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Hidden generic diversity in Neotropical birds: Molecular and anatomical data support a new genus for the "*Scytalopus*" *indigoticus* species-group (Aves: Rhinocryptidae)

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ABSTRACT

The genus *Scytalopus* is a species-rich and taxonomically complicated component of the Neotropical avian family Rhinocryptidae. Probably because *Scytalopus* is a superficially uniform assemblage, its monophyly has not been seriously questioned. We investigated phylogenetic relationships of a representative set of species in the genus using nuclear and mitochondrial DNA sequences as well as anatomical data, and provided the first test of its presumed monophyly by including in the analyses its hypothesized closest relatives (the genera *Myornis*, *Eugralla*, and *Merulaxis*) as well as most rhinocryptid genera. We found strong support for the paraphyly of the genus *Scytalopus*, with the *Scytalopus indigoticus* species-group forming a clade with *Merulaxis*. A well-supported clade including the genera *Eugralla*, *Myornis*, and the remaining *Scytalopus* was also recovered. Because these results were recovered independently and with strong support using mitochondrial and nuclear data, and were entirely consistent with anatomical data, we erect a new genus for the *S. indigoticus* species-group. These findings illustrate the importance of formally testing hypotheses of monophyly even for well-accepted groups of Neotropical birds.

1. Introduction

The Neotropical family Rhinocryptidae, as presently defined (Remsen et al., 2008), includes 11 genera of forest and non-forest birds with mainly terrestrial habits (Ridgely and Tudor, 1994; Krabbe and Schulenberg, 2003). It comprises a somewhat heterogeneous assemblage of species of medium to fairly large body size (19–24 cm of total length) in the genera *Pteroptochos*, *Scelorchilus*, *Acropternis*, *Rhinocrypta*, *Teledromas*, *Liosceles*, and *Merulaxis*, as well as a more uniform component which includes small (10–14 cm of total length), wren-like, gray or mainly gray taxa placed in the genera *Scytalopus*, *Eugralla*, and *Myornis*. The more divergent, small (c.13 cm), and rather slender *Psilorhamphus guttatus* was long placed in two diverse families, but is now accepted as a rhinocryptid (Plótnick, 1958; Krabbe and Schulenberg, 2003). *Pteroptochos*, *Scelorchilus*, *Eugralla*, *Acropternis*, and *Myornis* are confined to the Andes, *Rhinocrypta* and *Teledromas* inhabit central South American lowlands and foothills, *Liosceles* is restricted to the southwestern Amazonian basin, and *Psilorhamphus* and *Merulaxis* are endemic to the Atlantic forest of eastern South America. *Scytalopus* is by far the most widespread genus of all rhinocryptids, as it ranges along the entire Andean chain (and contiguous mountain systems) and, rather disjunctly, throughout eastern Brazil, and adjacent northeastern Argentina.

Whereas the other rhinocryptid genera include only one to three species, *Scytalopus* is among the most speciose genera of Neotropical birds, and has a growing number of recognized species, now reaching 40 (Krabbe and Schulenberg, 1997, 2003; Remsen et al., 2008). The limits of species and species-groups in *Scytalopus* have been traditionally difficult to establish, and although numerous taxonomic revisions have been published recently (e.g., Whitney, 1994; Krabbe and Schulenberg, 1997, 2003; Bornschein et al., 1998, 2007; Cuervo et al., 2005; Krabbe et al., 2005; Maurício, 2005; Raposo et al., 2006), further taxonomic investigations are still pending and several new species are likely to be discovered.

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On the basis of general observations on morphology, plumage coloration, and vocalizations, it has been suggested that the closest relatives of *Scytalopus* are the genera *Myornis*, *Eugralla*, and *Merulaxis* (Krabbe and Schulenberg, 1997, 2003). Except for the merger of *Myornis* into *Scytalopus* by Hilty and Brown (1986), a treatment that was firmly rejected subsequently (Fjeldså and Krabbe, 1990; Ridgely and Tudor, 1994; Krabbe and Schulenberg, 1997, 2003), the monophyly of *Scytalopus* has not been seriously questioned by any author, probably as a result of the uniformity in external morphology of all the members of the group. However, based on molecular phylogenetic analyses we have found that members of the genus *Scytalopus* are not as closely related as traditionally thought, a hypothesis we have further corroborated based on analyses of the internal anatomy of a variety of taxa. Here, we present molecular (nuclear and mitochondrial) and anatomical (syringeal and skeletal) data that strongly suggest that *Scytalopus*, as currently defined, is actually a paraphyletic group; two clades currently subsumed in *Scytalopus* are best considered separate genera, one of which we describe as new. These novel findings lead to a reinterpretation of morphological diversification in the Rhinocryptidae and illustrate the importance of formally testing hypotheses of monophyly even for well-accepted groups of Neotropical birds.

