Biology and parasites of *Pteronotus gymnonotus* from the Caatinga shrublands of Ceará (Brazil)

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Mormoopid bats are distributed from southern United States of America to Brazil and comprise the genera *Mormoops* and *Pteronotus*. Although forms of *Mormoops* in Bahia, Brazil were described for the Quaternary, only some of the extant species of *Pteronotus* occur in this country, including *P. gymnonotus*. The species distribution ranges from southeastern México to northeastern Bolivia and central Brazil. This work presents information about food preference, reproduction, and the ectoparasitological fauna of *P. gymnonotus* in the state of Ceará. Fieldwork took place over ten consecutive days in the rain and dry seasons, in 2000, 2012, 2013, and 2019, on trails within the Serra das Almas Private Natural Heritage Reserve. A total of 14 *P. gymnonotus* specimens were caught in the three main phytophysiognomies present in the region. They were inspected for ecto and endoparasites, and fecal material was collected and analyzed under a light microscope. Of the total collected females, four were pregnant and one was lactating in January, during the rain season. No food residues or evidence of endoparasites were observed in the gastric content of the animals. Regarding the fecal samples, fragments of insects were found and identified as belonging to the orders Coleoptera and Lepidoptera. Seven individuals were infested with ectoparasites of the subfamily Acari and newly described associations with Diptera genera are presented. Due to the scarcity of data about these bats in the Caatinga biome, data reported here help explain the ecological interactions of *P. gymnonotus* in this semiarid area in Brazil.

Los murciélagos Mormopidos se distribuyen desde el sur de los Estados Unidos de América hasta Brasil y comprenden los géneros *Mormoops* y *Pteronotus*. Aunque las formas de *Mormoops* son conocidas para el Cuaternario de Bahía, Brasil, solamente algunas de las especies recientes de *Pteronotus* se encuentran en este país. La distribución de la especie comprende desde el sureste de México hasta el noroeste de Bolivia y centro de Brasil. Este estudio presenta información sobre la preferencia alimentaria, reproducción y fauna ectoparasitológica de *P. gymnonotus* en el Estado de Ceará. El trabajo de campo se llevó a cabo durante diez días consecutivos en las estaciones lluviosa y seca en los años 2000, 2012, 2013 y 2019 en senderos dentro de la Reserva Privada del Patrimonio Natural Serra das Almas. Se capturaron un total de 14 especímenes de *P. gymnonotus* en las tres principales fitofisionomías presentes en la región. Fueron inspeccionados en busca de ecto y endoparásitos y el material fecal se recogió y analizó con un microscopio óptico. Del total de hembras recolectadas en enero, cuatro estaban preñadas y una en lactancia, durante la época de lluvias. No se observaron residuos de alimentos ni evidencia de endoparásitos en el contenido gástrico de los animales. En cuanto a las muestras fecales, se encontraron fragmentos de insectos que se identificaron como pertenecientes a los órdenes Coleoptera y Lepidoptera. Siete individuos encontraron infestados por ectoparásitos pertenecientes a la subfamilia Acari y se presentan nuevas asociaciones con géneros del orden Diptera. Debido a la escasez de datos sobre estos murciélagos en el Bioma de Caatinga, los datos presentados aquí ayudan a comprender las interacciones ecológicas de *P. gymnonotus* en una zona semiárida de Brasil.

Keywords: diet; ectoparasite; geographic distribution; hemoparasite; reproduction.

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Introduction

Of the 1,386 species of bats in the world (Burgin *et al.* 2018), the greatest biodiversity occurs in Brazil where there are 182 species (Nogueira *et al.* 2018; Quintela *et al.* 2020). The Mormoopidae was recognized as a family by Smith(1972); previously it was part of the family Phyllostomidae. This group of bats occurs from southern United States of America (U.S.A.) to Brazil and comprises the genera *Mormoops* and *Pteronotus*. Although forms of *Mormoops* in Bahia, Brazil were described for the Quaternary (Czaplewski and Cartelle 1998), only mormoopid extant species occur in this country: *Pteronotus alitonus*, *P. gymnonotus*, *P. personatus*, and *P. rubiginosus*.

