



Towards a phylogenetic framework for the evolution of shakes, rattles, and rolls in *Myiarchus* tyrant-flycatchers (Aves: Passeriformes: Tyrannidae)

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Abstract

A phylogeny of 19 of the 22 currently recognized species of *Myiarchus* tyrant-flycatchers is presented. It is based on 842 bp of mitochondrial DNA (mtDNA) sequences from the ATPase subunit 8 and ATPase subunit 6 genes. Except for the morphologically distinct *M. semirufus*, mtDNAs of the remaining 18 species fall into either of two clades. One comprises predominantly Caribbean and Central and North American taxa (Clade I), and the other is of predominantly South American taxa (Clade II). The phylogeny is only very broadly concordant with some vocal characters and also with the limited morphological diversity for which the group is well known. Paraphyly in several species (*M. swainsoni*, *M. tuberculifer*, *M. ferox*, *M. phaeocephalus*, *M. sagrae*, *M. stolidus*) suggests that morphological evolution, albeit resulting in limited morphological diversity, has been more rapid than that of mtDNA, or that current taxonomy is faulty, or both. A South American origin for *Myiarchus* is likely. Dispersal and vicariance both appear to have been involved in generating the present-day distribution of some species. Relatively recent dispersal events out of South America are inferred to have brought species of Clades I and II into broad sympatry. Jamaica has been colonized independently at least twice by members of Clades I and II. The phylogeny brings a historical perspective that in turn suggests that ecological study of closely related species from within each major clade where they are sympatric will be especially rewarding.

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1. Introduction

Tyrant-flycatchers are a diverse assemblage of several hundred species of suboscine passerine birds that range across the Americas (Ridgely and Tudor, 1994). Their taxonomic, ecological, and behavioural diversities have received much attention (e.g., Fitzpatrick, 1985; Lanyon, 1984; Smith, 1971; Vuilleumier, 1971). Systematic

research has traditionally focussed on higher-level relationships within the assemblage and has been based on internal and external anatomy (e.g., Lanyon, 1984, 1985, 1986; Traylor, 1977) with contributions from DNA hybridization (McKittrick, 1985; Sibley and Ahlquist, 1985). More recently, DNA sequencing has made it feasible to examine relationships among species in some of the larger, morphologically homogeneous genera (Chesser, 2000; Roy et al., 1999). The systematics and biogeography of one such genus, *Myiarchus* Cabanis, 1844, is the focus of the present paper.

Twenty-two species are currently recognized in *Myiarchus* (AOU, 1998; Lanyon, 1967, 1978). Their centre of diversity is the humid Neotropics though some species range north into temperate parts of the United States and south as far as Argentina and Uruguay. They are

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found mainly in secondary growth, rainforest edges and open, wooded habitats. *Myiarchus* has long been renowned for its extreme morphological homogeneity (e.g., Coues, 1872). Most species have a grey back and chest with yellow belly (white in two species), and varying degrees of rufous or brown in the wings and tail. *M. semirufus* of arid coastal Peru is the only exception being entirely rufous and black. Lanyon (1978) noted that the “remarkable uniformity of plumage coloration and pattern, a lack of appreciable sexual dimorphism, interspecific overlap in most mensural characters, a misunderstanding with respect to geographical variation, and inadequate or unreliable diagnostic characters have led many museum-based workers to despair that a reasonably satisfactory classification of the group would ever be achieved.” Lanyon (1967, 1978) analysed Caribbean and South American taxa for vocal characters that he termed rattles, rolls, hiccups, and so on. His analyses of vocal and plumage variations are the basis of current taxonomy of *Myiarchus* (AOU, 1998; Lanyon, 1967, 1978). Unless indicated otherwise, we follow Lanyon’s (1967, 1978) taxonomy. Development of a phylogenetic framework for *Myiarchus* within which the history of variation in these characters can be examined is therefore a challenge well-suited to molecular methods.

Lanyon (1985) formally rediagnosed *Myiarchus* with characters of the nest and structure of the nasal capsule and syrinx. He found that neither of three problematic species, *M. validus*, *M. magnirostris* and *M. semirufus*, each of which has at some time been assigned to a monotypic genus, had characters contradicting their placement in *Myiarchus*. *M. validus* is an unusually large but typically plumaged *Myiarchus* from Jamaica. *M. magnirostris* is an unusually small but also unexceptionally plumaged *Myiarchus* endemic to the Galapagos Islands. Relative to all other *Myiarchus* spp, *M. semirufus* from arid coastal Peru and Ecuador is uniquely rufous and black-plumaged though it is a typical *Myiarchus* in vocalizations, behaviour, nest and eggs (Lanyon, 1975). The circumscription of *Myiarchus* with respect to the inclusion of these species needs to be verified by independent sources of data, such as DNA sequences. Lanyon (1967) also offered specific hypotheses of the historical biogeography of Caribbean taxa, especially those of Jamaica. He proposed that *M. stolidus* evolved from a first, late Tertiary colonization of Jamaica, probably from Central America, and that *M. sagrae*, *M. antillarum*, and *M. oberi* were then derived from *M. stolidus*. He next proposed that *M. validus* and then *M. barbirostris* resulted from two later invasions in the early and late Pleistocene, respectively, the latter derived from Central American *M. tuberculifer*. Lastly, he proposed a fourth colonization of the Caribbean resulting in the evolution of *M. nugarator* of Grenada from *M. tyrannulus* in Venezuela.

This paper’s first main goal is to present a phylogeny of *Myiarchus* based on partial mitochondrial DNA

(mtDNA) sequences and explore its implications in testing Lanyon’s (1967, 1978, 1985) definitions of the monophyly of *Myiarchus*, species limits and historical biogeography. We also address the group’s ancestral distribution at a broad geographical scale (South America or North America or Central America or Caribbean) and review our data for instances of dispersal into and out of these broad regions. We plan separate, more detailed discussions of intraspecific phylogeography for later publications but have already examined the phylogeography and evolution of migration in the *M. swainsoni* complex (Joseph et al., 2003). Renewed concern has been expressed lately (e.g., Ballard et al., 2002; Weckstein et al., 2001) about making taxonomic changes based solely on mtDNA and so we offer our findings with this in mind.

2. Materials and methods

2.1. Specimens

Of the 123 sequences studied, 117 were from 19 nominal *Myiarchus* species (Appendix A). Mean sample size per species was six, with a range from 1 (for 10 species) to 49 (one species). Tissue samples of liver and heart were used for all DNA extractions except one, *M. venezuelensis* 01.VE, which was only available as blood. The remaining six sequences were outgroups chosen from within the Tyranninae (sensu Traylor, 1977) and on the basis of Lanyon’s (1982, 1985) assessments of the nearest relatives of *Myiarchus*. These were *Tyrannus melancholicus* ($n = 4$), *T. caudifasciatus* ($n = 1$), and *Rhytipterna immunda* ($n = 1$).

2.2. DNA isolation, PCR amplification, and sequencing

DNA extraction protocols and PCR conditions varied slightly in our two laboratories but were typically as follows (variant protocols are described in Joseph et al., 2003). DNA was extracted by digesting 0.1–0.5 g of ground tissue (pectoral muscle or liver) or 20–40 μ l of blood in 500 μ l of 2 \times CTAB buffer solution and 10 μ l of a 10 mg/ml Proteinase K solution at 54 $^{\circ}$ C for 6–14 h. A typical DNA extraction yielded a volume of 400 μ l of which 1 μ l was used to seed polymerase chain reaction (PCR) amplifications. Amplification was carried out in 50 μ l reactions under the following conditions: 1 μ l DNA (~15–20 ng), 1 \times PCR Buffer II (Perkin–Elmer, Forest City, CA), 2.0 mM MgCl₂, 2 μ M each of dATP, dCTP, dGTP, and dTTP, 2 pM of each primer, and 0.25 μ l of Amplitaq polymerase (Perkin–Elmer, Forest City, CA). Reactions were denatured for 3 min at 94 $^{\circ}$ C, followed by 25 thermal cycles of 94 $^{\circ}$ C denaturing for 45 s, 54 $^{\circ}$ C annealing for 45 s and 72 $^{\circ}$ C extension for 1 min, and terminated with a 5-min extension at 72 $^{\circ}$ C. We used the

primers COIIGQL (5'-GGACAATGCTCAGAAATC TGGCG-3') and COIIHMH (5'-CATGGGCTGGGG TCRACATGTG-3') developed by Seutin and Bermingham (details at <http://nmg.si.edu/bermlab.htm> to amplify the overlapping genes for subunits 8 and 6 of ATPase (hereafter ATPase 8 and 6).

2.3. Phylogenetic analyses

Prior to the phylogenetic analysis, Modeltest 3.06 (Posada and Crandall, 1998) was used in order to find the optimal model of DNA substitution. The model selected was F81 + Γ (Felsenstein, 1981) with base frequencies of $A = 0.4043$, $C = 0.1639$, $G = 0.16541$, $T = 0.26639$; and a gamma distribution shape parameter of $\alpha = 1.6296$. For phylogenetic reconstruction, we used MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). The program is based on Bayesian inference, an approach that performs exceptionally well in supporting correct groupings compared to traditional non-parametric bootstrap methods (Alfaro et al., 2003). It should be pointed out that Bayesian inference appears to be less conservative than bootstrap approaches and seems to be sensitive to small model mis-specifications (see Douady et al., 2003).

