ORNITOLOGÍA NEOTROPICAL
(2023) 34: 138–141
DOI: 10.58843/ornneo.v34i2.1237
SHORT NOTE

Sociedad de Ornitología Neotropical

MOLECULAR SEXING AND ITS RELATIONSHIP WITH THE SEMI-CONCEALED YELLOW CORONAL PATCH OF THE WHITE-THROATED SPADEBILL *PLATYRINCHUS MYSTACEUS CANCROMUS*

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Abstract · In sexually monomorphic species, sex identification by molecular techniques is an important alternative. In this study, we employed molecular sexing to determine the sex of seven adult females, two immature females, one female with missing age data, and 21 adult males of White-throated Spadebills *Platyrinchus mystaceus cancromus*. The sex and age of each individual were associated with the presence or absence of a semi-concealed yellow coronal patch, typical of the species. All males displayed the semi-concealed patch, and four out of seven adult females also exhibited it. One of the two juvenile females exhibited a patch. The female with missing age data also exhibited the patch. This finding disputes previous reports that juveniles of this species do not have coronal patches. We conclude that semi-concealed yellow coronal patches are not sexually dimorphic in White-throated Spadebills. Both sexes can have a patch, but adults who do not have them are females.

Resumen · Determinación molecular del sexo y su relación con la mancha amarilla semi-oculta en la corona del picoplano bigotudo *Platyrinchus mystaceus cancromus*

En especies sexualmente monomórficas, la determinación del sexo mediante técnicas moleculares es una alternativa importante. En este estudio, empleamos técnicas de determinación del sexo a nivel molecular para determinar el sexo de siete hembras adultas, dos hembras inmaduras, una hembra con datos de edad faltantes y 21 machos adultos del picoplano bigotudo *Platyrinchus mystaceus cancromus*. Posteriormente, el sexo y la edad de cada individuo se asociaron con la presencia o ausencia de una mancha coronal amarilla semiescondida típica de la especie. Todos los machos exhibieron la mancha coronal semiescondida, y cuatro de las siete hembras adultas también la mostraron. De las dos hembras juveniles, una presentó la mancha. La hembra con datos de edad faltantes también presentó la mancha. Este hallazgo contradice reportes anteriores de que los juveniles no tienen manchas coronales. Concluimos que las manchas coronales amarillas semiescondidas no son sexualmente dimórficas en el picoplano bigotudo. Ambos sexos pueden tener la mancha, pero las hembras normalmente no la presentan en la edad adulta.

Key words: *Avian sexing · CHD gene · Passerine · Sex chromosomes · Sex-linked markers · Tyrannidae*

Sex determination in birds is important for the study of population structure and dynamics, habitat selection, migration, mating systems, as well as sex-specific movement and behavior (Kelly & Wood 1996, Gonzalez et al. 2000, Evans & Day 2001, Noske 2003, Donohue & Dufty Jr. 2006, Shibuya et al. 2018). In addition, sex determination is necessary for conservation and breeding programs (Ong & Vellayan 2007) and wildlife DNA forensics (An et al. 2007). Frequently, in sexually monomorphic species and in immatures of sexually dichromatic species sex is difficult or impossible to determine by visual inspection, and alternative procedures are necessary (Koch et al. 2019, Rodrigues et al. 2019).

Sex can be determined in several ways, one of them is through laparoscopic. This method requires anesthesia and a small incision for the laparoscope to be inserted into the abdominal cavity, which implies the risk of accidental injury (Cerit & Avanus 2007). Fecal steroids also allow sexing, determining the ratio of estrogen to testosterone in feces. In adult birds, fresh feces are required from each individual, ideally during the breeding season (Swengel 1996, Cerit & Avanus 2007). Nevertheless, the use of a polymerase chain reaction (PCR) protocol has become the preferred option for sexing birds because it is a noninvasive technique, does not require anesthesia, and is relatively quick, accurate, and easy to perform since DNA can be extracted from blood, feces, feathers, or other tissues. This technique often uses chromo-helicase-DNA binding protein (CHD) gene markers (Griffiths et al. 1998). The CHD gene is used for sex determination in non-ratite birds because intron size is variable on the Z or W chromosomes and can be detected by one band for males (ZZ) and two bands for females (ZW) in gel electrophoresis (Griffiths et al. 1998).

Submitted 29 Aug 2023 · First decision 02 Sep 2023 · Acceptance 05 Oct 2023 · Online publication 21 Nov 2023
Communicated by María Andreína Pacheco

Figure 1. Sampled Spadebills *Platyrinchus mystaceus cancromus* during field research.

In this study, the sex of the White-throated Spadebill *Platyrinchus mystaceus cancromus* Temminck, 1820 was determined, and its association with age and the presence of a semi-concealed yellow coronal patch was evaluated.

Birds were captured by mist-netting under the approval of Brazilian biodiversity monitoring agencies (Chico Mendes Institute for Biodiversity Conservation/ICMBio, permission 60066-2, and the Brazilian National Center for Bird Conservation/CEMAVE, permission 4385). This study was approved by the Ethics Committee on Animal Use (CEUA, Portuguese acronym), Federal University of Ouro Preto (UFOP, Portuguese acronym), Brazil (Protocol No. 2017/45).

