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SHORT NOTE

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Helminths associated with the threatened Alcatrazes snouted treefrog *Ololygon alcatraz*

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ABSTRACT - *Ololygon alcatraz* is a threatened treefrog endemic to a single small island in Brazil. Research on ecological interactions for this species is scarce, and nothing is known about parasitism. Thus, we investigated the presence of macroparasites of the gastrointestinal system of *O. alcatraz*. We found only two types of helminth, nematodes and acanthocephalans, both in the larval stages. Our results suggest that individuals of *O. alcatraz* are part of these parasites' life-cycle, using them as paratenic or intermediate hosts.

INTRODUCTION

Amphibians are hosts to various parasites, with helminths being the most studied among them (Herczeg et al., 2021). Despite recent progress in understanding helminth communities in neotropical amphibians, knowledge is still scarce when considering the remarkable diversity of amphibians in this region, which is the highest in the world (Campião et al., 2015).

Amphibians have been recognised as definitive, intermediate or paratenic hosts of helminths (Herczeg et al., 2021). Depending on several factors, such as parasite prevalence, immune condition (Clayton, 2005) or life stage of the amphibian (Holland et al., 2007), some helminth species have the potential to cause diseases (Poynton & Whitaker, 2001). Amphibians are the most threatened group of vertebrates, with diseases being one of the major contributing factors alongside habitat losses and other synergetic effects (Hof et al., 2001; Becker et al., 2007; Fisher & Garner, 2020). Consequently, it is crucial to investigate the diversity of parasites and their interactions with amphibians, especially in the case of threatened species.

This study has documented the occurrence of helminths in *Ololygon alcatraz*, an insular Brazilian anuran that completes its life cycle exclusively within bromeliads (Brasileiro, 2008). Currently, *O. alcatraz* is categorised as Vulnerable in the national Red List (MMA, 2022), and there has been a captive breeding program for it since 2009 (Lisboa et al., 2021).

Ololygon alcatraz is endemic to Alcatrazes Island, a single small island covering 135 hectares located on the northern coast of the State of São Paulo, Brazil (24° 06' S, 45° 41' W). Despite its restricted range, the population is abundant and occurs throughout the entire island (Brasileiro, 2008). In 2016 we caught 15 individuals on the island that were used here to investigate the diversity of parasites in comparison to 15 captive-born individuals (F1 generation).

MATERIALS AND METHODS

The frogs were euthanised with isoflurane inhalation anesthetic (CFMV, 2013). We used scalpel and tweezers to assess the body cavity via a longitudinal ventral incision. We examined the stomach and intestines using a stereomicroscope (Nikon Smz 460); then we counted the parasites and recorded the site of infection. We fixed parasites in 70 % EtOH solution and placed them in Petri dishes for sorting. We applied chloridric carmine to stain acanthocephalans and clove oil (eugenol) to diaphanise them. For nematodes, we used lactophenol to clear them (adapted from Amato et al., 1991). We analysed morphometric, morphological and photomicrograph data of the helminths under an optical microscope (Leica DM-2500) with phase contrast system equipped with computerised image analysis V4 LAS (Leica Application Suite). We identified the helminths based on morphological traits (Amin, 1987; Anderson et al., 2009; Gibbons, 2010). We deposited voucher specimens of helminths in the Coleção Microbiológica de Genética de Biomas Brasileiros, São Paulo State, Brazil (SCP 294 - SCP 295).

We calculated ecological descriptors of parasitism: prevalence (% of the *O. alcatraz* population parasitised), mean abundance (MA) - total number of parasites divided by the total number of anurans examined, and mean intensity of infection (MII) - total number of parasites divided by the total number of anurans parasitised (according to Bush et al., 1997). We estimated MA and MII with bias-corrected and accelerated bootstrap intervals with 2000 replications, using the Quantitative Parasitology on the Web (Reiczigel et al., 2019). Mean values were expressed with standard deviation and 95 % confidence intervals.

