ARTÍCULO

Parásitos asociados a ofidios del Pacífico colombiano

Parasites associated with Ophidia of the Colombian Pacific

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ABSTRACT: This study describes the diversity of parasites in ophidian species from four ecoregions of Valle del Cauca and Gorgona Island (Colombian Pacific). For this, samples from necropsies, blood and feces were collected from 37 snakes belonging to the species *Bothrops asper* (terrestrial), *Leptodeira annulata* (semiarboreal), *Imantodes cenchoa* (arboreal), *Sibon nebulatus* (arboreal), *Boa constrictor* (semiarboreal), *Clelia clelia* (terrestrial), *Mastigodryas boddaerti* (terrestrial) and *Micrurus mipartitus* (terrestrial). Parasite detection was performed using stereoscopic and optical microscopy, and/or concentration and staining methods depending on the sample type; parasites were identified by morphological keys. Eleven snakes (29.7%) belonging to four species were infected with at least one of seven parasite taxa identified (six nematodes and one hemoparasite). Among nematodes, the families Rhabditidae and Oxyuridae, and the genus *Kalicephalus* were found for the first time in *L. annulata.* Worms of the order Strongylida, and the genera *Kalicephalus* and *Ophidascaris* were identified in *B. asper. Hepatozoon* spp. (hemoparasite) presented the highest prevalence of infection, which was only infecting arboreal and semi-arboreal snakes (*L. annulata*, *I. cenchoa* and *B. constrictor*), showing in addition morphological variability of the gametocytes. Our findings suggest that transmission of this parasite may be associated with snake habits and lifestyle.

Keywords: hemoparasites, nematodes, snakes, Colombian Pacific, Gorgona Island.

RESUMEN: El presente estudio describe la diversidad de endoparásitos en ocho especies de ofidios de las cuatro ecorregiones del Valle del Cauca e Isla Gorgona en Colombia. Para la detección e identificación de parásitos, se obtuvieron muestras de sangre, materia fecal, órganos y tejidos de 37 serpientes pertenecientes a las especies *Bothrops asper* (terrestre), *Leptodeira annulata* (semi-arborícola), *Imantodes cenchoa* (arborícola), *Sibon nebulatus* (arborícola), *Boa constrictor* (semi-arborícola), *Clelia clelia* (terrestre), *Mastigodryas boddaerti* (terrestre) y *Micrurus mipartitus* (terrestre). La búsqueda e identificación de parásitos se realizó mediante el uso de microscopia óptica y esteroscópica, y/o métodos de concentración y coloración dependiendo del tipo de muestra; los parásitos fueron identificados mediante claves morfológicas. Once serpientes (29,7%) pertenecientes a cuatro especies estuvieron infectadas con al menos uno de siete taxones parásitos identificados (seis nematodes y un hemoparásito). Entre los nematodes se reportan por primera vez las familias Rhabditidae y Oxyuridae y el género *Kalicephalus* en *L. annulata*; en *B. asper* se identificaron parásitos del orden Strongylida y de los géneros *Kalicephalus* y *Ophidascaris*. *Hepatozoon* spp. (hemoparásito) presentó la prevalencia de infección más elevada, y sólo se halló en *I. cenchoa, L. annulata* y *B. constrictor*, de hábitos arbóreo y semi-arbóreo, evidenciándose además variabilidad morfológica de los gametocitos. Los hallazgos indican que la transmisión de esta parasitosis puede estar relacionada con el hábito y modo de vida de estas serpientes.

Palabras clave: hemoparásitos, nematodes, serpientes, Valle del Cauca, Isla Gorgona.

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INTRODUCTION

Parasitism has an important impact on the structure of communities, either by competition among parasite species or by parasite-host interactions. A direct effect on host fitness may lead to phenotype changes or altered interactions with other individuals (Frainer *et al*., 2018). Factors such as climate conditions, eating habits, forced migration, and anthropic activities affect both parasite distribution and host interactions, and in some cases, these changes may endanger host populations (Frainer *et al*., 2018). Reptiles are excellent systems to determine patterns and processes influencing parasite communities (Aho, 1990; Polley and Thompson, 2015). Particularly, snakes are susceptible to being parasitized by nematode taxa (Souza *et al*., 2014; Mendoza-Roldan *et al*., 2020) and hemoparasites such as *Hepatozoon* spp. (Moço *et al*., 2002; Úngari *et al*., 2018; Bazzano *et al*., 2020).

Particularly in snakes from the Americas, several species of trematodes, cestodes and nematodes have been reported (Fernandes and Kohn, 2014; Oda *et al*., 2016; Peichoto *et al*., 2016; Carbaj al-Márquez *et al*., 2018; Fernandes De Carvalho *et al*., 2018; Soria-Díaz *et al*., 2019.

In Colombia, 327 snake species have been reported distributed widely in different microhabitats (Uetz *et al*., 2022); however, information on their parasite-host relationships is scarce (Duran-Peñaranda *et al*., 2020). Previous studies performed in *ex situ* organisms have found *Hepatozoon* spp. and Strongylida nematodes in *Boa constrictor* (Linnaeus) (Zamudio-Zuluaga and Ramírez-Monroy, 2007; Duran-Peñaranda *et al*., 2020). Likewise, the digenean *Ochetosoma heterocoelium* Travassos, 1921 was found in the oral cavity, Jacobson's organ, and esophagus of several snake species (Lenis *et al*., 2009). Studies on the snake parasites distribution are needed to know and analyze diversity in wild organisms and to evaluate the potential effects on hosts and environmental health.

The Colombian Pacific is considered by the World Wildlife Fund as one of the most biodiverse regions on the planet, and one of the 17 priority areas for conservation in the world (WWF, 2021), being the habitat of widely distributed snake species with different trophic and behavioral habits. The aim of this work was to know the parasites present in some snake species from the four ecoregions of Valle del Cauca, and from Gorgona Island in Colombia. Additionally, we analyzed the parasitemia by *Hepatozoon* and the morphological changes in the red blood cells parasitized.

MATERIALS AND METHODS

Study area and host sampling

The study was conducted in the four ecoregions of Valle del Cauca, namely: Pacific Region, Cordillera Occidental, Valle Interandino and Cordillera Central (Fig. 1a). In addition, Gorgona Island (02° 58' 03" N; 78° 10' 49" W) located 28 km off the Colombian Pacific Coast (National Park) was included (Fig. 1b). Snakes were collected in 2019 (July to December) and 2020 (October). Surveys were conducted between 8:00-11:30 h and 17:00-23:00 h, during free walking, and using the Visual Encounter Survey (VES) method (Angulo *et al*., 2006), two five-day sampling sessions were conducted in each locality. Each collected specimen was sexed and measured to face-cloacal length and total length.