First, we compared several independent runs using the default random tree option and the starting parameters suggested by Modeltest to monitor the convergence of the ln likelihoods of the trees. The ln likelihoods started at around -16,100 and converged upon a stable value of about -5900 after 75,000 generations. It can be assumed that once this stable value is reached, MrBayes is sampling trees according to their posterior probabilities. Based on these preliminary analyses, we did a final run using the Markov chain Monte Carlo approach without Metropolis-coupling (single chain) with 1,000,000 sampled generations and with the current tree saved at intervals of 10 generations. Note that there is no good criterion that could be used to determine the optimal number of generations necessary for obtaining a good consensus tree. However, given that the ln likelihood values reached a plateau after 75,000 generations, the number of 1,000,000 chosen here appears to be sufficient. Finally, a 50% majority rule tree was constructed from all sampled trees with the first 10,000 trees (= 100,000 generations) ignored as burn-in.

In order to test whether our dataset is suitable for assessing the relative timing of cladogenic events, we performed a log-likelihood ratio test (Huelsenbeck and Rannala, 1997) with our full dataset based on 1,000,000 sampled Bayes generations with and without the constraint of the molecular clock. The test clearly rejected the molecular clock ($\log L_0 = -5944.35$, $\log L_1 = -5862.64$, $-2 \log \Lambda = 163.43$, $df = 75.2$, $p = 0.00$), indicating that the rates among lineages are not equal. Moreover, as highly unequal rates were obvious

throughout the dataset (see Fig. 1), we did not attempt to remove problematic lineages or to otherwise correct for rate heterogeneity.

Given the potential limitations of Bayesian inference (see above), we also performed Maximum Parsimony (MP), Maximum Likelihood (ML), and Neighbor-joining (NJ) analyses in PAUP* v. 4.0b10 (Swofford, 2002) either on the whole dataset (NJ, Tamura and Nei (1993) distances) or on a reduced version in order to test whether different optimality criteria and different models of sequence evolution affect our phylogenetic hypothesis. TBR (tree bisection-reconstruction) branch swapping was used in MP and ML analyses. The reduced dataset comprised the minimum number of haplotypes to represent all clades found in all previous analyses ($n = 31$). It was analyzed with MP and ML under the best-fit model selected by Modeltest (HKY + Γ model with gamma shape parameter of 0.2442 and transition:transversion ratio of 10.7065). Individuals used in the reduced datasets are indicated in Appendix A. MP and ML analyses used 100 bootstrap pseudoreplicates.

2.4. Other analyses

The ancestral distribution of *Myiarchus* was reconstructed using parsimony in MacClade 4.0 (Maddison and Maddison, 2002). The distribution of the taxon to which each sample belonged was first coded as South America, Central America, North America, Caribbean, or combinations of these as appropriate using AOU (1998) and Lanyon (1967, 1978) (Appendix A). Distributions were then reconstructed on various MP (unweighted and 1:5 transition:transversion) and ML topologies. These analyses were repeated with Mexico coded as within North America, within Central America, or as a separate character state. Use of dispersal-variance analysis using DIVA 1.1 (Ronquist, 1996) was inhibited by that program's need for strictly bifurcating trees and the need to use all equally parsimonious trees where a consensus includes polytomies (Ronquist, 1996, 1997). This was impractical in the present case, e.g., 523 equally parsimonious trees from the $n = 31$ dataset. Accordingly, DIVA was used only to address the ancestral area of *Myiarchus* overall and not the number of dispersal events in the history of individual species. This was feasible because all phylogenetic analyses produced bifurcating trees at the base of *Myiarchus*.

3. Results

3.1. Sequence characteristics

Eight hundred and forty two base pairs (bp) were sequenced from the overlapping ATPase 8 (165 bp plus

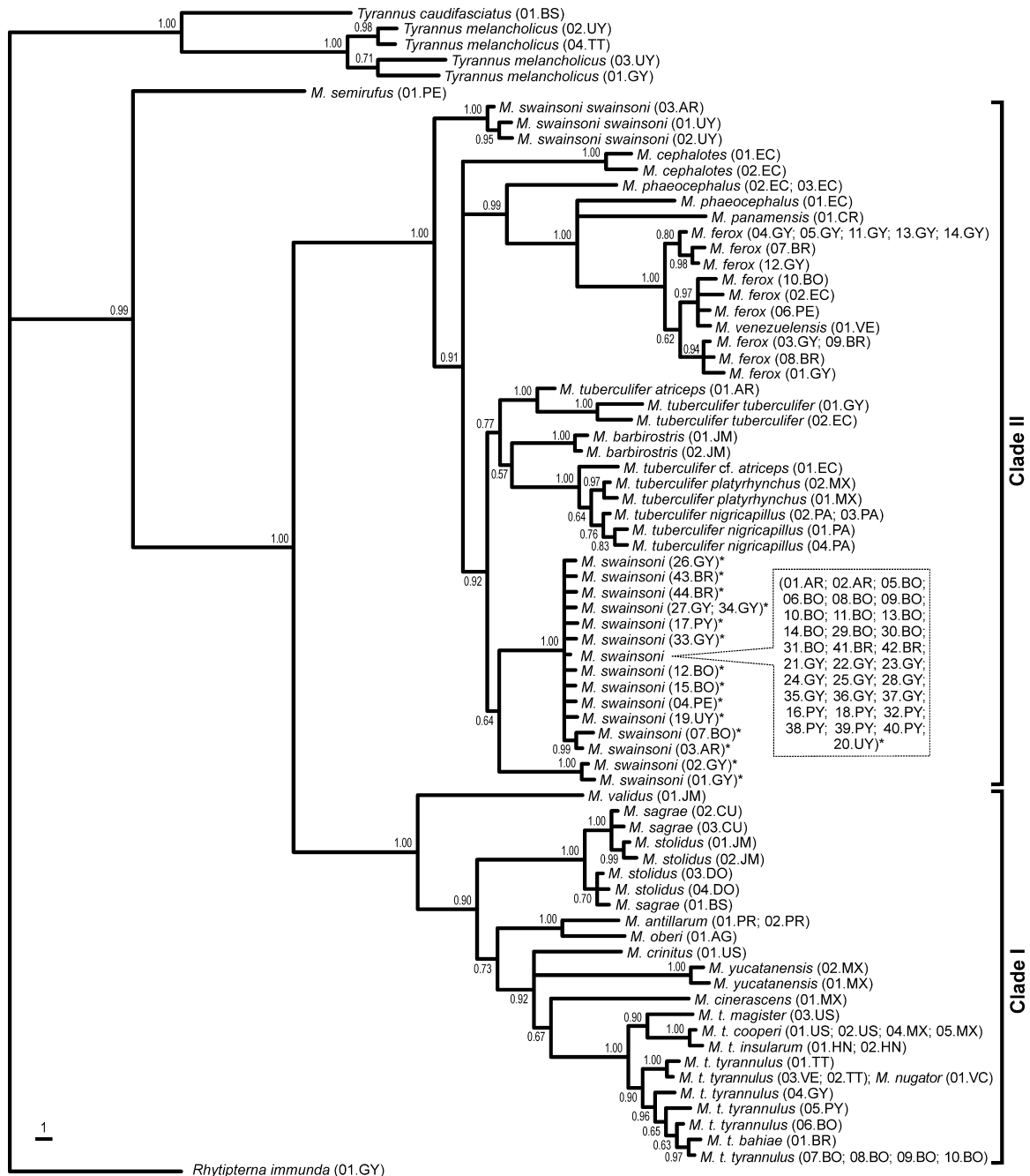


Fig. 1. Bayes phylogram for *Myiarchus* and outgroups based on 842 bp of mitochondrial sequences from the ATPase subunits 8 and 6 genes showing the 50% majority-rule consensus of topologies sampled during the Bayesian search. The scale bars indicate the expected number of substitutions according to the model of sequence evolution applied. Posterior probabilities are provided for each node. The phylogram shows the separation of *M. semirufus* from all other *Myiarchus* species and the division of the remaining species into two clades (numbered with Roman numerals). *For subspecies assignment see Joseph et al. (2003). Specimen codes are as in Appendix A.

TAA stop codon) and ATPase 6 genes (681 bp plus TAA). All sequences showed the expected 10 bp overlap in different reading frames (ATGAACYTAA) between the ATG start codon of ATPase 6 and the TAA stop codon of ATPase 8. Base frequencies were 0.29 (A), 0.34 (C), 0.08 (G), 0.28 (T), and showed the deficiency of guanine typical of avian mtDNA. All sequences were translated into amino acids and aligned with other avian

ATPase sequences in GenBank (results not shown). Relative to the homologous amino acid sequence in the chicken *Gallus gallus*, *Myiarchus* and outgroups had a single amino acid insertion in ATPase 8 between positions 44 and 45, as in other passerines (e.g., Håarlid and Arnason, 1999; Hunt et al., 2001; Lovette and Bermingham, 1999). This site was polymorphic for threonine (most *Myiarchus*, *R. immunda*), alanine (one

M. tuberculifer, ANSP 10481), isoleucine and leucine (*Tyrannus* spp). Among the 117 *Myiarchus* samples, 560 characters were constant, 193 were parsimony informative, and 89 were variable and parsimony uninformative. Percentages of variable sites were 35.1% in ATPase 8 and 33.1% in ATPase 6. These values are comparable with those reported by Hunt et al. (2001) for Caribbean mimids (33.9, 30.7%, respectively). Mean tree length of 10,000 random trees was 3164.22 ± 95.88 and the data contained significant phylogenetic signal ($g_1 = -0.32$, $p < 0.01$; Hillis and Huelsenbeck, 1992). GenBank accession numbers of the sequences used in this study are listed in Appendix A.

