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Metazoan parasite communities of three endemic cichlid fish species from the upper Grijalva River, Chiapas, Mexico

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Summary

We recorded the metazoan parasite communities in three endemic cichlids (*Chiapaheros grammodes*, *Vieja breidohri* and *V. hartwegi*) collected between November 2008 and July 2009 in the upper Grijalva River Basin (GRB), Chiapas, Mexico. In total, 6,287 individual parasites belonging to 18 taxa (1 monogenean, 6 digeneans, 1 cestode, 4 nematodes, 2 acanthocephalans, 1 hirudinean, 2 copepods and 1 pentastomid) were found. Eleven metazoans were adult forms and 7 larvae; moreover, 14 were endoparasites and 4 ectoparasites. Sixteen parasite taxa represent new geographical and host records. The helminth community in the three cichlids was characterized by higher number of generalists than specialists, as well as a higher proportion of autogenics than allogenics. The metazoan parasites showed prevalence and mean abundances moderate to high. The infracommunities and component community of metazoan parasites had low diversity, richness, and number of individuals and are similar to those reported for other cichlids in Southeastern Mexico, characterized by the presence of typical parasites of cichlids, with a high number of digeneans and generalist parasites. We report the introduced Asian parasitic copepod *Neoergasilus japonicus* parasitizing endangered or threatened endemic cichlids in the upper GRB. This copepod have been widespread in other freshwater fish species, mainly in Asia (China, India, Japan, Russia, Taiwan), Europe (France, Hungary, Italy, Turkey), and America (Cuba, Mexico, Peru, United States).
Keywords: *Chiapaheros grammodes; Vieja breidohri; Vieja hartwegi;* freshwater parasites*;* infection parameters

Introduction

In the neotropics, the family Cichlidae (Perciformes: Actinopterygii) constitutes a diverse group of freshwater fishes, with around 50 genera and 500 species (Kullander, 1998; Chakrabarty, 2004). In Mexico, cichlids are represented by 57 species belonging to 11 genera, whose diversity is mostly found in hydrological systems from southeastern Mexico, such as the Grijalva River Basin (GRB), which is considered a regional centre of endemism for cichlids (Miller *et al*., 2005; Soria-Barreto & Rodiles-Hernández, 2008; Lowe-McConnell, 2009; Gómez-González *et al*., 2015).
To date, more than 200 parasitic helminth species have been

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recorded in 237 species of cichlids from Africa and America (Vanhove *et al*., 2016). In Mexico, this fish family is well studied (Vidal-Martínez *et al*., 2001) and harbours the highest helminth richness (69 species), followed by Goodeidae (51) and Ictaluridae (40) (Garrido-Olvera *et al*., 2012; Martínez-Aquino *et al*., 2014). However, there are few data on the metazoan parasites and their communities in endemic cichlids of Mexico, such as *Chiapaheros grammodes* (Taylor & Miller, 1980), *Vieja breidohri* (Werner & Stawikowski, 1987) and *V. hartwegi* (Taylor & Miller, 1980). These cichlids inhabit the upper GRB and are considered endangered species (DOF, 2010; Salgado-Maldonado, 2011). In order to know the biodiversity of metazoan parasites from aquatic vertebrates in Mexico, the main goal of this study was to determine the metazoan parasite fauna of these three endemic cichlid species in the upper GRB, and to describe their metazoan community structure in terms of taxonomic composition, species richness and diversity. We hypothesize that metazoan communities of the three endemic cichlids have low species richness and diversity with a similar composition of species like that of their congeners in the Southern Mexico. Moreover, it will be characterized by the presence of high number of digenean and specialist parasites.

