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Morphological Variation in the Martinican Subspecies White-Breasted Thrasher (*Ramphocinclus brachyurus brachyurus*)

Steven Philippe Son^{1,2}, Thomas Alexandrine³, Sébastien Motreuil⁴, Daniel Imbert², Jean-Raphaël Gros-Désormeaux^{1*}

¹Laboratoire Caribéen de Sciences Sociales, Faculté de Droit et d'Economie, Martinique, France

Email: *jrmgrosdesormeaux@yahoo.fr

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Abstract

The White-breasted Thrasher (Ramphocinclus brachyurus) is the smallest passerine bird in the Mimids family. Ranked as "endangered" in the IUCN list, the demographic distribution of this species is spread unevenly across two islands in the Lesser Antilles. The Saint-Lucian subspecies, Ramphocinclus brachyurus sanctaeluciae, was estimated to number between 1200 and 1700 individuals, whereas the Martinique subspecies, Ramphocinclus brachyurus brachyurus, was estimated at between 200 and 400 individuals. As an endemic rare species with a high risk of extinction, the Martinique subspecies should be regarded as a conservation priority. The small size of the Martinique subspecies population, its restricted geographical range, and its narrow habitat tolerance make it quite difficult to study. The variability of morphological traits in this subspecies has not been biostatistically analyzed recently. In order to update knowledge and fill in any gaps, we have used the morphometric approach to study the morphological variability of the Martinique subspecies. The study site is a small peninsula located in the northeast of the island of Martinique (French West Indies) known as "la Caravelle". We captured 63 White-breasted Thrashers which were marked, measured, and sexed before being released. We did not observe any significant differences between the sexes, signifying an absence of sexual dimorphism. However, we were able to observe a significant variation in morphological traits between individuals measured inside and outside the national protected area of Caravelle. The body mass-tarsus ratio was equal at both sampled sites, something which could potentially be a clue to the bird's adaptation to dif-

²Université des Antilles, Site de Guadeloupe Campus de Fouillole, Guadeloupe, France

³Parc naturel de Martinique, Martinique, France

⁴Centre national de la Recherche Scientifique, Université de Bourgogne, Dijon, France

ferent habitats.

Keywords

Caribbean, Endemic Rare Species, Morphological Measurements, Sample Size Effect, Sexual Dimorphism, Subspecies

1. Introduction

The White-breasted Thrasher (*Ramphocinclus brachyurus*) is the smallest passerine bird in the Mimids family [1]. One of 25 bird families across the world with the highest percentage of endangered species, the Mimids family includes 20 globally endangered species and 9 species listed as "critically endangered" [2]. Birds belonging to this family have a wide geographical distribution, including in the "Neo-arctic" and "Neo-tropical" zones. Described as the smallest Mimid, the White-breasted Thrasher has physical characteristics inherent in *Mimidae*. It has a long tail, strong claws, strong legs and a curved beak that allows it to search and scrape the ground for potential prey [3].

According to the current classification, the *Ramphocinclus* genus only has one species made up of two subspecies, one endemic to Martinique (*Ramphocinclus brachyurus brachyurus*) and the other in Saint-Lucia (*Ramphocinclus brachyurus sanctaeluciae*). In addition, recently published research into the degree of genetic differentiation suggests that the two homologs should no longer be considered as two subspecies but rather as two distinct species [4]. Such a reconsideration would lead to the species being moved from the "endangered" list to the higher rank of "critically endangered". It appears supremely important to conserve the White-breasted Thrasher due to its rarity and its limited endemic character on the island of Martinique.

In 2016, the global population of White-breasted Thrashers was estimated to be less than 2000 individuals, 15% of which are located in Martinique [5]. Indeed, the population of *Ramphocinclus*. *b. sanctaeluciae* was estimated to be within the range of 1200 and 1700 individuals [5] whereas *Ramphocinclus b. brachyurus* [6] was estimated to be between 200 and 400 individuals [7].

