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RESEARCH ARTICLE

BODY CONDITION EVALUATION, EXTERNAL BIOMETRICS MEASUREMENTS AND MORPHOLOGY OF DIGESTIVE TUBE OF RED-COWLED CARDINALS

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ABSTRACT

Taxonomic Guerra R.R., Luna A.S.L., Siqueira R.A., Firmino M.O., Cleub C., Guerra M.V.S.F. and Cavalcanti T.A. 2016. [Body condition evaluation, external biometrics measurements and morphology of digestive tube of red-cowled cardinals (*Paroaria dominicana* Linnaeus, 1758) of wild life apprehended in Paraíba, Brazil: releasing projects subsidies.] *Paroaria dominicana* is a bird belonging to the Passeriforme order. Because of its song and color qualities is one of the main targets of wild animal trafficking in Brazil. This study aimed to evaluate the external and organ biometry of the digestive tube, as well as the histological aspects of this system and the body condition of specimens from apprehensions made by the Wild Animal Screening Center, in order to provide subsidies for taxonomic classification, clinical, nutritional and species preservation management, as well as for releasing projects. It were carried out body condition evaluation, external biometrics measurements and tube digestive morphological analysis of 20 *P. dominicana* apprehended in Paraíba, Brazil. The seized specimens presented unfavorable body conditions, which were related with loss of feathers; the visceral topography and histology of the digestive system was consistent with those of other birds, but the external and internal biometry presented differences. The results demonstrated that body condition analysis of seized animals is an important tool that should be considered in evaluations at Screening Centers, in order to achieve a better nutritional and/or clinical management before releasing projects, thus increasing the project success. The results will provide input for taxonomic classification, as well as corroborate further clinical, nutritional, preservationist and release project studies.

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INTRODUCTION

Paroaria dominicana has an average length of 17.5cm, lives in low, sparse and very sunny bushes (Sick 1997), typical of the interior of the Brazilian Northeast (Ridgely and Tudor, 2009). They are granivorous birds that forage in the soil and can feed on small arthropods and mollusks (Ridgely and Tudor 1989, Sick 1997). The *Paroaria* genus, of the Passeriforme order, of the Fringillidae family, recently transferred to the Thraupidae family (Klicka et al. 2007), allocates three species in Brazil (Sick 1997): *Paroaria dominicana* (red-cowled cardinal)

(Linnaeus, 1758), *Paroaria coronate* (cardinal) (Miller, 1776) and *Paroaria gularis gularis* (amazon cardinal) (Brisson, 1760). Because of their songbird and color qualities, this bird is targets of wild animal trafficking, and are one of the most trafficked species in Brazil (Sick 1997). In a survey performed at the Wild Animal Screening Center (CETAS) from Brazilian Institute of Environment and Renewable Resources (IBAMA) in Paraíba, Brazil, Pagano et al. (2009) showed that 79% of the apprehended birds belonged to the Passeriformes order. Among these birds, the red-cowled cardinal is third on the list of most trafficked species between August 2006 and July 2007. Biometrics helped by delimiting a specific pattern for each species or subspecies, providing support for observations in individual evolutionary, physiological, taxonomic (Cooper 2005) and ecological variations of each species (Dunning

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1993). These measures emphasize some physiological uniqueness of the species, e.g., the length of the digestive system is inferred in order to assess the rate of food passage and the period of exposure of the food to the intestinal villi, and this provides references for nutritional managements (Cherry and Siegel 1978). Another relevant evaluation is the histological description of wild species, because it provides a basis for classification of the animals in relation to the various ecological food peculiarities (Hofmann 1989, Heleno *et al.* 2011) and gives support to the feeding strategies that these birds may be submitted (Heleno *et al.* 2011). Furthermore, there is only one study with Brazilian wild bird histology (Siqueira *et al.* 2013). Despite *P. dominicana* conservation status be low concerned, the species trafficking made it to disappear from many areas which was native, been the reintroduction by releasing of animals seized one way to the restoration of their area original scope, provided that it is done responsibly. Thus, this study aimed to evaluate external biometry, digestive tube morphology, as well as the body condition of the red-cowled cardinals apprehended in Paraíba, Brazil in order to provide subsidies for taxonomic classification, clinical, nutritional and species preservation management, as well as for releasing projects.

