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Diet and nematode infection in *Proceratoprhys boiei* (Anura: Cycloramphidae) from two Atlantic rainforest remnants in Southeastern Brazil

THAÍS KLAION[1], MAURICIO ALMEIDA-GOMES[1], LUIZ E.R. TAVARES[2], CARLOS F.D. ROCHA[1] and MONIQUE VAN SLUYS[1]

[1]Departamento de Ecologia, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, Maracanã, 20550-013 Rio de Janeiro, RJ, Brasil
[2]Laboratório de Ictioparasitologia, Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, 23890-000 Seropédica, RJ, Brasil
Present address: Laboratório de Parasitologia Veterinária, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Mato Grosso do Sul, Avenida Costa e Silva, s/n, Cidade Universitária, 79070-900 Campo Grande, MS, Brasil

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ABSTRACT

Proceratophrys boiei is an endemic cycloramphid anuran inhabiting the leaf litter of Atlantic rainforests in Southeastern Brazil. We analyzed the whole digestive tract of 38 individuals of *Proceratophrys boiei* collected in two Atlantic Rainforest areas in the state of Rio de Janeiro, Brazil, to study the diet composition and the helminth fauna associated with this species. The main food items in *P. boiei'*s diet were Coleoptera, Orthoptera and Blattaria. Five nematode species were found: *Aplectana delirae*, *Cosmocerca parva*, *Oxyascaris oxyascaris*, *Physaloptera* sp. (larval stage only) and an unidentified nematode. Overall prevalence was 71% and mean infection intensity was 7.3 $\pm$ 5.8 nematodes per individual.

Key words: Anura, *Proceratoprhys*, diet, endoparasitism, neotropics.

INTRODUCTION

The neotropical genus *Proceratophrys* (Anura: Cycloramphidae) is distributed mainly in Brazil, with a few species also occurring in Paraguay and northern Argentina (Amaro et al. 2009). These frogs occur through a wide variety of biomes: from the Atlantic Rainforest domain (Izecksohn and Peixoto 1981, Kwet and Faivovich 2001, Prado and Pombal Jr 2008) to the Brazilian Cerrado (Eterovick and Sazima 1998, Amaro et al. 2009, Moreira et al. 2009), the semi-arid Caatinga (Rodrigues 2003), with one species reaching the Amazon rainforest (Giaretta et al. 2000). *Proceratophrys* species are important components of leaf litter communities in Neotropical forests (Rocha et al. 2001, Boquimpani-Freitas et al. 2002) and, because they are relatively large anurans, they can potentially feed upon a large spectrum of prey types and sizes. Studies on diet composition of *Proceratophrys* species (Giaretta et al. 1998, Boquimpani-Freitas et al. 2002, Teixeira and Coutinho 2002) report the consumption of arthropods, although small vertebrates (frogs) have also been found in some specimens (Boquimpani-Freitas et al. 2002), which may indicate their importance in regulating some species from the arthropod communities, as well as populations of small vertebrates of the leaf litter of tropical forests. Information on the endoparasitic fauna is restricted to only one species, *P. tupinamba* (= *P. appendiculata*; see Prado and Pombal Jr 2008), for which five nematodes and one cestode species were reported (Boquimpani-Freitas et al. 2001).

Correspondence to: Thaís Klaion Ferreira
E-mail: thaiskferreira@gmail.com

Proceratophrys boiei (Fig. 1) is endemic to the Brazilian Atlantic Forest domain (though also occurring in areas of transition with Cerrado) (Prado and Pombal Jr 2008), has horn-like appendages above the eyes and a cryptic morphology and coloration (Haddad and Sazima 1992, Costa et al. 2009). The Atlantic Forest supports high rates of anuran biodiversity and equally high threats of disappearance of species (Duellman 1999, Rocha et al. 2001, Young et al. 2004). Recent estimates of existing Atlantic Forest cover report about 11.7% of its original area, and most of it remains as small, relatively isolated fragments (Ribeiro et al. 2009). The Estação Ecológica Estadual do Paraíso (EEEP) and the Reserva Ecológica de Guapiaçu (REGUA) are two protected forest areas in the state of Rio de Janeiro where *P. boiei* occurs. In this study we evaluate to what extent diet composition and ecological parameters of its endoparasite fauna vary among these populations. Furthermore we present the first report on its endoparasitic fauna.