This uneven distribution is also reported in the scientific literature. Indeed, while the Martinique subspecies appears to be the most vulnerable because of its small population size and its restricted distribution of 5 km² on the Caravelle Peninsula, most published scientific research has been carried out into its counterpart in Saint-Lucia [8]. Published scientific articles on the Martinique subspecies consist of old articles describing the species [9], an article on the genetics of the species including samples from both subspecies [10], two articles on methods for detecting individuals and habitat characterization [11] [12], and an article describing the behavior of the *R. b. brachyurus* [13] Most of the information corresponds to institutional reports contained in expert reports [14]. None of the research provides a precise morphological description

of *R. b. brachyurus*. The articles which do mention it reproduce Vieillot's results from 1818.

The most recent published study into the biometric measurements of the Martinique subspecies is Storer's study from 1989. Storer quantified the level of dimorphism for Tremblers (*Cinclocerthia*) and White-breasted Thrashers (*Ramphocinclus*). His study published the measurements of different morphological traits such as tail length, wing length, tarsal length and beak size for *R. b. brachyurus*. All literature on this species presents morphological traits according to sex [9], something which may indirectly imply sexual dimorphism. We proposed analyzing the morphological traits of the birds, taking sex into account [15] in order to clarify the situation by verifying any monomorphic status or detecting a subtle dimorphic sexual character [16]. We used data collected during ringing sessions carried out in Martinique between 2016 and 2018 to achieve this. With regard to the morphometric measurements of the White-breasted Thrasher, significant differences were discovered between the two subspecies in terms of body size and plumage color [5].

There are many gaps in our knowledge about this species in terms of its physiology, reproductive system and living conditions. This study is a first step in improving the existing body of knowledge for an endemic species whose disappearance would contribute to eroding the world's biological diversity.

2. Methods

2.1. Study Area

In Martinique (**Figure 1**), the White-breasted Thrasher is often observed at the Caravelle Peninsula (14°45′N, 60°54′W).



Figure 1. Satellite image of the Caravelle peninsula in martinique. The yellow area shows the relative position of the peninsula to Martinique.

Observations of White-breasted Thrashers appear to be more frequent in sloping wooded areas in alluvial basins. This is why White-breasted Thrashers are considered highly specialized in this type of habitat [13]. Due to the steep topography of the area, we selected two accessible sampling sites: A protected area, the Caravelle Nature Reserve, and a location known as "Morne Pavillon" (Figure 2).

2.2. Sampling

Study of the individual characteristics of the White-breasted Thrasher required the capture-mark-recapture method (CMR.). This is a sampling technique commonly used in population ecology [17] [18] [19].

It is possible to estimate the size of a species by tagging individuals. Individuals are marked during an initial capture session. The marked individuals are then quantified during subsequent sessions. On the basis of the use of clues created by Lincoln and Petersen (1930), the first CMR models were criticized because it was impossible to integrate crucial elements that could have an impact on demography such as the movement of individuals, reproductive activities, the number of descendants of successive generations, and physiological and behavioral specificities [20] [21]. However, methodological advances have made it possible to construct models that are close to modern statistical models and that can integrate many parameters specific to the focal species, allowing for more precise estimates [22] [23] [24].

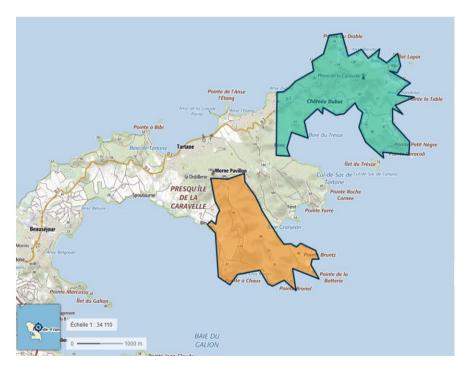


Figure 2. Sampling sites of the white-breasted thrasher in martinique between 2016 and 2018. The nature reserve is in green. "Morne pavillon" is in orange. This map was created by using IGN data.

