

## **A Molecular Phylogeny of Black-Tyrants (Tyrannidae: *Knipolegus*) Reveals Strong Geographic Patterns and Homoplasy in Plumage and Display Behavior**

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Source: *The Auk*, 129(1):156-167. 2012.

Published By: The American Ornithologists' Union

URL: <http://www.bioone.org/doi/full/10.1525/auk.2012.11101>

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## A MOLECULAR PHYLOGENY OF BLACK-TYRANTS (TYRANNIDAE: *KNIPOLEGUS*) REVEALS STRONG GEOGRAPHIC PATTERNS AND HOMOPLASY IN PLUMAGE AND DISPLAY BEHAVIOR

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**ABSTRACT.**—We present the first molecular phylogenetic hypothesis for *Knipolegus* (black-tyrants), a widespread genus of South American tyrant-flycatchers, based on nuclear and mitochondrial DNA sequences. Bayesian and maximum-likelihood analyses support three clades within *Knipolegus*, one confined to northern South America, one confined to southeast Brazil, and one confined to the Southern Cone and southern Andes. Within each clade, two or more species are broadly sympatric or parapatric, overlapping in general distribution but differing in habitat specialization. Maximum-likelihood ancestral state reconstructions using an equal-rate stochastic model support a single origin of austral migration in the southern group. Contrasting with these strong geographic patterns, ancestral state reconstructions of plumage and display evolution were more complex, with multiple inferred character-state changes. Ancestral state reconstructions suggest a sexually dimorphic ancestor of *Knipolegus*, and sexually similar plumages are the result of three independent character-state changes: one in male plumage and two in female plumage. Ancestral state reconstructions support the conclusion that flight displays with mechanical sounds originated in the *Knipolegus* ancestor, and loss of mechanical sounds in flight displays occurred twice. Received 7 May 2011, accepted 10 December 2011.

Key words: biogeography, *Knipolegus*, migration, plumage evolution, South America, tyrant-flycatchers.

### La Filogenia Molecular de *Knipolegus* (Tyrannidae) Revela Patrones Geográficos Fuertes y Homoplasia en el Plumaje y el Comportamiento de Despliegue

**RESUMEN.**—Con base en secuencias de ADN nuclear y mitocondrial, presentamos la primera hipótesis filogenética molecular para *Knipolegus* (Tyrannidae), un género ampliamente distribuido de mosqueros suramericanos. Análisis bayesianos y de máxima verosimilitud sustentan la existencia de tres clados dentro de *Knipolegus*, uno confinado al norte de Sur América, otro al sureste de Brasil y otro al cono sur y a los Andes del sur. Dentro de cada clado, dos o más especies son ampliamente simpátricas o parapátricas; aunque sus distribuciones generales se superponen, difieren en su especialización de hábitat. La reconstrucción ancestral de caracteres por máxima verosimilitud usando un modelo estocástico de tasas iguales sustenta un solo origen de la migración austral en el grupo. En contraste con estos fuertes patrones geográficos, la reconstrucción de caracteres ancestrales del plumaje y del comportamiento de despliegue fueron más complejas, con múltiples cambios de estado inferidos. Las reconstrucciones de caracteres ancestrales sugieren un ancestro sexualmente dimórfico en *Knipolegus*, y que los plumajes sin dimorfismo son el resultado de tres cambios independientes de estado de carácter: uno en el plumaje del macho y dos en el plumaje de la hembra. La reconstrucción de caracteres ancestrales sustenta la conclusión de que los despliegues en vuelo con sonidos mecánicos se originaron en el ancestro de *Knipolegus* y la pérdida de los sonidos mecánicos en el vuelo tuvo lugar dos veces.

THE GENUS *KNIPOLEGUS* (Passeriformes: Tyrannidae) comprises 10 recognized species of unobtrusive and little-studied tyrant-flycatchers distributed nearly throughout South America (Cory and Hellmayr 1927; Meyer de Schauensee 1970; Traylor 1979; Ridgely and Tudor 1994, 2009; Remsen et al. 2011). The English name for the genus, “black-tyrant,” refers to the blackish plumage of most male and some female *Knipolegus*, depending on the species. Their gross morphology is highly conserved and in many ways stereotypical of a tyrannid: small body size, upright posture,

and wide flattened bill. *Knipolegus* are members of the subfamily Fluvicolinae (Cory and Hellmayr 1927; Traylor 1977, 1979; Fitzpatrick 2004; Ohlson et al. 2008; Tello et al. 2009), a clade of tyrannids most diverse in open habitats of southern South America. *Knipolegus* occur in habitats as diverse as Amazon River island scrub, *várzea*, Andean montane forest edge, high-elevation arid valleys, open Pampas grassland, thorn forest, gallery woodland, and *monte* desert. No phylogenetic hypothesis exists for *Knipolegus*, although some authors have grouped species on the basis

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of primary feather morphology (e.g., *K. poecilocercus* and *K. hudsoni*; Ridgway 1905, Wetmore and Peters 1923, Cory and Hellmayr 1927, Traylor 1977) and plumage (e.g., *K. nigerrimus* and *K. lophotes*; Ridgely and Tudor 1994).

Classic paradigms explaining South American biogeography and diversification (e.g., Haffer 1969, Cracraft 1985) have focused on tropical forest species that are geographically partitioned by strong barriers to dispersal, such as Amazonian rivers and semiarid habitats. Phylogeographic studies of tropical forest species are largely congruent with these paradigms (Marks et al. 2002, Cheviron et al. 2005, Nyári 2007). Understanding phylogenetic relationships within *Knipolegus* would provide a novel perspective on South American biogeography because they utilize nonprimary forest habitats and are found in tropical, temperate, and semiarid biomes. *Knipolegus* species may form groups of allopatric replacements across South America, similar to birds in Amazonia (Cracraft and Prum 1988). Diversification could also be the result of processes independent of those that drive diversification in birds limited to the tropical zone. For example, seasonal migration in *Knipolegus* may facilitate diversification via long-distance colonization (Kondo et al. 2008), a mechanism that is not feasible for largely sedentary Amazonian birds. Hypothesized relationships between migratory and nonmigratory *Knipolegus* (e.g., *K. poecilocercus*–*K. hudsoni* and *K. aterrimus*–*K. a. franciscanus*) suggest that seasonal migration could play a role in *Knipolegus* diversification.

In addition to intriguing biogeographic patterns, *Knipolegus* show unusual patterns of plumage evolution. The majority of *Knipolegus* are sexually dimorphic, but some species have reduced sexual dimorphism or sexually monomorphic plumage. From a sexually dimorphic ancestor, two evolutionary routes can produce sexually monomorphic plumage (Wiens 2001, Badyaev and Hill 2003): a (frequently dull or cryptic) female phenotype may converge on a (frequently more elaborate) male phenotype, or a male phenotype may converge on a female phenotype. *Knipolegus* are notable in that monomorphic species embody either male or female phenotypes. For example, both sexes of *K. lophotes* have black plumage, typical of most male *Knipolegus*, whereas both sexes of *K. poecilurus* have brown and rufous plumage, more typical of female *Knipolegus*.

