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SYSTEMATICS AND EVOLUTION IN THE TITYRINAE (PASSERIFORMES: TYRANNOIDEA)

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Abstract.—We tested the monophyly and determined the phylogenetic relationships of the seven genera (*Laniisoma*, *Laniocera*, *Iodopleura*, *Pachyramphus*, *Schiffornis*, *Tityra*, and *Xenopsaris*) and 27 of the 31 recognized species of the subfamily Tityrinae using complete gene sequence data from the mitochondrial gene NADH dehydrogenase subunit 2. Monophyly of all seven genera was recovered using both weighted parsimony and Bayesian methods. Intergeneric relationships were nearly identical between the two methods and are largely in concordance with previous studies. Both analyses recovered two basal clades within the Tityrinae: one clade contained *Schiffornis*, *Laniocera*, and *Laniisoma*; the other clade consisted of *Iodopleura*, *Tityra*, *Xenopsaris*, and *Pachyramphus*. All genera in the Tityrinae that contained multiple species were monophyletic and are concordant with current taxonomy. We present the first phylogeny for *Pachyramphus* and suggest that *Platypsaris* is not valid. Character mapping of morphological, nest-construction, and breeding-system data on our phylogeny suggest conservative evolution of most characters. We recommend elevating the Tityrinae to family level. *Received 12 March 2005, accepted 19 November 2006.*

Key words: mitochondrial DNA, molecular systematics, NADH dehydrogenase subunit 2, *Schiffornis* assemblage, Tityrinae, Tyrannoidea.

Sistemática y Evolución de los Tityrinae (Passeriformes: Tyrannoidea)

Resumen.—Pusimos a prueba la monofilia y establecimos las relaciones filogenéticas de los siete géneros (*Laniisoma*, *Laniocera*, *Iodopleura*, *Pachyramphus*, *Schiffornis*, *Tityra* y *Xenopsaris*) y de 27 de las 31 especies reconocidas de la subfamilia Tityrinae, utilizando secuencias completas del gen mitocondrial de la subunidad 2 de la NADH deshidrogenasa. La monofilia de los siete géneros se recobró utilizando métodos de parsimonia con pesaje y de inferencia Bayesiana. Las relaciones intergenéricas observadas fueron casi idénticas entre los métodos y concuerdan en gran medida con estudios previos. Ambos análisis recobraron dos clados basales dentro de los Tityrinae: uno de ellos incluye a *Schiffornis*, *Laniocera* y *Laniisoma*, y el otro a *Iodopleura*, *Tityra*, *Xenopsaris* y *Pachyramphus*. Todos los géneros de Tityrinae para los que se incluyeron múltiples especies resultaron ser monofiléticos y concordantes con la taxonomía actual. Presentamos la primera filogenia para *Pachyramphus* y sugerimos que *Platypsaris* no es un género válido. El mapeo de caracteres morfólogicos, de construcción de nidos y de sistemas reproductivos sobre nuestra filogenia, sugiere que la mayoría de los rasgos evolucionan conservadoramente. Recomendamos que el taxón Tityrinae sea reconocido con el rango de familia.

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THE AVIAN SUBFAMILY Tityrinae (*sensu* Prum et al. 2000) consists of ~31 species (American Ornithologists' Union [AOU] 1998, Remsen et al. 2007) of New World suboscine passerines placed in seven genera (*Laniisoma*, *Laniocera*, *Iodopleura*, *Pachyramphus*, *Schiffornis*, *Tityra*, and *Xenopsaris*). The members of this group display impressive variation in nest architecture, plumage, and breeding systems (Prum and Lanyon 1989). The phylogenetic affinities of these genera are not completely understood, nor is the placement of this subfamily in relation to other tyrannoids. Recent studies have classified some genera as *incertae sedis* (Peters 1979, AOU 1998, Ericson et al. 2003, Remsen et al. 2007), or place them in various subfamilies of Tyrannidae (Sibley and Ahlquist 1990). Prum et al. (2000) suggested that these seven genera form a clade and should be recognized as the subfamily Tityrinae (we follow this nomenclature herein).

Recent phylogenetic work.—Ornithologists have long recognized Tyrannidae, Cotingidae, and Pipridae as separate but related lineages; historically, these families have been difficult to define (McKitrick 1985, Prum and Lanyon 1989). Recent work has confirmed monophyletic tyrannid, cotingid, and piprid radiations (Prum et al. 2000, Johansson et al. 2002) and clarified the position of some previously enigmatic genera. Unexpected, however, was the discovery of a previously unrecognized clade—the *Schiffornis* assemblage—that included genera previously assigned to each of these three families (Prum and Lanyon 1989). Evidence supporting the *Schiffornis* assemblage has accumulated (e.g., Prum et al. 2000), but several questions have remained unresolved—in particular, uncertainty over the relationship of *Tityra* to the *Schiffornis* assemblage, of the *Schiffornis* assemblage to other tyrannoids, and over relationships within the seven genera.

We used mitochondrial sequence data from 27 of the ~31 (AOU 1998, Remsen et al. 2007) species in the seven genera of the Tityrinae (*sensu* Prum et al. 2000) to address the following: (1) monophyly of the Tityrinae and each genus, (2) intergeneric relationships, (3) evolution of morphological characters and natural-history traits on the basis of our phylogeny, and (4) taxonomic changes suggested by our data.

METHODS

DNA sequencing and amplification.—We obtained genomic DNA from muscle tissue using QIAamp extraction kits following the manufacturer's protocols (Qiagen, Valencia, California). We generated mitochondrial sequence data from the NADH dehydrogenase subunit 2 (ND2) gene for at least one representative species from each of the seven genera in the Tityrinae, representatives of Pipridae (manakins), Cotingidae (cotingas), and Tyrannidae (flycatchers) (Table 1). These data were augmented with sequences from GenBank for a total of 63 taxa sampled. We lacked data for *Pachyramphus niger*, *Laniisoma buckleyi*, *Laniocera rufescens*, and *Iodopleura pipra*. We amplified the ND2 gene (1,041 base pairs [bp]) by polymerase chain reactions (PCR) using the primers L5215 (5′-TATCGGGCCCATACCCCGAAAAT-3′) (Hackett 1996) and H1064 (5′-CTTTGAAGG-CCTTCGGTTTA-3′) (Drovetski et al. 2004). Thermal cycling was done under the following profile: an initial 94°C hotstart for 150 s, and 35 cycles with 94°C denaturing for 30 s, 55°C annealing for 30 s, extension at 72°C for 70 s, and terminal extension at 72°C for 10 min. We purified PCR products using QIAquick PCR purification kits following the manufacturer's protocols (Qiagen). We amplified PCR products for sequencing using the above primers and an additional internal primer L347 (5′-CCATTCCACTTCTGATTCCC-3′) (Drovetski et al. 2004). Sequencing was done on an ABI-3700 automated sequencer (Applied Biosystems, Foster City, California), following the manufacturer's protocols. Sequences were aligned and edited with SEQUENCHER, version 3.1.1 (Gene Codes, Ann Arbor, Michigan). Aligned sequences were checked for internal stop codons and indels (insertions or deletions) to ensure against nuclear pseudogenes.

