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# DNA barcodes from northern birds of Colombia

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## Abstract

DNA barcode datasets are a useful tool for conservation and aid in taxonomic identification, particularly in megadiverse tropical countries seeking to document and describe its biota, which is dropping at an alarming rate during recent decades. Here we report the barcodes for several low elevation bird species from northern Colombia with the goal to provide tools for species identification in this region of South America. We blood sampled birds in a lowland tropical forest with various degrees of intervention using standard 10 × 12 mist-nets. We extracted DNA and sequenced the COI barcode gene using standard primers and laboratory methods. We obtained 28 COI sequences from 19 species, 10 families and 3 orders and found that barcodes largely matched (but not always) phenotypic identification (>90%) and they also facilitated the identification of several challenging passerine species. Despite our reduced sampling, our study represents the first attempt to document COI barcodes for birds (from blood samples) in this part of Colombia, which fills a considerable gap of sampling in this part of South America.

## Keywords

Aves, lowland tropical forest, mtDNA, northern Colombia

## Introduction

DNA barcode reference libraries are a useful tool for conservation and aid in taxonomic identification ([Gonzalez et al. 2009](#), Waugh 2007) for many biological groups (Hebert et al. 2003). Megadiverse countries such as Colombia are desperately in need to document and describe its biota, which is declining at an alarming rate during recent decades (Shaw et al. 2013), with emphasis on the putative cryptic diversity present in tropical areas (Crawford et al. 2012, Lohman et al. 2010, Stefan et al. 2018). Despite efforts to encourage sequence data collection and sharing through local and global initiatives (e.g. Barcode Life Data

System, BOLD), most taxa are underrepresented for barcodes. Naturally, although barcodes are particularly useful for advancing on the recognition of unknown diversity (in groups where alpha taxonomy is still developing or for highly diverse groups where many species remain to be described) it is also extremely useful for species identification in groups with better resolution in their taxonomy (Collins and Cruickshank 2012, Hebert and Gregory 2005, Hebert et al. 2003). Birds are one of the most well-known groups in terms of their taxonomy and systematics (Jarvis et al. 2014), but nonetheless new species are being described almost every year, particularly in the Neotropics (Avendaño et al. 2015), and some challenges still remain in the identification of species groups with very little phenotypic differentiation (Lara et al. 2012, Tavares et al. 2011, Cadena et al. 2016). Consequently, birds are an excellent group for implementing DNA barcoding for both species-identification and species-recognition purposes.

Barcode studies in Neotropical birds are on the increase particularly in Brazil and Argentina, where studies have focused on testing species limits and biogeographic patterns (Chaves et al. 2015, Kerr et al. 2009, Tavares et al. 2011, Vilaca et al. 2006). Despite that progress, a huge gap of information remains to be filled in northern South America, where very few studies have been completed (but see Mendoza et al. 2016). Here, we report the barcodes for several low elevation bird species from northern Colombia with the goal to provide tools for species identification and add to the existing gap of animal COI data in this part of South America.

## Materials and Methods

### Sample collection and processing

We sampled birds at “Hacienda Universidad de Antioquia”, in the Municipality of Caucasia, Department of Antioquia, Colombia (8.003143 N, -75.400716 W; 70 m a.s.l., Fig. 1), from the 26th to 29th of October 2017. The landscape at the study site is composed by remnants of lowland tropical forests with various degrees of intervention, immersed in a matrix of pastures, second growth forest, bushes and small streams. Birds were caught at forest edges and in open areas between forest fragments using standard 10x12 mist-nets and were blood sampled from the brachial vein using small gauge needles and non-heparinized capillary tubes (Pulgarín-R et al. 2018). All birds were processed and released in place. Blood samples were stored in 90-95% ethanol and kept at room temperature.

### Laboratory procedures

We extracted total DNA from blood using the PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer’s specifications. For blood samples, 20 µl of Proteinase K, 20 µl of RNase and 200 µl of PureLink® Genomic Lysis/Binding buffer were added during the digestion phase. Later, each sample was transferred to a spin column and two washes were performed with Wash Buffer 1 and Wash Buffer 2 to perform a final elution dividing the total volume in two consecutive sets of 50 µl with Elution Buffer.