MATERIALS AND METHODS

We conducted field samplings in two Atlantic rainforest areas in the state of Rio de Janeiro, Southeastern Brazil. Both constitute Conservation Units: the Estação Ecológica Estadual do Paraíso (EEEP, 22°29′S, 42°55′W; 40-290 meters high) and the Reserva Ecológica de Guapiaçu (REGUA, 22°24′S, 42°44′W; 40-400 m high). These forested areas are about 9 km far from each other, and both belong to a larger patch of continuous forests covering about 60,000 ha in the central portion of the state. The vegetation of the study areas is predominantly of low montane and sub-montane tropical forest. The climate in the region is wet and warm, with annual rainfall varying from 2000 to 2500 mm, and mean annual temperature is 24°C (Rocha et al. 2007, Attias et al. 2009).

We collected 15 individuals of *P. boiei* in September 2004 at EEEP and 23 individuals in October 2004 at REGUA. Frogs were collected by hand or in pitfall traps with drift fences.

Frogs were killed in 50% ethanol, fixed in 10% formalin and preserved in 70% ethanol. In the laboratory we weighted frogs with a digital balance (to the nearest 0.001g) and measured their snout-vent lengh (SVL ± SD) and jaw width (JW ± SD) with calipers (to the nearest 0.1 mm). Frogs were deposited in the Museu Nacional herpetological collection (MNRJ 68764-83, MNRJ 68795-809, MNRJ 68810-11 and in the UNIRIO herpetological collection (Uni-Rio 4297).

We removed the stomachs and examined their contents under stereomicroscope. Each food item was measured in its length and width (with calipers, to the nearest 0.1 mm), quantified and identified to the taxonomic level of Order, which was used to group them into categories. The volume of the preys (in mm^3) was estimated through the ellipsoid formula (Dunham 1983):

$$V = 4/3\pi \cdot (L/2) \cdot (W/2)^2 ,$$

where L = length and W = width.

We calculated prey consumption rates separately for each area. The frequency of occurrence (percentage of stomachs containing a given prey type) and percentage of the total number of items are given for each prey category. An index of relative importance (I_x) was calculated for each prey category as the sum of their numeric and volumetric proportions and frequency of occurrence divided by three (Howard et al. 1999).

We tested for the relationship between jaw width – JW and prey size (length of the largest intact prey found in the stomach) and number of prey per stomach using simple linear regression (Zar 1999). Because samples for each locality were too small, we performed this analysis for both sites pooled together.

Similarly, we considered the parasitism rates for each frog population separately. All worms found along the digestive tract (stomach, small and large intestines) and body cavities were fixed in ethanol 70 GL, cleared in solution of lactophenol and mounted on slides for identification under optical microscope. Helminths are currently deposited in a private collection at Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro.

Ecological parameters of parasitism such as prevalence (percentage of infected individuals) and mean intensity of infection (average number of parasites per host among infected individuals – expressed as arithmetic mean ± standard deviation) are given (*sensu* Bush et al. 1997). The relationship between frog body size and intensity of infection was tested using a randomization test because the data were not normally distributed

Fig. 1 – *Proceratophrys boiei*, Estação Ecológica Estadual do Paraíso, Rio de Janeiro, Brasil. Foto: Klaion, T.

and were not homocedastic (Zar 1999). Once again, we pooled data from both localities.