Phylogenetic methods.—Parsimony analyses were conducted in PAUP*, version 4.0b10 (Swofford 2002). *Grallaria squamigeria* was selected as the outgroup taxon on the basis of Chesser (2004) and Barker et al. (2004). We used two weighting schemes for third-position transitions versus transversions. First, we obtained the average ratio of third-position transitions: transversions (TI:TV) in pairwise comparisons of sequences. Second, we used one of two parsimonious trees obtained from an equally

TABLE 1. List of samples used in the present study. Those marked with asterisks identify multiple individuals of a particular taxon. GenBank numbers in bold were obtained from previous studies. Locality data are provided for newly acquired samples only.

Taxon	Collection	Tissue or preparation number	Country: state/department	GenBank number
Pachyramphus aglaiae	KU	2130	Mexico: Campeche	DQ363934
P. albogriseus	LSU	5154	Peru: Lambayeque Department	DQ363935
P. castaneus	KU	3756	Paraguay: Caazapa/Itapua	DQ363936
P. castaneus	LSU	10641	Peru:Ucayali	DQ363937
P. cinnamomeus	ANSP	3568	Ecuador: Azuay	DQ363938
P. homochrous	LSU	2306	Panama: Provincia de Darien	DQ363939
P. major	UWBM	70137	Nicaragua: Matagalpa	DQ363940
*P. marginatus**	AMNH	1407	Venezuela: Amazonas	DQ363941
*P. marginatus***	ANSP	8312	Guyana: Potaro-Siparuni; Iwokrama Reserve	DQ363942
P. marginatus	LSU	2951	Peru: Loreto	DQ363943
P. minor	ANSP	8345	Guyana: Potaro-Siparuni; Iwokrama Reserve	DQ363944
*P. polychopterus****	ANSP	7197	Panama: Veraguas; Cascajilloso	DQ363945
P. polychopterus	KU	3804	Paraguay: Caazapa/Itapua	DQ363946
*P. polychopterus****	LSU	9540	Bolivia: Pando Department	DQ363947
*P. polychopterus**	UWBM	RCF 2083	Bolivia: Santa Cruz Department	DQ363948
*P. polychopterus***	UWBM	70801	Argentina: Provincia de Corrientes	DQ363949
P. rufus	LSU	7299	Peru: Loreto Department	DQ363950
P. spodiurus	ANSP	5204	Ecuador: Loja; Zapotillo	DQ363951
P. surinamus	USNM	10368	Guyana: Sipu River	DQ363952
P. validus	KU	3314	Paraguay: Misiones	DQ363953
*P. validus****	KU	3562	Paraguay: Amambay	DQ363954
*P. validus***	UWBM	54450	Argentina: Provincia de Corrientes	DQ363955
*P. validus**	UWBM	VGR 276	Bolivia: Santa Cruz Department	DQ363956
P. versicolor	LSU	1702	Peru: Pasco Department	DQ363957
P. viridis	KU	3298	Paraguay: Misiones	DQ363958
*P. viridis***	KU	48	Paraguay: Concepcion	DQ363959
*P. viridis**	UWBM	70490	Argentina: Provincia de Corrientes	DQ363960
P. xanthogenys	ANSP	4533	Ecuador: Zamora-Chinchipe; La Chonta	DQ363961
Xenopsaris albinucha	ANSP	8359	Guyana: Potaro-Siparunit; Annai	DQ363962
*Tityra cayana**	LSU	9604	Bolivia: Pando Department	DQ363963
T. cayana	UWBM	54488	Argentina: Provincia de Misiones	DQ363964
*T. inquisitor**	LSU	18568	Bolivia: Santa Cruz Department	DQ363965

TABLE 1. Continued.

Taxon	Collection	Tissue or preparation number	Country: state/department	GenBank number
T. inquisitor	UWBM	70294	Argentina: Provincia de Misiones	DQ363966
T. semifasciata	LSU	18275	Bolivia: Santa Cruz Department	DQ363967
*T. semifasciata***	UWBM	69160	Nicaragua: Departmento de Granada	DQ363968
*T. semifasciata**	UWBM	RCF2077	Panama: Provincia de Panama	DQ363969
Schiffornis major	KU	1426	Peru: Madre de Dios	DQ363970
S. turdina	UWBM	SMB 229	Panama: Provincia de Panama	DQ363971
S. virescens	KU	332	Paraguay: Caazapa	DQ363972
*S. virescens**	FMNH	395452	Brazil: Sao Paulo	**AY136618**
Iodopleura fusca	ANSP	8331	Guyana: Potaro-Siparuni; Iwokrama Reserve	DQ363973
I. isabellae	ANSP	5127	Ecuador: Napo; Quindchiliaqui	DQ363974
Laniisoma elegans	ANSP	1558	Ecuador: Morona-Santiago; Santiago	DQ363975
Laniocera hypopyrra	KU	1408	Peru: Madre de Dios	DQ363976
Tyranneutes virescens	KU	1400	Guyana: Kurupukari	DQ363977
Neopelma chrysocephalum	KU	1376	Guyana: Iwokrama Reserve	DQ363978
Snowornis subalaris	LSU	5560	Peru: San Martin Department	DQ363979
Lipaugus fuscocinereus	ANSP	5039	Ecuador: Zamora-Chinchipe; San Andres	DQ363980
L. vociferans	LSU	12598	Bolivia: Santa Cruz Department	DQ363981
L. unirufus	ANSP	2399	Ecuador: Esmeraldas	DQ363982
Haematoderus militaris	KU	1348	Guyana: Iwokrama Reserve	DQ363983
Pipreola frontalis	LSU	5559	Peru: San Martin Department	DQ363984
P. pulchra	LSU	1625	Peru: Pasco Department	DQ363985
Piprites chloris	KU	1415	Peru: Madre de Dios	DQ363986
Manacus manacus	ANSP	2247	Ecuador: Esmeraldas; Alto Tambo	DQ363987
Chiroxiphia caudata				**AY136620**
Sayornis phoebe				**AF447641**
Sublegatus modestus				**AF447650**
Contopus cooperi				**AF447633**
Empidonax flaviventris				**AF447628**
Cnemotriccus fuscatus				**AF447649**
Mitrephanes phaeocercus				**AF447646**
Grallaria squamigera				**AY139637**

