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Body condition, external morphology, parasitology, and histological and biometrical study of the gastrointestinal tract of *Sporophila nigricollis* and *Sporophila caerulescens* seized from trafficking in Northeastern Brazil[1]

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ABSTRACT.- Nascimento C.J., Oliveira A.M.T., Santos W.M., Araújo J.L., Rola L.D. & Guerra R.R. 2024. **Body condition, external morphology, parasitology, and histological and biometrical study of the gastrointestinal tract of *Sporophila nigricollis* and *Sporophila caerulescens* seized from trafficking in Northeastern Brazil.** *Pesquisa Veterinária Brasileira 44:e07358, 2024*. Laboratório de Histologia Animal, Centro de Ciências Agrárias, Campus de Areia, PB-079 s/n, Bairro Centro, Areia, PB 58397-000, Brazil. E-mail: rromaoguerra@gmail.com

The objective was to analyze *Sporophila nigricollis* and *Sporophila caerulescens* in terms of body conditions, parasitological, bacteriological, external biometric measurements, and histological analysis of the gastrointestinal tract. We used 115 individuals apprehended from 2020 to 2021 and sent to the Wild Animal Triage Center, Paraíba, Brazil. Concerning weight, *S. nigricollis*, females and males presented 9.36±1.36g and 9.70±1.39g, and *S. caerulescens* with 10.5±0.70g and 11.75±1.28g, being the second heaviest. The body condition in 69.62% of *S. nigricollis* and 62.50% of *S. caerulescens* was good. In the fecal microbiological examination, 40.25% of *S. nigricollis* and 40% of *S. caerulescens* were positive for *Isospora* sp. *Dispharynx* sp. was reported for the first time in *S. nigricollis* and *S. caerulescens*. In fecal bacteriology, 95.65% of *S. nigricollis* were identified with Gram-positive cocci and 73.91% with Gram-positive bacilli. Forty-one animals died and were placed under refrigeration for 48 hours; of these, 31.16% of *S. nigricollis* and 37.50% of *S. caerulescens* presented inadequate body conditions. 19.51% had gastrointestinal tract engorgement with hemorrhagic points, and 14.63% had hepatic alteration due to yellowish coloration. The study contributes with subsidies for the taxonomic elucidation of the genus *Sporophila*, in addition to the knowledge of the conditions in which birds are found in sorting centers, once the destination of most of the seized animals is the release, thus, a potential source of pathogens to the natural environment.

INDEX TERMS: Anatomy, histology, microbiology, *Sporophila*, Yellow-bellied Seedeater.

RESUMO.- [**Condição corporal, morfologia externa, parasitologia e estudo histológico e biométrico do trato gastrointestinal de *Sporophila nigricollis* e *Sporophila caerulescens* apreendidos no Nordeste Brasileiro**]. O objetivo foi analisar *Sporophila nigricollis* e *Sporophila caerulescens* quanto às condições corporais, parasitológicas, bacteriológicas, medidas biométricas externas e análise histológica do trato gastrointestinal. Foram utilizados 115 indivíduos apreendidos entre 2020 e 2021 e encaminhados ao Centro de Triagem de Animais Silvestres, Paraíba, Brasil. Quanto ao peso, *S. nigricollis*, fêmeas e machos apresentaram, 9,36±1,36g e 9,70±1,39g, e *S. caerulescens* com 10,5±0,70g e 11,75±1,28g, sendo o segundo mais pesado. A condição corporal em 69,62% de *S. nigricollis* e 62,50% de *S. caerulescens* foi boa. No exame microbiológico fecal, 40,25% de *S. nigricollis* e 40% de S. *caerulescens* foram positivos para *Isospora* sp. *Dispharynx* sp. foi relatada pela primeira vez em *S. nigricollis* e *S. caerulescens*. Na bacteriologia fecal, 95,65% dos *S. nigricollis* foram identificados com cocos Gram-positivos e

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73,91% com bacilos Gram-positivos. Quarenta e um animais morreram e foram colocados sob refrigeração por 48 horas; destes, 31,16% de *S. nigricollis* e 37,50% de *S. caerulescens* apresentaram condições corporais inadequadas. 19,51% apresentaram ingurgitamento do trato gastrointestinal com pontos hemorrágicos e 14,63% alteração hepática devido à coloração amarelada. O estudo contribui com subsídios para a elucidação taxonômica do gênero *Sporophila*, além do conhecimento das condições em que as aves se encontram nos centros de triagem, uma vez que a maior parte dos animais apreendidos tem como destino a soltura, sendo assim, uma potencial fonte de patógenos para o ambiente natural.

TERMOS DE INDEXAÇÃO: Anatomia, histologia, microbiologia, *Sporophila*, papa-capim-capuchinho.

INTRODUCTION

Sporophila nigricollis is known as Yellow-bellied Seedeater (Vieillot, 1823) and inhabits the fields of culture and tall grasses; it is a widely distributed species (Sick 1997). *Sporophila caerulescens* is known as Double-collared Seedeater. This species is the most abundant in Brazil but does not seem to occur in the Northeast (Sick 1997). Despite not being mentioned as present in the state of Paraíba, its presence is observed in breeding, singing competitions, and animal trafficking in that state.

There has been a considerable increase in the number of legal breeders of these species for domestic purposes and singing tournaments. However, they are still victims of illegal breeding and trafficking, with a large part of these captured animals being sent to the "Centro de Triagem de Animais Silvestres" (Wild Animal Triage Center – CETAS) (Souza et al. 2014).

Wild animals are reservoirs of several infectious agents. Therefore, the parasitological study of the feces of animals that arrive at these CETAS is of great importance to generate relevant information about the state in which these animals arrive, as well as the agents circulating in the environment (Freitas et al. 2002).

Given the many birds designated as *Sporophila*, new studies contribute to tracing a characteristic pattern to relate the individuals' evolutionary, physiological, and taxonomic aspects (Dunning Jr. 2007). These morphological studies help to solve the problem of the *Sporophila* genus and the divergence regarding subspecies.

The length of the digestive tract is evaluated to verify the speed of food passage (Cherry & Siegel 1978). However, biometric data are scarce on *S. nigricollis* and *S. caerulescens,* and there are no such measures of the external morphology and histology of the organs of the gastrointestinal tract.

Therefore, the present study aimed to describe morphologically and histologically the gastrointestinal tract of *S. nigricollis* and *S. caerulescens*, comparing it between males and females, as well as verifying the bodily, microbiological and bacteriological conditions that these animals arrive at to CETAS/PB, providing subsidies for biometric and taxonomic classification, for clinical and nutritional procedures in triage centers, commercial creations, in reproduction and reintroduction programs in nature and sanitary release protocols.

MATERIALS AND METHODS

Animal Ethics. This research project was sent to the Biodiversity Authorization and Information System, protocol 75130/2020, and to the Ethics Committee on the Use of Animals (CEUA), number 9719240420.