Materials and Methods

Fishes were collected from the Angostura Dam (upstream) (16°06'28.4"N; 92°41'26.9"W) and Columbus Lakes (downstream) (15.2°42'31.9"N; 92°39'13"W), both located in the upper GRB (Fig. 1). A total of 84 *C. grammodes* (total length: 6.13 – 10.66 cm), 180 *V. breidohri* (9.5 – 14.56 cm) and 153 *V. hartwegi* (11.33 – 15.95 cm) were captured between November 2008 and July 2009 in both localities, using a 2 m diameter cast net with a 2.5 cm mesh. Living fish were transported in separated containers with artificial aeration to El Colegio de la Frontera Sur (ECOSUR), San Cristobal de las Casas, Chiapas, Mexico. Fishes were euthanized with a 100 mg/L of benzocaine until opercular movements ceased. Parasites were counted, preliminarily identified and fixed depending on the taxonomic group (Vidal-Martínez *et al*., 2001). Individual parasites were isolated from each organ, cleaned with physiological saline and preserved in vials with 4 % formalin or 70 % alcohol (Moravec *et al*., 1995).

Platyhelminthes (trematodes, cestodes), hirudineans and acanthocephalans were stained with Carmine as described by Vidal-Martínez *et al*. (2001). Nematodes and pentastomids were cleared in graded solutions of glycerine-water (Moravec *et al*., 1995; Silva *et al*., 2015). Infection parameters, such as prevalence, mean abundance and mean intensity were those proposed by Bush *et al*. (1997), whereas parasite species were classified as autogenic (i.e., mature in fishes or other aquatic hosts, are incapable of crossing land barriers between freshwater bodies) and allogenic (i.e., mature in mammal or bird hosts and use fish only as intermediate and paratenic host) (Esch *et al*., 1988; Salgado-Maldonado *et al*., 2016). Moreover, parasites were assorted

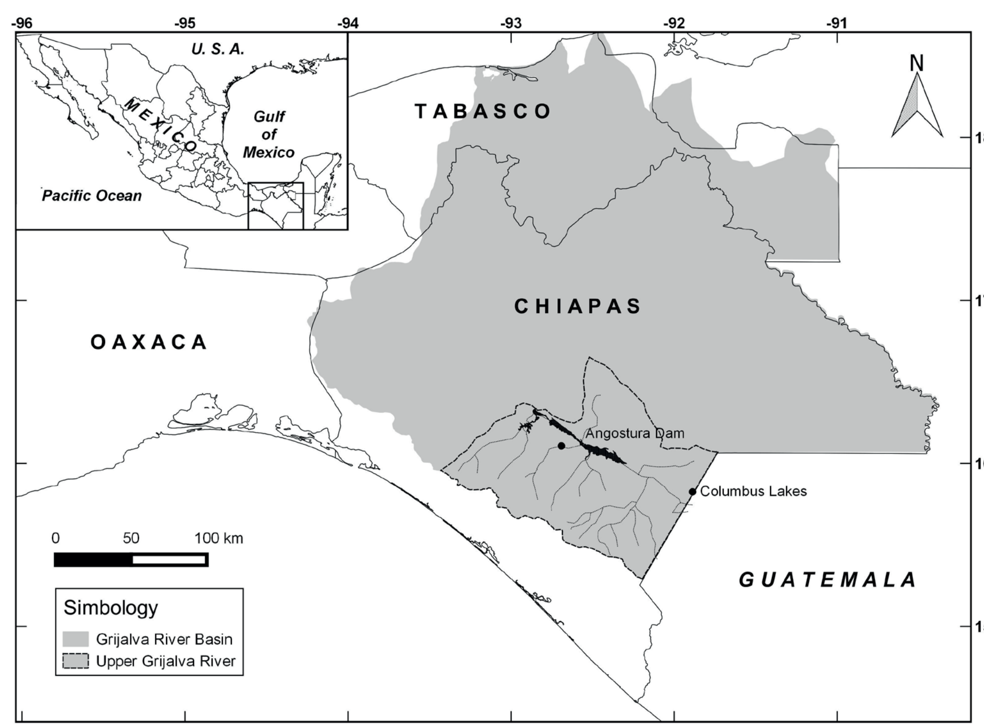

Fig. 1. Study area showing the two localities (Angostura Dam and Columbus Lakes) where fishes were collected.

as specialists (i.e., those restricted to one species, genus or host family) and generalists (i.e., those parasitizing several – two or more – host families) (Rohde, 1993).

