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SHORT-COMMUNICATION

A new population of the White Bellbird *Procnias albus* (Hermann, 1783) from lowland southern Brazilian Amazonia, with comments on genetic variation in bellbirds

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ABSTRACT: We report on a recently discovered population of the White Bellbird (*Procnias albus*) in southern Amazonia. Contrary to expectations based on geography and morphological analyses, a recently collected specimen from this new population is genetically closer to the northern subspecies, at the same time that it confirms the overall lack of genetic structure previously reported for the species. Our data reinforces the notion that the subspecies of *P. albus* may not be diagnosable by morphological and molecular characters. The discovery of a new *Procnias albus* population not far from the largest human settlement in Brazilian Amazonia underscores the need for more research to better understand avian distribution in this under-studied region.

KEY-WORDS: Cotingidae, distribution, phylogeography, subspecies, taxonomy.

The White Bellbird, *Procnias albus* (Cotingidae) has a disjunct distribution in Amazonia; the nominate subspecies occurs north of the Amazon River in Venezuela, the Guianas, and northernmost Brazil, whilst *P. a. wallacei* occurs south of the Amazon River on the Serra dos Carajás in southeastern Pará state, Brazil (Snow & Sharpe 2016). *Procnias a. wallacei* is listed as "Vulnerable" according to the Brazilian list of threatened species (MMA 2014). The species was first reported south of the Amazon River by Wallace (1889) near the city of Belém, more than 450 km north of Carajás, but no voucher specimen for this record has been found and until now there have been no other records from the region. Thus, Wallace's (1889) record has either been disregarded for lack of documentation (Moura *et al.* 2014) or, alternatively, considered an instance of vagrancy or even a currently extinct population (Snow 1982). According to Berv & Prum (2014), the two subspecies currently recognized in *P. albus* are not distinct genetically, but more study is needed to clarify the taxonomy of this patchily distributed species. Here, we report on a newly discovered southern Amazonian population of *P. albus*, and comment on the genetic structure of the species.

The new record was obtained during a biodiversity inventory of the northernmost part of the Xingu-Tocantins interfluve at the municipality of Bagre, near the Muratuba River (02°06'44.5''S; 50°22'15.8''W), *c.* 260 km (161 miles) to the west of Belém, in the state of Pará, Brazil. The region is covered by upland *terra firme* forest with canopy heights averaging 30 m, as well as *igapó* (black water forest), and *campinas* (white-sand forest). Four leks were found between 19 and 22 June 2015 in the municipality of Bagre (Fig. 1). Three leks were found in a *terra firme* forest area of approximately 25 km^2. These three leks combined contained at least nine adult males, a young male and some females. A fourth lek was located in *várzea* forest. One adult male from the first lek was collected on 22 June 2015 (MPEG 80706; Table 1). Digital sound files were deposited in Xeno-canto (www.xeno-canto.org: XC261271, XC263193) and photographs were deposited in WikiAves (www.wikiaves.com.br: WA1773951, WA1773933, WA1771028).

To compare morphometrics of this collected individual with those of known populations, S.M.D. measured six additional adult male specimens of *P. albus* deposited in the ornithological collection of Museu Paraense Emílio Goeldi (MPEG), as follows: *P. a. albus* (MPEG 32489 from Paru de Leste River, Aramapucú, state of Pará) and *P. a. wallacei* (MPEG 37213, 37214, 35042, 30543, all from Serra dos Carajás, Parauapebas, state of Pará). Measurements taken were bill length (exposed culmen), bill width at nostrils, bill height at nostrils, left tarsus length, left wing length, and tail length. Measurements were taken with a Vonder electronic caliper to the next 0.01 mm and a ruler. The morphometric data was analyzed by plotting each measured specimen with Principal Component Analysis (PCA) performed in XLSTAT (Addinsoft 2007). A Pearson's correlation matrix was used in the analysis, and the two factors that best explained the results were plotted against each other.

