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Helminth parasites of *Girardinichthys multiradiatus* (Pisces: Goodeidae) in the upper Lerma River sub-basin, Mexico

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Abstract Thirteen helminth species were recorded during a helminthological examination of 754 *Girardinichthys multiradiatus* (Meek) (Pisces: Goodeidae) collected from 20 localities in the upper Lerma River sub-basin on the highland plateau of Mexico. The study constitutes a complete and extensive inventory of the helminth parasites of this freshwater fish species across its entire current geographic distribution. The collected species included one adult trematode, three metacercariae, one monogenean, one adult cestode, three metacestodes, three nematode larvae and one cystacanth. The records of the metacercariae of *Tylodelphys* sp. and *Ochetosoma brevicaecum*, the larvae of the nematodes *Contracaecum* sp. and *Falcaustra* sp. and the cystacanth of *Polymorphus brevis* are all new records for *G. multiradiatus*. The metacercariae of *Tylodelphys* sp. were the most widespread and prevalent species in the sample, being collected from 15 of the 20 sampling localities, with a prevalence of 3.2–72.2%. The data indicate the helminth parasite community of *G. multiradiatus* to be relatively poor when compared with the helminth communities of freshwater fish from other parts of Mexico. This community is subject to colonization by generalist helminth species, mostly transported by birds. A further component of this community consists of helminth species that have been introduced anthropogenically.

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Introduction

Fishes of the family Goodeidae inhabit the shallow freshwater of the Mexican highland plateau (Goodeinae) and the western Great Basin of the United States (Empetrichthyinae; Webb 1998). It is thought this family of cyprinodontid, viviparous fish originated in the southern area of the Mexican highland plateau, the Mesa Central. Most species have a known distribution confined to the Mesa Central, with its center of abundance being the isolated basin of the Lerma River (Uyeno et al. 1983). The Goodeinae contain approximately 36 livebearing species in 17 genera, six of which are monotypic, distributed primarily across the Mesa Central, at elevations between 1000 m and 2300 m, although several species occupy Atlantic and Pacific systems which drain the margins of the southern highland plateau (Webb 1998).

The species *Girardinichthys multiradiatus* (Meek 1904) is a typical representative of the endemic Goodeid fish species of the upper Lerma River sub-basin (Díaz-Pardo et al. 1993). Occupying the far southeastern part of the Mesa Central, *G. multiradiatus* occurs in the headwaters of the Balsas and Lerma drainage areas and in the lake Laguna de Zempoala basin (Webb 1998). It is a key component in the aquatic food web and constitutes a major food item for many migratory bird species and other predators, like garter snakes. *G. multiradiatus* constitutes a good study system of the composition and structure of helminth parasite communities in these species. This species is highly resistant to pollution, widely distributed and is a typical species of highlands habitats. Its parasite fauna, however, is little studied, with a total of only nine helminth taxa reported from small numbers of *G. multiradiatus* examined (Lamothe-Argumedo 1970; Lamothe-Argumedo and Cruz-Reyes 1972; García-Prieto et al. 1987; León-Regagnon 1992; Astudillo-Ramos and Soto-Galera 1997; Salgado-Maldonado et al. 2001b, 2004a; Scholz and Salgado-Maldonado 2001; Pineda-López et al. 2004).

The objective of the present report is to provide basic survey information on the helminth parasites of *G. multiradiatus* from freshwater localities in the upper Lerma River sub-basin, covering the entire geographical range of this species. Data generated in this study are useful in exploring hypotheses on the origin and evolution of helminth communities in the fish of the Mexican highland plateau and in freshwater fish in general.

Materials and methods

Between June 1999 and November 2001, a total of 754 *Girardinichthys multiradiatus* (12–58 mm), collected from 20 sites in the upper Lerma River sub-basin in the states of Mexico, Morelos and Michoacan (Table 1, Fig. 1), were examined for helminth parasites. Samples were taken at every site where an established *G. multiradiatus* population existed, such that the species was sampled throughout its entire current geographical range. Collection sites included three lakes, seven reservoirs, three small channels and seven man-made ponds (locally called bordos) used to store water during dry seasons, which represent the totality of the current geographical distribution of this fish species. Fish were collected using an electrofishing device and nets or traps on the bottoms of different bodies of water. The numbers of fish examined at each locality and collection data are given in Table 1.