Abbreviations: KU = University of Kansas Museum of Natural History; LSU = Louisiana State University Museum of Natural History; UWBM = Burke Museum of Natural History, University of Washington; ANSP = Academy of Natural Sciences, Philadelphia; USNM = National Museum of Natural History, Smithsonian Institution, and FMNH = Field Museum of Natural History.

weighted analysis (not shown) and used the maximum-likelihood (ML) option in tree scores in PAUP* (Swofford 2002) to obtain an estimate of third-position TI:TV bias over this tree. These two weighting schemes were implemented in parsimony analyses using heuristic tree searches with 20 addition–sequence replicates and tree bisection-reconnection (TBR) branch-swapping. Nodal support for each weighting scheme was determined using nonparametric bootstraps with 500 pseudoreplicates (Felsenstein 1985), with heuristic searches, 10 random-addition sequences replicates, and TBR branch-swapping.

A Bayesian analysis was done in MRBAYES, version 3.0b4 (Huelsenbeck and Ronquist 2001). Optimal model parameters, as chosen by Akaike's Information Criterion (AIC) implemented in MODELTEST, version 3.06 (Posada and Crandall 1998), were used in conjunction with uniform priors. Four Markov chains were run for 5×10^6 generations with every 100 generations sampled for later analysis. Initial runs using the same priors and model parameters suggested that the burn-in (i.e., those generations obtained before log-likelihood values stabilized) was approximately 7×10^4. However, to ensure that subsequent analyses included only stabilized data, we set the burn-in at 1×10^6 generations. A 50% majority consensus tree was obtained from the remaining data, as were the average parameter values for the model. Posterior probabilities (proportion of sampled trees in which a given node appears) of nodes and tree parameters were estimated from the remaining trees.

Results

Molecular results.—The aligned data set consisted of 1,041 bp for 63 individuals. All new sequences were deposited in GenBank (accession numbers DQ363934–363987). No indels or internal stop codons were observed. The empirical base composition was [A] = 0.30464, [C] = 0.32640, [G] = 0.09676, and [T] = 0.27220. Of the 1,041 characters, 389 were constant, 78 were variable but uninformative, and 574 were potentially informative. By codon position, these were (1) first position = 153 constant, 38 variable but uninformative, and 156 potentially informative; (2) second position = 233 constant, 34 variable (uninformative), and 80 potentially informative; and (3) third postition = 3 constant, 6 variable (uninformative), and 338 potentially informative. The ML estimate of third-position TI:TV ratio over a parsimony tree (not shown) obtained by a heuristic search on equally weighted data was 10.0:1.0. The empirical TI:TV ratio of 2.85:1.0 was obtained by taking the average ratio in pairwise comparisons of third positions.

Phylogenetic results.—Two equally maximum-parsimony (MP) trees (Fig. 1; one MP tree shown) resulted from the analysis with third-position transversions weighted 10× more than transitions (TI:TV = 10.0/1). The two trees differed only in the placement of *Pachyramphus c. castaneus*). Likewise, the TI:TV = 2.85/1 analysis resulted in two equally MP trees (Fig. 2), with the only difference between the two being the placement of *P. c. castaneus*. *Pachyramphus c. castaneus* was either sister to *P. c. saturatus* or sister to a clade containing *P. cinnamomeus* and *P. c. saturatus*. Other than internally alternating positions of *P. c. castaneus*, the two weighted analyses were nearly identical, differing only in the placement of *P. major* and *P. marginatus*, discussed below. Hereafter, the two weighting schemes will be referred to as the 10.0 and 2.85 analyses.

Both weighting schemes recovered a monophyletic Tityrinae with moderate nodal support (70% and 79%, 10.0 [Fig. 1] and 2.85 [Fig. 2] analyses, respectively). Two primary clades within the Tityrinae were recovered. The first clade contained *Laniocera*, *Laniisoma*, and the three sampled species of *Schiffornis*. This clade has strong bootstrap support (94% and 99%, 10.0 and 2.85 analyses, respectively). The three sampled species of *Schiffornis* are monophyletic. *Laniocera hypopyrra* was sister to the *Schiffornis* species with weak nodal support, contrary to the Bayesian analysis (see below).

The remaining genera in the Tityrinae (*Pachyramphus*, *Tityra*, *Xenopsaris*, and *Iodopleura*) formed the other clade, with relatively weak (57% [10.0 analysis] and 69% [2.85 analysis]) bootstrap support. The two sampled species of *Iodopleura* are monophyletic (100% both weighting schemes) and sister to the clade containing *Xenopsaris*, *Tityra*, and *Pachyramphus*. In both weighting schemes, *Tityra* is sister to the monotypic *Xenopsaris* and *Pachyramphus*. The three species of *Tityra* are monophyletic, with 95% or greater nodal support under both weighting schemes, with *Tityra* inquisitor sister to *T. cayana* and *T. semifasciata* in both.

