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# EVOLUTION INTO AND OUT OF THE ANDES: A BAYESIAN ANALYSIS OF HISTORICAL DIVERSIFICATION IN *THAMNOPHILUS* ANTSHRIKES

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The Andean uplift played important roles in the historical diversification of Neotropical organisms, both by producing new high-elevation habitats that could be colonized and by isolating organisms on either side of the mountains. Here, we present a molecular phylogeny of *Thamnophilus* antshrikes, a clade of 30 species whose collective distribution spans nearly the entirety of lowland habitats in tropical South America, the eastern slope foothills of the Andes, and the tepuis of northern South America. Our goal was to examine the role of the Andes in the diversification of lowland and foothill species. Using parsimony and Bayesian ancestral state reconstructions of a three-state distribution character (lowland-restricted, lowland-to-highland, highland-restricted), we found that the Andes were colonized twice independently and the tepuis once from lowland-restricted ancestors. Over the entire evolutionary history of *Thamnophilus*, the highest transition rates were between highland-restricted and lowland-to-highland distributions, with extremely low rates into and out of lowland-restricted distributions. This pattern suggests lowland-restricted distributions are limited not by physiological constraints, but by other forces, such as competition. These results highlight the need for additional comparative studies in elucidating processes associated with the colonization of high-elevation habitats and the differentiation of populations within them.

**KEY WORDS:** Andes, biogeography, Neotropical, phylogeny, *Thamnophilus*.

Spanning nearly the entire length of western South America, the Andes mountain range provides a rich natural laboratory for studies of biogeography, geographic variation, and speciation (e.g., Chapman 1917; Remsen 1984; Hillis 1985; Graves 1988; Patton and Smith 1992; Young et al. 2002; Hall 2005). The Andean uplift played important roles in the historical diversification of Neotropical organisms both by isolating lowland organisms on either side of the mountains (Haffer 1967; Gentry 1982; Lynch and Duellman 1997) and by producing a mosaic of montane and inter-Andean valley habitats in which colonization and differentiation could occur (Graham et al. 2004; Hughes and Eastwood 2006). What re-

main poorly understood are the evolutionary dynamics surrounding the colonization and differentiation of populations in highland habitats as well as the subsequent re-colonization of lowland habitats.

Most Neotropical models of diversification invoke geographic isolation as the primary mode of differentiation (e.g., Haffer 1969; Capparella 1991; Nores 1999). Although the importance of geographic isolation in vertebrate speciation is still debated (reviewed in Fitzpatrick and Turelli 2006), the fact that most vertebrate Neotropical sister taxa are distributed parapatrically or allopatrically argues that geographic isolation should represent

the null diversification hypothesis for vertebrates in the Neotropics (Jordan 1905; Mayr 1942; Chesser and Zink 1994; Fjeldså 1994; Cheviron et al. 2005a). The most widely cited exception to allopatric speciation is a model of speciation via primary differentiation along ecological gradients (Endler 1977). Chapman (1926) anticipated this model in trying to explain the differentiation of foothill from lowland populations of birds in Ecuador. Although ecological gradients can produce genetic differentiation among bird populations, empirical demonstrations of speciation via such gradients remain few (Smith et al. 2005). Tests of these hypotheses in the Neotropics used different approaches (population genetic versus phylogenetic), but have generally corroborated geographic isolation as the dominant force in mammals (Patton and Smith 1992) and birds (Fjeldså 1994; Roy et al. 1997, 1999; García-Moreno and Fjeldså 2000; Dingle et al. 2006). In contrast, speciation in Andean anurans (Lynch and Duellman 1997; Graham et al. 2004) and insects (Willmott et al. 2001), which hold smaller ecological niches than birds and mammals, may be more heavily influenced by divergent ecological regimes. For the purposes of this study, we consider geographic isolation to be the primary mechanism of speciation in birds and, in the context of a well-resolved molecular phylogeny, explore potential vicariant processes that could have isolated birds in highland and lowland habitats.

Previous studies of Neotropical organisms suggest that habitat and elevation shifts may occur frequently (Bates and Zink 1994; García-Moreno and Silva 1997; Salazar-Bravo et al. 2001; Aleixo 2002; Donato et al. 2003; Weir 2006), so that studies of taxa composing a suite of habitats are required to provide a complete test of Neotropical diversification mechanisms. Whereas biogeographic analyses of exclusively highland or exclusively lowland species groups provide insights into the historical diversification processes operating within these regions, studies of species groups containing both highland and lowland representatives may offer an evolutionary snapshot of the processes underlying evolution into and out of the Andes. Phylogenetic relationships among Neotropical birds have been elucidated for a number of species groups, but many of these studies analyzed lowland (e.g., Brumfield and Capparella 1996; Aleixo 2002; Marks et al. 2002; Cheviron et al. 2005b; Porzecanski and Cracraft 2005) or high elevation taxa (e.g., Arctander and Fjeldså 1994; Roy et al. 1999; García-Moreno et al. 1999, 2001; Pérez-Emán 2005) exclusively. In cases where a phylogenetic study included taxa from both lowland and highland regions, the analysis did not include ancestral reconstructions of elevational distribution (Burns 1997; Lovette and Bermingham 2001) or, after the phylogenetic analysis, it was unclear if the highland and lowland taxa even represented a clade (Chesser 2000). To examine the interplay between highland and lowland regions explicitly, we present an analysis of historical diversification in an

avian genus (*Thamnophilus*) containing both highland and lowland species.

Nearly all core bird communities (Remsens 1994) of the lowland and foothill forests of Central America and tropical South America include at least one antshrike species from the genus *Thamnophilus* (Aves: Thamnophilidae) (Terborgh et al. 1990; Ridgely and Tudor 1994; Robinson et al. 2000; Zimmer and Isler 2003). Collectively, *Thamnophilus* species span humid and arid habitats of the eastern slope of the Andean foothills from Colombia to northern Argentina, and wet and dry lowland habitats in most regions of southern Central America and tropical South America (Zimmer and Isler 2003). Such geographic coverage and breadth of habitats, found in only a handful of other Neotropical suboscine genera (e.g., *Myrmotherula* antwrens, *Elaenia* flycatchers), make having a resolved phylogeny of *Thamnophilus* desirable insofar as the evolutionary relationships of its component species are likely to yield insights into general historical processes of diversification. Because of their widespread distribution, *Thamnophilus* antshrikes present a unique opportunity to examine the impact of the Andes on the historical diversification of organisms, including evolution both into and out of high elevation habitats (Bates and Zink 1994; García-Moreno and Silva 1997), as well as the diversification of lowland organisms isolated on either side of the mountains (Brumfield and Capparella 1996).

As currently recognized (Remsens et al. 2006), the genus *Thamnophilus* is composed of 27 species, all of which are insectivorous, socially monogamous, and sexually dichromatic, and which exist in the under- to mid-story of forest interiors, forest edge, or scrub (Table 1). The genus appears to be monophyletic based on similarities in vocalizations and behavior (Zimmer and Isler 2003), with the important caveat that one or more species from the antshrike genus *Sakesphorus* could fall within it. *Sakesphorus* has long been thought to be polyphyletic based on vocal and behavioral similarities of some of its species to *Thamnophilus* (Zimmer and Isler 2003). Within *Thamnophilus*, two main groups are readily recognizable by plumage pattern: (1) 7 barred species, the males of which have at least some horizontal black-and-white barring on the breast (the barring extends to other regions of the body in some taxa); and (2) 20 mostly solid species (ranging from pale gray to black), the males of which lack barring on the breast. As barred and solid plumages are found in other thamnophilid genera known to fall phylogenetically outside *Thamnophilus* (Irestedt et al. 2004), they are clearly homoplasious characters, but they provide a useful characterization of the two basic *Thamnophilus* plumage patterns. Variation within *Sakesphorus* includes solid plumage as well as a third readily recognizable plumage pattern exhibited by four of the six species (*S. canadensis*, *S. cristatus*, *S. melanotus*, and *S. bernardi*): solid plumage, but with a contrasting white belly. Plumage and morphological variation in

**Table 1.** List of tissue reference numbers, collecting localities, elevation, habitat, and collector and/or preparator of the vouchered specimens used in this study. Elevations that lack a range reflect lowland-restricted taxa whose entire distribution is no greater than 300 m above sea level. Unsampled were three "solid" lowland species (*T. sticturus*, *T. pelzelni*, and *T. ambiguus*).

| Species                            | Tissue number <sup>1</sup> | Locality  | Elevation <sup>2</sup> | Habitat <sup>3</sup>   | Collector or preparator |
|------------------------------------|----------------------------|---|------------------------|--|-------------------------|
| <i>Thamnomanes caesius</i>         | B9482 (LSUMZ)              | Guyana: Northwest District; Baramita  | L                      |  | R. T. Brumfield         |
| <i>Cymbilaimus lineatus</i>        | B18168 (LSUMZ)             | Bolivia: depto. Santa Cruz; Velasco; Parque Nacional Noel Kempff Mercado, 86 km ESE Florida | L                      |  | A. W. Kratter           |
| <i>Frederickena unduligera</i>     | B4281 (LSUMZ)              | Peru: depto. Loreto; Lower Río Napo region, E bank Río Yanayacu, ca. 90 km N Iquitos        | L                      |  | D. L. Dittmann          |
| <i>Sakesphorus canadensis</i>      | MBR6243 (KU)               | Guyana: along Washikunhmra River  | L                      | Tropical deciduous and gallery forest  | M. B. Robbins           |
| <i>Sakesphorus cristatus</i>       | B1188                      | Brazil: Minas Gerais; Bocauiua  | L                      | Tropical deciduous forest and arid scrub   | L. Carrara              |
| <i>Sakesphorus luctuosus</i>       | B7012 (USNM)               | Brazil: Para; 52 km SSW Altamira  | L                      | Flooded tropical evergreen and river-edge forest                                 | G. R. Graves            |
| <i>Sakesphorus bernardi</i>        | B5136 (LSUMZ)              | Peru: depto. Lambayeque; Las Pampas, km 885 Pan-American Hwy, 11 road km from Olmos         | L (0–1000 m)           | Tropical deciduous forest, arid lowland scrub, riparian thickets                 | D. L. Dittmann          |
| <i>Sakesphorus melanonotus</i>     | ML768                      | Venezuela: edo. Zulia; Campo Boscán, Hda. Grano de Oro                                      | L (0–500 m)            | Tropical deciduous forest  | M. Lentino              |
| <i>Sakesphorus melanothorax</i>    | B46298 (LSUMZ)             | Brazil: Amapá; Alto Rio Araguiri (collected in 1963)  | L (0–550 m)            | Flooded tropical evergreen forest  | M. M. Moreira           |
| <i>Thamnophilus atrinucha</i>      | B393 (USNM)                | Panama: prov. Bocas del Toro; Isla San Cristobal, Bocatorito                                | L (0–1500 m)           | Tropical lowland evergreen forest  | T. J. Parsons           |
| <i>Thamnophilus bridgesi</i>       | B16149 (LSUMZ)             | Costa Rica: prov. Puntarenas; 2 km SE Dominical   | L (0–1100 m)           | Tropical lowland evergreen, gallery, mangrove, and secondary forest              | S. J. Hackett           |
| <i>Thamnophilus doliatius</i>      | RTB390 (UWBM)              | Bolivia: depto. Santa Cruz; prov. Cordillera, 10.6 km E Abapo                               | LH (0–2000 m)          | River-edge forest, second-growth scrub, riparian thickets, river-island scrub    | R. T. Brumfield         |
| <i>Thamnophilus multistriatus</i>  | B52717 (LSUMZ)             | Colombia: depto. Santander; Gomez (collected in 1962)                                       | H (900–2200 m)         | Edge of montane evergreen and tropical deciduous forest, and second-growth scrub | M. A. Carriker, Jr.     |
| <i>Thamnophilus zarumae</i>        | B191 (LSUMZ)               | Peru: depto. Piura; km 34 on Olmos-Bagua Chica Hwy  | H (800–2100 m)         | Tropical deciduous forest  | J. P. O'Neill           |
| <i>Thamnophilus tenuipunctatus</i> | B1686 (ANSP)               | Ecuador: prov. Zamora Chinchipe; Zaruma, 1400 m   | H (500–2500 m)         | Montane evergreen forest   | B. Sliikas              |
| <i>Thamnophilus palliatus</i>      | MAB2 (UWBM)                | Bolivia: depto. Santa Cruz; prov. Florida, 23.2 km E Samaipata                              | LH (0–2200 m)          | Montane and tropical lowland evergreen forest, and second-growth scrub           | M. A. Blendinger        |

continued

Table 1. continued

| Species                             | Tissue number <sup>1</sup> | Locality  | Elevation <sup>2</sup> | Habitat <sup>3</sup>   | Collector or preparator |
|-------------------------------------|----------------------------|---|------------------------|--|-------------------------|
| <i>Thamnophtilus torquatus</i>      | B13900 (LSUMZ)             | Bolivia: depto. Santa Cruz; Serrania de Huachaca, 45 km E Florida                       | L (0–1000 m)           | Riparian thickets, gallery forest, cerrado                                     | G. H. Rosenberg         |
| <i>Thamnophtilus ruficapillus</i>   | RTB347 (UWBM)              | Bolivia: depto. Santa Cruz; prov. Cordillera, El Tambo, 14 km SE Comarapa               | LH (0–3050 m)          | Riparian thickets, montane evergreen forest, semihumid and humid montane scrub | R. T. Brumfield         |
| <i>Thamnophtilus schistaceus</i>    | B12559 (LSUMZ)             | Bolivia: depto. Santa Cruz; Velasco; 50 km ESE Florida, Arroyo de Encanto               | L (0–1100 m)           | Tropical and tropical flooded lowland evergreen forest                         | C. G. Schmitt           |
| <i>Thamnophtilus murinus</i>        | B9206 (USNM)               | Guyana: Northwest District; Baramita  | L (0–1000 m)           | Tropical lowland evergreen forest  | R. T. Brumfield         |
| <i>Thamnophtilus aethiops</i>       | B14649 (LSUMZ)             | Bolivia: depto. Santa Cruz; Serrania de Huachaca, 21 km SE Catarata Arco Iris           | LH (0–2000 m)          | Tropical lowland evergreen forest  | M. D. Cardeno           |
| <i>Thamnophtilus aroyae</i>         | RTB395 (UWBM)              | Bolivia: depto. Cochabamba; prov. Chapare, San Onofre, ca 43 km W Villa Tunari          | H (600–1700 m)         | Montane evergreen forest, secondary forest                                     | R. T. Brumfield         |
| <i>Thamnophtilus unicolor</i>       | B12144 (LSUMZ)             | Ecuador: prov. Pichincha; Mindo   | H (1200–2300 m)        | Montane evergreen forest   | J. Kennard              |
| <i>Thamnophtilus caeruleus</i>      | 395426 (FMNH)              | Brazil: Sao Paulo; Boraceia   | LH (0–2800 m)          | Montane, tropical lowland evergreen, gallery and tropical deciduous forest     | D. F. Stotz             |
| <i>Thamnophtilus cryptoleucus</i>   | B7285 (LSUMZ)              | Peru: depto. Loreto; Amazonas I. Pasto, 80 km NE Iquitos                                | L                      | River-edge and secondary forest  | A. P. Capparella        |
| <i>Thamnophtilus nigrocinereus</i>  | B20233 (LSUMZ)             | Brazil: Amazonas; Munic. Novo Airao; Arquipelago das Anavilhanas                        | L                      | Flooded tropical evergreen, river-edge, and gallery forest                     | M. Cohn-Haft            |
| <i>Thamnophtilus punctatus</i>      | B4172 (USNM)               | Guyana: Berbice; West bank Dubulay ranch  | L (0–1000 m)           | Tropical lowland evergreen and secondary forest                                | C. M. Milensky          |
| <i>Thamnophtilus stictocephalus</i> | B13850 (LSUMZ)             | Bolivia: depto. Santa Cruz; Serrania de Huachaca, ca 45 km E Florida                    | L (0–700 m)            | Tropical lowland evergreen and secondary forest                                | A. Castillo             |
| <i>Thamnophtilus nigriceps</i>      | 20238 (UAM)                | Panama: prov. Panama, Lago Bayano   | L (0–600 m)            | Tropical lowland evergreen and secondary forest                                | M. J. Miller            |
| <i>Thamnophtilus praecox</i>        | B3190 (ANSP)               | Ecuador: prov. Sucumbios; Imuya Cocha   | L                      | Flooded tropical evergreen forest  | F. Sornoza              |
| <i>Thamnophtilus amazonicus</i>     | B13045 (LSUMZ)             | Bolivia: depto. Santa Cruz; Velasco, W bank Río Paucerna, 4 km upstream from Río Itenez | L                      | Tropical and flooded lowland evergreen, river-edge, and gallery forest         | D. C. Schmitt           |
| <i>Thamnophtilus insignis</i>       | B7486 (LSUMZ)              | Venezuela: terr. Amazonas; Cerro de la Neblina camp VII                                 | H (900–2000 m)         | Montane evergreen and elfin forest   | J. P. O'Neill           |
| <i>Thamnophtilus divisorius</i>     | 228 (PNSD)                 | Brazil: Acre, Munic. Mâncio Lima, Parque Nacional da Serra do Divisor                   | L (500 m)              | Tropical lowland evergreen forest  | B. M. Whitney           |