2. Materials and methods

2.1. Taxonomic sampling

The Andean component of the genus *Scytalopus* comprises a large number of species that may be clustered into several groups, whereas the Brazilian one comprises only seven named taxa that are distributed into two discrete complexes, namely the *S. indigoticus* and the *S. speluncae* species-groups (Bornschein et al., 1998, 2007; Krabbe and Schulenberg, 1997, 2003; Maurício, 2005). We included representatives of the Andean component and of both Brazilian species-groups in addition to several outgroups in analyses aimed at assessing the deep phylogenetic relationships within *Scytalopus* and between *Scytalopus* and related genera. Tissue samples of *Merulaxis ater*, *Psilorhamphus guttatus*, and all named species in the *S. indigoticus* (*S. indigoticus* and *S. psychopompus*) and the *S. speluncae* (*S. pachecoi*, *S. novacapitalis*, *S. iraiensis*, and *S. speluncae*, but not the recently described *S. diamantinensis*) species-groups were obtained during field work throughout eastern Brazil. A representative set of the Andean *Scytalopus* (*S. magellanicus*, *S. canus*, *S. stilesi*, and *S. vicinior*) as well as the closely related taxa *Myornis senilis* and *Eugralla paradoxa*, in addition to *Scelorchilus rubecula*, were included in the analyses based on results from a comprehensive molecular phylogeny of the genus that is in preparation by Cadena and collaborators. A representative of the Grallariidae (*Hylopezus ochroleucus*), a family closely related to Rhinocryptidae (Irestedt et al., 2002; Chesser, 2004), was sampled as an outgroup. Sequences of different genes from the taxa mentioned above composed our main data set (Table 1). In addition, we used sequences of different loci obtained from GenBank for *Pteroptochos castaneus*, *P. tarnii*, *Liosceles thoracicus*, *Rhinocrypta lanceolata*, and *Scytalopus spillmanni* (Rhinocryptidae), and *Grallaria ruficapilla* (Grallariidae), in different analyses (Table 1; see below).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from tissue samples using either the Chelex 100 method (Karp et al., 1998) or a QIAamp DNA Mini Kit (Qiagen). Based on these extracts, we amplified and sequenced fragments of three mitochondrial protein-coding genes and two nuclear introns for different subsets of species and individuals (Table 1) following standard PCR protocols (see Cadena et al., 2007). The mitochondrial NADH-subunit 2 (ND2) was amplified for 22 individuals of 15 different species using the primers L5216 and H6313 (Sorenson et al., 1999), and sequenced with these two primers or with the internal primers L5758 and H5766 (Sorenson et al., 1999). For a total of nine individuals of nine species, we also amplified and sequenced a fragment spanning part of the cytochrome *b* and NADH-subunit five mitochondrial genes (cyt *b*-ND5) using primers L14764 and H15295 (Sorenson et al., 1999). For 22 individuals of 16 species, we amplified, and sequenced the nuclear β-fibrinogen intron 7 (FIB7) with primers FIB-BI7L and FIB-BI7U (Prychitko and Moore, 1997). Finally, we obtained sequences of the nuclear glyceraldehyde-3-phosphate dehydrogenase intron 11 (G3PDH) for nine individuals of nine species using primers G3PDH11F and G3PDH11R (Fjeldså et al., 2003). PCR products were purified with ExoSap-It (Amersham Biosciences), sequenced with DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences), and read on a MegaBACE 1000 automated sequencer (Amersham Biosciences) following the manufacturer's protocols.

2.3. Phylogenetic analyses

Sequence traces were checked by eye, edited manually using Bioedit 6.0.7 (Hall, 1999), and aligned using Clustal X 1.83 (Thompson et al., 1997). A partition homogeneity test (ILD, Farris et al., 1995) as implemented in PAUP*4.0b10 (Swofford, 2002) using 1000 random replications was undertaken to assess congruence in the phylogenetic signal between mitochondrial and nuclear genes. Saturation in the DNA sequences was examined by plotting the number of transition and transversion substitutions against *p*-distances for each pairwise comparison using the program Dambe (Xia and Xie, 2001).

We implemented different strategies to reconstruct phylogenies, using both independent and combined data sets. First, we maximized the number of species, and genera by analyzing a data set consisting of 15 taxa (21 individuals) and 1814 bp (930 bp of ND2 and 884 bp of FIB7), of which 661 were variable and 428 were parsimony-informative. Second, we maximized the number of molecular characters, with a data set that included nine taxa (nine sequences) and 2647 bp (930 bp of ND2, 462 of cyt *b*-ND5 and their intergenic spacer, 884 bp of FIB7, and 371 bp of G3PDH); 824 of these were variable and 420 were parsimony-informative. Note that in this data set, the representative of *S. iraiensis* was a composite sample, consisting of nuclear sequences of specimen MCP 957 and mitochondrial sequences of specimen MPEG 52945. We also analyzed a third set consisting only of FIB7 sequences, with 19 taxa (25 individuals) and 884 bp, of which 289 were variable and 136 were parsimony-informative (see Table 1 for the taxa included in each data set). Finally, we conducted additional analyses including only sequences of other genes (e.g., ND2), but because results were similar to those obtained with other, more comprehensive data sets, they are not reported here.

Phylogenies were estimated using maximum parsimony (MP) and maximum likelihood (ML) methods in PAUP* 4.0b10 and Bayesian analysis in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Maximum parsimony analyses were conducted based on heuristic searches using the TBR branch swapping algorithm with no upper limit set for the maximum number of trees saved. Gaps were treated as missing data. Branch support was evaluated using 1000 bootstrap replicates (Felsenstein, 1985). For the maximum likelihood analyses, we used the best-fit substitution models selected based on the Akaike Information Criterion (AIC) in ModelTest 3.7 (Posada and Crandall, 1998), as follows: FIB7 + ND2: TIM + I + G, ND2 + cyt *b*-ND5 + FIB7 + G3PDH: GTR + G, FIB7: