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Helminth communities of *Xiphophorus malinche* (Pisces: Poeciliidae), endemic freshwater fish from the Pánuco River, Hidalgo, Mexico

Comunidades de helmintos de *Xiphophorus malinche* (Pisces: Poeciliidae), especie dulceacuícola endémica del río Pánuco, Hidalgo, México

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Abstract. A total of 141 highland swordtails, *Xiphophorus malinche,* were collected from 2 localities in the Pánuco river drainage (Chicayotla and Malila) from Hidalgo, México. The parasite community structure of the 2 localities was examined and compared. Five taxa of helminths were recovered: 2 digeneans, adults of *Paracreptotrema* sp. and metacercariae of *Uvulifer* sp.; an adult monogenean, *Urocleidoides vaginoclastrum*; an adult cestode, *Bothriocephalus acheilognathi*, and an adult nematode, *Rhabdochona xiphophori.* Among them, *Ur. vaginoclastrum* was the most frequent and abundant species. The remaining species were rare and found at low mean abundance. Only 2 species of helminth were found at both localities. The observed species richness, individual abundance, and diversity were low at component community and infracommunity levels. Abundance of helminths and fish standard length were correlated. *Uvulifer* sp. was more abundant in small fish, but *Ur. vaginoclastrum* showed the opposite pattern, high abundance in larger fish. Differences observed in this study can be attributed to abiotic and biotic environmental factors resulting from the geographic separation of these localities during to the orogeny of the Sierra Madre Oriental that restricted fish to isolated headwaters.

Key words: component community, infracommunity, Sierra Madre Oriental, prevalence, abundance, diversity.

Resumen. Se compararon y examinaron las comunidades de helmintos de peces de *Xiphophorus malinche* de 2 localidades de la cuenca del río Pánuco (Chicayotla y Malila), Hidalgo, México. Se registraron 5 especies de helmintos: 2 digéneos, *Paracreptotrema* sp. y metacercarias de *Uvulifer* sp.; 1 especie de monogéneo adulto, *Urocleidoides vaginoclastrum*; 1 céstodo adulto, *Bothriocephalus acheilognathi*, y 1 nemátodo adulto *Rhabdochona xiphophori.* La especie de monogéneo resultó ser la más frecuente y abundante en las comunidades. Las demás especies se encontraron en baja abundancia. Sólo 2 especies de helmintos fueron encontradas en ambas localidades. La riqueza de especies, la abundancia y la diversidad se analizaron tanto a nivel de componente de comunidad como de infracomunidad. La abundancia de helmintos y la longitud patrón de los peces están correlacionadas. *Uvulifer* sp. fué más abundante en peces pequeños, pero *Ur. vaginoclastrum* fue más abundante en peces de talla grande. Las diferencias observadas en este estudio pueden ser atribuidas a factores bióticos y abióticos resultado de la separación geográfica de las localidades durante la orogenia de la sierra Madre Oriental, que restringió a los peces a cuerpos de agua aislados.

Palabras clave: componente de comunidad, infracomunidad, sierra Madre Oriental, prevalencia, abundancia, diversidad.

Introduction

The genus *Xiphophorus* (Pisces: Poeciliidae) nominally has 26 species, commonly called swordtails or platyfish, which are distributed in freshwater streams from northeastern México to eastern Honduras. In the Mexican drainages, the species are found in parts of the coastal plain and on the eastern to southeastern slopes of the Sierra Madre Oriental, but never beyond the barrier range of the Mexican plateau (Kallman and Kazianis, 2006). Several studies of the phylogeny of the genus have been conducted (e.g., Meyer et al., 1994; Marcus and McCune, 1999) and the results support the recognition of 3 principal clades. *Xiphophorus malinche* Rauchengerber, Kallman and Morizot, 1990 (Highland swordtail) is 1 of 9 members of the clade that inhabits the tributaries of the Río Pánuco

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basin. Its distribution is restricted to headwaters and highland streams of the Río Pánuco (Kallman and Kazianis, 2006; Culumber et al., 2011). *Xiphophorus malinche* has been the focus of the variety of behavioral ecology studies (Culumber et al., 2012; Verzijden et al., 2012), but, to date, only 4 species of *Xiphophorus* have been examined for helminth parasites (Salgado-Maldonado et al., 2004, 2005; Salgado-Maldonado, 2006), and *X. malinche* was not included in any of those studies. Furthermore, none of those studies described the parasite communities in a quantitative form. Therefore, the main goal of the present work is to record the helminth fauna of *X. malinche* and to describe the structure of its helminth community.

