Molecular Ecology Resources (2008) **8**, 1169–1177 doi: 10.1111/j.1755-0998.2008.02218.x

DNA BARCODING

Molecular taxonomy of Brazilian tyrant-flycatchers (Passeriformes: Tyrannidae)

A. V. CHAVES, C. L. CLOZATO, D. R. LACERDA, E. H. R. SARI* and F. R. SANTOS
Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, CP 486, 31270-901, Belo Horizonte, Minas Gerais, Brazil

Abstract

The tyrannids are one of the most diverse groups of birds in the world, and the most numerous suboscine family in the Neotropics. Reflecting such diversity, many taxonomic issues arise in this group, mainly due to morphological similarities, even among phylogenetically distant species. Other issues appear at higher taxonomic levels, mostly brought up by genetic studies, making systematics a rather inconclusive issue. This study looks into the use of DNA barcodes method to discriminate and identify Tyrannidae species occurring in the Atlantic Forest and Cerrado biomes of Brazil. We analysed 266 individuals of 71 tyrant-flycatcher species from different geographical locations by sequencing 542 bp of the mtDNA COI gene. The great majority of the analysed species showed exclusive haplotypes, usually displaying low intraspecific diversity and high interspecific divergence. Only *Casiornis fuscus* and *Casiornis rufus*, suggested in some studies to belong to a single species, could not be phylogenetically separated. High intraspecific diversity was observed among *Elaenia obscura* individuals, which can suggest the existence of cryptic species in this taxon. The same was also observed for *Suiriri suiriri*, considered by some authors to comprise at least two species, and by others to be divided into three subspecies. Additionally, the use of sequences from voucher specimens allowed us to correct four misidentifications that had happened in the field. Our findings suggest a great power of the COI barcodes to discriminate species of the Tyrannidae family that are found in Brazil.

Keywords: COI, DNA barcodes, molecular taxonomy, Tyrannidae, tyrant-flycatchers

Received 24 October2007; revision accepted 20 March 2008

Introduction

Tyrannidae is the largest bird family of the Neotropical region and belongs to the Suboscine lineage of the order Passeriformes. They are highly diverse and adapted to a wide variety of ecological niches in the South and Central American continents, although some species also reach North America (Ridgely & Tudor 1994). About 374 species distributed in 88 genera are estimated to be part of this family (Traylor-Jr 1977, 1979). In Brazil, 208 species belonging to 78 genera are traditionally grouped into four subfamilies: Pipromorphinae, Elaeninae, Fluvicolinae and Tyranninae (CBRO 2007).

Tyrannid birds were first grouped in subfamilies based on morphological features of the syringeal set of muscles (Ames 1971), foot structure and tarsal scutellation (Sclater 1888), presence of the internal syringeal cartilage (Lanyon 1986, 1988a, b, c), and presence of *Mm. obliqui ventrales* (Prum 1990). Those morphological criteria used so far showed a huge variability and discordance, lacking robust features to distinguish monophyletic lineages (Snow 1975; Traylor-Jr 1977, 1979; Birdsley 2002). Identification of tyrant-flycatcher species presents several taxonomic uncertainties, mostly due to the striking morphological similarities that exist between species, even those that are phylogenetically distant. Often, field identification is based on the birds' vocalizations or species exclusion using known geographical distribution.

Correspondence: Fabricio R. Santos, Fax: +55 31 3409 2570; E-mail: fsantos@icb.ufmg.br

*Current address: Department of Biology, University of Missouri–St Louis, Saint Louis, MO 63121, USA.

Furthermore, phylogenetic relationships within Tyrannidae and between Tyrannidae and other passerine families are difficult to establish (Ames 1971). At this point, scientific literature reveals many propositions coming from different fields, such as morphology, ethology and molecular biology, which conflict on several complicated genera (Traylor-Jr 1977; Birdsley 2002; Chesser 2004). The actual division of subfamilies remains informally assigned, partly because of the absence of some genera in systematic studies that could clarify these affinities (del Hoyo *et al*. 2004).

Recently, DNA barcodes based on sequences of the mtDNA cytochrome *c* oxidase subunit I (COI) gene have been applied to the identification of 93% of all North American birds (Kerr *et al*. 2007). Neotropical birds usually display higher genetic diversity within and between species than temperate species (Hackett & Rosenberg 1990; Bates 2000; Winker *et al*. 2000; Chesser 2004), and also exhibit impressive ecological, behavioural and morphological diversity. Therefore, theoretically, a higher discrimination power would be needed for species identification of suboscine birds, a diverse South American group that radiated into several hundred species since the Miocene (Futuyma 1997). Recently, our group showed that COI barcodes can be efficiently used to discriminate species of Thamnophilidae, another suboscine family occurring in the Neotropical region (Vilaça *et al*. 2006). In this work, DNA barcodes were applied to the identification of species of the highly diverse family Tyrannidae.

Materials and methods

We checked the discriminative power of COI barcode sequences applied to 266 individuals of 71 species belonging to 45 genera of Brazilian tyrannids (Table 1) classified according to the Brazilian Committee of Ornithological Register (CBRO 2007), the most updated list of Brazilian bird species. The number of analysed species is large, especially considering that most samples were collected from areas within the Atlantic Forest and Cerrado biomes (Fig. 1), two biodiversity hotspots from Brazil (Myers *et al*. 2000). Most species that are absent from this study are usually difficult to capture, rare, inhabitants of the canopy, or occur in the Amazon and other Brazilian biomes that were not sampled in this study.