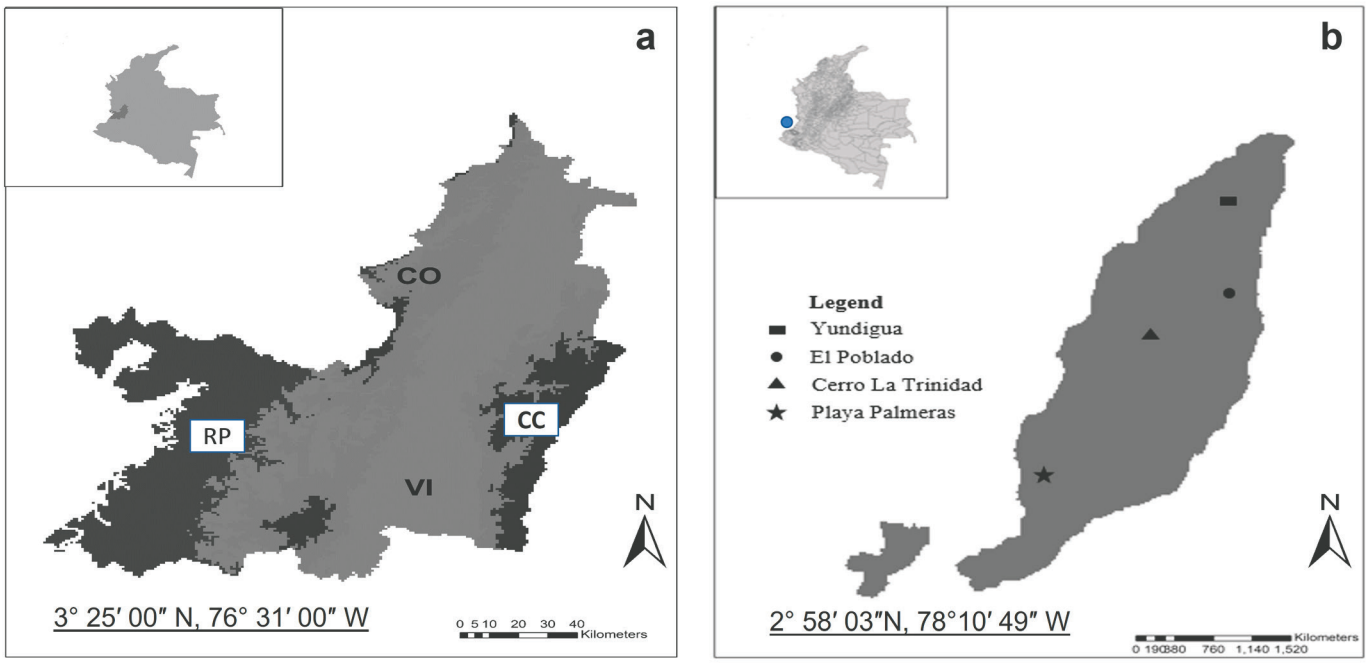

Figure 1. a) Ecoregions of Valle del Cauca. Pacific region (RP), Cordillera Occidental (CO), Valle Interandino (VI) and Cordillera Central (CC). b) Gorgona Island (right). Sampling areas are indicated with geometrical shapes.

The snakes were externally examined for tissue damage, macroscopic alterations, or the presence of ectoparasites. Blood and fecal matter samples were taken from all specimens. Specimens euthanized by roxicaine injection or found dead were immediately necropsied and examined under stereomicroscope. All snakes except those from Gorgona Island were necropsied.

This study was carried out in accordance with the guidelines issued by the National Environmental Licensing Authority (ANLA) Decree # 1076 (2015) and # 06553 (2016), permit # 1070 (2015).

Sampling and identification of parasites

Endoparasites

Stool samples from all collected specimens were obtained spontaneously or by massage in sterile containers and preserved in Schaudinn's fixative. Samples were subjected to microscopic examination with saline solution and the zinc sulphate flotation method was performed for parasite concentration. The thin top layer was examined using Lugol's iodine and saline solution. In addition, fecal smears were subjected to trichrome stain and to a modified acid-fast stain (Kinyoun), the latter in search of coccidian parasites (Ash and Orihel, 1987).

Necropsied specimens (n=21) were carefully examined for helminths attached to organs and tissues. The parasites found were fixed in 70% ethanol for 24 hours before being taken to alcohol-glycerol and then cleared and mounted in glycerol gelatin. The slides were observed under microscope (Olympus CX31 100x).

All parasites were studied using the research microscopes: Nikon ECLIPSE Ni-U 90 trinocular and Zeiss Axio imager A2, larger organisms were visualized in a Nikon SMZ-1500 trinocular stereo microscope. Parasites were measured using the imaging analysis system NIS ELEMENTS Br version 4.20, Nikon and Zen Lite version 3.1. Identification of parasites was made using nematode taxonomic keys (Anderson *et al.,*1974). The parasite intensity was determined following Bush *et al*., (1997). Parasite burden from fecal samples was expressed as eggs per gram of feces (epg). Parasites were deposited in the Collection of Parasitology UVPAR (Department of Microbiology, Universidad del Valle, Cali, Colombia/ Registered as #564 in Alexander von Humboldt Institute).

Hemoparasites

Blood samples were obtained by ventral tail venipuncture, thin blood smears were fixed with methanol for three minutes and stained with 0.75% Giemsa solution. The slides were examined under 100x magnification (Olympus CX31) and parasites identified using previous morphology descriptions (Moço *et al*., 2002).

Parasitemia (parasite density) was estimated according to Úngari *et al.* (2018). Severity of infection was defined based on the level of parasitemia. A low-density infection was defined when at least one hemoparasite was observed in three high-power fields (hpf), whereas up to three hemoparasites x hpf were considered a middle density infection and more than three parasites x hpf were defined as a high-density infection. A quantitative parasitemia was calculated by counting parasitized erythrocytes among 10.000 erythrocytes and expressed as % of parasitemia.

Morphology of hemoparasites was analyzed based on features such as gametocyte shape, cytoplasmic pigment and nucleus shape and location. Measurements (i.e. area, length, and width) from the parasite and their nucleus were taken using a Zeiss Axio imager A2 with Zen Lite software (version 3.1). For each infected snake, 10 hemoparasites at the same stage were examined as indicated above and compared with each positive specimen to detect relevant morphology variability. The data obtained were subjected to principal component (PCA) and clustering analyses using RStudio version 4.0.3.

As *Hepatozoon* infections have been associated with changes in the red blood cells, 10 infected and 10 non-infected red blood cells from each snake were also measured, including area, length and width of cells and the respective nucleus (Moço *et al*., 2002). Infected and non-infected erythrocytes were compared using a t-test, p values ≤ 0.05 were considered statistically significant. RStudio version 4.0.3 was used to perform statistical analysis.

