

## Research Note

**Helminth parasites and diet of *Leptodactylus petersii* (Steindachner, 1864) (Anura: Leptodactylidae) from Amapá state, Eastern Amazon, Brazil**

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**Article info**

 Received April 10, 2020  
 Accepted May 20, 2020

**Summary**

*Leptodactylus petersii* is a species of anuran found in both terrestrial and aquatic habitats and occurs from South America to southern North America and the West Indies. Studies involving the fauna of anuran parasites offer complementary information related to ecology. Thus, since there are few studies on the natural history of this species, this research aims to analyze the diet and the presence of endoparasitic helminths of *Leptodactylus petersii* from the state of Amapá, Brazil. We found 10 different taxonomic categories of prey in stomach contents, with the categories Hymenoptera (Formicidae) with 32.26 % (n = 12) being the most representative. Among the 12 individuals of *L. petersii* that were analyzed for helminth parasites, 83.3 % were infected with at least one species of helminths allocated to Phylum Nematoda. Our results report a new occurrence site for *Rhabdias breviensis*, originally described for *Leptodactylus petersii* in the state of Pará, as well as the second report of *Ortleppascaris* sp. in Brazil.

**Keywords:** Leptodactylidae; diet; nematodes, Amazon

**Introduction**

Studies about the parasite fauna of anurans may offer complementary information related to the ecology, behavior and feeding habits of the host, revealing the trophic interactions between the host and the environment, especially when considering that the life cycle of many parasite species are strictly linked to the food web interactions (Marcogliese, 2004; Dobson *et al.*, 2008). Thus, information about feeding habits and parasite infection are important aspects of interactions between the anuran and the environment. *Leptodactylus petersii* (Steindachner, 1864) is an anuran species found in many habitats, both terrestrial and aquatic, and occurs from South America to the south of North America and in Occidental Indias (Lima *et al.*, 2006). *L. petersii* is allocated in the species group *Leptodactylus melanonotus* and is characterized by having

snout vent length varying between 32 to 40 mm for males and between 35 to 45 mm for females, being of nocturnal habits and dwelling in forests and flooded pastures, occurring in the Amazon and in the Guiana shield region (De Sá *et al.*, 2014).

As there are scarce studies about the natural history of this species, this research aims to present and describe the endoparasite helminth composition and the diet of *Leptodactylus petersii* from the state of Amapá, Brazil.

**Material and Methods**
*Study area and sampling*

The specimens of *Leptodactylus petersii* were manually collected in floodplain areas, located in the state of Amapá. These areas are found mainly in river margins determined by the tides, char-

acterized for having a nutrient rich soil, however, they are fragile environments with origins linked to sediments deposition. These environments are used in plant extraction and, in the state of Amapá, the widest floodplain forests occur along with the amazonic waterfront (Amapá, 2002). From September 2017 until June 2018, 87 *L. petersii* individuals were collected through active and acoustic search (Heyer *et al.*, 1994)

#### Parasite collection and identification

Among 87 individuals, 12 were sent to the laboratory of Zoology of the Federal University of Amapá, where their weight and length were measured with a caliper (Mitutoyo®) and scales. The frogs were euthanized, and all their organs were dissected and analyzed for collection of gastrointestinal contents and endoparasites. The helminths found were killed with 70 % alcohol preheated at 85°C and preserved in the same solution at room temperature. All helminths collected were cleared in Aman Lactophenol and mounted in temporary microscope slides. The identification of the helminths was performed using taxonomic keys proposed by Anderson, (2000) and Vicente *et al.*, (1991) and species original description articles.

#### Diet composition analysis

The remaining 75 collected specimens went through the stomach flushing technique, which consists in stomach washing with the purpose of analyzing the diet items consumed by the individuals (Solé *et al.*, 2005), and were later returned to nature. Regarding the diet, the stomach contents were analyzed under stereomicroscope and the identification of the preys in the samples was done with the aid of the identification key proposed by

Rafael *et al.*, (2012) and the volume was measured through the ellipsoid formula (Magnusson *et al.*, 2003), where *V* represents prey volume, *l* = item length and *w* = item width.

$$V = \frac{4}{3} \pi \left(\frac{l}{2}\right) x \left(\frac{w}{2}\right)^2$$

The Importance Value Index (IVI) was also measured, through the equation below (Where *IVI* = Importance Value Index; *F%* = occurrence frequency; *N%* = numerical frequency; *V%* = volumetric frequency of each prey category in the diet):

$$IVI = \left(\frac{F\% + N\% + V\%}{3}\right)$$

The level of specificity of the diet was analyzed using the Levins Index (Pianka, 1986), where results from 0-0.50 show diet specificity and values from 0.51-1.00 show generalist feeding habits. Where *B* =Levins index (trophic niche breadth); *i* = prey category; *n* = number of categories; *pi* = numerical or volumetric proportion of the category of prey *i* in the diet.

$$B = 1 / \sum_{i=1}^n p_i^2$$

The correlations between body variables and largest prey volume from each individual were done through simple linear regression according to Zar (1999). We used 0.05 as the significance threshold.