Materials and methods

Between May 2008 and July 2009, 141 adult specimens of *X. malinche* were collected using minnow traps. Fish were collected from 2 localities; Chicayotla (*n*= 81; 20º55'30" N, 98º34''36" W) and Malila (*n*= 60; 20º44'2" N, 98º42'54" W), belonging to the Arroyo Xontla and Río Conzintla, respectively, in the Pánuco drainage in Hidalgo, Mexico. Fish were taken alive to the laboratory of Centro de Investigaciones Científicas de las Huastecas Aguazarca (CICHAZ), pithed, and internal and external organs were examined for helminths. Helminths were collected, counted, and processed according to Monks et al. (2005). Infection parameters were calculated and applied according to definitions proposed by Bush et al. (1997). Analyses were made at the levels of component community (i.e., all the helminths in all fish collected per site) and infracommunity (i.e., all the helminths in each individual host, Holmes, 1986; Zander, 2001). In order to determinate if the sample size was sufficient, accumulative species curve was plotted and the observed values fitted to the Clench Model to assess an asymptotic trend (Clench, 1979; Magurran, 2004). The non-parametric species richness estimator Bootstrap was calculated to estimate the number of missing species for each component community (Poulin, 1998). To describe the component community, the total number of species of helminth, the total number of individual helminths, the Simpson index (H), as a measure of diversity, and the Berger-Parker Index, as a measure of numerical dominance, were used (Magurran, 2004). Infracommunity level parameters were described by using the mean of number of species of helminth per fish, the mean number of individual helminths, and the mean Brillouin Diversity Index value per host. Finally, the infracommunities were compared qualitatively within the locality using the Jaccard similarity index and quantitatively using the Morisita-Horn index (Magurran, 2004). Possible differences in abundance for species recorded in the 2 localities were evaluated using Chi^2-test. Correlations were carried out using Spearman's Rank test. Voucher specimens of helminths were deposited in the Colección Nacional de Helmintos (CNHE), Universidad Nacional Autónoma de México, México D. F.; the Harold W. Manter Laboratory of Parasitology, University of Nebraska Lincoln, Lincoln, Nebraska, USA; and the Colección de Helmintos, (CHE), Universidad Autónoma del Estado de Hidalgo, Mexico. Specimens deposited include *Bothriocephalus acheilognathi* Yamaguti 1934 (CNHE-9267), *Paracreptotrema* sp. (CHE-9263 to 9266; HWML-75051 to 75054), *Urocleidoides vaginoclastrum* Jogunoori, Kritsky and Venkatanarasaiah, 2004 (CNHE4376, 9270 to 9275; HWML-75046 to 75050, 64628 to 64633), and *Rhabdochona xiphophori* Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2001 (CHE-9268, 9269; HWML-64624 to 64627).

Results

The helminthological record for *X. malinche* now comprises 5 species of helminth from 2 localities: 2 digeneans, adults of *Paracreptotrema* sp. and metacercariae of *Uvulifer* sp.; a monogenean, *Urocleidoides vaginoclastrum*; an adult cestode, *Bothriocephalus acheilognathi*, and adult nematodes, *Rhabdochona xiphophori*. The cumulative species curve and the nonparametric species richness estimator value indicate that the inventory presented here for both localities can be considered complete (Bootstrap: Chicayotla= 4.37; Malila= 3.05).

In Chicayotla, 2 067 individual helminths were collected, represented by 4 species; *Uvulifer* sp., *R. xiphophori*, *U. vaginoclastrum* and *B. acheilognathi*. Infection parameter values for each taxon are shown in Table 1. The data indicate that *U. vaginoclastrum*, as well as *R. xiphophori* and *Uvulifer* sp. were frequent (prevalence > 60%) and abundant (mean intensity > 3.8), and *B. acheilognathi* was the only rare species (infrequent and of low mean intensity). Of the 81 individual fish, 20 were infected with at least 1 species of helminth, 36 harbored 2 species, and 22 fish had a maximum of 3 species. The total number of individuals of all species per fish ranged from 1 to 161, with a mean number of helminths per host 25.51±33.35. The mean number of species per fish was 1.95±0.82. The Brillouin index ranged from 0 to 0.85, with a mean diversity value of 0.39±0.20. The Berger-Parker dominance index values ranged from 0.5 to 0.99, with a mean value of 0.83±0.12.

