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Endohelminth parasites of the freshwater fish *Zoogoneticus purhepechus* (Cyprinodontiformes: Goodeidae) from two springs in the Lower Lerma River, Mexico

Endohelmintos parásitos del pez dulceacuícola *Zoogoneticus purhepechus* (Cyprinodontiformes: Goodeidae) en dos manantiales de la cuenca del río Lerma bajo, México

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Abstract. In order to establish the helminthological record of the viviparous fish species *Zoogoneticus purhepechus*, 72 individuals were collected from 2 localities, La Luz spring (n= 45) and Los Negritos spring (n= 27), both in the lower Lerma River, in Michoacán state, Mexico. Twelve helminth taxa were recovered, 5 adults (the digeneans *Margotrema bravoae* and *Phyllodistomum* sp., the cestode *Bothriocephalus acheilognathi*, the nematode *Rhabdochona lichtenfelsi* and the acanthocephalan *Pomphorhynchus* cf. *bulbocolli*), and 7 larvae (the metacercariae of *Clinostomum complanatum*, the cysticercoid of Cyclophyllidea, the nematodes *Rhabdochona* sp., *Eustrongylides* sp., *Contracaecum* sp. and *Spiroxys* sp., and the cysthacanth of *Polymorphus brevis*). Of these, R. lichtenfelsi was the most prevalent and abundant species at La Luz spring with 15.6% and 0.33 individuals per analyzed host. The remaining species were relatively more rare and infrequent. The helminth parasite community of *Z. purhepechus* at Los Negritos spring was remarkably poor and abundance was very low. The omnivorous feeding habits, the position of the host species in the food web, and the environmental characteristics of each locality are suggested as the main factors determining the helminth parasite communities in this freshwater fish.

Key words: Goodeidae, *Zoogoneticus purhepechus,* helminth parasites, community structure, Mexico.

Resumen. Se examinaron 72 individuos del pez vivíparo *Zoogoneticus purhepechus* para establecer el registro helmintológico de la especie. Los huéspedes se recolectaron de los manantiales La Luz (n= 45) y Los Negritos (n= 27), ubicados en la porción baja del río Lerma, en el estado de Michoacán, México. El registro helmintológico consta de 12 especies, incluyendo como adultos los digéneos *Margotrema bravoae* y *Phyllodistomum* sp., el céstodo *Bothriocephalus acheilognathi*, el nemátodo *Rhabdochona lichtenfelsi*, y el acantocéfalo *Pomphorhynchus* cf. *bulbocolli*. Además, como estadios larvarios, se encontraron las metacercarias de *Clinostomum complanatum,* el cisticercoide de Cyclophyllidea, los nemátodos *Rhabdochona* sp., *Contracaecum* sp., *Eustrongylides* sp. y *Spiroxys* sp., y el cistacanto de *Polymorphus brevis*. De éstas, *R. lichtenfelsi* fue la especie más frecuente y abundante en el manantial La Luz, en tanto que las restantes fueron relativamente más raras. La comunidad de helmintos de *Z. purhepechus* en el manantial Los Negritos fue pobre y poco abundante. Se sugiere que los principales factores que determinan la estructura de la comunidad de helmintos son los hábitos alimentarios omnívoros de los huéspedes, la posición que éstos ocupan en la red trófica y las características ambientales de cada localidad.

Palabras clave: Goodeidae, *Zoogoneticus purhepechus***,** helmintos parásitos, estructura de la comunidad, México.

Introduction

The genus *Zoogoneticus* Meek (Cyprinodontiformes: Goodeidae) includes 3 species of livebearing freshwater fishes, *Z. quitzeoensis* Bean, *Z. tequila* Webb and Miller, and the recently described *Z. purhepechus* Domínguez-Domínguez, Pérez-Rodríguez and Doadrio. These fish species are restricted to particular river drainages in central Mexico (Domínguez-Domínguez et al., 2008) and are considered as endangered or critically endangered (De la Vega-Salazar et al., 2003; Domínguez-Domínguez

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et al., 2005, 2008). The helminth fauna of livebearing freshwater fishes of the family Goodeidae has been well documented and the depauperate nature of helminth parasite communities in these fishes has been suggested (Astudillo-Ramos and Soto-Galera, 1997; Rojas et al., 1997; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004, 2007, 2009; Sánchez-Nava et al., 2004; Martínez-Aquino, 2005; Mejía-Madrid et al., 2005; Romero-Tejeda et al., 2008). However, the only species of *Zoogoneticus* that has been studied to a certain extent is *Z. quitzeoensis* and no records of the helminth parasite fauna of the other 2 congeneric species had been established. The main objective of this work is to record the helminth parasite fauna of *Z. purhepechus* collected in 2 sites (Los Negritos and La Luz springs) located in the lower Lerma River Basin, and to describe the helminth community structure and the processes that determine such structure.