All the capture sessions took place at the Caravelle peninsula. Due to its steep topography, we selected several study sites where it would be possible to catch White-breasted Thrashers. These sites were selected on the basis of scientific knowledge describing the distribution of White-breasted Thrashers from the observations of various naturalists [25].

Three sampling sites had been tested beforehand: the Caravelle National Nature Reserve (RNNC), the Pointe Rouge domain and "Morne Pavillon". Two locations were sampled at each site. For the RNNC, the two locations were "Château Dubuc" and "Balata".

For the Pointe Rouge domain, the two locations consisted of the "Closed Trail" and the "interior forest". For the final site, the locations were "Morne Pavillon" and "Anse Spoutourne". Upon analysis of the results obtained during the test phase, attempts to capture individuals at the Pointe Rouge domain were abandoned. Our sampling was therefore carried out at the locations within the RNNC and the forest covering "Morne Pavillon" and "Anse Spoutourne" (Figure 2). The decision to choose sites within and beyond the reserve presented an opportunity to compare a location subject to strict regulatory protection with one subject to less regulation.

Capture sessions were held at each study site from sunrise at 0600 to sunset at 1800. This timeframe was selected on the basis of the results of a Data Mining approach carried out as part of a Master 2 internship in Applied Mathematics [26]. Sampling was carried out using Mist nets measuring approximately 516 m². As regulations prohibit the cutting and removal of vegetation, the locations were chosen on the basis of those areas where the vegetation allowed for optimal deployment of the nets. The eight nets were moved daily, avoiding areas already selected to prevent any habituation phenomenon. The birds were captured and handled in under 5 min. In 2016, each individual was marked with a combination of plastic marks colored according to a particular color code (Figure 3).



Figure 3. Marked white-breasted thrasher. On the left, an example of plastic marks used. On the right, an example of colored metallic marks.

During successive campaigns, the nets were placed in the previous locations with the aim of obtaining recaptures. Recaptures revealed that individuals captured with plastic marks before 2017 had removed them. Two marks had been placed on the right paw and one on the left paw. Of the nine individuals recaptured in 2016, six of them had lost one or two marks. Having observed the loss of these marks, marking was carried out with colored metallic marks from 2017 (Figure 3).

Due to their biology, vertebrates are difficult to count and birds all the more so because they are very mobile, small in size and live in closed habitats [27], something which is the case with regards to the White-breasted Thrasher (R. b. brachyurus). To respond to the arduous nature of catching this species, some sampling pressure was sought. Such sampling pressure can be measured differently depending on the capture method used. In order for sampling pressure to allow for inter-location comparisons, the sessions were to be performed by three groups, each of which carried out the standard procedure for installing and storing the nets. This task involved at least two positions allocated to individuals who had followed the Center for Research on the Biology of Bird Populations (CRBPO) bird ringing training and to individuals who were undergoing training in the method. Recovery of the birds caught with the net was attributed to a group composed of an individual who had undergone bird ringing training and a guard. Marking, morphological measurements and tissue samples were reserved for a group composed of qualified and trained individuals from the University of Burgundy, including a Research Engineer with a ringing license issued by the CRBPO.

For each bird, we took measurements including the length of the beak at the base of the skull, the height of the beak at the nostril, two dimensions for the right tarsus (length and thickness), the length of the wing, the length of the tail and the body mass. All these measurements were made with a digital caliper (precision: ± 0.2 mm), except for the tail and wing which were measured with a scale (precision: ± 1 mm) and the mass which was measured with a Pesola MS500 scale (precision: d = 0.1 g). Apart from body mass, we measured all the characteristics twice with the measurement tools removed between each measurement to reduce measurement errors by the scientist [28]. In addition, the scientist was always the same person for all the individuals measured.