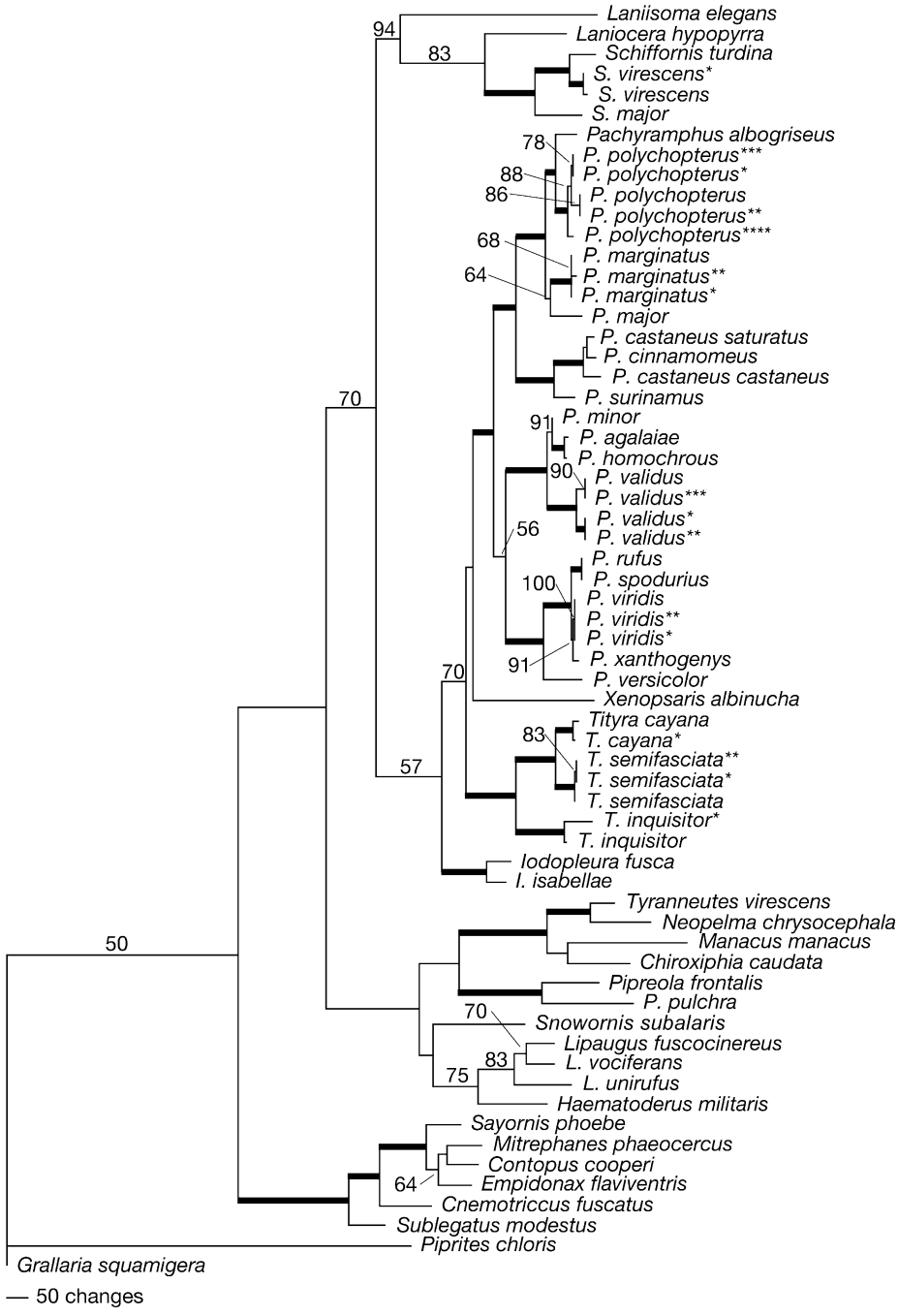

FIG. 1. An MP tree with third-position transversion weighted 10× more than transversions (TL = 8,643.0, CI = 0.324, RI = 0.712, RC = 0.231, HI = 0.676). Thickened branches indicate bootstrap support of >96%. Values of 50–96% are shown above branches. Branches with no values received <50% support. Bootstrap support was determined using 500 pseudoreplicates.

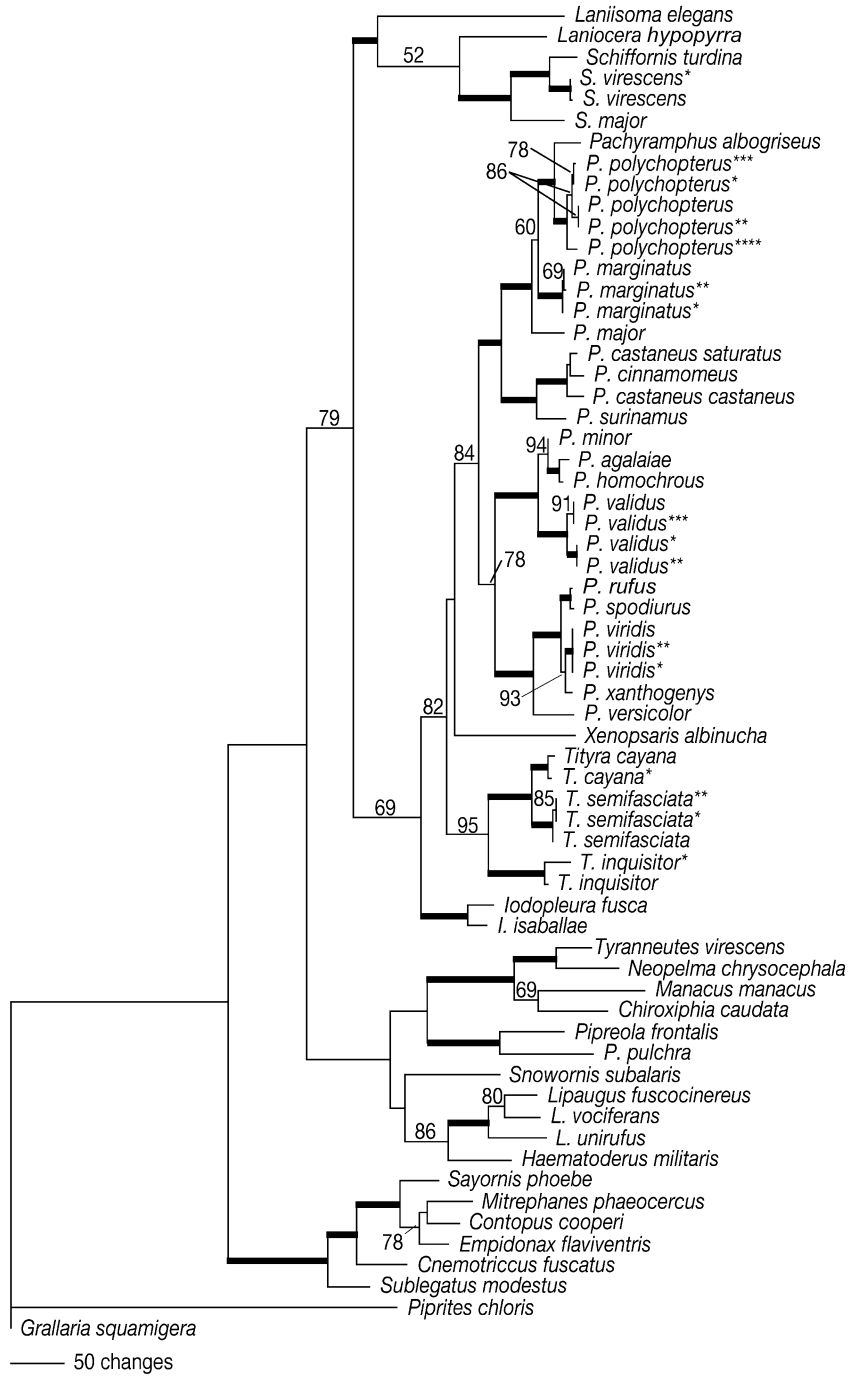

FIG. 2. An MP tree with third-position transversions weighted 2.85× more than transversions (TL = 3,387.60, CI = 0.328, RI = 0.697, RC = 0.229, HI = 0.672). Thickened branches indicate bootstrap support of >96%. Values of 50–96% are shown above branches. Branches with no values had <50% support. Bootstrap support was determined using 500 replicates.

