Nematode Parasites from Six Species of Marsupial *Gastrotheca* (Anura: Hemiphractidae) Frogs from the Peruvian Andean highlands

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Nematode Parasites from Six Species of Marsupial *Gastrotheca* (Anura: Hemiphractidae) Frogs from the Peruvian Andean highlands[1]

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Abstract: The present study examined the helminth fauna of marsupial frogs (*Gastrotheca* spp.). Forty-one individuals of six species of the genus *Gastrotheca* (*G. excubitor*, *G. griswoldi*, *G. marsupiata*, *G. monticola*, *G. peruana*, and *G. stictopleura*) from the Peruvian Andean highlands were analyzed for parasites. Eleven species of nematodes were found: *Rhabdias cff. elegans*, *Oswaldocruzia proencai*, *Aplectana hylambatis*, *A. membranosa*, *A. vellardi*, *Cosmocerca brasiliensis*, *C. cruzi*, *C. parva*, *Cosmocercoides lilloi*, *Parapharyngodon* sp., and *Oxyascaris* sp. All the recorded nematode species are generalists with a direct life cycle that can colonize hosts through cutaneous infection, often through contact with soil, suggesting that the terrestrial habitat of the hosts is the main factor responsible for the observed infection patterns. All these nematode species represent new parasite records for the host species studied.

Keywords: helminth parasites, terrestrial habitat, amphibians, marsupial frogs, *Gastrotheca*, Peru

SINCE THE PUBLICATION by Aho (1990), studies on amphibian parasites have increased. This is partly due to the interest in pathogens (e.g., fungi such as *Saprolegnia* spp. and *Batrachochytrium dendrobatidis*) related to the global decline in amphibians (Skerratt et al. 2007, Hayes et al. 2010); however, the most common invertebrate parasites of amphibians are helminths (González et al. 2012). Most of the knowledge about the parasites in this group of hosts has been derived from studies conducted in North America and mainly in hosts belonging to the Ranidae (e.g., McAlpine and Burt 1998, Paredes-Calderón et al. 2004, Velázquez-Urrieta and León-Règagnon 2018) and Bufonidae families (e.g., Goldberg et al. 1996, Bolek and Coggins 2003, Espínola-Novelo and Guillén-Hernández 2008, Muzzall and Andrus 2014). Information about the parasites of some amphibian groups from other geographic regions, such as South America, is still scarce.

Despite the high richness of amphibians in South America, few species have been examined for parasites. South America harbors approximately 2,599 amphibian species (Frost 2019), but there are parasitic helminth reports for only 185 (~7%) species (Campião et al. 2014). A similar picture is evident for Peru, with approximately 571 recorded amphibian species (Frost 2019), but parasitic helminths have only been reported for 68 (11%) of these species (Campião et al. 2014). In addition, parasites are an important component of any ecosystem, not only because of the number of species, but also because of the role they play in the trophic web (Dobson et al. 2008, Lafferty et al. 2008); they are also potentially

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useful as indicators of human impact on the environment (Lafferty 1997, Marcogliese 2005). Consequently, it is necessary to increase the number of studies on parasitic helminths in Peruvian amphibians since many species have yet to be examined (McAllister et al. 2010).

Marsupial frogs of the genus *Gastrotheca* are amphibians with terrestrial and arboreal habitats (Duellman 2015). They are distributed in Panamá, Venezuela, Colombia, Ecuador, Peru, Bolivia, Brazil, and Argentina (Ramírez and Rodríguez 2011, Duellman 2015). To date, 74 species have been described, of which 29 have been recorded in Peru (Frost 2019).

From a parasitological point of view, the marsupial frogs of the genus *Gastrotheca* have been poorly studied, and parasitic studies are limited to the taxonomic descriptions of two Platyhelminthes: *Polystoma touzeti* (Monogenea) from *Gastrotheca riobambae* (Vaucher 1987) and *Pseudosonsinotrema megalorchis* (Digenea) from *Gastrotheca pseustes* (Flowers et al. 2011), from Ecuador. Therefore, the aim of the present study is to report the nematode parasites of six species of marsupial frogs of the genus *Gastrotheca* from the Peruvian Andes.