We collected a 10 - $20~\mu L$ blood tissue sample from the brachial vein of the birds and stored it in a buffer solution for the purpose of determining the sex of the birds [29]. As the individuals appear to be monomorphic, it is not possible to determine their sex with the naked eye. A genetic tool is therefore the only way to achieve this. After handling, we released all the birds at the place of capture to reduce disturbance.

3. Molecular Methods

In order to verify the sex of the individuals we performed genotyping in all the captured birds. We performed genetic analyses using the method of Griffiths *et*

al. and determined the sex thanks to the CHD-W gene specific to the female [29].

3.1. Statistical Analysis

All statistical analyses were conducted using R version 4.0.5 software [30].

3.2. Sexual Dimorphism

Once it has obtained its adult plumage, the White-breasted Thrasher appears to be monomorphic visually. In order to clarify the situation by verifying its monomorphic status or detecting a subtle dimorphic sexual character [16], we analyzed the morphological traits of the birds, taking sex into account as a factor in a multivariate analysis of variance (MANOVA). Linear models seeking to explain the relationship between morphological traits (as a dependent variable) and sex (as an explanatory variable) were used to achieve this. The detection of an effect of sex on these traits would be an indication of the existence of subtle sexual dimorphism [15].

3.3. Datasets Analyses

We analyzed morphological traits using an analysis of variance (ANOVA), taking two factors into account: the year and the sampling site between the two study sites. To do this, we computed linear models seeking to explain the relationship between the morphological trait (dependent variable) and the following explanatory variables: a year effect, a site effect and their interaction [31] [32].

For each trait, the best model was selected using a nested models comparison which consists of sequentially removing nonsignificant variables from the complete model by following Akaike's information criterion (AIC).

Subsequently, comparisons of means were performed between the different datasets for four morphological traits: Body mass, Wing length, Tarsus length and Beak length [28]. As sample sizes were small, these analyses were supplemented with the computation of Cohen's d [33].

4. Results

4.1. Sampling

Overall, we captured 63 White-breasted Thrashers, 42 inside the reserve and 21 outside. **Table 1** shows the number of males and females captured at both sites.

There are no differences between the sex ratios of the two samples (Fisher exact, Odd-ratio = 0.74, P = 0.78, 95% CI = [0.21; 2.47]).

Table 1. Results of molecular sexing of 63 white-breasted thrashers.

	Males	Females
Inside the reserve	25	17
Outside the reserve	14	7

The two samples were considered as a population for statistical analyzes. The sex ratio is not significantly different from parity (Binomial test: Pobs = 0.62, P = 0.08, 95% CI = 0.49; 0.74).

4.2. Sexual Dimorphism

There is no sexual dimorphism in the Martinique White-breasted Thrasher for all the traits analyzed and these results are confirmed by the effect size measures **Table 2**.

Multivariate analysis (MANOVA) does not show any significant differences between sexes for all the traits considered (Table 3).

4.3. Datasets Analyses

ANOVA shows significant differences between the two study sites, except for wing length **Table 4**.

ANOVA does not show any significant differences between the body masses of the individuals captured depending on the year ($F_{2,59} = 1.43$, P = 0.25). However, it shows significant differences between those individuals captured within the reserve and those beyond it ($F_{1,60} = 6.13$, P = 0.02). The individuals living inside the reserve are heavier than those living outside (**Figure 4**).

ANOVA shows significant differences between Tarsus lengths depending on the study site ($F_{1,61} = 4.79$, P = 0.03). Indeed, the individuals captured inside the reserve have longer tarsus than individuals captured outside it (**Figure 5**).

ANOVA shows significant differences between the Beak lengths of the individuals captured during the different years (**Table 4**). It should be noted that within the reserve the individuals caught had longer beaks than those outside the reserve (**Figure 6**).

Above all, there was a marked difference in 2016: During that year, the individuals caught had smaller beaks on average than the individuals caught during the other years.

Table 2. Sample sizes, means and standard deviations for the traits of *R. b. brachyurus* measured (2016-2018). The measure of effect size computed between males and females shows no differences.