Infracommunities were described by the mean number of parasite species, individual helminths, and Brillouin diversity index (IDB) (Krebs, 1989). For component communities ecological descriptors were analysed at the diversity, richness and dominance levels (Holmes, 1987). Shannon-Wiener index (*H*) as a measure of diversity, Shannon evenness index (equitability of the species present) (*J*) and Berger-Parker index (IBP) as a measure of the numerical dominance, were also calculated (Krebs, 1989). A Kruskal-Wallis test (H) was used to determine the significant differences in the community component parameters among different host species (Sokal & Rohlf, 1995). The Spearman range coefficient (rs) was used to correlate the values of *J* with the diversity of the communities of the three cichlid species (Krebs, 1999).

Ethical Approval and/or Informed Consent

The research related to animal use has been complied with all the relevant institutional policies for the care and use of animals in Mexico (DOF, 2001).

Results

A total of 18 metazoan parasite taxa (11 adults and 7 larval stages): 1 Monogenea, 6 Digenea, 1 Cestoda, 4 Nematoda, 2 Acanthocephala, 1 Hirudinea, 1 Pentastomida and 2 Copepoda, were found in 417 fishes. Out of the hosts examined, 390 (90 %) were infected by at least one parasite taxon. Sixteen parasite taxa were present in *V. breidohri*, 15 in *V. hartwegi* and 14 in *C. grammodes*. Seven of the 18 parasite taxa were collected from the digestive tract (intestine and stomach), 5 in gills, 3 in fins, 2 in eyes, muscles and mesenteries and 1 in liver. Digeneans were the richest group in the three cichlid species, with 4 metacercariae and 2 adult forms. The monogenean *Sciadicleithrum bravohollisae* Kritsky, Vidal-Martínez & Rodríguez-Canul, 1994 and the nematode *Cucullanus angeli* Cabañas-Carranza & Caspeta-Mandujano, 2007 are considered as specialists, while the remaining 16 were generalists. Twelve taxa were cataloged as autogenic and 6 larval stages as allogenic; while 14 metazoans were endoparasites and 4 ectoparasites (Table 1).

Chiapaheros grammodes and *V. hartwegi* are new host records for 16 taxa, while all parasites in *V. breidohri* are new reports. Prevalence ranged 1–78 % in the three cichlids, with *Crassicutis cichlasomae* Manter, 1936 in *V. breidohri* and *V. hartwegi* (78 % and 72 %, respectively) and *Contracaecum* sp. type 2 and *Diplostomum* (*Austrodiplostomum*) cf. *compactum* (Lutz, 1928) in *C. grammodes* (69 % and 62 %, respectively) as those with the highest values. On the other hand, *Raillietnema kritscheri* Moravec, Salgado-Maldonado & Pineda-López, 1993 in *V. hartwegi* (8 ± 66.06 and 48.16 ± 144.5), *Posthodiplostomum* cf. *minimum* (4.02 ± 5.11 and 11 ± 7.60) and *C. cichlasomae* (9 ± 13.11 and 11.07 ± 14.1) in *V. breidohri*, and *Neoergasilus japonicus* (Harada, 1930) in *C. grammodes* (4 ± 6.35 and 16 ± 17.19) and *V. breidohri* (1.16 ± 3.98 and 23.33 ± 25.47), were the species with the highest values of mean abundance and intensity, respectively (Table 1).

Parasite infracommunities showed low diversity, number of species and individual metazoans in the three cichlid hosts. The mean species number was variable (1.94 ± 1.44–2.28 ± 1.21) and relatively higher in *C. grammodes*, while *V. hartwegi* showed higher number of individuals (17.11 ± 47.06) than the two other cichlid hosts. The IDB index was similar in the three cichlid species, but relatively higher in *C. grammodes* (0.03) than in *V. breidohri* and *V. hartwegi* (Table 2). The overall component community had values similar in all descriptors among cichlids (Table 2). Total species number was identical in *V. breidohri* (16), *V. hartwegi* (15) and *C. grammodes* (14). The average number of parasites per individual host was 19, with *V. hartwegi* as the host with the highest number of individual parasites (2984). The *H* index had little variation in the three cichlid species, although it was relatively higher for *C. grammodes* (2.97). The *J* index had a range of 0.48-0.50, but was relatively high in *C. grammodes*.