RESULTS

Five of the 38 individuals captured ($N_{EEEP} = 15$, $N_{REGUA} = 23$) were undoubtedly adults (SVL >50 mm, see Giaretta et al. 1998). The remaining 33 were presumably juveniles, and we could not determine their sex. There was no significant difference in biometric measures between the two sites, therefore we give such results jointly. Body size (SVL) of the adults ranged from 51.5 to 64.0 mm (mean = 56.6 ± 4.7 mm). Among the juveniles, SVL ranged from 21.2 to 48.2 mm (mean = 33.9 ± 8.3 mm). Body mass (preserved) ranged from 17.7 to 35.7g (mean = 23.0 ± 7.4g) among the adults, and from 0.7 to 17.6g (mean = 5.5 ± 4.6g) among juveniles. Seven of the 38 frogs analyzed had empty stomachs.

Fifty-four food items were recovered and categorized based on their taxonomical classification (level of order) (Table I). The diet of *P. boiei* was basically composed of arthropods, with some difference between the two sites. Five different arthropod orders were consumed at EEEP, and nine at REGUA. Only three of them coincided in both sites. At EEEP, the most important food category in terms of number (N = 6), frequency of occurrence (26.7%) and volume (82.3%) was Blattaria, thus yielding a large value of Index of Importance (*Ix* = 49.3%) (Table I). Coleoptera was the second most important food item at that site, with an *Ix* of 17.8%. On the other hand, at REGUA, Coleoptera was the most frequently consumed prey category, being found in 39.1% of the stomachs. Numerically, Coleoptera was again the most important item (N = 14), and in terms of volume it represented 48.6% of all the food found in stomachs of *P. boiei* collected at REGUA. Therefore, it also showed the highest *Ix* (41.3%). Orthoptera was the second most important food category at REGUA (*Ix* = 20.7%). Plant material was found in two individuals (one at each site) and consisted of leaf remains (Table I). An average of 2.24 ± 1.48 preys were found per stomach (range: 1-6; both sites pooled).

Prey size was significantly related to jaw width ($R^2 = 0.26$; $F_{1,21} = 7.3$; $P = 0.013$; Fig. 2). However, jaw width did not explain significantly the number of prey per frog stomach (P = 0.98).

A total of 197 nematodes were found in the digestive tract and (in one case) in the lungs of the frogs. Five nematode species were observed: *Aplectana delirae* (Fábio 1971), *Cosmocerca parva* Travassos, 1925, *Oxyascaris oxyascaris* Travassos, 1920, an unidentified *Physaloptera* sp. (found only as larvae), and one unidentified nematode found in a single specimen (Table II).

TABLE I

Number (N), frequency of occurrence (F), volume (V, in mm^3), and importance index (Ix) for each prey taxonomic category found in the stomachs of *Proceratophrys boiei* (N = 38) in Estação Ecológica do Paraíso and Reserva Ecológica de Guapiaçu, two Atlantic rainforest remnants in the State of Rio de Janeiro. Numbers in parenthesis are percentages.