<sup>1</sup>LSUMZ, Louisiana State University Museum of Natural Science; USNM, National Museum of Natural History; UWBM, University of Washington Burke Museum of Natural History; ANSP, Academy of Natural Sciences of Philadelphia; UAM, University of Alaska Museum; PNSD, Museo Paraense Emilio Goeldi; FMNH, Field Museum of Natural History; KV, University of Kansas Natural History Museum.

<sup>2</sup>Elevations are taken from Zimmer and Isler (2003) and Whitney et al. (2004).

<sup>3</sup>Habitat designations from Stotz et al. (1996) and Whitney et al. (2004).

*Thamnophilus* and *Sakesphorus* do not offer a rich palette of characters from which to reconstruct a robust phylogeny (Zimmer and Isler 2003). It is thus not surprising that previously published hypotheses of intrageneric relationships are few and largely relegated to statements of probable affinities in a nonphylogenetic context, or to taxonomic sequence in linear classifications. Most of the previously proposed phylogenetic relationships involve likely sister relationships between recently elevated allotaxa, with essentially no previously proposed hypotheses of higher level interspecific relationships.

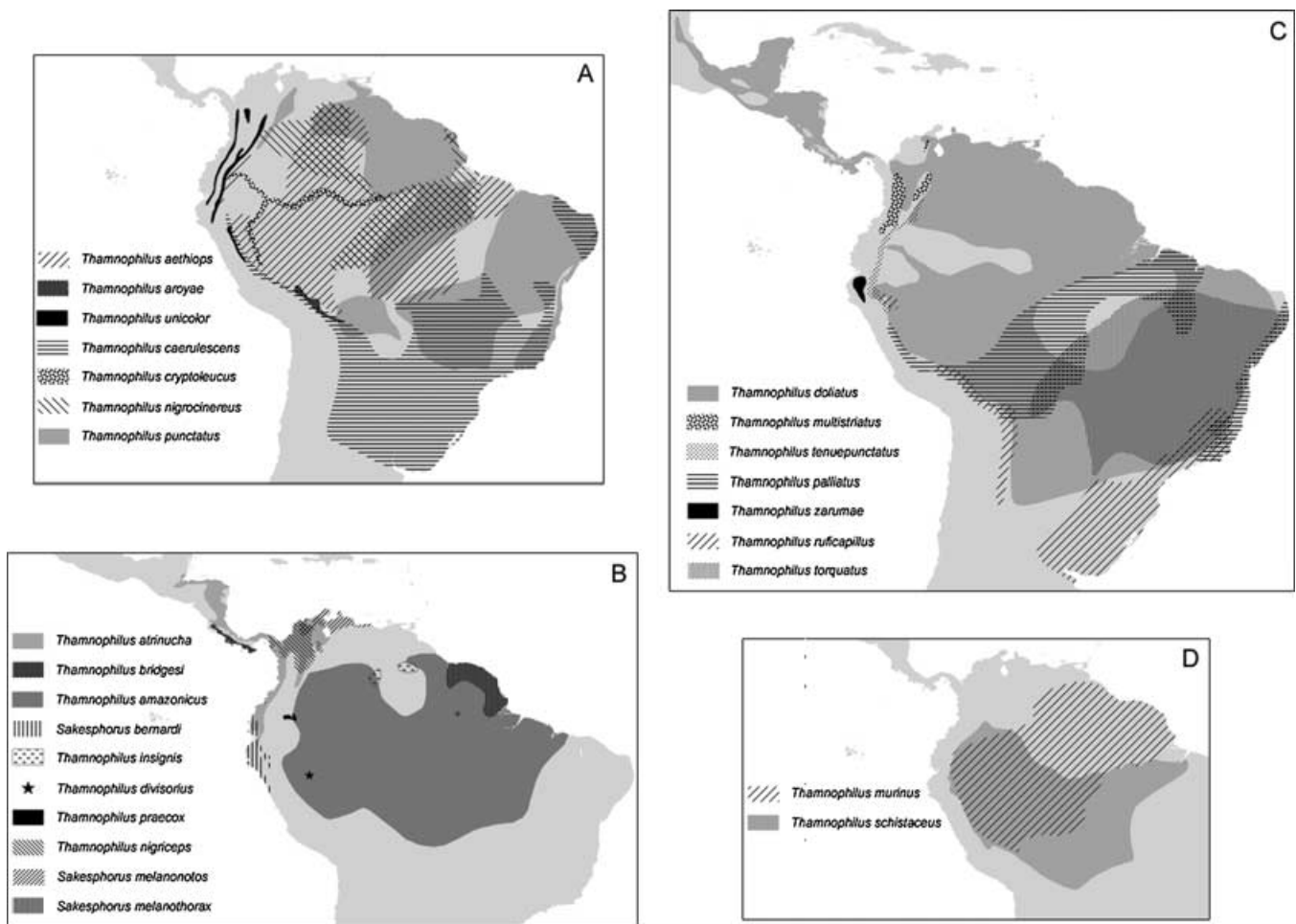
## Biogeography

### EVOLUTION INTO AND OUT OF THE ANDES

Six *Thamnophilus* species have distributions restricted to montane elevations (coded as highland-restricted in Methods), either on the dry western slope of the central Andes in southwestern Ecuador and northwestern Peru (*T. zarumae*), the humid eastern slope of the Andes (*T. tenuipunctatus*, *T. aroyae*), both slopes of

the northern Andes (*T. multistriatus*, *T. unicolor*), or in the tepuis of northern South America (*T. insignis*) (Zimmer and Isler 2003) (Fig. 1). A seventh recently described species (*T. divisorius*) could be considered highland-restricted, because it exists on an outlying, low-elevation (~500 m) Andean ridge (Acre Arch), but is not found in the surrounding lowland forest (Whitney et al. 2004). Of the five species whose distributions span both lowland and highland regions (coded as lowland-to-highland in Methods), one (*T. doliatus*) has a relatively widespread range that encompasses montane and lowland second-growth forests, one (*T. aethiops*) exists in tropical evergreen forests of the eastern Andean foothills and contiguously with the Amazon Basin, and three (*T. palliatus*, *T. ruficapillus*, *T. caeruleus*) occupy the humid montane forests on the eastern slope of the Andes but extend into the drier deciduous and gallery forests in lowland regions south and east of the Amazon Basin. All other *Thamnophilus* species ( $N = 16$ ) and all *Sakesphorus* species are restricted to the lowlands (Table 1).

Lynch (1986) categorized clades containing Andean taxa as (1) exclusively Andean, (2) Andean with some non-Andean



**Figure 1.** Schematic distributions of *Thamnophilus* species. The maps are divided into four panels to depict the distributions more clearly. Digital maps (Ridgely et al. 2003) for each species were downloaded from InfoNatura (2005).

taxa, and (3) non-Andean with some Andean taxa. *Thamnophilus* antshrikes would fall into the third category. It is generally assumed that most of the original colonists of the Andes were species from adjacent lowland populations that could tolerate the lower temperatures associated with higher elevations (Chapman 1926; Janzen 1967; Moritz et al. 2000). The distributions of such taxa would appear as Lynch's (1986) third clade type: a group of primarily lowland taxa but with some taxa whose distributions extend into the Andes. Because most thamnophilids have lowland distributions and all taxa that fall in the sister clade to *Thamnophilus* and *Sakesphorus* antshrikes are restricted to the lowlands (Irestedt et al. 2004, Brumfield, unpublished data), it seems reasonable to assume that the highland distribution of some *Thamnophilus* species is derived. We test this hypothesis with the molecular phylogeny.

In his monograph on the birds of Ecuador, Chapman (1926, p. 87) proposed that the majority of birds found in the Subtropical zone of the Andes (~1400 m to ~2900 m sensu Chapman 1926) were derived by primary differentiation of populations from the adjacent Amazonian lowlands, but that other foothill species must have come from noncontiguous subtropical regions elsewhere. The humid forests of the coastal ranges in southeastern Brazil are one such region, but it is isolated from the humid slopes of the Andes by several hundred kilometers of dry, open chaco, and cerrado habitats. A former connection between the two is suggested by the disjunct distributions of bird (e.g., *T. ruficapillus*, *T. palliatus*; Sick 1985, 1993), plant (Dusen 1903), mammal (Ribeiro 1906), and fish (Ribeiro 1906) species that are shared between them. Chapman (1926) proposed that a former corridor of humid forest between them accounts for the origin of some of the Andean foothill taxa. A current connection between the regions is exhibited by some species with broader habitat requirements, including *T. caerulescens*. Its distribution extends from the humid coastal ranges and lowlands of southeastern Brazil, through the dry intervening chaco and cerrado habitats, and into the humid eastern slope of the Andes. Chapman (1926) also proposed that subtropical regions to the north in Mexico may have been a source for some Andean taxa, but no *Thamnophilus* species exist there.

If colonization of the Andes occurred from the adjacent lowlands, the principal difficulty is identifying the vicariant event that subsequently isolated lowland from highland populations. Differentiation could occur via climatic perturbations that resulted in the removal of appropriate habitat between highland and lowland populations (e.g., refugia, Haffer 1969). Subsequent colonization and population differentiation at even higher Andean elevations could occur by this same mechanism (Lynch 1986). Assuming colonization and differentiation from higher to lower elevations never occur, this model of Andean diversification makes the phylogenetic prediction of a sister relationship between taxa in the two highest elevations with progressively more distant phylo-

genetic relationships moving downslope toward the base of the tree (Creighton 1985). Although this model has received empirical support (Bates and Zink 1994; Hall 2005; Pérez-Emán 2005), the simplifying assumption that colonization from higher to lower elevations does not occur is unrealistic (Lynch 1986; Burns 1997), so that the expected phylogenetic relationships are often much more complex and difficult to predict. The model also does not consider the horizontal differentiation of populations that occurs at the same elevation after the divergence of highland and lowland forms (e.g., if highland populations became isolated from one another on different montane peaks) (Knox and Palmer 1995; García-Moreno and Fjeldså 2000).

In attempting to explain the origin of birds that are found at higher elevations in the Andes (~2900 m to ~4500 m; sensu Chapman 1926), Chapman (1917) proposed three source populations that could have colonized newly available Andean habitats: (1) the Guianan highlands (Pantepui region), (2) the Central American highlands, and (3) the temperate lowlands of southern South America. Although Chapman's (1917) hypotheses were proposed to explain the colonization and subsequent differentiation of taxa in the High Andes (e.g., puna and páramo habitats) where *Thamnophilus* species do not exist, they can be applied to foothill forms. Once in the Andes, the initial differentiation from the source population would occur via allopatric differentiation (Vuilleumier 1969). Because no *Thamnophilus* antshrike occurs in the Central American highlands, the only alternatives for *Thamnophilus* are the adjacent lowlands and the Guianan highlands.

Our strategy was to test the basic predictions of the above models in their simplest form by estimating the geographic locality of the ancestral taxon in each *Thamnophilus* clade containing both high- and low-elevation taxa. Because we suspected the history of diversification among lower and higher elevation *Thamnophilus* taxa was complex, we also used a Bayesian, model-based method to estimate transition rates among different elevational distributions. This approach provides information about the relative overall transition rates among different elevations while building uncertainty about the phylogenetic relationships into the analysis.

#### **DIVERSIFICATION OF LOWLAND TAXA ON OPPOSITE SIDES OF THE ANDES**

Because the Andes and the associated arid lowland habitats near its northern (i.e., Caribbean) and eastern margins represent an insurmountable dispersal barrier for many birds and other organisms restricted to humid lowland forest (Chapman 1917; Janzen 1967; Gentry 1982), the Andean orogeny also had a profound impact on the diversification of lowland taxa (Prum 1988). That this barrier has had a demonstrable influence on the diversification of lowland organisms is evident both by current distributional

data, in which the Eastern Andes usually delineate the distributions of lowland organisms (Hershkovitz 1969; Hilty and Brown 1986; Ridgely and Tudor 1994), and by phylogeographic studies, which typically show a deep phylogenetic division between *trans*- (west of the Andes) and *cis*-Andean (east of the Andes) clades (Cracraft and Prum 1988; Prum 1988; Brumfield and Capparella 1996; Dick et al. 2003; Hoffmann and Baker 2003). Whether the Andes played a direct role in diversification through the vicariance of species' distributions that, before the Andean uplift, were continuous from the Amazon Basin to the Pacific Ocean (Chapman 1917), or an indirect role by creating a narrow lowland corridor around its northern rim that would allow across-Andes dispersal during humid climatic cycles (Haffer 1967) and/or low sea levels, but isolation during dry cycles and/or high sea levels (Nores 2004), is still debated. Differentiating these alternative hypotheses is complicated by the lack of unique testable predictions of area relationships (Brumfield and Capparella 1996) and by the poor fossil record for most organisms that makes good calibrations of molecular clocks elusive (Graur and Martin 2004). We thus tested in *Thamnophilus* antshrikes the basic prediction that the most basal divergence within relevant clades occurs between *trans*- and *cis*-Andean representatives.