3.2. Phylogeny: overview

Results of NJ, MP, and ML analyses were consistent with the Bayesian analysis of the entire dataset and usually with bootstrap values $>80\%$. For brevity, we here focus on the results of Bayesian analysis and cite MP, ML, and NJ analyses only where they produced relationships that differed from the Bayesian analysis with bootstrap support $>70\%$. Elsewhere we will present more detailed discussions of our data in terms of how they relate to the phylogeography and population history of *M. tyrannulus*, *M. tuberculifer*, and *M. ferox*.

All *Myiarchus* taxa except *M. semirufus* fell in either of two main clades. Clade I had 34 sequences from Caribbean, Central American and North American species (*M. validus*, *M. stolidus*, *M. sagrae*, *M. oberi*, *M. antillarum*, *M. yucatanensis*, *M. cinerascens*, and *M. nugator*) and all sampled populations of *M. tyrannulus*, which included widely dispersed South American samples. Clade II had 82 sequences from South American species (*M. swainsoni*, *M. ferox*, *M. phaeocephalus*, *M. cephalotes*) and *M. barbirostris* of Jamaica and all sequences of Central and South American *M. tuberculifer*. Tamura–Nei divergences between these clades and outgroups ranged from 13 to 20%. Estimates of average pairwise sequence divergence within Clades I and II made from the $n = 31$ dataset removed bias from multiple intraspecific comparisons in species with larger sample sizes. These were similar at 5.04 and 4.42%, respectively (Table 1). Pairwise divergences within Clade I were either less than 2% or greater than 4%.

M. semirufus was either the sister to all other *Myiarchus* (Bayesian analysis), sister to the outgroup *R. immunda* (ML), or one lineage of a trichotomy with *R. immunda* and all other *Myiarchus* (MP). Divergences between *M. semirufus* and the two *Myiarchus* clades ($14.00 \pm 0.37\%$, $13.99 \pm 0.72\%$) were the same as that between it and *R. immunda* (13.90%). *M. semirufus* was $18.47 \pm 1.27\%$ divergent from the two *Tyrannus* species.

Within Clade I, the pairs *M. oberi*–*M. antillarum* and *M. stolidus*–*M. sagrae* were strongly supported but *M. stolidus* and *M. sagrae* were each paraphyletic with respect to each other (Fig. 1). *M. nugator* nested within *M. tyrannulus*. Sequence divergences among Clade I species ranged from maxima between 4 and 9% (*M. validus*, *M. oberi*, and *M. antillarum* vs other Clade I species) to lower values of ca. 2% (*M. oberi* vs *M. antillarum*) and $<1\%$ with paraphyly (*M. stolidus* vs *M. sagrae*) or without it (*M. tyrannulus*). Divergences of 6% or greater in Clade I involved pairwise comparisons with *M. yucatanensis* and *M. validus*.

Within Clade II, *M. ferox*, *M. panamensis*, *M. venezuelensis*, and one *M. phaeocephalus* sample (ANSP 1687) were monophyletic (Fig. 1) thus rendering *M. phaeocephalus* paraphyletic. Next, *M. swainsoni* (except the nominate subspecies *M. s. swainsoni*) was sister to *M. tuberculifer* and *M. barbirostris*. With MP and ML, however, these same sequences formed a polytomy (72–75% MP, 70% ML). Divergences among these taxa were 2–2.75%. Closest relatives of *M. s. swainsoni* and *M. cephalotes* within Clade II could not be discerned in any analysis.

3.3. Phylogeny: individual species complexes

M. s. swainsoni is not closely related to the rest of the *M. swainsoni* complex. MtDNAs from remaining members of the *M. swainsoni* complex fell into two clades, which were more closely related to *M. tuberculifer*–*M. barbirostris* than to *M. s. swainsoni*. This complex has been discussed more fully elsewhere (Joseph et al., 2003; see Section 4).

M. tuberculifer was paraphyletic with respect to *M. barbirostris* although the posterior probability of 0.77 for uniting these two species was low (Fig. 1). Net divergence between the two clades of *M. tuberculifer* was 2.04%. *M. t. atriceps* was paraphyletic. *M. barbirostris*,

Table 1
Sequence divergence comparisons in Clades I and II

	Clade I		Clade II	
	Pairwise comparisons	Mean sequence divergence (%)	Pairwise comparisons	Mean sequence divergence (%)
Full dataset, $n = 123$	561	3.53 ± 2.09	3240	2.56 ± 1.84
Reduced dataset, $n = 31$	21	5.04 ± 1.24	66	4.42 ± 1.15

Note that the $n = 31$ dataset excludes 44 of 49 *M. swainsoni* individuals within which net diversity is zero.

though itself monophyletic, was sister to a predominantly Central and North American clade of *M. tuberculifer* samples.

The single *M. venezuelensis* sample nested within *M. ferox* making the latter paraphyletic (Fig. 1). The 0.2% divergence between *M. venezuelensis* and *M. ferox* was the same order of magnitude as that among *M. ferox* (0.5%). MP bootstrapping supported only a polytomy of three clades within *M. ferox* (62–81%) and posterior probabilities for two of these clades were relatively low at 0.80 and 0.62, also giving weak support for structure within this species. One had three western South American *M. f. ferox* and the single *M. venezuelensis*. The second had Guyanan and north-east Brazilian (Pará) samples from the zone of intergradation between *M. f. ferox* and *M. f. brunescens* identified by Lanyon (1978). The third had more Guyanan *M. f. ferox*–*M. f. brunescens* intergrades as well as a south central Brazilian (Rondônia) *M. f. ferox*.

One *M. phaeocephalus* sample, ANSP 1687, and *M. panamensis* were basal to *M. ferox*–*M. venezuelensis* (Fig. 1) in all analyses, thus making *M. phaeocephalus* paraphyletic. The associated voucher, ANSP 181729, is correctly identified as *M. phaeocephalus interior* (pers. obs., L.J.). Its mtDNA sequence is translatable and we are satisfied that a valid, uncontaminated mtDNA sequence has been obtained. Net divergence between ANSP 1687 and *M. ferox*–*M. venezuelensis* was 3.2% and that between ANSP 1687 and the two other *M. phaeocephalus*, with which it was paraphyletic, is 4.75%. Divergence between *M. ferox*–*M. venezuelensis* and the latter *M. phaeocephalus* is 4.26%.

M. nugator from the Lesser Antilles was nested within *M. tyrannulus* in a clade with samples of that species from Trinidad and Guaraúnos, Venezuela. This clade in turn was sister to non-Venezuelan South American samples. All remaining samples of *M. tyrannulus*, which were from North America and Central America (Honduras, Mexico, US) formed a further clade that was sister to the Trinidad and South American samples (Fig. 1). Maximum pairwise sequence divergence within it was 1.69%. Net divergence ranged from 0.60% between Caribbean–Venezuela and non-Venezuelan South America to 1.17% between North America–Central America and Caribbean–Venezuela. Net divergence among non-Venezuelan South America (Guyana and northern Brazil vs Paraguay, Bolivia) was zero and no phylogenetic structure was recovered among these samples.

Jamaican *M. stolidus stolidus* and Cuban *M. sagrae sagrae* formed a clade that was sister to another with Dominican Republic *M. stolidus dominicensis* and Bahaman *M. sagrae lucaysiensis* (Fig. 1). Divergences among *M. stolidus* and *M. sagrae* were <1% but both were at least 4% divergent from all other members of Clade I.

3.4. Biogeography

Reconstruction of distributions showed: a South American origin for *Myiarchus* and Clade II (arrow 1, Fig. 2), and dispersal out of South America to the Caribbean with the origin of Clade I (arrow 2, Fig. 2). How Mexico was coded did not affect these results. Reconstruction on the weighted transversions MP bootstrap topology with the $n = 31$ dataset yielded the same results except when Mexico was coded as a separate character state. Under that coding scheme, the ancestral distribution of *Myiarchus* generally and Clade I specifically were equivocal. Reconstructions on the unweighted MP bootstrap and ML topologies with the $n = 31$ dataset were similarly inconsistent; when an area of origin for *Myiarchus* was unequivocally suggested it was always South America. DIVA also found a South American origin for *Myiarchus* (not shown). Dispersal into and out of South America was evident in the history of some species. *M. tuberculifer* and *M. barbirostris* showed dispersal out of South America (arrows 3 and 4, Fig. 2). Whether the evolution of the Caribbean populations of *M. tyrannulus* involved dispersal into or out of South America is unclear (arrow 5, Fig. 2). A South American area of origin for the entire *M. tyrannulus* complex was never suggested. A relatively complex history of movement among South, North, and Central America is suggested for *M. tyrannulus* complex regardless of how Mexico was coded.