Item	Estação Ecológica Paraíso (EEP)				Reserva Ecológica de Guapiaçu (REGUA)				EEP + REGUA			
	N (%)	F (%)	V (%)	Ix	N (%)	F (%)	V (%)	Ix	N (%)	F (%)	V (%)	Ix
Coleoptera	3 (20)	3 (20)	456.4 (11.2)	17.80	**14 (35.9)**	**9 (39.1)**	**3473.2 (48.6)**	**41.29**	**17 (31.5)**	**12 (31.6)**	**3929 (35.0)**	**32.69**
Blattaria	**6.40**	**4 (26.7)**	**3361.4 (82.3)**	**49.28**	3 (7.7)	3 (13.0)	1581.7 (22.1)	13.58	**9 (16.7)**	**7 (18.4)**	**4943.1 (44.0)**	**26.37**
Orthoptera	4 (22.2)	1 (6.3)	152.4 (3.7)	10.73	6 (16.7)	6 (27.3)	1306.4 (18.3)	20.40	10 (18.5)	7 (18.4)	1.458.8 (13.0)	16.64
Hymenoptera	—	—	—	—	3 (8.3)	3 (13.6)	140.2 (2.0)	7.98	3 (5.5)	3 (7.9)	140.2 (1.2)	5.00
Odonata	1 (5.5)	1 (6.3)	106 (2.6)	4.80	—	—	—	—	1 (1.8)	1 (2.6)	106 (0.9)	1.81
Isoptera	—	—	—	—	1 (2.8)	1 (4.5)	3.6 (0.1)	2.46	1 (1.8)	1 (2.6)	3.6 (<0.1)	1.50
Hemiptera	1 (5.5)	1 (6.3)	8.7 (0.2)	4.01	—	—	—	—	1 (1.8)	1 (2.6)	8.7 (0.1)	1.52
Diptera	—	—	—	—	1 (2.8)	1 (4.5)	20.8 (0.3)	2.54	1 (1.8)	1 (2.6)	20.8 (0.2)	1.56
Larvas	—	—	—	—	6 (16.7)	3 (13.6)	319.7 (4.5)	11.6	6 (11.1)	3 (7.9)	319 (2.8)	7.28
Isopoda	—	—	—	—	3 (8.3)	3 (13.6)	201.7 (4.5)	8.26	3 (5.5)	3 (7.9)	201.7 (1.8)	5.08
Araneae	—	—	—	—	2 (5.1)	2 (13.6)	94.7 (1.3)	4.27	2 (3.7)	2 (5.3)	94.7 (0.8)	3.3
Material vegetal	—	1 (6.3)	—	—	—	1 (4.5)	—	—	—	2 (5.3)	—	—
Total	**15**	**10**	**5187.75**	**—**	**39**	**21**	**6039.15**	**—**	**54**	**31**	**11226.9**	**—**

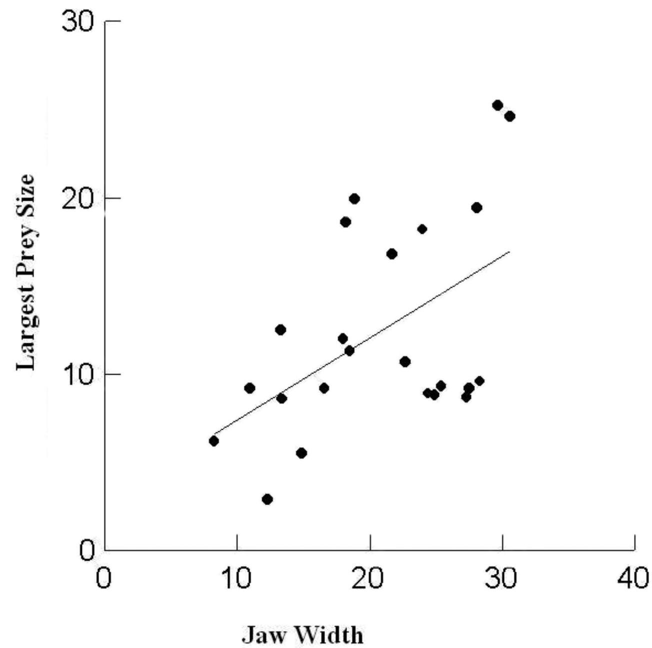

Fig. 2 – Relationship between jaw width (mm) and length (mm) of the biggest prey found in the stomach of *Proceratophrys boiei* in two Atlantic rainforest remnants in the State of Rio de Janeiro ($R^2 = 0.26$; $F_{1,21} = 7.3$; $P = 0.013$).

TABLE II

Prevalence (in percentage and absolute values), mean intensity of infection (± SD, with range in parenthesis) and number (quantity) of nematodes found in *Proceratophrys boiei* (N = 38) in EEEP and REGUA, two Atlantic rainforest remnants in the State of Rio de Janeiro. (a = found in the stomachs, b = found in the intestines and c = found in the lungs).