Fluvicoline flycatchers also exhibit a diversity of breeding display behaviors, and much of this variation is represented in *Knipolegus*. Display behaviors range from species with spectacular acrobatic displays featuring mechanical sounds or flight songs to species that have no known displays or vocalizations. The majority of *Knipolegus* species have male flight displays with a buzzy or ticking sound, perhaps a mechanical bill or wing sound as in other Tyrannids (Bostwick and Zyskowski 2001, Fitzpatrick 2004). These flight displays involve three phases: an upward flight, followed by an acrobatic turn in midair, and a return to a perch. All three of these phases are slightly different among species (Ridgely and Tudor 1994, Fitzpatrick 2004). However, four taxa (*K. lophotes*, *K. nigerrimus*, *K. a. franciscanus*, and *K. cyanirostris*) have drastically different display behaviors with no known mechanical sounds. The first, *K. lophotes*, has a unique flight song. Three remaining species are essentially silent; *K. cyanirostris* has a subtle flight display with no sound (Belton 1985, Fitzpatrick 2004), and *K. nigerrimus* and *K. a. franciscanus* have no known displays or vocalizations (Ridgely and Tudor 2004, 2009).

The goal of the present study is to create the first phylogenetic hypothesis for the *Knipolegus* black-tyrants based on DNA sequence data and to use the phylogeny and ancestral state reconstructions to examine this group's biogeographic and evolutionary history. The phylogeny will allow exploration of a variety of pertinent evolutionary questions in birds, such as the following. Do *Knipolegus* conform to a biogeographic pattern of many South American birds, a series of allopatric replacements distributed across the continent? Does migration play a role in diversification, isolating migratory and resident species pairs? Are plumage patterns and flight display behavior conserved, the result of single character-state changes in progression, or are they highly plastic, featuring losses of putative sexually selected traits? Results will provide a framework to broaden these same questions to Tyrannidae, one of the largest (Tello et al. 2009) and most diverse avian radiations.

## METHODS

*Taxon sampling.*—We obtained tissue samples from *Knipolegus* species, including multiple individuals and samples from geographically disparate regions when possible (Table 1). Although they are not currently recognized as species (Remsen et al. 2011), some taxonomists have treated *K. signatus cabanisi* (Meyer de Schauensee 1970, Traylor 1982, Ridgely and Tudor 1994) and *K. aterrimus franciscanus* (Silva and Oren 1992, Ridgely and Tudor 2009, van Perlo 2009) as species; here, we treat them as operational taxonomic units. Phylogenetic studies of Tyrannids based on morphology (Lanyon 1986) and molecular markers (Ohlson et al. 2008, Tello et al. 2009) have suggested several taxa as potential sister groups of *Knipolegus*; thus, we included *Pyrocephalus*, *Satrapa*, *Muscisaxicola*, *Hymenops*, *Xolmis*, *Agriornis*, and *Lessonia* as out-groups to account for this uncertainty.

*DNA extraction, amplification, and sequencing.*—We extracted genomic DNA from ethanol-preserved or frozen fresh muscle tissue using proteinase-K digestion following manufacturer's protocol (DNeasy tissue kit; Qiagen, Valencia, California). We used polymerase chain reaction (PCR) to amplify three coding mitochondrial genes and three unlinked nuclear introns. Mitochondrial genes included the entire NADH dehydrogenase-2 (hereafter ND2), the entire NADH dehydrogenase-3 (hereafter ND3), and a fragment of cytochrome-*c* oxidase-1 (hereafter CO1). Nuclear introns included beta-fibrinogen intron 5 (hereafter Fib-5), glyceraldehydes-3-phosphodehydrogenase intron 11 (hereafter GAPDH), and transforming growth factor beta-2 intron 5 (hereafter TGFb5). Primers used to amplify gene regions (Hackett 1996, Johnson and Sorenson 1998, Chesser 1999, Marini and Hackett 2002, Primmer et al. 2002, Fjeldså et al. 2003, Lohman et al. 2009) are summarized in Appendix 1. We purified PCR products with ExoSAP-IT (USB) and performed cycle sequencing of purified PCR products with BigDye Terminator Cycle Sequencing kits (Applied Biosystems, Foster City, California). Cycle sequencing reactions used the same primers as PCR, and we sequenced both strands for all PCR products. We purified cycle sequencing products using ethanol precipitation and analyzed sequences on an ABI 3730 automated capillary DNA sequencer (Applied Biosystems), and we reconciled chromatograms of complementary fragments using SEQUENCHER, version 4.9 (Genecodes, Ann Arbor, Michigan). We downloaded CO1 sequences of *K. nigerrimus* and

TABLE 1. Tissue samples and associated voucher specimens used to produce a phylogeny of black-tyrants in the genus *Knipolegus*.

Taxon	Institution <sup>a</sup>	Tissue	Locality
<i>Agriornis murinus</i>	KUNHM	11853	Argentina: Rio Negro
<i>Xolmis irupero</i>	KUNHM	2837	Paraguay: Presidente Hayes
<i>Muscisaxicola macloviana</i>	KUNMH	11768	Argentina: Rio Negro
<i>Lessonia rufa</i>	KUNHM	11806	Argentina: Rio Negro
<i>L. rufa</i>	KUNHM	11768	Argentina: Rio Negro
<i>L. oreas</i>	KUNHM	9841	Argentina: Jujuy
<i>Hymenops perspicillatus</i>	KUNHM	3221	Paraguay: Ñeembucú
<i>Knipolegus striaticeps</i>	LSUMNS	B38892	Bolivia: Santa Cruz
<i>K. striaticeps</i> <sup>b</sup>	MACN	1577	Argentina: Corrientes
<i>K. hudsoni</i>	KUNHM	11875	Argentina: Rio Negro
<i>K. hudsoni</i>	KUNHM	11902	Argentina: Rio Negro
<i>K. poecilocercus</i>	ANSP	18219	Ecuador: Sucumbíos
<i>K. poecilocercus</i>	MGB	Cn048	Brazil: Para
<i>K. poecilocercus</i>	MGB	Amz063	Brazil: Amazonas
<i>K. poecilocercus</i>	MGB	Cn1232	Brazil: Para
<i>K. poecilocercus</i>	MGB	Uhe106	Brazil: Para
<i>K. signatus</i>	LSUMNS	43921	Peru: San Martín
<i>K. signatus</i>	LSUMNS	44399	Peru: San Martín
<i>K. (s.) cabanisi</i>	KUNHM	9747	Argentina: Jujuy
<i>K. (s.) cabanisi</i>	LSUMNS	22645	Bolivia: La Paz
<i>K. cyanirostris</i>	CUMV	50658	Uruguay: Cerro Largo
<i>K. cyanirostris</i>	CUMV	50666	Uruguay: Cerro Largo
<i>K. poecilurus</i>	KUNHM	4088	Guyana: Cuyuni-Mazaruni
<i>K. poecilurus</i>	AMNH	223	Venezuela: Bolívar
<i>K. poecilurus</i>	AMNH	2005	Venezuela: Bolívar
<i>K. poecilurus</i>	LSUMNS	33221	Peru: Cajamarca
<i>K. poecilurus</i>	LSUMNS	8076	Peru: Pasco
<i>K. poecilurus</i>	KUNHM	17497	Peru: Junín
<i>K. poecilurus</i>	KUNHM	18722	Peru: Cuzco
<i>K. orenocensis</i>	LSUMNS	43080	Peru: Loreto
<i>K. orenocensis</i>	ANSP	19348	Ecuador: Sucumbíos
<i>K. aterrimus</i>	FMNH	433548	Peru: Cuzco
<i>K. aterrimus</i>	FMNH	335503	Bolivia: Cochabamba
<i>K. aterrimus</i>	KUNHM	9780	Argentina: Jujuy
<i>K. aterrimus</i>	KUNHM	9803	Argentina: Jujuy
<i>K. aterrimus</i>	KUNHM	11841	Argentina: Rio Negro
<i>K. (a.) franciscanus</i> <sup>c</sup>	UFMG	2211	Brazil: Minas Gerais
<i>K. (a.) franciscanus</i> <sup>c</sup>	UFMG	4337	Brazil: Minas Gerais
<i>K. lophotes</i>	CUMV	51149	Uruguay: Cerro Largo
<i>K. lophotes</i> <sup>c</sup>	UFMG	2456	Brazil: Minas Gerais
<i>K. nigerrimus</i> <sup>c</sup>	UFMG	4241	Brazil: Minas Gerais
<i>K. nigerrimus</i> <sup>b</sup>	UFMG	4242	Brazil: Minas Gerais
<i>Pyrocephalus rubinus</i>	KUNHM	9904	USA: Kansas