Studies by <u>Mares *et al.* (1981)</u>, <u>Willig (1983)</u> and <u>Willig and</u> <u>Mares (1989)</u> on the chiropterofauna of the Brazilian shrubland areas report the occurrence of *P. davyi* in the states of Ceará and Piauí. Later, <u>Simmons (2005)</u> and <u>Patton and</u> <u>Gardner (2007)</u> considered *P. gymnonotus* as a junior synonym of *P. davyi*, and thus these authors did not recognize the occurrence of this species in Brazil. The distribution of *P. gymnonotus* ranges from southeastern Mexico to northeastern Bolivia and central Brazil (<u>Pavan and Tavares 2020</u>). The first record of *P. gymnonotus* for the state of Ceará was published by <u>Mares *et al.* (1981</u>), who mistakenly identified specimens collected in the municipality of Crato as *P. davyi* (<u>Patton and Gardner 2007</u>). However, studies carried out in the Crato region, and the municipalities of Crateús and Aiuaba (<u>Novaes 2012</u>; <u>Novaes and Laurindo 2014</u>; <u>Silva *et al.* 2015; Feijó and Rocha 2017</u>), confirmed the occurrence of *P. gymnonotus* in this state. Mormopids are found up to 3,000 m elevation and inhabit caves, rock crevices, open areas and dry deciduous and humid forests (Herd 1983; Patton and Gardner 2007). They are classified as aerial insectivorous bats (Simmons and Conway 2001; Ladle *et al.* 2012) and their diet consists mainly of insects of the order Coleoptera, as well as other hexapods, such as those belonging to the orders Lepidoptera, Diptera, Orthoptera, Hymenoptera, Hemiptera and Odonata (Rolfe and Kurta 2012).

<u>Machado-Allison (1965)</u> highlighted the high degree of specificity between *Cameronieta* species (Acari) and their respective hosts and advocated for the removal of Chilonycterine bats from the family Phyllostomidae. Subsequently, <u>Smith (1972a)</u> showed that ectoparasites of spinturnicid mites and flies found in mormopids are different from those recorded in phyllostomid bats.

The list of ectoparasites associated with *P. gymnonotus* includes the mite *Cameronieta* sp. (Almeida *et al.* 2016), Dipterans *Trichobius johnsonae* and *Nycterophilia fairchildi* (Figueiredo *et al.* 2015; Dick *et al.* 2016), and the tick *Ornithodoros marinkellei* (Luz *et al.* 2016). Regarding endoparasitic fauna, there are no records of helminths infesting these bats, only the presence of protozoan hemoparasites (Lima *et al.* 2015). Due to the scarcity of data on ecological associations of mormoopid bats in the Caatinga biome (northeastern Brazilian shrublands), this work provides information on food preference, reproduction, and evidences of new ectoparasites in association to *P. gymnonotus* in the state of Ceará.

Material and Methods

Fieldwork took place over ten consecutive days in the rain and dry seasons of 2000, 2012, 2013 and 2019, on trails within the Serra das Almas Private Natural Heritage Reserve (SANR; -5° 5' to -5° 15' S, -40° 50' to -41° 00' W) in the western part of Ceará State. The reserve is 5,845 ha (Figure 1) and was recognized in 2018 by the Latin American Network for the Conservation of Bats (RELCOM) as the first "Area of Importance for the Conservation of Bats" (AICOM - A-BR-002) in the Caatinga biome. The climate of the region is BSh (semi-arid climate) according to the Köppen classification system (Alvares et al. 2013), with a dry summer and an average annual temperature of 26.5 °C. Rain is highly irregular, which is typical for this Brazilian semiarid zone. The average rainfall is 870 mm/year, the rain season occurs from January to May, and the dry season occurs from June to December (INMET 2020). Three main phytophysiognomies of Caatinga vegetation, in different successional stages, are in the reserve: dry forest, carrasco scrub, and arboreal caatinga (Associação Caatinga 2012).