For molecular typing, we targeted the Cytochrome c oxidase subunit 1 (COI) barcode region using the primer combination from Ivanova et al. (2007) with the unique difference that all primers were M13-tailed to facilitate the sequencing process (Table 1). PCR amplifications were performed in 35 µl reactions that contained: 2 mM of MgCl<sub>2</sub>, 1 × of buffer PCR 10 × with KCl, 0.2 mM of each dNTP, 0.14 µl of each primer cocktail, 1U of Taq DNA Polymerase (Fermentas) and 100 ng of DNA template. Thermal cycling conditions involved an initial denaturation at 95°C for 2 min followed by a single stage of 28 cycles that included denaturation at 95°C for 30 s, annealing at 52°C for 40 s, extension at 72°C for 1 min, and a final 10 min extension at 72°C. PCR products were visualized on a 1.5% agarose gel using a MiniBIS Pro-DNR Bio Imaging Systems. All amplification products were purified and sent to Macrogen (Seoul, Korea) to be sequenced on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

## Data analysis

Sequences were edited, assembled, and examined with reference to translated amino-acid sequences using Geneious PRO 6.1.6. Nucleotide-sequences and complementary information were deposited in BOLD ([www.barcodinglife.org](http://www.barcodinglife.org)) with the accession number data set (CANDE030-20 to CANDE055-20). For an initial sequence quality check and provisional identification, all assembled sequences were searched in the National Centre for Biotechnology Information (NCBI) database through BLAST (<http://BLAST.ncbi.nlm.nih.gov/BLAST.cgi>) using the Geneious Pro 6.1.6 match tool. We used the top-matching hit having the highest (>98%) maximal percent identity score as criteria for successful conspecific/congeneric identification. After the initial BLAST-based identification on the NCBI database, we used the Animal Identification (COI) tool from the BOLD Identification System (IDS) using the Species Level Barcode Records database. For all our sequences we recovered the species identification, closest matching BIN (Table 2), and a Neighbor-Joining topology using Kimura-2-Parameter (K2P) substitution model as implemented in the BOLD portal (Suppl. material 1).

## Results

We obtained 26 COI sequences from 18 species, 10 families and 4 orders, and when analyzed by BOLD, the species were grouped into 18 existing BINS (access numbers in Table 2). Most bird species were residents, but four species (*Catharus minimus*, *Catharus ustulatus*, *Myiodynastes luteiventris*, and *Parkesia noveboracensis*) were boreal migrants (Table 2). For all species, sequence length varied from 642 to 702 bp (Table 2). Since most bird species are under sampled for DNA barcodes (Mendoza et al. 2016) in this part of the tropics, our report represents an important contribution to expand the geographic sampling (for COI sequences) of several species in South America, and it also includes the first sequences for Colombia for the following species: buff-breasted wren (*Cantorchilus leucotis*), short-tailed swift (*Chaetura brachyura*), rufous-tailed jacamar (*Galbula ruficauda*), sulphur-bellied flycatcher (*Myiodynastes luteiventris*), rusty-margined flycatcher (

*Myiozetetes cayanensis*), yellow-olive flatbill (*Tolmomyias sulphurescens*), and the cocoa woodcreeper (*Xiphorhynchus susurrans*).