Evenness (*J*) was positively correlated with diversity values in the communities of the three cichlid species (rs = 8.43; $p < 0.03$), meaning that the most diverse communities were those with the higher uniformity in species abundance. The IBP oscillated between 0.21–0.39, but was relatively high in *V. hartwegi*. The dominant metazoan species for *C. grammodes*, *V. breidohri* and *V. hartwegi* were *P.* cf. *minimum*, *C. cichlasomae* and *R. kritscheri*, respectively (Table 2). There were significant differences in the mean individual numbers of parasite species among different host species (H = 99.21; p = 0.001).

Discussion

The parasite fauna of the three species of cichlids from the upper GRB is composed of 18 taxa, all of them have been previously reported in other cichlids from Southeastern Mexico (Campeche, Chiapas, Oaxaca, Quintana Roo, Tabasco, Veracruz and Yucatan) (Salgado-Maldonado *et al*., 1997, 2005; Vidal-Martínez *et al*., 2001; Violante-González & Aguirre-Macedo, 2007; Violante-González *et al*., 2008a; Salgado-Maldonado, 2008, 2011, 2016) and in other fish families (Atherinidae, Clupleidae, Eleotridae, Goodeidae, Mugilidae, Pimelodidae and Profundulidae) (Montoya-Mendoza *et al*., 2004; Salgado-Maldonado *et al*., 2005; Martínez-Aquino *et al*., 2014; Pinacho-Pinacho, 2015). Despite this fact, almost all parasites reported herein represent new host and geographical records probably due to hosts have been poorly parasitologically studied or not studied at all (e.g. *V. breidohri*) in the Grijalva river basin. The parasites species were shared among the three cichlid species, as a consequence of their phylogenetic relationships, similar feeding habits, and low host specificity of helminths (Salgado-Maldonado *et al*., 2005; Salgado-Maldonado, 2008).

Table 1. Metazoan parasites found in *Chiapaheros grammodes* (Cg, n = 84), *Vieja breidohri* (Vb, n = 153) and *V. hartwegi* (Vh, n = 180) from the upper Grijalva River Basin. Abbreviations: Au: Autogenic, Al: Allogenic, G: Generalist, P (%): Prevalence, S: Specialist, SD: Standard deviation.