A clade containing 16 of the 17 recognized species of *Pachyramphus* was recovered in both weighting schemes with moderate support (84%) in the 2.85 analysis and 100% nodal support in the 10.0 analysis (Figs. 1 and 2). More than half (14 of 25) of the nodes within *Pachyramphus* were recovered with >95% nodal support under both weighting schemes. All but one of the currently recognized species, for which we have more than one exemplar, were monophyletic in both analyses. *Pachyramphus castaneus* was paraphyletic with respect to *P. cinnamomeus* in one of the two MP trees under both weighting schemes (<50% support). *Pachyramphus surinamus* was sister to these taxa in both analyses, with 100% nodal support.

In both analyses, this clade was sister to a clade containing *P. major*, *P. marginatus*, *P. albogriseus*, and *P. polychopterus*. In the 10.0 analysis, *P. major* was sister to *P. marginatus*, with moderate support; however, in the 2.85 analysis, *P. major* was sister to a more inclusive clade containing *P. marginatus*, *P. albogriseus*, and *P. polychopterus*, again with only moderate bootstrap support. In both analyses, *P. albogriseus* was sister to *P. polychopterus*.

The remaining *Pachyramphus* species formed a clade with low (56%; 10.0 analysis) to moderate (78%; 2.85 analysis) bootstrap support, but identical topologies in both analyses. In one of the two primary clades within this group, *P. versicolor* was sister to four taxa (*P. xanthogenys*, *P. viridis*, *P. rufus*, and *P. spodiurus*). *Pachyramphus xanthogenys* was sister to *P. viridis*; these two taxa were sister to a monophyletic *P. rufus* and *P. spodiurus*. Internal nodal support was ≥91% for all nodes within this clade (Figs. 1 and 2). Sister to this group, with strong (≥90%) internal nodal support, were *P. validus*, *P. minor*, *P. aglaiae*, and *P. homochrous*. *Pachyramphus aglaiae* and *P. homochrous* were sister to *P. minor*. These three taxa were sister to *P. validus*.

Both weighting schemes placed the Tityrinae clade sister to a clade containing members of Cotingidae, Pipridae, and two putative piprids, *Tyranneutes* and *Neopelma*. However, nodal support for this relationship was weak (<50% for both weighting schemes). *Snowornis subalaris*, which was only recently removed from the genus *Lipaugus* (Prum 2001), was sister to a clade containing *Haematoderus militaris* and the three species of *Lipaugus*. The problematic *Piprites chloris* was sister to all other ingroup taxa.

The Bayesian analysis searched the Markov chains under a general time-reversal (GTR) model (Swofford et al. 1996) with the proportion of invariable sites (I) and gamma-shape parameter (G) allowed to vary as determined by AIC in MODELTEST (Posada and Crandall 1998). A total of 40,000 (4×10^6 generations) trees was analyzed after the burn-in was removed.

The Bayesian topology (Fig. 3) resulted in a monophyletic Tityrinae with a 1.00 posterior probability (PP). The relationships within the Tityrinae were nearly identical to the two weighted parsimony analyses, with only a few differences. First, *Laniisoma elegans* and *Laniocera hypopyrra* were sister taxa with a low posterior probability (PP = 0.80). Second, a polytomy of the genera *Xenopsaris*, *Tityra*, and *Pachyramphus* was recovered.

Unlike the two parsimony analyses, the Bayesian analysis placed Tityrinae sister to Tyrannids and *Piprites chloris*. However, the posterior probability for placing Tityrinae within the Tyrannids was weak (PP = 0.81), which was consistent with the parsimony analyses.

Discussion

Monophyly and intergeneric relationships of Tityrinae.—We have shown for the first time that phylogenetic analyses including all seven genera of the putative Tityrinae (*sensu* Prum et al. 2000) recovered monophyly of the group. This clade was robust to model and methodology choices. Two syringeal characters (constriction of the tracheobronchial junction and insertion of an intrinsic muscle on the A1 and B1 elements of the syrnix; Prum and Lanyon 1989) provide additional support for the monophyly of the Tityrinae. Before Prum and Lanyon's (1989) work, these genera were placed in three separate families. As observed by Prum et al. (2000), *Tityra* is within the Tityrinae; however, our data suggest that this genus is not closely allied with *Schiffornis*, as Prum et al. (2000) suggested. Placing *Tityra* within this clade suggests that the two syringeal characters were lost in this genus (Fig. 4)

Intergeneric relationships within the Tityrinae were consistent between the two analytical methods and with Chesser's (2004) topology. Our parsimony analyses and the results of Prum and Lanyon (1989) placed *Xenopsaris* sister to *Pachyramphus*. This relationship is supported

