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Rogelio Aguilar-Aguilar
Universidad Nacional Autónoma de México, raguilar@ciencias.unam.mx

Omar Lagunas-Calvo
Universidad Nacional Autónoma de México, raguilar@ciencias.unam.mx

Gerardo Pérez-Ponce de León
Universidad Nacional Autónoma de México, raguilar@ciencias.unam.mx

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HELMINTH COMMUNITIES OF *CYPRINODON ATRORUS* IN THE NATURAL PROTECTED AREA OF CUATRO CIÉNEGAS, COAHUILA, NORTHERN MEXICO

Rogelio Aguilar-Aguilar[1,3], Omar Lagunas-Calvo[1], and Gerardo Pérez-Ponce de León[2]

ABSTRACT.—The helminth fauna of the bolson pupfish *Cyprinodon atrorus* (Osteichthyes: Cyprinodontidae) consisted of 7 helminth taxa collected from 7 localities of Cuatro Ciénegas, a natural area protected by the Mexican government in Coahuila, northern Mexico. The helminth community is described at the infracommunity and component community levels. The acanthocephalan *Atactorhynchus duranguensis* was the most prevalent and abundant species. The remaining species were rare and found at low mean abundance values. The observed species richness, individual parasite abundance, and diversity were low at both component community and infracommunity levels. These findings are similar to those found in other freshwater fish species in the Nearctic part of Mexico; however, our study shows that the number of larval forms, in terms of species richness and abundance, is very low in comparison with previous studies in the area. Isolation of the Cuatro Ciénegas basin from the Bravo River (= Grande River) basins is suggested as the main factor determining the depauperate helminth assemblage. The low number of individual larval stages in this fish species is probably due to the lack of piscivorous birds in the area.

RESUMEN.—La fauna helmintológica del cachorrillo del bolsón, *Cyprinodon atrorus* (Osteichthyes: Cyprinodontidae) comprende siete taxa recolectados de siete localidades dentro de Cuatro Ciénegas, un área natural protegida por el gobierno mexicano ubicada en Coahuila, en el norte de México. En este trabajo se describe la comunidad helmintológica en los niveles de infracomunidad y comunidad componente. El acántocéfalo *Atactorhynchus duranguensis* fue la especie con valores mas altos de prevalencia y abundancia. Las especies restantes se consideran raras por encontrarse con bajos valores de abundancia. Tanto la riqueza específica, como la abundancia resultaron bajas, pero la comunidad está relativamente poco dominada. Estas características coinciden con aquellas descritas para otras especies de peces dulceacuícolas estudiadas en la porción neártica de México; sin embargo, a diferencia de estos trabajos previos, las especies de helmintos registradas como larvas se presentaron en muy bajos valores de prevalencia y abundancia, por lo que no forman una parte relevante de la comunidad. Se sugiere que el aislamiento de la cuenca de Cuatro Ciénegas con respecto a la cuenca del Río Bravo (= Río Grande), restringe la presencia de aves ictiófagas, reduciendo la presencia de estadios larvales, lo cual resulta en una comunidad depauperada y con un reducido número de larvas.

The bolson pupfish *Cyprinodon atrorus* (Miller, 1968) is a freshwater fish species endemic to the Cuatro Ciénegas region in the state of Coahuila, northern Mexico (Espinosa-Pérez et al. 1993, Carson 2009). It is an omnivorous fish that inhabits shallow, saline ponds and marshes (ciénegas) marked by extreme environmental variability (Miller et al. 2005, Carson 2009, Espinosa-Pérez 2014). The helminth fauna of this fish species, along with that of other fish in the same geographical area, was recently recorded by Aguilar-Aguilar et al. (2014). The record includes 7 helminth species collected from pupfishes distributed in 7 localities within the natural protected area. The helminth species composition in the fish examined within this region shows a series of singularities that make it very interesting in terms of parasite community ecology. The aim of this work is to describe the patterns of the helminth community structure at the infracommunity and component community levels, and to briefly discuss the possible causes that determine that structure.