It was used 115 specimens, 105 (91.30%) *Sporophila nigricollis* and 10 (8.61%) *Sporophila caerulescens* that arrived at the "Centro de Triagem de Animais Silvestres" (CETAS/IBAMA), Paraíba, Brazil.

The 41 animals that died were kept under refrigeration for external biometric and gastrointestinal tract analysis and histological analysis of these organs.

Subjects were weighed and physically restrained to analyze the skin extensions' body condition, plumage, and adipose tissue levels. Body condition was classified as good, lean, cachectic, and obese, according to the physical constitution and pectoral muscles. Observations were made regarding the loss or alteration of plumage (Firmino et al. 2013). The adipose tissue level in skin extensions was graded from 0 to 3, increasingly for the presence of fat.

Fresh fecal samples were collected for the parasitological and bacteriological study of the feces. The collections were carried out two times a day. Samples were analyzed using the direct technique and Gram staining (Bowman 2010).

The viscera were measured with a digital caliper. The sexual distinction was made through plumage coloring (Sick 1997).

The external biometric measurements were total length with feathers, total length without feathers, length of the tail, length of the tarsi, length of the wings with feathers, length of the wings without feathers, length of the closed wings, head length, head width, height length, beak width, beak length, length of the middle finger with the nail, length of the middle finger without a nail, the width of the thorax and width of the abdomen.

The biometric measurements of the gastrointestinal tract analyzed were the length of the esophagus, the length of the proventriculus, the length of the gizzard, the width of the gizzard, the thickness of the gizzard, the length of the intestine, and the sum of the entire length of the gastrointestinal tract (Firmino et al. 2013).

Samples were collected from the tongue, esophagus, ingluvium, proventricle, ventricle, and intestine, fixed in Metacarn and subjected to histological processing (Heleno et al. 2011). Hematoxylin-eosin (HE) staining was used, and the images were digitalized using an Olympus BX-60 microscope and a ZeissAxioCam camera, with the Ks-400 digital image capture program, for the histological description of the organs (Bacha Jr. & Bacha 2003).

For the morphological data of external and internal biometrics, weight, and body condition, statistical analysis was performed by the Mann-Whitney Test ($\alpha = 0.05$) and the T-student Test (GraphPad Prism 5).

RESULTS

The specimens studied were *Sporophila nigricollis* and *Sporophila caerulescens* (Sick 1997). Concerning weight (g), *S. nigricollis,* females and males presented 9.36±1.36g and 9.70±1.39g, respectively. *S. caerulescens,* females, and males presented 10.5±0.70g and 11.75±1.28g, respectively. Males and females of *S. caerulescens* were heavier than *S. nigricollis* (Table 1).

Regarding the body condition of the specimens, most were in good condition. In *S. nigricollis*, 38 females (77.55%) and 17 males (56.66%) presented good body condition; seven females (14.28%) and 10 males (33.33%) lean condition; one

female (2.04%) and three males (6.12%) obese condition; three females (6.12%) and none of the males with cachexia condition. In *S. caerulescens,* two females (100%) and five males (62.50%) were in good body condition, and three males (37.50%) were obese (Table 2).

Regarding the adipose tissue levels, the majority presented level 1 (low adipose tissue levels in skin). In *S. nigricollis,* two females (4.08%) had level 0 (weight: 9.50±2.12g), 30 females (61.22%) had level 1 (weight: 9.06±1.36g), 15 females (30.61%) level 2 (weight: 9.93±1.09g) and two females (4.08%) level 3 (10.5±2.12g). Among males, four males (13.33%) had level 0 (9.25±0.5g), 18 males (60.0%) had level 1 (9.35±1.03g), four males (13.33%) had level 2 (10.5 ± 1.37g) and four males (13.33%) level 3 (12.40 ± 0.89). In *S. caerulescens*, the two females (100%) presented level 1 (10.50±0.70g); 3 males (37.50%) level 1 (11.00±0.81g), two males (25.00%) level 2 (11.00±0g) and three males (37.50%) level 3 (13.00±1.00g) (Table 3).

Relating the level of adipose tissue in skin extensions to body condition, in *S. nigricollis*, in the classification of level 0, two animals (33.4%) had good body condition and four animals (66.6%) had lean condition and no animals were obese/cachectic; at level 1, 34 animals (43.03%) had good body condition, 11 animals (22.91%) had lean body condition, three animals (6.25%) had cachectic body condition, and none /obese. At level 2, 18 animals (62.06%) were in good condition, one (1.26%) was thin, and 0 were obese/cachectic. At level 3, one animal (16.6%) had a good body condition, one animal (16.16%) had a lean condition, four animals (66.6%) had an obese condition, and none had a cachectic condition. In *S. caerulescens*, at level 1, five animals (50%) presented good body condition; at level 2, two animals (20%) showed good condition; and at level 3, three animals (30%) were classified as obese (Table 3).

Eighty-seven coproparasitological examinations were carried out (77 in *S. nigricollis* and 10 in *S. caerulescens*). In *S. nigricollis*, 45 samples (58.44%) were negative for intestinal parasites, 31 samples (40.25%) had *Isospora* sp. oocysts, and in one sample (1.29%) eggs were found from *Dispharhynx* sp. In *S. caerulescens*, five samples (50%) were negative for parasites, four samples (40%) were positive for *Isospora* sp. oocysts, and one sample (10%) was positive for *Dispharynx* sp. eggs (Table 4).

Regarding the fecal bacteriological examination, 23 samples of *S. nigricollis* were performed, of which 22 samples (95.65%) were identified with Gram-positive cocci bacteria, 17 samples

Table 1. Weight classification (wt) and body condition of males and females of *Sporophila nigricollis* and *Sporophila caerulescens* apprehended and sent to CETAS from 2020 to 2021

	S. nigricollis		*S. caerulescens*	
	Female	Male	Female	Male
Average weight (wt)	9.36Bb	9.70Ba	10.50aB	11.75aA
Standard deviation (%)	1.36%	1.39%	0.70%	1.28%
Max weight (wt)	12	13	11	14
Min weight (wt)	7	8	10	10
Body condition				
Obese	1 (2.04%)	3 (6.12%)	-	3 (40%)
Good	38 (77.55%)	17 (56.66%)	2 (100%)	5 (60%)
Skinny	7 (14.28%)	10 (33.33%)	-	-
Gaunt	3 (6.12%)	-	-	-

T-student test $p>0.05$ between males and females of the same species; Mann Whitney U test $p<0.05$ between males and females of the two different species; Level of classification from highest to lowest weight between males and females: A,B = of the same species; a,b = of the two different species.