Traits	Males			Females	Cohen's d	
Traits	n	$x \pm sd$	п	$x \pm sd$	(95% CI)	
Body mass (g)	38	44.86 (3.33)	24	46.08 (3.19)	-0.38 (-0.99; 0.13)	
Wing length (mm)	39	97.37 (3.46)	24	96.23 (3.33)	0.34 (-0.17; 0.90)	
Tail length (mm)	39	92.01 (6.32)	24	93.96 (9.81)	-0.34 (-0.86; 0.24)	
Tarsus length (mm)	39	30.09 (2.02)	24	29.71 (1.07)	0.22 (-0.32; 0.58)	
Tarsus width (mm)	39	2.98 (0.30)	24	2.88 (0.37)	0.29 (-0.26; 0.81)	
Beak length (mm)	39	23.01 (4.60)	24	23.23 (4.38)	-0.05 (-0.56; 0.46)	
Beak height (mm)	39	5.97 (0.31)	24	5.98 (0.39)	-0.05 (-0.63; 0.51)	

Table 3. Results of the multivariate analysis of variance (MANOVA) on the morphological traits of *R. b. brachyurus*.

Morphological trait/Variable	Sum sq	Mean sq	F	df	P
Body mass					
Sex effect	22.01	22.015	2.0524	1	0.1572
Residuals	643.57	10.726		60	
Wing length					
Sex effect	16.54	16.537	1.4149	1	0.2389
Residuals	701.31	11.688		60	
Tail length					
Sex effect	71.7	71.735	1.2228	1	0.2732
Residuals	3519.8	58.664		60	
Tarsus length					
Sex effect	1.918	1.9184	0.5807	1	0.4490
Residuals	198.214	3.3036		60	
Tarsus width					
Sex effect	0.0908	0.090842	0.8908	1	0.3498
Residuals	6.1185	0.101975		60	
Beak length					
Sex effect	0.13	0.1324	0.0065	1	0.9361
Residuals	1224.97	20.4162		60	
Beak height					
Sex effect	0.0042	0.004227	0.0356	1	0.8511
Residuals	7.1325	0.118875		60	

Table 4. Results of the ANOVA on the morphological traits of *R. b. brachyurus*, measured between 2016 and 2018. Significant results are in bold.

Morphological traits/Variable	F	df	P
Mass			
Site effect	6.1252	1	0.01617*
Year effect	1.43	2	0.242
Interaction effect	2.0244	3	0.1204
Tarsus length			
Site effect	4.785	1	0.0326*
Year effect	1.978	2	0.147
Interaction effect	2.5703	3	0.06272
Beak length			
Site effect	187.35	1	<10 ⁻⁵
Year effect	85.065	2	<10 ⁻⁵
Interaction effect	119.16	3	<10 ⁻⁵
Wing length			
Site effect	0.1067	1	0.7450
Year effect	1.8950	2	0.1594
Interaction effect	1.2989	3	0.2833

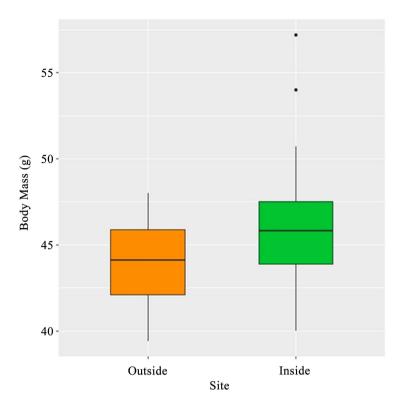


Figure 4. Body masses (in g) measured for white-breasted Thrashers (*R. b. brachyurus*) between the two sampling sites for the period between 2016 and 2018.