MATERIALS AND METHODS

This study was developed at the CETAS/IBAMA, Cabedelo, Paraíba, Brazil and Federal University of Paraíba, Brazil. The animals were provided for the project according to ethical procedures established in IBAMA agreement number 02019.00129/2009-12. Among the *Paroaria dominicana* (Linnaeus 1758) specimens that were seized in captivity and were apprehended during 2012 by inspectors of the CETAS/IBAMA, 20 males that have died by causes not known were utilized. The conditions of all birds were classified and analyzed by their body condition as being: Good, Lean, Cachectic and Obese, by evaluating skeleton, muscular mass and quantity of fat tissue in the back and other subcutaneous extensions (Sanches 2008) and by observing loss or not of plumage. The animals were weighed, and then measured with a digital pachymeter. The measurements taken were: total length with feathers, total length without feathers, length of the tail, length of the tarsus, length of the wings with feathers, length of the wings without feathers, length of the wings closed, length of the head, width of the head, height of the beak, width of the beak, length of the beak, length of the middle toe with claw, length of the middle toe without claw, width of the thorax, width of the abdomen, length of the esophagus, length of the proventriculus, length of the gizzard, width of the gizzard, thickness of the gizzard wall, length of the intestine, and sum of all the extension of the digestive tube (DT), in accordance with measurements used by Bierregaard (1988) and with the standard norms used for birds (Sick 1997, Baumel 1993). The biological samples de tongue, esophagus, ingluvies, pro-ventriculus, ventriculus and intestine were collected from all animals and fixed in formaldehyde 10%. Standard procedure for histology were performed. Hematoxylin-eosin staining and Masson's trichrome were carried out. Photographs were taken on an Olympus BX-60 (Olympus, Tokyo, JAP) microscope and a Zeiss AxioCam (Carl Zeiss, Jena, GER) camera coupled with the execution of

a Ks-400 (Carl Zeiss, Jena, GER) digital image capture program.

RESULTS AND DISCUSSION

The male specimens of *Paroaria dominicana* seized weighed 23.54 ± 5.44 g and had a total length with feathers of 16.94 ± 0.76 cm being minor (17.5cm) and lighter (25g) than described by Sick (1997) for wild specimens. The study of body conditions showed that 10% of the birds analyzed were obese, with great accumulation of fat tissue and 50% of these animals showed loss of plumage in at least some area of the body; 20% were in good condition, without loss of plumage and normal accumulation of fat tissue; 50% were lean, showing visible signs of maltreatment, with little accumulation of fat tissue and lack of plumage in several regions of the body; and 20% were in cachectic state also with lack of plumage in several regions of the body. The unfavorable body conditions observed are due how these animals are caged, type of cage used, population density, nutrition, hygiene conditions and consequently the health condition, which the animals are subjected. Even obese animals, are result of a wrong nutritional management and lack of space. The present study has demonstrated a positive relationship between unfavorable body condition (obese, lean and cachectic) and loss of feathers (83.42%) ($r > 0.047$) in the specimens studied, showing the poor nutritional and health status of the birds, thus interfering with the behavioral and clinical status, invalidating birds reproduction and possibly the reintroduction projects. Wolf *et al.* (2003) also describes the relationship between the loss of feathers rate and body mass, concluding that the nutritional needs influence the growth and maintenance of feathers.

In Brazil reintroduction by releasing is still challenged in scientific circles, contrary to what occurs in Europe (Yalden and Albarella 2010) for example; however, there are encouraging reports of success when they are performed with technical criteria (Wanjtal and Silveira 2000, Lima 2005, IBAMA 2006, 2009, 2012, Soorae 2010). Thus, extensive knowledge is required since screening until the selection of specimens, been essential that only individuals with good physical and health conditions has been chosen (IBAMA 2012). In São Paulo CETAS the index of nature reintegration (2003 to 2011) was 34.35% (IBAMA 2012), for Paraíba CETAS there are no such data. For *P. dominicana* releasing projects to repopulate areas in which this bird disappeared or to increase the gene flow of population, we suggest to use the methodology of body condition (Sanches 2008) employed in this work, as a complement to routine tests. In this methodology the animals are divided into obese, good lean or cachectic. Of these, only those with good condition would be destined for release; others would be redirected to a nutritional and clinical management, before being employed in releasing programs, since that reintroduction of animals in nature is a priority according to IBAMA (IBAMA 2012). Must be emphasized that despite *P. dominicana* do not be an endangered species (IUCN 2009), it is known that the results obtained with common species should not be overlooked once they may guide future management programs for endangered species (IBAMA 2012). Although the *P. dominicana* species has been widely studied in its ecological aspects, because of

animal trafficking (Sanches 2008), there is still a lack of data on external and internal biometrics. These data provide subsidies for a thorough taxonomic characterization of the species, and for a better nutritional and clinical management, and can be found respectively on Tables 1 and 2.