METHODS

We collected 108 adult specimens of *C. atrorus* by using seine nets in 7 localities (26°50′26″–26°55′15″N, 101°59′55″–102°11′22″ W) within the natural protected area of Cuatro Ciénegas. Fishes were taken alive to the laboratory and examined within 24 h after capture

[1]Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México, Apartado Postal 70-399, C.P. 04510, México D.F, Mexico.
[2]Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-153, C.P. 04510, México D.F, Mexico.
[3]E-mail: raguilar@ciencias.unam.mx

using standard procedures (Lamothe-Argumedo 1997). We counted all helminths found in each individual fish and fixed them for morphological study. Platyhelminthes and nematodes were fixed with 4% (steaming) formalin. Acanthocephalans were placed in distilled water for 24 h at 4 °C, and preserved in 100% ethanol. Platyhelminthes and acanthocephalans were stained with Mayer's paracarmine. Nematodes were cleared in glycerin for light microscopy and stored in 70% ethanol.

The following ecological terms were used following Bush et al. (1997): prevalence (number of hosts infected with ≥1 individuals of a particular parasite species divided by the number of hosts examined), mean abundance (total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined), and mean intensity (average intensity of a particular species of parasite among the infected members of a particular host species). To determine whether sample size was sufficient to produce an accurate estimate of the pool of parasites, we used a species-richness sample-effort curve. The nonparametric species-richness estimators of Chao 1 and Chao 2 were calculated following Colwell and Coddington (1994) and Escalante (2003), and are used to estimate the number of missing species for each component community. Infracommunities (a community of parasite infrapopulations in a single host; see Bush et al. 1997) is herein expressed as mean number of parasite species per host, the mean number of individual helminths, and the mean value of the Brillouin diversity index (used for fully censured communities) because 108 hosts were analyzed; we also calculated standard deviation and range for each parameter.

We determined the numerical dominance using the Berger–Parker dominance index (Southwood 1978). We compared infracommunities qualitatively within the locality using the Jaccard similarity index, and quantitatively using the Morisita–Horn index, as calculated in Magurran (1988). Component communities (all infrapopulations of parasites associated with some subset of a host species) are herein described by using total number of individual parasites, total number of species, and diversity calculated with Simpson's index. We grouped helminth species as dominant (high prevalence and abundance) and rare (low prevalence and abundance) after an Olmstead–Tukey corner test of association (Berry et al. 2014).

RESULTS

Infracommunities

Fifty-one of the analyzed hosts (47.2%) were uninfected. Most of the remaining infracommunities were composed of a single species, and only 15 had the maximum of 2 species. The mean number of species per host was 1.59 (SD 3.11). The Brillouin index for all infracommunities varied from 0 to 0.91, with a mean diversity value of 0.11 (0.22); whereas the Berger–Parker dominance index values varied from 0.5 to 1, with a mean of 0.47 (0.47). For comparative purposes, we calculated the Brillouin index for the 15 infracommunities composed of 2 species, which varied from 0.23 to 0.92, with a mean diversity value of 0.43 (0.22). The Berger–Parker dominance index values varied from 0.5 to 0.81, with a mean of 0.62 (0.1). The helminth infracommunities showed a Jaccard index that varied from 0 to 1 ($\bar{x}$ = 0.46, SD 0.42), and a Morisita–Horn index varying from 0 to 1 ($\bar{x}$ = 0.49, SD 0.44).

Component Community

We collected 172 individual helminths, representing 7 species (Table 1), from 108 hosts. The acanthocephalan *Atactorhynchus duranguensis* was the most abundant helminth species, accounting for approximately 46% of the worms collected. Based on the cumulative species curve and the value obtained from the nonparametric species-richness estimator (Chao 1 = 7 and Chao 2 = 7.5), no more than one missing species remains to be found at the component community level. The Simpson's index value was 0.332, indicating a relatively low level of dominance. According with the Olmstead–Tukey corner test of association, the species *A. duranguensis* was the dominant species because of its high values of prevalence and abundance.