Parasite species	Infection site	Status	Host	No. of individual parasites	Infected fish	P (%)	Mean intensity (± SD)	Mean abundance (± SD)
DIGENEA								
Adults								
Crassicutis cichlasomae Manter, 1936	Intestine	S/Au	Vh	458	110	72	4.16 ± 6.15	3 ± 10.22
			Vb	1550	140	78	11.07 ± 14.1	9 ± 13.11
			Cg	23	11	13	2.09 ± 1.60	0.27 ± 0.85
Genarchella isabellae (Lamothe-Argumedo, 1977)	Stomach	G/Au	Vh	46	18	12	2.55 ± 2.12	0.30 ± 1.04
			Vb	126	24	13	5.25 ± 4.01	0.70 ± 2.33
			Cg	205	26	31	8 ± 8.64	2.44 ± 4.83
Larvae								
Posthodiplostomum cf. *minimum* (MacCallum, 1921)	Gills, Muscle, Eyes	G/Al	Vh	429	46	30	9.32 ± 6.6	2.80 ± 3.72
			Vb	725	69	38	11 ± 7.60	4.02 ± 5.11
			Cg	304	25	30	12.16 ± 13.7	3.6 ± 5.5
Clinostomum cf. *complanatum* (Rudolphi, 1814)	Muscle	G/Al	Vh	112	28	18	4 ± 5.46	0.73 ± 2.70
			Vb	41	20	11	2 ± 1.58	0.22 ± 0.78
			Cg	20	4	5	5 ± 2.36	0.23 ± 0.92
Cladocystis cf. *trifolium* (Braun, 1901)	Gills	G/Al	Vh	499	58	38	9 ± 10.09	3.26 ± 7.09
			Vb	80	25	14	3.23 ± 2.04	0.44 ± 1.22
			Cg	5	2	2	3 ± 0	0.06 ± 0.43
Diplostomum (*Austrodiplostomum*) cf. *compactum* (Lutz, 1928)	Eyes	G/Al	Vh	25	24	16	1.04 ± 1.05	0.16 ± 0.4
			Vb	9	5	3	1.8 ± 1.01	0.05 ± 0.2
			Cg	343	52	62	7 ± 10.3	4.08 ± 3.98
MONOGENEA								
Adult								
Sciadicleithrum bravohollisae Kritsky, Vidal-Martínez & Rodríguez-Canul, 1994	Gills	S/Au	Vh	33	14	9	2.4 ± 1.05	0.21 ± 0.96
			Vb	54	36	20	1.5 ± 0.92	0.35 ± 0.70
			Cg	9	3	4	3 ± 2.86	0.10 ± 1.01
CESTODA								
Adult								
Schyzocotyle acheilognathi (Yamaguti, 1934)	Intestine	G/Au	Vb	2	1	1	2 ± 0	0.01 ± 0.32
			Cg	2	1	1	2 ± 0	0.02 ± 0.31
NEMATODA								
Adults								
Cucullanus angeli Cabañas-Carranza & Caspeta-Mandujano, 2007	Intestine	S/Au	Vh	16	11	7	1.45 ± 0.9	0.10 ± 0.7
			Vb	1	1	1	1 ± 0	0.005 ± 0.10
			Cg	3	1	1	3 ± 1.1	0.03 ± 0.46

Raillietnema kritscheri Moravec, Salgado-Maldonado & Pineda-López, 1993	Intestine	G/Au	Vh	1204	25	16	48.16 ± 144.5	8 ± 66.06
Goezia nonipapillata Osorio-Sarabia, 1982	Intestine	G/Au	Vh	19	6	3	3.16 ± 4.9	0.12 ± 1.11
			Vb	2	5	3	0.4 ± 0	0.01 ± 0.15
			Cg	3	3	4	1 ± 0	0.03 ± 0.46
Larva								
Contracaecum sp. type 2	Mesenteries, Liver	G/Al	Vh	73	43	28	1.7 ± 0.1	0.47 ± 1.6
			Vb	52	49	27	1.06 ± 1.11	0.28 ± 0.7
			Cg	172	58	69	3 ± 2.5	2.04 ± 2
ACANTHOCEPHALA								
Adult								
Neoechinorhynchus golvani Salgado-Maldonado, 1978	Intestine	G/Au	Vh	111	39	25	2.84 ± 2.01	0.72 ± 0.04
			Vb	59	32	18	1.84 ± 1.11	0.32 ± 1.09
			Cg	15	7	8	2.14 ± 1.7	0.17 ± 0.69
Larva								
Polymorphus brevis (van Cleave, 1916)	Stomach	G/Al	Vb	3	1	1	3 ± 4.66	0.01 ± 0.15
HIRUDINEA								
Adult								
Myzobdella sp.	Fins	G/Au	Vh	15	12	8	1.25 ± 0.67	0.09 ± 0.40
			Vb	20	24	13	0.85 ± 1	0.11 ± 0.40
			Cg	6	5	6	1.2 ± 0	0.07 ± 0.24
PENTASTOMIDA								
Larva								
Sebekia sp.	Mesenteries	G/Au	Vh	4	1	1	4 ± 5.32	0.02 ± 0.28
COPEPODA								
Adults								
Neoergasilus japonicus (Harada, 1930)	Gills, Pelvic fins	G/Au	Vh	403	31	20	13 ± 9.46	2.63 ± 7.07
			Vb	210	9	5	23.33 ± 25.47	1.16 ± 3.98
			Cg	314	20	24	16 ± 17.19	4 ± 6.35
Ergasilus sp.	Gills, Fins	G/Au	Vb	13	9	5	1.5 ± 0.57	0.07 ± 0.33

Table 2. Total values of the infracommunities and component community of metazoan parasites of the three cichlid fish species in the upper Grijalva River Basin.

