, Peru	EU231678	EU231776	EU231879
Arundinicola leucocephala	Tyrannidae	NRM 947 198	Alto Paraguay, Paraguay	EU231721	EU231819	EU231922
*Attila spadiceus**	Tyrannidae	ZMUC 125 869	Mato Grosso, Brazil	EU231697	EU231795	EU231898
Attila torridus	Tyrannidae	ZMUC 125 220	Loja, Ecuador	EU231698	EU231796	EU231899
Camptostoma obsoletum	Tyrannidae	NRM 947 111	Alto Paraguay, Paraguay	EU231689	EU231787	EU231890
Capsiempis flaveola	Tyrannidae	NRM 569 485	Amazonas, Brazil	EU231672	EU231770	EU231873
Casiornis rufus	Tyrannidae	NRM 976 656	Amambay, Paraguay	EU231703	EU231801	EU231904
*Cnemarchus erythropygius**	Tyrannidae	ZMUC 125 224	Huánuco, Peru	EU231742	EU231840	EU231943
Cnemotriccus fuscatus	Tyrannidae	NRM 966 767	Boquerón, Paraguay	EU231727	EU231825	EU231928
Cnipodectes subbrunneus	Tyrannidae	ZMUC 125 226	Guayas, Ecuador	EU231663	EU231761	EU231863

Appendix 1 *Continued.*

Taxon	Family	Sample ID	Provenance	G3P	Myoglobin	ODC
Colonia colonus	Tyrannidae	NRM 976 648	Amambay, Paraguay	EU231719	EU231817	EU231920
*Contopus sordidulus**	Tyrannidae	ZMUC 126 771	Apurímac, Peru	EU231731	EU231829	EU231932
Corythopis delalandei	Tyrannidae	NRM 937 282	Caazapa, Paraguay	DQ435463	AY065788	DQ435479
Culicivora caudacuta	Tyrannidae	NRM 986 699	Misiones, Paraguay	EU231680	EU231778	EU231881
Deltarhynchus flammulatus	Tyrannidae	NRM 569 487	Oaxaca, Mexico	EU231699	EU231797	EU231900
Elaenia flavogaster	Tyrannidae	NRM 966 970	Concepción, Paraguay	DQ435464	AY064254	DQ435480
Empidonax alnorum	Tyrannidae	ZMUC 125 779	Napo, Ecuador	EU231730	EU231828	EU231931
Empidonomus varius	Tyrannidae	NRM 956 628	Caazapa, Paraguay	EU231711	EU231809	EU231912
Euscarthmus meloryphus	Tyrannidae	NRM 976 685	Amambay, Paraguay	EU231684	EU231782	EU231885
Fluvicola albiventer	Tyrannidae	NRM 956 714	Alto Paraguay, Paraguay	DQ435465	DQ435517	DQ435481
Gubernetes yetapa	Tyrannidae	NRM 976 700	Concepción, Paraguay	AY336578	AY338739	DQ435483
Hemitriccus diops	Tyrannidae	NRM 956 601	Itapua, Paraguay	EU231668	EU231766	EU231868
Hemitriccus margaritaceiventer	Tyrannidae	NRM 966 805	Boquerón, Paraguay	EU231669	EU231767	EU231869
Hirundinea ferruginea	Tyrannidae	ZMUC 125 257	Cochabamba, Bolivia	EU231692	EU231790	EU231893
*Hymenops perspicillatus**	Tyrannidae	ZMUC 131 855	La Plata, Argentina	EU231732	EU231830	EU231933
Inezia inornata	Tyrannidae	NRM 947 139	Alto Paraguay, Paraguay	DQ435466	DQ435518	DQ435484
Knipolegus striaticeps	Tyrannidae	NRM 966 782	Boquerón, Paraguay	DQ435471	DQ435523	DQ435491
Lathrotriccus euleri	Tyrannidae	NRM 967 011	Concepción, Paraguay	EU231726	EU231824	EU231927
Legatus leucophaius	Tyrannidae	NRM 947 239	Amambay, Paraguay	EU231705	EU231803	EU231906
Leptopogon amaurocephalus	Tyrannidae	NRM 937 317	Caazapa, Paraguay	DQ435468	DQ435520	DQ435487
Lessonia rufa	Tyrannidae	ZMUC 131 858	La Plata, Argentina	EU231733	EU231831	EU231934
Lophotriccus pileatus	Tyrannidae	ZMUC 125 261	Zamora-Chinchipe, Ecuador	EU231667	EU231765	EU231867