Table 1. Weight (g) and external biometrics measurements (mm) of 20 *Paroaria dominicana*, males, apprehended by CETAS-IBAMA/PB. Mean \pm standard deviation

External biometric measurements	Male
	Mm
Total length with feathers	169.44 \pm 7.64
Total length without feathers	98.18 \pm 8.06
Length of tail	71.25 \pm 13.69
Length of the tarsus	37.54 \pm 2.05
Length of the wing with feathers	115.70 \pm 8.08
Length of the wing without feathers	56.82 \pm 3.17
Length of the closed wing	84.97 \pm 2.85
Length of the head	21.17 \pm 2.36
Width of the head	16.72 \pm 0.29
Beak height	10.26 \pm 0.58
Width of the beak	9.60 \pm 0.29
Length of the beak	15.86 \pm 1.44
Length of the middle toe with claw	18.29 \pm 1.39
Length of the middle toe without claw	13.72 \pm 1.52
Width of the tórax	21.39 \pm 2.02
Width of the abdomen	18.6 \pm 2.25
	G
Weight	23.54 \pm 5.44

Table 2. Digestive tube biometrics measurements (DT) (mm) of 20 *Paroaria dominicana* males, apprehended by CETAS-IBAMA/PB. Mean \pm standard deviation

Biometric measurements of the GIT	Male
	Mm
Length of the esophagus	49.90 \pm 4.37
Length of the proventriculus	14.38 \pm 2.61
Length of the gizzard	15.14 \pm 1.32
Width of the gizzard	15.14 \pm 1.32
Thickness of the gizzard	7.88 \pm 0.81
Length of the intestine	193.36 \pm 15.28
Sum of the lengths of the GIT	293.79 \pm 19.52

The size was superior to that of other Brazilian species of the type, such as *P. capitata* (Cavalaria) and *P. baeri* (Cardeal-de-Goiás) (Sick 1997), both with 16.5cm. The length of the wing with feathers of the *P. dominicana* (115.70 \pm 8.08mm) was greater than that found for other *Passeriformes* such as the *Thraupis sayaca* (Sanhaçu-cinzento) (92.1 \pm 2.4mm), *Thraupis bonariensis* (Sanhaçu-amarelo) (92.7 \pm 0.6mm) e and smaller than the of *Turdus rufiventris* (*Sabiá-laranjeira*) (119.8 \pm 3.4mm) and *Pitangus sulphuratus* (*Bem-te-vi*) (120.3 \pm 5.3mm) (Burgoni 2002). The tarsus can have significant variations even among strains or subspecies, as is observed in chickens (*Gallus gallus domesticus*) (Getty 1981). *P. dominicana* had larger tarsus (37.54 \pm 2.05mm) than other species of *Passeriformes* described by Burgoni (2002), such as *P. Sulphuratus* (25.3 \pm 2.3mm) and *T. rufiventris* (35.5 \pm 1.5mm), even though these birds are larger. The length of the tail (71.25 \pm 13.69mm) was similar to that of the male *Thraupis bonariensis* (72.3 \pm 2.1mm) and the female *Molothrus bonariensis* (Chupim) (70.0 \pm 2.3mm) (Burgoni 2002). Biometric measurements of the digestive tube (DT) of birds are limited in literature, which is mostly restricted to commercial birds. The proventriculus (14.38 \pm 2.61mm) size