Descriptor	*C. grammodes*	*V. breidohri*	*V. hartwegi*
Infracommunities			
Mean species number	2.28 ± 1.21	1.81 ± 1.28	1.94 ± 1.44
Mean individual number	15.64 ± 15.37	12.34 ± 18.17	17.11 ± 47.06
Brillouin Diversity Index	0.03	0.02	0.02
Component community			
Total species number	14	16	15
Total individual number	1307	1896	2984
Shannon-Wiener diversity Index	2.97	2.59	2.60
Shannon-Evenness Index	0.50	0.49	0.48
Berger-Parker Index	0.21	0.38	0.39
Dominant species	*P.* cf. *minimum*	*C. cichlasomae*	*R. kritscheri*

The number of parasites species recovered from *C. grammodes* (14), *V. breidohri* (16) and *V. hartwegi* (15) was relatively higher than those reported by Vidal-Martínez *et al*. (2001) and Salgado-Maldonado (2011) in *C. grammodes* (6) and *V. hartwegi* (1) from Chiapas and for others cichlids species; e.g., *Thorichthys helleri* (Steindachner, 1864) (5) from Tabasco, *Cichlasoma trimaculatum* (Guïnter, 1868) (12) in Guerrero and *C. fenestratum* (Guïnter, 1860) (11) in Veracruz (Jiménez-García, 1993). These differences could be a consequence of the abundance of intermediate hosts in the region, size, diet and geographic range of examined hosts. The feeding habits of *V. breidohri* and *V. hartwegi* consist mainly of aquatic plants, detritus and invertebrates (Rodiles-Hernández and González-Díaz, 2006, Ceballos *et al*., 2016) that could have favored the consumption of snails, such as *Pyrgophorus coronatus* (Pfeiffer, 1840), *Biomphalaria obstructa* Morelet, 1849 and *B. helophila* (d'Orbigny, 1835), which are present in the region and involved in the life cycles of many metazoans (see Aguirre-Macedo *et al*., 2011, 2016; Martínez-Aquino *et al*., 2017). On the other hand, *C. grammodes* feeds mainly on aquatic insects (e.g., Trichoptera, Odonata, Megaloptera) and small fish. Differences in the feeding items might generate variation on the species richness, diversity and infection parameters between host species (see Violante-González *et al*., 2008a, b; Gómez-González *et al*., 2015).

Digeneans were the most abundant and dominant group of helminths in the three endemic cichlids. This pattern agrees with that found by Violante-González *et al*. (2008a), who reported several digenean species in *C. trimaculatum*. Digeneans are also the numerically dominant group in many parasite communities of freshwater fishes in Mexico (Salgado-Maldonado *et al*., 2005; García-Prieto *et al*., 2014). Apparently, its high abundance in cichlids has been linked to the shallow waters and high productivity of aquatic systems that encourage the occurrence of intermediate (mollusks) and definitive hosts (fish-eating birds) (Aguirre-Macedo *et al*., 2011, 2016).

The higher proportion of adult stages (11) and autogenic species (12) suggests that these cichlids play an important role as definitive hosts for most helminth taxa in the upper GRB and that these metazoans mature within the aquatic environment. Occurrence of *Schyzocotyle acheilognathi* (Yamaguti, 1934) in the three cichlids represent new host records for this Asiatic parasite that was introduced into Mexico along with carp (*Cyprinus carpio* Linnaeus, 1758) (Salgado-Maldonado & Pineda-López, 2003; Suárez-Morales *et al*., 2010). *Neoergasilus japonicus* (also co-introduced along with *C. carpio*) has been recently reported from the same hosts/sites from the upper GRB (see Suárez-Morales *et al*., 2010), while *S. acheilognathi* has already been recorded in all Mexican states (with exception of Baja California) (Salgado-Maldonado & Rubio-Godoy, 2014).