Most COI barcodes matched our initial phenotypic identification, however, for 6 species (10 individuals) we found differences between our field identification, the query hits from BOLD's IDS and the NCBI BLAST search (Table 2). One bird species originally identified in the field as the streaked flycatcher (*Myiodynastes maculatus*, field ID LCA21) was positively identified as the sulphur-bellied flycatcher (*Myiodynastes luteiventris*, Fig. 2) by BOLD and NCBI analyses. Another passerine species correctly identified in the field (field ID's LCA31, LCA36, LCA38) and by the NCBI BLAST as the white-bearded manakin (*Manacus manacus*, Fig. 3) was recovered as the Central American restricted species, the orange-collared manakin (*Manacus aurantiacus*) by BOLD's IDS. A third species was identified in the field (and NCBI BLAST search) as the whooping motmot (*Momotus subrufescens*, Fig. 4), but BOLD IDS recovered its former nominal assignment, *Momotus momota*, the name of a widely distributed form of motmot before it was divided into five species-level taxa (Stiles 2009). Additionally, other three species (*Ramphocellus dimidiatus*, *Sporophila funerea*, *Xiphorhynchus susurrans*) were positively identified in the field and by BOLD but exhibited erroneous identifications by the NCBI BLAST apparently because of the absence of COI sequences for either species in the latter portal. Finally, in six instances, DNA sequences helped to confirm the identification of the Buff-throated Foliage-gleaner (*Automolus ochrolaemus*), the short-tailed swift (*Chaetura brachyura*) and the yellow-olive flatbill (*Tolmomyias sulphurescens*), which are all species difficult to identify in the field, even in hand, particularly the swifts.

## Discussion

Our assessment of species identification using the COI barcodes shows a strong correspondence (90%) with field identification based on research expertise and photo ID (Table 2, Suppl. material 1). However, DNA barcodes were able to help with the identification of challenging species that can be problematic even for trained neotropical ornithologists. This was the case of field ID LCA21, identified initially as the Striped Flycatcher (*Myiodynastes maculatus*), however, both NCBI BLAST and the BOLD identification tool later recovered it as the sulfur-bellied flycatcher (*Myiodynastes luteiventris*, Fig. 2). Similarly, barcodes might help to identify the breeding areas or population origin for species exhibiting migratory divide or genetic structure, as happened with passing through northern South America species, *Catharus minimus* and *Catharus ustulatus* (Topp et al. 2013, Pulgarín-R et al. 2018). Additionally, barcodes can be of great help in resident species with little phenotypic variation, such as the swifts in the genus *Chaetura*, which are hardly captured in mist-nets and hard to identify in the field.

We also found some discrepancies between IDs recovered by the NCBI BLAST tool, those recovered by BOLD and our initial identifications made in the field. For example, three specimens identified in the field (Fig. 3) and by the NCBI BLAST tool as the white-bearded manakin (*Manacus manacus*) were recovered by BOLD as the orange-collared manakin (*M. aurantiacus*). The BIN containing our sequences (Table 2) groups several phenotypes

that in the past have been treated as the same species (e.g., Snow 1975) and also as a superspecies with up to four species (*M. aurantiacus*, *M. candei*, *M. manacus* and *M. vitellinus*) (Snow et al. 2004). Taxonomy within this group is not fully resolved so far that *M. aurantiacus* has been considered a subspecies of *M. vitellinus* (Snow 1975), an independent allopatrically distributed species of the genus (Brumfield and Braun 2001, Brumfield et al. 2001, Brumfield et al. 2008), and even as a paraphyletic clade based on mtDNA (Brumfield and Braun 2001). Moreover, it has been found that species of *Manacus* can hybridize in areas of sympatric distribution with other species of genus and even the family (Brumfield and Braun 2001, Brumfield et al. 2001, Höglund and Shorey 2004). All the above-mentioned scenario indicates that although the phenotype of all our sequences corresponds to what is known as *M. manacus* (Fig. 3), in the absence of a clear phylogenetic arrangement and poor knowledge on the species limits within the genus *Manacus*, the DNA barcode by itself is not able to reconcile the morphological and molecular information and is only the reflection of a poorly understood taxonomy.

Another result that showed some inconsistencies was the identification of LCA4 and LCA7 sequences, which were recovered by BOLD as *Momotus momota*. This used to be a widely distributed species in Central and South America, until it was divided into five species-level taxa (*M. aequatorialis*, *M. bahamensis*, *M. lessonii*, *M. momota*, *M. subrufescens*) using a combined analysis of plumage, biometrics and voice (Stiles 2009). Currently, *Momotus momota* is considered a cis-Andean distributed species from eastern Colombia to southern Venezuela, Guianas, northwestern Argentina, and most of Brazil. Particularly, the specific epithet associated with the populations and phenotype obtained in this study corresponds to *M. subrufescens*, however, despite the presence of 8 different BINs that span much of the distribution of all the mentioned species within the genus, the taxonomy within the BOLD portal has yet to be updated and consequently our sequence is part of a BIN based on a haplotype with geographical proximity that bears the outdated *M. momota* taxon name.