Studied area	Estação Ecológica Paraíso (EEP)			Reserva Ecológica de Guapiaçu (REGUA)		
Nematode species	Prevalence % (n)	Mean intensity (range)	Number	Prevalence % (n)	Mean intensity (range)	Number
Aplectana delirae	6.7 (1)	2	2	39.1 (9/23)	3.6 (2.3; 1-7)	32
Cosmocerca parva	53.3 (8)	7.5 ± 5.0 (2-17)	60	43.5 (10/23)	7.2 (5.3; 1-17)	72
Oxyascaris oxyascaris	—	—	—	8.7 (2/23)	2.5 (2.1; 1-4)	5
Physaloptera sp.	13.3 (2)	5.5 ± 6.4 (1-10)	11	4.3 (1/23)	12	12
Unidentified species	—	—	—	4.3 (1/23)	3	3
Total	60 (9/15)	8.1 ± 7.3 (1-21)	73	78.3 (18/23)	6.7 (5.1; 1-17)	124

At EEEP, the overall prevalence was 60%. Seven out of the nine infected frogs hosts were parasitized by a single nematode species. Mean intensity of infection was 8.1 ± 7.3, ranging from 1 to 21. *O. oxyascaris* and the unidentified species were not found in this study site. Mean number of nematode species per host was 1.22 ± 0.44 (range 1-2).

At REGUA, 78.3% of the *P. boiei* examined were parasitized, and the mean intensity of infection was 6.7 ± 5.1, ranging from 1 to 17. Once again, few frogs had more than one nematode species in their digestive tract (4 out of 18). Mean number of nematode species per host was 1.28 ± 0.57 (range 1-3).

There was no relationship between frog body size and intensity of infection ($F_{1,25} = 0.610$; $P = 0.440$; data from both areas pooled).

DISCUSSION

In both Estação Ecológica Estadual do Paraíso and Reserva Ecológica de Guapiaçu, Coleoptera, Blattaria and Orthoptera were the most constant and important prey items consumed by the *Proceratophrys boiei* analyzed. These arthropods usually present large body size and mass, and presumably are important to provide an appropriated amount of food and energy for a frog as *P. boiei*, which is one of the largest frog species in the leaf litter of the Atlantic rainforest. This idea has been previously suggested by Boquimpani-Freitas et al. (2002) for *P. tupinamba* at Ilha Grande. In fact, the main prey items of *Proceratophrys* frogs in other Atlantic rainforest areas are arthropods having large size and mass (see Giaretta et al. 1998 for *P. boiei*; Boquimpani-Freitas et al. 2002 for *P. tupinamba*; Teixeira and Coutinho 2002 for *P. paviotti*), which reinforces this idea. We found a slight difference in diet composition between the two sites studied. While at EEEP *P. boiei* fed predominantly on Blattaria, and then Coleoptera, at REGUA it fed predominantly on Coleoptera, followed by Orthoptera. Moreover, a richer spectrum of prey was found in REGUA (nine arthropod orders) when compared to EEEP (five orders), and Blattaria, Coleoptera and Orthoptera were the only orders found as prey items at both sites. Further studies would be necessary in order to determine whether this is a consequence of differences in local availability of food or if there is some sort of selection happening.

Proceratophrys frogs are carnivorous, primarily arthropod consumers. However, some variation in the relative importance of each arthropod group exists. The diet of *P. tupinamba* studied by Boquimpani-Freitas et al. (2002) was largely dominated by orthopterans, whereas *P. paviotti* (= *P. boiei*; Prado and Pombal Jr 2008) studied by Teixeira and Coutinho (2002) showed a more diversified diet, mainly composed of blattaria, aranae and lepidopteran larvae. Specifically for *P. boiei*, in two previous studies of diet composition, Coleoptera and Orthoptera were the dominant items in terms of number, frequency and volume over other arthropod groups (Moreira and Barreto 1996, Giaretta et al. 1998). These variations may result from differences in the composition of the arthropod fauna available at the leaf litter of each studied site.