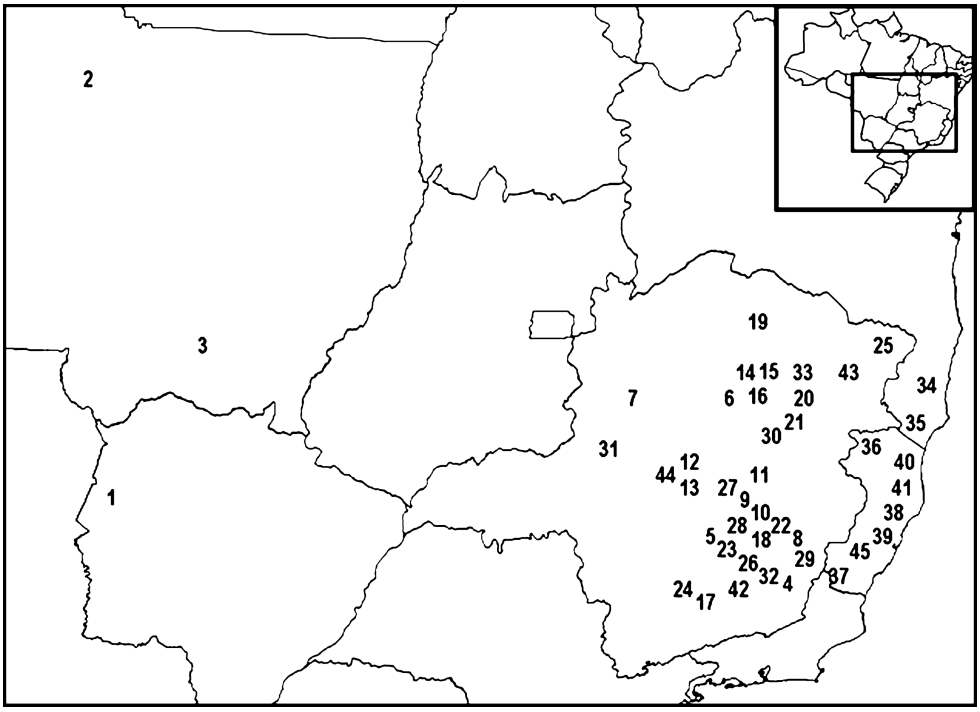

Fig. 1 Map showing the sampling sites in Brazil (states in parentheses: MS, Mato Grosso do Sul; MT, Mato Grosso; MG, Minas Gerais; BA, Bahia; ES, Espírito Santo): 1 Corumbá (MS), 2 Aripuanã (MT), 3 Nossa Senhora do Livramento (MT), 4 Araponga (MG), 5 Belo Horizonte (MG), 6 Bocaiúva (MG), 7 Brazilândia de Minas (MG), 8 Caratinga (MG), 9 Cardeal Mota (MG), 10 Catas Altas (MG), 11 Conceição do Mato Dentro (MG), 12 Diamantina (MG), 13 Felixlândia (MG), 14 Francisco Sá (MG), 15 Grão Mogol (MG), 16 Itacambira (MG), 17 Itumirim (MG), 18 Jaboticatubas (MG), 19 Janaúba (MG), 20 José Gonçalves de Minas (MG), 21 Leme do Prado (MG), 22 Marliéria (MG), 23 Nova Lima (MG), 24 Perdões (MG), 25 Salto da Divisa (MG), 26 Santa Bárbara (MG), 27 Santana do Riacho (MG), 28 São Bartolomeu (MG), 29 Simonésia (MG), 30 Turmalina (MG), 31 Vazante (MG), 32 Viçosa (MG), 33 Virgem da Lapa (MG), 34 Alcobaça (BA), 35 Mucuri (BA), 36 Água Doce do Norte (ES), 37 Alfredo Chaves (ES), 38 Aracruz (ES), 39 Santa Teresa (ES), 40 São Mateus (ES), 41 Sooretama (ES), 42 Ouro Preto (MG), 43 Jequitinhonha Valley (MG), 44 Morada Nova de Minas (MG), 45 Domingos Martins (ES), 46 Divino (MG).

Table 1 Species of Tyrannidae and codes, subfamilies (CBRO 2007), number of individuals (*N*) analysed and haplotypes observed (*H*) for each species, sampling sites and number of intraspecific variable (V), parsimony informative (PI) and singleton (S) sites