A final group of inconsistencies between identification methods corresponds to 3 species for which no COI sequence data is available at the NCBI portal and consequently their closest matching sequences are inconsistent with their correct field- and BOLD-based identifications. In the case of the genera *Ramphocellus* and *Xiphorhynchus*, the BLAST search tool identified our samples as the cis-Andean distributed congeneric species (*Ramphocellus carbo* and *Xiphorhynchus guttatus*) and not as the correct trans-Andean species (*R. dimidiatus* and *X. sussurrans*). For the genus *Sporophila*, although the chestnut-bellied ceed-finch (*Sporophila angolensis*) and the thick-billed seed-Finch (*Sporophila funerea*) can show sympatric distributions, the morphology exhibited by their males is strikingly different and leaves no room to discussion on their morphological identification.

Even though we found some discrepancies between our identification methods compared to BOLD's IDS, a close inspection to the K2P trees from BOLD (Fig. 4) showed that individuals across all sampled species are closely related to other individuals from nearby populations/areas. This is an important fact because even in the presence of outdated or incorrect assignment of names to a barcode sequence (and consequently to its

corresponding BIN) the K2P topologies are able to group individuals that based even on geography itself can putatively represent the current taxonomical treatment of the species (as is the case with the Whooping Motmot in Fig. 4).

## Conclusions

Despite our reduced sampling, this study represents the first attempt to document COI barcodes for birds (from blood samples) in this part of Colombia, which fills a considerable gap of sampling in northwestern South America. Particularly, a call for broader sampling for barcodes might provide hints on cryptic species across barriers (Barreira et al. 2016) or might facilitate the identification of highly-traded species in Colombia such as parrots (Mendoza et al. 2016, Restrepo-R and Pulgarin-R 2017).

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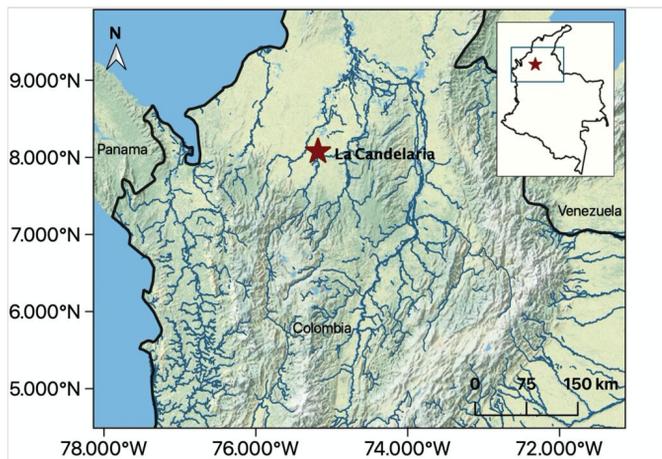


Figure 1.

Study area in the lowlands of northern Colombia.



Figure 2.

The sulphur-bellied flycatcher (*Myiodynastes luteiventris*), a boreal migrant, was initially identified in the field as *Myiodynastes maculatus* but was subsequently correctly reidentified with the help of its COI barcode.



Figure 3.

The white-bearded manakin (*Manacus manacus*) was identified as a different manakin species according to BOLD.

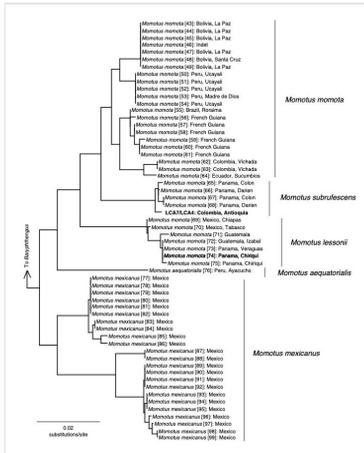


Figure 4. Kimura-2-parameter tree of the "*Momotus momota* complex" indicates that barcodes can group together recently split species.