## Methods

### SAMPLING AND DATA COLLECTION

Individuals from 24 of the 27 described *Thamnophilus* species were sampled (Table 1). Assuming monophyly of each species allowed us to collect more sequence data per taxon. Three species in the *T. punctatus* species group (*T. sticturus*, *T. pelzelni*, and *T. ambiguous*) were not included in this study. A phylogeographic study of the entire *T. punctatus* species complex is desirable (Isler et al. 1997), but was beyond the scope of this investigation. As allo-replacements that were elevated only recently from subspecies status based on vocal differences, the species in this group form a clade (Isler et al. 1997; R. T. Brumfield, unpubl. data). Most tissues used in this study were vouchered, frozen-preserved pectoral muscle samples obtained during fieldwork by RTB in Bolivia and Guyana or borrowed from the genetic resources collections of the Louisiana State University Museum of Natural Science (LSUMZ), the University of Washington Burke Museum of Natural History (UWBM), the United States National Museum of Natural History (USNM), the Academy of Natural Science of Philadelphia (ANSP), the University of Kansas Natural History Museum (KU), the University of Alaska Museum (UAM), or the Field Museum of Natural History (FMNH). Because we were unable to borrow a tissue of the Colombian endemic *T. multistriatus*, we extracted DNA from a skin housed at the LSUMZ (Table 1). Sequences of *T. divisorius* are from Whitney et al. (2004). The outgroup taxa *Cymbilaimus lineatus*, *Thammomanes caesius*, and *Frederickena*

*unduligera* were chosen because they fall within the sister clade to *Thamnophilus* and *Sakesphorus* antshrikes (Irestedt et al. 2004).

In our initial phylogenetic analyses of the sequences it became clear that some *Sakesphorus* species fall within *Thamnophilus*. We thus sought to include samples from all five *Sakesphorus* species. DNA sequences of *S. cristatus* were provided by Daniela Rodrigues Lacerda of the Universidade Federal de Minas Gerais (UFMG). Jorge Pérez-Emán of the Universidad Central de Venezuela sent us PCR amplifications of *S. melanonotus* that we sequenced at LSU. Because we could not locate a tissue sample of *S. melanothorax* in any museum worldwide, we extracted DNA from a museum study skin housed at the LSUMZ (Table 1).

To achieve phylogenetic resolution at multiple divergence levels across the evolutionary tree, we sequenced three mitochondrial genes plus two introns from a more slowly evolving nuclear gene (Hillis 1987). Although this multilocus approach is often advocated in the literature, it is not without pitfalls (Barker 2004). In particular, combining DNA sequences that evolved under markedly different rates can lead to errors in phylogenetic estimation (Bull et al. 1993), and different genes can have intrinsically conflicting phylogenetic histories (Edwards et al. 2006). To address these possibilities, we analyzed each gene separately in addition to a combined analysis.

DNA was extracted from tissues using DNeasy kits (Qiagen, Valencia, CA). DNA sequences were obtained from the mitochondrial genes *cytochrome b* (*cytb*), *nicotinamide dehydrogenase subunit 2* (ND2), and *nicotinamide dehydrogenase subunit 3* (ND3), and from introns 5 and 7 of the nuclear  $\beta$ -*fibrinogen* gene (hereafter  $\beta$ f5 and  $\beta$ f7) (Kocher et al. 1989; Desjardins and Morais 1990; Prychitko and Moore 1997). To minimize co-amplification of nuclear copies of mitochondrial genes, DNA was extracted solely from mitochondrial-rich pectoral muscle tissue. Each gene was amplified using the polymerase chain reaction (PCR) in a PTC-200 thermocycler (MJ Research, Waltham, MA), purified using polyethylene glycol (PEG) precipitation, and sequenced directly with an ABI Prism cycle sequencing kit (Applied Biosystems, Foster City, CA) and ABI 377, 3100, or 3700 Genetic Analyzers. Primer combinations for amplification were ND2 (L5215/H6313), ND3 (L10755/H11151), *cyt b* (L14990/H16065),  $\beta$ f5 (FIB5L/FIB5H), and  $\beta$ f7 (FIB-B17U/FIB-B17L) (Table 2). Internal sequencing primers for ND2 were L5758 and H5766, plus a primer designed specifically for *T. bridgesi* (L5758bridge), for *cyt b* were *cytb.intf* and *cytb.intr*, and for  $\beta$ f7 were BF7intF and BF7intR. Amplification and sequencing primers for the toepad samples were ND3 (L10755/H10902b, L10907b/H11014b, L11023a/H11151) and ND2 (L5215/H5366, L5326/H5478, L5457/H5605, L5912/H6037). Sequencing reactions were purified with AutoSeq G-50 spin columns (Amersham Biosciences, Piscataway, NJ). Genes were amplified from total



**Table 2.** List of primers used in PCR amplification and cycle sequencing. The mitochondrial primers are numbered according to location in the *Gallus* genome (Desjardins and Morais 1990) and the  $\beta$ -fibrinogen external primers with regard to a *Gallus* cDNA sequence (Weissbach et al. 1991).

| Gene         | Primer      | Sequence (5' → 3')                    | Location                      |
|--------------|-------------|---------------------------------------|-------------------------------|
| cyt <i>b</i> | L14990      | CCA TCC AAC ATC TCA GCA TGA TGA AA    | L14990 (cyt <i>b</i> )        |
| cyt <i>b</i> | H16065      | AAC TGC AGT CAT CTC CGG TTT ACA AGA C | H16065 (tRNA <sub>Thr</sub> ) |
| cyt <i>b</i> | cyt.intf    | CAC GAR ACY GGR TCY AAY AAY CC        | L15496 (internal)             |
| cyt <i>b</i> | cyt.intr    | GGR TTR TTR GAY CCR GTY TCG TG        | H15496 (internal)             |
| ND2          | L5215       | TAT CGG GCC CAT ACC CCG AAA AT        | L5193 (tRNA <sub>Met</sub> )  |
| ND2          | H6313       | CTC TTA TTT AAG GCT TTG AAG GC        | H6313 (tRNA <sub>Trp</sub> )  |
| ND2          | L5758       | GGN GGN TGR RBH GGN YTD AAY CAR AC    | L5733 (internal)              |
| ND2          | L5758bridge | GGN GGN TGA GCY GGA YTN AAY CAR AC    | L5733 (internal)              |
| ND2          | H5766       | DGA DGA RAA DGC YAR RAY YTT DCG       | H5766 (internal)              |
| ND2          | H5366       | RGG NAG RAT RGC TAG GTG TTR           | H5366 (internal)              |
| ND2          | L5326       | AAG YAA YCA YTG AAT CAT AGC           | L5326 (internal)              |
| ND2          | H5478       | TRT CTC ATT GYC CRG TRT RYC           | H5478 (internal)              |
| ND2          | L5457       | CYT CHG CWC TAC TCY TAT TYT           | L5457 (internal)              |
| ND2          | L5912       | ACC CTH RAY WCA ATC AAA ACC           | L5912 (internal)              |
| ND2          | H6037       | TGY TTG GTT AGY TCT TGR ATR AT        | H6037 (internal)              |
| ND3          | L10755      | GAC TTC CAA TCT TTA AAA TCT GG        | L10733 (tRNA <sub>Gly</sub> ) |
| ND3          | H11151      | GAT TTG TTG AGC CGA AAT CAA C         | H11154 (tRNA <sub>Arg</sub> ) |
| ND3          | L10907b     | CTC TCC CCC TAC GAA TGT GG            | L10907 (internal)             |
| ND3          | L11023a     | CMT GAG CYA CYC AAC TTC A             | L11012 (internal)             |
| ND3          | H10902b     | CGA AGC CAC ATT CGT AGG G             | H10902 (internal)             |
| ND3          | H11014b     | GAG GGG AAA TTC AAG TTG AGT           | H11014 (internal)             |
| $\beta$ f5   | FIB5L       | CGC CAT ACA GAG TAT ACT GTG ACA T     | S713                          |
| $\beta$ f5   | FIB5H       | GCC ATC CTG GCG ATC TGA A             | AS767                         |
| $\beta$ f7   | FIB-B17U    | GGA GAA AAC AGG ACA ATG ACA ATT CAC   | S1093                         |
| $\beta$ f7   | FIB-BI7L    | TCC CCA GTA GTA TCT GCC ATT AGG GTT   | AS1240                        |
| $\beta$ f7   | BF7intF     | TTG TAA AGT ACA TAA CTG AGC           |                               |
| $\beta$ f7   | BF7intR     | GTG CTC AGT TAT GTA CTT TAC AA        |                               |

DNA in PCR volumes of 25  $\mu$ L, with 0.1  $\mu$ L AmpliTaq polymerase (Applied Biosystems), 1X Tris buffer with MgCl<sub>2</sub> included (Applied Biosystems), dNTPs (each dNTP 50  $\mu$ M), and 2.5  $\mu$ L (~50 ng) of template. Primer concentrations were 125 nM for cyt *b*, and 500 nM for all other loci. Individuals heterozygous for indels in  $\beta$ f7 (*T. murinus*, *T. punctatus*, *S. canadensis*) were sequenced by TOPO cloning the PEG-purified amplifications (Invitrogen), and cycle sequencing the mini-prepped clones with M13 primers and the internal primers (Table 2).

### SEQUENCE ANALYSIS

Sequences were aligned manually using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, MI). Protein-coding mitochondrial sequences were translated into amino acids to verify the absence of stop codons or other anomalous residues. All phylogenetic analyses were performed using a portable UNIX version of PAUP\*4.0b10 on Macintosh G5 computers with two parallel processors (Swofford 2003). Homogeneity in base frequencies across taxa was tested in each dataset (separate loci plus combined) using a  $\chi^2$  analysis of base frequencies across taxa.

Maximum-likelihood and Bayesian methods were the primary methods of phylogenetic analysis, but equal-weighted parsimony was also used to estimate phylogenetic relationships from each dataset. All tree searches under parsimony were performed heuristically with 10 random addition replicates and TBR branch-swapping. Confidence in nodes was assessed using 100 bootstrap replicates, with each replicate composing a heuristic search with 10 random-addition replicates. To determine the optimal ML model for each dataset (each locus plus combined), we used the Akaike Information Criterion implemented in the program ModelTest 3.5 (Posada and Crandall 1998; Posada and Buckley 2004). Using PAUP\*, likelihood scores for input into ModelTest were estimated on neighbor-joining trees inferred for each dataset from an uncorrected “*p*” distance matrix. The best-fit ML model (Table 3) for ND3 was K81uf +  $\Gamma$  + INV, for  $\beta$ f5 and  $\beta$ f7 was GTR +  $\Gamma$ , and for cyt *b*, ND2 and the combined dataset was GTR +  $\Gamma$  + INV (Swofford et al. 1996). Starting with the model parameters estimated on the neighbor-joining tree in ModelTest, a relatively fast PAUP\* search was first performed by setting the addition sequence of taxa to “as-is.” After re-estimating

**Table 3.** Summary of model parameters and tree scores for maximum-likelihood and Bayesian analyses for all taxa. The maximum-likelihood parameters were used in bootstrap analyses of the data. NA indicates parameters that were not estimated.

|                    | ND2    | ND3    | <i>cytb</i> | $\beta 5$ | $\beta 7$ | Combined |
|--------------------|--------|--------|-------------|-----------|-----------|----------|
| Maximum-likelihood |        |        |             |           |           |          |
| No. bp             | 1046   | 367    | 1045        | 532       | 970       | 3960     |
| No. Trees          | 1      | 3      | 2           | 1         | 1         | 1        |
| $-\ln(L)$          | 8292.4 | 2834.4 | 6921.7      | 1738.1    | 2767.8    | 23352.3  |
| $r_{AC}$           | 0.3999 | 1.000  | 0.977       | 1.026     | 2.102     | 0.965    |
| $r_{AG}$           | 23.199 | 12.106 | 15.391      | 2.622     | 3.211     | 9.330    |
| $r_{AT}$           | 0.776  | 0.387  | 1.315       | 0.275     | 0.792     | 0.804    |
| $r_{CG}$           | 0.760  | 0.387  | 0.087       | 1.153     | 1.009     | 0.469    |
| $r_{CT}$           | 12.594 | 12.106 | 22.654      | 4.053     | 5.679     | 14.972   |
| $\alpha$           | 1.628  | 3.497  | 1.815       | 0.401     | 0.303     | 0.797    |
| $p_{iv}$           | 0.486  | 0.547  | 0.573       | NA        | NA        | 0.515    |
| freq(A)            | 0.342  | 0.299  | 0.290       | 0.303     | 0.345     | 0.309    |
| freq(C)            | 0.363  | 0.337  | 0.355       | 0.164     | 0.170     | 0.289    |
| freq(G)            | 0.063  | 0.119  | 0.120       | 0.210     | 0.176     | 0.140    |
| freq(T)            | 0.232  | 0.245  | 0.235       | 0.323     | 0.309     | 0.263    |
| Bayesian           |        |        |             |           |           |          |
| $-\ln(L)$          | 4304.2 | 2554.6 | 6958.2      | 1790.0    | 2811.8    | 23440.0  |
| $r_{AC}$           | 0.474  | 1.036  | 1.065       | NA        | 2.285     | 0.845    |
| $r_{AG}$           | 45.938 | 19.858 | 17.705      | NA        | 3.393     | 8.493    |
| $r_{AT}$           | 1.183  | 0.293  | 1.458       | NA        | 0.826     | 0.817    |
| $r_{CG}$           | 1.756  | 0.692  | 0.110       | NA        | 1.072     | 0.401    |
| $r_{CT}$           | 21.052 | 12.695 | 25.774      | NA        | 6.305     | 13.858   |
| Kappa              | NA     | NA     | NA          | 4.332     | NA        | NA       |
| $\alpha$           | 1.588  | 2.637  | 1.773       | 0.387     | 0.330     | 0.207    |
| $p_{iv}$           | 0.485  | 0.468  | 0.556       | NA        | NA        | 0.003    |
| freq(A)            | 0.371  | 0.296  | 0.290       | 0.286     | 0.343     | 0.307    |
| freq(C)            | 0.339  | 0.358  | 0.358       | 0.181     | 0.173     | 0.302    |
| freq(G)            | 0.063  | 0.091  | 0.117       | 0.213     | 0.176     | 0.141    |
| freq(T)            | 0.228  | 0.255  | 0.235       | 0.320     | 0.309     | 0.251    |

the parameters on the most-likely tree from this initial search, a final search was performed with 10 random-addition replicates of the taxa. Using these same parameters, a bootstrap analysis (number of reps = 100) was performed using 10 random-addition replicate.

Using the optimal ML model inferred from the AIC tests, Bayesian analyses were performed on each dataset using the program MrBayes (version 3.0b4; Huelsenbeck and Ronquist 2001). Because the K81uf substitution model is not implemented in MrBayes, we replaced it with GTR for the analysis of ND3. On the combined dataset, the data were partitioned by gene and analyzed under the best-fit ML model for each gene. Uniform interval priors were assumed for the parameters, except for base frequencies, which were assigned a Dirichlet prior (Huelsenbeck and Ronquist 2001). Four heated chains were run for  $2.0 \times 10^6$  generations, and sampled every 1000. After visually examining burn-in plots from each run using Tracer (Rambaut and Drummond 2003) to ensure the chain had reached stationarity, trees from the first 100,000 generations were discarded, with the remaining trees used to estimate

posterior probabilities of tree topology and other ML parameters. Three independent runs with different random seeds were performed to ensure the posterior probabilities were stable.