Table 2. Classification of adipose tissue levels in male and female *Sporophila nigricollis* and *Sporophila caerulescens* skin extensions seized and sent to the CETAS from 2020 to 2021, concerning weight (wt) of the animals, No. of specimens, mean weight (wt), standard deviation (SD %), and maximum-minimum

	S. nigricollis			
	Female (wt)		Male (wt)	
	No. specimens	Mean ± SD; max-min.	No. specimens	Mean ± SD; max-min.
Level 0	2 (4.08%)	9.50g ± 2.12; 11-8	4 (13.33%)	9.25g ± 0.5; 10-9
Level 1	30 (61.22%)	9.06g ± 1.36; 12-7	18 (60%)	9.35g ± 1.03; 12-8
Level 2	15 (30.61%)	9.93g ± 1.09; 12-8	4 (13.33%)	10.50g ± 1.37; 12-8
Level 3	2 (4.08%)	10.50g ± 2.12; 12-9	4 (13.33%)	12.40g ± 0.89; 13-11
		S. caerulescens		
Level 0	-	-	-	-
Level 1	2 (100%)	10.50g ± 0.70; 11-10	3 (37.5%)	11g ± 0,81; 12-10
Level 2	-	-	2 (25%)	11g ± 0; 11-11
Level 3	-	-	3 (37.5%)	13g ± 1; 14-12

Grading levels according to adipose tissue in skin extensions of the thorax, sternal notch and coelomic cavity: Level 1 = Animal with low levels of adipose tissue in skin extensions, Level 2 = animal with medium levels of adipose tissue in skin extensions, Level 3 = animal with accentuated levels of adipose tissue in skin extensions.

Table 3. Adipose tissue levels in skin extensions concerning body condition of *Sporophila nigricollis* and *Sporophila caerulescens* seized and sent to the CETAS from 2020 to 2021

	S. nigricollis			
	Level 0	Level 1	Level 2	Level 3
Good	2 (2.53%)	34 (43.03%)	18 (22.78%)	1 (1.26%)
Skinny	4 (5.06%)	11 (13.92%)	1 (1.26%)	1 (1.26%)
Obese	-	-	-	4 (5.06%)
Gaunt	-	3 (3.79%)	-	-
	S. caerulescens			
	Level 0	Level 1	Level 2	Level 3
Good	-	5 (50%)	2 (20%)	-
Skinny	-	-	-	-
Obese	-	-	-	3 (30%)
Gaunt	-	-	-	-

Grading levels according to adipose tissue in skin extensions of the thorax, sternal notch and coelomic cavity: Level 1 = Animal with low levels of adipose tissue in skin extensions, Level 2 = animal with medium levels of adipose tissue in skin extensions, Level 3 = animal with accentuated levels of adipose tissue in skin extensions.

Table 4. Results of coproparasitological exams performed on *Sporophila nigricollis* and *Sporophila caerulescens* seized and sent to the CETAS from 2020 to 2021, results of fecal bacteriological exams performed and their classification regarding the degree of fecal bacterial proliferation in *S. nigricollis*

Coproparasitological exams of *S. nigricollis* and *S. caerulescens*		
	S. nigricollis	*S. caerulescens*
Negatives	45 (58.44%)	5 (50%)
Isospora sp.	31 (40.25%)	4 (40%)
Dispharhynx sp.	1 (1.29%)	1 (10%)
Bacteriological examination *S. nigricollis*		
	Gram-positive cocci	Gram-positive bacilli
Samples	22 (95.65%)	17 (73.91%)
Degree of proliferation		
(+)	7 (30.43%)	11 (48.82%)
(++)	5 (21.73%)	5 (21.73%)
(+++)	10 (43.7%)	1 (4.34%)

(73.91%) were identified with Gram-positive bacilli and one sample (4.34%) was negative (Table 4). In the quantitative analysis of bacterial proliferation in *S. nigricollis*, 11 samples (48.82%) had grade 1+ bacilli, five samples (21.73%) grade 2+ bacilli, and one sample (4.34%) grade 3+ bacilli; seven samples (30.43%) grade 1+ cocci, five samples (21.73%) grade 2+ cocci, and 10 samples (43.7%) grade 3+ cocci (Table 4).

The external biometric measurements (Table 5) showed differences regarding gender for *S. nigricollis*, being higher in males for total length with feathers, tail length, wing length with feathers, length of the closed wings, the height of the beak, length of the middle finger with nail and width of the abdomen. However, there were no differences regarding gender for the internal biometric measurements of the gastrointestinal tract (Table 6).

In the macroscopic analysis, eight animals (19.51%) presented gastrointestinal tract engorgement with hemorrhagic points, and six animals (14.63%) presented hepatic alteration due to yellowish color. Caseous nodules in different body regions were observed in four animals (9.75%), and in two animals (4.87%), there was loss/alteration of plumage. There was one case of engorgement of the kidneys, neoplastic nodule adjacent to the kidneys, neoplastic nodule adjacent to the duodenum, yellowish lungs, hemorrhage in the oral cavity, ingluvial stasis, and aspergilloma plaques.

Microscopically, the tongue presented keratinized stratified squamous epithelium (KSSE) (Fig.1), and just below it, we can see the lamina propria (LP) with taste buds (Fig.2). In the esophagus (Fig.3-4), the KSSE was observed, and below LP with very voluminous mucous glands followed by a muscular layer of the mucosa (MLM). Then comes the submucosa with connective tissue (CT) and glands, followed by the muscular layer (ML) with skeletal striated muscle formed by two muscle bundles (MB). The ingluvium (Fig.5-6) showed the presence of longitudinal folds with stratified squamous epithelium in the mucosa, followed by LP glands and MLM; the submucosa was histologically similar to that of the esophagus but thicker; then came the ML.

In the proventriculus (Fig.7-8), a simple prismatic epithelium (SPE) pleated was observed included in the submucosal lamina propria, which presented gastric groove multi-tubular glands and the ML presented smooth muscle (SM). The ventricle

Table 5. Weight (wt) and external biometric measurements (mm) of *Sporophila nigricollis* seized by the CETAS from 2020 to 2021 concerning average, standard deviation (SD) and maximum-minimum