<sup>a</sup>Abbreviations: ANSP = Academy of Natural Sciences, Philadelphia; AMNH = American Museum of Natural History; CUMV = Cornell Museum of Vertebrates; FMNH = Field Museum of Natural History; KUNHM = University of Kansas Natural History Museum; LSUMNS = Louisiana State University Museum of Natural Science; MGB = Museu Paraense Emílio Goeldi; MACN = Museo Argentino de Ciencias Naturales; and UFMG = Universidade Federal de Minas Gerais.

<sup>b</sup>COI sequence only, from Kerr et al. 2009.

<sup>c</sup>COI sequence only, from Chaves et al. 2008.

*K. a. franciscanus*, for which we had no tissue samples, and additional samples of *K. lophotes* and *K. striaticeps* (Chaves et al. 2008, Kerr et al. 2009; Table 1) from GenBank and added them to our sequence data set; we lack all other gene sequences for these samples. Alignments were reconstructed for each gene using the on-line version of MUSCLE (Edgar 2004).

**Phylogenetic analysis.**—We estimated phylogenetic relationships with Bayesian and maximum-likelihood (ML) inference for mitochondrial and nuclear data sets separately (Appendix 2) to

examine the potential pitfall of combining data sets with conflicting phylogenetic signal. We observed no well-supported conflicts in topology ( $P > 0.95$  posterior probability) among in-group samples; subsequently we used a concatenated data set for analyses. MODELTEST, version 3.7 (Posada and Crandall 1998), using Akaike's information criterion (AIC), selected a general time-reversible model with gamma-distributed rates among sites and invariant sites (GTR+I+G) for mitochondrial genes, the HKY+G model for nuclear introns G3PDH and Fib-5, and the HKY+I+G

model for TGFb5. We implemented Bayesian analysis with MRBAYES, version 3.1 (Ronquist and Huelsenbeck 2003), with two independent Markov-chain Monte Carlo (MCMC) runs of 20 million generations each, which we sampled every 10,000 generations. Each run included eight chains, seven heated and one unheated. Chain heating was adjusted with the “temp” setting in MRBAYES to 0.05 (Beiko et al. 2006), until the proportion of accepted swaps between adjacent chains was in the range of 0.20 to 0.70. We used the 2,000 trees, minus a burn-in of 500 (after runs had reached stationarity), to create consensus trees and a set of topologies for ancestral state reconstructions. To examine stationarity, effective sample size, and convergence of parameter estimates between runs, we used TRACER, version 1.5 (Rambaut and Drummond 2007). To examine stationarity and convergence of tree topology estimates, we used the “cumulative,” “slide,” and “compare” functions in the online version of AWTY (Wilgenbusch et al. 2004). Summarized posterior probabilities of all nodes in the two runs were similar (identical or within 0.01). In addition to Bayesian methods, we used ML tree estimation including 1,000 bootstrap replicates on the partitioned data set under the GTR+G model invoked in RAXML, version 7.0.3 (Stamatakis 2006, Stamatakis et al. 2008).

**Ancestral state reconstruction.**—To reconstruct evolutionary histories of migratory behavior, plumage, and display behavior, we used ML ancestral state reconstruction on the Bayesian tree set. Character series (Appendix 3) included seasonal migratory behavior, plumage phenotype (both sexes), and display behavior. We coded migratory behavior as sedentary or migratory following Chesser (2005). Partially migratory species were coded as migratory. We categorized uniform blackish or slate-gray plumage phenotypes as elaborate, and paler, brownish-washed birds with wingbars, tail markings, contrasting rumps, streaked underparts, and pale bellies as cryptic. Plumage character states are based on observations of the University of Kansas Natural History Museum ( $n = 22$ ) and the Louisiana State University Museum of Natural Science ( $n = 280$ ) specimens augmented with color plates from Fitzpatrick (2004) and Ridgely and Tudor (2009). We coded two species that are slightly sexually dimorphic as monomorphic with elaborate plumage: *K. nigerrimus* (female plumage is uniform blackish, similar to the male except for the gorget, which is streaked faintly with brown), and *K. orenocensis* (in subspecies *orenocensis* and *xinguensis*, females are slaty-blackish, resembling males; but subspecies *sclateri* is slightly dimorphic, with females showing a brownish rump and a whitish belly). We coded flight-display behavior as four different states: mechanical display (flight display featuring putative mechanical sounds found in many *Knipolegus*), flight song (a display flight with a true song), silent display (a flight display without vocalizations or putative mechanical sounds), and no display (no known display). Display character states were based on the literature (Belton 1985, Ridgely and Tudor 1994, Fitzpatrick 2004, Schulenberg et al. 2007), augmented with personal observations (P. A. Hosner and M. Robbins pers. comm.). We chose to code the display of nominate *K. signatus* as “no data” because this taxon’s behavior is virtually unknown in life, and all data published on its behavior actually pertain to *K. s. cabanisi*, with which it is frequently lumped.