Table 1.

Primers used for the amplification of COI sequences obtained in this study.

Name	Sequence + M13	Ratio	Source
LepF1_t1-M13FWD	GTAAAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG	1	Ivanova et al. 2007
VF1_t1-M13FWD	GTAAAACGACGGCCAGTTTCTCAACCAACCACAAAGACATTGG	1	Ivanova et al. 2007
VF1d_t1-M13FWD	GTAAAACGACGGCCAGTTTCTCAACCAACCACAARGAYATYGG	1	Ivanova et al. 2007
VF1i_t1-M13FWD	GTAAAACGACGGCCAGTTTCTCAACCAACCAIAAIGAIATIGG	3	Ivanova et al. 2007
LepRI_t1-M13REV	CAGGAAACAGCTATGACCTAACTTCTGGATGTCCAAAAATCA	1	Ivanova et al. 2007
VR1d_t1-M13REV	CAGGAAACAGCTATGACCTAGACTTCTGGGTGGCCRAARAAYCA	1	Ivanova et al. 2007
VR1_t1-M13REV	CAGGAAACAGCTATGACCTAGACTTCTGGGTGGCCAAAGAATCA	1	Ivanova et al. 2007
VR1i_t1-M13REV	CAGGAAACAGCTATGACCTAGACTTCTGGGTGICCAIAAICA	3	Ivanova et al. 2007
M13REV	CAGGAAACAGCTATGACC	NA	Beckman Coulter, Inc 2020
M13FWD	GTAAAACGACGGCCAGT	NA	Beckman Coulter, Inc 2020

Table 2.

Individuals sampled and barcoded in this study. Individuals with \* represent boreal migrants. Bolded taxa represent inconsistencies between our identification methods (see text).