OBSERVATIONS AND DISCUSSION

We observed two types of parasites infesting the gastrointestinal system of *O. alcatraz*: nematodes

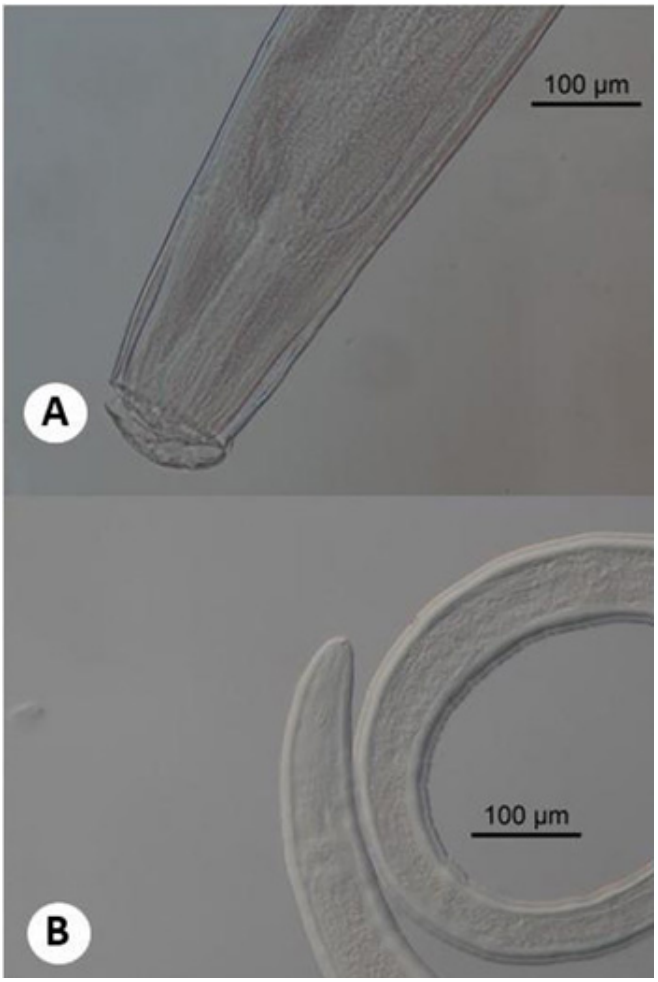

Figure 1. Encysted larva of an acuariid nematode – **A.** anterior end, **B.** caudal region

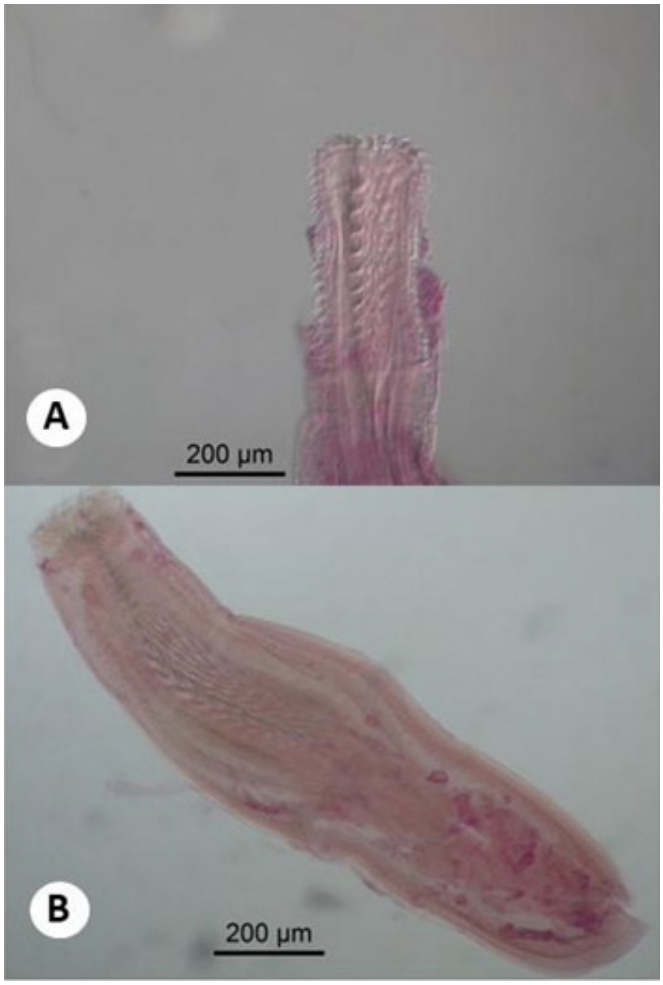

Figure 2. Cystacanth of the echinorhynchid acanthocephalan – **A.** proboscis detail, **B.** total view

(Acuariidae) (Fig. 1) and acanthocephalans (Echinorhynchidae) (Fig. 2), both in larval stages. Captive individuals, however, did not present any parasites. From the 15 wild-caught individuals of *O. alcatraz*, ten were infested with at least one type of parasite, resulting in an overall prevalence of 66.7 %, and three had both parasite species (prevalence of 30 %). Nematodes were exclusively found inside the stomach, while acanthocephalans were collected from the stomach attached to its external wall, and also found in contact with the body cavity (Fig. 3). The prevalence of nematodes was 46.7 %, the MA was 1 ± 1.41 (0.4 – 1.8) and the MII was 2.14 ± 1.35 (1.29 – 3.14). In comparison, the prevalence of acanthocephalans was 40 %, the MA was 0.47 ± 0.64 (0.13 – 0.73) and the MII was 1.17 ± 0.41 (1 – 1.5).