RESULTS

A total of 37 snakes belonging to the following eight species were analyzed: *Boa constrictor* (L.), *Bothrops asper* (Garman), *Clelia clelia* (Daudin), *Imantodes cenchoa* (L.), *Leptodeira annulata* (L.), *Mastigodryas boddaerti* (Sentzen), *Micrurus mipartitus* (Duméril, Bibron and Duméril), and *Sibon nebulatus* (L.) (Table 1). Most of them (43.2%) were collected in the tropical humid forest of Gorgona Island, the remaining snakes were collected from the Pacific region (32.4%), Cordillera Occidental (21.6%), and Cordillera Central (2.7%) (Table 1). Thirteen of the total snakes analyzed (35.1%) were females, 12 were males (32.4%), and the remaining 12 were undetermined (32.4%).

Eleven snakes (29.7%) were infected with at least one of the seven parasite taxa identified (i.e. six nematode taxa: Strongylida, Rhabditidae, Oxyuridae, Capillariidae, *Kalicephalus* sp., *Ophidascaris* sp., and one hemoparasite taxon: *Hepatozoon* sp.). No ectoparasites or enteric protozoa (coccidians or any other) were observed in any of the snakes analyzed in this study. Snakes of the species *S. nebulatus*,

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Tabla 1. Characteristics and distribution of the snakes studied from the Colombian Pacific. n= host analyzed. Total parasite prevalence for each host population is indicated (P). When P is absent, parasites were not found.

Host species	Habits and habitat	Cordillera Occidental	Pacific Region	Cordillera Central	Gorgona Island	Total
Bothrops asper (Garman)	nocturnal/ terrestrial	n= 3 P=33%	n=6 P=33%	-	n=5	n=14 P=21.4%
Imantodes cenchoa (Linnaeus)	nocturnal/ arboreal	n= 1	n= 3	n= 1	n=7 P=86%	n=12 P=50%
Sibon nebulatus (Linnaeus)	nocturnal/ arboreal	n=2	n=2	-	-	n=4
Leptodeira annulata (Linnaeus)	nocturnal/ semi-arboreal	n=2 P=50%	n=1	-	-	n=3 P=33%
Boa constrictor (Linnaeus)	nocturnal/ semi-arboreal	-	-	-	n=1 P=100%	n=1 P=100%
Mastigodryas boddaerti (Sentzen)	diurnal	-	-	-	n=1	n=1
Micrurus mipartitus (Duméril, Bibron and Duméril)	nocturnal	-	-	-	n=1	n=1
Clelia clelia (Daudin)	nocturnal/ terrestrial	-	-	-	n=1	n=1
Total		n=8 P=25%	n=12 P=16.6%	n=1	n=16 P=43.8%	n=37 P=29.7%

M. boddaerti, *M. mipartitus*, and *C. clelia* were not parasitized (Table 1).

Total parasite prevalences for each host population analyzed are shown in Table 1. Seven snake specimens (18.9%) were infected only by hemoparasites; three specimens (8.1%) were parasitized only by intestinal nematodes, whereas only one snake (2.7%) was infected by both hemoparasites and nematodes.

Three out of 14 *B. asper* analyzed (21%) were infected by four nematode taxa (see Table 2, Fig. 2a, b, c, d). One out of three *L. annulata* was co-infected with hemoparasites and three nematode taxa (Table 2, Fig. 2e, f). The single *B. constrictor* analyzed and six out of 12 *I. cenchoa* (50%) were infected by *Hepatozoon* spp. (Fig. 3a, b, c).

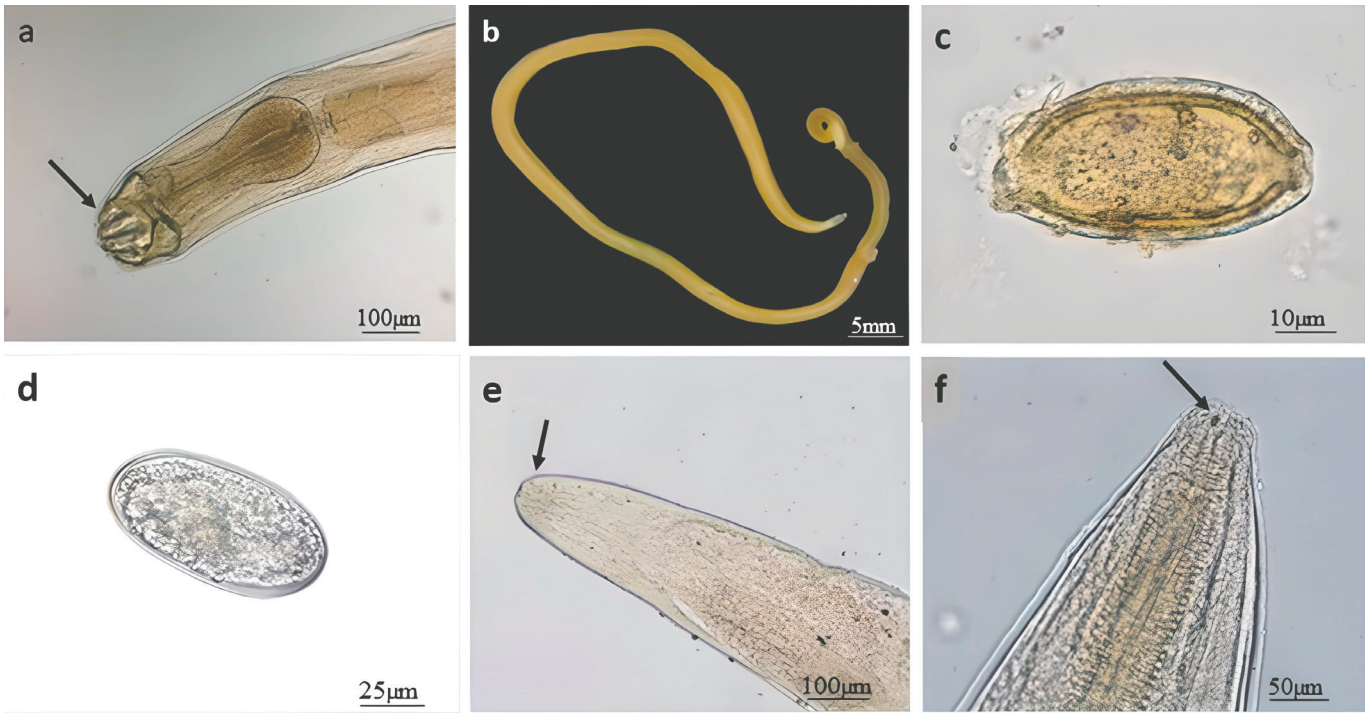

Figure 2. a-d) Parasites in *B. asper* from the Colombian Pacific. a) *Kalicephalus* sp. adult male, anterior end with buccal capsule showing four pairs of sclerotized plates (arrow). b) *Ophidascaris* sp. adult female. c) Capillariidae egg. d) Strongylida egg. e-f) Parasites in *L. annulata* from the Colombian Pacific. e) Rhabditidae, adult female, arrow indicates cylindrical buccal cavity. f) Oxyuridae, adult female, arrow indicates oral aperture with three lips.