External biometric measurements	Female (mm)	Male (mm)
Full-length of feathers	99.04±9.18b; 107.29-84.01	102.36±5.17a; 112.14-92.21
Full-length without feathers	58.64±7.27; 69.80-33.09	59.8±4.66; 66.2-51.36
Length from the tail	42.42±2.78b; 46.94-33.36	45.99±2.79a; 50-41.03
Length of the tarsus	12.11±1.62; 14.29-6.63	12.80±1.37; 13.76-9.56
Length of the wing with feathers	64.95±5.69 b; 75.34-50.73b	69.47±3.10a; 73.80-62.02
Length of the featherless wing	32.23±2.90; 37.6-26.27	33.34±4.35; 45-27.80
Length of closed wings	50.87±2.30b; 56.80-45.57	53.84±1.36a; 55-50.94
Head length	14.03±1.36; 18.9-11.53	14.21±1.23; 16.22-11.13
Head width	11.65±1.20; 13-6.78	11.82±0.61; 12.40-10.42
Beak height	5.97±0.99b; 7.10-1.58	6.47±0.32a; 7.26-5.94
Beak width	6.13±1.03; 7.30-2,36	6.41±0.55; 6.93-4.77
Beak length	8.14±1.19; 10.79-5.41	8.37±0.68; 10.14-7.40
Length of middle finger with nail	13.07±1.25b; 15.50-9.76	13.8 ±1.16a; 15.54-11.6 b
Length of middle finger without nail	9.11±1.10; 10.90-5.08	9.34±0,61; 10.16-7.81
Chest width	11.51±2.16; 17.01-6.18 a	12.82±1.77; 16.11-9.34 b
Abdomen width	11.10±2.24b; 15.60-6.73	12.13±1.04a; 14.84-10.96
Weight (g)	6.70±0.86; 9-5*	7.21±0.86; 9-6*

T-student test $p>0.05$; * Weights during necropsy are lower than live weight due to dehydration during freezing.

Table 6. Internal biometric measurements (mm) of *Sporophila nigricollis* seized by the CETAS from 2020 to 2021 concerning mean ± standard deviation (SD) and maximum-minimum

Biometric measurements of the alimentary canal	Female (mm)	Male (mm)	T-student
Esophagus length	37.81±3.91; 49.04-31.5	38.52±2.87; 44.72-35.02	0.5686
Proventriculus length	9.7±2.35; 12.86-4.47	10.64±2.03; 12.74-5.64	0.2619
Gizzard length	8.89±1.1; 10.78-5.78	9.35±1.28; 11.297.92	0.3463
Gizzard width	7.84±1.29; 10.18-5.03	8.11±0.93; 10-6.88	0.5184
Gizzard thickness	5.38±0.77; 7.92-4.37	6.12±1.8; 11.2-4.9	0.2581
Bowel length	53.74±12.35; 82.39-39.28	52.73±11.75; 71.93-38	0.8333
Sum of the length of the gastrointestinal tract	105.53±13.01; 135.5-85	102.9±14.4; 123.23-86	0.6528

T-student test $p>0.05$.

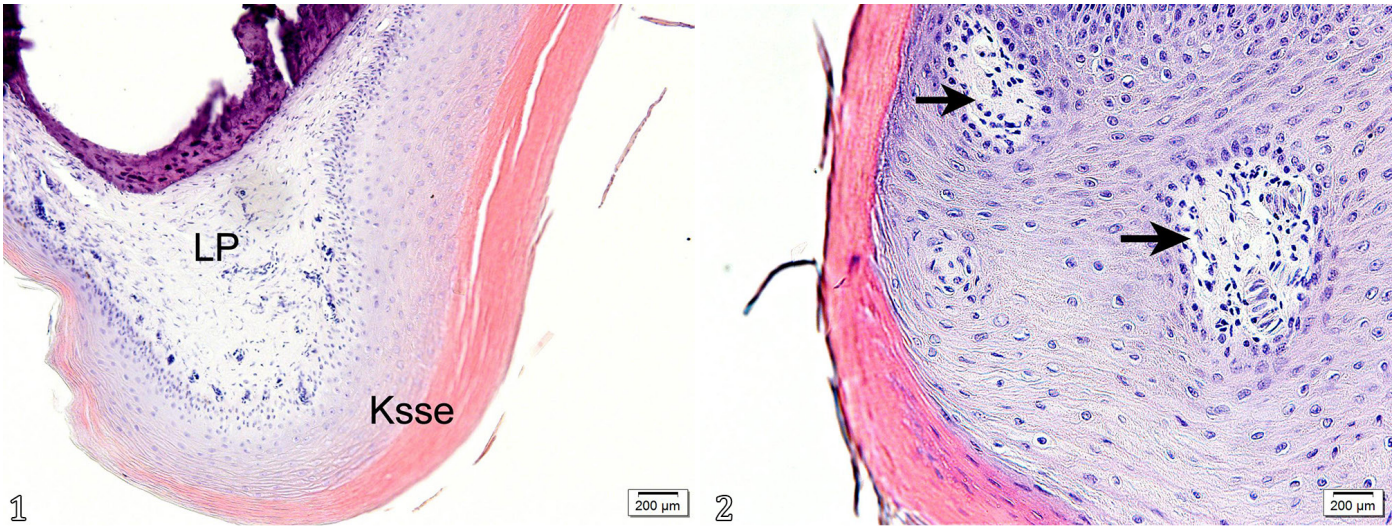

Fig.1-2. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**1**) Tongue: keratinized stratified squamous epithelium (Ksse) and lamina propria (LP). (**2**) Tongue: fungiform taste buds (arrow). HE, bar = 200µm.

(Fig.9-10) presented pleated mucosa formed by SPE. Above the epithelium was a rough cuticle of chitin produced by the deep, simple tubular glands that lie beneath the epithelium in the lamina propria. This organ is also characterized by the great thickness of the ML due to the organ's maceration function. The intestine (Fig.11-12) showed an SPE forming digitiform villi with enterocytes and, to a lesser extent, goblet cells; below came the LP followed by the MLM; then came the submucosa and finally, the ML with SM, being two MB. The most distal portion of the intestine (Fig.13-14) was distinguished by having only one MB in the ML. All organs were covered by a serosa formed by CT and mesothelium.

Morphometrically, in *S. nigricollis*, the mucosal thickness (MT) of the initial portion of the intestine was greater in males, whereas in the middle portion, such thickness was greater in females. There was no difference in MT for the final portion. Females showed less MT in the initial portion of the intestine than in the other portions. Crypt depth showed no differences in the gender of the specimens. The muscle layer thickness (MLT) in males was thicker than in females in the initial and final portions of the intestine. In females, the MLT was less thick in the initial portion, and males, in the middle portion, about the other portions (Table 7).