Once collected, the fish specimens were stored live and immediately transported to the laboratory for examination within the next 12 h. A complete examination of each specimen for helminth parasites was conducted. External surfaces, including scales, skin and fins, were examined for ectoparasites, using a stereomicroscope. Gills were recovered and the arches examined individually. The buccal cavity, opercula and eyes were examined separately. The external surfaces of the internal organs (heart, liver, spleen, gall bladder, digestive tract, gonads, swim bladder, kidney, entire body cavity, mesentery) were inspected for free or encapsulated parasites and then separated and examined individually. The intestine was opened longitudinally. The liver, spleen, kidney and heart were compressed between glass plates and examined for parasites. The body musculature was removed from the vertebral column, the skin removed from the fillets and the fillets compressed between glass plates and inspected for helminths, using a stereomicroscope. All collected helminths were sorted by taxon, cleaned and counted by organ.

Trematodes (adults, metacercariae), monogeneans, cestodes (adults, metacestodes) and nematodes were fixed in hot 4% formalin. Acanthocephalan cystacanths were placed in distilled water, refrigerated overnight (6–12 h) to evert the proboscis and then fixed in hot 4% formalin. Trematodes, monogeneans, cestodes and acanthocephalans were stained with Mayer's paracarmine or Ehrlich's haematoxylin, dehydrated using a graded alcohol series, cleared in methyl salicylate and mounted whole. To study sclerotized parts, several specimens of each species of monogenean were fixed following Malmberg's semi-permanent mount method (see Ergens 1969; Vidal-Martínez et al. 2001). Nema-

Table 1 Codes and features of the localities at which hosts were collected. Aproximate location of localities without coordinates are shown in Fig. 1. *B* Bordo or man-made pond, *C* channel or small stream, *D* dammed lake, *L* natural lake

Code	Locality name	Habitat type	Coordinates	Sample size (*n*)	Collection date
Estado de México					
Chic	Chicnahuapan, Almoloya del Río	L	19°11′20″N, 99°29′30″W	92	December 1999
				52	January 2001
Almo	Almoloya, Almolya del Río	C	19°11′20″N, 99°29′30″W	20	September 2000
Lagu	La Lagunilla, Tianguistenco	B	19°05′42″N, 99°24′0″W	50	June 1999
Cimm	El CIMMYT, Metepec	B	19°13′55″N, 99°33′05″W	7	September 2000
Sala	Salazar, Ocoyoacac	L	19°18′34″N, 99°23′45″W	12	September 2000
Igna	Ignacio Ramírez, Almoloya de Juarez	D	19°26′54″N, 99°54′39″W	75	July 1999
Vict	Villa Victoria, Villa Victoria	D	19°27′30″N, 99°59′39″W	5	July 1999
Trin	Trinidad Fabela, Atlacomulco	D	19°49′27″N, 99°47′12″W	31	July 1999
Atla	Atlacomulco, Atlacomulco	B	19°47′61″N, 99°51′74″W	15	January 2001
Pedr	San Pedro del Rosal, Atlacomulco	B	Not located	25	January 2001
Tepe	Tepetitlan, San Felipe del Progreso	D	19°37′50″N, 99°58′27″W	1	June 1999
Juan	San Juanico, Acambay	D	19°55′30″N, 99°46′94″W	53	June 2000
				58	January 2001
				50	July 2001
Vent	Rancho La venta, Acambay	B	Not located	40	January 2001
Huap	Huapango, Timilpan	D	Not located	1	June 2000
Tiac	Santiago Tiacaque, Ixtlahuaca	D	19°40′22″N, 99°42′28″W	11	October 2000
Asan	Santiago Tiacaque, Ixtlahuaca	C	19°40′22″N, 99°42′28″W	49	October 2000
Sier	Parque Sierra Morelos, Toluca	B	19°18′31″N, 99°41′18″W	30	November 2001
Mina	Mina, Toluca	B	Not located	21	October 2000
Morelos State and Estado de México					
Zemp	Zempoala	L	19°03′00″N, 99°18′42″W	20	March 2001
Michoacán State					
Porv	Canal El Porvenir, Ciudad Hidalgo	C	19°40′29″N, 100°38′25″W	36	February 2001