Materials and methods

On July 2009, 72 adult specimens of *Z. purhepechus* were collected using nets in 2 localities, La Luz spring (*n*= 45) (19° 56' 10.4'' N, 102° 17' 57.8'' W; 1 616 m) and Los Negritos spring (*n*= 27) (20° 03' 23.1'' N, 102° 36' 38.3'' W; 1 539 m), in Michoacán state, central Mexico. Hosts were taken alive to the laboratory, pithed and examined individually for intestinal helminths. Other organs (eyes, gall bladder, liver, spleen, swim and urinary bladders) as well as body cavity and musculature, were examined under a stereomicroscope in separate Petri dishes with 0.65% saline. Digeneans, nematodes and cestodes were fixed with 4% (steaming) formalin. Acanthocephalans were maintained at 4°C for 24 hrs. in distilled water, and then fixed in 100% ethanol. Platyhelminths and acanthocephalans were stained with Meyer's paracarmine. Nematodes were cleared with glycerin for light microscopy and stored in 70% ethanol. Voucher specimens were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (Table 1); when accession number is not provided, specimens were collected for DNA work or scanning electron microscopy. Use of prevalence (% infected) and abundance (mean number of parasites of a single species in the sample) follows Bush et al. (1997). The non-parametric species richness estimators Chao 1 and Chao 2, were calculated following Colwell and Coddington (1995) and Escalante (2003), and were

Table 1. Endohelminth parasites of *Zoogoneticus purhepechus* in 2 springs of the Lower Lerma River, Mexico. CNHE= Accession number; HI= Number of infected hosts; %= Prevalence; Ab= Abundance; SD= Standard deviation; MI= Mean intensity

Helminth (Infection site / CNHE)*	*La Luz spring (n= 45)* *HI / % / Ab ± SD / MI ± SD*	*Los Negritos spring (n= 27)* *HI / % / Ab ± SD / MI ± SD*
Adult Digenea		
Margotrema bravoae (I)	1 / 2.2 / 0.02 ± 0.15 / 1	
Phyllodistomum sp. (Ub / 7791)	1 / 2.2 / 0.02 ± 0.15 / 1	
Larval Digenea		
Clinostomum complanatum (Bc, M / 7792)	5 / 11.1 / 0.13 ± 0.4 / 1.2 ± 0.45	
Adult Cestoda		
Bothriocephalus acheilognathi (I, 7793)		2 / 7.4 / 0.07 ± 0.27 / 1
Larval Cestoda		
Cyclophyllidea gen. sp. (Gb)		1 / 3.7 / 0.04 ± 0.19 / 1
Adult Nematoda		
Rhabdochona lichtenfelsi (I, 7794)	7 / 15.6 / 0.33 ± 0.98 / 2.14 ± 1.57	
Larval Nematoda		
Rhabdochona sp. (I, 7108)		1 / 3.7 / 0.04 ± 0.19 / 1
Contracaecum sp. (L, 7105)		1 / 3.7 / 0.07 ± 0.38 / 2
Eustrongylides sp. (L, 7106)		1 / 3.7 / 0.04 ± 0.19 / 1
Spiroxys sp. (M)	4 / 8.9 / 0.13 ± 0.5 / 1.5 ± 1	1 / 3.7 / 0.11 ± 0.58 / 3
Adult Acanthocephala		
Pomphorhynchus cf. *bulbocolli* (I, 7795)	6 / 13.3 / 0.13 ± 0.34 / 1	
Larval Acanthocephala		
Polymorphus brevis (M, 7796)		1 / 3.7 / 0.04 ± 0.19 / 1

*Infection site: Bc= Body cavity; Gb= Gall bladder; Ub= Urinary bladder; I= Intestine; L= Liver; M= Mesentery

used to estimate the number of missing species for each component community. Infracommunities include all the helminth species in an individual host, and were described by using the mean number of parasite species per host, the mean number of individual helminths, and the mean value of the Brillouin diversity index. The numerical dominance at the infracommunity level was determined using the Berger-Parker dominance index (Southwood, 1978). Infracommunities were compared qualitatively within the locality using Jaccard similarity index and quantitatively using the Morisita-Horn index, as calculated in Magurran (1988).