In the locality of Malila 417 individual helminths of 3 species were collected: *Ur. vaginoclastrum*, *R. xiphophori*, and *Paracreptotrema* sp. Infection parameters for each

Table 1. Ecological parameters for the helminths of *Xiphophorus malinche*, from 2 localities in Hidalgo, México. *n*= total number of helminths collected; %= prevalence; Ab= abundance; MI= mean intensity; RI= range of intensity

Helminths (site of infection)	*Chicayotla (n= 81)*					*Malila (n= 60)*				
	n	*%*	*Ab*	*MI*	*RI*	*n*	*%*	*Ab*	*MI*	*RI*
Digenea										
Paracreptotrema sp. (intestine)						5	6.6	0.08±0.5	1.3	1-2
Uvulifer sp. (skin and fins)	189	60.4	2.3±4.2	3.8	1-21					
Cestoda										
B. acheilognathi (intestine)	1	1.2	0.01	1	1					
Monogenea										
Ur. vaginoclaustrum (gills)	1684	74.0	20.7± 35.4	28	1-161	407	56.6	6.7±14.6	11.9	1-67
Nematoda										
R. xiphophori (intestine)	193	59.2	2.3±4.4	4	1-23	5	6.6	0.08±0.5	1.3	1-2

taxa of helminth are given in Table 1. *Urocleidoides vaginoclastrum* had the highest prevalence and abundance (57% and 6.7 helminths per fish, respectively), and the remaining species were rare and infrequent. The total number of individual helminths of all species per fish ranged from 1 to 67, with a mean number of 6.95±12.44 individuals per host. The infracommunity in fish of Malila was species-poor; of 60 fish, 35 were infected with 1 species of helminth, 3 fish had 2 species, and 1 fish had a maximum of 3 species. The mean number of species per host was 0.70±6.95. It was not possible to calculate the Brilloin index because the number of infracommunities with more than 2 species of helminth was low. The Berger-Parker dominance index values ranged from 0.50 to 0.98, with a mean of 0.75±0.17.

The comparison between the helminth fauna of both study sites indicated that there are differences in the composition of species present in each site. *Uvulifer* sp. and *B. acheilognathi* were found only in fish of Chicayotla, and *Paracreptotrema* sp. was collected only from fish of Malila. *Rhabdochona xiphophori* and *U. vaginoclaustrum* were the species common to both component communities (Table 1), resulting in a Jaccard value of 0.40 and Morisita-Horn value of 0.97. The latter value showed that the component communities were highly influenced by the abundance of the dominant species. The Simpson diversity index was 0.31 in Chicayotla and 0.04 in Malila; the community at Chicayotla was more diverse than that at Malila. Evenness values were 0.46 in Chicayotla and 0.37 in Malila, relatively similar values. The Berger-Parker dominance index values were 0.81 in Chicayotla and 0.97 in Malila, and both localities were numerically dominated by *Ur. vaginoclastrum.*

No differences were observed between the sex of fish and the infection parameters or richness for any of the recorded helminth species. However, a significant correlation between mean abundance and fish size was observed. In Chicayotla, host size had a negative correlation with mean abundance of the metacercariae, *Uvulifer* sp. (r_s= -1; p< 0.01); and in Malila, mean abundance of *U. vaginoclastrum* was positively correlated with host size (r_s= 0.34; p< 0.04); none of the remaining species had a significant correlation in either population.

Discussion

Most of the species of helminths registered in this study have been recorded previously as parasites of species of *Xiphophorus* in other bodies of water in México (Salgado-Maldonado, 2006; Mendoza-Palmero and Aguilar-Aguilar, 2008). However, this is the first report of the digenean *Paracreptotrema* in *Xiphophorus*, although *P. heterandriae* has been reported recently from a species of Poeciliidae (*Heterandria bimaculata* [Heckel, 1848], *sensu* Salgado-Maldonado et al. 2012; =*Pseudoxiphophorus* "*bimaculata*" *sensu* Agorreta et al. 2013), *P. profundulusi* from 2 species of Profundulidae (*Profundulus punctatus* [Günther, 1866] and *P. balsanus* Ahl, 1935), and *P. blancoi* Choudhury, Pérez-Ponce de León, Brooks, and Daverdin, 2006 from *P. punctatus*, *P. balsanus*, and *P. oaxacae* (Meek, 1902) (Salgado-Maldonado et al., 2011). It should be noted that Salgado-Maldonado et al. (2011) did not find specimens of *Paracreptotrema* in individuals of *X. hellerii* Heckel, 1848 sympatric with *P. balsanus* and *P. punctatus.*

