PROGRESS REPORT

23522-2 Genetic diversity of an endangered and endemic bird of Yucatan Peninsula (*Campylorhynchus yucatanicus*) for conservationist aims II

Anay Serrano-Rodríguez

Study area and sample collect

Fieldwork covered 14 sites that encompass historical distribution of species on the northern coast of Yucatan Peninsula in Mexico (Fig. 1). In each sites we walk in transects for three days from 07:00-11:00 and from 16:00-19:00 hours. We realized intensive searches of individuals, making stops 6 minutes each 100m, with help of speaker playback. For this study, we capture 186 individuals, corresponding to 12 individuals per sampling site. Individuals were captured using mist nets, marked with unique combinations of colored rings and georeferenced (Fig. 3A and B). From the brachial vein were extracted 2-3µl of blood. We made sure that when releasing individuals they not where have any bleeding. Blood was collect in tubes with K_3EDTA at 15% and the samples were store at -20 ° C until processing in the laboratory. Access to protected areas and collections was made with the SGPA / DGVS / 06821/14 and SGPA / DGVS / 007765/15 authorization.

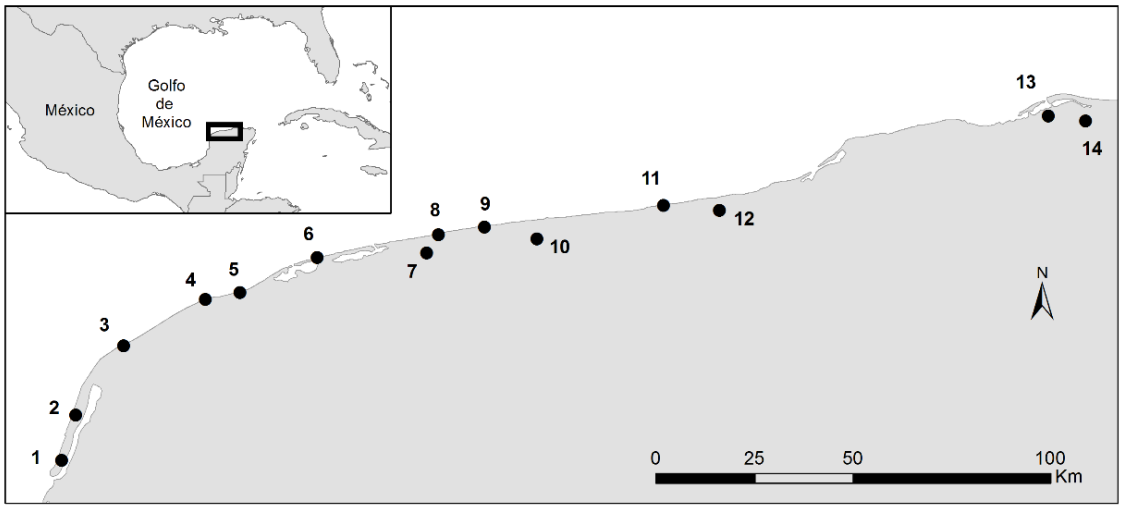

Figure 1. Sampled sites location to analyze genetic diversity of *C. yucatanicus* populations: (1) southwest Celestún, (2) northeast Celestún, (3) El Palmar, (4) west Sisal, (5) east Sisal, (6) Chuburná, (7) Capilla, (8) west Chixchulub, (9) San Benito, (10) Xcambo, (11) Santa Clara, (12) Dzilam, (13) west Ría Lagartos y (14) east Ría Lagartos.