4. Discussion

4.1. Monophyly and composition of *Myiarchus*

The uniquely rufous- and black-plumaged *M. semirufus* from arid coastal Peru and Ecuador was consistently sister to all other *Myiarchus*, or allied with the outgroup *R. immunda* making *Myiarchus* paraphyletic. Further, *M. semirufus* is as divergent from other *Myiarchus* (14%) as they are from outgroups (13–20%). We provisionally retain it in *Myiarchus* because our taxon sampling cannot determine whether it should be placed in monotypic *Muscifur* Bangs and Penard, 1921, or elsewhere. Zimmer's (1938) recommendation of treating *Muscifur* as a subgenus would be an appropriate interim taxonomic measure. The relationships of *M. semirufus* need to be clarified with respect to taxa such as the similarly rufous-plumaged species of *Rhytipterna*, *Attila*, and *Casiornis* and the *Myiarchus*-like *Deltarhynchus flammulatus* (Lanyon, 1982), which we have been unable to examine. Our data affirm with strong support the monophyly of all other *Myiarchus* taxa examined and furthermore the inclusion of *M. validus* in *Myiarchus* from our data is unremarkable. Furthermore, it is part of Clade I and consistently the sister to all remaining

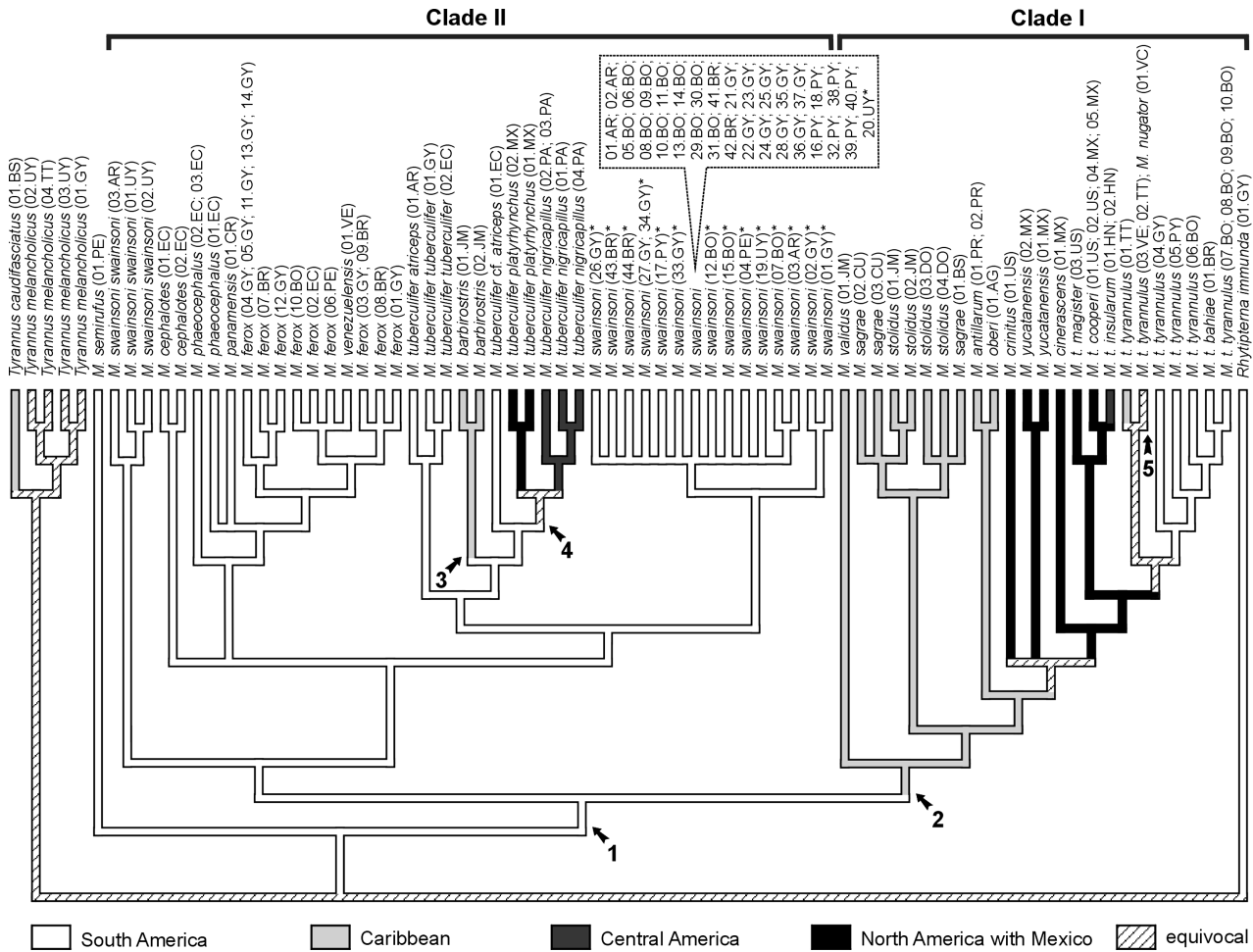


Fig. 2. Reconstruction of distributional changes in *Myiarchus* on the Bayesian tree derived from the full dataset. Five arrows point to distributional shifts found in all or most analyses and discussed in Section 3. Specimen codes are as in Appendix A.

taxa in that clade. We have been unable to examine *M. nuttingii* (Central America) and *M. apicalis* (Colombia) but predict on the basis of morphology and distribution that they will be members of Clades I and II, respectively (AOU, 1998; Lanyon, 1978). Similarly, we were unable to examine the unusually small but again otherwise unexceptionally plumaged *M. magnirostris* endemic to the Galapagos Islands. We predict from Lanyon (1978) that its closest relative will be within the *M. tyrannulus* complex (Clade I).

Congruence among characters of mtDNA, voice, and plumage is limited. The phylogenetic structure in mtDNA of Clades I and II being sisters and *M. semirufus* being their sister is paralleled in other characters only in plumage. Thus *M. semirufus* is distinctively rufous and black in plumage and all other species are essentially similar to each other in being dorsally and pectorally grey, and yellow or creamy bellied. Although *M. semirufus* is the only strictly South American species having a dawn song with what Lanyon (1967, 1978) termed “*huit*” notes and “rasping whistles,” Jamaican *M. stolidus* from Clade I, for example, does have mod-

ified *huit* notes in its dawn song (Lanyon, 1967). Few other characters show any such degree of congruence. Notable among them is extensive cinnamon (= Antique Brown of Lanyon, 1978) being present in the inner vanes of the rectrices in *M. semirufus* and Clade I but absent in Clade II. In contrast, simple, unmodulated whistles in the dawn song and the roll, an element of the vocal response to intruding conspecifics, show no correlation with membership of Clade I or II (see Lanyon, 1978, pp. 443–448) whereas “*hiccup*” notes are present in Clade II and variable in Clade I (e.g., absent in *M. tyrannulus*, present in *M. antillarum*). All members of Clade II lack what Lanyon (1978) termed “*rapid huit*” notes, “*whay-burg*” notes and a dawn song with *huits* but no rasping or plaintive whistles though each is present in *M. tyrannulus* (Clade I). Despite Lanyon’s (1967, 1978) herculean work in documenting vocal characters, we are reluctant to attempt further mapping of vocal characters on to the phylogeny. Homologies in many of the vocal elements involved are not readily identifiable without a broader phylogeny of tyrannids. The problem is further compounded by a formal nomenclature of the different

elements of the *Myiarchus* vocal repertoire being more readily available for South American species (Lanyon, 1978) than for Caribbean ones (Lanyon, 1967) though we have attempted to circumvent this in some of the observations reported above. Finally here, we note that the basic plumage pattern shown by most *Myiarchus* species except *M. semirufus* (i.e., dorsal and pectoral grey, yellow belly) is probably ancestral. It occurs in *R. immunda* and *T. melancholicus* as well as in *Deltarhynchus flammulatus*, a species we have not examined but which Lanyon (1967, 1985) considered not closely related to *Myiarchus*, and still other tyrannids.

4.2. Species-level systematics

Despite limited morphological diversity among *Myiarchus* species, the mtDNA phylogeny is often not concordant with current taxonomy. Paraphyly is widespread and suggests in some taxa a decoupling of mtDNA and morphological evolution. To illustrate concordances and discordances between mtDNA and morphology, we review cases of paraphyly and their systematic implications. Also, because renewed concern has been expressed lately (e.g., Ballard et al., 2002; Weckstein et al., 2001) about making taxonomic changes based solely on mtDNA, we offer our findings as a focus for further questions that should be addressed with characters from nuclear DNA, morphology and voice.

4.2.1. *M. swainsoni*

We have discussed this complex in detail elsewhere (Joseph et al., 2003). Here it is worth noting Lanyon's (1978, pp. 472–474) finding that playback of *M. s. ferocior* elicited a behavioural response in Argentinean *M. tuberculifer atriceps* and that our mtDNA data have identified a similar, unexpected relationship between *M. tuberculifer* and all subspecies examined of *M. swainsoni* except its nominate subspecies *M. s. swainsoni*. Playback results may have indicated a response to plesiomorphic vocal characters (Ryan and Rand, 1995). Pending acquisition of nuclear DNA (nDNA) data, we note that taxonomic changes will almost certainly be needed in the *M. swainsoni* complex. Even if *M. s. swainsoni sensu* Lanyon (1978) is separated as *M. swainsoni* Cabanis and Heine, 1859, however, as seems reasonable based on its mtDNA and on morphological differences that presumably reflect nDNA, taxonomic changes to the remaining members of the group depend on: (1) examination of the single *M. swainsoni* subspecies we were unable to study, *M. s. phaeonotus*, (2) resolution of why two Guyanan individuals are so divergent for mtDNA, and (3) determination of priority among the epithets *ferocior*, *pelzelni*, and *phaeonotus*, all of which were described in 1883.