We reconstructed ancestral states of these discrete characters series using the ML criterion in the multistate module invoked

in BAYESTRAITS, version 1.0 (Pagel et al. 2004), over 1,500 trees from the first 20-million-generation MRBAYES run. Using the Bayesian tree set rather than a single Bayesian consensus tree or ML tree accounts for phylogenetic uncertainty by considering a posterior distribution of trees rather than a point estimate. We used a conservative single-rate model for each of the four character series because likelihood-ratio tests (Pagel 1999) showed that more complex multiple-rate models were not a significantly better fit (Appendix 4). For each character series, we summarized and averaged the 1,500 ML estimates for each node within the in-group of the *Knipolegus* phylogeny.

## RESULTS

**Sequence attributes.**—The aligned sequences yielded a data matrix of 3,722 characters (1,041 ND2, 351 ND3, 671 COI, 425 GAPDH, 594 Fib-5, and 640 TGFb5) from 44 in-group samples. Of these characters, 984 were variable (451 ND2, 133 ND3, 221 COI, 40 G3PDH, 73 Fib-5, and 66 TGFb5) and 712 were parsimony informative (341 ND2, 107 ND3, 178 COI, 17 G3PDH, 36 Fib-5, and 33 TGFb5). The Fib-5 intron contained two informative indels, a 7-bp deletion in *Agriornis*, *Hymenops*, and *K. signatus*, and a 7-bp insertion in *Agriornis*, *Xolmis*, *Muscisaxicola*, and *K. aterrimus*. The TGFb5 intron contained three informative indels: a 3-bp insertion in *K. cyanirostris* and *K. lophotes*, and a 2-bp deletion and a 3-bp insertion in *Hymenops* and *Xolmis*. All DNA sequences generated are archived in GenBank (accession numbers JQ288110–JQ288303).

**Phylogenetic analysis.**—Maximum-likelihood and Bayesian analyses (Fig. 1) recovered *Knipolegus* monophyly with strong support (0.99 posterior probability, 100% of ML bootstrap replicates), although its position among out-groups was poorly resolved. The most likely sister group to *Knipolegus* was *Lessonia*, although this relationship was not strongly supported (0.78 posterior probability, <50% ML bootstrap replicates); alternative sister groups received <4% of the posterior distribution of trees, and <15% of the ML bootstrap replicates. Analyses recovered three major clades within *Knipolegus* that correspond to South American geography (Fig. 1). The first clade comprises three species confined to northern South America: Amazonian *K. poecilocercus* and *K. orenocensis* and Andean–Tepui *K. poecilurus*; this northern clade was sister to all other *Knipolegus*. The second clade includes three species largely restricted to eastern Brazil: *K. a. franciscanus*, *K. nigerrimus*, and *K. lophotes*. This southeast Brazil clade was sister to the third clade, which contained species largely restricted to the Southern Cone (during breeding) and the Andes as far north as Peru: *K. signatus*, *K. s. cabanisi*, *K. cyanirostris*, *K. striaticeps*, *K. hudsoni*, and *K. aterrimus*. Within this southern clade, three species that occur in dry forest and arid scrub (*K. striaticeps*, *K. hudsoni*, and *K. aterrimus*) formed a clade, although with low support. The northern and southern clades received high support (1.0 posterior probability, 98% of ML bootstrap replicates; 0.99 posterior probability, 71% of ML bootstrap replicates, respectively), the southeast Brazil clade was supported marginally (0.94 posterior probability, 70% of ML bootstrap replicates).

**Ancestral state reconstructions.**—Maximum-likelihood ancestral state reconstruction of migration (Fig. 2) strongly supported a sedentary ancestor of *Knipolegus* ( $P = 0.99$ ), with a

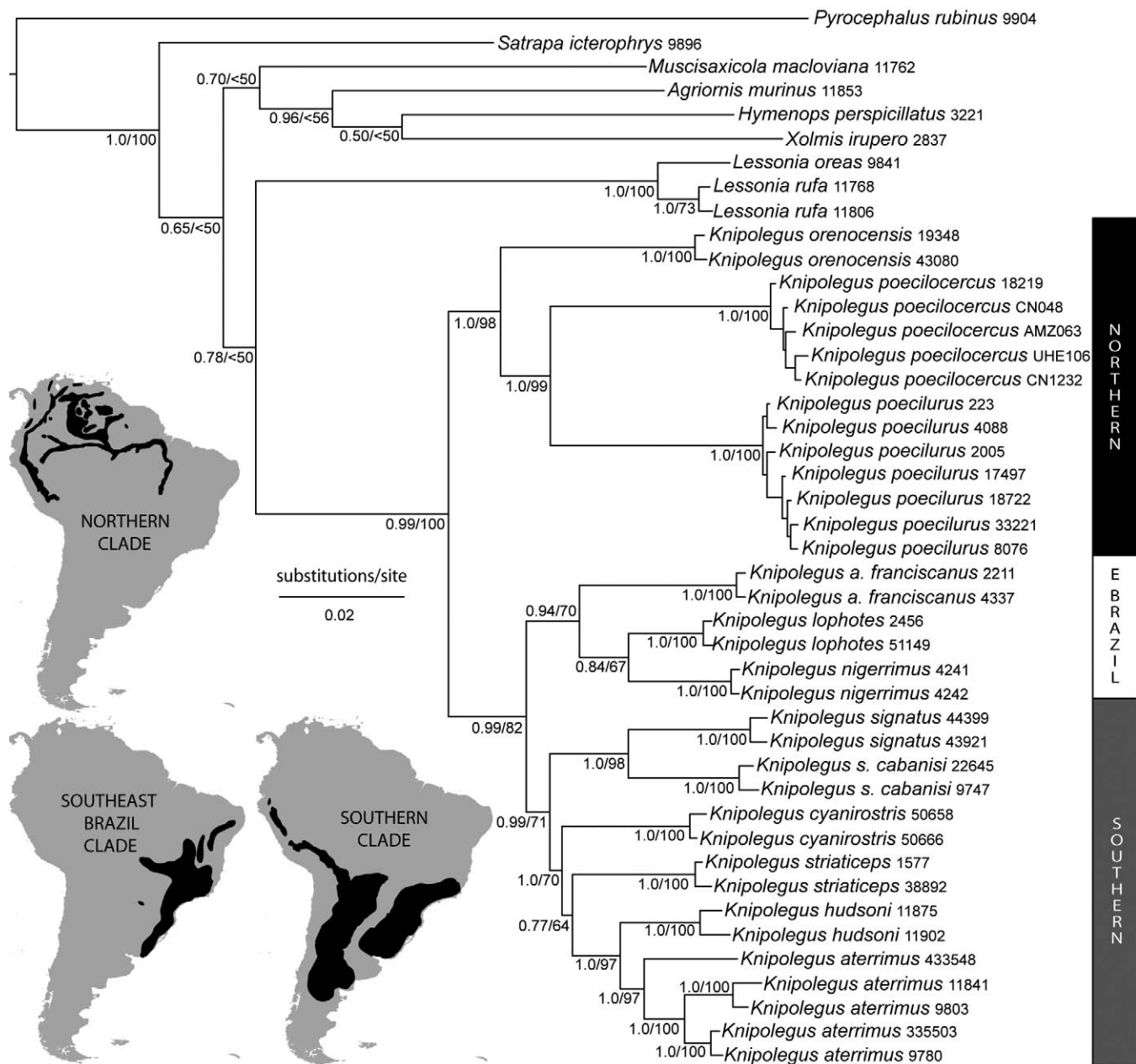


FIG. 1. Bayesian consensus tree (MRBAYES, version 3.1) of *Knipolegus* inferred from mitochondrial and nuclear genes. Values on nodes are Bayesian posterior probabilities or maximum-likelihood bootstrap percentages in support of each node. Values of poorly supported nodes within species are not reported. Distribution maps show pooled approximate distributions of the species in each *Knipolegus* clade, adapted from Fitzpatrick (2004) and Ridgely and Tudor (2009). For austral migrants, only the breeding distribution is shown.