Machetornis rixosus	Tyrannidae	NRM 967 068	Concepción, Paraguay	EU231706	EU231804	EU231907
Mecocerculus calopterus	Tyrannidae	ZMUC 125 265	Loja, Ecuador	EU231687	EU231785	EU231888
Mecocerculus leucophrys	Tyrannidae	ZMUC 125 277	Ancash, Peru	EU231676	EU231774	EU231877
Mecocerculus poecilocercus	Tyrannidae	ZMUC 125 281	Carchi, Ecuador	EU231688	EU231786	EU231889
Megarynchus pitangua	Tyrannidae	NRM 966 981	Concepción, Paraguay	EU231709	EU231807	EU231910
Mionectes rufiventris	Tyrannidae	NRM 966 944	Paraguari, Paraguay	EU231662	EU231760	EU231862
Mitrephanes phaeocercus	Tyrannidae	ZMUC 126 161	Esmeraldas, Ecuador	EU231729	EU231827	EU231930
Muscigralla brevicauda	Tyrannidae	ZMUC 125 316	Lambayeyque/Piura, Peru	EU231712	EU231810	EU231913
Muscisaxicola alpinus	Tyrannidae	ZMUC 125 319	Chimborazo, Ecuador	EU231735	EU231833	EU231936
Muscisaxicola maculirostris	Tyrannidae	ZMUC 125 322	Oruro, Bolivia	EU231736	EU231834	EU231937
Myiarchus tyrannulus	Tyrannidae	NRM 937 173	Presidente Hayes, Paraguay	DQ435469	DQ435521	DQ435489
Myiobius villosus	Tyrannidae	ZMUC 126 095	Esmeraldas, Ecuador	EU231654	EU231752	EU231854
Myiodynastes maculatus	Tyrannidae	NRM 966 928	Presidente Hayes, Paraguay	EU231710	EU231808	EU231911
Myiopagis caniceps	Tyrannidae	NRM 976 683	Amambay, Paraguay	DQ435475	DQ435527	EU231871
Myiopagis viridicata	Tyrannidae	NRM 986 779	Misiones, Paraguay	DQ435470	DQ435522	DQ435490
Myiophobus cryptoxanthus	Tyrannidae	ZMUC 127 826	Morona-Santiago, Ecuador	EU231724	EU231822	EU231925
Myiophobus fasciatus	Tyrannidae	NRM 956 696	Alto Paraguay, Paraguay	EU231725	EU231823	EU231926
Myiophobus flavicans	Tyrannidae	ZMUC 125 347	Carchi, Ecuador	EU231714	EU231812	EU231915
Myiophobus lintoni	Tyrannidae	ZMUC 128 898	Zamora-Chinchipe, Ecuador	EU231695	EU231793	EU231896
*Myiophobus ochraceiventris**	Tyrannidae	ZMUC 127 036	La Paz, Bolivia	EU231696	EU231794	EU231897
Myiophobus phoenicomitra	Tyrannidae	ZMUC 127 836	Morona-Santiago, Ecuador	EU231713	EU231811	EU231914
Myiophobus pulcher	Tyrannidae	ZMUC 125 787	Sucumbios, Ecuador	EU231694	EU231792	EU231895
Myiophobus roraimae	Tyrannidae	ZMUC 126 361	Zamora-Chinchipe, Ecuador	EU231715	EU231813	EU231916
Myiornis auricularis	Tyrannidae	NRM 967 077	Amambay, Paraguay	EU231670	EU231768	EU231870
Myiotheretes fumigatus	Tyrannidae	ZMUC 125 354	Loja, Ecuador	EU231737	EU231835	EU231938
Myiotriccus ornatus	Tyrannidae	ZMUC 125 759	Napo, Ecuador	EU231691	EU231789	EU231892
Myiozetetes similis	Tyrannidae	NRM 976 708	Concepción, Paraguay	EU231708	EU231806	EU231909
Neopipo cinnamomea	Tyrannidae	USNM B11797	Gunn's Landing, Guyana	EU231658	EU231756	EU231858
Neoxolmis rufiventris	Tyrannidae	ZMUC 135 470	Patagonia, Argentina	EU231739	EU231837	EU231940
*Ochthoeca cinnamomeiventris**	Tyrannidae	ZMUC 125 360	Cuzco, Peru	EU231717	EU231815	EU231918
Ochthoeca diadema	Tyrannidae	ZMUC 125 782	Imbabura/Esmeraldas, Ecuador	EU231716	EU231814	EU231917