4.2.2. *M. tuberculifer*

M. tuberculifer comprised two clades in most analyses. While not closest relatives, their relationships to each other and to *M. swainsoni* (except its nominate subspecies *M. s. swainsoni*) are unresolved. Caribbean *M. barbirostris* was most closely related to the predominantly Central and North American clade of *M. tuberculifer*.

We plan a more complete discussion of the *M. tuberculifer* group elsewhere but the paraphyly of *M. t. atriceps* warrants mention here. The taxonomic status of *M. t. atriceps* is among the most challenging problems in South American *Myiarchus* (Lanyon, 1978) and our findings reinforce this. Lanyon's (1978) arguments for treating *M. t. atriceps* as a subspecies of *M. tuberculifer* were complex but carefully reasoned interpretations of playback experiments and altitudinal effects on morphological and vocal characters. The central, novel issue arising in our study is the paraphyly of mtDNA from opposite ends of *M. t. atriceps*'s range, in Ecuador and Argentina. Obviously, more complete sampling will be needed before this can be explained but some possibilities emerge. MtDNA of *M. t. atriceps* at the northern and southern ends of its range could be introgressed from adjoining populations of other subspecies. This would cause paraphyly in *M. t. atriceps* because those adjoining populations' mtDNAs are themselves paraphyletic (Fig. 1). Alternative explanations include incomplete sorting of mtDNA, and inaccurate taxonomy. We will examine these possibilities more fully elsewhere.

4.2.3. *M. ferox*

Paraphyly of *M. ferox* with respect to *M. venezuelensis* and the divergence between them (0.24%), which is the same order of magnitude as among *M. ferox* alone (0.55%), suggest either incomplete sorting or that they are conspecific or that the *M. venezuelensis* specimen is descended from hybridization between female *M. ferox* and male *M. venezuelensis* at an unknown time in the past. The locality of the *M. venezuelensis* sample (Guaraúnos, Sucre, Venezuela: 10°33'N, 63°07'W) is from within a zone of overlap between that species and *M. ferox* (Lanyon, 1978) so hybridization is feasible. (The *M. venezuelensis* sample was available only as a blood sample and so we have been unable to check its external phenotype.) A close relationship among these taxa, however, is expected (see Lanyon, 1978).

4.2.4. *M. phaeocephalus*

Hybridization between *M. phaeocephalus* and *M. ferox* might explain the paraphyly of *M. phaeocephalus*. The sample causing it (ANSP 1687) is of the subspecies *M. p. interior*, which is locally sympatric with *M. ferox* (Lanyon, 1978, Figs. 1 and 3). We consider

hybridization unlikely, however, because ANSP 1687 is consistently basal to *M. ferox* rather than nested within it as would be expected from hybridization of *M. venezuelensis* unless, perhaps, the hybridization event was an ancient one relative to the divergence of these species from their common ancestor. Furthermore, the associated voucher shows no phenotypic tendency towards *M. ferox* (Fig. 3). Alternatively, mtDNAs of *M. phaeocephalus* and *M. ferox* may be incompletely sorted. The close relationship of *M. panamensis* to *M. ferox* and *M. venezuelensis* was not unexpected (Lanyon, 1978). Arguably, the mtDNAs of *M. phaeocephalus*, *M. panamensis*, *M. ferox*, and *M. venezuelensis* are all incompletely sorted. Field study of ecological interactions and niche separation among these four taxa where they are parapatric and sympatric would be rewarding in light of

the mtDNA data suggesting paraphyly due to hybridization and/or incomplete sorting.

4.2.5. *M. tyrannulus*

Separation of Central and North American mtDNAs within the *M. tyrannulus* complex supports their recognition either as *M. cooperi* Baird, 1858 or two species *M. magister* Ridgway, 1884 and *M. cooperi* (see AOU, 1998; Cardiff and Dittmann, 2000). We cannot address whether Pacific slope populations of Central America should be further separated as *M. brachyurus* Ridgway, 1887 (e.g., AOU, 1998) because our Central American samples are from the Atlantic coast. Our two samples of *M. t. insularum* from the island of Utila, Honduras, were monophyletic. On the basis of voice playback experiments and minor differences in colour of mouth-lining

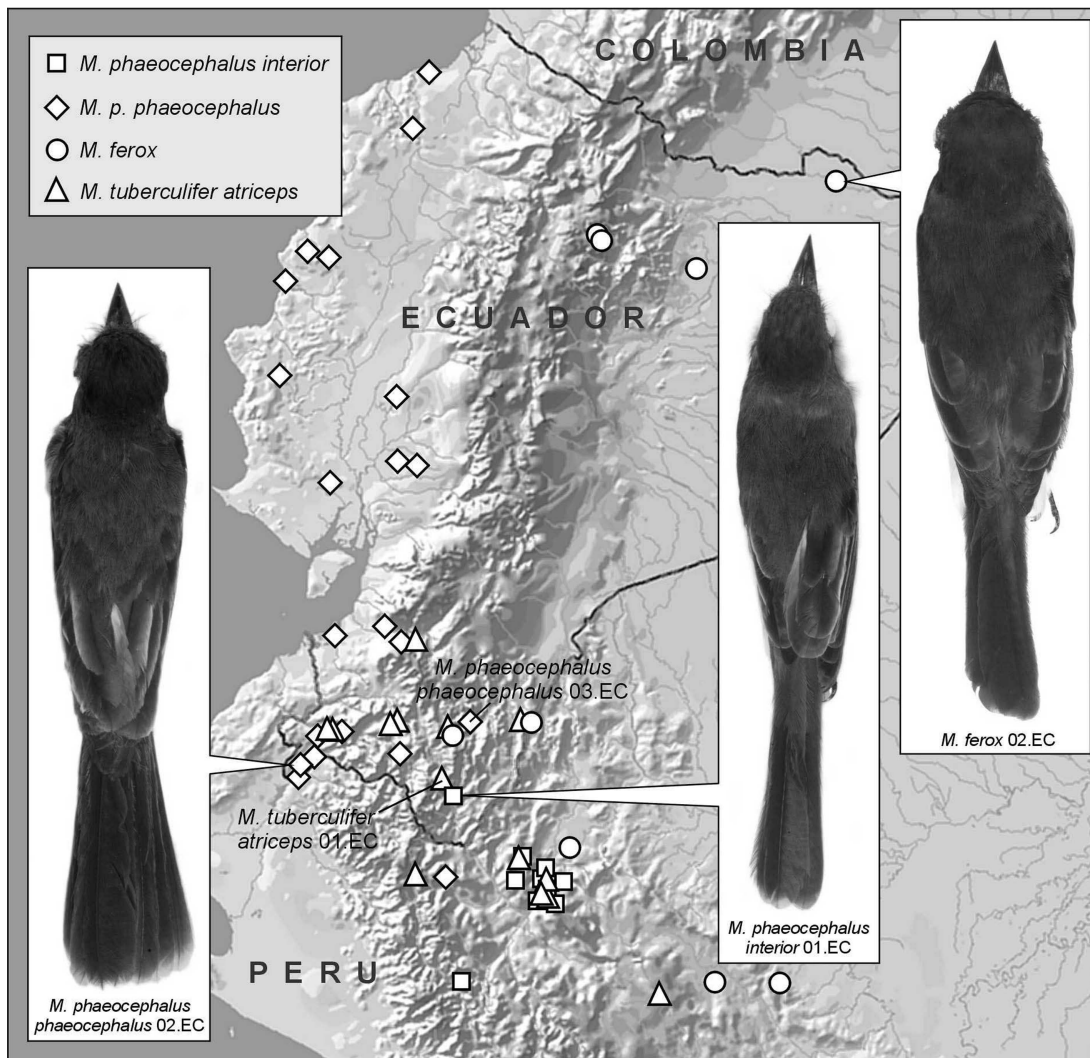


Fig. 3. Map of Ecuador and adjacent parts of Peru showing distributions of *M. ferox*, *M. phaeocephalus*, and *M. tuberculifer* in that region. The mtDNA of sample *M. phaeocephalus interior* 01.EC (= skin ANSP 181729 = tissue ANSP 1687) is more closely related to that of *M. ferox* than to other *M. phaeocephalus* (Fig. 1). Note, however, that the same bird is phenotypically typical of *M. phaeocephalus*, e.g., its bill is narrow as in *M. phaeocephalus*, not broad as in *M. ferox*, despite its proximity to *M. ferox* localities. Localities are derived from Lanyon (1978) except where indicated. Specimen codes are as in Appendix A.

and wing feathers, Lanyon (1967) argued that *M. nugator* of the southern Lesser Antilles is specifically distinct from *M. tyrannulus*. Divergence in these characters has likely been extremely rapid and recent given *M. nugator*'s 0.03% divergence from its closest relatives in *M. tyrannulus* from nearby Trinidad and Venezuela. Relevant to the status of the Caribbean endemic *M. nugator* is that our Venezuelan sample of *M. tyrannulus* was unexpectedly more closely related to *M. nugator* and Trinidad *M. tyrannulus* than to *M. tyrannulus* samples from Guyana and the rest of South America and that biogeographic reconstruction suggested this clade had dispersed into northern South America. The possibility arises that *M. nugator* Riley, 1904 and Venezuelan and Trinidad populations should all be recognized as *M. erythrocerus* Sclater and Salvin, 1868 (type locality Caracas, Venezuela). *M. tyrannulus* (Muller, 1776) (type locality Cayenne, French Guiana) would then apply to remaining South American populations. The respective ranges in northern South America of *M. erythrocerus* and *M. tyrannulus* as so construed need to be clarified. The mtDNA data again imply that phenotypic divergence in the *M. tyrannulus* complex has been rapid.