Codes	Species	Subfamily	*N*	*H*	Sampling sites*	V	PI	S
Mru	*Mionectes rufiventris*	Pipromorphinae	6	5	4,21,26,29,30	6	1	5
Lam	*Leptopogon amaurocephalus*	Pipromorphinae	9	9	4,6,7,8,13,23,25,29,43	25	20	5
Cde	*Corythopis delalandi*	Pipromorphinae	5	2	6,24,28,32	2	0	2
Hdi	*Hemitriccus diops*	Pipromorphinae	3	3	4,23,43	2	0	2
Hst	*Hemitriccus striaticollis*	Pipromorphinae	2	2	7	1	0	1
Hni	*Hemitriccus nidipendulus*	Pipromorphinae	1	1	10	—	—	—
Hma	*Hemitriccus margaritaceiventer*	Pipromorphinae	6	2	1,6,13	2	0	2
Tpl	*Poecilotriccus plumbeiceps*	Pipromorphinae	2	2	26,46	2	0	2
Tla	*Poecilotriccus latirostris*	Pipromorphinae	1	1	31	—	—	—
Tci	*Todirostrum cinereum*	Pipromorphinae	2	2	1,25	3	0	3
Pfa	*Phyllomyias fasciatus*	Elaeniinae	4	4	6,26	4	1	3
Mga	*Myiopagis gaimardii*	Elaeniinae	2	2	2,3	2	0	2
Mca	*Myiopagis caniceps*	Elaeniinae	1	1	8	—	—	—
Mvi	*Myiopagis viridicata*	Elaeniinae	6	5	6,7,21,43	3	1	2
Efl	*Elaenia flavogaster*	Elaeniinae	6	5	7,12,13,18,38	4	0	4
Eab	*Elaenia albiceps*	Elaeniinae	2	2	12	1	0	1
Epa	*Elaenia parvirostris*	Elaeniinae	1	1	1	—	—	—
Emes	*Elaenia mesoleuca*	Elaeniinae	2	2	13	1	0	1
Ecr	*Elaenia cristata*	Elaeniinae	9	2	9,2,13,16	2	2	0
Ech	*Elaenia chiriquensis*	Elaeniinae	3	3	6,9,13	11	0	11
Eob	*Elaenia obscura*	Elaeniinae	6	6	9,12,13,42	63	58	5
Cob	*Camptostoma obsoletum*	Elaeniinae	4	1	7,13,30	0	0	0
Ssu	*Suiriri suiriri*	Elaeniinae	8	4	3, 7,13,44	44	3	41
Pmu	*Phaeomyias murina*	Elaeniinae	7	7	6,7,12,25,26	7	1	6
Cfl	*Capsiempis flaveola*	Elaeniinae	4	4	25,31	11	4	7
Psu	*Polystictus superciliaris*	Elaeniinae	5	1	9,27	0	0	0
Pac	*Pseudocolopteryx acutipennis*	Elaeniinae	1	1	1	—	—	—
Eme	*Euscarthmus meloryphus*	Elaeniinae	6	2	15,25,38	2	0	2
Smo	*Sublegatus modestus*	Elaeniinae	1	1	3	—	—	—
Iin	*Inezia inornata*	Elaeniinae	2	2	1	2	0	2
Ccau	*Culicivora caudacuta*	Elaeniinae	1	1	23	—	—	—
Mau	*Myiornis auricularis*	Elaeniinae	1	1	21	—	—	—
Tsu	*Tolmomyias sulphurescens*	Elaeniinae	5	3	6,7,13,14,36	10	9	1
Tfl	*Tolmomyias flaviventris*	Elaeniinae	7	4	6, 14, 25, 38	3	2	1
Pmy	*Platyrinchus mystaceus*	Elaeniinae	4	3	4,13,21,43	9	7	2
Ppl	*Platyrinchus platyrhynchos*	Elaeniinae	1	1	2	—	—	—
Mfa	*Myiophobus fasciatus*	Fluvicolinae	2	2	14,26	1	0	1
Mba	*Myiobius barbatus*	Fluvicolinae	6	4	6,7,30	2	2	0
Mat	*Myiobius atricaudus*	Fluvicolinae	5	3	4,26,28,39	4	1	3
Leu	*Lathrotriccus euleri*	Fluvicolinae	11	4	4,6,8,13,23,25,28,43	4	0	4
Cnfu	*Cnemotriccus fuscatus*	Fluvicolinae	8	4	5,6,7,13,43,45	4	2	2
Cci	*Contopus cinereus*	Fluvicolinae	1	1	7	—	—	—
Kcy	*Knipolegus cyanirostris*	Fluvicolinae	2	2	4,23	14	0	14
Kfr	*Knipolegus franciscanus*	Fluvicolinae	2	1	6	0	0	0
Klo	*Knipolegus lophotes*	Fluvicolinae	1	1	9	—	—	—
Kni	*Knipolegus nigerrimus*	Fluvicolinae	2	1	10,27	0	0	0
Xve	*Xolmis velata*	Fluvicolinae	1	1	26	—	—	—
Gye	*Gubernetes yetapa*	Fluvicolinae	1	1	15	—	—	—
Fne	*Fluvicola nengeta*	Fluvicolinae	1	1	18	—	—	—
Cco	*Colonia colonus*	Fluvicolinae	3	3	17,17,33	8	0	8
Lle	*Legatus leucophaius*	Tyranninae	1	1	26	—	—	—
Mcay	*Myiozetetes cayanensis*	Tyranninae	4	2	21,26	2	2	0
Msi	*Myiozetetes similis*	Tyranninae	3	1	14,22	0	0	0
Pisu	*Pitangus sulphuratus*	Tyranninae	5	3	25,29,40	2	0	2
Pli	*Philohydor lictor*	Tyranninae	2	1	22	0	0	0
Mma	*Myiodynastes maculatus*	Tyranninae	6	3	7,13,25,38	7	4	3

Table 1 *Continued*

Codes	Species	Subfamily	*N*	*H*	Sampling sites*	V	PI	S
Mpi	*Megarynchus pitangua*	Tyranninae	4	1	6,7,14,38	0	0	0
Eva	*Empidonomus varius*	Tyranninae	1	1	26	—	—	—
Gau	*Griseotyrannus aurantioatrocristatus*	Tyranninae	2	2	1,13	3	0	3
Tme	*Tyrannus melancholicus*	Tyranninae	6	4	2,13,25,38	16	11	5
Tsa	*Tyrannus savana*	Tyranninae	1	1	15	—	—	—
Ssi	*Sirystes sibilator*	Tyranninae	2	2	7,37	2	0	2
Cru	*Casiornis rufus*	Tyranninae	8	7	1,6,7,13	13	10	3
Cfu	*Casiornis fuscus*	Tyranninae	6	4	19,25,43	8	1	7
Mtu	*Myiarchus tuberculifer*	Tyranninae	2	2	38	3	0	3
Msw	*Myiarchus swainsoni*	Tyranninae	7	3	6,7,21,24	4	0	4
Mfe	*Myiarchus ferox*	Tyranninae	10	5	4,6,7,8,13,22,23,33	5	2	3
Mty	*Myiarchus tyrannulus*	Tyranninae	8	2	1,7,25,26,35,38	1	0	1
Aph	*Attila phoenicurus*	Tyranninae	1	1	2	—	—	—
Aru	*Attila rufus*	Tyranninae	3	3	4,29,32	2	0	2
Asp	*Attila spadiceus*	Tyranninae	1	1	41	—	—	—

*See Fig. 1 for geographical location of sampling sites.
(—) dashes indicate that intraspecific variable positions cannot be computed.