Our results show that wild individuals of *O. alcatraz* are prone to gastrointestinal parasitism with a high prevalence of both nematodes and acanthocephalans. On the contrary, captive-born individuals were free of parasites. Both nematodes and acanthocephalans are common in amphibians, especially nematodes that normally have a higher prevalence than any other helminths (Poynton & Whitaker, 2001; Aguiar et al., 2021), as observed in this study for *O. alcatraz* as well. In this study the species richness of helminths was low, this may be a reflection of the small body size of *O. alcatraz* (Campião et al., 2015)

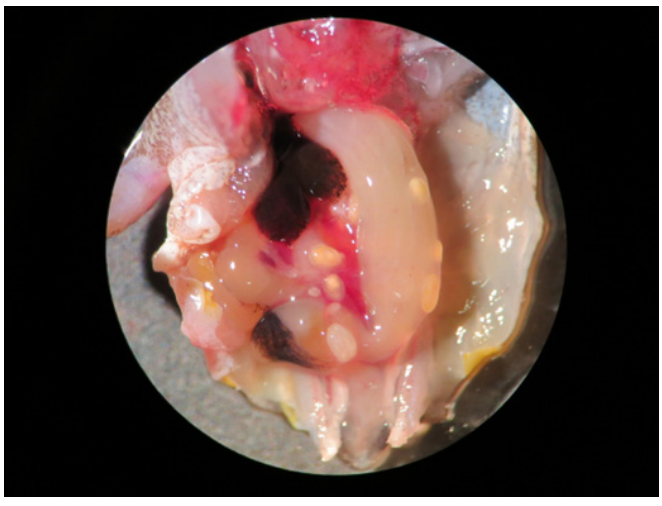

Figure 3. The gastrointestinal system of a necropsied *Ololygon alcatraz* with nematode larvae and acanthocephalan larvae encysted *in situ*

and/or by the fact that the species inhabits an isolated island habitat (Poulin, 2004), although we cannot rule out the fact that our results are based on only a small sample. The overall prevalence of helminths in the population of *O. alcatraz* was high but the abundance and intensity of

infection of each parasite species were low. Insular effects can also influence some of these parameters by increasing (Aguiar et al., 2020) or decreasing them (Roca et al., 1999); however, as this species is endemic to one very small island there are no populations of this species elsewhere to which they could be compared.

As the helminths were observed only in immature stages it was not possible to identify them to genus and species (Aguiar et al., 2021). Amphibians frequently serve as intermediate or even paratenic host for larval helminths life-stages (Campião et al., 2015), as these animals eat arthropods that carry infective larvae and are prey for several vertebrates where the life-cycle of the parasite can be completed (Poynton & Whitaker, 2001). Acanthocephalans of the Echinorhynchidae family always need at least one intermediate host (heteroxenic life-cycles), and the definitive hosts are usually fish and, sporadically, amphibians and reptiles (García-Varela & Andrade-Gomez, 2021). The nematodes of the Acuariidae family also have heteroxenic life-cycles but their definitive hosts are mainly aquatic birds (Schramm et al., 2018).

The types of parasites we found in *O. alcatraz* may be explained by the vertebrate diversity of Alcatrazes Island which consists of amphibians, reptiles (snakes and lizards) and seabirds, while mammals are absent (ICMBio, 2017). Some seabirds and the snake *Bothrops alcatraz* are potential predators of *O. alcatraz*. Thus, these anurans could be part of the parasites' strategy to complete the life-cycle in an ecosystem with an abundance of birds and snakes. The absence of these parasites in captive individuals suggest that the cycles of these two parasites have been interrupted, however, we emphasise the need for further studies to confirm the dynamics of the parasites' life-cycles both in nature and in captivity.

Our study presents the first record of helminths infecting *O. alcatraz*. Our findings suggest that individuals of *O. alcatraz* serve as an intermediate or paratenic host of these helminths (Loiseau et al., 2017). Overall, this study highlights the significance of basic science in describing the interactions between organisms and underscores the necessity for further research to understand the consequences of these interactions for both organisms. The knowledge gained from this research could help support conservation management practices for *O. alcatraz* and other threatened species.

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