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Mitochondrial introgression obscures phylogenetic relationships among manakins of the genus *Lepidothrix* (Aves: Pipridae)

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ABSTRACT

Lepidothrix is the most diverse genus of the family Pipridae, with eight recognized species. Although the genus' monophyly has been supported by both molecular and morphological characters, phylogenetic relationships and species limits within *Lepidothrix* remain uncertain. In the present study, we combined molecular sequences of mitochondrial (ND2 and COI) and nuclear (MYO, G3PDh and I5BF) markers in a multilocus analysis, to evaluate relationships and inter-specific limits among *L. iris*, *L. nattereri*, and *L. vilasboasi*, which are known to hybridize in eastern Amazonia. The results revealed a complex pattern, whereby events of secondary contact and gene flow after isolation and genetic and phenotypic differentiation prevented the recuperation of reciprocal monophyly among the studied taxa. The mitochondrial data indicate that *L. nattereri* is divided into two non-sister groups, one monophyletic, and the other, paraphyletic, with *L. iris iris* being more closely related to one of the two *L. nattereri* groups, while *L. iris eucephala* forms an undifferentiated clade with *L. vilasboasi*, probably resulting from an extensive process of mitochondrial introgression. In agreement with a previous study based on Single Nucleotide Polymorphism (SNP) data, mitochondrial haplotype networks also support that *L. vilasboasi* does not represent a recent "hybrid swarm" between *L. iris* and *L. nattereri*, but instead a genetically divergent lineage with a separate species status. Finally, the sister relationship recovered herein between *L. iris iris* and some western populations of *L. nattereri* currently in allopatry is also apparently explained by mitochondrial introgression, as also supported for nuclear genes by SNP data, indicating a complex scenario of past contact and gene flow between currently geographically distant *Lepidothrix* lineages.

1. Introduction

The genus *Lepidothrix* currently includes eight species, which are widely distributed in Central and South America (Snow, 2017). The plumage of males in this genus is predominantly black or green, otherwise varying mainly on the crown and rump, which provide the basic characteristics for the diagnosis of taxa. The monophyly of *Lepidothrix* has been confirmed through both morphological (Prum, 1992) and molecular analyses (McKay et al., 2010; Rêgo et al., 2007; Tello et al., 2009), although some relationships within the genus remain poorly resolved, in particular those within the *coronata* group, which includes *L. coeruleocapilla*, *L. coronata*, *L. iris*, *L. isidorei*, *L. nattereri*, and *L. vilasboasi*, generating considerable taxonomic and phylogenetic uncertainties (Cheviron et al., 2005; Ohlson et al., 2013). Up until recently, *Lepidothrix vilasboasi* was the only taxon in the genus that had not been included in a molecular study, and its evolutionary relationships with the other species of the genus were unclear (Bandeira et al., 2008). Former studies suggested that it was closely related to *L. iris* and *L. nattereri* according to morphological characters (Prum, 1997; Sick, 1997), and a recent study based on Single Nucleotide Polymorphism (SNP) data showed that *L. vilasboasi* in fact consists on an independent species of hybrid origin resulting from ancient introgression between *L. iris* and *L. nattereri* (Barrera-Guzmán et al., 2018).

The most recent phylogeny proposed for the Pipridae (Ohlson et al., 2013) recovered *L. iris* and *L. nattereri* as sister taxa, although no specimens of *L. vilasboasi* were included in this study. *Lepidothrix iris* has two disjunct populations, which have been described as distinct subspecies (Snow and Sharpe, 2017). The males and females are green, with a silvery-opalescent cap in the adult males. One of the subspecies, *L. iris iris*, is found in eastern Pará (to the east of the Xingu River) and northwestern Maranhão, Brazil, while *L. iris eucephala* occurs in western Pará, limited to the west by the Tapajós River (Ridgely and Tudor,