Bats were captured using eight mist nets extended from 18 to 24h, at elevations varying from 569 to 700 masl, in the three main phytophysiognomies in the region. Fecal samples were placed in plastic microtubes, ectoparasites were removed with fine-tipped tweezers and hematological slides were made according to <u>Baptista and Esbérard (1997)</u> to verify the occurrence of hemoparasites. For each specimen body measurements (total lenght, forearm, foot, ear, tibia, calcaneus and tail) were obtained using a digital caliper; weight and reproductive condition were also recorded.

Abdominal and thoracic cavities of necropsied specimens were examined to search for endoparasites. The fragments of insects found in the feces were mounted on permanent slides. Blood smears were prepared by using the "Panotic" rapid staining method (NewProv®) and observed using a Biofocus optical microscope (model Bio1600 BA-H; 100x and 400x). Dipterans were identified using a stereomicroscope (40x) and based on descriptions and keys by Wenzel et al. (1966) and Guerrero (1993; 1994; 1995). Mites were prepared using a clarification method following the Flechtmann (1990) technique, mounted on slides with a cover slip, and identified using the taxonomic key by Machado-Allison (1965). The bat specimens collected as vouchers are in the Adriano Lúcio Peracchi Collection (ALP-UFRRJ), currently housed at the Federal Rural University of Rio de Janeiro (UFRRJ / RJ), and the slides with the ectoparasites were deposited in the Acarological Collection at the Oswaldo Cruz Foundation -FIOCRUZ (Rio de Janeiro - RJ). The capture and collection of specimens was authorized by the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) of the Brazilian government (authorization number 32684-1).

Results

From a sampling effort (Straube and Bianconi 2002) of 103,313 m² h, a total of 14 *P. gymnonotus* specimens (12 females and two males) were caught between 620 and 700 masl elevation in the three phytophysiognomies in the region (Appendix 1). Of the total collected females (n= 12), four were pregnant and one was lactating (producing milk in the breast) in January, during the rain season. Two males and seven females were sexually inactive and were collected in both dry and rain seasons. Biometric data from these specimens are available in Table 1.



Figure 1. Location of Serra das Almas Private Natural Heritage Reserve in Crateús (Ceará, Brazil; Software ArcGis 10.1- Esri Maps; Prepared by: Sergio Carvalho Moreira, modified by Shirley Seixas Pereira da Silva).

 Table 1. Mean biometric (in mm) data from *P. gymnonotus* specimens from Crateús (Ceará, Brazil).

	Pteronotus gymnonotus		
	Males (<i>n</i> = 2)	Females (<i>n</i> = 12)	
Weight (g)	11.0 - 13.0	10.0 - 17.0	
Total lenght	46.6 - 54.1	48.8 - 57.4	
Forearm	48.7 - 51.0	48.4 - 51.2	
Ear	12.8 - 17.6	11.9 - 18,7	
Tibia	21.0 - 21.6	18.1 - 22.0	
Foot	9.5 - 10.7	9.1 - 11.5	
Tail	20.6 - 22.7	16.1 - 21.3	

Of the *P. gymnonotus* specimens captured, it was possible to analyze the gastrointestinal content and fecal samples of three and 13 specimens, respectively. For three bats, both gastrointestinal and fecal samples were analyzed. No food residue or evidence of endoparasites was observed in the gastric content of the animals. Regarding the fecal samples, fragments of insects were found and identified as belonging to the orders Coleoptera and Lepidoptera (Figure 2). Some fragments were crushed, which made it impossible to identify them (38.2 %). The Coleoptera families consumed by bats in SANR were identified as Carabidae and Elateridae (Figure 3). Regarding the order Lepidoptera, the wing scales found were associated with the family Sphingidae (hawk moths: Figure 3).