The source of the DNA was blood or tissue samples from recently captured individuals or corneal tissue of the tarsus from museum specimens. Blood and tissue samples were kept in absolute ethanol until DNA extraction. Collected skin specimens were deposited in the Ornithological Collection of the Universidade Federal de Minas Gerais, Brazil. Most of the 46 sampling sites were in the Brazilian states of Minas Gerais (MG), Bahia (BA) and Espírito Santo (ES). There was also a single Pantanal site in the Mato Grosso do Sul (MS), and a single Amazon forest site in the Mato Grosso (MT) State of Brazil (Fig. 1). DNA from 21 species was obtained exclusively from blood or fresh tissue samples, for another 20 species DNA was obtained exclusively from voucher samples, and for the remaining 30 species DNA was obtained from both source materials. One sample of *Synallaxis frontalis* (Furnariidae) was used as an outgroup for phylogenetic reconstructions.

For DNA extraction from blood and fresh tissue samples, we used a modified phenol–chloroform–isoamilic alcohol protocol (Vilaça *et al*. 2006). For ancient voucher specimen skin, total DNA was extracted using the DNeasy Kit (QIAGEN). Next, DNA samples were stored in the collection (BD-LBEM) of the Universidade Federal de Minas Gerais, recognized by the Brazilian Ministry of Environment.

The COI gene was entirely amplified (1550 bp), to avoid amplification of numts (Sorenson & Quinn 1998), using primers L6615 and H8121 (Folmer *et al*. 1994). The polymerase chain reactions (PCRs) were done under the following conditions: 94 °C for 2 min, 35 cycles of 63 °C for 40 s, 72 °C for 2 min, 94 °C for 40 s and a final extension of 10 min at 72 °C. The amplifications were carried out in 12.5 μL reactions containing 0.5 U of *Taq* polymerase (Phoneutria), 1× buffer with 1.5 mM $MgCl_2$ (Phoneutria), 200 μM of dNTPs, 0.5 μM of each primer and 2 μL of genomic DNA (~40 ng). The amplification products were purified by precipitation in PEG 8000 (20% polyethyleneglycol, 2.5 M NaCl) and finally dissolved in ultrapure water.

The sequencing reactions consisted of 35 cycles of 95 °C for 25 s, 50 °C for 15 s, 60 °C for 3 min in a total volume of 10 μL, which contained 4 μL of the sequencing Kit (ET DYE Terminator Kit for MegaBACE, Amersham Biosciences), 3 μL of ultrapure water, 2 μL of purified PCR product and 1 μL of primer (0.5 μM final concentration). The following primers were used for sequence reactions: socoiF1 5′-TTCTACAAACCATAAAGATATTGGCA-3′ (modified from Hebert *et al*. 2004), LCO1490 and HCO2198 (Folmer *et al*. 1994), and H6035COI_Tyr 5′-CCTCCTGCAGGGTCAAAGAATGT-3′ (designed for the present study). Sequencing products were purified using ammonium acetate and ethanol, then dissolved with formamide-EDTA buffer and run in the automatic sequencer MegaBACE 1000 (Amersham Biosciences).

Contig alignments were obtained from three to five forward and reverse sequences, derived from at least two different PCR products, using programs PHRED version 0.20425 (Ewing *et al*. 1998), PHRAP version 0.990319 (http://www.phrap.org) and CONSED version 14.0 (Gordon *et al*. 1998). High quality consensus COI sequences presented at least a PHRED 20 score (99% confidence) for every nucleotide position. Final consensus sequences for each individual are deposited at the Barcode of Life Data Systems (Accession nos BBB048-07 to BBB318-07) and at GenBank (Accession nos EU232761 to EU233026).

The alignments of the consensus sequences for all individuals and species were built using CLUSTAL W algorithm available in the software MEGA 3.1 (Kumar *et al*. 2004).

MEGA 3.1 was also used to estimate the number intraspecific polymorphisms, the divergence between different haplotypes using the Kimura 2-parameter's (K2P) nucleotide substitution model, and to build the neighbour joining (NJ) trees with K2P distances, tested with 1000 bootstrap replications. Besides, the program DNASP 4.0 (Rozas *et al*. 2003) was also used to check synonymous and nonsynonymous substitution sites.

Results

Consensus sequences of 542 bp of the COI mtDNA gene were obtained for all individuals. The sequences did not contain insertions, deletions, stop codons or any high quality ambiguities that could suggest the presence of numts (Sorenson & Quinn 1998). We obtained 252 variable sites, from which 78 corresponded to species' autapomorphic sites. Forty-six species showed at least one autapomorphy, and the number of variable sites that were exclusive of single species varied from one to four (Table S1, Supporting Information). We also found 54 nonsynonymous substitution sites, 32 of which were exclusively found in 19 species. Intraspecific nucleotide variation was found for 45 species (Table 1), while amino-acid intraspecific changes were observed in 21 species (data not shown).

The differences between COI sequences were usually higher among than within species. Intraspecific haplotypes clustered in monophyletic clades with 99–100% bootstrap support in an NJ tree (Fig. 2). *Casiornis rufus* and *C. fuscus* were the only case in which different species shared COI haplotypes, suggesting that they do not represent reciprocally monophyletic species (Fig. 3). Interspecific divergences ranged from 2% between *Myiobius atricaudus* and *Myiobius barbatus*, to 23.2% between *Platyrinchus mistaceus* and *Polystictus superciliares*, averaging 17.3%. The intraspecific diversities were calculated for 52 species and varied from 0 for several species, to 6.7% for *Elaenia obscura*, averaging 0.6%. This high diversity observed among *E. obscura* individuals was mostly due to the inclusion of two specimens from Diamantina region (cluster 1) that were highly divergent (12%) from a separate clade containing four specimens sampled in other distant localities (cluster 2). Besides, these two *E. obscura* clades join with a bootstrap support of only 53% (Fig. 2).