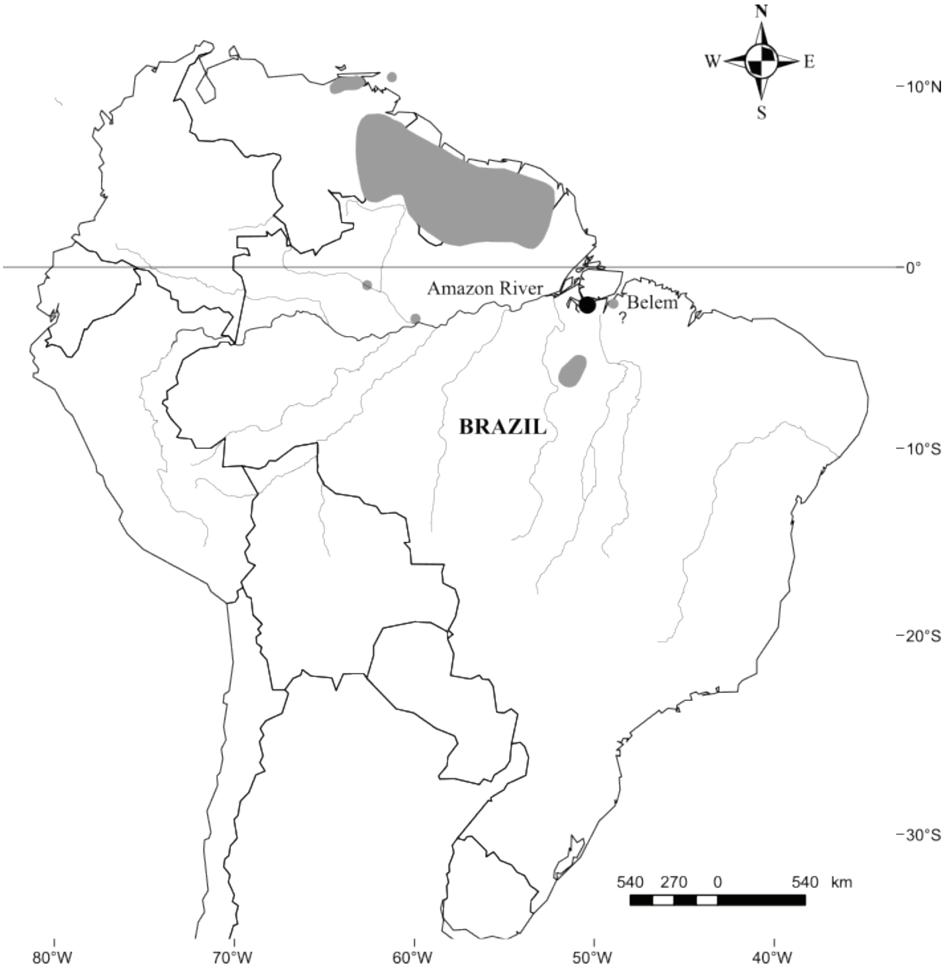

Figure 1. Currently known distribution of *Procnias albus* (gray), with the new location where the species has been documented shown in black. Question mark signs a location (Belém) where there is an historical report. Source: www.birdlife.org.

Plumage and soft color parts of MPEG 80706 were the same as in the nominate and *P. a. wallacei* subspecies (Oren & Novaes 1985), which shared a pure white plumage and tiny white plumes on the black wattle. The maxilla was black with a greyish edge and the mandible was grayish with a black tip, and the feet were gray. Females and one young male observed at the second lek were streaked yellow and olive below as in other *P. albus* populations (Kirwan & Green 2011).

To explore possible genetic differences the DNA of MPEG 80706, the specimen collected during our avifaunal survey at Bagre, was extracted using a phenol-chloroform protocol (Sambrook & Russel 2001) and PCR-amplified for the mitochondrial gene NADH Dehydrogenase Subunit 2 (ND2); the PCR product was purified using a solution of 20% polyethylene glycol 8000 (PEG) and Sanger sequencing was performed on an ABI PRISM 3130 (Applied Biosystems®). Sequences of ND2 for all *Procnias* species and outgroups were downloaded from Genbank (Berv & Prum 2014) and used in the analysis. The downloaded sequences of *P. albus* were from samples AMNH 12002, KUNHM 1244 (both from subspecies *P. a. albus*) and MPEG 37214 (*P. a. wallacei*).

We conducted a phylogenetic analysis using Bayesian Inference (BI) with BEAST (Drummond & Rambaut 2007) and Maximum Likelihood (ML) using RAxML-7.0.3 (Stamatakis 2006). The best fitting model selected by jModelTest 2.1.3 (Darriba *et al.* 2012) was HKY (ti/tv = 5.1282). We also constructed a median-joining network (Bandelt *et al.* 1999) using NETWORK 4.5.1.0 (www.fluxus-engineering.com).