When comparing diet between males and females of *P. gymnonotus* in our sample at SANR, both consumed Lepidoptera in dry and rain periods; only males consumed coleopterans in the rain season; the Coleoptera / Lepidoptera combination was consumed only by females in the rain season.

Seven individuals of *P. gymnonotus* were infested with ectoparasites. Specimens of the order Diptera and subfamily Acari were collected after checking the coat, patagium and uropatagium of the bats (Figure 3). Dipterans of Streblidae were identified as *Nycterophilia parnelli* (ALP 10194), *Megistopoda aranea* (ALP 10390), and *Trichobius johnsonae* (ALP 10386; ALP 10389; ALP 10422). Mite specimens were identified as *Cameronieta* sp. (Spinturnicidae).

Three bats were infested with both mites and dipterans, and one specimen had two Diptera species in its coat (Table 2). Regarding endoparasitic fauna, there were



Figure 2. Food preferences of 13 P. gymnonotus specimens from Crateús (Ceará, Brazil).

no records of infestation by helminths in the three bats inspected, and the analysis of the hematological slides did not show presence of hemoparasites.

Table 2. Ectoparasites found in association with *P. gymnonotus* from Crateús (Ceará, Brazil).

Ectoparasites				
Bats	Spinturnicidae		Streblidae	
	Cameronieta sp.	Trichobius johnsonae	Nycterophilia parnelli	Megistopoda aranea
ALP 10194			2♀	
ALP 10386	18	1♀		
ALP 10389		1♂ / 2♀		
ALP 10390				1♂/1♀
ALP 10422	1 pn / 3 🕉	2♂/3♀		
REG 188	1 pn		1්	
REG 201	1්			
REG 314		1♀		
REG 362	aph	1♂/1♀	1♀	

pn = protonymph

Discussion

Pteronotus gymnonotus is an aerial insectivorous bat species that uses closed spaces as a foraging and feeding strategy, in the lower strata of the canopy and understory, and occurs in dry environments at elevations up to 1,000 masl (Norberg and Rayner 1987; Bredt *et al.* 1999; Denzinger and Schnitzler 2013; Novaes and Laurindo 2014; Pavan and Tavares 2020). In the Serra das Almas Private Natural Heritage Reserve, individuals were collected in the understory in all three phytophysiognomies in the area, at elevations ranging from 620 to 700 masl, confirming the pattern found for the species by previous studies.

Silva et al. (2015) publication on the biodiversity of bats in SANR included *P. gymnonotus*, but these authors did not record the new area of distribution of the species in Ceará State. The records of specimens near to the border of Piauí State extends its distribution to the north by 167 km in relation to the municipality of Aiuaba and 266 km in relation to the municipality of Crato, locations in Ceará where the occurrence of the species has already been registered.

Measurements taken from SANR P. gymnonotus specimens are in accordance with those previously published (Pavan and Tavares 2020). The reproductive pattern known for mormoopid bats is the seasonal monoestrous type, where pregnancy occurs from January to May and the offspring is born at the end of the rain season, giving birth to one young per pregnancy (Nowak 1994; Reis et al. 2006; Pavan and Tavares 2020). Feijó and Rocha (2017) registered pregnant females at the end of December in the municipality of Aiuaba (Ceará), which corresponds to the end of the dry season and the beginning of the rain season. The results presented here confirm this pattern; pregnant females were collected in January at the beginning of the rain season. However, the record of a lactating female in January differs from that reported by Nowak (1994), who recorded births in May at the end of the rain season.