Table 1. Number of items found in the diet (N%), occurrence (F%), volume (V%) and Importance Value Index (IVI) in the diet of *Leptodactylus petersii*.

Prey item	N	N (%)	F	F (%)	V	V (%)	IVI
<b>Phylum Arthropoda</b>							
<b>Class Arachnida</b>							
Acari	8	22.86	7	22.58	0.78	0.41	15.28
<b>Class Insecta</b>							
Diptera	1	2.86	1	3.23	0.36	0.19	2.09
Collembola	1	2.86	1	3.23	0.03	0.02	2.03
Coleoptera	7	20.00	6	19.35	52.52	27.55	22.30
Hymenoptera	1	2.86	1	3.23	8.57	4.50	3.53
Formicidae	12	34.29	10	32.26	9.36	4.91	23.82
Coleoptera larvae	1	2.86	1	3.23	40.92	21.47	9.18
Diptera larvae	2	5.71	2	6.45	16.28	8.54	6.90
Orthoptera	1	2.86	1	3.23	7.31	3.83	3.31
<b>Class Malacostraca</b>							
Isopoda	1	2.86	1	3.23	54.5	28.59	11.56
<b>Total</b>	<b>35</b>	<b>100</b>	<b>31</b>	<b>100</b>	<b>190.63</b>	<b>100</b>	<b>100</b>

## Ethical Approval and/or Informed Consent

All applicable institutional, national and international guidelines for the care and use of animals were followed. Host specimens were collected under permits ICMBio (# 48102-2).

## Results

We collected 87 individuals (36 adults and 51 juveniles), with the 36 adults showing SVL of 17.56 – 46.85 mm ( $33.77 \pm 6.53$  mm) and weighting 0.5 – 11.00g ( $4.23 \pm 2.55$ g), while the 51 juvenile individuals had SVL of 13.38 – 26.5 mm ( $16.37 \pm 2.30$  mm) and weighted 0.2 – 1.6 g ( $0.48 \pm 0.22$  g).

Out of the 87 *Leptodactylus petersii*, 64 (73.6 %) had empty stomachs and only 23 (26.4 %) had at least one diet item in their gastrointestinal content. These consumed 10 different taxonomic categories of preys, but Hymenoptera (Formicidae) and Acari had the highest frequency in the diet of *L. petersii*, representing 32.26 % (n=12) and 22.58 % (n=8), respectively of prey items. Though, when it comes to volume, Isopoda (28.59 %) contributed with the highest volume in the diet, followed by Coleoptera (27.55 %). The Importance Value Index (IVI) shows that the most representative category was Hymenoptera (Formicidae) with 23.82 % (Table 1). The niche width value was 0.39.

We did not find significant correlation between SVL and prey volume ( $r_s=0.2402$ ,  $p=0.2695$ ) and between mandible width and prey volume ( $r_s=0.2443$ ,  $p=0.2613$ ), however, the correlation between the weight of the animal and volume of the largest prey consumed was significant ( $r_s=0.4322$ ,  $p=0.0394$ ).

Among the 12 *L. petersii* individuals that were analyzed for helminths parasites, 83.3 % were infected by at least one helminth species of Nematoda, no other groups of parasites were found. In total, 12 nematodes of three taxa were found, with prevalence and abundance of female *Cosmocercidae* gen. sp. (7 out 12; 58.3 % of prevalence) collected from the large and small intestines, followed by *Rhabdias brevisensis* (4 out of 12; 33.3 %) from the lungs and one host was infected by *Ortleppascaris* sp. larvae found encysted in the liver tissue.

## Discussion

The individuals consumed some preys like the ones found in the work by Teles *et al.*, (2018), where the diet of *Leptodactylus macrosternum* was analyzed and had the categories Coleoptera and Hymenoptera (Formicidae) as the most frequent items. The diet of these specimens showed similarities with various works involving the diet of Leptodactylids, such as the work by Piatti & Souza (2011), which showed the Orders Coleoptera and Hymenoptera as the most frequent for *Leptodactylus podicipinus* individuals from a rice field in the wetlands.

In this study, we observed differences in relation to previous studies, for instance, the one by Ceron *et al.*, (2018), which also ana-

lyzed the diet of *Leptodactylus podicipinus*, belonging to the same *Leptodactylus melanonotus* species group, where the prey category with the highest IVI was Coleoptera; however, the *L. petersii* individuals analyzed in this study had Hymenoptera (Formicidae) as the most representative item.

Regarding the niche width value (0.39), it can be related to the availability of ants in the environment, as those have a great biomass in the tropical forests (Holldobler & Wilson, 1990), becoming then, the most consumed item by the individuals. The majority of Leptodactylidae species have generalist habits and are sit and wait foragers, feeding of large and soft body preys, but that is not the case with *L. petersii*, that fed mainly of ants and mites. Records of ants in the diet of species from of the Genus *Leptodactylus* are also found in the work by Ferreira *et al.*, (2007), about the diet of *L. natalenses*. These differences between diets of species of Leptodactylids can be attributed to the availability of preys, reflecting a generalist behavior in this species. Therefore, there is no feeding pattern for this group, as opposing to the observed in Dendrobatids, which showed preference for a determined type of prey (Toft, 1980; Grant *et al.*, 2006).

