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Helminth parasites of the Neotropical treefrog *Itapotihyla langsdorffii* in southeastern Brazil

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Abstract: *Itapotihyla langsdorffii* is a large-bodied hylid frog endemic to the Atlantic Forest, occurring in Brazil, Paraguay and northern Argentina. Information on its endoparasite fauna is currently restricted to one old report of a nematode (*Aplectana* sp.). In the present study we analyse for the first time the endoparasite assemblage associated with a population of *Itapotihyla langsdorffii*, assessing its infection parameters. We examined 27 specimens from an Atlantic Forest remnant in the State of Espírito Santo, Brazil, of which 85% were infected by helminths. Ten helminth species were recorded in the hosts (plus indeterminate cosmocercids and larval nematodes that could potentially represent additional species). The helminths occurring with the highest prevalences (48% and 37%, respectively) were *Polystoma* sp. (Platyhelminthes, Monogenea) and Cosmocercidae indet. (Nematoda). Helminth richness was positively influenced by host body size and was greater in females than in males. On the other hand, host size did not seem to influence the infection intensity of the most common parasites. The present survey indicated that *Itapotihyla langsdorffii* has one of the richest endoparasite faunas among South American hylids, including both heteroxenous and monoxenous species (although they seemingly serve as final hosts only for the latter).

Key words: Anura, Atlantic Forest, Endoparasites, Hylidae, Infection intensity, Prevalence.

INTRODUCTION

Parasite diversity inventories are a fundamental first step to understanding the dynamics of host-parasite ecology and evolution and can provide substantially greater information about ecosystems than the study of free-living organisms alone (Hoberg 2002, Poulin & Jorge 2019). In studies with amphibians, information about their interaction with parasites has provided insights concerning their habitat (Campião et al. 2016, Euclydes et al. 2021), foraging mode (Brito et al. 2014), behavior and physiology (Moretti et al. 2017), reproductive biology (Madelaire et al. 2013), trophic interactions (Orlofske et al. 2012), and phylogeography (Hoskin & McCallum 2007). Despite all that, only a small proportion of amphibian species have been surveyed for parasite diversity (Martins et al. 2021).

Until recently, very little was known of the helminth communities associated with anuran amphibians from South America, with most pre-2000 studies being dedicated mainly to descriptions of new taxa or to reporting isolated new host records (e.g. Vicente et al. 1990, Ben Slimane & Durette-Desset 1996a, b, Torres & Puga 1996, Hamann & Pérez 1999). Among South American anurans, the richest helminth faunas are usually observed in medium and large-bodied, widely distributed terrestrial/semi-aquatic species of the families Bufonidae and Leptodactylidae, and in members of the strongly aquatic hylid genus *Pseudis* (Campião et al. 2014, González & Hamann 2015, da Graça

et al. 2017, Toledo et al. 2018, Chero et al. 2023). Among more typical, arboreal/semi-arboreal hylids, the richest helminth communities have been recorded for species with wide distribution but varied sizes, including the large-bodied *Boana faber*, the medium-sized *B. raniceps*, and the small-sized *Dendropsophus nanus*, *Scinax fuscovarius* and *S. nasicus* (Campião et al. 2014, Parra et al. 2019, Machado et al. 2022, Silva et al. 2022). Thus, although Campião et al. (2015) reported helminth richness in South American anurans to be positively influenced by body size but not by the geographic range of hosts, this may not apply to hylid frogs, in particular.

Itapotihyla langsdorffii is one of the largest hylid frogs occurring in cis-Andean South America, with females occasionaly exceeding 100 mm in snout-vent length (Izecksohn & Carvalho-e-Silva 2001, Vrcibradic et al. 2009). This species is endemic to the Atlantic Forest domain, where it has an extensive distribution, occurring between latitudes 11° and 30° S in eastern Brazil and westward to eastern Paraguay and northern Argentina at the southern portion of its range (Silva et al. 2011, Airaldi-Wood et al. 2021). It is the only species in the genus *Itapotihyla*, with no close relatives within the tribe Lophyohylini, though its phylogenetic position in that clade remains unclear (see Blotto et al. 2021). Despite its phylogenetic uniqueness within the Lophyohylini, *I. langsdorffii* has a generalized ecology, with eggs laid in ponds and opportunistic feeding habits, which represent the standard pattern for hylid frogs (Pimenta & Canedo 2007, Vrcibradic et al. 2009).