DISCUSSION

Most of the helminth taxa found in the bolson pupfish *Cyprinodon atrorus* from Cuatro Ciénegas have been previously recorded in several species of freshwater fish in northern Mexico (e.g., Martínez-Aquino et al. 2007, Martínez-Aquino and Aguilar-Aguilar 2008,

TABLE 1. Helminth parasites of the bolson pupfish *Cyprinodon atrorus* (*n* = 108) in Cuatro Ciénegas, Coahuila, Mexico. D = adult digenean, d = metacercariae, M = monogenean, n = larval nematode, A = adult acanthocephalan. SD = standard deviation.

Helminth taxa	Number	Prevalence (%)	Abundance (SD)	Mean intensity (SD)
Microphallus opacus (D)	4	1.85	0.04 (0.3)	2 (1.4)
Posthodiplostomum minimum (d)	59	5.56	0.55 (2.7)	9.8 (6.8)
Gyrodactylus sp. (M)	1	0.93	0.01 (0.1)	1
Salsuginus sp. (M)	12	5.56	0.11 (0.3)	2 (1.6)
Spiroxys sp. (n)	9	6.48	0.08 (0.4)	1.3 (0.8)
Atactorhynchus duranguensis (A)	79	39.81	0.73 (1.3)	1.8 (1.5)
Leptorhynchoides thecatus (A)	8	6.48	0.07 (0.3)	1.1 (0.4)

Pérez-Ponce de León et al. 2009, 2010, Méndez 2013; Aguilar-Aguilar et al. 2014). However, the trematode *Microphallus opacus* and the acanthocephalan *Leptorhynchoides thecatus,* which are widely recorded from diverse North American freshwater fish species (Hoffman 1999), were recently found in Mexican freshwater (Aguilar-Aguilar et al. 2014). Only the acanthocephalan *A. duranguensis* appears to be specific to fishes of the genus *Cyprinodon* (Salgado-Maldonado et al. 2005, Martínez-Aquino and Aguilar-Aguilar 2008).

Most of the helminth assemblages described from freshwater fish species in Mexico are dominated by larval forms that mature in fish-eating vertebrates (Pérez-Ponce de León and Choudhury 2010). However, in this study, only 2 of the 7 helminth taxa parasitizing *C. atrorus* were larval stages and they exhibited very low prevalence and abundance values. The record of 5 adult helminth species in *C. atrorus,* where an apparently native adult acanthocephalan is predominant, contrasts with the parasite community structure described in other freshwater fish species in the region. This could be explained as a result of the isolation of the studied region. Here the aquatic and semiaquatic habitats are mostly surrounded by extensive arid zones, restricting the presence of fish-eating birds nesting in the area (Contreras-Balderas 1984, Hendrickson et al. 2008); because these birds act as definitive hosts for many helminth larvae, their absence in the area results in a reduced number of larval forms of helminths in their freshwater fish intermediate hosts. Predominance of adult taxa within the helminth parasite fauna of a freshwater fish appears to be a more frequent pattern for Nearctic elements inhabiting more northern localities in North America (e.g., Hensley and Nahhas 1975—ictalurid fishes; Weichman and Janovy 2000—cyprinid *Pimephales promelas;* Muzzall and Madenjian 2013—salmonid *Coregonus hoyi;* see also Muzzall and Whelan [2011] for diverse fish species from the Great Lakes).

The helminth community of *C. atrorus* exhibits low values of species richness at the infra- and component community levels, and resembles those of other freshwater fish species inhabiting epicontinental waters in the Nearctic part of Mexico (see Choudhury and Dick 2000; but also Pérez-Ponce de León et al. 2000, Martínez-Aquino et al. 2004, 2007, 2009,

2011, Sánchez-Nava et al. 2004, Martínez-Aquino and Aguilar-Aguilar 2008, Romero-Tejeda et al. 2008, Aguilar-Aguilar et al. 2010). A second characteristic shared among the helminth community of *C. atrorus* and helminth communities of other freshwater fishes occurring in northern Mexico is the numerical dominance presented by a single species of parasite; in this case, the acanthocephalan *A. duranguensis* was the dominant species at both infracommunity and component community levels.