MATERIALS AND METHODS

Forty-one marsupial frogs were borrowed from the herpetological collection of the Museo de Historia Natural-UNMSM, Lima, Peru and examined for parasitic infections (see Table S1 [online supplemental material]). The frogs were collected between July 1952 and March 2013 from different locations in the Peruvian Andes (Figure 1) and field-fixed in 10% formalin. The frogs were transported to the Department of Protozoology, Helminthology and Related Invertebrates of the Natural History Museum UNMSM, Peru, where they were measured and sexed. Subsequently, the internal organs (lungs, urinary bladder, heart, and intestines) were removed and dissected for the examination for parasitic helminths by stereoscope microscopy. To conduct taxonomic identification, nematodes were removed, and the samples were placed on a glass slide, cleared in a drop of Aman's lactophenol, and covered with a cover slip. All the nematodes were deposited in the Parasitological Collection of the Museo de Historia Natural-UNMSM (see Table S2 [online supplemental material]). The parameters of infection (prevalence, mean abundance and mean intensity) for each species of parasite were calculated according to Bush et al. (1997).

RESULTS

Five hundred and eighty-two helminths from 11 nematode species in four families were found: *Rhabdias cff. elegans* Gutierrez, 1945 (Rhabdiasidae); *Oswaldocruzia proencai* Ben Slimane and Durette-Desset, 1995 (Molineidae); *Parapharyngodon* sp. (Pharyngodonidae); *Cosmocerca parva* Travassos, 1925, *C. cruzi* Rodrigues and Fabio, 1970, *C. brasiliensis* Travassos, 1925, *Cosmocercoides lilloi* Ramallo et al. 2007; *Aplectana membranosa* Schneider, 1866; *A. vellardi* Travassos, 1926; *A. hylambatis* Travassos, 1925; and *Oxyascaris* sp. (=*Paraoxyascaris*) (Cosmocercidae).

The infection parameters for each nematode species are shown in Table 1. Cosmocercids were the most prevalent (>30%) as opposed to species from the other families which were found less frequently (<10%).

DISCUSSION

This study represents what appears to be the first report of parasitic nematodes in *Gastrotheca* spp. Previous reports include two Platyhelminthes (Vaucher 1987, Flowers et al. 2011), consequently, all nematodes species reported here represent the first records in these host species.

Eight (72.7%) of the 11 nematodes species found belonged to the family Cosmocercidae. Members of this family are intestinal parasites of amphibians and reptiles (Anderson 2000). To date, the genus *Cosmocerca* consists of 27 known species (Bursey et al. 2015, Sou and Nandi 2015), ten have been reported in South America (Campião et al. 2014). The life cycle of many species is still unknown. According to Anderson (2000), cosmocercids have a direct