With respect to the other species of helminth, *Urocle idoides vaginoclaustrum* is a species originally described from *Xiphophorus hellerii* Heckel, 1848, a commonly kept aquarium species native to southern México and Central America that had been introduced to India (Jogunoori et al., 2004). It is possible that this monogenean originated in the neotropics, where its host also originated. Recently, Mendoza-Palmero and Aguilar-Aguilar (2008) made the first report of *U. vaginoclaustrum* in a natural habitat, parasite of the gills of *X. hellerii* introduced to the Los Berros spring, Durango, México. However, the authors observed that there were slight morphological differences in the sclerotized structures of the haptor in their specimens with respect to the specimens described from India, which they speculated might be due to differences in the fixation method used in their study. Although the specimens collected in the present study conform generally to previous descriptions, the fine morphology of the worms collected in Malila and Chicayotla should be reviewed and compared to the specimens of *U. vaginoclaustrum* from Durango and those from India to determine whether morphological differences are real or just artifacts of fixation methods. *Xiphophorus hellerii* also has been introduced to Hidalgo (Culumber et al., 2013), although its helminthofauna has not been reported.

Species of *Rhabdochona* are well known for host specialization at the family, genus and species level (Sánchez-Álvarez et al., 1998; Mejía-Madrid and Pérez-Ponce de León, 2003). *Rhabdochona xiphophori* originally was described in *Xiphophorus* sp. from the Pánuco drainage (Caspeta-Mandujano et al., 2001) and is now considered a specialist parasite of the Poeciliidae because it has been reported only from water bodies inhabited by *X. hellerii*, infecting only that species of fish (Mejía-Madrid et al., 2005). However, Mejía-Madrid et al. (2005) reported *R. xiphophori* as a parasite of 2 species of goodeids (*Allotoca catarinae* [de Buen, 1942] and *Xenotoca eiseni* [Rutter, 1896]), those authors suggested that this infection likely occurred due to ecological host-extension.

The tapeworm *B. acheilognathi* is an introduced species, now widely distributed throughout México (Salgado-Maldonado and Pineda-López, 2003), and is particularly common in the region (Gutiérrez-Cabrera et al., 2005). However, despite the fact that *B. acheilognathi* is widespread and found in high abundance in native fish species in many drainages, such as the Lerma-Santiago and Rio Balsas (Salgado-Maldonado and Pineda-López, 2003), it appears that it is not widely dispersed or common in native fishes in the Pánuco. Salgado-Maldonado et al. (2004), in their list of helminth parasites in the Pánuco, only registered this cestode in 5 of 17 native species that they examined. In the present study, only a single immature individual of *B. acheilognathi* was recovered from 1 fish.

Habitat characteristics, both biotic and abiotic, may facilitate the establishment and spread of helminths in certain populations of host. Some variables, such as the size of the water body, physical-chemical characteristics of the water, and the distance between each body of water in the region, are factors that have been associated with the number of species of helminth in fish populations and the mean abundance of a given species of helminth (Bagge et al., 2004).

Another aspect that affects the community structure of parasites are the characteristics of the host (body size, age, geographic distribution, food habits, etc.), which can make certain potential hosts more susceptible to colonization by new parasites, in addition to the fact that evolutionarily older species have typically accumulated more parasites than comparatively younger species (Poulin, 1997). In the case of *X. malinche*, the species is restricted to headwaters of the Pánuco drainage, a habitat/locality that was formed during the uplifting and folding of the Sierra Madre Oriental. During this process populations became isolated in the headwaters of multiple, disconnected streams (Kallman and Kazianis, 2006). Differences in the habitat or environment, such as the aforementioned water chemistry, distance to other populations, etc., of these disconnected stream populations may account for differences in the parasite communities. However, more populations of fish must be sampled consistently in order to elucidate a strong hypothesis about structuring factors.