Results

Twelve helminth taxa were recovered from the 2 sampled localities. The helminthological record comprises 3 digenean species: *Margotrema bravoae* Lamothe-Argumedo, 1970, *Phyllodistomum* sp., and the metacercariae of *Clinostomum complanatum* (Rudolphi, 1819); 2 cestodes: the cysticercoid of Cyclophyllidea gen. sp., and *Bothriocephalus acheilognathi* Yamaguti, 1934; 2 acanthocephalans: the cystacanth of *Polymorphus brevis* (Van Cleave, 1916) and *Pomphorhynchus* cf. *bulbocolli* Van Cleave, 1919; the adult nematode *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García-Prieto and Pérez-Ponce de León, 1998, and larval forms of *Contracaecum* sp., *Spiroxys* sp., *Eustrongylides* sp., and *Rhabdochona* sp. *La Luz spring*. Thirty-four individual helminths were collected from 45 hosts from La Luz spring. These helminths represent 6 species: *M. bravoae*, *Phyllodistomum* sp. (adult), *C. complanatum* (metacercariae), *R. lichtenfelsi* (adult), *Spiroxys* sp. (larvae), and *P.* cf. *bulbocolli* (adult). Infection site, number of infected fish, prevalence, abundance, and mean intensity for each helminth taxa are shown in Table 1. The adult nematode *R. lichtenfelsi* was the most abundant helminth species, accounting for about 42.8% of the worms collected in this locality; this nematode reached a prevalence value of 15.6% and abundance of 0.33 worms per analyzed host. The remaining species were rare, infrequent, and reached very low abundance values (Table 1). Of the 45 analyzed individual hosts, 19 were infected with at least 1 species of helminth in La Luz spring. Only 5 of the 45 hosts harbored 2 or more helminth species. The total number of individuals of all species per host varied from 1 to 7, with a mean intensity of 0.82 ± 1.41. The non-parametric species richness estimators (Chao1 and Chao 2) reached a value of 6. The helminth infracommunities were species-poor. Mean number of species per host was 0.6 ± 0.8. The Brillouin index for all infracommunities varied from 0 to 0.599, with a mean diversity value of 0.099 ± 0.21, while the Berger-Parker dominance index values varied from 0.33 to 1, with a mean of 0.91 ± 0.2. The helminth infracommunities displayed a low level of similarity. The corresponding Jaccard index varied from 0 to 1 (mean of 0.18 ± 0.4) and the Morisita-Horn index varied from 0 to 1 (mean of 0.2 ± 0.37).

Los Negritos spring. Only 11 individual helminths were collected from Los Negritos spring. The 7 species recovered were *B. acheilognathi* (adult), 1 specimen of Cyclophyllidea (cysticercoid), the larval nematodes *Rhabdochona* sp., *Contracaecum* sp., *Eustrongylides* sp., and *Spiroxys* sp., and *Polymorphus brevis* (cystacanth). Infection site, number of infected fish, prevalence, abundance, mean intensity for each helminth species are shown in Table 1. Only *B. acheilognathi* was found in 2 hosts, while the remaining species were even more rare and infrequent (Table 1). The total number of individual helminths of all species per host varied from 1 to 7, with a mean number of 0.4 ± 1.4 individuals per host. The value obtained from the non-parametric species richness estimators (Chao 1= 11 and Chao 2= 25), shows that apparently several missing species remaining to be found at the component of community level. The helminth infracommunities were also species-poor; 2 infracommunities had just 1 species and 1 had a maximum of 4. Mean number of species per host was 0.29 ± 0.87. The Brillouin index for all infracommunities varied from 0 to 0.86, with a mean diversity value of 0.3 ± 0.41, while the Berger-Parker dominance index values varied from 0.43 to 1, with a mean of 0.73 ± 0.31. The helminth infracommunities show a low level of similarity. The corresponding Jaccard index varied from 0 to 0.5 (mean of 0.08 ± 0.2) and the Morisita-Horn index varied from 0 to 0.67 (mean of 0.11 ± 0.27).