Infracommunities showed low species richness, number of individuals and diversity, like those in *Cichlasoma istlanum* (Jordan & Snyder, 1899), *C. synspilum* Hubbs, 1935 and *C. trimaculatum* from Guerrero and Campeche (Vidal-Martínez & Kennedy, 2000; Violante-González *et al*., 2008a). Probably, the main factors structuring the infracommunities were the feeding habits and variation in the availability of infective larval stages. These factors structured the infracommunities through the accumulation of free-living cercariae (e.g., *D.* (*A.*) cf. *compactum*, *C.* cf. *complanatum* and *P.* cf. *minimum*), or ingestion of infected preys, such as snails (e.g., *C. cichlasomae*) or small crustaceans (e.g., *Contracaecum* sp. type 2) (Aguirre-Macedo *et al*., 2011, 2016). The feeding habits of *V. breidohri* and *V. hartwegi* consist mainly of aquatic plants and a wide variety of snails (Gómez-González *et al*., 2015), that could have favoured the parasite infections of intermediate hosts (e.g., snails *P. coronatus*, *B. obstructa* and *B. helophila*), involved in the life cycles of many metazoans (Aguirre-Macedo *et al*., 2011, 2016; Martínez-Aquino *et al*., 2017). On the other hand, *C. grammodes* feeds mainly on aquatic insects (e.g., Trichoptera, Odonata, Megaloptera) and small fish that might produce little variation on their community descriptors (species richness, diversity) and infection parameters (mean abundance) between host species (Violante-González *et al*., 2008a,b; Gómez-González *et al*., 2015).

Overall component communities in the three cichlids were poor in species and number of metazoans, when comparing with other tropical cichlids: *Mayaheros urophthalmus* (Günther, 1862) group (= *Cichlasoma urophthalmum*) (38 species), *Parachromis managuensis* (34 species), *Thorichthys helleri* (32 species), *Vieja fenestrata* (= *Cichlasoma fenestratum*) (37 species) and *Petenia splendida* (27 species) (Salgado-Maldonado & Kennedy, 1996; Salgado-Maldonado *et al*., 2005). Generally, helminth communities in freshwater fish are isolationist in structure and stochastic in composition, thus reflecting their low species richness and diversity (see Vidal-Martínez & Kennedy, 2000; Kennedy, 2006; Salgado-Maldonado *et al*., 2019). This pattern is probably due to several factors, such as geography and geology (contemporary and past) of regions and drainages, different environmental conditions, historical ecology of both hosts and parasites, feeding behavior and specializations of the host, which contribute to the structuration of the communities of parasites (Choudhury & Dick, 2000).

Helminth species reported herein have clearly a Mesoamerican affinity and some with relatively high host specificity (i.e., *C. angeli* and *S. bravohollisae*), parasitizing almost exclusively cichlid fishes (Aguilar-Aguilar *et al*., 2008; Salgado-Maldonado, 2008). Others (i.e, *P.* cf. *minimum*, *Contracaecum* sp. type 2, *D.* (*A.*) cf. *compactum*, *Ergasilus* sp.), due to their low host specificity, have extended their distribution to some other regions of the Mexican highlands; while some others represent introduced and broadly distributed parasites (i.e., *S. acheilognathi* and *N. japonicus*). In conclusion, the parasite community of *C. grammodes*, *V. breidohri* and *V. hartwegi* is similar to that reported for other cichlids in Southeastern México and characterized by the presence of typical parasites of cichlids, with a high number of digenean and generalist parasites.

Conflict of Interest

The authors fully declare that there is no financial or other potential conflict of interest.

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