Tabla 2. Parasite taxa found in the positive snakes (11/37) from different localities in the Colombian Pacific.

Locality Host species Taxa parasite	Infected hosts / total hosts (Prevalence)	stage, intensity of infection	Source/ Site
Cordillera occidental			
B. asper	1/3 (33%)		
Strongylida	1/3 (33%)	eggs, 6 epg	feces
L. annulata	1/2 (50%)		
Kalicephalus sp. (Strongylida)	1/2 (50%)	adult, 1	muscular tissue
Rhabditidae	1/2 (50%)	adult, 1	rectum
Oxyuridae	1/2 (50%)	adult, 1	rectum
Hepatozoon sp.	1/2 (50%)	gametocyte, 0.06%*	blood
Pacific Region Cordillera occidental			
B. asper	2/6 (33%)		
Kalicephalus sp.	1/6 (16.6%)	adult, 6	colon
Ophidascaris sp. (Ascarididae)	1/6 (16.6%)	adult, 1	small intestine
Capillaridae	1/6 (16.6%)	eggs, 2 epg	feces
Strongylida	1/6 (16.6%)	adult, 1	large intestine
Gorgona Island			
I. cenchoa	6/7 (85.7%		
Hepatozoon sp.	6/7 (85.7%	gametocyte, 0.02- 0.36%*	blood
B. constrictor	1/1 (100%)		
Hepatozoon sp.	1/1 (100%)	gametocyte, 1.7 %*	blood

epg= eggs per gram of feces. *Parasite burden of hemoparasites expressed as parasitemia level (percentage of parasitized erythrocytes calculated on 10000 erythrocytes).

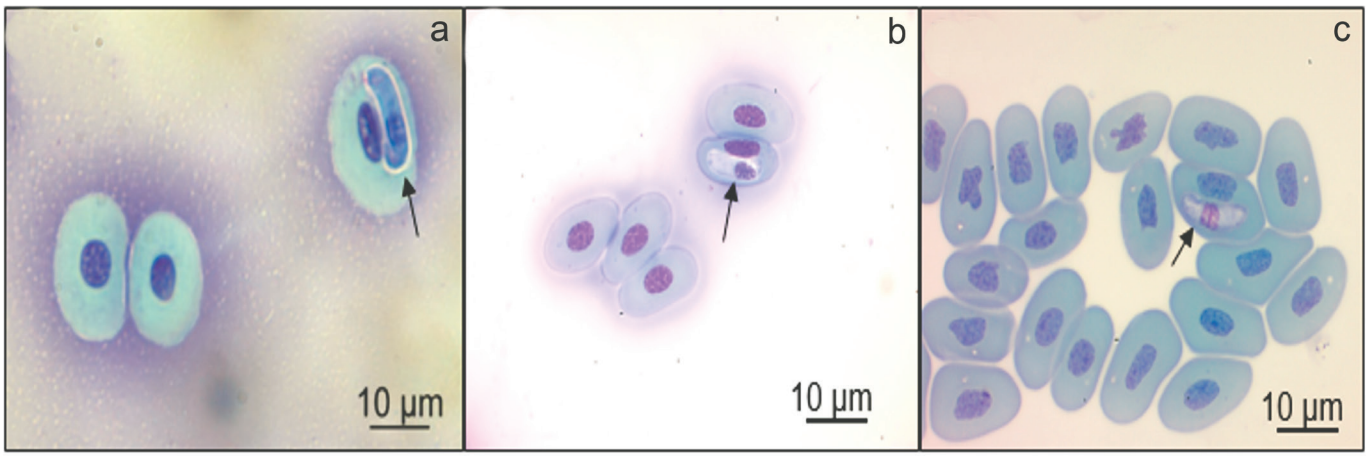

Figure 3. Morphology of *Hepatozoon* gametocytes (arrows) found in *B. constrictor* (a), *I. cenchoa* (b) and *L. annulata* (c) from the Colombian Pacific. Displacement of erythrocyte nucleus is seen in b and c.

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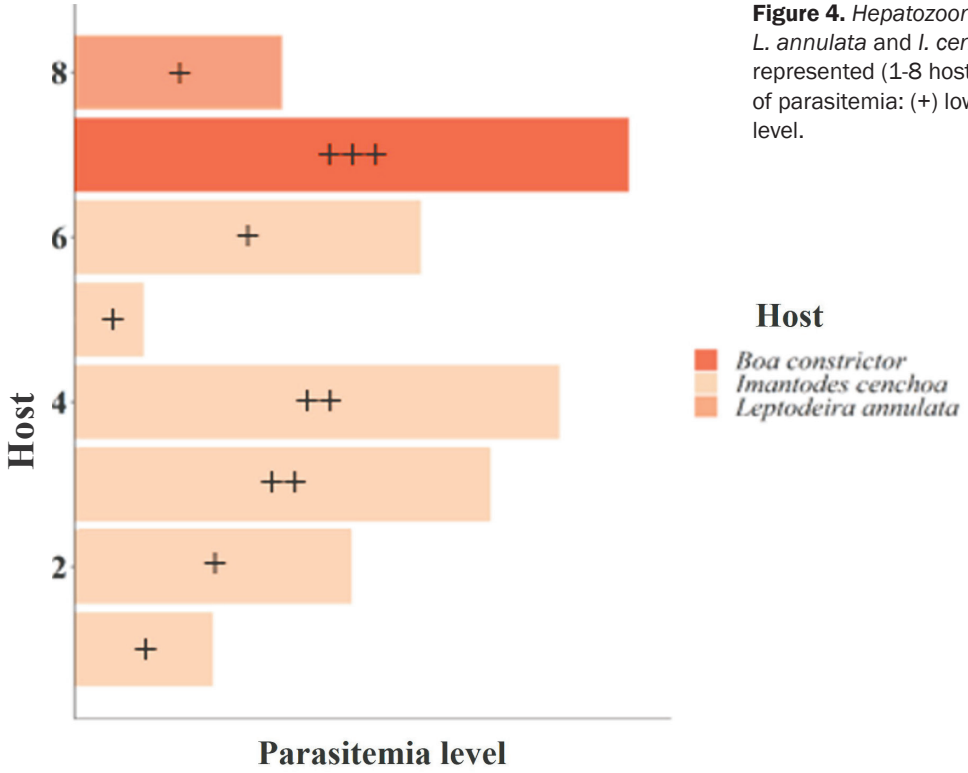

Figure 4. *Hepatozoon* spp. parasitemia in *B. constrictor*, *L. annulata* and *I. cenchoa*. A total of 8 infected snakes are represented (1-8 hosts). The crosses represent the level of parasitemia: (+) low level, (++) medium level, (+++) high level.