Topological congruence among the phylogenies reconstructed from each dataset was evaluated in a parsimony framework by performing a partition homogeneity test ( $nreps = 1000$ ) as implemented in PAUP\* (Farris et al. 1995). We also evaluated potential differences by testing for statistical significance of strongly incongruent nodes (Shimodaira and Hasegawa 1999). "Strong" incongruence was identified by the presence of a well-supported node in one phylogeny that was in direct conflict to a well-supported node in another phylogeny, with a well-supported node defined as one with either a 75% or higher bootstrap (ML or parsimony) value or a 0.95 or higher posterior probability. Nodes in conflict were evaluated by performing Shimodaira–Hasegawa (SH) tests (Shimodaira and Hasegawa 1999) with full optimization and 1000 bootstrap replicates between trees with the node in conflict, and constrained trees in congruence with phylogenetic relationships from other datasets.

### BIOGEOGRAPHIC ANALYSIS

To reconstruct the evolutionary history of Andean colonization, we coded the distribution of each ingroup species as lowland-restricted (L, 0–1500 m), lowland-to-highland (LH, 0–3050 m), or highland-restricted (H, 500–2500 m), based on the elevations at which each was most commonly observed in the field (Zimmer and Isler 2003). This discrete coding scheme does not account for variance in habitat distributions, but captures what would be considered the “optimal” elevation for each. The only ambivalent coding was of the recently described species *Thamnophilus divisorius*, which is known only from a single locality in a low-lying ridge (~500 m elev.) in western Amazonia (Whitney et al. 2004). This species could be considered lowland-restricted in that it exists at an elevation no greater than 600 m, or highland-restricted, in that it does not exist in the lowland habitats adjacent to the ridge. To address both possibilities, we performed separate analyses with the two different codings. We did not code it as lowland-to-highland because it is not distributed continuously between these elevations where it exists.

Ancestral character-state reconstructions for this multi-state character were inferred using parsimony (Maddison 1990; Maddison and Maddison 2000) as well as a Bayesian method that builds into the analysis uncertainty about both the process of character change and the phylogeny (Mooers and Schluter 1999; Huelsenbeck et al. 2003; Pagel et al. 2004; Ronquist 2004). Parsimony reconstructions were performed using MacClade (Maddison and Maddison 2000) and assuming unordered character states. With the ML tree from the combined analysis (Fig. 2F), we used the program DIVA (Ronquist 1997) with default parameters to reconstruct ancestral areas from the three-character binary matrix (lowland-restricted, lowland-to-highland, or highland-restricted). The Bayesian method was performed using the program BayesMultistate (Pagel et al. 2004; Buschbom and Barker 2006). On the same set of 1,900 trees sampled from the posterior distribution and used to estimate nodal confidence in the phylogeny (Huelsenbeck and Ronquist 2001), BayesMultistate was used to estimate ML rates of character change and ancestral character states after excluding outgroup taxa. The method accounts for phylogenetic uncertainty by averaging the ML estimates across the set of trees. Ancestral state posterior probabilities for a given node were estimated by multiplying the mean ancestral character state probability at that node across all trees by the proportion of the trees in which the node was found (Pagel et al. 2004). Average tree lengths were calculated using Tracer on a second set of 1900 trees from a MrBayes analysis performed using the same protocol described above but excluding the outgroup taxa.

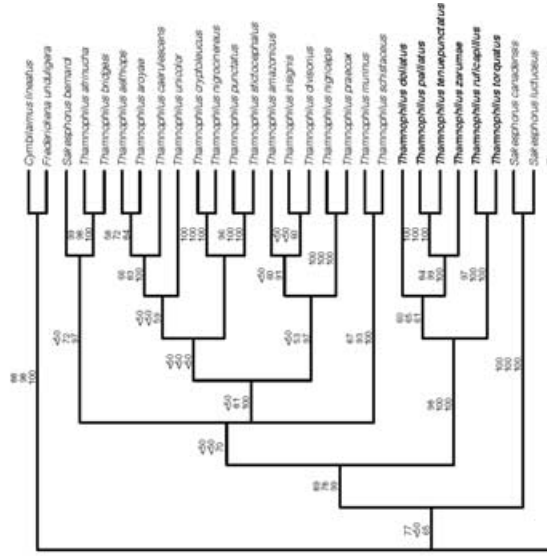
We sought to test whether the most basal divergence within *Thamnophilus* clades occurred between *trans*- and *cis*-Andean taxa. Because there were few previously proposed clades in *Thamnophilus* beyond those of obvious sister taxa, we could not

set up testable hypotheses a priori. Instead, we used the phylogenetic hypotheses generated from the molecular data presented herein to set up a series of a posteriori tests. To measure whether *Thamnophilus* clades were consistent with a basal split between *trans*- and *cis*-Andean groups, we first defined paralogy-free subtrees (Nelson and Ladiges 1996). Beginning at the tips of the tree, relevant paralogy-free subtrees were identified by moving toward the base of the tree until reaching the first node that contained representatives from both *trans*- and *cis*-Andean regions (Nelson and Ladiges 1996). We used SH tests with full optimization and 1000 bootstrap replicates to compare the most-likely tree with trees constrained to the alternative hypothesis. For example, if a subtree had a basal divergence between *cis*- and *trans*-Andean taxa, we performed a second ML analysis in which this relationship was constrained to not occur, and used the SH test to compare the constrained and unconstrained trees.

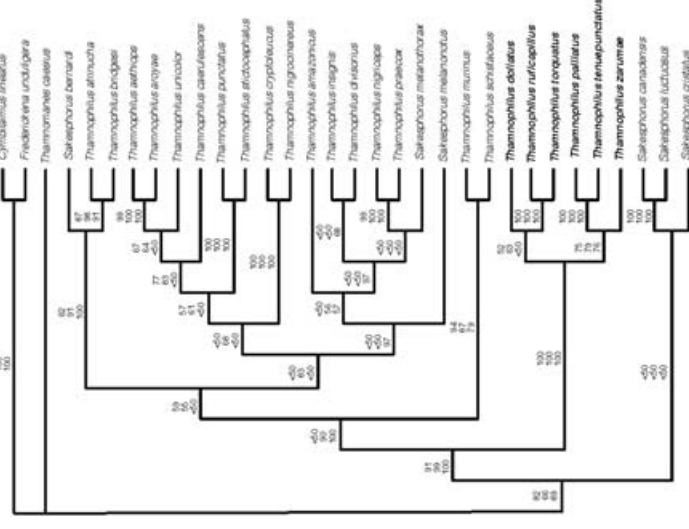
To make approximate estimates of divergence times (Graur and Martin 2004; Lovette 2004; Ho et al. 2005; Ho and Larson 2006), we converted ML genetic distances for *cyt b* and ND2 to time measures. A molecular clock calibration for *cyt b* variation in birds (Fleischer et al. 1998) equates 1.6% sequence divergence with one million years of evolution. The calibration is based on *cyt b* variation in Hawaiian oscine passerines and is probably more applicable to suboscine passerine antshrikes than a 2.0% rate (Shields and Wilson 1987) derived from mitochondrial RFLP variation in geese. Recently, Arbogast et al. (2006) estimated 4.0% sequence divergence per million years based on ND2 evolution in Galapagos populations of oscine mockingbirds. To date relevant ancestral nodes in the *Thamnophilus* phylogeny, we used a parametric bootstrap to examine first whether genetic variation at *cyt b* and ND2 was consistent with a molecular clock. On the ML phylograms reconstructed from the two genes, parameters in the GTR + G + INV substitution model were estimated with and without a clock constraint and the likelihood calculated. The test statistic is twice the absolute difference in log likelihood scores. *S. melanothorax* and *T. multistriatus* were excluded from the ND2 analysis and *S. melanothorax*, *S. cristatus*, *S. melanonotus*, and *T. multistriatus* from the *cyt b* analysis because of missing data. Any site in which the remaining taxa had missing data was deleted. To generate a null distribution, we used the program Seq-Gen (Rambaut and Grassly 1997) to evolve 100 sequences of 951 bp (*cyt b*; sequence length shorter than in

**Figure 2.** Maximum-likelihood trees for each gene analyzed separately (A)–(E) and combined (F). Nodal support above branches based on 100 bootstraps under equal-weighted maximum parsimony (top) and maximum likelihood (middle). Posterior probabilities ( $\times 100$ ) from Bayesian analysis also presented (bottom). Tree for ND3 is a strict consensus of three ML trees; tree for *cyt b* is a strict consensus of two ML trees. Names of “barred” *Thamnophilus* species in bold.

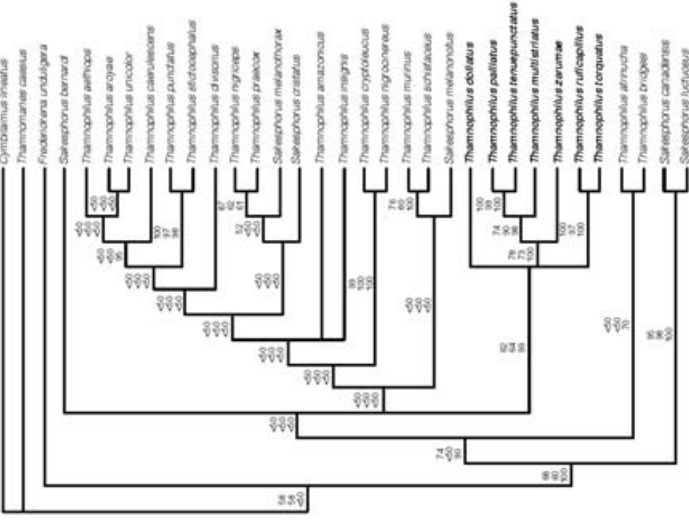
C. *cyt b*



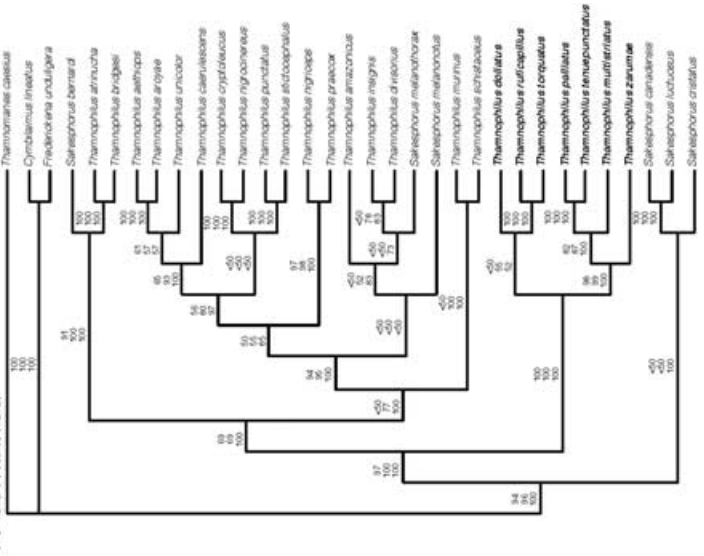
B. ND2



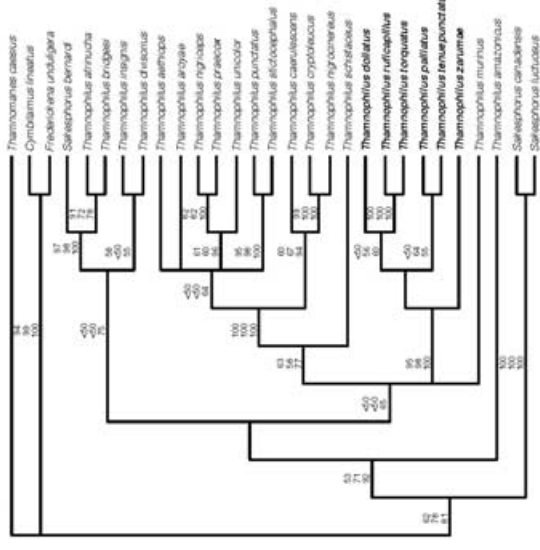
A. ND3



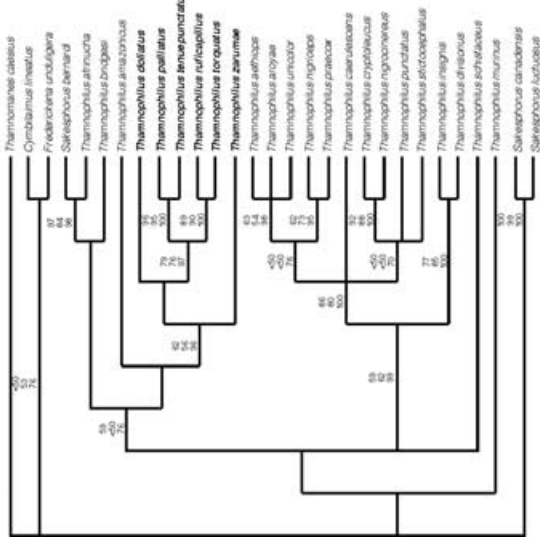
F. combined



E.  $\beta 7$



D.  $\beta 5$



full analysis because sites with missing data excluded) or 1025 bp (ND2) along the tree according to the substitution model parameters that had been optimized on the unconstrained trees (Table 3). On each simulated dataset, the model parameters were optimized with and without a clock constraint and the likelihoods calculated. Statistical significance at a level of  $\alpha = 0.95$  is indicated if the test statistic falls into the top 5% of values in the simulated distribution.

## Results

### SEQUENCE VARIATION

All data matrices and phylogenetic trees from this study were deposited into TreeBASE (Accession No. S1617). Sequences of *cyt b* (1,045 bp, corresponding to bases 14,991–16,035 of the chicken mitochondrial genome), ND2 (1,046 bp, corresponding to bases 5,220–6,265), ND3 (367 bp, corresponding to bases 10,795–11,161),  $\beta f5$  (532 bp), and  $\beta f7$  (979 bp) were obtained for most taxa, creating a total aligned dataset of 3,969 bp (GenBank Accession nos. EF030192–EF030351 and AY962685–AY962686). Partial datasets of ND3 were collected from *T. divisorius* (332 bp), *S. cristatus* (333 bp), and *S. melanothorax* (342 bp), of ND2 from *T. divisorius* (1025 bp) and *S. melanothorax* (571 bp), and of *cyt b* from *T. divisorius* (951 bp). Nine base-pairs of difficult-to-align  $\beta f7$  sequence were excluded from all analyses. The ND3 sequences included 35 base pairs of noncoding tRNA<sub>Arg</sub> on the 3' end, and the ND2 sequences included 21 base pairs of tRNA<sub>Met</sub> on the 5' end. These small noncoding regions were not treated as a separate data partition in phylogenetic analyses. No stop codons or other anomalous amino acid residues were detected in the mitochondrial protein-coding sequences. Homogeneity of base frequencies across all taxa was not rejected for any of the datasets ( $P > 0.05$ ). The number of parsimony-informative characters was

higher in the mitochondrial genes than in the nuclear genes: 314 for *cyt b*, 373 for ND2, 123 for ND3, 48 for  $\beta f5$ , and 86 for  $\beta f7$ .