DISCUSSION

There was no significant difference between the weights of males and females of *Sporophila nigricollis* and *Sporophila caerulescens*, however, the specimens of the second species were heavier. Both species were lighter than *Sicalis flaveola brasiliensis* (Siqueira et al. 2013), *Cyanoloxia brissonii*, and

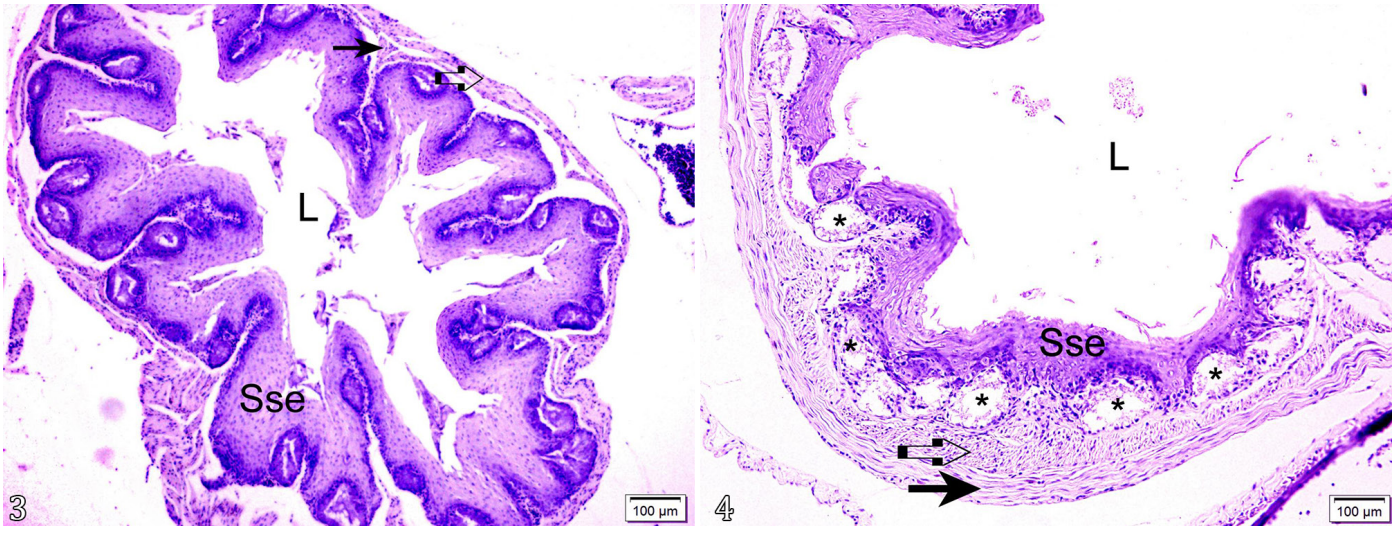

Fig.3-4. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**3**) Esophagus: lumen (L), non-keratinized stratified squamous epithelium of the mucosa (Sse), internal muscle layer (arrow), and external muscle layer (leaked arrow). (**4**) Esophagus: Lumen (L), non-keratinized stratified squamous epithelium of the mucosa (Sse), mucosal glands (*) formed by cubic cells from submucosa, and internal muscle layer (arrow), and external muscle layer (leaked arrow). HE, bar = 100µm.

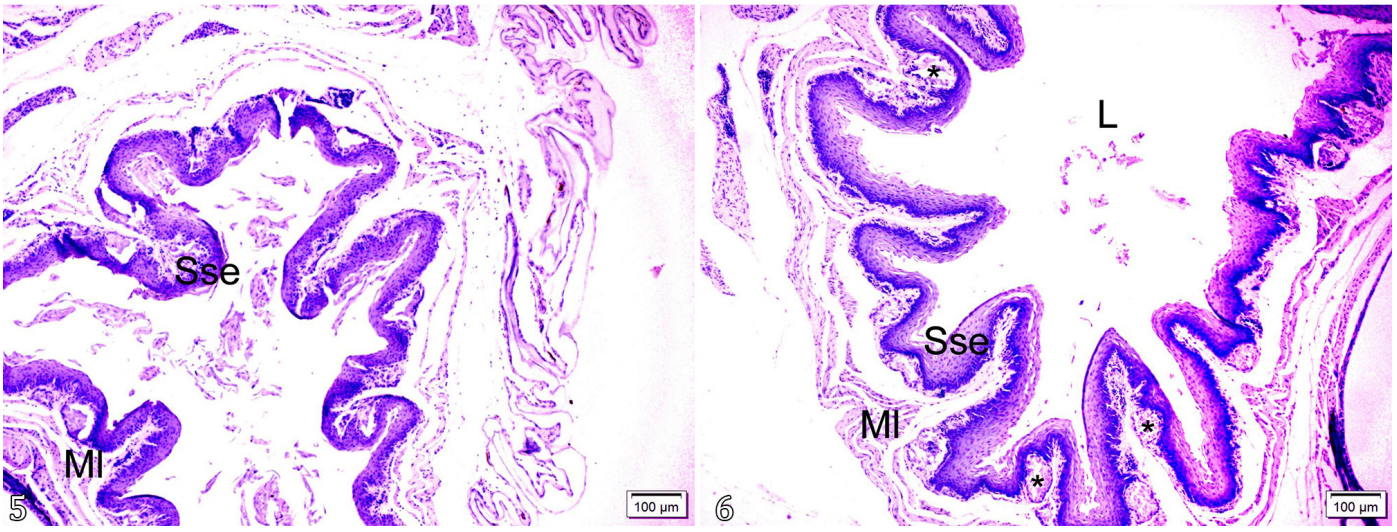

Fig.5-6. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**5**) Ingluvium: non-keratinized stratified squamous epithelium of the mucosa (Sse) and muscle layer (Ml). (**6**) Ingluvium: lumen (L), non-keratinized stratified squamous epithelium of the mucosa (Sse), mucosal glands (*) of the lamina propria, and muscle layer (Ml). HE, bar = 100µm.

Paroaria dominicana (Guerra et al. 2016). However, *S. nigricollis* proved to be lighter, and *S. caerulescens* heavier than *Volatinia jacarina* (Caetano 2013).

In evaluating the animals' body condition, most female and male *S. nigricollis* specimens (77.55 and 56.66%, respectively) were classified as having good body condition. In *S. caerulescens*, 100% of the females and 60% of the males had good body condition. Both species did not present cachectic male specimens, and male *S. caerulescens* did not present lean specimens either. The better body condition of the specimens of these species seized is because they are highly valued for their singing in Brazil, being used in championships. Therefore, those who capture them in nature usually take better care of their food than other species. Despite their good body condition, this does not mean they are in good health.

This is confirmed in the study because of the 41 *S. nigricollis* that underwent necropsy; 26 animals presented cachectic body condition, 11 had the thin condition, and only one had a good condition. Of this amount, 15 were still evaluated in life. Of these, nine animals showed good body condition, whereas seven showed cachectic body condition during necropsy, one in lean condition and another in good condition. The remaining six animals, in life, presented lean body condition; during necropsy, four were in lean condition, and two were in cachectic condition. This leads us to consider that those who died were being affected by infectious agents and nutritional impairments compromising their body state.