cannot be compared once there are not data in the literature for other *Passeriformes*. The DT/body size ratio of *P. dominicana* (1.73) is lower than that of the saffron finch (Siqueira *et al.* 2013), and is 1.81. The visceral topography analysis (Fig.1) revealed similarities to that described by Dyce *et al.* (2004) for domestic birds and for Australian parakeet (*Melopsittacus undulatus*) (Matsumoto *et al.* 2009). The tongue was found long and thin, projected through the beak. The esophagus was like a distensible tube, relatively fine, thicker in the thoracic abdominal cavity where the ingluvies (crop) is located. This is the saccular dilatation of the esophagus in the distal portion near the pro-ventriculus (König and Liecbich 2004). The pro-ventriculus was along the median plane, showing a spindle shape as described by Coelho (2002). It had a darker color; located subsequent to this was the gizzard. The gizzard was ventral to the pro-ventriculus, and connected to the small intestine. The liver was dark, had two lobes, and was connected cranially by a dorsal bridge to the heart. The intestines occupied the caudal part of the body cavity, with intimate contact with the gizzard and the reproductive organs. There was no presence of caeca, which are found in some domestic birds (König and Liecbich 2004, Monteiro *et al.* 2009).

The histological structure of the DT for Brazilian *Passeriformes* are rare, there is just one species described (Siqueira *et al.* 2013). The histology of *P. dominicana* DT had similarities to that described for other birds such as the Australian parakeet (Matsumoto *et al.* 2009), saffron finch (Siqueira *et al.* 2013) and partridges (Rossi *et al.* 2005). The tongue (Fig.2A) presented keratinized stratified squamous epithelium and hyaline cartilage through its entire length, comparing to endochondral bone described in other birds, such as chicken (Bacha and Bacha 2003). As in the ostrich (Monteiro *et al.* 2009) the esophagus of *P. dominicana* (Fig.2B) showed the mucosa covered by a keratinized stratified squamous epithelium, lamina propria composed of less evident loose connective tissue and with more mucous secretion glands than chickens (Bacha and Bacha 2003), and muscularis mucosae. The submucosa with connective tissue and glands was followed by the muscular layer (striated skeletal muscle) showed two segments, the inner circular and outer longitudinal, similar to those described for ostriches (Monteiro *et al.* 2009).

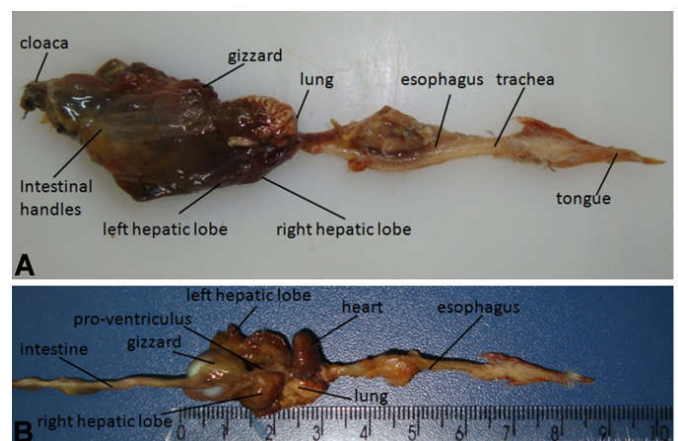


Fig. 1. The digestive system of *Paroaria dominicana* apprehended by CETAS-IBAMA/PB. (A,B) Digestive tube morphology

In the ingluvies (Fig.2C), after the serosa, the muscle layer formed by circular (inner) and longitudinal (external) bundles, similar to the domestic chicken (Bacha and Bacha 2003) and young ostriches (Monteiro *et al.*, 2009) were found. The submucosa was thicker than the esophagus, however similar histologically. The mucosa possessed stratified squamous epithelium and glands in the lamina propria.