single origin of migratory behavior in the most recent common ancestor (MRCA) of *K. cyanirostris*, *K. striaticeps*, *K. hudsoni*, and *K. aterrimus* ( $P = 1.00$ ), which form a strongly supported clade. The evolutionary history of plumage dimorphism was more complex (Fig. 3). In males, ancestral state reconstruction strongly supported a blackish elaborate plumage in the MRCA of *Knipolegus* ( $P = 1.00$ ), with a single change to a brown-and-rufous cryptic plumage in *K. poecilurus*. In females, analysis supported a cryptic plumage in the MRCA of *Knipolegus* ( $P = 0.95$ ), with two

origins of elaborate plumage in *K. orenocensis* and in the MRCA of *K. lophotes* + *K. nigerrimus* ( $P = 1.00$ ). Taken together, these analyses imply that the MRCA of *Knipolegus* was sexually dimorphic and that reduced sexual dimorphism—sexually monomorphic plumage evolved three separate times. All three origins of reduced sexual dimorphism—sexually monomorphic plumage occurred in sedentary species; all migratory species retained conserved sexually dimorphic plumage. The ML ancestral state reconstruction of display behavior (Fig. 4) marginally supported

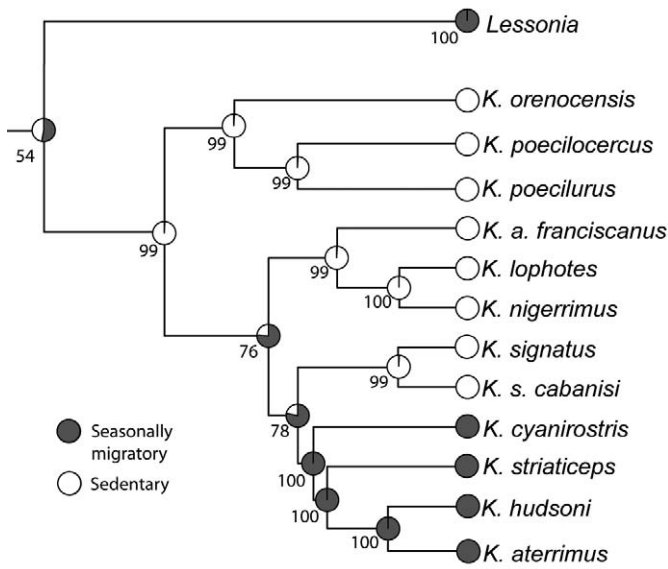


FIG. 2. Maximum-likelihood ancestral state reconstructions (BAYESTRAITS, version 1.0) of seasonal migratory behavior in *Knipolegus* under a single-rate model. Pie diagrams at each node indicate the proportional likelihood supporting each character state; white denotes “sedentary” and gray denotes “migratory.” Values associated with pie diagrams are the likelihood of the most likely character state at each ancestral node. Branches are not proportional to lengths. Although all out-groups were used in analysis, only *Lessonia* is shown.

a *Knipolegus* MRCA with a flight display–mechanical sound ( $P = 0.92$ ), with two losses—one in the MRCA of *K. a. franciscanus*, *K. lophotes*, and *K. nigerrimus* ( $P = 0.97$ ) and a second in *K. cyanirostris* (Fig. 4).

DISCUSSION

**Taxonomy.**—Two phylogenetic results have important taxonomic implications that alter the interpretation of *Knipolegus* biogeography and evolutionary history. One currently recognized species, *K. aterrimus*, is paraphyletic in our study; subspecies *franciscanus* is unrelated to other *K. aterrimus* samples and instead is recovered in the southeast Brazil clade. Our analysis corroborates plumage differences and supports recognition of *K. franciscanus* as a species, Caatinga Black-tyrant (Silva and Oren 1992, Ridgely and Tudor 2009, van Perlo 2009). *Knipolegus signatus* and *K. s. cabanisi* have a convoluted taxonomic history, originally described as separate species but lumped in recent taxonomic treatments (Cory and Hellmayr 1927; Meyer de Schauensee 1970; Traylor 1979, 1982; Ridgely and Tudor 1994; Remsen et al. 2011). We found substantial genetic differentiation between these taxa (5.7–5.9% ND2 p-distance and fixed differences in two introns), which corroborates plumage and body size differences (Ridgely and Tudor 1994). The consistency of these results supports recognition of *K. signatus* and *K. cabanisi* as separate species. The English name of *K. cabanisi* can revert to Plumbeous Tyrant (Meyer de Schauensee 1970), and the English name of *K. signatus* can become Jelski’s Black-Tyrant, a slight modification of the name used by Meyer de Schauensee (1970).