These data corroborate other studies, confirming that most individuals who die have a fair to poor body condition, as demonstrated in other CETAS in São Paulo, Brazil (Godoy & Matushima 2010). In the study, cachexia was considered responsible for most deaths caused by non-infectious diseases. Free-living passerines from trafficking were studied, including *S. nigricollis* and *S. caerulescens*, and sent to the rehabilitation

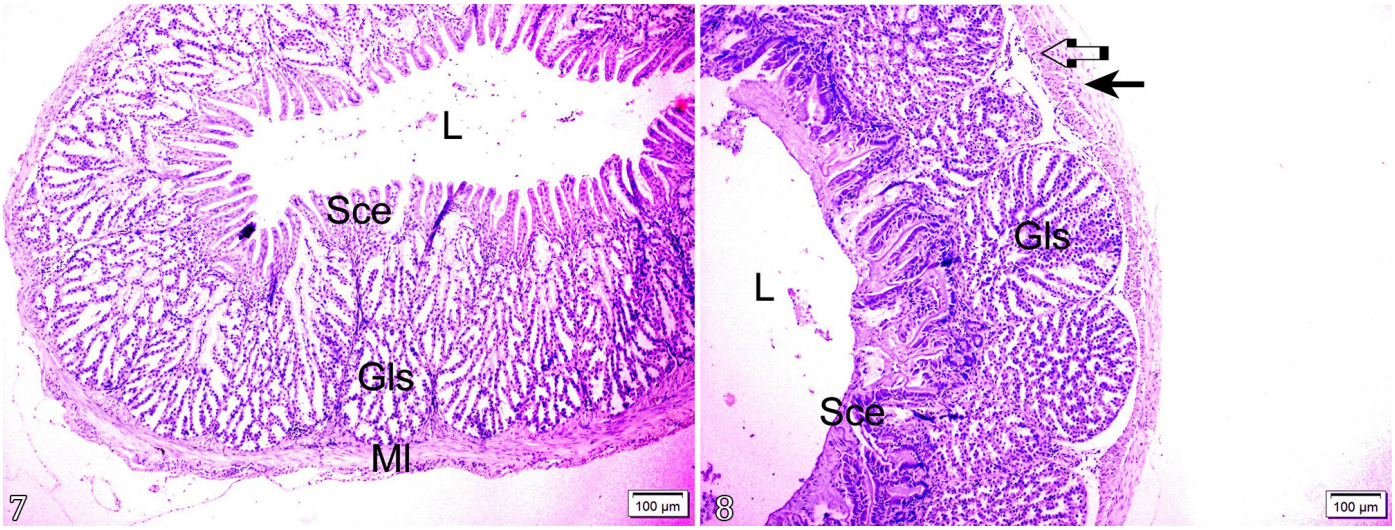

Fig.7-8. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**7**) Proventricle: lumen (L), simple columnar epithelium (Sce) pleated and digestory glands (Gls) from submucosal lamina propria, and muscular layer (Ml). (**8**) Proventricle: lumen (L), simple columnar epithelium (Sce) pleated and digestive glands (Gls) from submucosal lamina propria, and internal muscle layer (arrow), and external muscle layer (leaked arrow). HE, bar = 100µm.

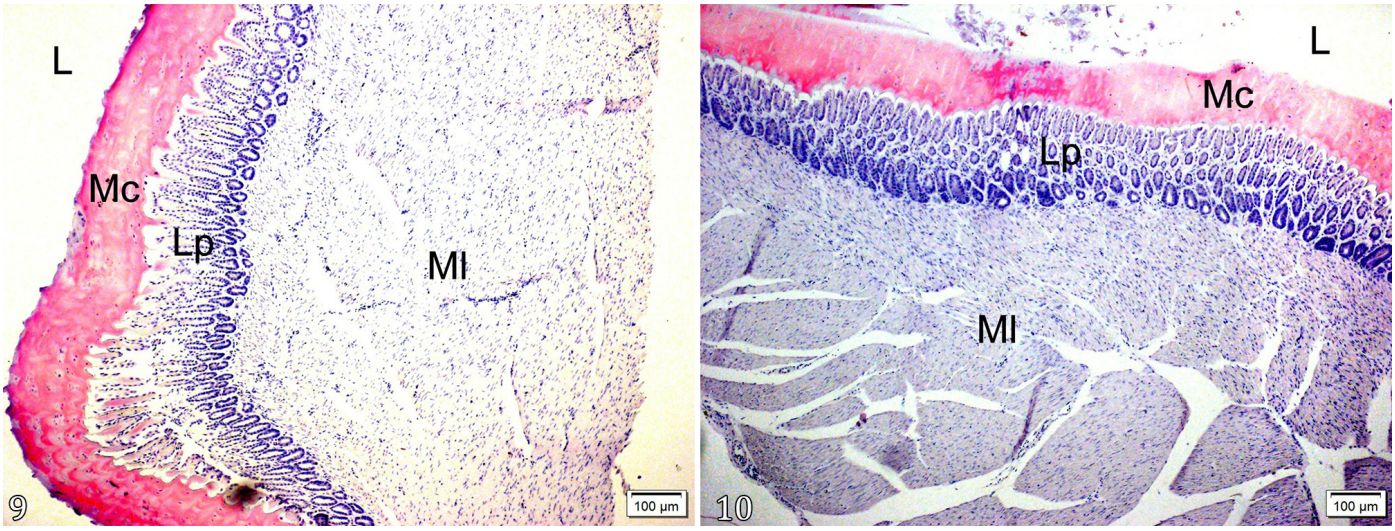

Fig.9-10. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**9**-**10**) Ventricle: lumen (L), mucosal cuticle formed by keratinoid (Mc), lamina propria with glands (Lp), and the thick muscular layer (Ml). HE, bar = 100µm.

center. This mortality and adverse condition come from the stress of capture, transport, storage, and poor food and sanitary conditions, favoring immunosuppression (Sanches 2008).

Assessing the body condition of individuals who arrive at triage centers is important to program adequate food/health management. Nutritional disorders in free-living and trafficked passerines comprise 47.62% of the causes of mortality due to poor conditions of trafficking and handling (Sanches 2008). Echenique et al. (2020) carried out a study of the causes of death in wild birds in the Rio Grande do Sul, finding that 13.31% of mortality corresponded to metabolic or nutritional diseases, with passerines being the most affected order.

Corroborating Firmino et al. (2013) and Rodrigues et al. (2019), the present study demonstrates the importance of nutritional protocols once inadequate nutritional management is the main cause of compromised body condition. Allied with the nutritional factor and body condition, the sanitary and microbiological factor of the individuals is important. This is confirmed since the microbiological examination of the feces showed that in *S. nigricollis* and *S. caerulescens*, 40.25% and 40% were positive for oocysts of coccidia of the genus *Isospora* sp. A study by Sanches (2008) shows even higher values, where 66.66% of passerines had *Isospora* sp. oocysts; 84.21% came from bird trafficking. Boll (2016) describes *Isospora* sp. in 55.6% of the positive enclosures for the parasitosis; in *S. caerulescens*, *Isospora* sp. was present in 3/4 of the samples.