Three specimens of *Suiriri suiriri* analysed in this study were captured as a single family group, such as those described in Zimmer *et al*. (2001) and Lopes & Marini (2005), in Brazilândia de Minas. One of those birds was a white-bellied form (dz4972), and the other two were yellow-bellied forms (dz4970 and dz4971). Our genetic analysis showed that the individual dz4970 was highly divergent from the others, resulting in an increase of the mean genetic divergence obtained for the entire *S. suiriri* complex (4.6%). With the exclusion of dz4970 from the analysis, the intraspecific divergence between the other seven *S. suiriri* specimens dropped to only 0.2%. Interesting to note is the well-supported clade containing the white-bellied dz4972 together with yellow-bellied specimens

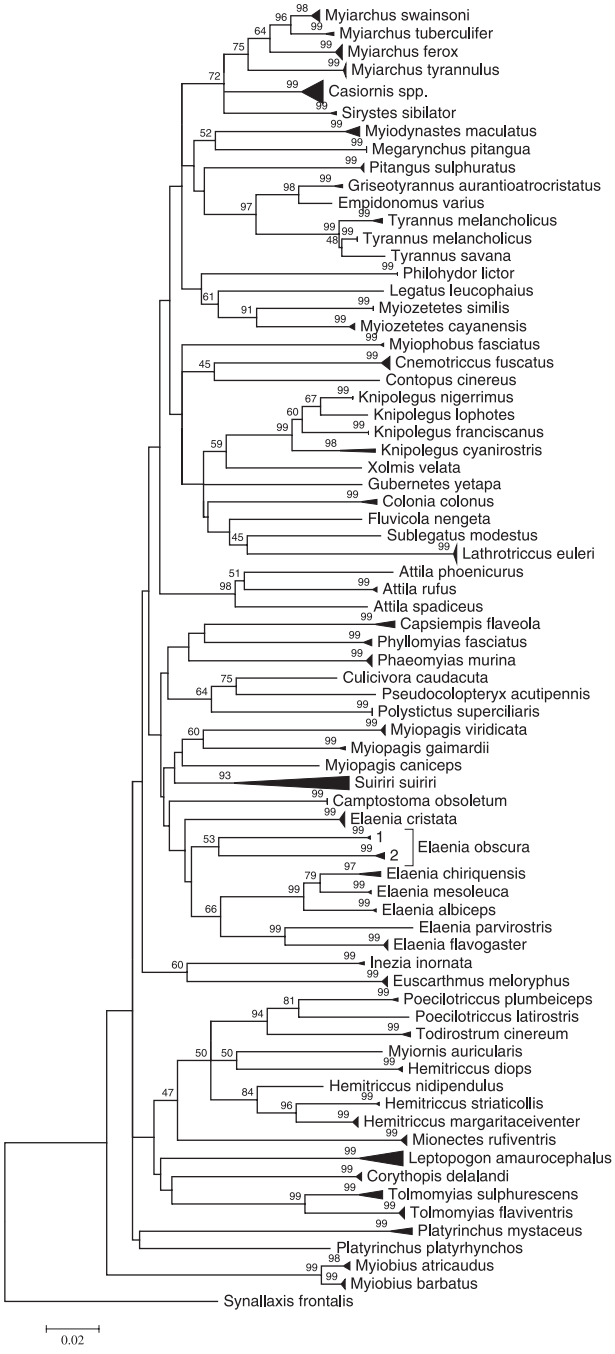

Fig. 2 Neighbour-joining tree using Kimura 2-parameters model for 266 COI sequences from 71 Tyrannidae species. Bootstrap support values are indicated on the branches (values below 45 were omitted) and sequences of each species were collapsed. We used *Synallaxys frontalis* (Furnariidae) as outgroup.

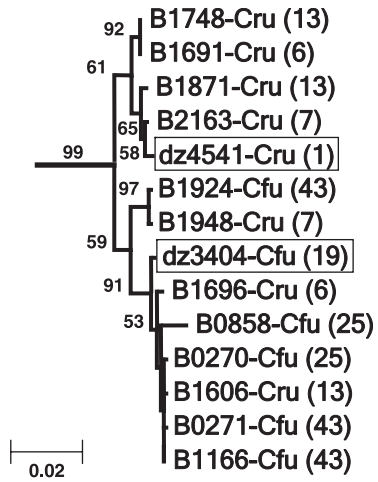

Fig. 3 Details of the neighbour-joining tree for the *Casiornis* species (see species codes in Table 1). Numbers below the branches show the bootstrap support values. Numbers between parentheses indicate the sampling localities (Fig. 1). The individual codes within the squares are voucher specimens.

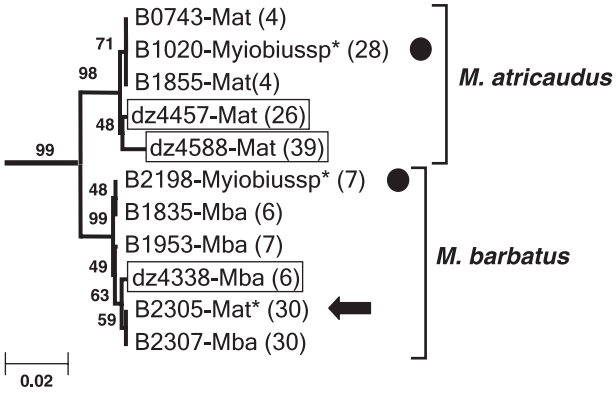

Fig. 5 Details of the neighbour-joining tree for the *Myiobius* species (see species codes in Table 1). Numbers below the branches show the bootstrap support values. Numbers between parentheses indicate the sampling localities (Fig. 1). The individual codes within the squares are voucher specimens. Black circles mark individuals that could not be assigned to any *Myiobius* species in the field. The arrow marks an individual that was misidentified as *Myiobius atricaudatus*, but COI data indicate that it is *Myiobius barbatus*.