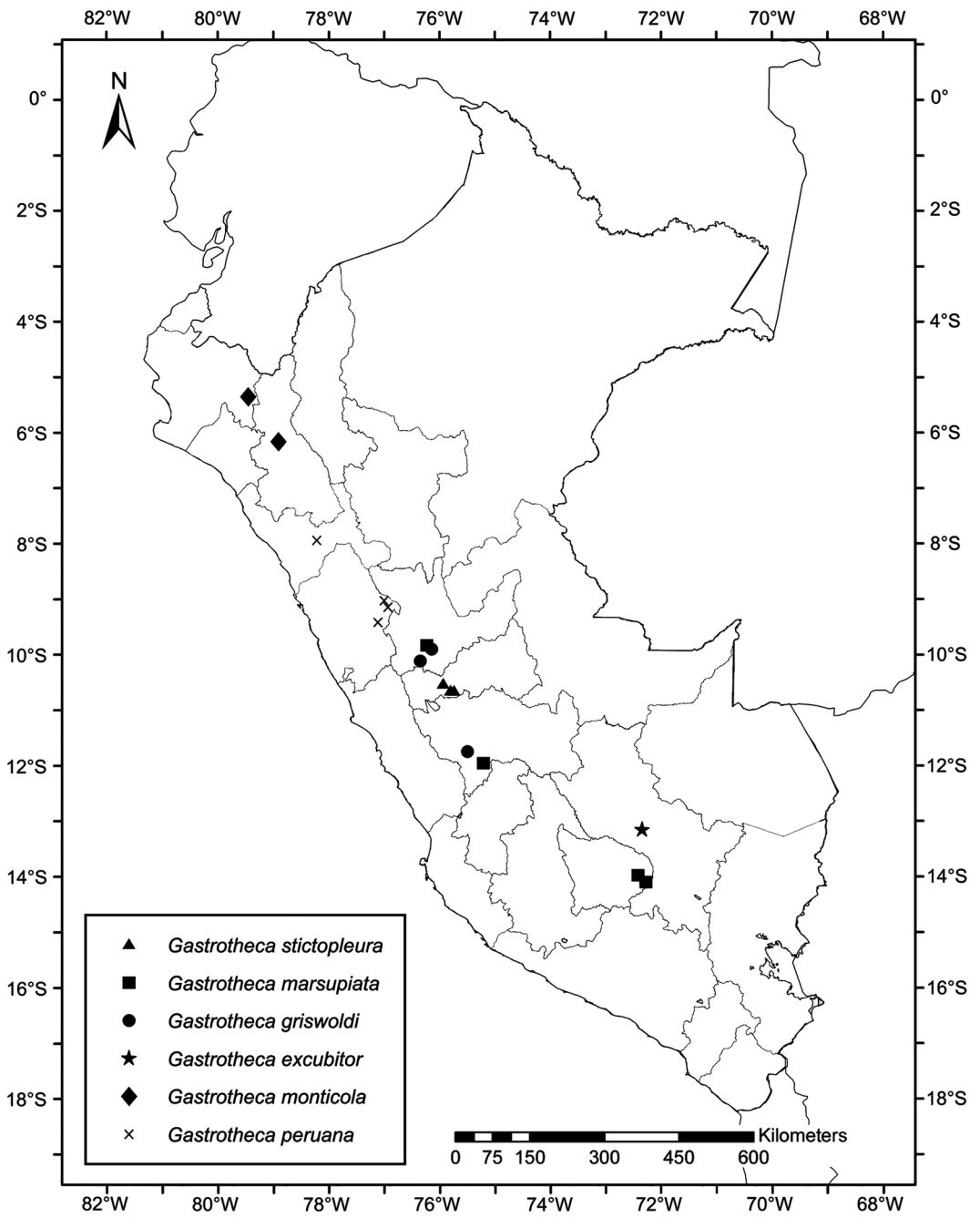

FIGURE 1. The locations in the Peruvian Andes where the marsupial frogs used in this study were collected.

TABLE 1

Prevalence (P %) and Mean Intensity (MI ± SD) for Nematodes in 41 Marsupial Frogs from Peruvian Andean highlands: *Gastrotheca excubitor* (*n* = 4), *Gastrotheca griswoldi* (*n* = 6), *Gastrotheca marsupiata* (*n* = 6), *Gastrotheca monticola* (*n* = 5), *Gastrotheca peruana* (*n* = 17), and *Gastrotheca Stictopleura* (*n* = 3).

Parasites	S.I.	*G. excubitor*		*G. griswoldi*		*G. marsupiata*		*G. monticola*		*G. peruana*		*G. stictopleura*	
		P %	MI ± SD	P %	MI ± SD	P %	MI ± SD	P %	MI ± SD	P %	MI ± SD	P %	MI ± SD
Cosmocercidae													
Cosmocerca cruzi	I	25.0	1.0	–	–	–	–	–	–	–	–	–	–
Cosmocerca brasiliensis	I	–	–	–	–	–	–	–	–	–	–	33.3	6.0
Cosmocercoides lilloi	I	75.0	6.6 ± 4.7	16.6	3.0	–	–	40.0	10 ± 8.5	17.6	14.3 ± 15.3	–	–
Aplectana hylambatis	I	–	–	16.6	1.0	50.0	95.0	–	–	35.5	35.7 ± 49.2	–	–
Aplectana membranosa	I	–	–	–	–	16.7	31 ± 32.1	–	–	–	–	–	–
Aplectana vellardi	I	–	–	–	–	–	–	–	–	5.9	2.0	–	–
Oxyascaris sp.	I	–	–	–	–	–	–	–	–	5.9	1.0	–	–
Molineidae													
Oswaldocruzia proencai	I	–	–	–	–	–	–	–	–	5.9	4.0	–	–
Pharyngodonidae													
Parapaharyngodon sp.	I	–	–	16.6	1.0	–	–	–	–	–	–	–	–
Rhabdiasidae													
Rhabdias cff. elegans	L	–	–	16.6	1.0	–	–	–	–	5.9	3.0	–	–

S.I. = site of infection; I = intestine; L = lung.

life cycle and infect anurans by skin penetration. *Cosmocerca cruzi*, *C. brasiliensis*, and *C. parva* are generalist species reported in hosts of the families Bufonidae, Ceratophryidae, Craugastoridae, Dendrobatidae, Hylidae, and Leptodactylidae (Dos Santos and Amato 2013, Campião et al. 2014). Another cosmocercid found was *C. lilloi*; of the 15 species in the *Cosmocercoides* genus, only *C. lilloi* has been reported in South America *in Rhinella arenarum* (Bufonidae) (Ramallo et al. 2007).