Onychorhynchus occidentalis	Tyrannidae	ZMUC 126 915	Guayas, Ecuador	EU231653	EU231751	EU231853
Ornithion brunneicapillus	Tyrannidae	ZMUC 125 428	Guayas, Ecuador	EU231690	EU231788	EU231891
Phaeomyias murina	Tyrannidae	ZMUC 125 431	Lambayeque, Peru	EU231673	EU231771	EU231874
*Phyllomyias fasciatus**	Tyrannidae	ZMUC 128 819	Southeast Brazil	EU231674	EU231772	EU231875
Phyllomyias griseiceps	Tyrannidae	ZMUC 127 947	Morona-Santiago, Ecuador	EU231675	EU231773	EU231876

Appendix 1 *Continued.*

Taxon	Family	Sample ID	Provenance	G3P	Myoglobin	ODC
Phyllomyias uropygialis	Tyrannidae	ZMUC 125 434	Imbabura, Ecuador	EU231685	EU231783	EU231886
*Phyllomyias virescens**	Tyrannidae	ZMUC 127 231	Caazapa, Paraguay	EU231686	EU231784	EU231887
Phylloscartes ventralis	Tyrannidae	ZMUC 126 247	Chuquisaca, Bolivia	EU231659	EU231757	EU231859
Pitangus sulphuratus	Tyrannidae	NRM 976 752	Presidente Hayes, Paraguay	EU231707	EU231805	EU231908
Platyrinchus mystaceus	Tyrannidae	NRM 976 724	Concepción, Paraguay	DQ435473	DQ435525	DQ435497
Poecilotriccus ruficeps	Tyrannidae	ZMUC 125 976	Napo, Ecuador	EU231666	EU231764	EU231866
Pogonotriccus poecilotis	Tyrannidae	ZMUC 84-04-18	Morona-Santiago, Ecuador	EU231660	EU231758	EU231860
Polioxolmis rufipennis	Tyrannidae	ZMUC 125 448	Apurímac, Peru	EU231741	EU231839	EU231942
*Polystictus pectoralis**	Tyrannidae	ZMUC 127 227	Cordilera, Paraguay	EU231679	EU231777	EU231880
Pseudocolopteryx acutipennis	Tyrannidae	ZMUC 125 459	Cotopaxi, Ecuador	EU231681	EU231779	EU231882
*Pseudotriccus ruficeps**	Tyrannidae	ZMUC 89-06-08	Huánuco, Peru	EU231661	EU231759	EU231861
Pyrocephalus rubinus	Tyrannidae	NRM 965 730	Alto Paraguay, Paraguay	EU231720	EU231818	EU231921
Pyrrhomyias cinnamomeus	Tyrannidae	ZMUC 125 477	Loja, Ecuador	EU231693	EU231791	EU231894
*Ramphotrigon megacephalum**	Tyrannidae	ZMUC 127 218	Alto Paraná, Paraguay	EU231700	EU231798	EU231901
*Ramphotrigon ruficauda**	Tyrannidae	ZMUC 125 895	Mato Grosso, Brazil	EU231701	EU231799	EU231902
Rhynchocyclus fulvipectus	Tyrannidae	ZMUC 127 832	Morona-Santiago, Ecuador	EU231664	EU231762	EU231864
Rhytipterna simplex	Tyrannidae	ZMUC 125 482	Sucumbios, Ecuador	EU231704	EU231802	EU231905
Satrapa icterophrys	Tyrannidae	NRM 996 681	Alto Paraguay, Paraguay	EU231734	EU231832	EU231935
Sayornis nigricans	Tyrannidae	ZMUC 125 483	Zamora-Chinchipe, Ecuador	EU231728	EU231826	EU231929
Serpophaga munda	Tyrannidae	NRM 947 171	Alto Paraguay, Paraguay	EU231682	EU231780	EU231883
Sirystes sibilator	Tyrannidae	NRM 956 578	Itapua, Paraguay	EU231702	EU231800	EU231903
Stigmatura budytoides	Tyrannidae	NRM 966 804	Boquerón, Paraguay	DQ435476	DQ435528	DQ435503
Sublegatus modestus	Tyrannidae	NRM 996 668	Alto Paraguay, Paraguay	EU231723	EU231821	EU231924
Suiriri suiriri	Tyrannidae	NRM 976 762	Presidente Hayes, Paraguay	EU231671	EU231769	EU231872
Tachuris rubrigastra	Tyrannidae	ZMUC 135 914	Junín, Peru	EU231657	EU231755	EU231857