We did not observe significant correlation between SVL and volume and mandible width and volume, presenting that both variables do not influence in the volume of the prey, which may be related to the type of prey consumed and, this type of influence may be much more common in frogs that feed of larger preys than in those that consume smaller preys, such as ants and mites, which is also observed in the work by Camera *et al.*, (2014), where the authors also did not find significant correlation between SVL of *L. mystaceus* and prey volume. The correlation between weight of animal and volume showed different results, as it was significant. In our study we found only nematodes and an incredibly low parasite species richness (three species), and the reduced parasite species richness among *Leptodactylus* spp. was also observed by Goldberg *et al.*, (2009), that found only two species of nematodes when they surveyed parasites from 31 specimens of *L. petersii* (*C. podicipinus* with 52 % of prevalence and *Physaloptera* larvae 23 % of prevalence) collected in Tocantins state, Brazil and also Bursey *et al.* (2001) that reported only *Cosmocerca brasiliense* infecting *L. petersii* from Peru.

In other hand, some authors reported a higher parasite diversity when studying the parasite community of Leptodactylids, for instance Goldberg *et al.*, (2002) studying the parasite fauna of *Leptodactylus melanonotus* (same species group for *L. petersii*) found 11 different species of parasites and the studies performed by Campião *et al.*, (2012, 2014, 2016) Hamann *et al.*, (2012), González & Hamann, (2006), González & Hamann, (2016), Teles *et al.*, (2014) found an average of 12 species of helminths infecting different *Leptodactylus* spp. hosts.

According to Campião *et al.*, (2015) the main determinants of parasite richness are related to the anurans body size. However, if we compare the body size and parasite richness of hosts studied by those authors, it's possible to observe that they all belong to small

moderate body size, indicating that other factors might be influencing in the parasite richness of *Leptodactylus* spp. from those studies. For example, another important determinant of parasite richness is the host habitat (Bush *et al.*, 1990, Campião *et al.*, 2016), and this might be related to the differences found in studies performed in Amazon Region (present study, Bursey *et al.*, 2001 and Goldberg *et al.*, 2009) and in other regions, considering that fragmentation and habitat differences can be a factor influencing the parasitic community (Gibb & Hochuli, 2002; Hamann *et al.*, 2006). We also observed females of *Cosmocercidae* gen. sp. as the most prevalent parasite (58.3 %), however, as the morphology of these nematodes are conserved and uniform, it is very difficult to differentiate them and we could not even allocate these specimens among the genera. The second most prevalent parasite was *Rhabdias breviensis*, which had already been recorded for *L. petersii* in Breves, Pará (Nascimento *et al.*, 2013). The adult nematodes found in this study presented direct life cycle and infect the hosts through different modes, including oral infection and through active penetration of infective larvae, when those come into contact with the soil. Thus, as *Leptodactylus petersii* is a terrestrial species, their contact with the soil makes infection possible by nematode larva.

*Ortleppascaris* sp. larvae had already been reported parasitizing *Prismantis cf. terraebolivaris* (Moravec & Kaiser, 1995), from Tobago, *Leptodactylus bufonius* and *Rhinella fernandezae* (Gonzalez; Hamann, 2006; Gonzalez & Hamann, 2007) in Argentina and *Rhinella marina* in Belém, Brazil (Silva *et al.*, 2013), while the adult nematodes were reported parasitizing the intestines of crocodylians (genera *Crocodylus*, *Caiman* and *Alligator*) (Sprent, 1978; Goldberg *et al.*, 1991; Waddle *et al.*, 2009). Although the life cycle of these nematodes is yet to be known, the presence of these larvae in anurans suggests that the infection route for these helminths is through the ingestion of the prey, which indicates that the anurans participate as potential intermediate/paratenic hosts. The parasites recorded for *L. petersii* up until this moment are *Cosmocerca podicipinus*, *Physaloptera* sp. in Tocantins (Goldberg *et al.*, 2009), *Rhabdias breviensis* from the Breves municipality, Pará (Nascimento *et al.*, 2013), and *Cosmocerca brasiliense* in the reserve of Cuzco, in the Peruvian Amazon (Bursey *et al.*, 2001). Thus, our results report a new occurrence location for *Rhabdias breviensis*, as well as the second report of larvae of *Ortleppascaris* sp. in Brazil, raising the hypothesis that these anurans are acting as intermediate/paratenic hosts in the life cycle of these parasites.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Acknowledgements

We thank Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO/ICMBio # 48102-2), for issued permits. We are also

grateful to Universidade Federal do Amapá and Universidade Federal do Pará for their support. This work was supported by PPG-BAIP/UFGA the National Council for Scientific and Technological Development (CNPq) (grant number 431809/2018-6 Universal); CNPq Research grant productivity to MELO, F. T. V. (Process N° 304955/2018-3)

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