The comparison between the helminth parasite fauna of both study sites, exhibits a very low similarity. Table 1 shows that only 1 taxon (*Spiroxys* sp.) is shared between component communities, resulting in a Jaccard value of 0.17, and a Morisita-Horn value of 0.23. However, it is possible that the larval stage of *Rhabdochona* found in Los Negritos might be conspecific with *R. lichtenfelsi*; although, we were unable to identify this larval satge up to species.

Discussion

Most of the helminth species found in this study have been previously recorded in diverse freshwater fish species in central and northern Mexico (Mejía-Madrid et al., 2005; Pérez-Ponce de León et al., 2007, 2009, 2010; Martínez-Aquino and Aguilar-Aguilar, 2008; Romero-Tejeda et al., 2008). Two of these species, the digenean *M. bravoae* and the nematode *R. lichtenfelsi*, are commonly found

in goodeid fishes, and have been considered as a part of the core parasite fauna for this fish family (Mejía-Madrid et al., 2005; Pérez-Ponce de León and Choudhury, 2005; Martínez-Aquino et al., 2009).

Other helminth species recorded herein as adults were *Phyllodistomum* sp., *P.* cf. *bulbocolli*, and *B. acheilognathi*. The finding of *Phyllodistomum* sp. in this study represents a new host record for fishes of the family Goodeidae. To date, 6 species of *Phyllodistomum* have been recorded as a parasite of marine and freshwater fishes in Mexico (Pérez-Ponce de León et al., 2007). In this work, we collected 1 single specimen of this digenean, which hinders the accurate taxonomic determination. Considering the host associations of species of *Phyllodistomum* in freshwater fishes of North America (Hoffman, 1999), we may speculate that this represents an undescribed species. Further analysis of this worm and those collected from other goodeids in central Mexico (Martínez-Aquino, unpublished data) will allow the proper taxonomic identification of this digenean by using both, morphological and molecular data.

The acanthocephalan *P.* cf. *bulbocolli* is a parasite of freshwater fishes frequently recorded in North America (Hoffmann, 1999), and it was recently found in Mexico as a parasite of the catostomid *Catostomus nebuliferus* Garman and the cyprinid *Gila conspersa* Garman (Pérez-Ponce de León et al., 2009, 2010). The presence of this acanthocephalan in *Z. purhepechus* from La Luz spring represents the third published record for this species in Mexico, and the first record in freshwater fishes of the family Goodeidae. The cestode *B. acheilognathi* is an introduced species, which currently is widely distributed in freshwater fishes of Mexico (Rojas-Sánchez and García-Prieto, 2008), including several species of goodeids (Peresbarbosa-Rojas et al., 1994; Pérez-Ponce de León et al., 2000; Sánchez-Nava et al., 2004; Romero-Tejeda et al., 2008; Pérez-Ponce de León et al., 2009).

The remaining 7 helminth species found in *Z. purhepechus* in both localities were larval stages frequently recorded in the freshwater fish helminth fauna of Mexico and North America. These species were *C. complanatum*, the cysticercoid of Cyclophyllidea, *Contracaecum* sp., *Eustrongylides* sp. and *P. brevis*, all of them maturing in piscivorous birds, and the nematode *Spiroxys* sp., which reaches the maturity mainly in freshwater turtles. One single immature specimen of *Rhabdochona* sp. was found in Los Negritos spring. This nematode most likely belongs to the species *R. lichtenfelsi*, however, absence of reproductive structures prevent its accurate taxonomic determination.