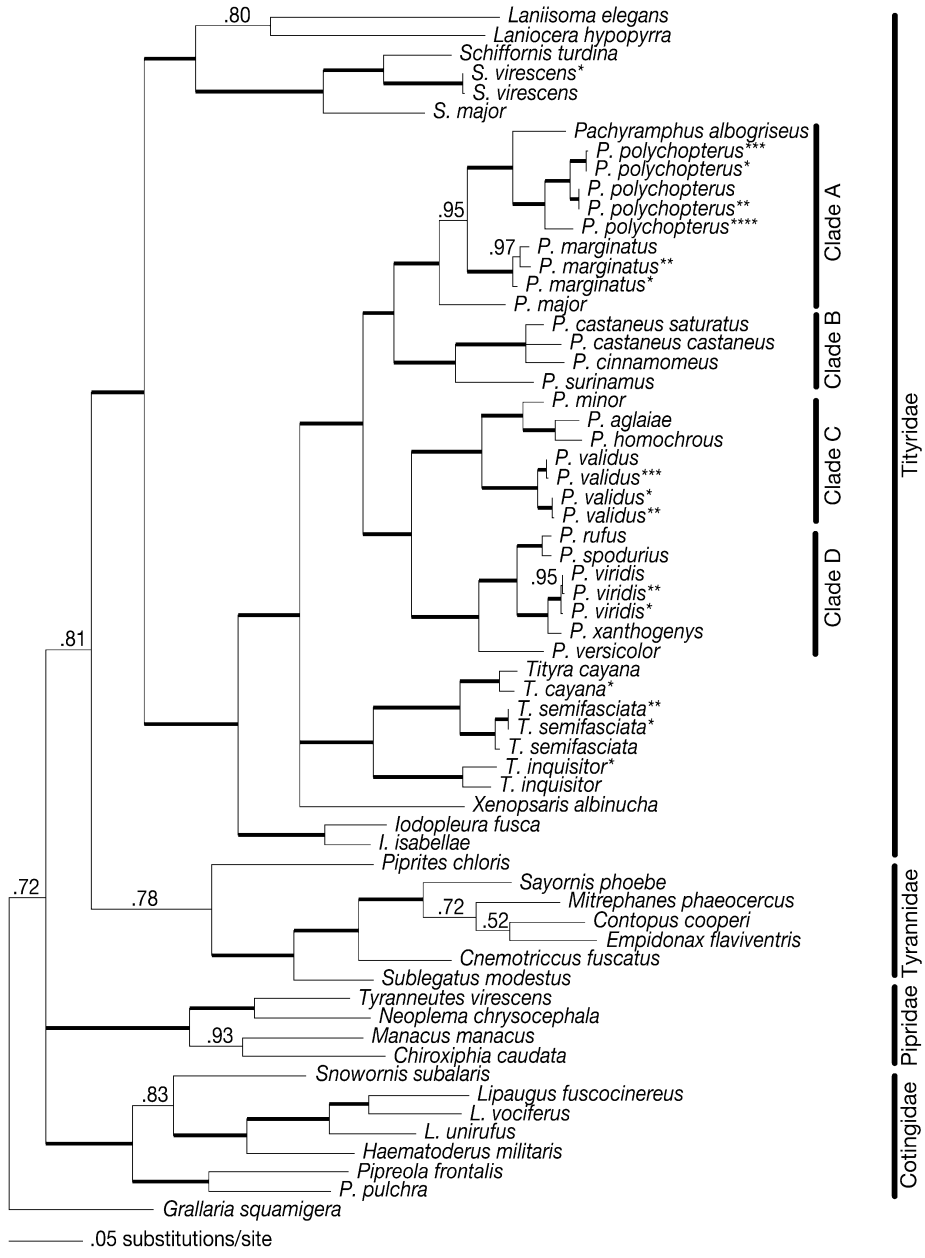

FIG. 3. A 50% Bayesian consensus tree obtained from the remaining 40,000 trees retained after burn-in data were removed. Averaged model parameters of post-burn-in data points were (mean ± SD): –lnL = 18,144.026 ± 8.76, TL = 8.7214 ± 0.448, r[G-T] = 1.0 ± 0.0, r[C-T] = 5.5732 ± 1.234, r[C-G] = 0.6652 ± 0.201, r[A-T] = 0.6329 ± 0.159, r[A-G] = 15.1823 ± 3.193, r[A-C] = 0.2607 ± 0.065, p[A] = 0.3454 ± 0.11, p[C] = 0.3545 ± 0.009, p[G] = 0.0514 ± 0.003, p[T] = 0.2486 ± 0.007, α = 0.8486 ± 0.057, pinvar = 0.3198 ± 0.017. Posterior probabilities were calculated from the 40,000 trees remaining after burn-in trees were removed. Thickened branches indicate posterior probabilities of >0.96. Values between 0.50–0.96 are shown above branches. Branches with no values had <0.50 posterior probabilities. Labeled bars refer to clades discussed in text.

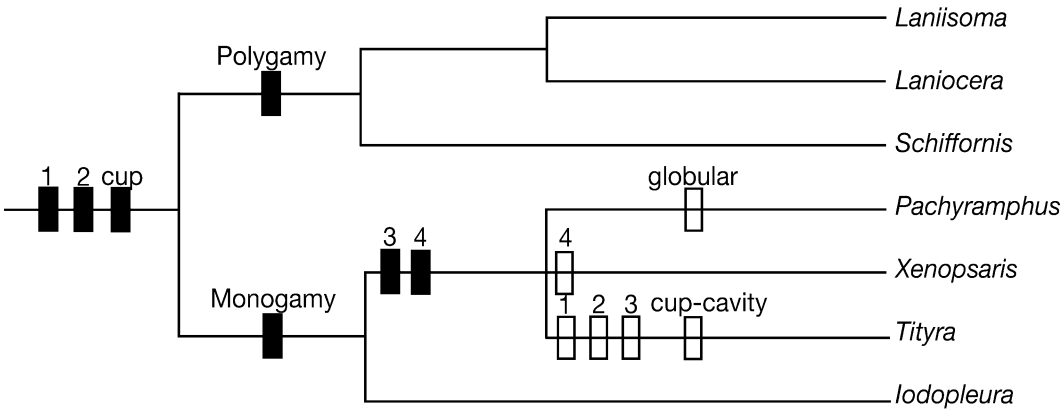

FIG. 4. Simplified cladogram of the generic-level relationships and characters within Tityridae. Characters: 1 = constriction of the tracheobronchial junction, 2 = insertion of the intrinstic muscle on the A1 and B1 elements of the syrinx, 3 = insertion of the *tracheolateralis* muscle on the ventral end of the A1 element of the syrinx, 4 = truncated ninth primary. Hypothesized nest of Tityridae is a cup nest. "Globular" refers to the globular-shaped nest of the members of the genus *Pachyramphus*, and "cup-cavity" refers to the cup placed inside a cavity nest by the three species of *Tityra*. The nest of *Laniisoma* spp. is unknown but hypothesized to be a cup. The ancestral breeding system of the group is unknown, as is the system of *Xenopsaris* and *Tityra*. A solid rectangle represents the origin of a character. An empty rectangle indicates loss or change from a previous character state. Characters 1–4 are from Prum and Lanyon (1989).

by one morphological synapomorphy (insertion of the *tracheolateralis* muscle on the ventral end of the A1 element of the syrinx; Prum and Lanyon 1989). The low bootstrap value in our parsimony analyses and the polytomy observed in the Bayesian analysis suggest little confidence in the relationships of *Xenopsaris*, *Pachyramphus*, and *Tityra*. Morphology does not provide a resolution of this polytomy. For example, if *Tityra* were sister to *Pachyramphus*, it would require either the secondary loss of this character in *Tityra* or two independent origins in *Pachyramphus* and *Xenopsaris* (Fig. 4). Thus, placing *Xenopsaris* sister to *Pachyramphus* is the most parsimonious reconstruction of this character, requiring only one origin and one loss. By contrast, *Tityra* and *Pachyramphus* both possess truncated ninth primaries. Prum and Lanyon (1989) suggested that this character is not homologous, because the two conditions differ slightly in the extent of the truncation and narrowing of the feather. Our results suggest that this condition is likely homologous, having arisen in a *Tityra–Pachyramphus* ancestor or a *Tityra–Pachyramphus–Xenopsaris* ancestor, then secondarily lost in *Xenopsaris* (Fig. 4). However, placing *Tityra* sister to *Pachyramphus* on the basis of this character conflicts with the syringeal character described above. Additional data will be needed to resolve this node and determine the evolution of these characters.