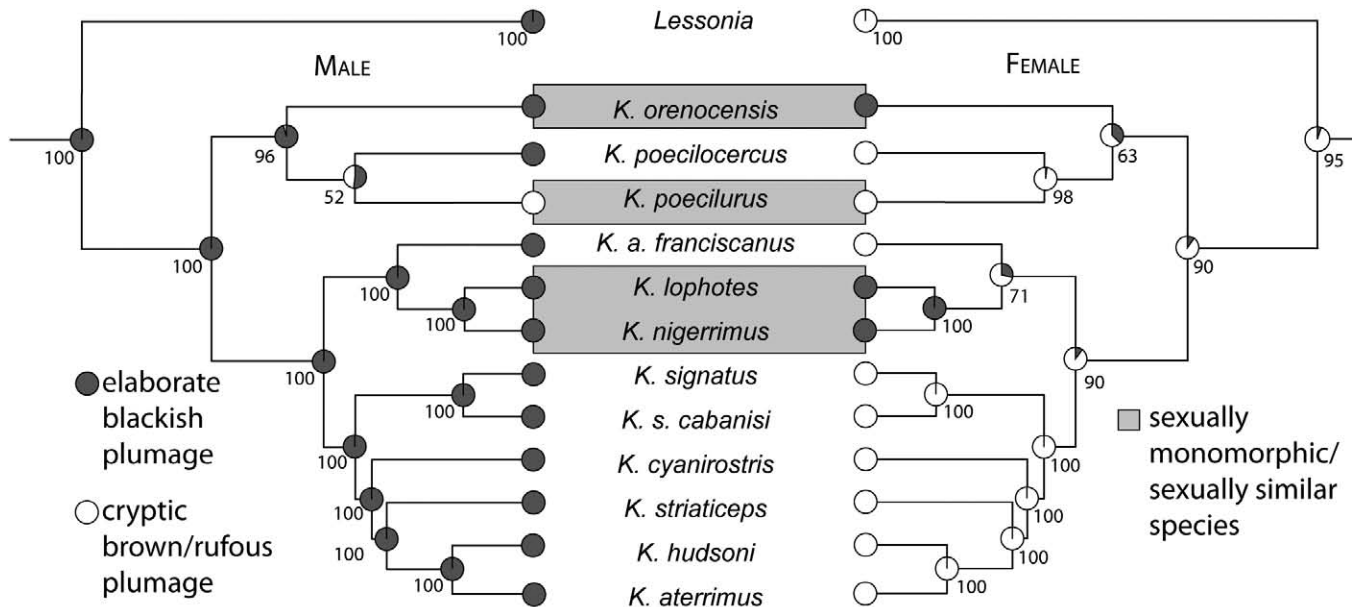


FIG. 3. Maximum-likelihood ancestral state reconstructions (BAYESTRAITS, version 1.0) of the evolution of plumage dimorphism in *Knipolegus* under a single-rate model. Male plumage is reconstructed on the left tree, female plumage on the right tree. Pie diagrams at each node indicate the proportional likelihood supporting each character state; white denotes a cryptic brown-and-rufous plumage; gray denotes elaborate blackish plumage. Values associated with pie diagrams are the likelihood of the most likely character state at each ancestral node. Species with sexually monomorphic–sexually similar plumage are highlighted with a gray box. Branches are not proportional to lengths. Although all out-groups were used in analysis, only *Lessonia* is shown.

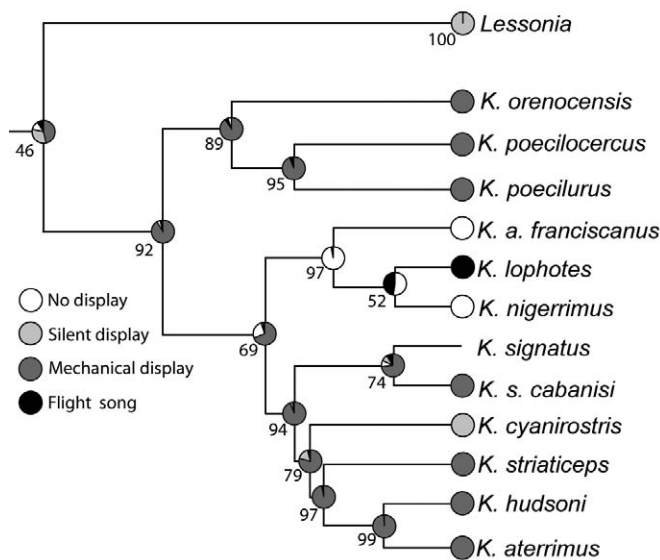


FIG. 4. Maximum-likelihood ancestral state reconstructions (BAYES-TRAITS, version 1.0) of the evolution of mechanical sounds in breeding displays in *Knipolegus* under a single-rate model. Pie diagrams at each node indicate the proportion of the maximum likelihood supporting each character state; white denotes “no display,” light gray denotes “silent display,” dark gray denotes “mechanical display,” and black denotes “flight song.” Branches are not proportional to lengths. Although all out-groups were used in analysis, only *Lessonia* is shown.

**Biogeography.**—In contrast to a common pattern in which Neotropical bird species form a series of allopatric replacements across broad areas (Cracraft and Prum 1988), three *Knipolegus* clades appear to have been split by initial vicariance events, followed by subsequent diversification within regions, resulting in geographic clusters of closely related species. The northern *Knipolegus* group consists of three species of habitat specialists found in naturally disturbed nonprimary habitats. *Knipolegus orenocensis* and *K. poecilocercus* are sympatric over a large part of their distribution but are segregated by habitat, with *K. orenocensis* in young second growth or scrub on river islands and river edges, and *K. poecilocercus* in seasonally flooded *várzea* forest (Ridgely and Tudor 1994, 2009; Fitzpatrick 2004).

Despite isolation by >500 km of unsuitable habitat and slight but fixed phenotypic differences, Andean and Tepui populations of *K. poecilurus* are undifferentiated in markers used in our study. Samples varied by <1.0% p-distance in the ND2 gene. This result contrasts strongly with other phylogenetic studies, which have found strong genetic differentiation between Andean and Tepui bird populations or that Tepui taxa are not sister to Andean taxa (Pérez-Emán 2005, Rheindt et al. 2008, Mauck and Burns 2009, Bonaccorso et al. 2011). Two scenarios possibly explain the lack of genetic differentiation in *K. poecilurus*: either colonization of the Andes from the Tepuis (or vice versa) has been recent, or there has been ongoing gene flow across lowland areas currently uninhabited by *K. poecilurus*. Presence of *K. poecilurus* on isolated Tepuis and on isolated outlying ridges of the Andes suggests that this species may have good dispersal capabilities, perhaps also related to its preference for early-successional vegetation following

landslides. Because suitable secondary vegetation may be present in each landslide for only a few generations, birds may frequently need to disperse in search of suitable habitat, resulting in a highly mobile species throughout its range.

In the southeast Brazil clade, *K. nigerrimus* and *K. lophotes* have previously been hypothesized to be relatives (Ridgely and Tudor 1994), but placement of *K. franciscanus* with them is a novel result that highlights (1) the importance of southeast Brazil as an area of endemism and (2) that geography is often a better predictor of relationships than presumably fast-evolving plumage characters (e.g., Brumfield and Edwards 2007). *Knipolegus nigerrimus* and *K. lophotes* have largely overlapping distributions and a preference for open habitats, although *nigerrimus* is typically found at higher elevations. *Knipolegus franciscanus* is found in dry *caatinga* woodland and is the only *Knipolegus* other than the *K. striaticeps*–*hudsoni*–*aterrimus* group to inhabit semiarid habitats (Ridgely and Tudor 2009).

The southern group is the largest *Knipolegus* clade in terms of species diversity and geographic range. The *K. signatus*–*cabanisi* pair and *K. cyanirostris* inhabit humid montane forest edge and gallery forest edge, respectively, whereas *K. striaticeps*, *K. hudsoni*, and *K. aterrimus* are found in more arid regions. The phylogeny suggests that the southern clade colonized the Andes twice: by the humid-forest-adapted *K. signatus*–*cabanisi* ancestor, and by semiarid-adapted *K. aterrimus*, which is distributed in the Andes as well as the lowlands in the southern part of its range. Unlike in northern and southeast Brazil clades, the area of endemism diversification model (Cracraft 1985) explains some biogeographic patterns in the southern clade. The Rio Apurímac Valley, the geographic break between the East Peruvian and South Peruvian subcenters (Cracraft 1985), isolates *K. signatus* and *K. cabanisi*. *Knipolegus hudsoni* and *K. striaticeps* are distributed in the adjacent Chaco and Patagonian subcenters in the breeding season and form a clade with the widespread Andean *K. aterrimus*, which geographically replaces them in similar semiarid habitats throughout the southern Andes.