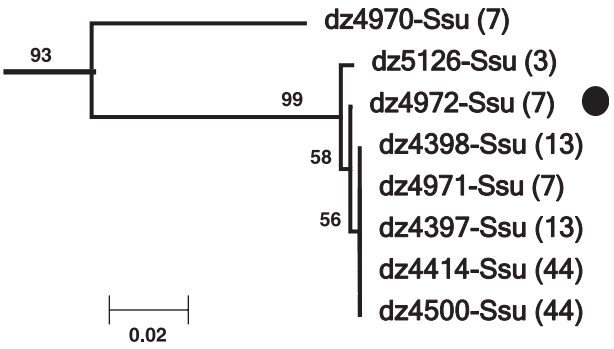

Fig. 4 Details of the neighbour-joining tree for the *Suiriri suiriri* species. Numbers below the branches show the bootstrap support values. Numbers between parentheses indicate the sampling localities (Fig. 1). All sequences were obtained from voucher specimens (dz#). The black circle marks the white-bellied *S. suiriri*; the remaining ones are yellow-bellied *S. suiriri*.

classified as *S. s. affinis* (Fig. 4), suggesting that genetic data may not reflect morphological differences that are often used for subspecies classification.

The efficiency of the COI gene sequences for species identification was also tested through the analysis of samples with questionable field identifications. This was the case of two samples that, after grouping with high bootstrap support with five unambiguously identified *Phaeomyias murina* (including one voucher specimen), were definitely assigned to this species, confirming the uncertain field identification (tree not shown). Additionally, two samples that were previously classified as *Myiobius* sp., could be safely assigned to *M. atricaudus* (B1020*) and *M. barbatus* (B2198*), after forming strongly supported clades with sequences obtained from vouchers of both species (Fig. 5). Finally, phylogenetic reconstructions showed that one sample tagged in the field as *M. atricaudus* (B2507*), was indeed an *M. barbatus* (Fig. 5), and another sample tagged as *Myiarchus ferox* was shown to belong to the species *Myiarchus swainsoni* (tree not shown). Both examples show that the barcode technique applied to sequences from voucher specimens can be useful to correct earlier misidentifications that often occur in the field, especially when congeneric species occur in the same locality.

Discussion

The present study showed that the DNA barcodes technique is a simple and direct method for identifying Neotropical tyrant-flycatchers. The 542-bp fragment from the COI mtDNA gene displayed a high discrimination power providing interspecific divergences that were, on average, 26-fold higher than intraspecific ones. Additionally, the analysis of COI barcodes using voucher specimens allowed us to test the relative accuracy of this method. Field identification of tyrant-flycatchers is often prone to mistakes, resulting mostly from problems in diagnosing the subtle morphological differences used to define species that, frequently, show gradual variation across their distribution. Although some taxonomic confusion could potentially arise by hybridization and introgression, we have not observed any suggestive evidence of their occurrence in the present data set. This is not unexpected since mtDNA markers should not be easily blurred by hybridization in birds, because hybrid F_1 female birds would rarely reproduce and transfer their mitochondria to the progeny if we assume

that the Haldane's rule applies to tyrant-flycatchers. Haldane's rule states that in the hybrid offspring of two different animal races or species, if one of the sexes is absent, rare or sterile, it is usually going to be the heterozygous sex, i.e. females, in the case of birds (Haldane 1922).

Although based on small sample sizes, intraspecific diversities observed in tyrannids were coherent and even lower than the ones found in other bird species (Hebert *et al*. 2004; Kerr *et al*. 2007), including Thamnophilidae, another Neotropical passerine family (Vilaça *et al*. 2006). DNA barcodes that show high intraspecific diversity may create or reinforce suspicions of hidden diversity (Hebert *et al*. 2003, 2004). This means that samples may have been incorrectly assigned to the same species based on inaccurate evaluation of morphological markers or differences in morphology may simply not reflect the differences in genetic parameters. The high intraspecific diversity we found among samples of *Elaenia obscura* contrasted with the low values found for all other species. Although a detailed morphological analysis of the samples of *E. obscura* is not possible (vouchers were not available), we suspect that this result suggests the existence of cryptic species within this taxon, which should be not uncommon for Neotropical taxa.

Taxonomic classification within the genus *Suiriri* is the focus of an intense debate. Currently, it includes *S. islerorum*, a recently described species (Chapada flycatcher; Zimmer *et al*. 2001), and the *S. suiriri* complex, which according to many authors (Short 1975; Sibley & Ahlquist 1990; Sibley & Monroe 1990; Lopes & Marini 2005) should be divided in two species, *S. suiriri* and *S. affinis*. In a recent taxonomic review (Hayes 2001), *S. suiriri* is considered as a single species divided in three distinct morphological forms or subspecies occurring in parapatry. The first form, the *S. s. suiriri* (South Suiriri), is a short-billed and white bellied form that is found in the southwest (southern Bolivia, southern Brazil, Paraguay, Uruguay and northern Argentina). The *S. s. affinis* (Cerrado-Suiriri) is a longer-billed and yellow-bellied form that is found in the Cerrado and southern Amazon from Brazil, southern Surinam and eastern Bolivia. Finally, *S. s. bahiae* includes both white and yellow-bellied forms that are found in the Caatinga from northeastern Brazil. There is a hybrid zone between the Chaco and Cerrado biomes (Brazil, Bolivia and Paraguay) where specimens exhibit intermediate morphometric measures and increased plumage variability between *affinis* and *suiriri* forms (Laubmann 1940; Zimmer 1955; Hayes 1995) and also similar vocalizations (Zimmer *et al*. 2001). The existence of a second hybrid zone has been suggested between Cerrado and Caatinga, where *S. s. affinis* and *S. s. suiriri* would have originated the poorly known *S. s. bahiae* (Hayes 2001). The variability in plumage and intermediate sizes exhibited by some *S. s. bahiae* vouchers available, as well as the similarity between the *S. s. suiriri* and the white-bellied form of *S. s. bahiae*, supports the hypothesis of a hybrid origin of *S. s. bahiae* (Hayes 2001). In this study, one of the yellow-bellied *S. suiriri* (dz4970) was genetically very divergent from the others, while clearly different forms of *S. suiriri* grouped together in the phylogenetic reconstruction (Fig. 4). Re-evaluation of the vouchers from the *S. suiriri* collected in Brazilândia de Minas confirms that both yellow-bellied dz4970 and dz4971 are within the morphological continuum of the *affinis* form (Lopes & Marini 2005 and unpublished morphological data from Leonardo Lopes, personal communication, UFMG, Belo Horizonte, Brazil). In addition, the white-bellied dz4972 was collected in the same sampling site, which is also within the range of the *bahiae* form, was genetically similar to other yellow-bellied forms (Fig. 4). Therefore, to investigate in detail the actual status of this species complex, what is fundamentally needed is a broader morphological and genetic analysis that includes sampling all forms and also potential hybrid zones.