Studies on free-living animals are necessary to analyze which impacts are generated and which coccidia are most important for each species, especially in areas where releases from CETAS occur. In free-living *S. caerulescens* at Acre, 13 fecal samples were analyzed, and 12 samples (92.3%) had parasites, where coccidia was the most prevalent (69%) (Souza et al. 2019). Such data demonstrate that free-ranging animals can be important reservoirs of pathogens from reintroduced birds.

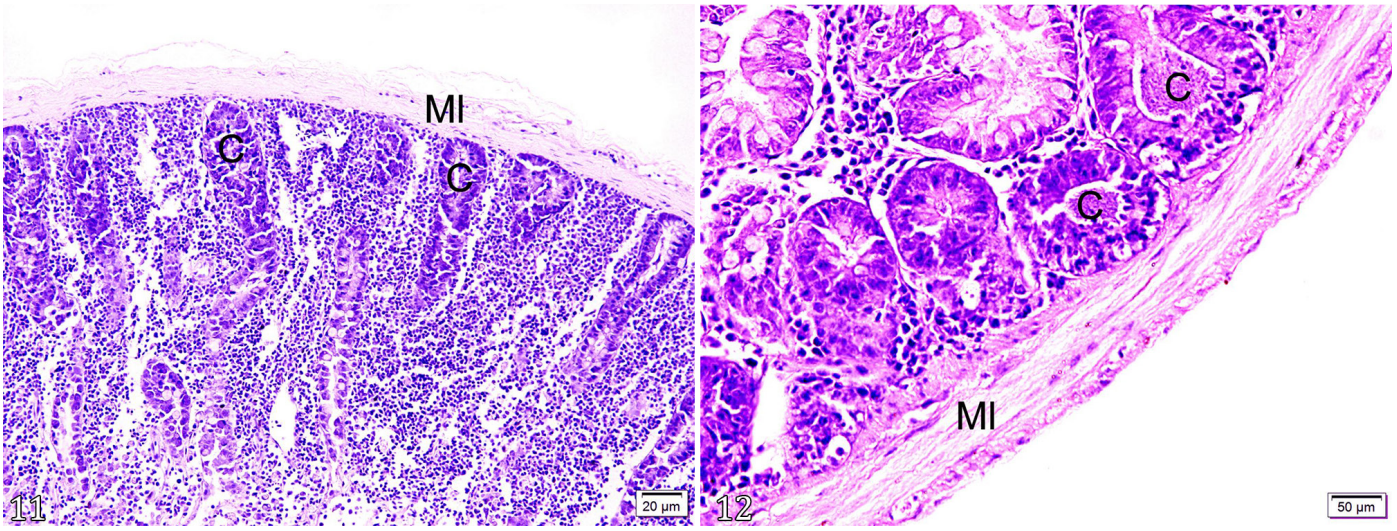

Fig.11-12. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**11-12**) Proximal portion of the intestine: Lieberkühn's crypts (C) in the base of the villi and the muscular layer (Ml) and villus (V). (**11**) HE, bar = 20µm. (**12**) HE, bar = 50µm.

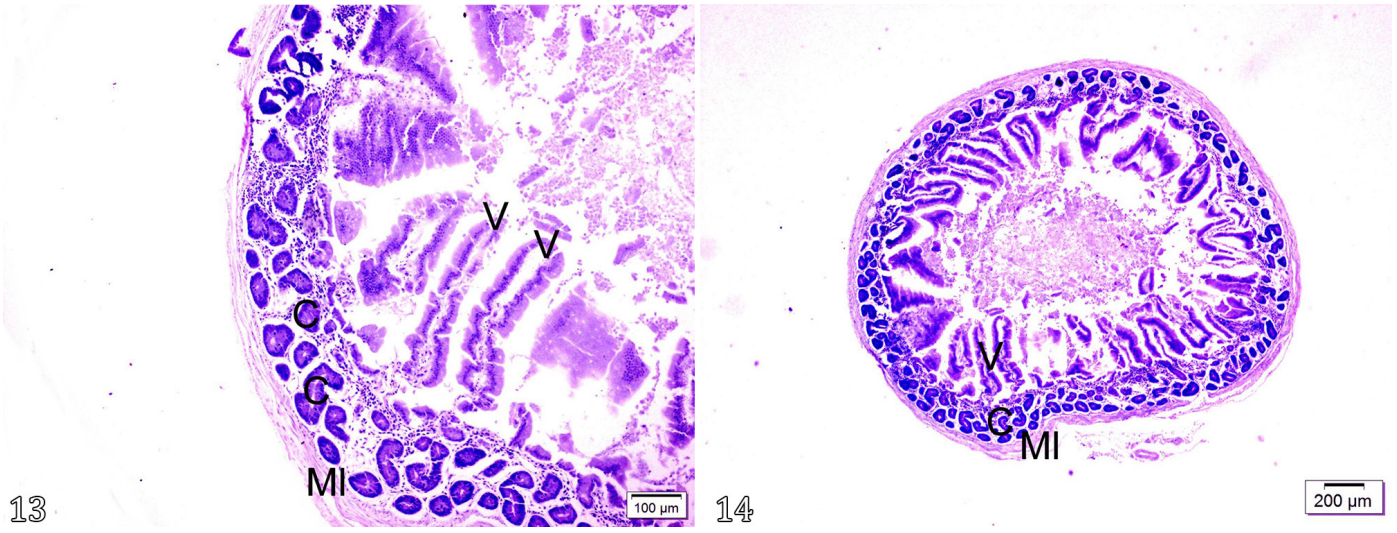

Fig.13-14. Photomicrographs of *Sporophila nigricollis* digestive tract organs. (**13-14**) Distal portion of the intestine: villus (V), Lieberkühn's crypts (C), muscle layer (Ml). Hematoxylin-eosin staining. (**13**) HE, bar = 100µm. (**14**) HE, bar = 200µm.

According to Sanches (2008), the decrease in immunity combined with the regular to bad body conditions observed in most birds favors the contagion of infections. *Isospora* sp. can live harmoniously with the host, causing diseases when there is immunosuppression. Early diagnosis is essential for the correct sanitary management of these animals, collaborating with reducing deaths since the agglomeration of birds facilitates the spread of infection (Barreto 2014).

This study reported the occurrence of *Dispharynx* sp. in *S. nigricollis* and *S. caerulescens* for the first time. According to Cubas et al. (2014), small amounts of Gram-positive cocci or rods, few yeasts, and rare Gram-negative bacteria are normal in passerine feces. In this study, only Gram-positive bacteria were observed, where Gram-positive cocci were more predominant than Gram-positive bacilli.

Of the 41 animals at the necropsy, only 15 underwent coproparasitological examinations while alive. Of these, 11 animals (73.33%) were identified with *Isospora* sp. oocysts, of which seven animals (63.63%) were in cachexia during necropsy, and four animals (36.36%) were in a thin condition, demonstrating a relationship between deaths, the occurrence of parasitism by coccidia and body condition from fair to poor. Of the 41 animals, 19 (43.90%) had feces adhered to the cloaca during the necropsy, suggesting involvement by digestive diseases suggesting that more individuals who did not undergo the examination could be infected.