The first two axes of the PCA explained 81.23% of the morphometric variability among the examined specimens (eigenvalues F1 = 3.187 and F2 = 1.69) and were plotted against each other (Fig. 2). Axis 1 failed to discriminate between specimens of *P. a. albus* and *P. a. wallacei*, whereas axis 2 completely separated specimens of *P. a. albus* (with negative values) and *P. a. wallacei* (with positive values).

The Bagre specimen (MPEG 80706) grouped on axis 2 with the only *P. a. albus* specimen included in the analysis (Fig. 2), which is explained by the fact that these specimens shared similar tarsus and bill length values, the characters with the highest autocorrelation with axis 2 (*i.e.* 0.905 and 0.900, respectively).

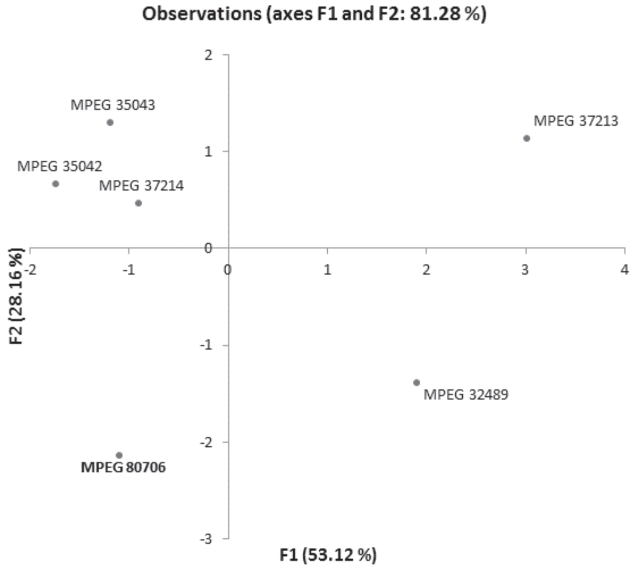

Figure 2. Result of Principal Component Analysis (PCA). The first two axes of PCA are plotted against each other.

The ND2 sequence for MPEG 80706 is deposited in GenBank (Accession No. KY563658.1). Both ML and BI phylogenies, and the haplotype network recovered the same pattern of overall lack of genetic differentiation between subspecies of *P. albus* (Fig. 3). All specimens examined share the same haplotype (Fig. 3B) and although the clade uniting all *P. albus* specimens is strongly supported (Fig. 3A) the lack of haplotype variation within the species does not allow an assessment of relationships among these different populations/subspecies of *P. albus* and likely suggests that these subspecies are not real evolutionary entities.

This paper reports the second confirmed population of *P. albus* south of the Amazon River, the other being the Carajás population known since the 1980's (Roth *et al.* 1984). The presence of a young male and several singing males in at least four leks points to a breeding population rather than wandering individuals. However, according to some local people interviewed, the bellbirds sing only during the onset of the dry season (*i.e.* between May–July) and then either disappear or remain silent and inconspicuous. Thus, whether birds remain in the region outside the dry season remains to be established. Also, the Bagre record lies about 260 km west of Belém, therefore suggesting that the old Wallace (1848) record from near Belém is likely valid.

The PCA analysis indicated that bill and tarsus length are able to distinguish male specimens of *P. albus* subspecies (Fig. 3), corroborating Oren & Novaes (1985). The PCR analysis grouped the Bagre specimen (MPEG 80706) closer to the northern subspecies, *c.* 500 km far and separated by the Amazonas River, than to *P. a. wallacei* from Carajás, which are found *c.* 440 km to the south and not separated by any apparent geographic barrier. Therefore, our morphometric analyses suggest that the newly discovered population south of the Amazon belongs to the nominate form. However, these results should be interpreted with caution because we analyzed only one specimen of the nominate subspecies and therefore purported morphometric diagnoses between both *P. albus* subspecies may not hold when a larger series of specimens are analyzed. The genetic data presented herein suggest that the latter hypothesis is more likely to be correct. Despite the Carajás individual (MPEG 37214) have separated from the others in the ND2 tree, only one haplotype was recovered for *P. albus*. A possible explanation for this result is that the sequence for MPEG 37214, extracted from skin (Berv & Prum 2014), was much shorter than the others.