Laboratory process

DNA extraction was performed using a modification of cell lysis method and phenol-chloroform isoamyl alcohol (Bello *et al*. 2001). Success of extraction were examine in 1.5% agarose gel. Optimal temperatures for PCRs had to be evaluate in new laboratory conditions. To identify correct amplification temperature of microsatellites using ten selected markers, it were used two DNA samples and subjected to six different temperatures in a range of 45°C to 60°C. PCRs were performed for 10 individuals using 10 microsatellites described for *C. brunneicapillus* (Barr *et al*. 2015). Optimal alignment temperature during PCR of 3 markers is

still being evaluated (4-03, 4-06 and 4-10). We used polymerase chain reaction (PCR) for amplification of specific DNA fragments. For amplification, was use a mixture of 1uL of DNA, 3uL of master mix Taq DNA Polymerase InvitrogenTM, 2.7uL of water and 0.3uL of primer, for a final volume of 6uL. PCRs reactions were preheat of 94 ° C for 3 min and 39 cycles were performed by following steps: denaturation at 94 ° C for 1 min, alignment at a specific temperature for each primer (Table 1) for 1 min and extension to 72°C for 1 min. After chains extension, was maintain temperature of 72°C for 10 min and it was allowed cooling to 10 ° C. Products of PCRs were visualized in 2% agarose gels, through an electrophoretic 100V run and 30 min of duration (Fig. 2). Reads were made by capillary electrophoresis in an Applied Biosystems automatic sequencer, using Peak Scanner program version 1.0.

Table 1. Microsatellite loci information, total number of alleles (AT), and alignment temperature (TA) to analyze the genetic diversity of *C. yucatanicus*.

ID	Locus[1]	Repetition units	Primer sequence (5´-3´)	AT	Length (pb)	TA (°C)
Locus1	CACW3-01	$(ATT)^5G(TTA)^4(TTG)^6TTATTG(TTGTTA)^3(TCA)^9$	F: ACTGTTCACCCTTGGACCTG R: TGTCTGGAAACCACTGAAGAAC	6	168-188	54
Locus2	CACW3-03	$(CTA)^5CTG(CTA)^8(ATA)^{10}$	F: TCCTGAAATGTAATTCAGACACC R: CAGAGTGCTACTTAAATTGATTCTTTC	5	259-279	57.6
Locus3	CACW3-05	$(TGT)^5$	F: GATGCATATTGTCAGAGTTCCAC R: CTGGACTGAGCTAACAAATGATG	5	131-149	57.6
Locus4	CACW3-11	$(ATA)^5..(AAC)^6AAT(AAC)^4(AAT)^3AG(TAA)^4$	F: TTCTCCTCCCTCTACCTCCTTT R: GTGACAACAGAAAATTCCCTTTA	8	180-204	54
Locus5	CACW4-01	$(GTAT)^6GAATCTG(TCTA)^{11}$	F: TTTTGCCTAATAAACTGGCTGAC R: CACAGAACCACAACCTACATGG	3	122-133	54
Locus7	CACW4-04	$(TCTA)^{14}$	F: TCTCACGTCTTACCATCCTGTG R: TTGATACTTGAAACTCTCCTTCTGTC	5	241-257	57.6
Locus9	CACW4-09	$(GATG)^{22}$	F: GCTAACTGAAAGGGATTGTTGG R: TTTCTGGCATGTTTCCTGTC	5	92-116	59

[1] Barr *et al*. 2015

Landscape composition and genetic diversity: node level

We established 14 parcels that resulted from a buffer area of 2 km around capture points in each site. In order to characterize composition and structure of landscape were use three images of Sentinel 2 satellite (20m spatial resolution, April 2016). We digitized manually fragments and they were classified in four habitat with different types of vegetation and land use: (1) adequate habitat preserved, (2) disturbed habitat, (3) unsuitable habitat, (4) secondary vegetation habitat, with some human intervention. First category is composed of conserved fragments with spiny coastal scrub of dune vegetation complex. Disturbed habitat includes human settlements, roads and areas with bare soil because deforestation. In third category were included habitats where species were not registered according to literature and our field observations. Finally, we considered in fourth category types of secondary vegetation that maintain elements of original vegetation of spiny coastal scrub such as agaves and cacti, and are subject to different human uses such as livestock.

Structure and composition of landscape was recorder in parcels, as well as the degree of fragmentation of it in Patch Analyst.