The highest level of parasitemia for *Hepatozoon* spp. was recorded in the single *B. constrictor* analyzed (Table 2), whereas the lowest level was observed in one *I. cenchoa* (Fig. 4).

Morphometric analysis of *Hepatozoon* and parasitized cells

All intracellular gametocytes were elongated and had variable size. A compact nucleus and blue-colored cytoplasm were observed, in some cases, the parasite displaced the host cell nucleus (Fig. 3). Cluster analysis using the *Hepatozoon* morphometric data showed two groups, 1) parasites smaller than the mean, 2) parasites larger than the mean (Fig. 5). *Hepatozoon* spp. infection was mainly seen in arboreal and semi-arboreal snakes. As shown by the t- test analysis, changes in the nucleus and shape of the erythrocytes infected by *Hepatozoon* sp. were observed in most of the parasitized snakes *L. annulata*, *B. constrictor*, and *I. cenchoa* (Table 3).

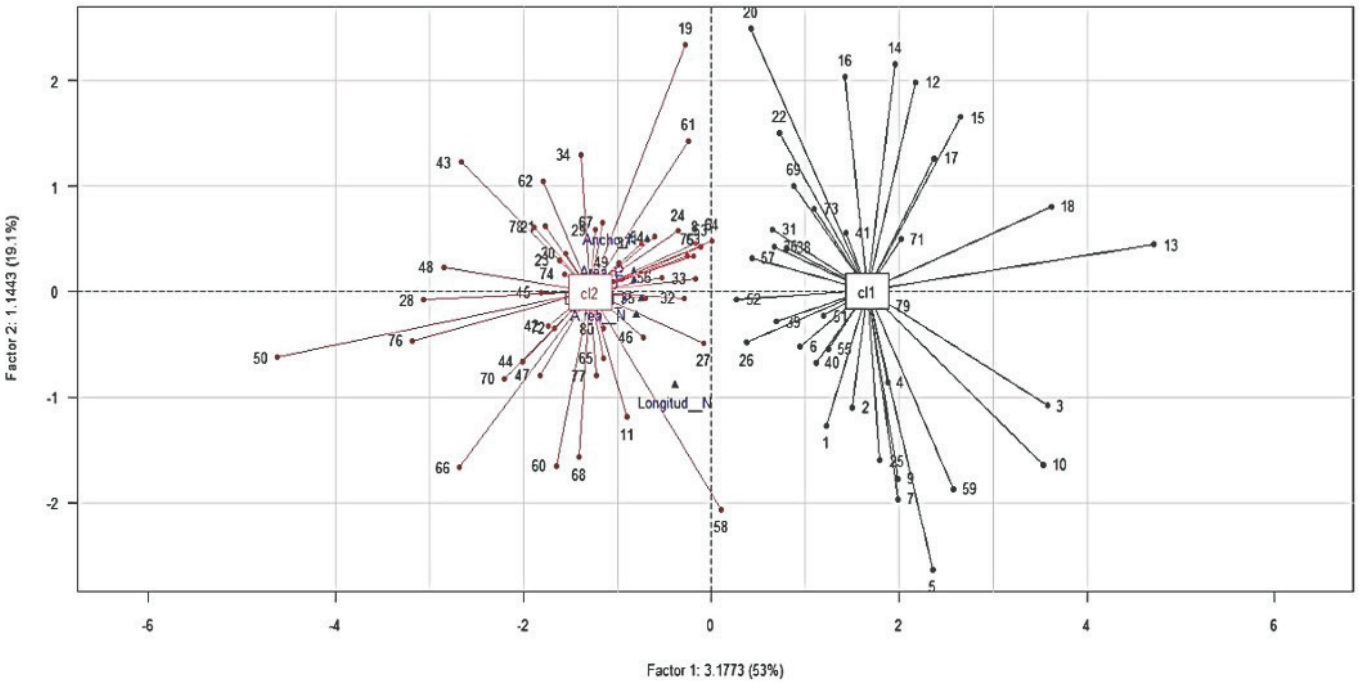

Figure 5. Morphometric analysis of *Hepatozoon* sp. from *L. annulata*, *I. cenchoa* and *B. constrictor*. Data subjected to PCA were visualized using Biplot (R studio) and parasites were assigned into two clusters (cl1 and cl2) indicating two morphology patterns. Eighty parasites were analyzed; each number corresponds to one parasite.

Tabla 2. Morphometric analysis of *Hepatozoon* infected and non-infected erythrocytes

Snake specimen	Parameter	Non infected erythrocyte (µm; SD)	Infected erythrocyte (µm; SD)	t-test p value
L. annulata	E area	184.8; 16.1	171.9; 15.6	0.09
	length	18.2; 1.0	19.2; 1.2	0.04
	width	12.8; 1.1	11.5; 0.8	0.01
	N area	24.0; 3.3	22.3; 3.4	0.28
	length	6.7; 0.7	8.0; 0.7	0.00
	width	4.5; 0.4	3.6; 0.4	0.00
B. constrictor	E area	172.1; 17.0	188.3; 10.3	0.02
	length	19.4; 0.9	19.1; 1.5	0.53
	width	11.1; 0.9	13.2; 1.8	0.00
	N area	23.0; 3.9	24.3; 3.3	0.42
	length	7.5; 0.4	7.0; 0.6	0.06
	width	4.2; 0.5	4.3; 0.3	0.39
I. cenchoa 1	E area	210.2; 23.4	203.8; 29.2	0.60
	length	19.6; 1.5	18.7; 1.7	0.21
	width	13.5; 1.5	13.5; 1.5	0.95
	N area	24.7; 4.2	24.1; 6.2	0.48
	length	7.8; 1.2	8.8; 0.8	0.02
	width	4.1; 0.4	3.7; 0.6	0.09
I. cenchoa 2	E area	205.2; 12.8	196.5; 18.0	0.23
	length	20.5; 1.7	20.3; 0.7	0.74
	width	12.6; 1.2	12.0; 1.9	0.36
	N area	26.6; 3.4	29.6; 5.6	0.24
	length	7.4; 0.5	8.8; 1.4	0.02
	width	4.6; 0.5	4.2; 0.8	0.11
I. cenchoa 3	E area	183.3; 24.9	184.5; 33.2	0.93
	length	18.5; 2.2	19.1; 1.6	0.50
	width	12.7: 2.0	12.3; 2.2	0.72
	N area	26.0; 3.0	25.6; 3.1	0.78
	length	7.7; 1.0	9.2; 1.0	0.00
	width	3.5; 0.7	4.7; 0.9	0.01
I. cenchoa 4	E area	207.3; 36.3	182.8; 14.4	0.07
	length	21.0; 2.0	20.0; 6301.8	0.24
	width	12.7; 1.8	12.2; 0.9	0.54
	N area	31.3; 3.5	25.9; 4.0	0.00
	length	8.5; 1.1	9.1; 0.9	0.39
	width	4.7; 0.6	3.7; 0.4	0.00
I. cenchoa 5	E area	185.1; 20.6	191.5; 15.6	0.44
	length	19.1; 1.3	19.2; 1.8	0.93
	width	12.2; 1.6	12.5; 1.4	0.68
	N area	26.3; 2.6	24.3; 4.1	0.21
	length	7.7; 2051.1	8.9; 0.9	0.00
	width	4.6; 0.7	3.9; 0.8	0.08
I. cenchoa 6	E area	197.2; 30.1	196.6; 14.1	0.58
	length	20.0; 1.9	20.3; 1.0	0.67
	width	12.5; 1.0	12.0; 0.8	0.30
	N area	25.7; 2.7	27.3; 2.4	0.16
	length	7.9; 0.7	9.6; 0.5	0.00
	width	4.6; 0.4	3.5; 0.4	0.00