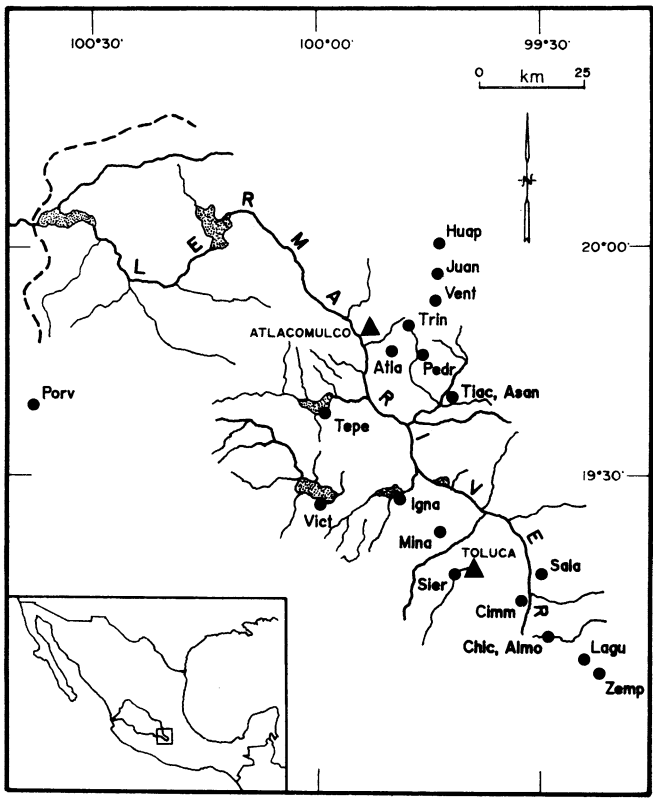

Fig. 1 Location of each locality at which hosts were collected

todes were cleared with glycerin for light microscopy and stored in 70% ethanol. Voucher specimens of all taxa have been deposited in the National Helminth Collection (Colección Nacional de Helmintos, Institute of Biology, National Autonomous University of Mexico, Mexico City).

The infection parameters utilized are those proposed by Bush et al. (1997), i.e. prevalence (% infected) and mean intensity of infection (number of parasites per infected fish).

Results

The parasites encountered, their collection locations, infection sites, prevalence and intensity of helminth species are summarized in Table 2.

A total of 13 helminth species were collected, including: one adult trematode, three metacercariae, one monogenean, one adult cestode, three metacestode, three nematode larvae and one cystacanth (Table 2). The records of the metacercariae of *Tylodelphys* sp. and *Ochetosoma brevicaecum*, the larvae of the nematodes *Contracaecum* sp. and *Falcaustra* sp. and the cystacanth of *Polymorphus brevis* are all new records for this host.

The most widely distributed and abundant helminth species recorded in this study was *Tylodelphys* sp. The metacercariae of this species were collected from 15 of the 20 sampling localities and its prevalence varied from 3.2% to 72.7% (Table 2). The metacestode of *Valipora campylancristrota* were the second most widely distributed, with specimens collected from 11 of the sampling localities. The adult cestode *Bothriocephalus acheilognathi* was third, with specimens collected from ten of the localities.

The number of helminth species collected at each locality varied between one and eight. The highest number of species was collected at Lago Chicnahuapan, although the localities of San Juanico, Rancho La Venta and Parque Sierra Morelos were also rich in parasites.

Discussion

This study constitutes a complete and extensive inventory of the helminth parasites in a freshwater fish species across its entire current geographic distribution.

The data indicate a poor helminth community with few specialist species, plus a number of wide-distribution generalist species. A third component are two anthropogenically introduced species.

The only helminth species that has developed a close relationship with *Girarninichthys multiradiatus* is *Margotrema bravoae*, a trematode only found parasitizing Goodeidae, endemic to but infrequent in the study area (Lamothe-Argumedo 1970; Pérez 2001; Salgado-Maldonado et al. 2001b, 2004a; Pineda-López et al. 2004).

The metacercariae of *Tylodelphys* sp. is the most frequent species in this host throughout its geographic distribution range. Widely distributed Nearctic generalist species, such as *Posthodiplostomum minimum*, *Ligula intestinalis*, *Cyclustera ralli*, *Valipora campylancristrota*, *Contracaecum* sp. and *Polymorphus brevis* enrich the community. The presence of all these species can be related to predation of the Goodeidae, small fish that live in shallow waters, by migratory birds from Nearctic climes (Salgado-Maldonado et al. 2001b).