Unlike other species of *Knipolegus*, samples of *K. aterrimus* had divergent mitochondrial sequences. A single sample of *K. aterrimus anthracinus* from Cuzco, Peru (FMNH 43358), was 4.2–4.5% (ND2 p-distance) divergent from all other samples of nominate *aterrimus* from Bolivia and Argentina and also contained autapomorphic substitutions in TGFb5 and Fib-5 sequences, which suggests phylogeographic structure related to dry inter-Andean valley systems.

Additionally, haplotypes 2.7–2.8% (ND2 uncorrected p-distance) divergent were found at the same locality in Jujuy, Argentina. These two haplotype groups correspond to the 1.9% mitochondrial break in the COI gene in *K. aterrimus* in Argentina previously reported by Kerr et al. (2009). The lack of differentiation in nuclear introns and plumage characters suggests that this result is best explained as two divergent haplotype groups within one population, rather than two divergent populations.

**Evolution of seasonal migration.**—Maximum-likelihood ancestral state reconstructions strongly supported a sedentary ancestor of *Knipolegus*, with a single origin of migratory behavior in the MRCA of *K. aterrimus*, *K. hudsoni*, *K. cyanirostris*, and *K. striaticeps*. Conserved migratory behavior in *Knipolegus* contrasts with previous phylogenetic studies of migratory behavior



in birds (Joseph et al. 1999, 2003; Outlaw et al. 2003; Joseph 2005; Omland and Hofmann 2006; Kondo and Omland 2007), in which seasonal migratory behavior (frequently correlated with dramatic temperate–tropical range shifts) was inferred to be rapidly evolving and highly plastic. The phylogeny rejects previous treatments that suggested close relationships between migratory and resident species and indicates that migration has not directly influenced recent diversification in *Knipolegus*. The origin of migration in the southern clade appears to be correlated with expansion into seasonally favorable breeding habitats (Rappole 1995) in southern South America at latitudes south of 30°S (Chesser 1994, 2005). Alternatively, seasonal migration is widespread in the Fluvicolinae and, therefore, evolution of migratory behavior in *Knipolegus* may be more a question of modification and maintenance rather than a true origin (Zink 2002). Under a plastic, rapidly evolving model of migration (e.g., Joseph 2005, Milá et al. 2006), simultaneous recent range expansion in southern *Knipolegus* species, correlated with multiple recent origins of migration in response to a warming climate, would produce the same results that we observed in our ancestral state reconstructions. Sedentary populations of *K. aterrimus* in Peru and Bolivia provide some support for this alternative hypothesis.

*Evolution of plumage phenotypes.*—Plumage changes in both male and female *Knipolegus* contributed to losses of sexual dimorphism. Similar to general trends that have been observed in other birds (Badyaev and Hill 2003), sexual dimorphism in *Knipolegus* is ancestral, and female plumage is more labile ( $q = 0.00150$ , two changes) than male plumage ( $q = 0.00056$ , one change). Previous studies have documented increased loss of female elaboration correlated with evolution of seasonal migration in New World orioles (Kondo and Omland 2007, Friedman et al. 2009) and ducks (Omland and Hofmann 2006). In *Knipolegus*, migrant species retained ancestral sexual dimorphism, but four of eight resident species lost sexual dimorphism from both loss of male elaboration (*K. poecilurus*) and gains of female elaboration (*K. lophotes-nigerrimus* MRCAs; *K. orenocensis*).

*Evolution of display flights and mechanical sounds.*—Maximum-likelihood character mapping analysis inferred that evolution of flight displays was complex and likely involved multiple origins or reversals of character states. The most likely scenario (though only marginally supported) is that displays with mechanical sounds were ancestral in *Knipolegus* flycatchers, and two losses or modifications of display behavior occurred, one in the southeast Brazil clade and one in *K. cyanirostris*. Flight displays of *Knipolegus* and other Fluvicolinae flycatchers are thought to replace an advertisement song to attract a female (Fitzpatrick 2004). Many other phylogenetic studies support similar loss of sexually selected traits in vertebrates (Wiens 2001).

In two *Knipolegus* species that lack mechanical sounds, it appears that other methods of advertisement have evolved. In *K. cyanirostris*, a silent flight display (the male flies back and forth in front of females) has replaced the typical mechanical sound display. The spectacular flight song of *K. lophotes*—a long, arcing flight over an open area with a loud, complex song at the peak (the only true song of any *Knipolegus*)—apparently evolved from an ancestor with no flight display or mechanical sound ( $P = 0.97$ ). Its sister species, *K. nigerrimus*, as well as the *lophotes-nigerrimus* sister, *K. a. franciscanus*, lack any described display behavior.

Therefore, our results suggest that the display of *K. lophotes* originated independently from and is not homologous with other *Knipolegus* flight displays.

Future research on the evolution of *Knipolegus* displays would benefit from use of high-speed video of display behaviors for all species. These data would allow key insights. For instance, displays could be broken down into homologous sections for rigorous ethological studies in phylogenetic context, and mechanical sounds could be analyzed to reveal whether multiple methods are involved in their production. The fact that some species, such as *K. aterrimus*, create mechanical sounds at the peak height in the flight display, whereas other species, such as *K. striaticeps*, create mechanical sounds at the lowest height in the flight display, suggests that multiple methods of sound production are involved (Fitzpatrick 2004, P. A. Hosner pers. obs.).

#### ACKNOWLEDGMENTS

We thank the museum collectors and field workers who acquired specimens used in this study. The Academy of Natural Sciences of Philadelphia, the American Museum of Natural History, the Cornell Museum of Vertebrates, the Field Museum of Natural History, the Louisiana State University Museum of Natural Science, and the Museu Paraense Emílio Goeldi kindly provided supplemental tissue grants. M. Robbins, M. Andersen, C. Oliveros, H. Owens, and R. Jones provided helpful comments on drafts of the manuscript and figure design. Research was supported by funds from the KUNHM Panorama fund and the University of Kansas Center for Research.