Figure 3. Material associated to *P. gymnonotus* from Crateús (Ceará, Brazil). Hexapoda fragments found in fecal samples: Lepidoptera proboscis (a), scales (b, c), antennae (d, e), and leg (f); Elateridae (Coleoptera) leg and antennae (g, i); undetermined Coleoptera leg (h); Carabidae (Coleoptera) leg (j). Ectoparasites: *Cameronieta* sp. (male; k); *Nycterophilia parnelli* (female; l); *Trichobius johnsonae* (male; m); *Megistopoda aranea* (male; n); *Megistopoda aranea* (female; o; Photos of fragments by Flávia Silva Severino and ectoparasites by Juliana Cardoso de Almeida).

With a strictly insectivorous diet, Mormopid bats food items include mainly coleopterans and lepidopterans, and smaller quantities of dipterans, orthopterans, homopterans, odonates and hymenopterans (Howell and Burch 1974; Whitaker and Findley 1980; Mancina 2005; Rolfe and Kurta 2012; Pavan and Tavares 2020). According to Rolfe and Kurta (2012), factors such as sex, age and reproductive condition could influence the feeding preference of insectivorous bats.

The consumption of Lepidoptera (15.4 %) and Coleoptera (15.4 %) in SANR reinforces the preference for these groups of Hexapoda. Some individuals consumed a combination of prey that included Coleoptera + Lepidoptera, which represented the highest percentage of fragments found (31%). These results differ from those found by Whitaker and Findley (1980), who recorded a preferential intake of Coleoptera, followed by a much smaller amount of Hymenoptera and, lastly Lepidoptera; the Coleoptera family consumed was not mentioned by the authors. The difference in diet found in this study with that recorded by Whitaker and Findley (1980) may be related to the local entomofauna where the studies were carried out. Studies conducted by Molinari et al. (2000) in Venezuela indicated that P. gymnonotus consumes 35 families of insects from ten orders, of which Coleoptera is the most representative. Howell and Burch (1974) observed the ingestion of only lepidopterans by P. gymnonotus in Costa Rica. In the case of SANR food residues, only the consumption of moths from family Sphingidae was verified.

Parasitological studies on South America Mormopids bats were conducted by <u>Guerrero (1985)</u>, <u>Guerrero et al.</u> (2003; 2006) and <u>Travassos (1965)</u>. These authors identified microfilaria (Nematoda) in the systemic blood and intestine of *P. parnellii*. The necropsied individuals of *P. gymnonotus* from SANR did not display any endoparasites. Helminthic infestations for this species reported in the literature are unknown. There are only records of *Trypanosoma wauwau* hemoparasites in the states of Pará and Sergipe (Lima et al. 2015; Pavan and Tavares 2020).

Studies on P. gymnonotus ectoparasites collected in the Caatinga biome are scarce. The only records are cited by Almeida et al. (2016) and Luz et al. (2016). The former was conducted in SANR and lists Cameronieta sp. (Spinturnicidae) in association with this species in the area. Regarding Diptera, the results of the present study are significant considering that Frank et al. (2014) reported Nycterophilia parnelli in association with P. davyi and P. parnellii in Belize, Costa Rica and Venezuela; Trichobius johnsonae parasitizing P. davyi in Belize, Costa Rica and Venezuela; P. gymnonotus in Venezuela and Panama; P. parnellii in Belize; and P. personatus in Venezuela and Panama. That is, all of these fly species are new records for P. gymononotus in Brazil. The association of Megistopoda aranea with P. gymnonotus is a new evidence that makes this mormopid bat a potential new host for this Diptera species. Although a protocol to avoid contamination was implemented, further investigation is required.

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Appendix 1

Examined material (Abbreviations: ALP - catalogue number for the Adriano Lúcio Peracchi Collection, UFRRJ; REG - specimen released): *Pteronotus gymnonotus* – ALP 10192, ALP 10194, ALP 10386, ALP 10389, ALP 10390, ALP 10422, REG 107, REG 138, REG 140, REG 141, REG 188, REG 201, REG 314, REG 362.

BIOLOGY AND PARASITES OF P. GYMNONOTUS