### PHYLOGENETICS

The only “strong” (see Methods for definition) incongruence among phylogenies of the six datasets (five loci analyzed independently plus all loci combined) was a strongly supported sister relationship between *Sakesphorus bernardi* and *Thamnophilus atrinucha* in the  $\beta f5$  phylogeny that was absent in all other phylogenies. In these, *S. bernardi* was sister to a clade of *T. atrinucha* and *T. bridgesi* (Fig. 2D). To evaluate the strength of the incongruent  $\beta f5$  node, a SH test (number of bootstrap replicates = 1000) was performed between the most-likely  $\beta f5$  tree ( $-\ln L = 1738.1$ , Fig. 2D) and the most-likely tree from a search in which *T. atrinucha* and *T. bridgesi* were constrained as sisters (= 1740.1). The two topologies were statistically indistinguishable ( $P = 0.285$ ). A partition homogeneity test did not reveal any significant differences in topology among the genes.

In the phylogeny recovered from the ML analysis of the combined dataset (Fig. 2F), monophyly of *Thamnophilus* was supported strongly (ML bootstrap 96%), with the caveat that it included *Sakesphorus bernardi*, *S. melanothorax*, and *S. melanothorax*. These three species are treated hereafter as *Thamnophilus* species. The barred plumage group formed a strongly supported clade (ML bootstrap 100%). Placement of *T. doliatus* within the barred clade was unresolved, but the sister relationship of *T. ruficapillus* and *T. torquatus* was corroborated (ML bootstrap 100%) (Table 4). Somewhat surprisingly, *T. zarumae* was sister not to *T. doliatus*, as was proposed (Chapman 1926), but to a clade of *T. multistriatus*, *T. tenuipunctatus*, and *T. palliatus*.

The solid species were found in a sister clade to the barred clade with moderately strong bootstrap support (ML bootstrap

**Table 4.** SH tests of previously proposed interspecific phylogenetic relationships in *Thamnophilus*. SH test probabilities are based on comparison of the most-likely unconstrained tree (Fig. 1F) with a constrained tree in which the proposed relationship was constrained.

| Relationship   | Citation                | $-\ln(L)$ | SH test ( $P$ ) |
|--|-------------------------|-----------|-----------------|
| <i>(T. ruficapillus, T. torquatus)</i> <sup>1</sup>  | Zimmer and Isler (2003) | 23382.8   | 0.001           |
| <i>(T. doliatus, T. zarumae)</i> <sup>2</sup>  | Chapman (1926)          | 23380.8   | 0.016           |
| <i>(T. palliatus, T. tenuipunctatus)</i> <sup>1</sup>  | Zimmer (1933)           | 23360.7   | 0.102           |
| <i>(T. praecox, T. nigriceps)</i> <sup>1</sup>   | Zimmer (1937)           | 23364.3   | 0.127           |
| <i>(T. cryptoleucus, T. nigrocinereus)</i> <sup>1</sup>                                      | Peters (1951)           | 23413.9   | 0.0001          |
| <i>(T. aethiops, T. unicolor)</i> <sup>2</sup>   | Zimmer and Isler (2003) | 23376.6   | 0.010           |
| <i>(T. schistaceus, T. murinus)</i> <sup>1</sup>   | Zimmer and Isler (2003) | 23377.0   | 0.006           |
| <i>((T. punctatus, T. stictocephalus), T. amazonicus)</i> <sup>2</sup>                       | Carriker (1934)         | 23427.8   | 0.0001          |
| <i>((T. punctatus, T. stictocephalus), (T. nigrocinereus, T. cryptoleucus))</i> <sup>1</sup> | Isler et al. (1997)     | 23352.6   | 0.448           |
| <i>((T. punctatus, T. stictocephalus), T. insignis)</i> <sup>2</sup>                         | Isler et al. (1997)     | 23443.2   | 0.0001          |
| <i>(T. caerulescens, T. amazonicus)</i> <sup>2</sup>   | Short (1975)            | 23452.4   | 0.0001          |

<sup>1</sup>Constrained to not occur.

<sup>2</sup>Constrained to occur.

**Table 5.** Character transition rate ( $q$ ) matrix (means, SD) among lowland-restricted, lowland-to-highland, and highland-restricted *Thamnophilus antshrikes*. Top half of table are rates estimated under asymmetrical model of evolution; bottom half are estimates from symmetrical model.

|              |                          | To                     |                          |                         |
|--------------|--------------------------|------------------------|--------------------------|-------------------------|
|              |                          | Lowland-restricted (L) | Lowland-to-highland (LH) | Highland-restricted (H) |
| Asymmetrical |                          |                        |                          |                         |
| Asymmetrical | Lowland-restricted (L)   | –                      | 0.0±0.3                  | 4.2±1.6                 |
| From         | Lowland-to-highland (LH) | 17.9±10.3              | –                        | 291.1±146.6             |
|              | Highland-restricted (F)  | 2.4±8.7                | 250.2±118.1              | –                       |
| Symmetrical  |                          |                        |                          |                         |
|              |                          | From/To                |                          |                         |
|              |                          | Lowland-restricted (L) | Lowland to highland (LH) | Highland-restricted (H) |
| From/To      | Lowland-restricted (L)   | –                      | 0.1±0.9                  | 5.5±0.9                 |
|              | Lowland-to-highland (LH) | –                      | –                        | 381.9±139.9             |

69%). Solid plumage is not diagnostic for the clade, however, because it includes *T. bernardi* and *T. melanonotus*, both of which have the third plumage type of solid plumage with a contrasting white belly. Within the clade there was strong support for a basal split of *trans*-Andean (ML bootstrap 100%) and primarily *cis*-Andean (ML bootstrap 77%) taxa (Fig. 2F); the *trans*-Andean species *T. nigriceps* fell within the *cis*-Andean clade. The strongly supported (ML bootstrap 100%) *trans*-Andean clade that included *T. atrinuca*, *T. bridgesi*, and *T. bernardi* (this species formerly in *Sakesphorus*) was somewhat expected, because the loudsong of *T. bernardi* is similar to those of the other two species (Isler and Whitney 2002). The placement of *T. melanothorax* and *T. melanonotus* (both formerly in *Sakesphorus*) was unexpected, however, because their loudsongs differ qualitatively from all other *Thamnophilus* species (Isler and Whitney 2002). Unfortunately, too little sequence data were available for *T. melanonotus* and *T. melanothorax* to make a strong statement about their phylogenetic position within *Thamnophilus*.

Also noteworthy in the phylogeny was the distant relationship of *trans*-Andean *T. atrinuca* to members of the *cis*-Andean *T. punctatus* species complex, of which it was a member formerly. The sister taxon to the *T. punctatus* species complex (here represented by *T. punctatus* and *T. stictocephalus*) remained unresolved, but a sister relationship to *T. amazonicus*, *T. caerulescens*, *T. nigrocinereus* (including *T. cryptoleucus*), and *T. insignis* was not supported (Table 4). Among the remaining taxa, most previously proposed sister relationships (e.g., *T. cryptoleucus*/*T. nigrocinereus*, *T. murinus*/*T. schistaceus*, *T. nigriceps*/*T. praecox*) were corroborated (Table 4). An exception was the strongly supported sister relationship of *T. aroyae* and *T. aethiops* (ML bootstrap 100%) that was in conflict with a previously proposed sister relationship between *T. unicolor* and *T. aethiops* (Zimmer and Isler 2003). *T. aroyae* and *T. aethiops*, along with *T. unicolor*

and *T. caerulescens*, formed a well-supported clade (ML bootstrap 93%). The recently described species *T. divisorius* was sister to *T. insignis* (ML bootstrap 78%) (Whitney et al. 2004).

Equal-weighted parsimony analysis of the  $\beta$ f5 data resulted in 2040 most-parsimonious trees. Other parsimony searches of the data produced 1776 most-parsimonious trees from  $\beta$ f7, 1 for NDII, 11 for *cyt b*, and 31 for NDIII. Confidence in nodes based on 100 parsimony bootstrap replicates was lower, in general, than support inferred from ML (Fig. 2). There was no significant disagreement between the parsimony and ML results.

## BIOGEOGRAPHY

### *Evolution into and out of the Andes*

Our biogeographic analysis was restricted to species currently placed in the genus *Thamnophilus*, including the three species formerly in *Sakesphorus*. In the Bayesian analysis we found that the more parameter-rich model with asymmetric transition rates estimated on the ML tree ( $\ln L = -20.5$ ) did not provide a significantly better fit to the data than did a simpler model with symmetric ( $\ln L = -21.6$ ) transition rates ( $\chi^2 = 2.2$ ,  $df = 1$ ,  $P > 0.05$ ). Although transition rates estimated under both models are presented (Table 5), the asymmetric parameter estimates should be considered with caution. The most striking results, evident in the transition rates estimated under both models, were the low rates (range 0.0 to 5.5) from or into lowland-restricted distributions, and the relatively high rates (range 291.1 to 381.9) between highland-restricted and lowland-to-highland distributions (Table 5). This same pattern was evident in the phylogeny, in which species whose distributions included the Andes tended to cluster together (see the barred and *Thamnophilus caerulescens* clades in Fig. 3). The rate coefficients from the symmetric model can be translated to absolute numbers by multiplying them by the average tree length of the 1900 Bayesian trees. Applying the average values of the

symmetric rate coefficients to the average tree length of  $1.08 \pm 0.001$  SD implies there have been zero transitions between lowland-restricted and lowland-to-foothill distributions, five transitions between lowland-restricted and highland-restricted distributions, and 412 transitions between highland-restricted and lowland-to-highland distributions. The relatively high number of transitions are interpreted as multiple changes along a branch.

Parsimony ancestral character-state reconstructions (CI 0.21, RI 0.41, RC 0.09) are not presented, but were ambiguous at most internal nodes from which taxa with more than one character state descended. The exceptions were a reconstructed lowland-restricted common ancestor of *T. insignis* and *T. divisorius* that was resolved when the latter taxon was coded as lowland-restricted, and a reconstructed highland-restricted ancestor of *T. palliatus* and *T. tenuipunctatus*. Ancestral area reconstructions from the DIVA analysis (not presented) were similar to the parsimony reconstructions except that all internal nodes from which more than one character state descended were ambiguous. The Bayesian reconstructions provided some resolution at nodes where the parsimony and DIVA analyses were equivocal, although the Bayesian results were not statistically significant (i.e., posterior probability < 0.95). For example, the common ancestor of *T. ruficapillus* and *T. torquatus* was ambiguous in the parsimony and DIVA analyses, but Bayesian support was high (posterior probability = 0.80; Fig. 3) so that the ancestor was either highland-restricted (posterior probability = 0.32) or distributed from the lowlands to the highlands (posterior probability = 0.48). Similarly, the character state at the root of the *Thamnophilus* phylogeny was ambiguous in the parsimony and DIVA analysis, but the Bayesian analysis indicated stronger support for a lowland-restricted species (Fig. 3).

The Bayesian reconstruction of ancestral distribution suggests that from a lowland-restricted ancestor, evolution into highland elevations occurred independently in the barred clade, the *T. caerulescens* clade, and in *T. insignis* (Fig. 3). Beginning with a highland-restricted ancestral species in the barred clade, the most probable reconstruction indicates there were two reversals to species having lowland-to-highland distributions (*T. doliatus* and *T. ruficapillus*), with one of these lineages leading to the lowland-restricted species *T. torquatus*. Beginning with a highland-restricted ancestral species in the solid clade, there were two independent reversals to species having lowland-to-highland distributions (*T. aethiops* and *T. caerulescens*). If *T. divisorius* is coded as lowland-restricted, the most probable common ancestor of *T. insignis* and *T. divisorius* is ambiguously resolved as either lowland-to-highland or highland-restricted ( $P = 0.66$ ). If *T. divisorius* is coded as highland-restricted instead, the support for lowland-to-highland or highland-restricted became even higher ( $P = 0.83$ ).

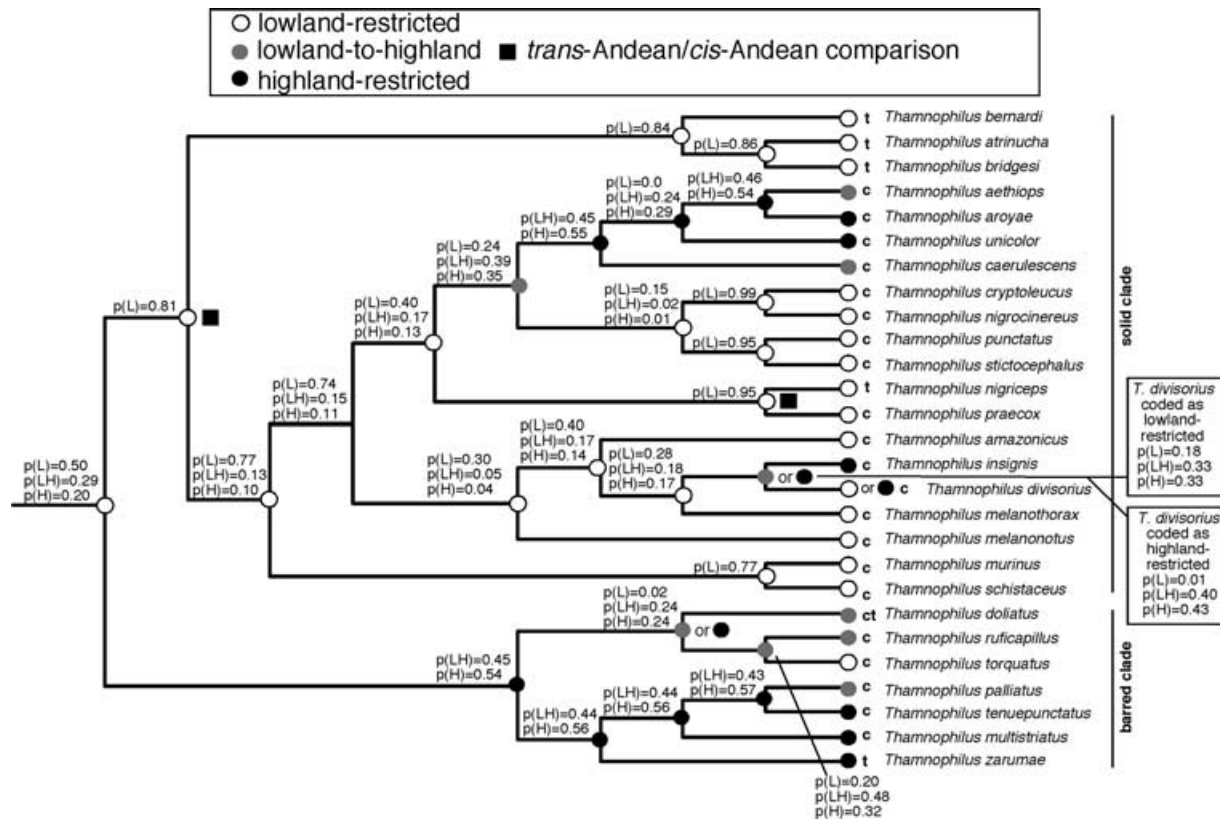
### Diversification of lowland taxa on opposite sides of the Andes

We identified four paralogy-free subtrees, three of which we could test with the available samples. The first subtree represented the basal split in the solid clade between a *trans*-Andean clade (i.e., *T. atrinucha*, *T. bridgesi*, and *T. bernardi*) and a primarily *cis*-Andean clade (Fig. 3). The *cis*-Andean clade included one *trans*-Andean species, *T. nigriceps*. Because all other species in this clade exist east of the Andes, we interpret the *trans*-Andean distribution of *T. nigriceps* as a secondary invasion of the *trans*-Andean lowlands that occurred after the basal split. The second subtree contained only two species, representing the sister relationship between *T. nigriceps* (eastern Panama east to Magdalena Valley of Colombia) and *T. praecox* (known only from the Río Napo and its tributaries in northern Ecuador) (Fig. 3). The third subtree (not illustrated in Fig. 3) contained *trans*- and *cis*-Andean populations of *Thamnophilus doliatus*. As noted earlier, a population-level analysis of *T. doliatus* was beyond the scope of this study. We included a fourth subtree composed of montane instead of lowland taxa. *T. zarumae* exists on the dry western slope of the central Andes in southwestern Ecuador and northwestern Peru, and is replaced on the eastern slope of the central Andes by a clade composed of *T. palliatus*, *T. tenuipunctatus*, and *T. multistriatus*.