In spite of its large body size and relatively wide geographic distribution, there is practically no information on the endoparasite fauna associated with *I. langsdorffii* in the literature, except for one report of a taxon referred as *Aplectana* sp from an unspecified locality in Brazil by Walton (1947; host referred as *Hyla langsdorffii*). In the present study, we report for the first time the endoparasite assemblage associated with a population of the large-bodied hylid frog *Itapotihyla langsdorffii*, based on a sample from a forest fragment in southeastern Brazil. We also assess some infection parameters of the helminths such as prevalence and intensity of infection, and their relationship with body size and sex of the hosts.

MATERIALS AND METHODS

We examined 27 specimens of *I. langsdorffii* collected in September, November, and December 2002 in the Estação Biológica de Santa Lúcia (hereafter EBSL; 19°58'S, 40°32'W), in the municipality of Santa Teresa, State of Espírito Santo, southeastern Brazil. The EBSL comprises a remnant of montane Atlantic Rainforest of approximately 440 ha, with altitudes varying from 550 to 950 m (Mendes & Padovan 2000). The mean annual rainfall in the area is 1868 mm, with November being the rainiest month and June the driest (Mendes & Padovan 2000). The mean annual temperature is 19.9° C, with the warmest period being January-February and the coolest being June-July (Thomaz & Monteiro 1997).

The specimens examined for the present study have been previously used for an analysis of the species' diet and reproduction (Vrcibradic et al. 2009) and, on that occasion, had their snout-vent lengths (SVL) measured with digital calipers and were dissected for examination of their gonads and removal of stomachs (with all helminths found in the stomachs being removed and kept for future analyses). For the present study, we also examined the body cavity, stomach, intestines, liver, lungs, and urinary bladder of those frogs, which were removed and analyzed under a stereomicroscope to check for endoparasites. All parasites found in

the frogs, including those previously recovered from stomachs during the dietary analysis of Vrcibradic et al. (2009), were mounted on temporary slides and identified under a microscope. In this process, the nematodes and acanthocephalans were cleared, respectively, with lactophenol and lactic acid; monogeneans were rinsed in tap water for one hour, stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene, and mounted using Dammar gum.

The relationship of both helminth richness and helminth infection intensity (for the helminth species with the greatest prevalences) with host body size (SVL) was tested by simple regression analyses. Differences between males and females in mean helminth richness was tested using one-way analyses of variance (ANOVA) after testing for homoscedasticity of distributions. Basic statistics given in the text represent arithmetic means ± one SD. Throughout the text, we follow the parasitological terminology of Bush et al. (1997).

The frogs examined for this study are currently deposited at the amphibian collection of the Universidade Federal do Estado do Rio de Janeiro (voucher numbers: UNIRIO 6724-50). Vouchers of the helminth species recorded in the frogs were deposited in the parasitological collections of the Departamento de Zoologia, Universidade Federal do Paraná (DZUP), of the Instituto Oswaldo Cruz (CHIOC) and of the Universidade Federal do Ceará (CPUFC) (see Appendix).

RESULTS

Of the 27 individuals examined, comprising 20 males (73.3-92.1 mm SVL) and seven females (92.0-112.1 mm SVL), 23 (85.2%) were infected by at least one helminth. Overall infection prevalence was 80% in males and 100% in females. The species of helminths recorded included the nematodes *Cosmocerca parva*, *Oswaldocruzia chabaudi*, *Oxyascaris* sp., *Parapharyngodon* sp., *Physaloptera* sp., Ascaridida gen. sp., and Spirurida gen. sp., the monogenean trematode *Polystoma* sp., an indeterminate cestode, and immature forms of centrorhynchid acanthocephalans (Table I). The Ascaridida, the Spirurida, some indeterminate larval nematodes, and several individuals of Cosmocercidae could not be identified to genus because we only found females (in the case of cosmocercids) or immature stages, which lack morphological characteristics that are taxonomically informative.

The number of helminth species per individual host averaged 2.22 ± 1.09 (range: 1-5) and was positively and significantly correlated with host SVL (r^2 = 0.240, p = 0.018, n = 23). Helminth richness per host was significantly higher in females (3.29 ± 0.95; range: 2-5; n = 7) than in males (1.75 ± 0.78; range: 1-3; n = 16; $F_{1,21}$ = 16.72, p = 0.001). There was no significant relationship between the infection intensity of cosmocercid nematodes (i.e. pooled data for *C. parva* and indeterminate Cosmocercidae) and host SVL (r = 0.521, p = 0.082, n = 12) nor between the infection intensity of *Polystoma* sp. and host SVL (r = 0.475, p = 0.101, n = 13).