Data in grey shadows are statistically significant, suggesting differences between infected and non-infected erythrocytes. E: Erythrocyte, N: Nucleus, SD: Standard deviation.

DISCUSSION

Most snakes were collected from Gorgona Island followed by the Valle del Cauca region where the herpetofauna is distributed mainly in the Pacific region and the Cordillera Occidental (Castro-Herrera and Vargas-Salinas, 2008). By contrast, in the Valle Interandino, which is a dry and very dry tropical forest, no snakes were found. This may suggest that the sampling effort requires longer times as reptile density in this ecosystem has been associated with prey availability and environmental and structural factors in the habitat (Bolívar-García *et al*., 2019). The high anthropic disturbance generated by agricultural activities and deforestation in this area, leads to a reduction of prey items, like amphibians (Cardona-Botero *et al.*, 2013; Lynch *et al.*, 2014).

Species of *Kalicephalus* have been found in the gastrointestinal tract and feces of snakes in South America (Schad, 1962; Siqueira *et al.*, 2009; González *et al*., 2018). *Kalicephalus* spp. has been associated with esophageal abscesses, chronic stomatitis, and oral infections (Matt *et al*., 2020). Although transmission of *Kalicephalus* spp. is still unresolved, it is known that the infective stage is the free-living third-stage larva. The snake could be infected by exposure of its tongue when the reptile checks the chemistry of the environment or through ingestion of paratenic hosts (Anderson, 2000). In this study, *Kalicephalus* sp. adults were observed in colon and one granuloma in somatic musculature, in the latter particular case, infection probably occurred by cutaneous perforation as suggested by Flynn (1973) and Siqueira *et al*. (2009).

The remaining nematode taxa found (i.e. strongylids, capillariids and *Ophidascaris* species), are commonly distributed among ophidians (Sprent, 1988; Fraser, 1993; Lichtenfels, 2009; Šlapeta *et al*., 2017). Capillariidae nematodes are commonly found in wetland reptiles (Šlapeta *et al*., 2017) and previously reported in colubrid and viperid species from Europe (Kvapil *et al.*, 2017). Likewise, *Ophidascaris* has been reported in *B. jararaca* from America, from the Neotropics (Siqueira *et al*., 2009).

In agreement with studies by Medrano-Tupiza *et al*. (2017) in wild snakes from ecological reserves in Ecuador, we did not find ectoparasites in the snakes examined. The absence of ectoparasites in our study may be due to the sampling of young snakes (based on size) which tend to have a higher growth rate (Webb and Whiting, 2005) and thus molt more frequently (Lynch *et al*., 2014) affecting somewhat the recovery of ectoparasites.

Hepatozoon spp. was the most frequent parasite in this work. Previous studies in Neotropical realm have reported *Hepatozoon* spp. in *B. constrictor* from Brazil (Moço *et al*., 2002; Úngari *et al*., 2018) and Colombia (Zamudio-Zuluaga and Ramírez-Monroy, 2007). High prevalence of *Hepatozoon* sp. has been reported in copperheads (50%) and non-viperid snakes (up to 100%) from urban old-growth forest in Memphis, Tennessee (Davis *et al.*, 2012). It is thought that snakes are infected by ingestion of intermediate hosts, invertebrate vectors, or mosquito bites (*Culex pipiens*) (Zamudio-Zuluaga and Ramírez-Monroy, 2007; Tomé *et al*., 2012). Here, *Hepatozoon* sp. were mainly identified in blood from arboreal and semi-arboreal snakes from Gorgona Island, where Culicidae mosquitoes have been described and may be potential vectors (González-Obando *et al*., 2012).

High levels of parasitemia by *Hepatozoon* spp. cause lack of oxygenation in muscle tissue (Caudell *et al*., 2002), malnutrition and delayed growth, impairing the ability for competition and reproduction (Úngari *et al*., 2018). However, in this study *Hepatozoon* parasitemia was under the threshold level associated with disease (Úngari *et al*., 2018). Several studies based on morphology analyses of *Hepatozoon* have suggested mixed infections by *Hepatozoon* spp. in *Hydrodynastes gigas* (Moço *et al*., 2002) and *Crotalus durissus* (Úngari *et al.,* 2018). However, rather than a mixed infection, morphometric variability may be driven by intraspecific variation and differential developmental stages in *Hepatozoon* spp. (Úngari *et al*., 2018). Many efforts have been made to identify *Hepatozoon* based on gametocyte morphometry, however, intraspecific polymorphism is one of the limitations of morphology-based studies (Bazzano *et al*., 2020). Morphology variability of *Hepatozoon* parasitizing *L. annulata*, *B. constrictor*, and *I. cenchoa* was observed in the present study. Although mixed infections are still possible, the nature of this variability needs to be further validated by molecular methods.

Analyses of infected and non-infected erythrocytes for each host species showed significant variations in particular features, so the effect induced by parasites on the red blood cells of snakes cannot be generalized. Moço *et al.* (2002) suggested that the degree of alteration of erythrocytes may be useful for characterization of *Hepatozoon* species, but our results indicate that morphometric features of erythrocytes are not suitable for identification to the species level.