Helminth communities described herein for *C. atrorus* show some similarities with those described for the congeneric species *Cyprinodon meeki*, the Mezquital pupfish. The helminth community structure of this species was studied by Martínez-Aquino and Aguilar-Aguilar (2008), who analyzed 21 individuals in the Abraham Gonzalez spring in Durango State. Both fish species harbored poor parasite communities but were dominated by *A. duranguensis* (see Martínez-Aquino and Aguilar-Aguilar 2008). Predominance of adult helminth species in *C. atrorus* seems to be a characteristic feature, whereas the list of helminth taxa for other species of the genus *Cyprinodon*, such as *C. meeki* or the sheepsheas minnow (*Cyprinodon variegatus*), is characterized by a high number of larval stages (Mayberry et al. 2000, Martínez-Aquino and Aguilar-Aguilar 2008). Pérez-Ponce de León et al. (2010) studied 33 specimens of the Nazas pupfish (*Cyprinodon nazas*) from the San Juan River, a tributary of the Nazas River, and found only a larval nematode (*Spiroxys* sp.). Meanwhile the sister species, the Cuatro Cienegas pupfish (*Cyprinodon bifasciatus*), was studied by Aguilar-Aguilar et al. (2014), who analyzed 28 individuals and 6 hybrids of *C. atrorus* × *C. bifasciatus*. Interestingly, *C. bifasciatus* harbored no helminth parasites, whereas the hybrid was parasitized by the acanthocephalan *A. duranguensis*, the larval digenean *Centrocestus formosanus*, and the monogenean *Salsuginus* sp.

The historical biogeography of the New World pupfish genus *Cyprinodon* was studied by Echelle et al. (2005). This study examined approximately 30 species that occur in arid regions of southwestern North America, where they are primarily distributed allopatrically as endemics to relatively small bodies of water. Molecular phylogenetic analysis of pupfishes shows that *C. meeki* and *C. nazas* are sister taxa and that they form a monophyletic clade along with other species, including *C. atrorus* from Cuatro Ciénegas (Echelle et al. 2005). This indicates an ancient connection between the Bravo, Nazas, and Mezquital river basins. Actually, the freshwater fish fauna of the upper Mezquital River has been biogeographically associated with typical Nearctic elements because it has been proposed that rather than flowing to the Pacific coast of Mexico as the river does today, it formerly flowed to the Bravo River (= Grande River; Conant 1963, Miller and Smith 1986, Echelle et al. 2005). The presence of the acanthocephalan *A. duranguensis* in both *C. meeki* and *C. atrorus* reinforces that proposal. Examination of specimens of the closely related *C. nazas* failed to yield any acanthocephalan. Interestingly, *C. variegatus*, the only other species of *Cyprinodon* for which parasites have been studied to a certain extent, is parasitized with *Atactorhynchus verecundus* in Texas, Florida, New York, and Alabama (Salgado-Maldonado et al. 2005). *Atactorhynchus verecundus* seems to be very host-specific, even though *C. variegatus* and *C. atrorus* are not closest relatives according to the phylogenetic analysis by Echelle et al. (2005).

Cuatro Ciénegas is a very complex area, geographically located in the Nearctic realm. The area is considered a transitional zone of Neotropical and temperate climate zones, and so is a mixing point where different species with diverse biogeographical affinities may co-occur (Aguilar-Aguilar et al. 2014, Quiroz-Martínez et al. 2014). However, excepting those taxa parasitizing the pupfish as larval stages that show a wide distribution in Mexico, the remaining taxa could be considered typically Nearctic. These Nearctic taxa have numerous records in diverse localities in North America (Hoffman 1999, Salgado-Maldonado et al. 2005, Martínez-Aquino and Aguilar-Aguilar 2008), without any endemic component. According to our results, helminth communities are not particularly rich or diverse. However, the data we provide in the present paper represent an additional piece of information on the parasite species composition and patterns of parasite community structure of freshwater fishes in North America, particularly from a very interesting region in terms of biodiversity and endemism. More data should be gathered in order to describe general patterns of host–parasite associations and to fully understand the processes that shape the historical biogeography

and community structure of the freshwater-fish parasite fauna in North America.

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