Despite slight differences, as expected, Coleoptera, Blattaria and Orthoptera were together the three most important orders in *P. boiei* diet in both EEEP an REGUA, jointly accounting for an Index of Importance of over 75.

Proceratophrys boiei is a relatively large-bodied, sit-and wait forager (Giaretta et al. 1998). Thus, it might be advantageous for the species to prey upon large mobile preys (Boquimpani-Freitas et al. 2002) such as beetles, cockroaches and grasshoppers, as we found in this study. Giaretta et al. (1998) studied *P. boiei* in another Atlantic forest area and found frog remains (*Ischnocnema parva*) in the stomachs of two of the 38 individuals analyzed. Boquimpani-Freitas et al. (2002) also reported remains of an undetermined eleuthrodactyline frog in the stomach of a specimen of *P. tupinamba*. These records suggest that *Proceratophrys* species may be relatively common predators of small anurans inhabiting the leaf litter in the forest floor. Our sample was small and largely composed of juveniles, which probably may help to explain the absence of anurans in the stomachs that we analyzed.

Deliberate consumption of plant material is a relatively rare event for anurans, although it has been observed for some species (Silva et al. 1989, Das 1996). None of the studies that analyzed food habits of *Proceratophrys* frogs (*P. tupinamba*, Boquimpani-Freitas et al. 2002; *P. paviotii*, Teixeira and Coutinho 2002; *P. boiei*, Giaretta et al. 1998) reported the consumption of plants. In our study, only two of the 38 *P. boiei* analyzed had ingested plant material. Probably, these plant remains were accidentally ingested during prey capture attempts.

The relationship between frog jaw width and prey size reflects the fact that anurans are gape-limited because they are usually not able to tear or chew their food (Lima and Moreira 1993). It also indicates that there might be an ontogenetic shift in the diet composition of *P. boiei*, as the mean size of individuals differs among the arthropodan orders (Lima and Moreira 1993). We found a significant relationship between jaw width and prey size in the two populations studied. This result was similar to the one found by Boquimpani-Freitas et al. (2002) for *P. tupinamba*. Using different parameters and analysis – relationship between frog size and the log-transformed volumes of the largest and smallest prey plus the relationship between the most important component in a previous PCA analysis (on the variations in proportions of each prey category) and frog size, Giaretta et al. (1998) also found similar results for *P. boiei*. However, for *P. paviotti* (Teixeira and Coutinho 2002), no such relationship was found.

Nematode species of the genus *Aplectana* Railliet & Henry, 1916 are intestinal parasites of reptiles and amphibians (Travassos 1931, Yorke and Maplestone 1926, Baker 1980). Species from the genus *Aplectana* are monoxenous, and infection pathway is through the ingestion of infecting larvae (Anderson 2000). *Aplectana delirae* was originally described parasitizing *Rhinella ornata* (= *Bufo crucifer*, see Baldissera et al. 2004) and was later registered in *Crossodactylus gaudichaudii* (Fábio 1971, Vicente et al. 1990). It has also been reported in *P. tupinamba* (Boquimpani-Freitas et al. 2001), where it was considered core species. All these accounts come from areas within the Atlantic rainforest Biome in Rio de Janeiro State.

Cosmocerca parva was first described in *Hylodes nasus* from Angra dos Reis, in Rio de Janeiro State, and later reported parasitizing *Leptodactylus marmoratus*, *L. latrans*, *L. fuscus*, *L. mystaceus* and *Physalaemus soaresi* in Brazil, *Rhinella schneideri*, *Scinax fuscovarius*, *Leptodactylus chaquensis* and *L. elenae* in Paraguay, and *Rhinella granulosa* in Argentina (Fábio 1981, Baker and Vaucher 1984, Mordeglia and Digiani 1998). Species of *Cosmocerca* are monoxenous, and infection occurs through active skin penetration (and also, occasionally, by ingestion) by infecting larvae (Anderson 2000). *Cosmocerca parva* was found in the lungs of one of the frogs at REGUA, though in most cases they were found in the intestines. Although *C. parva* is usually found in the intestines of their hosts, the congeneric *C. podicipinus* has been described parasitizing the lungs of an anuran host (*Pseudopaludicola falcipes*) (Gonzalez and Hamann 2004). In fact, some species of this genus (e.g. *C. variabilis*) need to undergo through a period of development in their host's lungs before establishing themselves in their intestines (Anderson 2000). We found *C. parva* occurring with relatively high prevalence in *P. boiei* at both REGUA and EEEP, acquiring core status (Aho 1990) in the later area. Boquimpani-Freitas et al. 2001 also found one *Cosmocerca* species (*C. brasiliense*) in *P. tupinamba* from Ilha Grande, also in Rio de Janeiro State.