4.2.6. *M. stolidus* superspecies

Lanyon (1967) recognized *M. antillarum*, *M. oberi*, *M. sagrae*, and *M. stolidus* as the *M. stolidus* superspecies to emphasize close inferred relationship. In the present study, the four formed a single monophyletic clade only in the Bayesian and NJ analyses of all data. Individually, *M. sagrae* and *M. stolidus* were not monophyletic. Bahaman *M. sagrae lucaysiensis* aligns with Dominican Republic *M. stolidus dominicensis* (Fig. 1) and in this mtDNA and morphology are concordant to some extent. They share smaller size and paler yellow bellies relative to *M. stolidus stolidus* (Lanyon, 1967). Cuban *M. sagrae sagrae* aligns with Jamaican *M. stolidus stolidus*. In this alignment, mtDNA and morphology are discordant. *M. stolidus stolidus* is larger and more brightly yellow-bellied than *M. sagrae sagrae* (Lanyon, 1967). Pallor of the belly increases in the sequence *M. stolidus dominicensis*–*M. sagrae lucaysiensis*–*M. antillarum*, the last-named having an almost white belly. Rather than interpret the paraphyly of *M. stolidus* and *M. sagrae sensu* Lanyon (1967) as being solely due to incomplete sorting and obvious decoupling of the evolution of mtDNA and morphology, we suspect that the taxonomy requires re-examination by way of a systematic reassessment of the entire *M. stolidus* “superspecies.”

4.3. Biogeography

A South American origin of *Myiarchus* is plausible given the diversity of Tyrannidae there. Our data reveal

two major radiations within *Myiarchus*. One is primarily of West Indian and Central and North American species (Clade I), and the other is primarily of South American species (Clade II). The former comprises all West Indian endemics as well as *M. tyrannulus*, which is widely distributed across North, Central, and South America. Similarly, the South American radiation comprises all other species occurring there that we have examined except *M. semirufus*, which is not closely related to other *Myiarchus*. We do find support for Lanyon's suggestion of *M. barbirostris* having evolved through relatively recent colonization from a *M. tuberculifer*-like ancestor. This represents dispersal of Clade II into the Caribbean from South or Central America. However, in the history of Clade I and with respect to *M. stolidus* and *M. validus*, we find support for a sequence of evolution that is the reverse of what Lanyon (1967) proposed, i.e., that *M. validus* is the older species. In the *M. tyrannulus* complex, a more complex history is suggested. Colonization of the Caribbean has clearly been involved in the evolution of *M. nugator*. From our data, we cannot rule out the possibility that *M. tyrannulus* has not itself back colonized far northern mainland South America from the Caribbean. It appears highly likely that the occurrence of *M. tyrannulus* elsewhere in South America other than far northern Venezuela is the result of an earlier, independent colonization. Thus Clade I has secondarily entered South America at least once.

Rejection of a molecular clock in our dataset precludes most attempts to estimate the timing of cladogenic events in the history of *Myiarchus*. Accordingly, we only make the following statements. If non-control region mtDNA is evolving at roughly 2% per million years (e.g., Fleischer et al., 1998; Krajewski and King, 1996; see also Avise and Walker, 1998), then sequence divergences suggest that speciation within Clades I and II began before the Pleistocene (Table 1). *M. validus* is likely the oldest member of Clade I, and *M. s. swainsoni* and *M. cephalotes* likely are among the oldest of Clade II. Maximum pairwise divergences in Clade I (6%–10%) suggest a longer history of diversification in that Clade (Table 1). Identification of factors causing speciation within Clades I and II and the history of speciation in both clades are topics beyond the scope of the present paper.

5. Conclusions

The monophyly of *Myiarchus* has been robustly supported with the only uncertainty being the placement of the uniquely rufous and black *M. semirufus* among the group's close relatives. Evolution within a primarily South American clade and a primarily Caribbean/North American one has occurred over broadly

similar periods of time, as judged by similar levels of divergence within the two clades. Some species of the primarily South American clade have dispersed out of that continent whereas *M. tyrannulus* of a primarily Caribbean and North American clade has secondarily entered it. Closer resolution of relationships and rates of evolution within both clades of *Myiarchus* will require study of nuclear DNA and loci with a range of evolutionary rates.

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Appendix A

Details of specimens examined with latitudes and longitudes where available from primary specimen data. An asterisk (*) indicates individuals used in the $n = 31$ dataset. Full details of the 49 specimens examined from the *M. swainsoni* complex are in Joseph et al., 2003; for brevity, they are here accompanied only with taxon codes corresponding to those used in Figs. 1 and 2 of this paper, their GenBank accession codes, and their museum accession numbers. Coding used for biogeographic analyses shown in Fig. 2 are: SA, South America; NA, North America; CA, Central America; Car, Caribbean; MX, Mexico. See Section 2 for details of how Mexico was incorporated into various coding schemes. Museum and collection acronyms: AMNH, American Museum of Natural History; ANSP, Academy of Natural Sciences; FMNH, Field Museum of Natural History; KU, University of Kansas; LSUMZ, Louisiana State University Museum of Zoology; MBM, Marjorie Barrick Museum of Natural History; STRI, Smithsonian Tropical Research Institute; USNM, United States National Museum.

Appendix A

Taxon and code in Fig. 1	Locality	Distribution code	GenBank	Museum code
<i>M. antillarum</i> 01.PR*	Puerto Rico: Guanica	Car	AY115182	STRI PR MAN 2
<i>M. antillarum</i> 02.PR	Puerto Rico: Guanica	Car	AY115181	STRI PR MAN 1
<i>M. barbirostris</i> 01.JM	Jamaica: Luana Point	Car	AF497962	STRI JA-MBA 1
<i>M. barbirostris</i> 02.JM*	Jamaica: Paradise Park	Car	AY266207	STRI JA-MBA 5
<i>M. cephalotes</i> 01.EC*	Ecuador: Depto Napo, 12 km NNE El Chaco, Mirador	SA	AF497966	ANSP 4807
<i>M. cephalotes</i> 02.EC	Ecuador: Napo, 3 km N Cosanga	SA	AY266183	ANSP 5124
<i>M. cinerascens</i> 01.MX*	Mexico: Queretaro, Pena Bernal	Mex	AY266222	FMNH 394371
<i>M. crinitus</i> 01.US*	Honduras: Cochino Pequeno	CA	AY266197	STRI HA MCR HA168
<i>M. ferox</i> 01.GY*	Guyana: Surama, 04°10'N, 59°05'W	SA	AY266184	ANSP 8369
<i>M. ferox</i> 02.EC	Ecuador: Sucumbios, ca 14 km N Tigre Playa, 00°20'N, 76°40'W	SA	AY266185	ANSP 5498

Appendix A (continued)