Cod e	Field ID	Bold ID	BOLD hit <sup>1</sup> (%)	NCBI ID	NCBI hit <sup>1</sup> (%)	Consensus sp BOLD ID	Seq length (bp)	BIN
LCA35	<i>Automolus ochrolaemus</i>	<i>A. ochrolaemus</i>	100	<i>A. ochrolaemus</i>	99.10	<i>A. ochrolaemus</i>	671	<a href="#">BOLD:ADM4531</a>
LCA9	<i>Cantorchilus leucotis</i>	<i>C. leucotis</i>	100	<i>C. leucotis</i>	95.55	<i>C. leucotis</i>	690	<a href="#">BOLD:ABX4224</a>
LCA12	<i>Catharus minimus</i>	<i>C. minimus</i>	100	<i>C. minimus</i>	100	<i>C. minimus</i> *	657	<a href="#">BOLD:AAA9441</a>
LCA30	<i>Catharus minimus</i>	<i>C. minimus</i>	100	<i>C. minimus</i> *	100	<i>C. minimus</i> *	660	<a href="#">BOLD:AAA9441</a>
LCA3	<i>Catharus ustulatus</i>	<i>C. ustulatus</i>	100	<i>Catharus ustulatus</i>	100	<i>C. ustulatus</i> *	702	<a href="#">BOLD:AAA9440</a>
LCA26	<i>Chaetura sp</i>	<i>C. brachyura</i>	100	<i>C. brachyura</i>	100	<i>C. brachyura</i>	644	<a href="#">BOLD:AAK0488</a>
LCA27	<i>Chaetura sp</i>	<i>C. brachyura</i>	100	<i>C. brachyura</i>	100	<i>C. brachyura</i>	642	<a href="#">BOLD:AAK0488</a>
LCA28	<i>Chaetura sp</i>	<i>C. brachyura</i>	100	<i>C. brachyura</i>	100	<i>C. brachyura</i>	652	<a href="#">BOLD:AAK0488</a>
LCA24	<i>Coereba flaveola</i>	<i>C. flaveola</i>	100	<i>C. flaveola</i>	100	<i>C. flaveola</i>	651	<a href="#">BOLD:AAA4006</a>
LCA33	<i>Dendrocicla fuliginosa</i>	<i>D. fuliginosa</i>	99.85	<i>D. fuliginosa</i>	99.15	<i>D. fuliginosa</i>	673	<a href="#">BOLD:ABZ6107</a>
LCA20	<i>Elaenia flavogaster</i>	<i>E. flavogaster</i>	99.85	<i>E. flavogaster</i>	98.93	<i>E. flavogaster</i>	681	<a href="#">BOLD:AAB3859</a>
LCA6	<i>Elaenia flavogaster</i>	<i>E. flavogaster</i>	100	<i>E. flavogaster</i>	99.39	<i>E. flavogaster</i>	696	<a href="#">BOLD:AAB3859</a>
LCA18	<i>Galbula ruficauda</i>	<i>G. ruficauda</i>	100	<i>G. ruficauda</i>	97.55	<i>G. ruficauda</i>	675	<a href="#">BOLD:ABX4491</a>
LCA31	<i>Manacus manacus</i>	<i>M. aurianticus</i>	100	<i>M. manacus</i>	100	<i>M. aurianticus</i>	667	<a href="#">BOLD:AAB9291</a>
LCA36	<i>Manacus manacus</i>	<i>M. aurianticus</i>	100	<i>M. manacus</i>	100	<i>M. aurianticus</i>	663	<a href="#">BOLD:AAB9291</a>
LCA38	<i>Manacus manacus</i>	<i>M. aurianticus</i>	100	<i>M. manacus</i>	100	<i>M. aurianticus</i>	667	<a href="#">BOLD:AAB9291</a>
LCA4	<i>Momotus subrufescens</i>	<i>M. momota</i>	100	<i>M. momota</i>	96.92	<i>M. momota</i>	681	<a href="#">BOLD:ABX4186</a>
LCA7	<i>Momotus subrufescens</i>	<i>M. momota</i>	100	<i>M. momota</i>	97.41	<i>M. momota</i>	657	<a href="#">BOLD:ABX4186</a>
LCA21	<i>Myiodinastes maculatus</i>	<i>M. luteiventris</i>	100	<i>M. luteiventris</i> *	100	<i>M. luteiventris</i> *	651	<a href="#">BOLD:AAF5348</a>

LCA22	<i>Myiozetetes cayanensis</i>	<i>M. cayanensis</i>	99.85	<i>M. cayanensis</i>	98.77	<i>M. cayanensis</i>	660	<a href="#">BOLD:AAE6211</a>
LCA13	<i>Parkesia noveboracensis</i>	<i>P. noveboracensis</i>	100	<i>P. noveboracensis</i> *	99.85	<i>P. noveboracensis</i> *	658	<a href="#">BOLD:AAB0401</a>
LCA15	<i>Ramphocellus dimidiatus</i>	<i>R. dimidiatus</i>	100	<i>R. carbo</i>	99.39	<i>R. dimidiatus</i>	681	<a href="#">BOLD:AAD5047</a>
LCA40	<i>Ramphocellus dimidiatus</i>	<i>R. dimidiatus</i>	100	<i>R. carbo</i>	99.23	<i>R. dimidiatus</i>	654	<a href="#">BOLD:AAD5047</a>
LCA1	<i>Sporophila funerea</i>	<i>S. funerea</i>	100	<i>S. ang ol ensis</i>	98.92	<i>S. funerea</i>	687	<a href="#">BOLD:AAE5360</a>
LCA19	<i>Tolmomyias sulphurens</i>	<i>T. sulphurens</i>	99.85	<i>T. sulphurens</i>	97.89	<i>T. sulphurens</i>	666	<a href="#">BOLD:ACI3658</a>
LCA8	<i>Xiphorhynchus susurrans</i>	<i>X. susurrans</i>	99.54	<i>X. guttatus</i>	98.15	<i>X. susurrans</i>	670	<a href="#">BOLD:ACF1637</a>

## Supplementary material

### Suppl. material 1: Supplementary Information

**Authors:** Pulgarin et al.

**Data type:** DNA sequences, data Tables.

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