To gauge the support for monophyly of the *trans*- and *cis*-Andean clades within the subtrees, we performed SH tests between the most-likely tree (Fig. 3) and constrained trees. For the first subtree, we tested the most-likely unconstrained tree ( $-\ln L = 23352.3$ ) against the most-likely tree ( $-\ln L = 23379.7$ ) from a search in which the *trans*-Andean clade was constrained to not occur. We found that the two trees differed significantly in length ( $P < 0.05$ ), indicating a strong support for the *trans*-Andean clade. A similar test was performed between the most-likely unconstrained tree and the most-likely tree from a search in which the *cis*-Andean clade was constrained to not occur. The test of the second subtree also found a significant difference ( $P < 0.05$ ) between the unconstrained tree and a tree in which *T. nigriceps* was constrained to monophyly with the clade of the three other *trans*-Andean species ( $-\ln L = 23594.5$ ). To test the fourth subtree, we performed a search in which the three eastern slope species (including *T. multistriatus*) were constrained to not exist as a clade. The resulting constrained tree was not significantly different ( $P > 0.05$ ) in length than the most-likely tree.

### Divergence time estimates

Parametric bootstrap tests did not reject a molecular clock for sequence variation at *cyt b* and ND2. Divergence time estimates for relevant nodes were made using 1.6% and 4.0% sequence divergence per year calibrations for *cyt b* and ND2, respectively (see



**Figure 3.** Bayesian posterior probabilities of elevational distribution at which species most commonly encountered, mapped onto ancestral nodes of the maximum-likelihood tree (Fig. 1F). Underlying transition model was symmetric between the three states (L, lowland-restricted; LH, lowland-to-highland; H, highland-restricted). Species with *trans*-Andean distribution marked “t,” *cis*-Andean marked “c,” and those existing on both sides of Andes “ct.”

online Appendix S1). Because we lacked data from these genes for *T. multistriatus*, we substituted ND3 to calculate divergence times for this species using both the 1.6% and 4.0% calibrations.

## Discussion

### EVOLUTION INTO AND OUT OF THE ANDES

Despite the importance of understanding the evolutionary colonization of high-elevation habitats and reversals into low-elevation habitats toward a general hypothesis of diversification processes in the Neotropics, relatively few empirical studies have addressed these processes explicitly (e.g., Chapman 1917; Creighton 1985; Lynch 1986; Patton and Smith 1992; Bates and Zink 1994; García-Moreno and Silva 1997; Voelker 1999; Richardson et al. 2001; Salazar-Bravo et al. 2001; Burns and Naoki 2004; Hall 2005; Weir 2006). This paucity reflects the lack of unique and testable predictions that would facilitate such investigations (Lynch 1986) as well as the practical difficulty of acquiring genetic material from remote regions. Reconstructed ancestral distributions in the *Thamnophilus* phylogeny help shed some light on the historical diversification of species in lowland and highland habitats.

The two *Thamnophilus* clades whose distributions included high-elevation Andean habitats (barred clade, *T. caerulescens* clade) were both derived from lowland-restricted ancestors (Fig. 3). We thus rejected strongly the Guianan highlands as the ancestral source population for “solid” *Thamnophilus* species of the Andean foothills (Chapman 1917). Based on a molecular clock, divergence of the primarily Andean barred clade and the primarily lowland-restricted solid clade occurred between 5.5 to 3 million years ago (Mya) based on *cyt b* and ND2 estimates, respectively. The divergence of the montane *T. caerulescens* clade from its lowland-restricted sister clade occurred more recently between and 3.6 and 1.6 Mya based on *cyt b* and ND2, respectively. These time estimates undoubtedly have large errors associated with them (Graur and Martin 2004; Ho et al. 2005; Ho and Larson 2006), but likely postdate the availability of habitat on the eastern slope of the Andes that could have been colonized by *Thamnophilus*.

Analyses of paleobotanical data, crustal shortening estimates, and erosion surfaces from southern Peru and Bolivia suggest the Eastern Cordillera of the Andes had reached 50% of its current elevation by 10 Mya (Gregory-Wodzicki 2000). Thus, by 10 Mya a proto-Eastern Cordillera mountain range existed that



was approximately 2000 m in elevation (Hartley 2003). At that time, the now arid Bolivian Altiplano (~4000 m elev.) was also approximately 2000 m in elevation and supported a subtropical-dry forest (Gregory-Wodzicki 2002) that could also have been colonized by *Thamnophilus*. The hyperaridity of the western slope of the Andes from northern Chile to southwestern Ecuador was evident by 4 to 3 Mya (Hartley and Chong 2002), with mesic conditions on the eastern slope due to moisture-laden air carried by the southeastern tradewinds from Amazonia (García 1994). Collectively, these data suggest the Andes would have been inhabitable several million years before their initial colonization by *Thamnophilus*.

The two principal models attempting to explain the speciation of Andean colonists from lowland ancestors are primary differentiation and allopatric speciation. Chapman's (1926) hypothesis that the divergence of highland species occurred in response to selection acting along environmental gradients from the highlands to the lowlands was a harbinger of Endler's (1977) formalized model of primary differentiation. Vertebrate studies that have tested primary differentiation as a mechanism for speciation between populations occupying different elevations have not yielded corroborating evidence. In their analysis of diversification in Andean *Akodon* mice, Patton and Smith (1992) examined a series of paired highland-lowland species replacements. They found that highland populations had as their closest relatives other highland populations, rejecting an elevational gradient as the mechanism underlying the divergence of highland and lowland populations. Similarly, Dingle et al. (2006) found that the phylogeny of Andean and lowland populations of *Henicorhina* wrens did not support a model of primary differentiation.

Pinpointing vicariant events that could have isolated Andean colonist populations from source lowland populations is problematic because of the long and dynamic history of the region, with many climatic fluctuations that impacted habitats in a nonuniform manner (Gosling and Bush 2005). The elevation of the Andes has changed little over the past 2 million years (Gregory-Wodzicki 2000), but the distribution of plant species and habitats has undoubtedly cycled with climatic changes (Graham et al. 2001). Any isolation of appropriate habitat for a sufficient duration could have resulted in allopatric speciation of highland from lowland forms. Aridity associated with Pleistocene glacial cycles has been implicated in producing geographic isolation of organisms found in humid South American forests (Haffer 1969), but it remains unclear whether the climate of tropical South America was colder and drier during the Last Glacial Maximum (LGM) or colder and wetter than current conditions (Bush et al. 2004). Studies (Betts and Ridgway 1992; Bush et al. 2001) concur that average temperatures were reduced during the LGM and that Andean habitats descended to lower elevations as a result (Flenley 1998), but evidence of drier climates that could have fragmented humid habitats

during the cool cycles has proved elusive. One analysis of a pollen profile collected from the eastern slope of the Andes in Bolivia supported a drier climate during the LGM (Mourguiart and Ledru 2003), but the same data were interpreted subsequently as consistent with a humid climate (Baker et al. 2003). A recent study (Urrego et al. 2005) of a pollen record from a locality in the humid foothills of the eastern Andes in Peru (elev. 1360 m) found evidence for humid conditions throughout the LGM.

Current distributions of *Thamnophilus* antshrikes may provide some clues concerning potential vicariant events, assuming extinction rates and post-vicariance distributional changes are minimal. For example, a former connection between Andean habitats and those of the coastal ranges of southeastern Brazil is suggested by disjunct distributions of bird (e.g., *T. ruficapillus*, *T. palliatus*, Sick 1985, 1993), plant (Dusen 1903), mammal (Ribeiro 1906), and fish (Ribeiro 1906) species. The distribution of *T. caerulescens* bridges this gap, because it occurs in the forests of southeastern Brazil and the Andes, but also in the intervening dry, lowland chaco scrub. Hypothesizing that the divergence of *T. caerulescens* from the common ancestor of the (*T. unicolor*, *T. aroyae*, *T. aethiops*) clade was associated with the disruption of a forest bridge between southeastern Brazil (*T. caerulescens*) and the Andes (*T. unicolor*, *T. aroyae*, *T. aethiops*), one can predict that the Andean distribution of *T. caerulescens* represents a secondary invasion. Consistent with this hypothesis, a recent population-level mitochondrial analysis of *Thamnophilus caerulescens* found that the ancestral distribution of this widespread species was probably in the lowlands of eastern Brazil (Brumfield 2005). The hypothesis also predicts that the existence of *T. aethiops* in the Amazonian lowlands represents a reversal from Andean-distributed ancestors (Fig. 2F). A comparative phylogeographic study of variation within *T. aethiops* could test this, illustrating how microevolutionary processes can inform phylogenetic patterns at higher taxonomic levels, and vice versa (Edwards 1997). We note that additional intraspecific sampling could uncover paraphyly of taxa (e.g., *T. aroyae* nested within *T. aethiops*) that would further elucidate historical patterns of diversification obscured by the current taxonomic treatment (Funk and Omland 2003).

Unfortunately, geological studies shed little light on when a humid forest corridor may have existed between the Andes and eastern Brazil. The Pebas and Paranan Seas that isolated the Andes from the adjacent lowlands ~11 Mya were gone before *Thamnophilus* occupied the Andes (Roddaz et al. 2006), and all of the current drainage systems were in place ~8 Mya (Potter 1997). What remain as plausible vicariant mechanisms of differentiation between highland and lowland regions are Pliocene and Pleistocene changes in the distribution of humid and dry habitats, as well as changes in the distribution of *Thamnophilus* that were linked to or independent of these changes. The palynological record from the Quaternary suggests that much of the dry

caatinga scrub may have been covered by rain forests during humid climatic cycles (Ledru 1993; Ledru et al. 1996), but, as noted earlier, significant debate surrounds how the distribution of forest and nonforest habitats was impacted by Pliocene and Pleistocene climatic oscillations.

The phylogenetic relationships of *Thamnophilus* antshrikes corroborate other studies of Neotropical organisms in finding that species whose distributions include high elevations (i.e., highland-restricted and lowland-to-highland species) tend to be closely related (Patton and Smith 1992; Bates and Zink 1994; Salazar-Bravo et al. 2001; Burns and Naoki 2004; Weir 2006). This pattern is reflected in the Bayesian elevation transition rate estimates, with significantly greater rates between highland-restricted and lowland-to-highland distributions than rates into or out of lowland-restricted distributions (Table 5). The extremely high transition rates between highland-restricted and lowland-to-highland *Thamnophilus* distributions imply that physiological constraints probably do not restrict highland-restricted species from the lowlands. Other mechanisms, such as competition, could be playing a significant role in limiting reversals to lowland-restricted distributions (Remsen and Graves 1995). The influence of competition is evident in the distributions of many Neotropical birds (Terborgh and Weske 1975). For example, the widespread lowland-tropical flycatcher *Leptopogon amaurocephalus* exists up to 600 m in the Andes, where it is replaced by its upper-tropical zone congener, *L. superciliaris*. In the Pantepui region where *L. superciliaris* is absent, *L. amaurocephalus* exists up to 1200 m (Ridgely and Tudor 1994). Conversely, *L. superciliaris* ranges below 600 m on the west slope of the Andes where *L. amaurocephalus* is absent (Bates and Zink 1994). Competition may also explain habitat or elevational segregation in *Thamnophilus* at a microgeographic scale. *T. caerulescens* and *T. aroyae* exist sympatrically in the Río Paracti Valley (~1500 m) of Cochabamba Department, Bolivia. Although the elevational distributions of these two species overlap broadly, in the Río Paracti valley they appeared to segregate into structurally similar hilltop and slope habitats, respectively (Brumfield, pers. obs.). Detailed population-level studies are needed to clarify the role of competition in defining the elevational limits of taxa.

#### DIVERSIFICATION OF LOWLAND TAXA ON OPPOSITE SIDES OF THE ANDES

The impact of the Andean uplift on the historical diversification of lowland antshrikes was illustrated by a basal divergence between *trans*- and *cis*-Andean solid antshrikes. That this same basal split between *trans*- and *cis*-Andean taxa was revealed in previous analyses of *intraspecific* genetic variation in 13 other avian species groups (Brumfield and Capparella 1996) suggests that the Andes have shaped genetic structure at multiple taxonomic depths. Assuming a positive association exists between taxonomic level and

age of divergence, these results imply that the Andes' efficacy as a geographic isolating barrier has had some periodicity over its evolutionary history, in contrast to the final closure of the Panamanian Isthmus that permanently isolated populations of Pacific and Atlantic marine organisms on either side (Coates et al. 1992; Knowlton et al. 1993).

Three hypotheses have been proposed to clarify the role of the Andes in the divergence of lowland organisms. Chapman (1917) proposed that the final phase of the Andean uplift approximately 6–2.7 million years ago (Gregory-Wodzicki 2000) fragmented populations that were previously continuous from the Amazon Basin to the Pacific Ocean. Haffer (1967) proposed that the Andes only had an indirect effect by creating a narrow dispersal corridor around their northern end. Cyclical climatic perturbations during the Pliocene and Pleistocene produced habitat changes (Haffer 1967) or marine transgressions (Haq et al. 1987) that prevented access to this corridor by organisms of humid lowland forest. A third hypothesis is that dispersal directly across the Andes resulted in the isolation of forms on either side (Chapman 1926).