The larvae of *Ochetosoma brevicaecum* and *Spiroxys* sp. are two helminth species that mature in reptiles. The larvae of *Spiroxys* sp. are found frequently in freshwater fish of Neotropical Mexico (Moravec 1998; Salgado-Maldonado et al. 2001a, 2001b, 2004, 2004a, 2004b), suggesting that reptiles prey extensively on fish. The presence of *O. brevicaecum* in *G. multiradiatus* is thus probably an accidental infection. This helminth is common in the Mexican Mesa Central. The most common intermediate hosts of the metacercariae are amphibians (García-Altamirano et al. 1993; unpublished data), although metacercariae have also been collected from fish (Peresbarbosa et al. 1994). Adult stages parasitize several species of snakes of the genus *Thamnophis* (Caballero y Caballero 1941; Bravo-Hollis 1943; Pérez et al. 2001).

Larvae of the nematode *Falcaustra* sp. are found infrequently. Very little is known about the transmission of this nematode, except that they are intestinal parasites of reptiles, frogs and freshwater fishes (Moravec 1998).

Table 2 Parasite–host list of helminths collected from *Girardinichthys multirdiatus* in the upper Lerma River sub-basin of Central Mexico

Parasite	Infection site(s)	Locality	Number of hosts examined	Number of hosts parasitized	Prevalence (%)	Intensity	
						Mean ± SD	Range
Adult Trematoda: Family Allocreadiidae Stossich, 1903							
Margotrema bravoae Lamothe-Argumedo,1970	Intestine	Vict	5	2	40.0	22.5 ± 6.4	1–27
		Porv	36	2	5.6	1	1
Larval Trematoda: Family Diplostomidae Poirier, 1886							
Posthodiplostomum minimum (MacCallum, 1921)	Mesentery, liver	Chic	52	1	1.9	8	8
		Juan	53	3	5.7	1.3 ± 0.6	1–2
		Juan	58	3	5.2	1	1
		Juan	50	1	2.0	1	1
		Porv	36	1	2.8	3	3
		Vent	40	1	2.5	1	1
		Sier	30	2	6.7	1	1
		Tiac	11	1	9.1	1	1
Tylodelphys sp.	Body cavity, mesentery, eyes	Chic	92	42	45.7	6.2 ± 9.2	1–44
			52	15	28.8	4.6 ± 4.1	1–12
		Igna	75	11	14.7	3 ± 2.4	1–10
		Lagu	50	10	20.0	2.6 ± 3.1	1–11
		Vict	5	1	20.0	1	1
		Trin	31	1	3.2	1	1
		Sala	12	8	66.7	4.2 ± 3.6	1–11
		Almo	20	14	70.0	5.1 ± 4.5	1–15
		Juan	53	37	69.8	4.5 ± 5.2	1–31
			58	2	3.4	4.0 ± 4.2	1–7
		Huap	1	1	100.0	4	4
		Asan	49	35	71.4	8.9 ± 16.0	1–62
		Tiac	11	8	72.7	6.0 ± 3.9	1–12
		Mina	21	4	19.0	1	1
		Vent	40	3	7.5	1	1
		Atla	15	1	6.7	2	2
		Zemp	20	8	40.0	4.4 ± 2.4	2–9
Larval Trematoda: Family Plagiorchiidae Lühe, 1901							
Ochetosoma brevicaecum (Caballero y Caballero, 1941)	Mesentery	Asan	49	1	2.0	1	1
		Porv	36	1	2.8	1	1
		Pedr	25	1	4.0	2	2
		Juan	58	1	1.7	2	2
		Chic	52	4	7.7	1.3 ± 0.5	1–2
			92	3	3.3	1.3 ± 0.6	1–2
Monogenea: Family Gyrodactylidae Cobbold, 1864							
Gyrodactylus elegans Nordmann, 1832	Fins	Chic	92	28	30.4	1.7 ± 1.5	1–8
			52	4	7.7	1.3 ± 0.5	1–2
		Sala	12	2	16.7	1.5 ± 0.7	1–2
		Juan	53	9	16.7	1.2 ± 0.4	1–2
			58	20	34.5	2.6 ± 1.0	1–9
			50	1	2.0	1	1
		Cimm	7	1	14.3	1	1
		Asan	49	4	8.2	2.3 ± 2.5	1–6
		Pedr	25	6	24.0	2.3 ± 1.9	1–6
		Sier	30	3	10.0	1.7 ± 1.1	1–3
Adult cestoda: Family Bothriocephalidea Blanchard, 1849							
Bothriocephalus acheilognathi Yamaguti, 1934	Intestine	Chic	92	2	2.2	1	1
			52	1	1.9	1	1
		Igna	75	2	2.7	1	1
		Lagu	50	13	26.0	2.5 ± 1.8	1–7
		Sala	12	1	8.3	1	1
		Juan	53	13	24.5	1.7 ± 1.6	1–6
			58	4	6.9	1	1
			50	4	8.0	1	1
		Cimm	7	3	42.9	1.7 ± 0.6	1–2
		Vent	40	5	12.5	1.2 ± 0.4	1–2
		Atla	15	2	13.3	1	1
		Pedr	25	1	4.0	1	1
		Sier	30	3	10.0	1	1
Metacestodes: Family Diphylobothriidae Lühe, 1910							
Ligula intestinalis (Gmelin, 1970)	Body cavity	Lagu	50	2	4.0	1.5 ± 0.7	2
		Juan	53	1	1.9	1	1
		Igna	75	1	1.3	1	1
		Zemp	20	11	55.0	2.7 ± 1.7	1–6