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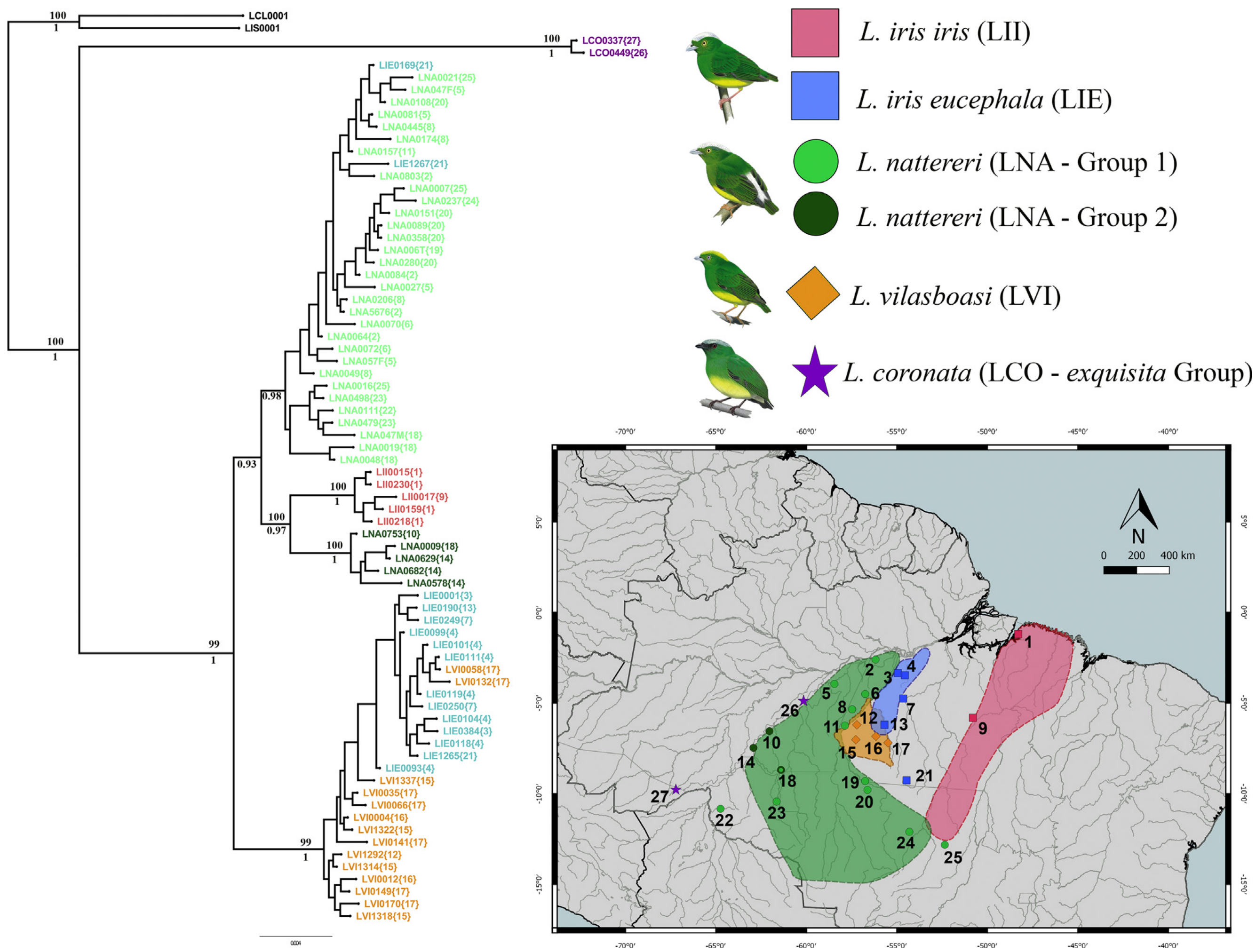

Fig. 1. Multilocus topology and map. Left: Bayesian Inference obtained with the concatenated nuclear and mitochondrial markers with an outgroup consisting of *L. coronata*, *L. isidorei* and *L. coeruleocapilla.* Codes: LCO: *L. coronata,* LCL: *L. coeruleocapilla,* LIE: *L. i. eucephala*, LII: *L. i. iris*, LIS: *L. isidorei*, LNA: *L. nattereri*, LVI: *L. vilasboasi.* The bootstrap values for the ML analysis are shown above the branches, and the posterior probabilities for the BI analysis, below the branches. The branches with no ML bootstrap were not recuperated in the ML analysis. Values within braces "{}" indicate sampling points that correspond to those on the map to the right. The colors of the terminal taxa correspond to those of the symbols that represent the taxa on the map. Right: Map of the distribution of *Lepidothrix iris iris, L. iris eucephala*, *L. nattereri* and *L. vilasboasi*. The numbered points represent the collecting localities of the samples analyzed in the present study. The identification of the taxa and the scale are shown in the image. Symbols with the same shape indicate the collecting localities of specimens with the same phenotype. The species distributions are adapted from the BirdLife International and Handbook of the Birds of the World (2016a, b, c). Images of the species are adapted from those available in the Handbook of the Birds of the World.

1994) (Fig. 1). The latter subspecies, *L. i. eucephala*, is the taxon involved in the proposed hybridization with *L. nattereri* that would have produced *L. vilasboasi* (Barrera-Guzmán et al., 2018). *Lepidothrix nattereri* differs morphologically from *L. iris* and *L. vilasboasi* due to the shiny white coloration of the feathers on the top of the head and rump, and is widely distributed in south-central Brazilian Amazonia (Sick, 1997), with some records from Bolivia (Bates et al., 1989) (Fig. 1).

Although recent studies showed that reproductive isolation between these otherwise phenotypically distinct taxa is still incomplete (Barrera-Guzmán et al., 2018; Weir et al., 2015), no explicit phylogenetic hypothesis is available for *L. iris*, *L. nattereri*, and *L. vilasboasi*. In the present study, specimens of *L. iris*, *L. nattereri* and *L. vilasboasi* were analyzed using sequences of mitochondrial and nuclear markers to establish the phylogenetic relationships among these taxa and determine whether the molecular data is consistent with species limits based on current taxonomy. We also evaluated the level of genetic differentiation between the two *L. iris* subspecies and the possible presence of distinct evolutionary units in *L. nattereri.*

2. Material and methods

2.1. Sampling

The present study was based on the analysis of samples of muscle tissue provided by a total of 70 specimens from 25 different localities, including 20 *L. iris* (five *L. iris iris* and 15 *L. iris eucephala*), 36 *L. nattereri,* and 14 *L. vilasboasi* (Fig. 1, Table 1S). To root the phylogenies, we included samples from two specimens of *L. coronata*, representing the sister group of this species complex, according to the findings of Ohlson et al. (2013), as well as sequences of species *L. coeruleocapilla* and *L. isidorei* obtained from GenBank (Table 1S).

2.2. Isolation and sequencing of molecular markers

The genetic material (DNA) was extracted using the reagents of the Wizard® Genomic Purification kit (Promega), according to the maker's instructions. We used the Polymerase Chain Reaction (PCR) to amplify the sequences of the target regions, using specific primers for the mitochondrial and nuclear regions (Table 2S). The mitochondrial markers