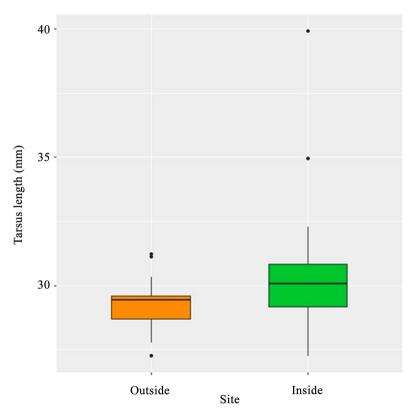


Figure 5. Tarsus length (in mm) measured for white-breasted thrashers (*R. b. brachyurus*) between the two sampling sites for the period between 2016 and 2018.

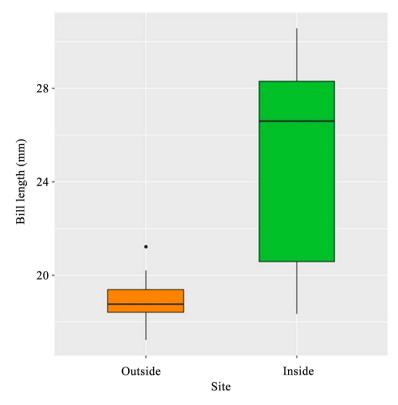


Figure 6. Bill length (in mm) measured for white-breasted thrashers (*R. b. brachyurus*) between the two sampling sites for the period between 2016 and 2018.

ANOVA does not show any significant differences between the Wing lengths of the individuals captured depending on the year ($F_{2,59} = 1.90$, P = 0.16). Nor does ANOVA show any significant differences between the Wing lengths of individuals caught inside the reserve and those caught outside it (**Table 4**).

5. Discussion

Several elements reduced the interpretative margins of this study during analysis of the data. It is possible that measurements made in the same way may also contain errors. With regards to the Beak length measurements, the way the scientist perceives the end of the upper mandible hidden at the base of the fore-head can induce bias. We observed this with the U-shape distribution of this trait. Such a distribution could be explained by the existence of groups hidden in our data but several analyses are required to verify this hypothesis. Therefore, it is possible that the Beak measurements could be biased despite the preventative action taken (measurements, mathematical transformations, etc.). Finally, we consider the other measurements, the other datasets and their comparisons to be reliable because of the low error measurement we found.

Our results confirm that there is no sexual dimorphism with regards to the traits of the Martinique White-breasted Thrasher we studied. However, the data collected by Vieillot (1818) and Storer (1989) specify the sex of the individuals captured [6] [9]. A dimorphic trait could exist in this species but it is possible

that we did not measure it. Further research is required to determine which one. If there is no dimorphism, as is the case in several bird species [34], the question of how the birds recognize each other would be an important one. Additional behavioral studies would be required to answer that question [35]. Indeed, possible discriminating behavior during the breeding period, especially during display, could highlight behavioral differences specific to males and females.

Moreover, individuals inside the reserve were larger and heavier than those outside it. On the basis of the theory of ecogeographic adaptation for birds [36], it can be assumed that birds living in better quality locations must have better living conditions. In birds and in an island context, Clegg and Owens observed that, in conditions with a great abundance of resources, some species displayed larger morphological characters than those with a lower abundance of resources [37]. In an island environment, the differentiation of certain morphological traits seems to be more apparent than in a continental environment [38].

6. Conclusion

In line with Storer's results, this study confirms the absence of sexual dimorphism in the White-breasted Thrasher. However, we found significant differences for Tarsus lengths and for Body mass between study sites. Indeed, the individuals captured inside the reserve have longer tarsus than individuals captured outside it. The individuals living inside the reserve are heavier than those living outside. This was an initial contribution to the assessment of the conservation status of the Martinique White-breasted Thrasher and further research is required to properly advise the decision makers responsible for species conservation. Those responsible for the conservation of this endemic bird must implement methods and/or protocols to facilitate monitoring of the species' demoecology. Considering the "endangered" status of the White-breasted Thrasher and the recent results of genetics analyses advising that the conservation status of the Martinique White-breasted Thrasher be upgraded to "critically endangered", studying and assessing sex-ratio and morphometric variability appears to be essential for the conservation status of the White-breasted Thrasher.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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