The timing of the completion of the Andean uplift provides an upper bound on the divergence age based on Chapman's (1917) hypothesis that the orogeny itself resulted in the isolation of lowland populations. Using the corrected pairwise genetic distances (see online Appendix S1), a rough approximation of the divergence between *cis* and *trans*-Andean solid antshrikes is 5.2 (cyt *b*) to 2.4 (ND2) million years ago. The divergence of *cis*-Andean *T. praecox* and *trans*-Andean *T. nigriciceps* occurred comparatively recently at approximately 1.1 (cyt *b*) to 0.6 (ND2) million years ago. With all of the assumptions underlying the estimates of these absolute ages, their confidence limits are undoubtedly large, but as relative ages they support a periodicity to the Andean role in divergence. Although the initial Andean lift undoubtedly resulted in the diversification of some *trans*- and *cis*-Andean taxa, we conclude that subsequent climatic fluctuations and/or marine transgressions that periodically interrupted and reconnected the dispersal corridor around the northern end of the Andes also played an important role. Divergence of western slope *T. zarumae* from its eastern slope sister clade occurred 3.1 (cyt *b*) to 1.1 Mya (ND2). Because the proto-Andes were 2000 m high 10 Mya (Hartley 2003), it seems likely that the western slope distribution of *T. zarumae* reflects across-Andes dispersal. Both the Marañón valley in northern Peru and the Loja valley in southern Ecuador have been proposed as possible dispersal corridors across the central Andes (Chapman 1926).

#### DIVERSIFICATION IN OTHER REGIONS

Nearly 15% of the terrestrial Amazonian avifauna is restricted to river-associated habitats (Remsen and Parker 1983). Sequences of the morphologically divergent river-island specialists *T. cryptoleucus* and *T. nigrocinereus* were weakly differentiated (ND2 =

0.011; *cyt b* = 0.013) (Rosenberg 1990), suggesting either a very recent divergence or that introgression is currently occurring between them where they come into contact. Detailed sampling along their contact zone should elucidate the evolutionary processes shaping these genetic patterns. Interestingly, these two species provide yet another example in which species restricted to river-created habitats (e.g., river islands, *varzea*) have relatively long phylogenetic branches. Aleixo's (2002) investigation of phylogenetic relationships in *Xiphorhynchus* woodcreepers found that the two species indigenous to the seasonally flooded Amazonian *varzea* forests (*X. kiernii* and *X. obsoletus*) both had long branches relative to other species in the study. The placement of *X. kiernii* as sister to the widespread *X. picus* species group and the level of divergence between these two groups (uncorrected divergence 10.5–10.6% based on *cyt b*, ND2, and ND3) were reminiscent of the relationship between *T. nigrocinereus* and *T. cryptoleucus* and its possible *T. punctatus* sister clade (uncorrected divergence 8.7–9.2% based on *cyt b*, ND2, and ND3). Aleixo (2002) found that *X. obsoletus* also had a long branch that was sister to a large clade of *Xiphorhynchus* species not found in river-created habitats; the range of uncorrected genetic divergence values between *X. obsoletus* and these species was 7.7–9.6%. Collectively, these results suggest the divergence of river-habitat specialists was a relatively old event that preceded much of the large-scale diversification in Amazonia and the Andes (Moritz et al. 2000). Studies of additional species groups will be needed to determine if “long branches” are typical of organisms restricted to river-created habitats, and whether a common vicariant mechanism, perhaps associated with the ancient Amazonian lake system (Marroig and Cerqueira 1997; Wesselingh et al. 2002), underlies their diversification.

The (*T. insignis*, *T. divisorius*) clade comprises two broadly disjunct species, the former a highland-restricted species in the Pantepui region of northern South America, the latter known from a single locality on a low-lying ridge along the eastern flank of the Peruvian Andes (Fig. 1). Whitney et al. (2004) noted that despite their elevational differences both species occupy “structurally and physiognomically similar habitats that have especially nutrient-poor soils.” Whether the tiny distribution of *T. divisorius* is due to long-distance dispersal or is simply a relict of a once more expansive distribution remains unclear, but given the limited dispersal capabilities of *Thamnophilus* antshrikes, the relict hypothesis seems more probable. Interestingly, the relatively high genetic distance between *T. insignis* and *T. divisorius* (*cyt b*: 10.8%; ND2: 9.5%), consistent with a divergence 6.7 to 2.3 Mya, is remarkably similar to that found between the Pantepui endemic thamnophilid *Percnostola saturata* and its possible lowland sister replacement *P. leucostigma* (ND2: 9.2 to 9.6%) (Braun et al. 2005). The distinctive sandstone tepuis that characterize the Pantepui region were formed by Miocene (~24 to 5 Mya) erosion of a once more extensive elevated mantle that extends discontinuously to the eastern

flank of the Andes in Ecuador (Maguire 1970). Our data suggest *T. divisorius* could represent a relict of this ancient sandstone system, and that it and *T. insignis* were derived from lowland-restricted species in northern South America. The generality of these patterns awaits the availability of additional comparative studies.

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## LITERATURE CITED

- Aleixo, A. 2002. Molecular systematics and the role of the “varzea”—“terra-firme” ecotone in the diversification of *Xiphorhynchus* woodcreepers (Aves: Dendrocolaptidae). *Auk* 119:621–640.
- Arbogast, B. S., S. V. Drovetski, R. L. Curry, P. T. Boag, G. Seutin, P. R. Grant, B. R. Grant, and D. J. Anderson. 2006. The origin and diversification of Galapagos mockingbirds. *Evolution* 60:370–382.
- Arctander, P., and J. Fjeldså. 1994. Andean tapaculos of the genus *Scytalopus* (Aves, Rhinocryptidae): a study of speciation using DNA sequence data. Pp. 205–225 in V. Loeschcke, J. Tomiuk, and S. K. Jain, eds. Conservation genetics. Birkhäuser Verlag, Basel, Switzerland.
- Baker, P. A., M. Bush, S. Fritz, C. A. Rigsby, G. Seltzer, and M. Silman. 2003. Last glacial maximum in an Andean cloud forest environment (Eastern Cordillera, Bolivia): comment and reply. *Geol. Forum* 31:e26–e27.
- Barker, F. K. 2004. Monophyly and relationships of wrens (Aves: Troglodytidae): a congruence analysis of heterogeneous mitochondrial and nuclear sequence data. *Mol. Phylogenet. Evol.* 31:486–504.
- Bates, J. M., and R. M. Zink. 1994. Evolution into the Andes: molecular evidence for species relationships in the genus *Leptopogon*. *Auk* 111:507–515.
- Betts, A. K., and W. Ridgway. 1992. Tropical boundary-layer equilibrium in the last ice-age. *J. Geophys. Res.* 97:2529–2534.

- Braun, M. J., M. L. Isler, P. R. Isler, J. M. Bates, and M. B. Robbins. 2005. Avian speciation in the Pantepui: the case of the Roraiman Antbird (*Pernostola [Schistocichla] "leucostigma" saturata*). *Condor* 107:327–341.
- Brumfield, R. T. 2005. Mitochondrial variation in Bolivian populations of the Variable Antshrike (*Thamnophilus caerulescens*). *Auk* 122:414–432.
- Brumfield, R. T., and A. P. Capparella. 1996. Historical diversification of birds in northwestern South America: a molecular perspective on the role of vicariant events. *Evolution* 50:1607–1624.
- Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, and D. L. Swofford. 1993. Partitioning and combining data in phylogenetic analysis. *Syst. Zool.* 42:384–397.
- Burns, K. J. 1997. Molecular systematics of tanagers (Thraupinae): evolution and biogeography of a diverse radiation of neotropical birds. *Mol. Phylogenet. Evol.* 8:334–348.
- Burns, K. J., and K. Naoki. 2004. Molecular phylogenetics and biogeography of Neotropical tanagers in the genus *Tangara*. *Mol. Phylogenet. Evol.* 32:838–854.
- Buschbom, J., and D. Barker. 2006. Evolutionary history of vegetative reproduction in *Porpidia s. l.* (lichen-forming Ascomycota). *Syst. Biol.* 55:471–484.
- Bush, M. B., M. R. Silman, and D. H. Urrego. 2004. 48,000 years of climate and forest change in a biodiversity hot spot. *Science* 303:827–829.
- Bush, M. B., M. Stute, M. P. Ledru, H. Behling, P. A. Colinvaux, P. E. De Oliveira, E. C. Grimm, H. Hooghiemstra, S. Haberle, B. W. Leyden, M. L. Salgado-Labouriau, and R. Webb. 2001. Paleotemperature estimates for the lowland Americas between 30S and 30N at the Last Glacial Maximum. In V. Markgraf, ed. *Interhemispheric climate linkages: present and past interhemispheric climate linkages in the Americas and their societal effects*. Academic Press, New York.
- Capparella, A. P. 1991. Neotropical avian diversity and riverine barriers. *Acta Cong. Int. Ornithol.* 20:307–316.
- Carriker, M. A. 1934. Description of new birds from Peru, with notes on the nomenclature and status of other little-known species. *Proc. Acad. Natl. Sci., Philadelphia* 86:317–334.
- Chapman, F. M. 1917. The distribution of bird-life in Colombia. *Bull. Am. Mus. Nat. Hist.* 36:1–729.
- . 1926. The distribution of bird-life in Ecuador. *Bull. Am. Mus. Nat. Hist.* 55:1–784.
- Chesser, R. T. 2000. Evolution in the high Andes: the phylogenetics of *Muscisaxicola* ground-tyrants. *Mol. Phylogenet. Evol.* 15:369–380.
- Chesser, R. T., and R. M. Zink. 1994. Modes of speciation in birds: a test of Lynch's method. *Evolution* 48:490–497.
- Cheviron, Z. A., A. P. Capparella, and F. Vuilleumier. 2005a. Molecular phylogenetic relationships among the *Geositta* miners (Furnariidae) and biogeographic implications for avian speciation in Fuego-Patagonia. *Auk* 122:158–174.
- Cheviron, Z. A., S. J. Hackett, and A. P. Capparella. 2005b. Complex evolutionary history of a Neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for historical hypotheses of the origin of Neotropical avian diversity. *Mol. Phylogenet. Evol.*: 338–357.
- Coates, A. G., J. B. C. Jackson, L. S. Collins, T. M. Cronin, H. J. Dowsett, L. M. Bybell, P. Jung, and J. A. Obando. 1992. Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geol. Soc. Am.* 104:814–828.
- Cracraft, J., and R. O. Prum. 1988. Patterns and processes of diversification: speciation and historical congruence in some Neotropical birds. *Evolution* 42:603–620.
- Creighton, G. K. 1985. Phylogenetic inference, biogeographic interpretations, and the pattern of speciation in *Marmosa* (Marsupialia: Didelphidae). *Acta Zool. Fennica* 170:121–124.
- Desjardins, P., and R. Morais. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *J. Mol. Biol.* 212:599–634.
- Dick, C. W., K. Abdul-Salim, and E. Bermingham. 2003. Molecular systematic analysis reveals cryptic Tertiary diversification of a widespread tropical rain forest tree. *Am. Nat.* 162:691–703.
- Dingle, C., I. J. Lovette, C. Canaday, and T. B. Smith. 2006. Elevational zonation and the phylogenetic relationships of the *Henicorhina* woodwrens. *Auk* 123:119–134.
- Donato, M., P. Posadas, D. R. Miranda-Esquivel, E. O. Jaureguizar, and G. Cladera. 2003. Historical biogeography of the Andean region: evidence from *Listroderina* (Coleoptera: Curculionidae: Rhytirhinini) in the context of the South American geobiotic scenario. *Biol. J. Linn. Soc.* 80:339–352.
- Dusen, D. K. H. 1903. Sur la flora de la Serra do Itatiaia. *Arq. Mus. Nac. Rio de J.* 13:1–119.
- Edwards, S. V. 1997. Relevance of microevolutionary processes to higher level molecular systematics. Pp. 251–278 in D. P. Mindell, ed. *Avian molecular evolution and systematics*. Academic Press, San Diego.
- Edwards, S. V., W. B. Jennings, and A. M. Shedlock. 2006. Phylogenetics of modern birds in the era of genomics. *Proc. Royal Soc. Ser. B* 272:979–992.
- Endler, J. A. 1977. *Geographic variation, speciation, and clines*. Princeton Univ. Press, Princeton.
- Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10:315–319.
- Fitzpatrick, B. M., and M. Turelli. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. *Evolution* 60:601–615.
- Fjeldså, J. 1994. Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodivers. Conserv.* 3:207–226.
- Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 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.
- Flenley, J. R. 1998. Tropical forests under the climates of the last 30,000 years. *Climatic Change* 39:177–197.
- Funk, D. J., and K. E. Omland. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annu. Rev. Ecol. Syst.* 34:397–423.
- García, N. O. 1994. South American climatology. *Quaternary Int.* 21:7–27.
- García-Moreno, J., P. Arctander, and J. Fjeldsa. 1999. Strong diversification at the treeline among *Metallura* hummingbirds. *Auk* July 116:702–711.
- García-Moreno, J., and J. Fjeldså. 2000. Chronology and mode of speciation in the Andean avifauna. *Bonner Zool. Monogr.* 46:25–46.
- García-Moreno, J., J. Ohlson, and J. Fjeldsa. 2001. MtDNA sequences support monophyly of Hemispingus tanagers. *Mol. Phylogenet. Evol.* 21:424–435.
- García-Moreno, J., and J. M. C. D. Silva. 1997. An interplay between forest and non-forest South American avifaunas suggested by a phylogeny of *Lepidocolaptes* woodcreepers (Dendrocolaptinae). *Stud. Neotrop. Fauna Environ.* 32:164–173.
- Gentry, A. H. 1982. Phylogeographic patterns as evidence for a Chocó refuge. Pp. 112–136 in G. T. Prance, ed. *Biological diversification in the tropics*. Columbia Univ. Press, New York.
- Gosling, W. D., and M. B. Bush. 2005. A biogeographic comment on: Wüster et al. (2005) Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). *Mol. Ecol.* 14:3615–3617.