Taxon and code in Fig. 1	Locality	Distribution code	GenBank	Museum code
<i>M. ferox</i> 03.GY	Guyana: Iwokrama, ca. 41 road km SW Kurupukari, 04°20'N, 58°51'W	SA	AY266186	KU B 1233
<i>M. ferox</i> 04.GY	Guyana: Gunn's Landing, 01°39'N, 58°57'W	SA	AY266187	USNM B 10968
<i>M. ferox</i> 05.GY	Guyana: Berbice River, Dubulay Ranch, 05°40'N, 57°53'W	SA	AY266788	USNM B 4377
<i>M. ferox</i> 06.PE	Peru: Dpto Ucayali, 65 km ENE Pucallpa	SA	AY266189	LSUMZ B 10730
<i>M. ferox</i> 07.BR	Brazil: Rondonia, Cachoeira Nazare, W bank Rio Jiparaná,	SA	AY266191	FMNH 389972
<i>M. ferox</i> 08.BR	Brazil: Para, Monte Alegre, Colonia do Erere,	SA	AY266192	FMNH 392547
<i>M. ferox</i> 09.BR	Brazil: Para, Monte Alegre, Colonia do Erere,	SA	AF497965	FMNH 392548
<i>M. ferox</i> 10.BO	Bolivia: Laguna Suarez, 14°48'S, 64°46'W	SA	AY266193	FMNH 394469
<i>M. ferox</i> 11.GY	Guyana: 01°39'N, 58°37'W	SA	AY266194	USNM B 10972
<i>M. ferox</i> 12.GY	Guyana: near Iwokrama Reserve, 04°10'N, 59°05'W	SA	AY266195	ANSP 8162
<i>M. ferox</i> 13.GY	Guyana: 01°39'N, 58°37'W	SA	AY266196	USNM B 10971
<i>M. ferox</i> 14.GY	Guyana: Gunn's Landing: 01°39'N, 58°57'W	SA	AY266190	USNM B 11551
<i>M. nigator</i> 01.VC*	St. Vincent: Cumberland Valley	Car	AY266198	STRI SV MOB 1
<i>M. oberi</i> 01.AG*	Antigua and Barbuda: 5 km E Cadrington	Car	AY115173	STRI BU MOB 1
<i>M. panamensis</i> 01.CR*	Costa Rica: Puntarenas, 1 km NW Tarcoles	CA	AF497959	LSUMZ B9935
<i>M. phaeocephalus</i> 01.EC*	Ecuador: Zaruma, 04°53'S, 79°08'W	SA	AF497963	ANSP 1687
<i>M. phaeocephalus</i> 02.EC*	Ecuador: 3 km N Zapotillo, ca. 04°02'S, 79°01'W	SA	AF497964	ANSP 4147
<i>M. phaeocephalus</i> 03.EC	Ecuador: Loja, 16 road km SW Sabanilla	CA	AY266171	ANSP 1745
<i>M. sagrae</i> 01.BS*	Bahamas: Airport	Car	AY266203	STRI BH MAN 1
<i>M. sagrae</i> 02.CU*	Cuba: Pinar del Rio	Car	AY266204	STRI CU MAN 5546
<i>M. sagrae</i> 03.CU	Cuba: Pinar del Rio	Car	AY266205	STRI CU MAN 5549
<i>M. semirufus</i> 01.PE*	Peru: Lambayeque: Las Pampas, km 885 Pan American Hwy, 11 road km from Olmos	SA	AY266170	LSUMZ B 5203
<i>M. stolidus</i> 01.JM*	Jamaica: Luana Point	Car	AY266199	STRI JAMYS 1
<i>M. stolidus</i> 02.JM	Jamaica: Portland Ridge	Car	AY266200	STRI JAMYS 2
<i>M. stolidus</i> 03.DO*	Dominican Republic: Club Dominic	Car	AY266201	STRI RDMSYS 1
<i>M. stolidus</i> 04.DO	Dominican Republic: 8 December 1995	Car	AY266202	STRI RDMSYS 7
<i>M. tuberculifer platyrhynchus</i> 01.MX	Mexico: Campeche, 24 km S Silvituc, 18°14'N, 90°12'W	MX	AY266172	KU B 1932
<i>M. tuberculifer tuberculifer</i> 01.GY	Guyana: Sipu River, 01°25'N, 58°57'W	SA	AY266173	USNM B 10481
<i>M. tuberculifer atriceps</i> 01.AR*	Argentina: Tucumán	SA	AF497961	USNM B 5785
<i>M. tuberculifer nigricapillus</i> 01.PA*	Panama: Bocas del Toro	CA	AY266174	USNM B 458
<i>M. tuberculifer nigricapillus</i> 02.PA	Panama: Bocas del Toro, Cayo Agua, near Punta Limon	CA	AY266175	USNM B 1157
<i>M. tuberculifer nigricapillus</i> 03.PA	Panama: Bocas del Toro, Rio Changuinola	CA	AY266176	USNM B 1917
<i>M. tuberculifer cf. atriceps</i> (see text) 01.EC	Ecuador: 6 km NW San Andres, E slope Cord. Lagunillas	SA	AF497960	ANSP 5043
<i>M. tuberculifer nigricapillus</i> 04.PA	Panama: Chiriqui, Fortuna Dam, 08°45'N, 82°15'W	CA	AY266177	USNM B 5343
<i>M. tuberculifer platyrhynchus</i> 02.MX	Mexico: Yucatan, 18 km E Dzilam de Bravo, 21°28'N, 88°34'W	MX	AY266178	USNM B 1860
<i>M. tuberculifer tuberculifer</i> 02.EC	Ecuador: Imuya Cocha, Sucumbios, 00°34'S, 75°17' W	SA	AY266179	ANSP 3241
<i>M. tyrannulus tyrannulus</i> 01.TT*	Trinidad and Tobago: Chacachacare Island	Car	AY115170	STRI CC-MTY1
<i>M. tyrannulus tyrannulus</i> 02.TT	Trinidad and Tobago: Chacachacare Island	Car	AY115169	STRI CC-MTY2
<i>M. tyrannulus insularum</i> 01.HN*	Honduras: Utila, Bay Islands	CA	AY266217	STRI HA-MTY-HA214
<i>M. tyrannulus insularum</i> 02.HN	Honduras: Utila, Bay Islands	CA	AY266218	STRI HA-MTY-HA215
<i>M. tyrannulus cooperi</i> 01.US	USA: Louisiana: Cameron Parish	NA	AY266219	STRI US-MTY4069
<i>M. tyrannulus cooperi</i> 02.US	USA: Louisiana: Cameron Parish	NA	AY266220	STRI US-MTY5678

Appendix A (continued)

Taxon and code in Fig. 1	Locality	Distribution code	GenBank	Museum code
<i>M. tyrannulus magister</i> 03.US*	USA: Arizona: Cochise County, Silver Creek, near Paradise	NA	AY266221	LSUMZ B 4075
<i>M. tyrannulus tyrannulus</i> 03.VE	Venezuela: Guarapo	SA	AY115171	STRI VE MTY 1
<i>M. tyrannulus tyrannulus</i> 04.GY*	Guyana: Mahaica-Berbice, Coast, near Onverwagt, 06°25'N, 57°37'W	SA	AF497958	ANSP 8399
<i>M. tyrannulus tyrannulus</i> 05.PY	Paraguay: San Luis N.P., 22°48'S, 57°21' W	SA	AY266214	KU B 176
<i>M. tyrannulus cooperi</i> 04.MX	Mexico: Campeche, 24 km S Silvituc, 18°14'N, 90°12'W	MX	AY266215	KU B 2094
<i>M. tyrannulus cooperi</i> 05.MX	Mexico: Campeche, 24 km S Silvituc, 18°14'N, 90°12'W	MX	AY266216	KU B 2112
<i>M. tyrannulus bahiae</i> 01.BR	Brazil: Amapá, Fazenda Casemiro	SA	AY266208	FMNH 391520
<i>M. tyrannulus tyrannulus</i> 06.BO	Bolivia: Beni, 5 km SW Laguna Suárez, 04°50'S, 64°51'W	SA	AY266209	FMNH 396030
<i>M. tyrannulus tyrannulus</i> 07.BO	Bolivia: Chiquitos, 69 Km. N of San José de Chiquitos, 17°40'S, 59°42'W	SA	AY266210	FMNH 396038
<i>M. tyrannulus tyrannulus</i> 08.BO	Bolivia: Beni, 5 km SW Laguna Suárez, 04°50'S, 64°51'W	SA	AY266211	FMNH 396037
<i>M. tyrannulus tyrannulus</i> 09.BO	Bolivia: Beni, 5 km SW Laguna Suárez, 04°50'S, 64°51'W	SA	AY266212	FMNH 396051
<i>M. tyrannulus tyrannulus</i> 10.BO	Bolivia: near El Tuná, 16°33'S, 59°39'W	SA	AY266213	AMNH 25665
<i>M. validus</i> 01.JM*	Jamaica: Portland Ridge	Car	AY266206	STRI JA MVL 1
<i>M. venezuelensis</i> 01.VE*	Venezuela: Guaraunos, 110°33'N, 63°07'W	SA	AY266182	STRI VE MVE 1
<i>M. yucatanensis</i> 01.MX*	Mexico: Campeche, 24 km S Silvituc, 18°14'N, 90°12'W	MX	AY266180	KU B 1950
<i>M. yucatanensis</i> 02.MX	Mexico: Yucatan, 18 km E Dzilam de Bravo, 21°28'N, 88°34'W	MX	AY266181	KU B 1880
<i>Rhytipterna immunda</i> 01.GY*	Guyana: Rupununi, 02°12'N, 59°22'W	SA	AF497967	USNM B 12786
<i>Tyrannus caudifasciatus</i> 01.BS*	Bahamas: Abaco, 19 October 1993	Car	AF497968	STRI AB-TCF 1
<i>Tyrannus melancholicus</i> 01.GY	Guyana: Iwokrama, 04°45'N, 59°01'W	SA	AY266223	ANSP 8341
<i>Tyrannus melancholicus</i> 02.UY	Uruguay: 15 km N Mercedes, 33°07'S, 58°01'W	SA	AY266224	ANSP 10316
<i>Tyrannus melancholicus</i> 03.UY	Uruguay: 15 km N Mercedes, 33°07'S, 58°01'W	SA	AY266225	ANSP 10317
<i>Tyrannus melancholicus</i> 04.TT*	Trinidad and Tobago: Caroni Swamp	Car	AY266226	STRI TRTML 4
<i>M. swainsoni</i> (includes <i>M. s. ferocior</i> , <i>M. s. pelzelni</i> and <i>M. s. swainsoni</i> × <i>M. s. pelzelni</i> intergrades, <i>M. s. ferocior</i> × <i>M. s. pelzelni</i> intergrades, and all but two <i>M. s. phaenotus</i> × <i>M. s. pelzelni</i> intergrades). See Joseph et al. (2003) for further details.	01 AR*, AF497948, MBM 5343; 02 AR, AF497949, MBM 5500; 03 AR, AF497950, MBM 5501; 04 PE, AF497927, LSUMZ B 10644; 05 BO, AF497928, LSUMZ B 9563; 06 BO, AF497930, AMNH 833439; 07 BO, AF497931, AMNH 833291; 08 BO, AF497937, AMNH 833292; 09 BO, AF497932, AMNH 25670; 10 BO, AF497939, AMNH 833440; 11 BO, AF497933, AMNH 25671; 12 BO, AF497938, AMNH 833437; 13 BO, AF497934, AMNH 833438; 14 BO, AF497935, AMNH 25668; 15 BO, AF497936, AMNH 25669; 16 PY, AF497922, KU B 4397; 17 PY, AF497946, KU B 137; 18 PY, AF497947, KU B 102; 19 UY, AF497951, ANSP 10921; 20 UY, AF497952, ANSP 10331; 21 GY, AF497921, KU B 4336; 22 GY, AF497909, USNM B 10975; 23 GY, AF497910, USNM B 10986; 24 GY, AF497911, USNM B 10993; 25 GY, AF497912, USNM B 10980; 26 GY, AF497913, USNM B 11108; 27 GY, AF497914, USNM B 10963; 28 GY, AF497915, USNM B 10312; 29 BO, AF497940, FMNH 334508; 30 BO, AF497941, FMNH 394470; 31 BO, AF497929, LSU B 9458; 32 PY, AF497942, KU B 3415; 33 GY, AF497916, USNM B 11387; 34 GY, AF497917, USNM B 11554; 35 GY, AF497918, USNM B 11908; 36 GY, AF497919, USNM B 11964; 37 GY, AF497920, USNM B 12220; 38 PY, AF497945, KU B 2984; 39 PY, AF497944, KU B 2986; 40 PY, AF497943, KU B 3101; 41 BR, AF497926, FMNH 391521 = MPEG 53569; 42 BR, AF497923, FMNH 391516; 43 BR, AF497924, FMNH 391517; 44 BR, AF497925, FMNH 391518			
<i>M. s. phaenotus</i> × <i>M. s. pelzelni</i> intergrades	01.GY, AF497954, USNM B 11581; 02.GY*, AF497953, USNM B 10990			
<i>M. swainsoni swainsoni</i>	01.UY*, AF497955, ANSP 10250; 02.UY, AF497956, ANSP 10269; 03.AR, AF497957, USNM B 5967			