The *Casiornis* genus has been previously grouped with the *Myiarchus* genus based on morphological data (Ames 1971). In the present study, our data support that close relationship and also the clustering (72% bootstrap) of those two genera with *Sirystes sibilator* (Fig. 2). Within *Casiornis,* the existence of 'intermediate forms' suggests that *C. rufus* and *C. fuscus* may be a single species (Snow 1973; Traylor-Jr 1979; Sibley & Monroe 1990; Ridgely & Tudor 1994). Although these two taxa, theoretically, do not have overlapping distributions, with *C. rufus* occurring in northeastern Brazil (Caatinga) and *C. fuscus* occurring in central Brazil (Cerrado), many ornithologists have found both of them in the same area, sometimes even in the same field net, suggesting they are widely sympatric (Vasconcelos *et al*. 2006). Field ornithologists use several methods to distinguish these two *Casiornis* species, which differ by several subtle plumage traits (Ridgely & Tudor 1994). In the present study, our COI barcode phylogeny showed a single well-supported monophyletic group, suggesting that *C. fuscus* and *C. rufus* may be considered as a single species. Still, more detailed genetic and morphological studies based on a broader geographical sampling area would help to understand their current taxonomic status.

The genus *Myiarchus*, with 22 species, is the largest in the Tyrannidae family. Factors such as the uniformity in the plumage patterns and colours, interspecific overlap of most measurable characters, confusing geographical variation and incompletely understood geographical distribution of species, render it difficult to distinguish several forms within this genus (Lanyon 1978). In the present study, the COI barcodes allowed us to update the identification of one specimen (B2173*), a *Myiarchus swainsoni* that was misclassified in the field as *M. ferox*. Those two taxa are good examples of polytypic species of the genus *Myiarchus*, both displaying migratory and resident

populations, and bearing extremely diverse patterns of morphology and vocalization. Because of such diversity, a clear distinction between both species may be compromised. With that goal, Lanyon (1978) has proposed a method based on an index of proportions of the tail/wing length and a formula that relates the length of the primary remiges P5-P9 and P4-P10. However, it has been suggested that Lanyon's method does not apply to birds from some localities, especially from the region of the Jequitinhonha Valley in Brazil (Lucas Carrara, personal communication, UFMG, Belo Horizonte, Brazil). Because B2173* was sampled in Bocaiúva, a city close to the Jequitinhonha Valley, it is possible that the use of these continuous characteristics have taken to the misidentification.

Our comparisons of intraspecific and interspecific divergences, and the phylogenetic analyses using COI barcodes, confirmed that this method is a precise and quick way for the identification of Tyrannidae species from Brazil. Therefore, we believe that it can be a valuable auxiliary tool for taxonomic inventories and for identifying cryptic species in this group. Our current COI analysis in a limited number of species and other phylogeographical studies from our group (Lacerda *et al*. 2007; unpublished data) suggest the existence of large amounts of genetic diversity in presently recognized bird species in the Neotropics, which may be indicative of the existence of cryptic species. Clearly, detailed phylogeographical and morphological studies based on large samples from a broad geographical area are needed to build a more complete and less- biased database for Neotropical species.

Acknowledgements

This work received financial support from CNPq and FAPEMIG (Brazil). We thank Marcos Rodrigues and his students Lílian M. Costa, Guilherme Freitas, Jordana D. Ferreira, Vinícius Cerqueira and Elisa Mesquita for the biological material (vouchers, blood and tissue samples), to Marcelo Ferreira de Vasconcelos, Leonardo Lopes and Lucas Carrara for providing some samples, voucher information and important comments on the manuscript. We also thank Rodrigo A. F. Redondo, Leandro M. Freitas, Fabiano A. Assunção and Sibelle T. Vilaça for technical help. Finally, we thank Iris Levin for revising the language use in the manuscript. All samples were collected with the Brazilian government permit nos 055/2007, 153/2004, 019/2004, 207/2003 and 070/2002 issued by IBAMA/MMA.

References

Ames PL (1971) The morphology of the syrinx in passerine birds. *Bulletin of Peabody Museum of Natural History, Yale University*, **37**, 1–194.

Bates JM (2000) Allozyme genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. *Condor*, **102**, 770–783.

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

CBRO (2007) Listas das aves do Brasil. Brazilian Committee of Ornithological Register (CBRO), Version 08/16/2007. http://www.cbro.org.br.Accessed 8/27/2007.

Chesser RT (2004) Molecular systematics of New World suboscine birds. *Molecular Phylogenetics and Evolution*, **32**, 11–24.

Ewing B, Hillier L, Wendi M, Green P (1998) Base-calling of automated sequencer tracer using PHRED I: accuracy assessment. *Genome Resource*, **8**, 175–185.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Futuyma DJ (1997) *Evolutionary Biology*, 3rd edn. Sinauer Associates, Sunderland, Massachusetts.

Gordon D, Abajian C, Green P (1998) CONSED: a graphical tool for sequence finishing. *Genome Resource*, **8**, 195–202.