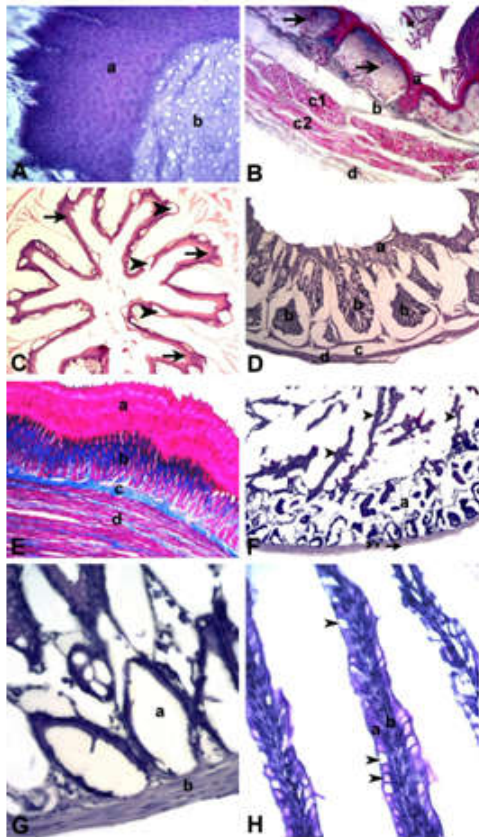


Fig. 2. *Paroaria dominicana* digestive tube. (A) Tongue: a- keratinized stratified squamous epithelium; b- hyaline cartilage with chondrocytes. Hematoxilin-eosin stain, obj.40x. (B) Esophagus: a- keratinized stratified squamous epithelium; arrow- secretion glands of lamina propria mucosae; b- submucosa; c1- inner circular muscular layer; c2- outer longitudinal muscular layer (both striated skeletal muscle); d- Serosa; asteristic- esophagus content; magnification: 200x; Masson trichrome stain. (C) Inguvies (crop): arrow- stratified squamous epithelium; arrow head- mucous glands of lamina propria of mucosa; magnification: Hematoxilin-Eosin stain, obj.10x. (D) Pro-ventriculus: a- mucosa with simple prismatic epithelium; b- multitubular glands of submucosa (c); d- muscular layer involved by serosa, magnification: Hematoxilin-eosin stain, obj.10x. (E) Gizzard (ventriculus): a- keratinoid; b- tubular glands that produce keratinoid with simple prismatic epithelium; c- submucosa; d- muscular layer; magnification: 100x; Masson trichrome stain. (F) Initial portion of intestine: arrow head- intestinal villi; a- crypt of Lieberkuhn; broach- inner circular bundle of muscular layer; arrow- outer longitudinal bundle of muscular layer. Hematoxilin-eosin stain, obj.10x. (G) Final portion of intestine: a- Lieberkuhn glands of villi crypt; b- muscular layer with a unique bundle. Hematoxilin-eosin stain, obj.10x. (H) Intestinal villis with a simple prismatic epithelium (a) including globet cells (arrow heads) and a lamina propria (b). Hematoxilin-eosin stain, obj.40.x

The pro-ventriculus (Fig.2D) showed folds in its lumen, which was coated with simple prismatic epithelium. The lamina

propria was composed of connective tissue with blood vessels, as seen in *Paroaria gularis gularis* (amazon cardinal) (Catroxo *et al.* 1997). The submucosa exhibited multitubular glands also corroborating Catroxo *et al.* (1997). The muscle layer showed smooth muscle and the serosa was rich in blood vessels, such as in domestic chickens (Bacha and Bacha 2003).

The structure of the gizzard (Fig.2E) was similar to that found for *P. gularis gularis* (Catroxo *et al.* 1997), Australian parakeet (Matsumoto *et al.* 2009) and domestic chickens (Bacha and Bacha 2003); the muscular layer being voluminous because of its biological function. The mucosa showed low folds, lined by simple prismatic epithelium. The lamina propria was formed by dense connective tissue, occupied by numerous simple deep tubular glands that produce the keratinoid, as in other domestic birds (Bacha and Bacha 2003, Samuelson 2003). The initial portion of the intestine (Fig.2F) showed serosa; muscular layer with smooth muscle bundles, followed by a layer of submucosa and a not very thick muscularis mucosae layer. In the lamina propria of the mucosa secretory units were found. The lumen of this organ was formed by villi with simple columnar epithelium including goblet cells similar to domestic birds (Samuelson 2003). The final portion of the intestine (Fig.2G,H) differed from the initial portion because it had only one muscle bundle in the muscle layer, the inner circular, corroborating that of the Australian parakeet (Matsumoto *et al.* 2009). Thus, the unprecedented data obtained by evaluating body condition, external biometrics and morphological analysis of the DT of *P. dominicana* apprehend can provide subsidies for a more individual taxonomic classification, as well as, corroborate posterior clinical, nutritional, and preservationist studies, and release projects in Screening Centers. The results also demonstrated that there are a positive relationship between unfavorable body condition and loss of feathers and that the body conditions study of animals seized can be an important tool and should be considered in evaluations at Screening Centers in order to have a better nutritional and/or clinical management, before releasing projects.

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