This study describes the parasites affecting wildlife snakes in the Colombian Pacific and provides supporting evidence of hemoparasite and nematode infections of these reptiles in the ecoregions included for survey. The families Rhabditidae and Oxyuridae, and the genus *Kalicephalus* were found for the first time in *L. annulata.* Strongylida nematodes and the genera *Kalicephalus* and *Ophidascaris* were identified in *B. asper.* As stated by other authors (Aho, 1990; Martinez and Merino, 2011; Auld *et al.*, 2014; Frainer, 2018), it seems that parasite prevalence shows

drastic variations depending on host, parasite taxa, and environmental factors from each region.

Despite the small sample size, in the present study *Hepatozoon* sp. were detected in *L. annulata*, *B. constrictor*, and remarkably in *I. cenchoa* from Gorgona Island. Further research and monitoring are needed to elucidate the route of transmission of *Hepatozoon* and to determine the infection effects on *I. cenchoa* and other hosts and the ecological processes in Gorgona Island. Overall, this work contributes to the knowledge of reptile parasitic species from the sampled areas in Colombia.

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LITERATURE CITED

Aho, J. M. (1990). Helminth communities of amphibians and reptiles: comparative approaches to understanding patterns and processes. In: Esch, G.W., Bush, A.O., Aho, J.M. (eds) Parasite Communities: Patterns and Processes (157-195). Springer, Dordrecht.

Anderson, R. C. (2000). Nematode parasites of vertebrates: their development and transmission. CABI Digital Library.

Anderson, R. C., Chabaut, A. G., and Willmott, S. (1974). CIH Keys to the nematode keys to the nematode parasites of vertebrates. Commonwealth Agricultural Bureaux. https://books.google.com.co/books?id=z4wOAQAAMAAJ

Angulo, A., Rueda-Almonacid, J. V., Rodríguez-Mahecha, J. V., and La Marca, E. (2006). Técnicas de inventario y monitoreo para los anfibios de la región tropical andina. In Conservación Internacional. Serie Manuales de Campo Nº 2. Panamericana Formas e Impresos S.A., Bogotá D.C. (298). https://www.amphibians.org/wp-content/uploads/2018/12/Monitoreo-de-anfibios-baja-final.pdf

Ash, L. R., and Orihel, T. C. (1987). Parasites: A Guide to Laboratory Procedures and Identification. American Society of Clinical Pathologist .

Auld, S. K., Hall, S. R., Housley Ochs, J., Sebastian, M., and Duffy, M. A. (2014). Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. The American Naturalist, 184(S1), S77-S90.

Bazzano, V., Félix, M. L., Parodi, P., Carvalho, L. A., Nava, S., Armúa-Fernández, M. T., and Venzal, J. M. (2020). Phylogenetic analysis of *Hepatozoon* spp. (Apicomplexa: Hepatozoidae) infecting *Philodryas patagoniensis* (Serpentes: Dipsadidae) in Uruguay. Parasitology Research, 119(3), 1093–1100.

Bolívar-García, W., Gómez-Figueroa, A., Blanco-Torres, A., and Giraldo, A. (2019). Biología de los anfibios y reptiles en el bosque seco tropical del norte de Colombia (p. 247). Tunja UPTC.

Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology, 83(4), 575–583.

Carbajal-Márquez, R. A., González-Solís, D., and Cedeño-Vázquez, J. R. (2018). Endoparasites of *Crotalus tzabcan* (Serpentes: Viperidae), with a checklist in rattlesnakes. Journal of Parasitic Disesases, 42(2), 303–314.

Cardona-Botero, V. E., Viáfara-Vega, R. A., Valencia-Zuleta, A., Echeverry-Bocanegra, A., Hernández-Córdoba, O. D., Jaramillo-Martínez, A. F., Galvis-Cruz, R., Gutiérrez, J. A., and Castro-Herrera, F. (2013). Diversidad de la herpetofauna en el Valle del Cauca (Colombia): un enfoque basado en la distribución por ecorregiones, altura y zonas de vida. Biota Colombiana, 14 (2), 156-233.

Castro-Herrera, F., and Vargas-Salinas, F. (2008). Anfibios y reptiles en el departamento del Valle del Cauca, Colombia. Biota Colombiana, 9 (2), 251-277.

Caudell, J. N., Whittier, J., and Conover, M. R. (2002). The effects of haemogregarine-like parasites on brown tree snakes (*Boiga irregularis*) and slatey-grey snakes (*Stegonotus cucullatus*) in Queensland, Australia. International. Biodeterioration and Biodegradation, 49(2–3), 113–119.

Davis, J. R., Boyle, S. A., Khan, A. A., Gay, A. L. J., Grisham, J. M., and Luque, L. E. (2012). Snake parasitism in an urban old-growth forest. Urban Ecosystems, 15(3), 739–752.

Duran Peñaranda, N. D., Franco Gutiérrez, M., Guimarães Riva, H., and Flórez Gelvez, J. (2020). Prevalencia de endoparásitos y ectoparásitos en serpientes ex situ en Barranquilla, Colombia. Revista

MVZ Córdoba, 25(1), 1537.

Fernandes, B. M. M., and Kohn, A. (2014). South American trematodes parasites of amphibians and reptiles. Rio de Janeiro: Oficina de Livros, 2014, 228p.

Fernandes De Carvalho, E. F., Ferreira Da Silva-Neta, A., De Sousa Silva, C., De Oliveira, C. R., Da Cunha Xavier Nunes, J., Goncalves De Souza, T., and Ávila, R. W. (2018). Helminths infecting the cat-eyed snake *Leptodeira annulata* Linnaeus, 1758 (Squamata: Dipsadidae) in a semiarid region of Brazil. Helminthologia, 55(4), 281–285.

Flynn, R.J. 1973. Parasites of laboratory animals. p 159-161. Iowa State University Press. Ames, USA.

Frainer, A., McKie, B. G., Amundsen, P. A., Knudsen, R., and Lafferty, K. D. (2018). Parasitism and the Biodiversity-Functioning Relationship. Trends in Ecology and Evolution, 33(4), 260–268.

Fraser, C. M. (1993). El manual Merk de veterinaria (Océano. España (ed.); 4ª ed).

González-Obando, Ranulfo Armbrecht, Inge Montoya-Lerma, James Carrejo, N., Zuñiga, M., Ulloa-Chacón, P., Posso, C., Torres, D., Calero Mejía, H., Mendivil, J., González Córdoba, M., Valdés-Rodríguez, S., and Sarria, F. (2012). Artropofauna del Parque Nacional Natural Gorgona. In A. Giraldo and B. Valencia (Eds.), Isla Gorgona, paraíso de Biodiversidad y Ciencia. (149–192). Universidad del Valle.

González, C. E., Schaefer, E. F., and Duré, M. I. (2018). Presence of *Kalicephalus subulatus* Molin, 1861 (Nematoda, Diaphanocephalidae) in Wagler's snake, *Xenodon merremi* from Argentina. Annals of Parasitology, 64(4), 51–56.