Hackett SJ, Rosenberg WV (1990) A comparison of phenotypic and genetic differentiation in South American antwrens (Formicariidae). *Auk*, **107**, 473–489.

Haldane JBS (1922) Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, **12**, 101–109.

Hayes FE (1995) Status, distribution and biogeography of the birds of Paraguay. *American Birding Association. Monographs in Field Ornithology*, **1**, 1–230.

Hayes FE (2001) Geographic variation, hybridization, and the leapfrog pattern of evolution in the Suiriri flycatcher (*Suiriri suiriri*) complex. *Auk*, **118**, 457–471.

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 313–321.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. *Public Library of Science, Biology*, **2**, 1657–1663.

del Hoyo J, Elliot A, Christie DA, eds. (2004) *Handbook of the Birds of the World, 9. Cotingas to Pipits and Wagtails*. Lynx Edicions, Barcelona, Spain.

Kerr KCR, Stoeckle MY, Dove CJ, Weigt LA, Francis CM, Hebert PDN (2007) Comprehensive DNA barcode coverage of North American birds. *Molecular Ecology Notes*, **7**, 535–543.

Kumar S, Tamura K, Nei M (2004) MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Lacerda DR, Marini MA, Santos FR (2007) Mitochondrial DNA corroborates the species distinctiveness of the Planalto (*Thamnophilus pelzelni*) and the Sooretama (*T. ambiguus*) Slaty-antshrikes (Passeriformes: Thamnophilidae). *Brazilian Journal of Biology*, **67**, 873–882.

Lanyon WE (1978) Revision of the *Myiarchus* flycatchers of South America. *Bulletin of the American Museum of Natural History*, **161**, 429–627.

Lanyon WE (1986) A phylogeny of the thirty-three genera of the *Empidonax* assemblage of tyrant flycatchers. *American Museum Novitates*, **2846**, 1–64.

Lanyon WE (1988a) A phylogeny of the thirty-two genera in the *Elaenia* assemblage of tyrant flycatchers. *American Museum Novitates*, **2914**, 1–57.

Lanyon WE (1988b) The phylogenetic affinities of the flycatcher genera *Myiobius* Darwin and *Terenotriccus* Ridgeway. *American Museum Novitates*, **2915**, 1–11.

Lanyon WE (1988c) A phylogeny of the flatbill and tody-tyrant assemblage of tyrant flycatchers. *American Museum Novitates*, **2923**, 1–41.

Laubmann A (1940) *Wissenschaftliche Ergebnisse der Deutschen Gran Chaco-Expedition. Die Vogel Von Paraguay*, Vol. 2. Strecker und Schroder, Stuttgart, Germany.

Lopes LE, Marini MA (2005) Biologia reprodutiva de *Suiriri affinis* e *S. islerorum* (Aves: Tyrannidae) no cerrado do Brasil Central. *Papéis Avulsos de Zoologia*, **45**, 127–141.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Prum RO (1990) A test of the monophyly of the manakins (Pipridae) and of the cotingas (Cotingidae) based on morphology. *Occasional Papers of the Museum of Zoology, University of Michigan*, **723**, 1–44.

Ridgely RS, Tudor G (1994) *The Birds of South America: the Suboscine Passerines*, Vol. 2. University of Texas Press, Austin, Texas.

Rozas J, Sanchez-De I, Barrio JC, Messeguer X, Rozas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Sclater PL (1888) *Catalogue of the Birds in the British Museum (Natural History)*, Vol. 14. British Museum, London.

Short LLJR (1975) A zoogeographic analysis of the South American Chaco avifauna. *Bulletin of the American Museum of Natural History*, **154**, 163–352.

Sibley CG, Ahlquist JE (1990) Ordem passeriformes. In: *Phylogeny and Classification of Birds: a Study in Molecular Evolution*, pp. 576–591. Yale University Press, New Haven, Connecticut.

Sibley CG, Monroe BL Jr (1990) *Distribution and Taxonomy of the Birds of the World*. Yale University Press, New Haven, Connecticut.

Snow DW (1973) The classification of the Cotingidae (Aves). *Breviora*, **409**, 1–27.

Snow DW (1975) The classification of the manakins. *Bulletin of the British Ornithological Club*, **95**, 20–27.

Sorenson MD, Quinn TW (1998) Numts: a challenge for avian systematics and population biology. *Auk*, **115**, 214–221.

Traylor-Jr MA (1977) A classification of the tyrant flycatchers (Tyrannidae). *Bulletin of the Museum of Comparative Zoology*, **148**, 129–184.

Traylor-Jr MA (1979) Family Tyrannidae. In: *Check-List of Birds of the World*, Vol. 8 (ed. Traylor-Jr MA), pp. 1–245. Museum of Comparative Zoology, Cambridge, UK.

Vasconcelos MF, Neto SD' A, Kirwan GM, Bornschein MR, Diniz MG, Silva JF (2006) Important ornithological records from Minas Gerais State, Brazil. *Bulletin of British Ornithologists' Club*, **126**, 212–238.

Vilaça ST, Lacerda DR, Sari EHR, Santos FR (2006) DNA-based identification applied to Thamnophilidae (Passeriformes) species: the first barcodes of Neotropical birds. *Ararajuba. Revista Brasileira de Ornitologia*, **14**, 7–13.

Winker K, Graves GR, Braun MM (2000) Genetic differentiation among populations of a migratory songbird: *Limnothlypis swainsonii*. *Journal of Avian Biology*, **31**, 319–328.

Zimmer J (1955) Further notes on tyrant flycatchers (Tyrannidae). *American Museum Novitates*, **1749**, 1–24.

Zimmer KJ, Whittaker A, Oren DC (2001) A cryptic new species of flycatcher (Tyrannidae: *Suiriri*) from the Cerrado region of central South America. *Auk*, **118**, 56–78.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Autapomorphic characters for 43 species of the Tyrannidae

Please note: Blackwell Publishing are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.