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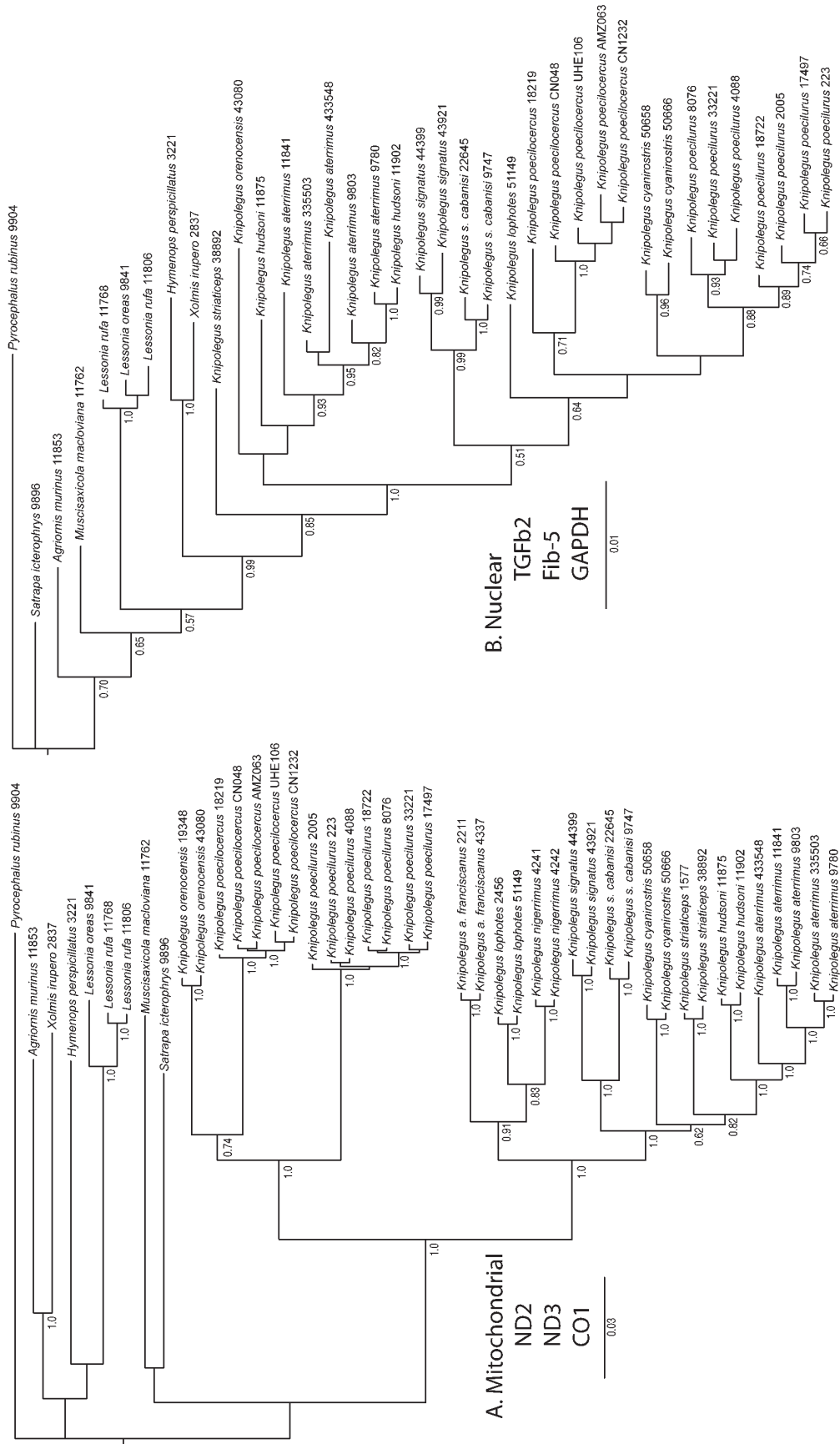
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Associate Editor: L. Joseph

APPENDIX 1. Primers used in PCR amplifications. All primers were used in PCR and sequencing reactions (sequence of KNIP398L: 5' CATAAAATCCCCCAACTAT 3').

	L primer	H primer
ND2 (1st half)	L5215(Hackett 1996)	H5766 (Hackett 1996)
ND2 (2nd half)	KNIP398L (present study)	H6313 (Johnson and Sorenson 1998)
ND3	L10755 (Chesser 1999)	H11151 (Chesser 1999)
COI	birdR1 (Lohman 2009)	birdF1 (Lohman et al. 2009)
G3PDH	G3P13b (Fjeldså et al. 2003)	G3P14b (Fjeldså et al. 2003)
TGFb5	TGF5 (Primmer et al. 2002)	TGF6 (Primmer et al. 2002)
Fib5	Fib5 (Marini and Hackett 2002)	Fib6 (Marini and Hackett 2002)

APPENDIX 2. (A) Mitochondrial and (B) nuclear DNA sequences analyzed separately using MRBAYES, version 3.1; methods, settings, and partitioning follow those used for analyzing the combined mitochondrial–nuclear data set. No well-supported conflicts in topology (posterior > 0.95) were observed between mitochondrial and nuclear data in in-group samples. Placement of out-group *Xolmis* differs in mitochondrial and nuclear trees, which perhaps contributes to the poor resolution among out-groups in the combined data set. The in-group mitochondrial topology (A) is very similar to the combined data-set topology, but support is lower at some deeper in-group nodes. The nuclear-only tree (B) is poorly resolved, perhaps because there are relatively few informative characters compared to the mitochondrial data set and incomplete lineage sorting. Maximum-likelihood topologies from RAXML, version 7.0.3 (not shown), inferred from each data set were identical to their respective Bayesian consensus topologies.



APPENDIX 3. Character states for all *Knipolegus* species used in ancestral state reconstructions (M = migratory, S = sedentary, E = elaborate, C = cryptic, ND = no display, SD = silent display, MD = mechanical display, FS = flight song, and na = not applicable [unclear homology for distant out-groups]).

Taxon	Migration	Male plumage	Female plumage	Display
<i>Knipolegus striaticeps</i>	M	E	C	MD
<i>K. hudsoni</i>	M	E	C	MD
<i>K. poecilocercus</i>	S	E	C	MD
<i>K. signatus</i>	S	E	C	MD
<i>K. s. cabanisi</i>	S	E	C	MD
<i>K. cyanostris</i>	M	E	C	SD
<i>K. poecilurus</i>	S	C	C	MD
<i>K. orenocensis</i>	S	E	E	MD
<i>K. aterrimus</i>	M	E	C	MD
<i>K. a. franciscanus</i>	S	E	C	ND
<i>K. lophotes</i>	S	E	E	FS
<i>K. nigerrimus</i>	S	E	E	ND
<i>Lessonia oreas</i>	M	E	C	SD
<i>L. rufa</i>	M	E	C	SD
<i>Hymenops perspicillatus</i>	M	E	C	MD
<i>Agriornis murinus</i>	M	na	na	SD
<i>Muscisaxicola macloviana</i>	M	na	na	SD
<i>Xolmis irupero</i>	M	na	na	ND
<i>Satrapa icterophrys</i>	M	na	na	ND
<i>Pyrocephalus rubinus</i>	M	E	C	FS

APPENDIX 4. Likelihood-ratio test statistics (Pagel 1999) justifying use of one-rate models (null) over rate-matrices (alternative) in BAYESTRAITS, version 1.0. Test statistics are compared to a chi-square distribution.

Character series	Null	Alternative	Test statistic	df	P
Migration	-8.58	-8.47	2.025974026	1	0.154
Male plumage	-3.95	-3.49	2.263610315	1	0.132
Female plumage	-9.57	-7.08	2.703389831	1	0.1
Display	-24.75	-16.17	3.06122449	11	0.999