Pineda-López et al. (2005) pointed out that an important component in the determination of helminth parasite community composition of Mexican poeciliids is the dominance of generalist, allogenic species that use freshwater fish as a secondary intermediate host or as a paratenic host. Salgado-Maldonado (2006) documented the helminths parasites of 24 species of poeciliids and 100% of the communities of helminths of those hosts were dominated proportionally by larval stages (larval Digenea, Cestoda, Nematoda, and Acanthocephala). The only 4 species of *Xiphophorus* for which parasites were reported (Salgado-Maldonado, 2006) had the same pattern; the only other mention of the helminths of a species of *Xiphophorus* does not provide complete data (Salgado-Maldonado et al., 2011).

However, our data does not fit their prediction. The component community of *X. malinche* is dominated by adult helminths (mostly monogeans), although an allogenic species is present (1 of 5 species; 189 of 2067 individuals= 9%). One possible explanation for the low number of allogenic species and of larval parasites in general in our data is that fish-eating birds are rarely observed at the localities included in this study (CEBH personal observation). Migratory waterfowl play an important role on the transmission and spread of allogenic species (Pineda-López et al., 2005; Salgado-Maldonado, 2006). Martínez-Morales et al. (2007) reported few piscivorus birds within the distribution of *X. malinche*. The absence of large, ictiophagus birds in the region could be because of the relative lack of open areas around the upstreams of the Pánuco drainage and abiotic factors (i.e. water speed, type of perching area, etc.). However, 4 species of kingfisher have been reported in the Huasteca region (Martínez-Morales et al., 2007), although their helminths have not been studied. These species could be the definitive hosts of the larval digenean, *Uvulifer* sp., reported herein.

The infracommunities studied herein were generally species-poor (on average, only 1-2 species of helminth per fish) and were dominated by the monogenean *Urocleidoides vaginoclaustrum*. This is consistent with infracommunities reported for other freshwater fishes in México (Martínez-Aquino et al., 2004, 2007; Martínez-Aquino and Aguilar-Aguilar, 2008). Kennedy (1989) suggested that infracommunities with low rates of colonization, low numbers of species and of individuals, and with low or no presence of interspecific interactions is a general pattern in freshwater fishes. As well, the diet of a species of fish may also influence parasite species richness; this may be an influencing factor in the present study because 3 of the 5 species reported here for *X. malinche* infect the fish via dietary habits.

In the present study, *U. vaginoclaustrum* was the dominant species, but the abundance of the species varied significantly between the 2 localities (χ^2= 7.11; p< 0.05). One factor that may determine the abundance of monogeneans on a host is the parasite's body size. Several studies have observed that as helminth body length increases, its abundance decreases, with the largest species of parasites naturally requiring more space and resources (Blackburn and Gaston, 1997; Poulin and Justine, 2008; Randhawa and Poulin, 2009). However, Poulin et al. (2008) has argued that this pattern does not always determine helminth parasite communities and those differences may be attributed to other interspecific factors.

Across all fish from Chicayotla, the metacercariae of *Uvulifer* sp. was found to have a negative correlation between parasite abundance and host body length. Lemly and Esch (1984) suggested that metacercariae of *Uvulifer ambloplitis* base their survival success and the completeness of their life cycle on the size of fish that it infects, such that the smaller the size of the host, the greater the abundance of metacercariae. Violante-González et al. (2009) found a similar pattern in cultivated fish, *Oreochromis niloticus* (Linnaeus, 1758), infected by *Diplostomum* (*Austrodiplostomum*) *compactum* (Lutz, 1928) Dubois, 1970. The authors suggested that this negative correlation might be due to behavioral differences between fingerlings and adults. Their reasoning was that small fish could get refuge among the aquatic vegetation, where they would be in constant proximity to snails (intermediate hosts), greatly increasing the probability of becoming infected (Violante-González et al., 2009).

The infections of *U. vaginoclaustrum* in fish from Malila revealed a positive correlation between parasite abundance and host body length. Öztürk and Altunel (2006) and Cable and van Oosterhout (2007) suggested independently that the abundance of this species of helminth increased with the age/size of the fish, since larger (and older) fish have a greater gill surface area, which permits abundance to increase over time, correlated with the increasing size of the host.

Because this is the first ecological study of the parasites of *X. malinche*, we cannot offer conclusive hypotheses, but, overall, our data indicate that the parasite communities of this species of fish are different in structure at the component level in each locality. We suggest that this is likely attributed to differences in local abiotic and biotic factors that determine the pool of species present in each locality. However, more solid hypotheses must await studies from additional localities and from sympatric species.

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