- Graham, A., K. M. Gregory-Wodzicki, and K. L. Wright. 2001. Studies in Neotropical botany. XV. A Mio-Pliocene palynoflora from the Eastern Cordillera, Bolivia: implications for the uplift history of the Central Andes. *Am. J. Bot.* 88:1545–1557.
- Graham, C. H., S. R. Ron, J. C. Santos, C. J. Schneider, and C. Moritz. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution* 58:1781–1793.
- Graur, D., and W. Martin. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet.* 20:81–86.
- Graves, G. R. 1988. Linearity of geographic range and its possible effect on the population structure of Andean birds. *Auk*: 47–52.
- Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: a review. *Bull. Geol. Soc. Am.* 112:1091–1105.
- . 2002. A late Miocene subtropical-dry flora from the northern Altiplano, Bolivia. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 180:331–348.
- Haffer, J. 1967. Speciation in Colombian forest birds west of the Andes. *Am. Mus. Novit.* 294:1–57.
- . 1969. Speciation in Amazonian forest birds. *Science* 165:131–137.
- Hall, J. P. W. 2005. Montane speciation patterns in *Ithomiola* butterflies (Lepidoptera: Riodinidae): are they consistently moving up in the world? *Proc. Royal Soc. B-Biol. Sci.* 272:2457–2466.
- Haq, B. U., J. Hardenbol, and P. R. Vail. 1987. Chronology of fluctuating sea levels since the Triassic. *Science* 235:1156–1167.
- Hartley, A. J. 2003. Andean uplift and climate change. *J. Geol. Soc. Lond.* 160:7–10.
- Hartley, A. J., and G. Chong. 2002. A late Pliocene age for the Atacama Desert: implications for the desertification of western South America. *Geology* 30:43–46.
- Hershkovitz, P. 1969. The evolution of mammals on southern continents, VI: the recent mammals of the neotropical region: a zoogeographic and ecological review. *Q. Rev. Biol.* 44:1–70.
- Hillis, D. M. 1985. Evolutionary genetics of the Andean lizard genus *Pholidobolus* (Sauria, Gymnophthalmidae)—phylogeny, biogeography, and a comparison of tree construction techniques. *Syst. Zool.* 34:109–126.
- . 1987. Molecular versus morphological approaches to systematics. *Annu. Rev. Ecol. Syst.* 18:23–42.
- Hilty, S. L., and W. L. Brown. 1986. A guide to the birds of Colombia. Princeton Univ. Press, Princeton, NJ.
- Ho, S. Y. W., and G. Larson. 2006. Molecular clocks: when times are a-changin'. *Trends Genet.* 22:79–83.
- Ho, S. Y. W., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Mol. Biol. Evol.* 22:1561–1568.
- Hoffmann, F. G., and R. J. Baker. 2003. Comparative phylogeography of short-tailed bats (*Carollia*: Phyllostomidae). *Mol. Ecol.* 12:3403–3414.
- Huelsenbeck, J. P., R. Nielsen, and J. P. Bollback. 2003. Stochastic mapping of morphological characters. *Syst. Biol.* 52:131–158.
- Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. *Biometrics* 17:754–755.
- Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. *Proc. Natl. Acad. Sci. U.S.A.* 103:10334–10339.
- InfoNatura: birds, mammals, and amphibians of Latin America [web application]. 2005. Version 4.1. NatureServe, Arlington, VA. Available at <http://www.natureserve.org/infonatura>.
- Irestedt, M., J. Fjeldså, J. A. Nylander, and P. G. P. Ericson. 2004. Phylogenetic relationships of typical antbirds (Thamnophilidae) and test of incongruence based on Bayes factors. *BMC Evol. Biol.* 4:23.
- Isler, M. L., P. R. Isler, and B. M. Whitney. 1997. Biogeography and systematics of the *Thamnophilus punctatus* (Thamnophilidae) complex. *Ornithol. Monogr.* 48:355–381.
- Isler, P. R., and B. M. Whitney. 2002. Songs of the antbirds. Macaulay Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, NY.
- Janzen, D. H. 1967. Why mountain passes are higher in the tropics. *Am. Nat.* 101:233–249.
- Jordan, D. S. 1905. The origin of species through isolation. *Science* 22:545–562.
- Knowlton, N., L. A. Weight, L. A. Solórzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260:1629–1632.
- Knox, E. B., and J. D. Palmer. 1995. Chloroplast DNA variation and the recent radiation of the giant senecios (Asteraceae) on the tall mountains of eastern Africa. *Proc. Natl. Acad. Sci. U.S.A.* 92:10349–10353.
- Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* 86:6196–6200.
- Ledru, M. P. 1993. Late Quaternary environmental and climatic changes in central Brazil. *Quaternary Res.* 39:90–98.
- Ledru, M. P., P. I. S. Braga, F. Soubies, M. Fournier, L. Martin, K. Suguio, and B. Turcq. 1996. The last 50,000 years in the Neotropics (Southern Brazil): evolution of vegetation and climate. *Palaeogeogr. palaeoclimatol. palaeoecol.* 123:239–257.
- Lovette, I. J. 2004. Mitochondrial dating and mixed-support for the “2% rule” in birds. *Auk* 121:1–6.
- Lovette, I. J., and E. Bermingham. 2001. Mitochondrial perspective on the phylogenetic relationships of the *Parula* wood-warblers. *Auk* 118:211–215.
- Lynch, J. D. 1986. Origins of the High Andean herpetological fauna. Pp. 478–499 in F. Vuilleumier, and M. Monasterio, eds. High altitude tropical biogeography. Oxford Univ. Press, New York.
- Lynch, J. D., and W. E. Duellman. 1997. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: systematics, ecology, and biogeography. Univ. Kansas Spec. Publ. No. 23. Univ. of Kansas Printing Service, Lawrence, Kansas.
- Maddison, W. P. 1990. A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree. *Evolution* 44:539–557.
- Maddison, D. R., and W. P. Maddison. 2000. MacClade: analysis of phylogeny and character evolution. Vers. 4.0. Sinauer, Sunderland, MA.
- Maguire, B. 1970. On the flora of the Guayana highland. *Biotropica* 2:85–100.
- Marks, B. D., S. J. Hackett, and A. P. Capparella. 2002. Historical relationships among Neotropical lowland forest areas of endemism as inferred from mitochondrial DNA sequence variation in the Wedge-billed Woodcreeper (Dendrocolaptidae: *Glyphorhynchus spirurus*). *Mol. Phylogenet. Evol.* 24:155–167.
- Marroig, G., and R. Cerqueira. 1997. Plio-Pleistocene South American history and the Amazon lagoon hypothesis: a piece in the puzzle of Amazonian diversification. *J. Comp. Biol.* 2:103–119.
- Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.
- Mooers, A. O., and D. Schluter. 1999. Reconstructing ancestor states with maximum likelihood: support for one- and two-rate models. *Syst. Biol.* 48:623–633.
- Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annu. Rev. Ecol. Syst.* 31:533–563.

- Mourguiart, P., and M. P. Ledru. 2003. Last Glacial Maximum in an Andean cloud forest environment (Eastern Cordillera, Bolivia). *Geology* 31:195–198.
- Nelson, G., and P. Y. Ladiges. 1996. Paralogy in cladistic biogeography and analysis of paralogy-free subtrees. *Am. Mus. Novit.* 3167:1–58.
- Nores, M. 1999. An alternative hypothesis for the origin of Amazonian bird diversity. *J. Biogeogr.* 26:475–485.
- . 2004. The implications of Tertiary and Quaternary sea level rise events for avian distribution patterns in the lowlands of northern South America. *Global Ecol. Biogeogr.* 13:149–161.
- Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* 53:673–684.
- Patton, J. L., and M. F. Smith. 1992. MtDNA phylogeny of Andean mice: a test of diversification across ecological gradients. *Evolution* 46:174–183.
- Pérez-Emán, J. L. 2005. Molecular phylogenetics and biogeography of the Neotropical redstarts (*Myioborus*; Aves, Parulinae). *Mol. Phylogenet. Evol.* 37:511–528.
- Peters, J. L. 1951. Check-list of birds of the world. Vol. 7. Museum of Comparative Zoology, Harvard Univ. Press, Cambridge, MA.
- Porzecanski, A. L., and J. Cracraft. 2005. Cladistic analysis of distributions and endemism (CADE): using raw distributions of birds to unravel the biogeography of the South American aridlands. *J. Biogeogr.* 32:261–275.
- Posada, D., and K. A. Crandall. 1998. MODEL-TEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53:793–808.
- Potter, P. E. 1997. The Mesozoic and Cenozoic paleodrainage of South America: a natural history. *J. South Am. Earth Sci.* 10:331–344.
- Prum, R. O. 1988. Historical relationships among avian forest areas of endemism in the Neotropics. *Acta XIX Cong. Int. Ornithol.* 1988:2562–2572.
- Prychitko, T. M., and W. S. Moore. 1997. The utility of DNA sequences of an intron from the  $\beta$ -fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). *Mol. Phylogenet. Evol.* 8:193–204.
- Rambaut, A., and N. C. Grassly. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Comput. Appl. Biosci.* 13:235–238.
- Rambaut, A., and A. Drummond. 2003. Tracer v1.0, available from [www.evolve.zoo.ox.ac.uk](http://www.evolve.zoo.ox.ac.uk).
- Remsen, J. V., Jr., 1984. High incidence of “leapfrog” pattern of geographic variation in Andean birds: implications for the speciation process. *Science* 224:171–173.
- . 1994. Use and misuse of bird lists in community ecology and conservation. *Auk* 111:225–227.
- Remsen, J. V., Jr., and T. A. Parker, III 1983. Contribution of river-created habitats to bird species richness in Amazonia. *Biotropica* 15:223–231.
- Remsen, J. V., Jr. and W. S. V. Graves. 1995. Distribution patterns of Buarremon brush-finches (Emberizinae) and interspecific competition in Andean birds. *Auk* 112(111):225–236.
- Remsen, J. V., Jr., A. Jaramillo, M. A. Nores, J. F. Pacheco, M. B. Robbins, T. S. Schulenberg, F. G. Stiles, J. M. C. da Silva, D. F. Stotz, and K. J. Zimmer. 2006. A classification of the bird species of South America. American Ornithologists’ Union, version 29 June 2006. Published online at [www.museum.lsu.edu/~Remsen/SACCBaseline.html](http://www.museum.lsu.edu/~Remsen/SACCBaseline.html).
- Ribeiro, A. M. 1906. Vertebrados do Itatiaia (Peixes, Serpentes, Saurios, Aves e mamíferos). *Arq. Mus. Nac. Rio de J.* 13:165–190.
- Richardson, J. E., R. T. Pennington, T. D. Pennington, and P. M. Hollingsworth. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* 293:2242–2245.
- Ridgely, R. S., and G. Tudor. 1994. The birds of South America: the suboscine passerines. Univ. Texas Press, Austin, TX.
- Ridgely, R. S., T. F. Allnutt, T. Brooks, D. K. McNicol, D. W. Mehlman, B. E. Young, and J. R. Zook. 2003. Digital distribution maps of the birds of the western hemisphere, version 1.0. NatureServe, Arlington, VA.
- Robinson, W. D., J. D. Brawn, and S. K. Robinson. 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. *Ecolog. Monogr.* 70:209–235.
- Roddaz, M., S. Brusset, P. Baby, and G. Hérail. 2006. Miocene tidal-influenced sedimentation to continental Pliocene sedimentation in the forebulge-backbulge depozones of the Beni-Mamore foreland basin (northern Bolivia). *J. South Am. Earth Sci.* 20:351–368.
- Ronquist, F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* 46:195–203.
- . 2004. Bayesian inference of character evolution. *Trends Ecol. Evol.* 19:475–481.
- Rosenberg, G. H. 1990. Habitat specialization and foraging behavior by birds of Amazonian river islands in northeastern Peru. *Condor* 92:427–443.
- Roy, M. S., J. M. C. D. Silva, P. Arctander, J. Garcia-Moreno, and J. Fjeldså. 1997. The speciation of South American and African birds in montane regions. Pp. 325–343 in D. P. Mindell, ed. *Avian molecular evolution and systematics*. Academic Press, New York.
- Roy, M. S., J. C. Torres-Mura, and F. Hertel. 1999. Molecular phylogeny and evolutionary history of the tit-tyrants (Aves: Tyrannidae). *Mol. Phylogenet. Evol.* 11:67–76.
- Salazar-Bravo, J., J. W. Drago, D. S. Tinnin, and T. L. Yates. 2001. Phylogeny and evolution of the neotropical rodent genus *Calomys*: inferences from mitochondrial DNA sequence data. *Mol. Phylogenet. Evol.* 20:173–184.
- Shields, G. F., and A. C. Wilson. 1987. Calibration of mitochondrial DNA evolution in geese. *J. Mol. Evol.* 24:212–217.
- Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16:1114–1116.
- Short, L. L. 1975. A zoogeographical analysis of the South American chaco avifauna. *Bull. Am. Mus. Nat. Hist.* 154:1–188.
- Sick, H. 1985. Observations on the Andean-Patagonian component of southeastern Brazil’s avifauna. *Ornithol. Monogr.* 36:233–237.
- . 1993. *Birds in Brazil*. Princeton Univ. Press.
- Smith, T. B., R. Calsbeek, R. K. Wayne, K. H. Holder, D. Pires, and C. Bardeleben. 2005. Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. *J. Evol. Biol.* 18:257–268.
- Stotz, D. F., J. W. Fitzpatrick, T. A. Parker III, and D. K. Moskovits. 1996. Neotropical birds: ecology and conservation. Univ. Chicago Press, Chicago.
- Swofford, D. L. 2003. PAUP\*. Phylogenetic Analysis Using Parsimony (\* and Other Methods). Sinauer Associates, Sunderland, MA.
- Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 in D. M. Hillis, C. Moritz, and B. K. Mable, eds. *Molecular systematics*. Sinauer Associates Inc., Sunderland, MA.
- Terborgh, J., and J. S. Weske. 1975. The role of competition in the distribution of Andean birds. *Ecology* 56:562–576.
- Terborgh, J., S. K. Robinson, T. A. Parker, C. A. Munn, and N. Pierpont. 1990. Structure and organization of an Amazonian forest bird community. *Ecolog. Monogr.* 60:213–238.
- Urrego, D. H., M. R. Silman, and M. B. Bush. 2005. The Last Glacial Maximum: stability and change in a western Amazonia cloud forest. *J. Quaternary Sci.* 20:693–701.
- Voelker, G. 1999. Molecular evolutionary relationships in the avian genus *Anthus* (Pipits: Motacillidae). *Mol. Phylogenet. Evol.* 11:84–94.
- Vuilleumier, F. 1969. Pleistocene speciation in birds living in the High Andes. *Nature* 223:1179–1180.

- Weir, J. T. 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. *Evolution* 60:842–855.
- Weissbach, L., C. Oddoux, R. Procyk, and G. Grieninger. 1991. The  $\beta$  chain of chicken fibrinogen contains an atypical thrombin cleavage site. *Biochemistry* 30:3290–3294.
- Wesselingh, F. P., M. E. Räsänen, G. Irion, H. B. Vonhof, R. Kaandorp, W. Renema, L. R. Pittman, and M. Gingras. 2002. Lake Pebas: a palaeoecological reconstruction of a Miocene, long-lived lake complex in western Amazonia. *Cainozoic Res.* 1:35–81.
- Whitney, B. M., D. C. Oren, and R. T. Brumfield. 2004. A new species of *Thamnophilus* antshrike (Aves: Thamnophilidae) from the Serra do Divisor, Acre, Brazil. *Auk* 121:1031–1039.
- Willmott, K. R., J. P. W. Hall, and G. Lamas. 2001. Systematics of *Hypanartia* (Lepidoptera: Nymphalidae: Nymphalinae), with a test for geographical speciation mechanisms in the Andes. *Syst. Entomol.* 26:369–399.
- Young, K. R., C. U. Ulloa, J. L. Luteyn, and S. Knapp. 2002. Plant evolution and endemism in Andean South America: an introduction. *Bot. Rev.* 68:4–21.
- Zimmer, J. T. 1933. Studies of Peruvian birds 9. The Formicarian genus *Thamnophilus*. Part 1. *Am. Mus. Novit.* 646:1–22.
- . 1937. Studies of Peruvian birds 25. Notes on the genera *Thamnophilus*, *Thamnocharis*, *Gymnophis*, and *Ramphocaenus*. *Am. Mus. Novit.* 917:1–16.
- Zimmer, K. J., and M. L. Isler. 2003. Family Thamnophilidae (typical antbirds). Pp. 448–681 in J. del Hoyo, A. Elliott, and D. A. Christie, eds. *Handbook of the birds of the world*. Vol. 8. Broadbills to Tapaculos. Lynx Edicions, Barcelona.

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