The genus *Aplectana* consists of intestinal parasites of amphibians and reptiles (Bursey et al. 2006). Of the 56 described species (Sou et al. 2018), 23 have been recorded in South America (Piñeiro-Gomez et al. 2017). Members of genus *Aplectana* infect their definitive host through skin penetration or via predation when tadpoles feed on nematode larvae, and adults can also become infected if they consume infected tadpoles (Anderson 2000). *Aplectana hylambatis*, *A. membranosa*, and *A. vellardi* are generalist species, reported in hosts of the families Bufonidae, Brachycephalidae, Hylodidae, Leptodactylidae, and Telmatobiidae (Campião et al. 2014, Chero et al. 2014, 2015, Teles et al. 2018).

The genus *Oxyascaris* (=*Paraoxyascaris*) infects anurans of the families Bufonidae, Eleutherodactylidae, Leptodactylidae, Hylidae, and Odontophrynidae (Baker 1987, Bursey and Goldberg 2007, Campião et al. 2014, Lins et al. 2017). We found only one female individual of *Oxyascaris* sp. As males are required to determine the species, we are unable to identify the species of this specimen.

Species of the genus *Rhabdias* are common parasites of the lungs of amphibians and reptiles (Bursey et al. 2003, Martínez-Salazar and León-Règagnon 2007). Of the 80 species described (Kuzmin and Tkach 2019), 17 have been recorded in South America (Kuzmin et al. 2016), including *R. elegans* from toads in the genus *Rhinella* from Argentina, Brazil, Peru, and Uruguay.

Members of the genus *Oswaldocruzia* comprise more than 88 species and parasitize amphibians and reptiles (Bursey and Goldberg 2011, Larrat et al. 2018). To date, 33 species have been reported in South America (Larrat et al. 2018). *Oswaldocruzia proencai* has been reported in 11 anuran species of the genus *Leptodactylus* and *Rhinella* (Campião et al. 2014).

The genus *Parapharyngodon* contains 54 valid species (Santos et al. 2019), and 11 have been found in South America (Pereira et al. 2017, Santos et al. 2019) in anurans in the families Bufonidae, Eleutherodactylidae, Hylidae, Microhylidae, and Phyllodactylidae (Bursey and Goldberg 2015, De Araujo Filho et al. 2015).

All the nematodes found have a direct life cycle (Anderson 2000). The habitat occupied by the hosts can explain the presence of these species. Except for *Gastrotheca stictopleura*, which is mainly arboreal, the remaining species studied are predominantly terrestrial (Duellman 2015), increasing the probability of contact with nematode larvae that develop in the soil and colonize their hosts by cutaneous penetration (Anderson 2000). Although *G. stictopleura* is arboreal, the presence of *C. brasiliensis* suggests that at least some part of its life is spent in contact with the soil, probably in the reproductive periods.

The terrestrial habitats of *Gastrotheca* species also would explain the absence of parasites with indirect life cycles. Anurans which live in aquatic habitats are more easily infected with digeneans than the terrestrial anurans (Guillén-Hernández et al. 2000). In this regard, Espínola-Novelo et al. (2017) suggest that due to terrestrial anurans spend most of their lives outside of water bodies they a have decreased likelihood of feeding aquatic invertebrates (e.g., snails and aquatic insects), which act as intermediate hosts of digeneans, acanthocephalans, and cestodes.

Differences in the number of taxa found in each host species could relate to the number of hosts analyzed. According to Campião et al. (2015), the known parasite richness for a host species is related to the sampling effort. In our study, the highest number of species was obtained from *Gastrotheca peruana* (7 species, $n = 17$) and the lowest number of species was obtained from *G. stictopleura* (1 species, $n = 3$) (Table 1).

Most of the nematodes that presented high prevalence values (> 30%) belonged to the Cosmocercidae family, while the lowest values

belonged to other families (<10%); this suggests a close relationship between anurans of the genus *Gastrotheca* and cosmocercid nematodes. However, it is necessary to analyze a greater number of hosts to confirm this pattern.

In conclusion, the marsupial frogs of the genus *Gastrotheca* that we examined were generally infected by a helminth fauna of generalist nematodes that also infect anurans of other families. All the nematodes recorded have a direct life cycle and colonize their hosts by cutaneous penetration, generally through contact with larvae in the soil, suggesting that the terrestrial habitat of the hosts is the main factor responsible for this pattern.

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