References

- Alfaro, M.E., Zoller, S., Lutzoni, F., 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Mol. Biol. Evol.* 20, 255–266.
- AOU [American Ornithologists Union], 1998. Check-list of North American Birds, seventh ed. American Ornithologists Union, Washington, DC.
- Awise, J.C., Walker, D., 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. *Proc. R. Soc. Lond. B* 265, 457–463.
- Ballard, J.W.O., Chernoff, B., James, A.C., 2002. Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. *Evolution* 56, 527–545.
- Cardiff, S.W., Dittmann, D.L., 2000. Brown-crested Flycatcher (*Myiarchus tyrannulus*). In: Poole, A., Gill, F. (Eds.), *The Birds of*

- North America, No. 496. The Birds of North America, Inc, Philadelphia, PA.
- Chesser, R.T., 2000. Evolution in the high Andes: the phylogenetics of *Muscisaxicola* ground-tyrants. *Mol. Phylogenet. Evol.* 15, 369–380.
- Coues, E., 1872. Studies of the Tyrannidae—Part I. Revision of the species of *Myiarchus*. *Proc. Acad. Nat. Sci. Phil.* 24, 56–81.
- Douady, C.J., Delsuc, F., Boucher, Y., Doolittle, W.F., Douzery, E.J.P., 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Mol. Biol. Evol.* 20, 248–254.
- Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17, 368–376.
- Fitzpatrick, J.W., 1985. Form, foraging behavior, and adaptive radiation in the Tyrannidae. *Orn. Monogr.* 36, 447–470.
- Fleischer, R., McIntosh, C., Tarr, C., 1998. Evolution on a volcanic conveyor belt, using phylogeographic reconstructions and K–Ar based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Mol. Ecol.* 7, 533–545.
- Häarlid, A., Arnason, U., 1999. Analyses of mitochondrial DNA nest ratite birds within the Neognathae: supporting a neotenus origin of ratite morphological characters. *Proc. R. Soc. Lond. B* 266, 305–309.
- Hillis, D.M., Huelsenbeck, J.P., 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *J. Hered.* 83, 189–195.
- Huelsenbeck, J.P., Rannala, B., 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* 276, 227–232.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Hunt, J.S., Bermingham, E., Ricklefs, R., 2001. Molecular systematics and biogeography of Antillean thrashers, termblers, and mockingbirds (Aves: Mimidae). *Auk* 118, 35–55.
- Joseph, L., Wilke, T., Alpers, D., 2003. Independent evolution of migration on the South American landscape in a long-distance temperate-tropical migratory bird, Swainson's Flycatcher *Myiarchus swainsoni*. *J. Biogeogr.* 30, 925–937.
- Krajewski, C., King, D.G., 1996. Molecular divergence and phylogeny: rates and patterns of cytochrome *b* evolution in cranes. *Mol. Biol. Evol.* 13, 21–30.
- Lanyon, W.E., 1967. Revision and probable evolution of the *Myiarchus* flycatchers of the West Indies. *Bull. Am. Mus. Nat. Hist.* 136, 329–370.
- Lanyon, W.E., 1975. Behavior and generic status of the Rufous Flycatcher of Peru. *Wilson Bull.* 87, 441–608.
- Lanyon, W.E., 1978. Revision of the *Myiarchus* flycatchers of South America. *Bull. Am. Mus. Nat. Hist.* 161, 429–627.
- Lanyon, W.E., 1982. Behavior, morphology, and systematics of the flammulated flycatcher of Mexico. *Auk* 99, 414–423.
- Lanyon, W.E., 1984. A phylogeny of the kingbirds and their allies. *Am. Mus. Novitat.* 2797, 1–28.
- Lanyon, W.E., 1985. A phylogeny of the myiarchine flycatchers. *Orn. Monogr.* 36, 360–380.
- Lanyon, W.E., 1986. A phylogeny of the thirty-three genera in the *Empidonax* assemblage of tyrant flycatchers. *Am. Mus. Novitat.* 2846, 1–29.
- Lovette, I.J., Bermingham, E., 1999. Explosive speciation in the New World *Dendroica* warblers. *Proc. R. Soc. Lond. B* 266, 1629–1636.
- Maddison, D.R., Maddison, W.P., 2002. MacClade 4.0. Sinauer, Sunderland.
- McKittrick, M.C., 1985. Monophyly of the Tyrannidae (Aves: comparison of morphology and DNA). *Syst. Zool.* 34, 35–45.
- Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Ridgely, R.S., Tudor, G., 1994. The Birds of South America: Oscines. Oxford University Press, Oxford.
- Ronquist, F., 1996. DIVA, ver. 1.1. Computer program available by anonymous ftp via <<http://www.ebc.uu.se/systzoo/staff/ronquist.html>>. Uppsala University, Uppsala.
- Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* 46, 195–203.
- Roy, M.S., Torres-Mura, J.C., Hertel, F., 1999. Molecular phylogeny and evolutionary history of the tit-tyrants (Aves: Tyrannidae). *Mol. Phylogenet. Evol.* 11, 67–76.
- Ryan, M.J., Rand, A.S., 1995. Female responses to ancestral advertisement calls in Túngara frogs. *Science* 269, 390–392.
- Sibley, C.G., Ahlquist, J.E., 1985. Phylogeny and classification of New World subsocial passerine birds (Passeriformes: Oligomyodi: Tyrannides). *Orn. Monogr.* 36, 396–428.
- Smith, J.W., 1971. Behavior of *Muscisaxicola* and related genera. *Bull. Mus. Comp. Zool.* 141, 23–268.
- Swofford, D., 2002. PAUP 4.0b10. Phylogenetic Analysis Using Parsimony. Sinauer, Sunderland.
- Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512–526.
- Traylor, M.E., 1977. A classification of the tyrant flycatchers (Tyrannidae). *Bull. Mus. Comp. Zool.* 148, 129–184.
- Weckstein, J.D., Zink, R.M., Blackwell-Rago, R.C., Nelson, D.A., 2001. Anomalous variation in mitochondrial genomes of white-crowned (*Zonotrichia leucophrys*) and golden-crowned (*Z. atricapilla*) sparrows: pseudogenes, hybridization, or incomplete lineage sorting? *Auk* 118, 231–236.
- Vuilleumier, F., 1971. Generic relationships and speciation patterns in *Ochthoeca*, *Myiotheretes*, *Xolmis*, *Neoxolmis*, *Agriornis*, and *Muscisaxicola*. *Bull. Mus. Comp. Zool.* 141, 181–232.
- Zimmer, J.T., 1938. Studies of Peruvian birds. No 29. The genera *Myiarchus*, *Mitrephanes*, and *Cnemotriccus*. *Am. Mus. Novitat.* 994.