Regarding the macroscopic findings, of the 41 animals, eight animals (19.51%) had gastrointestinal tract engorgement with hemorrhagic points, six animals (14.63%) had hepatic alterations due to yellow coloration, and two animals (4.87%) showed loss or alteration of plumage. These findings corroborate Monteiro (2019), who observed that lesions associated with malnutrition (27%) and digestive lesions (26%) are the most observed in passerines, with the main digestive lesion observed being hemorrhagic enteritis (82%).

Sanches (2008) shows that in animals affected by coccidia, 61.53% have intestinal alterations compatible with *Isospora* sp., such as mucosal hyperemia, serous congestion, and blackened mucous and fecal content. Microscopically, 92.30% have enteritis; of these, 84.61% had structures compatible with oocysts, and all had a fair/poor body condition.

The 14.63% of specimens in this study with hepatic alteration due to yellowish color were suggestive of hepatic lipidosis. Caseous nodules were observed in four animals (9.75%), and in two animals (4.87%), there was loss or alteration of plumage. This is in agreement with other studies where the animals showed high levels of bodily injury and alteration in plumage and flight feathers, as in Firmino et al. (2013).

In smaller numbers, engorgement of the kidneys, neoplastic nodule adjacent to the kidneys, neoplastic nodule adjacent to the duodenum, yellowish lungs, hemorrhage in the oral cavity, ingluvium stasis and aspergilloma plaques in the air sacs and lungs were seen. These results demonstrate the importance of autopsies in triage centers to close the diagnosis of death and guide health, clinical and nutritional protocols.

Regarding the external biometric measurements of *S. nigricollis*, five variables were found in which males had higher values, demonstrating sexual dimorphism for the species, not only feather coloration.

Regarding the total length of feathers, when compared to other Brazilian passerines, such as *C. brissonii cyanoides* (Firmino et al. 2013) and *S. flaveola brasiliensis* (Siqueira et al. 2013), *S. nigricollis* showed lower values. The feathered wing length measurements of *S. nigricollis* are also smaller than those of *S. flaveola brasiliensis* (Siqueira et al. 2013) and *S. luteola* (Bugoni et al. 2002). The tarsi length in *S. nigricollis* was shorter compared to *S. luteola, S. flaveola brasiliensis,* and *C. brissonii cyanoides* (Bugoni et al. 2002, Firmino et al. 2013, Siqueira et al. 2013). *S. nigricollis* presented similar measurements to the digestive tube to *S. flaveola brasilienses* (Siqueira et al. 2013) and *Cyanoloxia brissonii cyanoides* (Firmino et al. 2013).

As for the proventricle, the length, thickness, and width of the gizzard of *S. nigricollis* have a similar measurement to that of *S. flaveola brasilienses* and *C. brissonii cyanoides* and has an intestine that is extremely smaller than that of these species (Firmino et al. 2013, Siqueira et al. 2013). We verified no difference in the internal biometric measurements between males and females of *S. nigricollis*.

Histologically, the tongue presented KSSE with taste buds similar to *Rupornis magnirostris* (Silva 2016). In the esophagus, very voluminous mucous glands are formed by cubic cells similar to those found in *C. brissonii* (Firmino et al. 2013), and *Pimelodus maculatus* (Santos et al. 2007) were observed in the LP. The ingluvium had the presence of glands in the lamina propria of the mucosa and other features similar to other birds (Sousa et al. 2015).

The proventriculus presented columnar epithelium and submucosal lamina propria with histological characteristics that resemble those of *C. brissonii* (Firmino et al. 2013), *S. flaveola brasiliensis* (Siqueira et al. 2013), *Falco sparverius* (Campi et al. 2020) and *Crypturellus parvirostris* (Silva et al. 2013). In the ventricle, where the mechanical digestion of food occurs, a rough and pleated cuticle was replenished by the underlying glands, also observed in *Crypturellus parvirostris* (Silva et al. 2013). The LP is formed by glands, also identified in *Rupornis magnirostris* (Silva 2016), *Rhynchotus rufescens* (Rossi et al. 2005), and *Crypturellus parvirostris* (Silva et al. 2013).

Table 7. Morphometric measurements of crypt depth (µm), mucosal thickness and muscle thickness of the food duct segments of *Sporophila nigricollis*, seized by the CETAS from 2020 to 2021, concerning mean ± standard deviation (SD) and maximum-minimum

Segments	Crypt depth		Mucosal thickness		Muscle thickness	
	Females	Males	Females	Males	Females	Males
Initial	78.95±36.42	75.11±25.77	313.78±79.26bB	315.77±119.08A	46.08±23.33bB	56.69±33.55bcA
Medium	82.69±40.30	89.69±36.03	395.35±157.49aA	288.42±130.02B	52.37±27.40a	55.82±31.93c
Final	84.70±40.19	110.05±63.28	408.13±175.44a	363.66±325.44	55.63±29.38aB	68.27±25.83abA

Mann-Whitney U test $p<00.5$ between males and females; A,B = Demonstrate a significant difference; Kruskal-Wallis test between bowel portions; a,b,c = Denote a significant difference between the portions of the intestine.

The four layers present in the intestine were similar to those of other birds (Bacha Jr. & Bacha 2003). The villi are important in nutrient absorption, being the same long and fingerlike as in *Caracara plancus* (Almeida et al. 2016) and *R. magnirostris* (Silva 2016).

The destination of seized animals until their reintroduction is an issue that needs to be analyzed because wild passerines are hosts of several infectious agents. Many animals are asymptomatic, but they are efficient transmitters in nature, which can cause outbreaks of diseases that can lead to population decline. Wild animals, making it extremely important to follow pre-established health protocols (Sanches 2008).

This study demonstrates the importance of microbiological and sanitary factors, where there was a high occurrence of intestinal protozoa of sanitary importance. In addition to presenting *Isospora* sp. oocysts, most of the animals that died showed signs of poor body condition. It is, therefore, necessary to adopt sanitary protocols, such as quarantine measures. It is extremely important to carry out complementary exams, identifying pathological agents to establish a safe therapeutic protocol for disease control. It also brings the biometric measurements of external organs of the gastrointestinal tract for the first time, serving as a subsidy for taxonomic studies between species and clinical, reproductive, and nutritional management.

CONCLUSIONS

The present study reveals that most *Sporophila nigricollis* and *Sporophila caerulescens* seized and alive have good body conditions. However, most specimens that died had microbiological infestations and inadequate body conditions.

This seems to be the first report of positivity for *Dispharynx* sp. in *S. nigricollis* and *S. caerulescens*. The same also brings the biometric sexual dimorphism of *S. nigricollis* and also, for the first time, a histological study of the digestive tract of the species.

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