The study was carried out at Brigida Farm (20°21'39"S; 43°30'39"W - 250 ha), an experimental farm belonging to UFOP. The farm is in a transitional zone between the Cerrado and Atlantic Forest domains. The vegetation is a semi-deciduous seasonal forest, riparian woodland, and rupestrian fields. Access to the farm is restricted with no logging or burning within its limits. The climate of the region is humid subtropical (Cwb according to the Köppen climate classification), with dry winters and mild summers, an average annual temperature of 20°C, and an average annual rainfall of 1,250 mm (Alvares et al. 2013).

The White-throated Spadebill is a small Neotropical flycatcher (Tyrannidae) recognized by its broad, flat bill, mustache, and its solitary behavior. The species is insectivorous and is found in a variety of habitats, including forest understory, riparian forests, and forest-edge bamboo thickets (del Hoyo et al. 2020). The breeding season of the species occurs during the rainy season, from October to December. It has been proposed (del Hoyo et al. 2020) that adult males have a semi-concealed yellow coronal patch (Figure 1), whose feathers can be erected in a notable fan, whereas in adult females, the coronal patch is small or absent. There are no reports of semi-concealed yellow coronal patch in juveniles so far (del Hoyo et al. 2020).

White-throated Spadebills were sampled between June 2018 and September 2019. Birds were captured using Ecotone mist-nets (18 x 3 m, five shelves and 19 mm mesh), opened at 06:00 a.m., checked every 30 min, and closed at 12:00 p.m. All captured birds were tagged with individual aluminum bands provided by CEMAVE/ICMBio (Permission 4385). The presence of a semi-concealed yellow coronal patch and the age of the birds were recorded. We assigned them as adults or juveniles, based on the yellowish labial commissure and the absence of a whitish spot at the base of the mandible, which is only seen in juveniles (Sick 1997, Macario et al. 2017). Blood samples were collected by puncturing the brachial vein with a sterile needle (13 x 4.5 mm). Blood was stored on filter paper (Unifil®) at -4°C until DNA extraction.

Genomic material was extracted from the blood samples using the phenol-chloroform method, followed by isopropanol precipitation (Sambrook & Russell 2001). The DNA pellet was resuspended in 50 µL of TE 1X (10 mM Tris-HCl, pH 7.4; 1 mM EDTA, pH 8.0). Sex determination was conducted through Polymerase Chain Reaction (PCR) by amplifying the CHD gene marker using P2 (5'TCTGCATCGCTAAATCCTTT3') and P8 (5'CTCCCAAGGATGAGRAAYTG3') primers (Griffiths et al. 1998). As a positive control, samples of both sexes of Swallow-tailed Manakin *Chiroxiphia caudata*, a dimorphic species, were used in each PCR.

CHD amplification was performed for sex determination in 15 μL volume containing 1.5 µM of each primer, 250 µM of each dNTP, 1 unit of Taq DNA polymerase (Phoneutria®, MG, Brazil), 1×reaction buffer (10 mM Tris-HCl pH 8.4, 50 mM KCl, 0.1% Triton X-100), 4 mM of $MgCl_2$, and 10.0 ng of template DNA. PCR amplifications were perfomed using a PE9700 thermal controller (Applied Biosystems, CA, USA) under the following conditions: 94°C for 1 min 30 s (one cycle), 95°C for 30 s, 52°C for 30 s, 72°C for 30 s (35 cycles), and 72°C for 5 min (one cycle). PCR products were detected on a 6% acrylamide gel (Sanguinetti et al. 1994) and molecular sexing results were as-

Figure 2. CHD gene amplification of Spadebills *Platyrinchus mystaceus cancromus*. Columns with single bands (ZZ) correspond to males, and columns with double bans (ZW) to females (ZW). Individuals with asterisks (*) were recaptured birds analyzed more than once.

sociated with the presence of a semi-concealed yellow coronal patch registered in the field.

We captured 32 White-throated Spadebills and sexed them by PCR. PCR products were single-banded for males (ZZ) and double-banded for females (ZW). Bands were inconclusive for one bird; therefore, its sex was not determined (Figure 2, column 16). Of the successfully sexed birds, 10 were female and 21 were male (Figure 2). The sex of the 12 recaptured birds was confirmed on their second capture (individuals indicated by an asterisk in Figure 2).

After molecular sex determination, we examined the possible association between sex and age, and the presence or absence of a semi-concealed yellow coronal patch. All 21 males were adults and exhibited a semi-concealed yellow coronal patch. Seven adult and one juvenile female exhibited patches. One female of uncertain age also exhibited a patch, and one juvenile female did not have a patch.

We conclude that the semi-concealed coronal of White-throated Spadebills is not an unequivocal sexually dimorphic trait. This finding supports previous observations that this trait is present in all males but also in some females (del Hoyo et al. 2020). However, our study also provides novel information by recording the presence of a coronal patch in juvenile females. Regrettably, the Brazilian field guides (Gwynne et al. 2010, Sigrist 2013, Ridgely et al. 2015) do not provide precise information on the presence or absence of a semi-concealed yellow coronal patch in this species. Semi-concealed coronal patches are frequent among species of Tyrannidae (del Hoyo et al. 2020), and our results support that distribution across sexes and immatures should not be overlooked in flycatchers.

ACKNOWLEDGMENTS

The authors thank the Federal University of Ouro Preto (UFOP) for logistical support and the Brazilian National Center for Bird Conservation (CEMAVE/ICMBio) for supplying aluminum leg bands. JAB Cabral and VL Lopes thank the UFOP and the Coordination for the Improvement of Higher Education Personnel (CAPES) for providing scholarships, respectively.

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