Kvapil, P., Kastelic, M., Dovc, A., Bártová, E., Cížek, P., Lima, N., and Štrus, Š. (2017). An eight-year survey of the intestinal parasites of carnivores, hoofed mammals, primates, ratites and reptiles in the Ljubljana zoo in Slovenia. Folia Parasitologica (Praha), 64, 1–6.

Lenis, C., Arredondo, J. C., and Calle, J. I. (2009). *Ochetosoma heterocoelium* (Digenea: Plagiorchiidae) in snakes from Colombia. Revista Mexicana de Biodiversidad, 80(3), 603–609.

Lichtenfels, J. R. (2009). Strongylida: Diaphanocephaloidea and Ancylostomatoidea. In *Keys to the nematode parasites of vertebrates: archival volume* (pp. 44-68). Wallingford UK: CABI.

Lynch, J. D., Angarita-Sierra, T., and Ruíz-Goméz, F. J. (2014). Programa nacional para la conservación de las serpientes presentes en Colombia. Ministerio de Ambiente y Desarrollo Sostenible. Colombia.

Martinez, J., and Merino, S. (2011). Host-parasite interactions under extreme climatic conditions. Current Zoology, 57(3), 390-405.

Matt, C. L., Nagamori, Y., Stayton, E., and Brandão, J. (2020). *Kalicephalus* hookworm infection in four corn snakes (*Pantherophis guttatus*). Journal of Exotic Pet Medicine, 34, 62–66.

Medrano-Tupiza, E., Morales-Arciniega, S., Santander-Parra, S., Núñez-Naranjo, L., and Puga-Torres, B. (2017). Absence of Hemoparasites in Wildlife Snakes, Located in the Ecological Reserves Cota 70, Cotacachi-Cayapas and Sumaco-Napo-Galeras in Ecuador. Research in Zoology, 7(1), 7–10.

Mendoza-Roldan, J. A., Modry, D., and Otranto, D. (2020). Zoonotic Parasites of Reptiles: A Crawling Threat. Trends in Parasitology, 36(8), 677–687.

Moço, T. C., O'Dwyer, L. H., Vilela, F. C., Barrella, T. H., and Da Silva, R. J. (2002). Morphologic and morphometric analysis of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) of snakes. Memorias do Instituto Oswaldo Cruz, 97(8), 1169–1176.

Oda, F.H., Borteiro, C., da Graça,E. J., Tavares, L.E. R, Crampet, A., Guerra, V., Lima, F.S., Bellay, S., Karling, L.C., Castro, O., Takemoto, R. M. and Pavanelli,,G. C. (2016). Parasitism by larval tapeworms genus *Spirometra* in South American amphibians and reptiles: new records from Brazil and Uruguay, and a review of current knowledge in the region. Acta Tropica. 64,150-164.

Peichoto, M. E., Sánchez, M. N., López, A., Salas, M., Rivero, M. R., Teibler, P., Toledo, G. de M., and Tavares, F. L. (2016). First report of parasitism by *Hexametra boddaertii* (Nematoda: Ascaridae) in *Oxyrhopus guibei* (Serpentes: Colubridae). Veterinary Parasitology, *224*, 60–64.

Polley, L., and Thompson, A. (2015). Parasites and wildlife in a changing world. Trends in Parasitology, 31(4), 123–124.

Schad G.A. 1962. Studies on the genus *Kalicephalus* (Nematoda: Diaphanocephalidae). II. A taxonomic revision of the genus *Kalicephalus* Molin, 1861. Canadian Journal of Zoology 49: 1035-1165.

Siqueira, L. R., Panizzutti, M. H. M., Muniz-Pereira, L. C., and Pinto, R. M. (2009). Gross lesions induced by nematodes of *Bothrops jararaca* and *Bothrops alternatus* in Brazil with two new records. Neotrop Helminthol, 3(1):29.

Šlapeta, J., Modrý, D., and Johnson, R. (2017). Reptile parasitology in health and disease. In B. Doneley, D. Monks, R. Johnson, and B. Carmel (Eds.), Reptile Medicine and Surgery in Clinical Practice, (pp. 425–439). Wiley Blackwell.

Soria-Díaz, L., Rábago-Castro, J. L., Domínguez-Vega, H., Gómez-Ortíz, Y., Manjarrez, J., Garrido-Olvera (2019). Parasites in feces of the endemic rattlesnake, *Crotalus triseriatus* (Serpentes: Viperidae), from Mexican highlands. Zoologia, 36, 1–6.

Souza, J. L. de, Barbosa, A. da S., Vazon, A. P. Uchôa, C. M. A., Nunes, B. C., Cortez, M. B. V., Silva, V. L. da, Más, L. B., Melgarejo, A. R., Bastos, O. M. P., (2014). Parasitological and immunological diagnoses from

feces of captive-bred snakes at Vital Brazil Institute. Revista Brasileira de Parasitologia Veterinária, 23(2), 123–128.

Sprent, J. F. A. (1988). Ascaridoid nematodes of amphibians and reptiles: *Ophidascaris baylis* Systematic Parasitology, 11, 253–287.

The World Wide Fund for Nature (WWF). (2021). https://www.wwf.org.co/_donde_trabajamos_/pacifico/, consultado [20 de febrero de 2021].

Tomé, B., Maia, J. P., and Harris, D. J. (2012). *Hepatozoon* infection prevalence in four snake genera: influence of diet, prey parasitemia levels, or parasite type?. The Journal of Parasitology, 98(5), 913–917.

Uetz, P., Freed, P. and Hošek, J. (eds.) (2022). The Reptile Database, http://www.reptile-database.org, consultado [25 de agosto de 2022]

Úngari, L. P., Santos, A. L. Q., O'Dwyer, L. H., da Silva, M. R. L., Rodrigues Santos, T. C., da Cunha, M. J. R., de Melo Costa Pinto, R., and Cury, M. C. (2018). Molecular characterization and identification of Hepatozoon species Miller, 1908 (Apicomplexa: Adeleina: Hepatozoidae) in captive snakes from Brazil. Parasitology Research, 117(12), 3857–3865.

Webb, J. K., and Whiting, M. J. (2005). Why don't small snakes bask? Juvenile broad-headed snakes trade thermal benefits for safety. Oikos, 110(3), 515-522.

Zamudio-Zuluaga, N., and Ramírez-Monroy, M. (2007). Presencia de *Hepatozoon* spp. en serpientes del Centro de Atención y Valoración de Fauna Silvestre (CAV) del Área Metropolitana del Valle de Aburra, Barbosa - Antioquia. Revista CES Medicina Veterinaria y Zootecnia, 2(2), 33–36.

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