Terenotriccus erythrurus	Tyrannidae	ZMUC 126 692	Napo, Ecuador	EU231655	EU231753	EU231855
Todirostrum cinereum	Tyrannidae	NRM 947 036	Alto Paraguay, Paraguay	AY336575	AY338740	DQ435506
*Tolmomyias flaviventris**	Tyrannidae	ZMUC 125 587	Bahia, Brazil	EU231665	EU231763	EU231865
Tumbezia salvini	Tyrannidae	ZMUC 125 508	Lambayeque/Piura, Peru	EU231718	EU231816	EU231919
Tyrannus savana	Tyrannidae	NRM 976 722	Concepción, Paraguay	AY336579	AY165826	DQ435507
Xolmis irupero	Tyrannidae	NRM 947 038	Alto Paraguay, Paraguay	EU231740	EU231838	EU231941
Zimmerius chrysops	Tyrannidae	ZMUC 125 511	Zamora-Chinchipe, Ecuador	EU231683	EU231781	EU231884
Iodopleura isabellae	Tityridae	ZMUC 125 762	Napo, Ecuador	DQ435467	DQ435519	DQ435485
Laniisoma elegans buckeyi	Tityridae	ZMUC 127 782	Morona-Santiago, Ecuador	EU231649	EU231747	EU231848
*Laniocera hypopyrrha**	Tityridae	ZMUC 125 879	Mato Grosso, Brazil	DQ470527	DQ470554	EU231849
Pachyramphus castaneus	Tityridae	NRM 976 715	Concepción, Paraguay	EU231652	EU231750	EU231852
Pachyramphus polychopterus	Tityridae	NRM 967 032	Concepción, Paraguay	AY336573	AY338747	DQ435493
Pachyramphus validus	Tityridae	NRM 947 250	Amambay, Paraguay	EU231651	EU231749	EU231851
Schiffornis turdina	Tityridae	USNM B05097	Essequibo, Guyana	EU231648	EU231746	EU231847
Schiffornis virescens	Tityridae	NRM 937 315	Caazapa, Paraguay	AY336574	AY338741	DQ435501
Tityra cayana	Tityridae	NRM 956 584	Itapua, Paraguay	AY336580	AY338742	DQ435505
Tityra inquisitor	Tityridae	NRM 967 083	Amambay, Paraguay	EU231650	EU231748	EU231850
Oxyruncus cristatus	Oxyruncidae	NRM 967 078	Amambay, Paraguay	AY336572	AY338745	DQ435492
Chiroxiphia caudata	Pipridae	NRM 956 620	Caazapa, Paraguay	DQ435462	DQ435516	DQ435477
Lepidothrix coronata	Pipridae	ZMUC 126 073	Napo, Ecuador	EU231647	EU231745	EU231846
*Neopelma pallescens**	Pipridae	ZMUC 125 609	Bahia, Brazil	EU231646	EU231744	EU231845
Pipra fasciicauda	Pipridae	NRM 947 271	Amambay, Paraguay	AY336583	AY065787	DQ435495
Tyranneutes stolzmanni	Pipridae	ZMUC 126 866	Pastaza, Ecuador	EU231645	EU231743	EU231844
*Ampelioides tschudii**	Cotingidae	ZMUC 127 031	Guayas, Ecuador	DQ470516	DQ470543	EU231841
Pyroderus scutatus	Cotingidae	NRM 967 030	Concepción, Paraguay	AY336582	AY065786	DQ435498
Rupicola peruvianus	Cotingidae	ZMUC 126 003	Zamora-Chinchipe, Ecuador	DQ435474	DQ435526	DQ435500
*Tijuca atra**	Cotingidae	ZMUC 128 821	Southeast Brazil	DQ470540	DQ470567	EU231843
Zaratornis stresemanni	Cotingidae	ZMUC 125 021	Lima, Peru	DQ470542	DQ470569	EU231842
Piprites chloris	Incerta sedis	ZMUC 127 972	Morona-Santiago, Ecuador	EU231656	EU231754	EU231856
Piprites pileatus	Incerta sedis	ZMUC 128 817	Southeast Brazil	DQ435472	DQ435524	DQ435496
Furnarius cristatus	Furnariidae	NRM 966 772	Boquerón, Paraguay	AY590066	AY064255	DQ435482
Thamnophilus caerulescens	Thamnophilidae	NRM 967 007	Concepción, Paraguay	AY336587	AY065783	DQ435504