Schiffornis, *Laniocera*, and *Laniisoma* are monophyletic in both analyses. The position of *Laniocera hypopyrra* was unstable between the two methods. The relatively long branches of *Laniocera* and *Laniisoma* (e.g., Fig 3) may have caused *Laniocera* to group with *Schiffornis* in both of the parsimony analyses. Prum and Lanyon (1989) recovered a clade containing these three taxa, with *Schiffornis* sister to *Laniisoma* and *Laniocera*.

Monophyly of the 16 species of *Pachyramphus* was observed under both parsimony and Bayesian methods. Within *Pachyramphus*, we recognize four groups (Fig. 3) that all received strong bootstrap values and posterior probabilities. Clade A consists of four species of sexually dimorphic species: *P. major*, *P. marginatus*, *P. polychopterus*, and *P. albogriseus*. Males in this clade possess black crowns, white-tipped wing coverts and rectrices (rusty color in females), and, in *P. major* and *P. polychopterus*, white scapulars. In clade B, *P. castaneus* and *P. cinnamomeus* are sexually monochromatic, with

males mostly cinnamon and rufous in color; and *P. surinamus* is sexually dichromatic, with males a glossy-black above and white below. Clade C is composed of *P. minor*, *P. aglaiae*, *P. homochrous*, and *P. validus*, which are robust, sexually dimorphic, uniformly colored becards with larger bills than other species. Three (*P. viridis*, *P. xanthogenys*, and *P. versicolor*) of the five species in Clade D can be characterized by the presence of yellow plumage either in the cheek or upper chest and olive-colored backs (black in *P. versicolor*) in males. By contrast, male *P. spodurius* and *P. rufus* have black crowns, with *P. spodurius* nearly uniform black above and grayish below, whereas *P. rufus* is grayer above. The females of both species tend to be a uniform rufous. Males of *P. versicolor* are unique among becards in having gray barring on the cheeks continuing down to the undertail coverts. Biogeography, vocalizations, and morphological character evolution in *Pachyramphus* spp. will be discussed in greater detail in another publication (B. R. Barber unpubl. data).

Higher-level Tyrannoid relationships.—The placement of *Snowornis subalaris* outside of a clade containing *Lipaugus fuscocinerus*, *L. unirufus*, *L. vociferous*, and *Haematoderus militaris* in our analysis supports the recommendation of Prum (2001).

The genera *Tyranneutes* and *Neopelma* were found to be sister taxa, supporting the results of Lanyon (1985) and Prum (1990). Our results and those of Lanyon (1985) and Prum (1990) contained different representatives of these genera (Lanyon [1985] included *T. stolzmanni* and *N. sulphureiventer*; Prum [1990] *N. aurifrons*), providing strong evidence of the close relationship of the two genera and the inclusion of all the species that constitute them. This clade was sister to a manakin clade in our study, a relationship first identified by Lanyon (1985) and supported by Chesser (2004). Thus, the genera *Tryanneutes* and *Neopelma* should be recognized as members of the Pipridae.

Piprites chloris exhibits one of the longest branches in this data set (e.g., Fig. 2), which possibly explains its instability between the two analytical methods. This taxon has a problematic taxonomic history, and additional data will be required to firmly establish its phylogenetic affinities within Tyrannoidea.

Evolution of natural-history traits.—The genera in the Tityrinae have an impressive diversity of nest architecture, plumage, and breeding systems (Prum and Lanyon 1989). The nests of most species in the Tityrinae are a variation of the cup-like pattern observed in most other Tyrannoids. Enigmatic for the group are the nests of *Tityra* spp., which are loosely constructed cup-like nests that are placed in tree cavities (Skutch 1969), and the globular nest of *Pachyramphus* spp. with side and bottom entrances, which are often placed near beehives (Skutch 1967, 1969). Nests of *Laniocera* spp. and *Schiffornis* spp. are bulky cup nests constructed of leaves (Skutch 1969, Londoño and Cadena 2003). The nest of *Laniisoma* spp. remains undescribed, but it is likely an open-cup nest. *Iodopleura* spp. also make a cup nest, but it is very tidy and made primarily from cobwebs and fungus, often described as resembling a hummingbird nest (Smith 1971, DiGiacomo and Leiberman 2000). *Xenopsaris* spp. also make a compact cup-like nest, but it is constructed from fine dry grasses and fibers and may include spider webs (Skutch 1969). Given that most other Tyrannoids and at least three of the Tityrinae genera make some variation of cup-like nest, we reconstructed the ancestral nest condition of Tityrinae to be a cup-like nest (Fig. 4). Thus, the globular nest of *Pachyramphus* spp. and the cup-in-a-cavity nest of *Tityra* spp. evolved from a cup nest. This is perhaps an example of diverging nest construction, with the darkened cavity retained, as in the Furnariidae (Zyskowski and Prum 1999). If, in fact, *Xenopsaris* is sister to *Pachyramphus*, the enclosed condition of *Tityra* and *Pachyramphus* evolved independently, or it evolved in a *Tityra–Pachyramphus–Xenopsaris* ancestor with the ancestral cup nest regained by *Xenopsaris*. However, a *Tityra* and *Pachyramphus* relationship would be the MP evolution of an enclosed condition, requiring only one appearance of this character and no secondary loss of the cup-like condition.

Biparental care (and apparent monogamy) with both sexes constructing the nest is known for species in *Pachyramphus* and *Iodopleura* (Snow 1982). In *Iodopleura*, nonbreeding young from previous clutches have been observed helping at a nest, a behavior nearly unique to the Cotingidae (*Querula purpurata*; Snow 1982). By contrast, *Schiffornis* is polygynous, leaving nest construction and nestling care and rearing to females (Skutch 1969). The recently described breeding behavior of *Laniocera* suggests that just

one parent (probably the female) spends time at the nest (Londoño and Cadena 2003). There is anecdotal evidence that males of *Laniocera* spp. (Hilty and Brown 1986) and *Laniisoma* spp. (Snow 1982) sing from dispersed–solitary perches, which suggests a polygynous breeding system. The breeding system of *Xenopsaris* is not known but is likely monogamous on the basis of our phylogeny (Fig. 4).