The data we present here suggest that the helminth parasite species composition, and the helminth infracommunity structure in *Z. purhepechus* is consistent with the pattern that shows a depauperate parasite fauna in goodeid fishes inhabiting freshwaters in the Nearctic part of Mexico such as *Alloophorus robustus* Bean, *Goodea atripinnis* Jordan, *Allotoca diazi* Meek, *Chapalichthys encaustus* Jordan and Snyder, *Characodon audax* Smith and Miller, *Skiffia lermae* Meek, *Girardinychthys multiradiatus* Meek, *Xenotoca variata* Bean, *Xenotaenia resolanae* Turner, and *Z. quitzeoensis* Bean (Astudillo-Ramos and Soto-Galera, 1997; Rojas et al., 1997; Pérez-Ponce de León et al., 2000; Martínez-Aquino et al., 2004, 2007, 2009; Sánchez-Nava et al., 2004; Martínez-Aquino, 2005; Romero-Tejeda et al., 2008). The factors that determine the helminth community structure herein described are concordant with those described in the aforementioned studies (the feeding habits, omnivorous in this case, and the position in the food web of this species of host), i.e., 11 of the 12 helminth species infect their host when it feeds upon some species of crustacean or insect. Likewise, 7 of the 12 helminth species are larval forms that complete their life cycle when the fish is consumed by a definitive host, either a fish-eating bird or a reptile.

On the basis of the values obtained through the species richness estimators used in this study (Chao 1 and Chao 2), it seems that we sampled all the helminth fauna in La Luz spring, since the observed and estimated richness value was very similar; however, values obtained for the same richness estimators in Los Negritos spring indicate that various helminth species apparently remain to be found. This result was surprising, since following the idea of Pérez-Ponce de León and Choudhury (2010) that the inventory of the freshwater fish helminth fauna in Mexico is nearing completion, and not many additional species are expected to be found, and particularly in goodeids, since this is a group of hosts that has been extensively studied for helminths in the last years. Thus, this apparently high number of species remaining to be found at Los Negritos spring, could be the result of an artifact derived from very low abundance and mean intensity values for each helminth taxa. Both nonparametric estimators Chao 1 and Chao2 are sensitive to the presence of rare species (Escalante, 2003), and that is probably the reason of the apparently high number of missing species in that particular locality.

The comparison between the 2 study sites showed that both helminth assemblages had very low numbers of individuals and consequently, remarkable low prevalence and abundance values. However, the helminth community of *Z. purhepechus* at La Luz spring included at least 2 of the species considered specialists for the fish family Goodeidae, the digenean *M. bravoae* and the nematode *R.*

lichtenfelsi (Pérez-Ponce de León and Choudhury 2005). Likewise, the helminth assemblage of Los Negritos spring is comparatively species-poor, specialist species are lacking, and is conformed only by larval stages and the introduced tapeworm *B. acheilognathi*. This pattern results in very low levels of similarity between the component communities. It is noteworthy the absence of digeneans in Los Negritos spring in conjunction with extremely low abundance values for the helminth species found in that locality. On the other hand, in La Luz, helminth species composition includes at least 3 species of digeneans, even though no tapeworms were found, and abundance values of the helminth parasite fauna are slightly higher than in Los Negritos. We may speculate that this is the result of different environmental conditions of the sampling sites, e.g., primary productivity and associated physicochemical parameters (personal observation), and it is possible that the absence of digeneans in Los Negritos could be the result of the lack of molluscs that are the first intermediate host in their life cycle, however this needs to be determined with the proper sampling in the locality.

Of the 3 recognized species of *Zoogoneticus*, the helminth parasite fauna has been studied for 2 of them, *Z. quitzeoensis*, and now, *Z. purhepechus*. We compared the helminth parasite fauna of *Z. purhepechus* and its putative sister species, *Z. quitzeoensis* (see Domínguez-Domínguez et al., 2008), and some differences were found. The endohelminth fauna of both *Zoogoneticus* species comprises 16 taxa, including 7 digeneans (4 adults and 3 metacercariae), 2 cestodes (1 adult and 1 cysticercoid), 5 nematodes (1 adult and 4 larvae), and 2 acanthocephalans (1 adult and 1 larvae) (see Martínez-Aquino, 2005; Mejía-Madrid et al., 2005; Romero-Tejeda et al., 2008). Only 3 of the 16 taxa (*M. bravoae*, *B. acheilognathi*, and *R. lichtenfelsi*) are shared between both species. Considering that they are sister species, and even though they do not occur in sympatry, the differences in helminth parasite fauna are a sampling artifact due to the fact that these species have not been studied along their entire distributional range, in the Lower and Middle Lerma River, respectively. We predict that the result of a detailed survey work in other localities along the distribution range for both species, will allow us to find the species that have not yet been documented, increasing as a result the levels of similarity among the parasite fauna.

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