Oxyascaris oxyascaris was described for the first time in reptiles (Travassos 1920), and then a new species, *O. necopinus* (sinonym of *O. oxyascaris* – Baker and Vaucher 1985, Bursey and Goldberg 2007), was proposed by Freitas (1958) for specimens found in the small intestine of the frogs *Leptodactylus ocellatus*, *L. sibilatrix* and *Pleuroderma diplolistris*. This species was found with low prevalence at REGUA and was not recorded at EEEP.

Physaloptera species have been reported in all classes of terrestrial vertebrates, including several anuran species (Vicente et al. 1990). Boquimpani-Freitas et al. (2001) suggested that, since only larval specimens of *Physaloptera* are found in amphibians, these nematodes might not be able to complete their cycles in such hosts that probably act as intermediate/paratenic hosts of these parasites. Our results support the contention of Boquimpani-Freitas et al. 2001 because we also found only larvae of *Physaloptera* infecting *P. boiei*. However, according to Anderson (2000), the development of larvae through subsequent stages depends on the presence of a high quantity of food in the stomach of their host. The acquisition of *Physaloptera* parasites by the hosts occurs through the ingestion of infected insects, mainly Orthoptera.

The overall helminth prevalence for both *P. boiei* populations studied was relatively high. *Cosmocerca parva* was the only species that reached core status in one of our localities. Aho (1990) presented mean values of nematode richness (3.54) and mean number of species per hosts (0.98) in a study that covered 83 amphibian populations, mostly from North America. In this study, nematode fauna richness at REGUA (5) and the mean number of species per host in both sites were above the values found by Aho, but still within the ranges (0-9 and 0-2.08, respectively) reported by that author.

We conclude that *Proceratophrys boiei* is carnivorous, basically consuming arthropods. Coleoptera, Orthopteran and Blattaria are its main prey. Five species of nematodes were found parasitizing *P. boiei* in the studied areas. These are well known widely distributed amphibian nematodes previously reported in many species of anurans. This is the first report of nematodes for

P. boiei, which represents a new host record for *Aplectana delirae*, *Cosmocerca parva*, *Oxyascaris oxyascaris* and the genus *Physaloptera*.

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RESUMO

Proceratophrys boiei é um anuro da familia Cycloramphidae que vive no folhico e é endêmico de areas de floresta na Mata Atlantica do Sudeste do Brasil. Nós analisamos o trato digestivo de 38 indivíduos de *Proceratophrys boiei* provenientes de duas áreas de Mata Atlântica no Estado do Rio de Janeiro, Brasil, para estudar a composição da dieta e a fauna helmíntica associada a esta espécie. Os principais itens alimentares na dieta de *P. boiei* foram Coleoptera, Orthoptera e Blattaria. Cinco espécies de nematóides foram encontradas: *Aplectana delirae*, *Cosmocerca parva*, *Oxyascaris oxyascaris*, *Physaloptera* sp. (apenas larvas) e uma espécie de nematóide não identificada. A prevalência total foi de 71% e a intensidade media de infecção foi de 7,3 ± 5,8 nematóides por indivíduo.

Palavras-chave: Anura, *Proceratophrys*, dieta, endoparasitismo, neotrópico.

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