Conclusions and Taxonomic Recommendations

Using parsimony and Bayesian methods, we recovered a monophyletic Tityrinae that is distinct from both the Tyrannidae and a clade containing members of the Pipridae and Cotingidae. In addition to our data, the monophyly of this group is supported by two syringeal characters (Prum and Lanyon 1989). Furthermore, Tityridae exhibit unique morphological characters, behavior, and nest construction. These data warrant elevation of the subfamily Tityrinae to family level, thus becoming Tityridae. Two subfamilies within the Tityridae should be recognized: (1) Laniisominae (type genus *Laniisoma*; Swainson 1831), including *Schiffornis*, *Laniocera*, and *Laniisoma*; and (2) Tityrinae (type genus *Tityra*; Vieillot 1816), comprising *Tityra*, *Pachyramphus*, *Iodopleura*, and *Xenopsaris*. These clades were also recovered by Chesser (2004) using independent loci.

Some authors (e.g., Ridgely and Greenfield 2001) have suggested that *Pachyramphus homochrous*, *P. minor*, *P. aglaiae*, and *P. validus* (our clade C; Fig. 3) are best placed in the genus *Platypsaris* (Sclater 1857); recognizing this genus would make *Pachyramphus* paraphyletic; thus, *Platypsaris* is not valid.

The results presented here provide the first molecular assessment that includes all seven genera of this group and the most complete phylogenetic hypothesis for *Pachyramphus* to date. Relationships were nearly identical between parsimony and Bayesian approaches and to the topology of Prum and Lanyon (1989). Mapping of morphological, nest-construction, and breeding-system data provided insight into evolution in this group. The trichotomy of *Xenopsaris*, *Pachyramphus*, and *Tityra* caused uncertainy in the evolution of nest construction, truncation of the ninth primary, and insertion of a unique syringeal muscle. Additional data will be required to resolve this trichotomy and provide insight into the polarity of the evolution of nest construction (e.g., cup → cup-in-a-cavity → globular) and the evolution of other characters in this group. We lack sequence data for *P. niger*. This species is a robust, uniformly colored becard that is phenotypically similar to species in the former genus *Platypsaris* (clade C). In addition to having similar plumages, vocalizations of *P. niger* are similar to those of *P. aglaiae* (B. R. Barber and N. H. Rice unpubl. data). We posit that *P. niger* is closely related to *P. aglaiae*. The phylogeny presented here provides a preliminary framework for further study of the biogeography, plumage, and vocal evolution of this group.

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Literature Cited

AMERICAN ORNITHOLOGISTS' UNION. 1998. Checklist of North American Birds, 7th ed. American Ornithologists' Union, Washington, D.C.

BARKER, F. K., A. CIBOIS, P. SCHIKLER, J. FEINSTEIN, AND J. CRACRAFT. 2004. Phylogeny and diversification of the largest avian radiation. Proceedings of the National Academy of Sciences USA 101:11040–11045.

CHESSER, R. T. 2004. Molecular systematics of New World suboscine birds. Molecular Phylogenetics and Evolution 32:11–24.

DIGIACOMO, A. G., AND J. LEIBERMAN. 2000. Description of the nestling of *Xenopsaris albinucha*. Hornero 15:129–130.

DROVETSKI, S. V., R. M. ZINK, I. V. FADEEV, E. V. NESTEROV, E. A. KOBLIK, Y. A. RED'KIN, AND

S. Rohwer. 2004. Mitochondrial phylogeny of *Locustella* and related genera. Journal of Avian Biology 35:105–110.

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

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

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

Hilty, S. L., and W. L. Brown. 1986. A Guide to the Birds of Colombia. Princeton University Press, Princeton, New Jersey.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

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

Lanyon, S. M. 1985. Molecular perspective on higher-level relationships in the Tyrannoidea (Aves). Systematic Zoology 34:404–418.

Londoño, G. A., and C. D. Cadena. 2003. The nest and eggs of the Cinereous Mourner (*Laniocera hypopyrra*). Wilson Bulletin 115: 115–118.

McKitrick, M. C. 1985. Monophyly of the Tyrannidae (Aves): Comparison of morphology and DNA. Systematic Zoology 34: 35–45.

Peters, J. L. 1979. Check-list of Birds of the World, vol. 8. Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

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

Prum, R. O. 1990. A test of the monophyly of the manakins (Pipridae) and of the contigas (Cotingidae) based on morphology. Occasional Papers of the Museum of Zoology of the University of Michigan, no. 72.2

Prum, R. O. 2001. A new genus for the Andean Green Pihas (Cotingidae). Ibis 143:307–309.

Prum, R. O., and W. E. Lanyon. 1989. Monophyly and phylogeny of the *Schiffornis* group (Tyrannoidea). Condor 91:444–461.

Prum, R. O., N. H. Rice, J. A. Mobley, and W. W. Dimmick. 2000. A preliminary phylogenetic hypothesis for the cotingas (Cotingidae) based on mitochondrial DNA. Auk 117: 236–241.

Remsen, J. V., Jr., C. D. Cadena, A. Jaramillo, M. Nores, J. F. Pacheco, M. B. Robbins, T. S. Schulenberg, F. G. Stiles, D. F. Stotz, and K. J. Zimmer. 2007. A classification of the bird species of South America. American Ornithologists' Union. [Online.] Available at www.museum.lsu.edu/~Remsen/SACCBaseline.html.

Ridgely, R. S., and P. J. Greenfield. 2001. The Birds of Ecuador: Status, Distribution, and Taxonomy, vol. 1. Cornell University Press, Ithaca, New York.

Sclater, P. L. 1857. Review of the species of the South American subfamily Tityrinae. Pages 67–80 *in* Proceedings of the Zoological Society of London, part XXV.

Sibley, C. G., and J. E. Ahlquist. 1990. Phylogeny and Classification of Birds: A Study in Molecular Evolution. Yale University Press, New Haven, Connecticut.

Skutch, A. F. 1967. Life Histories of Central American Highland Birds. Publications of the Nuttall Ornithological Club, no. 7.

Skutch, A. F. 1969. Life Histories of Central American Birds, vol. III. Pacific Coast Avifauna, no. 35.

Smith, W. J. 1971. Behavioral characteristics of serpophaginine tyrannids. Condor 73:259–286.

Snow, D. W. 1982. The Cotingas: Bellbirds, Umbrellabirds, and Other Species. Comstock Publishing, Ithaca, New York.

Swofford, D. S. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0b10. Sinauer, Sunderland, Massachusetts.

Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pages 407–514 *in* Molecular Systematics, 2nd ed. (D. M. Hillis, C. Moritz, and B. K. Mable, Eds.). Sinauer Associates, Sunderland, Massachusetts.

Zyskowski, K., and R. O. Prum. 1999. Phylogenetic analysis of the nest architecture of Neotropical ovenbirds (Furnariidae). Auk 116:891–911.

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