DISCUSSION

Itapotihyla langsdorffii represents a new host record for all helminth taxa reported here. Overall prevalence was high, but each parasite taxon showed a prevalence of less than 50% and most had a low intensity of infection. The endoparasite component community was represented by various parasite lineages that vary in their life cycles. This suggests that the feeding habits and habitat use patterns of *I. langsdorffii* contribute to its relatively diverse

Table I. Helminth parasites infecting *Itapotihyla langsdorffiii* (N = 27) from Santa Teresa, in southeast Brazil. Mode of transmission (MT) either trophic (i.e. via ingestion of infected intermediate hosts by the frogs) or direct (i.e. via penetration of larvae through skin or mucosa or by accidental ingestion of eggs or larvae directly by hosts), Prevalence (P), mean intensity of infection ± one standard deviation (MI, with range in parentheses), and site of infection of helminths are also given.

Species	MT	P (%)	MI (range)	Site of infection
Acanthocephala Centrorhynchidae gen. sp. (cystacanth)	trophic	18.5	10.20 ± 12.76 (1-32)	coelom, stomach wall
Nematoda Ascaridida gen. sp. (larva)	trophic	7.4	3.00 (2-4)	stomach wall
Cosmocerca parva	direct	7.4	1.50 (1-2)	large intestine, small intestine
indeterminate Cosmocercidae	direct	37.0	2.40 ± 2.17 (1-8)	large intestine, small intestine
Oswaldocruzia chabaudi	direct	22.2	3.83 ± 6.01 (1-16)	small intestine, large intestine
Oxyascaris sp.	direct	11.1	1.33 ± 0.58 (1-2)	small intestine, large intestine
Parapharyngodon sp.	direct	7.4	9.50 (1-18)	large intestine, small intestine
Physaloptera sp. (larva)	trophic	11.1	1.0	stomach
Spirurida gen. sp. (larva)	trophic	3.7	2	stomach
indeterminate Nematoda (larva)	trophic	7.4	6.50 (1-12)	stomach wall, intestine wall, small intestine, stomach
Platyhelminthes Cestoda gen. sp.	trophic	3.7	2	urinary bladder
Polystoma sp.	direct	48.1	2.92 ± 2.06 (1-7)	urinary bladder

endoparasite fauna, including helminths acquired trophically, as well as through contact with soil or water.

Among nematodes, *Oswaldocruzia chabaudi* has previously been reported from five species of hylid frogs (*Boana boans*, *B. geographica*, *B. fasciata*, *B. wavrini*, and *Osteocephalus cabrerai*) from the Amazon Region in Ecuador (Campião et al. 2014) and Brazil (Willkens et al. 2021, Neves et al. 2024). Our record adds a sixth hylid species to its host list and provides the first record of *O. chabaudi* for southeastern Brazil and for the Atlantic Forest domain.

Cosmocerca parva is a common parasite of anurans and has been reported in several frog species from different families in Brazil and various other South American countries (Campião et al. 2014, 2016, Martins-Sobrinho et al. 2017, Silva et al. 2018, Chero et al. 2023, Martins et al. 2024). Nematodes of the genera *Oxyascaris*, *Parapharyngodon*, and *Physaloptera* (the latter usually found as larvae) have also been previously reported for various anurans from different families in Brazil and other countries in South America (Campião et al. 2014, 2016, Aguiar et al. 2014, Almeida-Santos et al. 2017, da Graça et al. 2017, Gómez et al. 2020, de Oliveira et al. 2022a, b, Martins et al. 2024, Neves et al. 2024) and are here recorded for the first time in *I. langsdorffii*.

Notably, nematodes of the genus *Rhabdias*, which typically infect the lungs of anuran hosts, were not recorded in our sample of *I. langsdorffii*. This finding is somewhat surprising, as members of this genus have been reported from several species of hylids in Brazil (da Graça et al. 2017, de Oliveira et al. 2022b, Machado et al. 2022, Silva et al. 2022). Infective larval stages of *Rhabdias*

spp. live in the soil and enter the bodies of amphibian hosts by actively penetrating their skin or mucosa (Baker 1979). The absence of those parasites in our sample of *I. langsdorffii* may suggest that this species could spend less time on the ground than other hylids, which reduces the probability of contact with infective *Rhabdias* larvae.