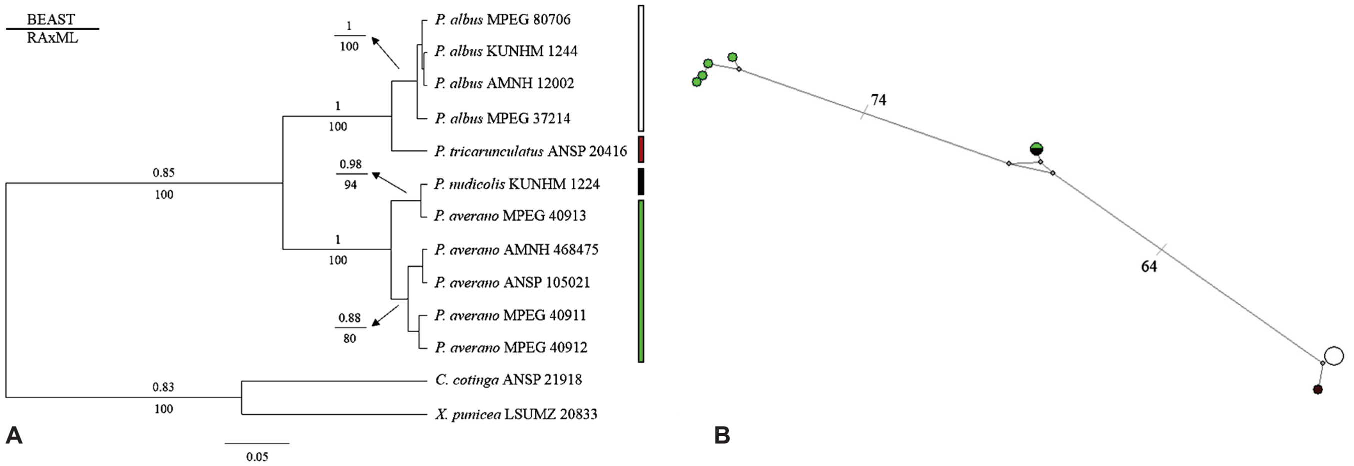

Figure 3. (**A**) Gene tree generated by BEAST based on 1,001 bp of ND2 sequences of all *Procnias* species, including both *P. albus* subspecies (AMNH 12002 and KUNHM 1244 - *P. a. albus*/MPEG 37214 - *P. a. wallacei*) and outgroups. Numbers above and below branches are Bayesian posterior probabilities and RaxML bootstrap support values, respectively. (**B**) Median joining network of all haplotypes. The size of the circles is proportional to haplotype frequency and the colors correspond to the species colors on the tree in (**A**). Numbers under or below bars mean number of mutations between haplotypes.

The lack of genetic differentiation between the Bagre specimen and other *P. albus* populations both south and north of the Amazon agree with Berv & Prum's (2014) suggestion that these subspecies "… are unlikely to be distinct evolutionary lineages that should be recognized as species". The nominate subspecies is a short distance migrant, and vagrants have been recorded in Brazil and Trinidad (Novaes 1980, Snow & Sharpe 2016). Therefore, it seems likely there are occasional dispersal events between both populations known south of the Amazon, which would be consistent with the lack of genetic divergence between the samples currently available.

The subspecies *P. a. wallacei* has recently been included in the Brazilian list of threatened species in the "Vulnerable" category (MMA 2014), in part because it has been documented only for Carajás in the Xingu area of endemism (*sensu* Silva *et al.* 2005), which is the second-most severely deforested Amazonian forest area of endemism (the Belém region is first, Silva *et al.* 2005, Bird *et al.* 2012). Therefore, despite the lack of consistent morphological and genetic differentiation between currently recognized subspecies of *P. albus*, it is premature to synonymize these taxa without analyzing a larger sample of specimens with a broader screen of molecular markers. This cautious approach is justified from a conservation standpoint. The Bagre region's forests and *campinas* have been exploited heavily for timber and sand extraction among other impacts, and there is much need for the creation of conservation units there, which would protect the second well documented populations of *P. albus* south of the Amazon as well as other remarkable codistributed species in a region that is continuing to yield significant ornithological discoveries (Lees *et al.* 2014).

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