Genetic distances and landscape resistance

Resistance offered by landscape to movement of individuals was evaluated using circuit theory in Circuitscape. Term resistance was used as an antonym of landscape connectivity, which we define as degree to which landscape facilitates or impedes movement of individuals that can move randomly between two sites or nodes.

In this context, nodes can be habitat fragments, populations or points in landscape, among which we are interested in evaluating connectivity. To establish focal nodes in this study was used centroid of set of capture points of each collection site. A map cut of Yucatan Peninsula that it contained potential distribution obtained was area analyzed, which it allowed acquiring more accurate resistance models. Resistance layer used to estimate cost or resistance between nodes was one used in previous studies for the same species.

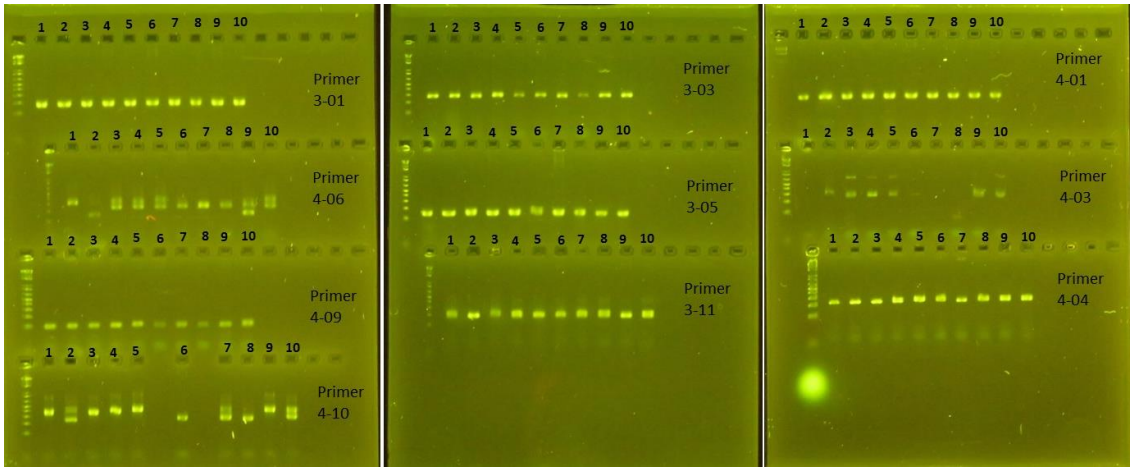

Figure 2. Agarose gels (1.5%) showing PCR product for 10 individuals of 9 different locations using 10 microsatellite markers described for *C. brunneicapillus*.

Figure 3. Fieldwork (A and B), re-avistations of ringed individual of C. yucatanicus by birdwatchers (C) and partial presentation of results in doctoral thesis (D).

Environmental education and training

We work with local bird watchers and involve protected area technicians in the capture and monitoring of individuals. We distribute informative material about the species and its habitat, which address recommendations on how to monitor and maintain relational connectivity between remnant patches of habitat. Birdwatchers report location and identity of individuals of *C. yucatanicus* that they observe in their field trips (Fig. 3C).

We participated in bird festival and in Environmental Education Program on protection of *C. yucatanicus* and its habitat. For that, we elaborate divulgation materials in coordination with national and international artists (Fig. 4). We carry out talks in Sisal, Ría Lagartos, Celestún and Progreso about importance of conserving connectivity of landscape for this species and other important natural resources in Yucatan Peninsula.

Divulgation of our results

We prepare reports for protected areas that will help them to monitor marked individuals. We recommend some management actions for species conservation and its habitats that can be incorporate into plans of protected areas. We present results of this project as part of my doctoral thesis at El Colegio de la Frontera Sur (Fig. 3D), where I successfully qualified as an Honorable Mention.

Figure 4. Illustrations by artist Paul Sosa Moya, which were used to promote *C. yucatanicus* conservation and its habitats.