Nearly half of the helminth taxa reported in the present survey comprise species with heteroxenous life cycles. These findings may reflect the generalist food habits of *I. langsdorffii*. Additionally, this frog species has a large body size, which allows it to consume prey of various sizes and types, including smaller frogs (Vrcibradic et al. 2009). The heteroxenenous helminths were present mostly as immature forms and are likely unable to complete their cycle inside the frogs, which may function as paratenic hosts for them. Anurans tend to serve as hosts to a great diversity of helminths in immature stages, as they are frequently preyed on by vertebrates of other groups that are the final hosts to those parasites (Campião et al. 2015). Heteroxenenous helminths infecting *I. langsdorffii* also had lower prevalences, in general, compared to the species that infect their hosts directly. This may reflect the fact that, although *I. langsdorffii* includes a relatively wide variety of prey in its diet, individuals tend to present only one to three prey items in the stomach, suggesting that they do not feed very frequently (Vrcibradic et al. 2009).

The richness of parasites with monoxenous life cycles (which are generally acquired through accidental ingestion or contact of the host's skin with eggs/larvae on the soil) was similar to those with heteroxenous cycles. However, as already mentioned, some monoxenous taxa (especially cosmocercid nematodes) occurred with relatively high infection prevalence. Also, helminths with monoxenous life cycles were primarily found in adult stages, indicating that they use the *I. langsdorffii* as their final hosts. These findings suggest that, despite being mainly arboreal, *I. langsdorffii* may also spend some time on the ground and/or water, as supposed by Vrcibradic et al. (2009) based on the finding of a terrestrial frog (*Physalaemus crombiei*) in the stomach of one specimen. The large size attained by *I. langsdorffii* may provide an excellent opportunity for infection by nematodes that are directly transmitted through contact with the soil, as observed in the aforementioned high prevalence of cosmocercid nematodes.

The high prevalence (48%) of monogenean trematodes (*Polystoma* sp.) in *I. langsdorffii* may appear unusual for an anuran with predominantly arboreal habits. However, this species breeds in puddles, where individuals may congregate in large numbers during reproductive events (Vrcibradic et al. 2009). Thus, these treefrogs could get infected when they enter the water to reproduce. The time of the year when the *I. langsdorffii* individuals in our sample were collected (i.e., between September and December) mostly coincides with the reproductive period of the species (August to November), as indicated by data from other studies (Toledo et al. 2003, Narvaes et al. 2009, Vrcibradic et al. 2009, Vilela et al. 2011), which suggest the frogs may indeed have acquired the monogeneans during breeding events.

Our results suggest that larger specimens of *I. langsdorffii* tend to have richer parasite infracommunities, which is reflected in an inter-sexual difference in this parameter, since the largest individuals are female. However, the infection intensities of the most prevalent heminths do not seem to be influenced by host size. It is possible that the sample size could have been insufficient to reveal this relationship. Nevertheless, a number of studies on different

species of Neotropical frogs have not detected a relationship between parasite abundance/intensity of infection and host body size (e.g. Klaion et al. 2011, Silveira et al. 2022, Coimbra et al. 2023), indicating that our finding may not be so unusual.

With at least eleven species of associated helminths reported so far (Walton 1947; this study), most of them from a single population, *Itapotihyla langsdorffii* currently has one of the richest endoparasite faunas known among South American hylids (Campião et al. 2014, 2015, Machado et al. 2022). In fact, the richness of the component community reported here may be underestimated, as the indeterminate larval nematodes and female cosmocercids could potentially contain one or more additional species. This rich helminth fauna may reflect the large body size of *I. langsdorffii*, lending further support to the findings of Campião et al. (2015) regarding the positive influence of host body size on parasite richness among anuran species.

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Appendix. Voucher specimens of helminths recovered from *Itapotihyla langsdorffii.*

Ascaridida: DZUP 541917; Centrorhynchidae: CPUFC 218, 484-85, DZUP 542390; Cestoda: CPUFC 486; *Polystoma* sp.: CHIOC 39912-14, DZUP 542389; *Cosmocerca parva* DZUP 541919; Cosmocercidae indet.: DZUP 541916; *Parapharygodon* sp.: DZUP 541914; *Physaloptera* sp.: DZUP 541918; *Oswaldocruzia chabaudi*: DZUP 541920; *Oxyascaris* sp.: DZUP 541915; Spirurida: DZUP 541922

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