Table 2 (Contd.)

Parasite	Infection site(s)	Locality	Number of hosts examined	Number of hosts parasitized	Prevalence (%)	Intensity	
						Mean ± SD	Range
Metacestodes: Family Dilepididae Railliet and Henry, 1909							
Cyclustera ralli Underwood and Dronen, 1986	Mesentery	Chic	92	5	5.4	1	1
			52	3	5.8	1	1
		Porv	36	13	36.1	1.6 ± 0.6	1–3
		Vent	40	2	5.0	3.0 ± 2.8	1–5
		Sier	30	1	3.3	2	2
		Juan	50	6	28.0	2.8 ± 1.7	1–5
		Mina	21	1	4.8	1	1
Valipora campylancristrota (Wedl, 1855)	Gall bladder	Chic	92	3	3.1	1.7 ± 0.6	1–2
			52	1	1.9	1	1
		Igna	75	7	9.3	1.9 ± 1.0	1–3
		Lagu	50	1	1.0	1	1
		Trin	31	1	3.2	1	1
		Juan	53	11	20.8	1.8 ± 1.0	1–4
			58	7	12.1	1.1 ± 0.4	1–2
			50	3	6.0	1.7 ± 0.6	1–2
		Almo	20	1	5.0	1	1
		Asan	49	3	6.1	1	1–5
		Tiac	11	1	9.1	1	1
		Mina	21	2	9.5	1.5 ± 0.7	1–2
		Vent	40	5	12.5	1	1
		Sier	30	1	3.3	2	2
Larval nematodes: Family Anisakidae Railliet and Henry, 1912							
Contracaecum sp.	Mesentery	Vict	5	1	20.0	1	1
		Igna	75	1	1.3	1	1
		Chic	52	1	1.9	1	1
		Vent	40	2	5.0	2.5 ± 2.1	1–4
		Juan	58	1	1.7	2	2
			50	2	4.0	1	1
		Pedr	25	2	8.0	1	1
		Sier	30	3	10.0	1	1
		Almo	20	1	5.0	1	1
Larval nematodes: Family Kathlaniidae Lane, 1914							
Falcaustra sp.	Mesentery, intestine	Lagu	50	6	12.0	10.0 ± 13.8	1–36
		Tepe	1	1	100.0	4	4
Larval nematodes: Family Gnathostomidae Railliet, 1895							
Spiroxys sp.	Mesentery	Porv	36	1	2.8	1	1
Acantocephala larvae: Family Polymorphidae Meyer, 1931							
Polymorphus brevis (Van Cleave, 1916)	Mesentery	Sala	12	1	8.3	1	1
		Tiac	11	2	18.2	1	1

The recorded helminth parasites also include two anthropogenically introduced species, *Bothriocephalus acheilognathi* and *Gyrodactylus elegans*. The Asian fish tapeworm, *B. acheilognathi* is widely dispersed among the freshwater fish of Mexico, being recorded to date in 49 fish species from 26 genera, seven families and five orders (see Salgado-Maldonado and Pineda-López 2003). The monogenean *G. elegans* is a common species in Europe, although its occurrence in North America needs more study. Several unidentified species of *Gyrodactylus* have been reported from Mexican freshwater fishes (Salgado-Maldonado et al. 1997, 2001a, 2001b; Vidal-Martínez et al. 2001). The genus *Gyrodactylus* includes more than 150 species and its taxonomy is very complicated (Vidal-Martínez et al. 2001).

The taxonomic composition of the helminth communities of the Goodeidae suggests a depauperate helminth community open to invasion, which developed in association with evolutionarily young and isolated fish communities. These characteristics have also been observed in other fish helminth communities in the Mexican Mesa Central, especially in the Lerma River basin (Salgado-Maldonado et al. 2001b). The helminth communities of these fish are poor, with the richest documented community components reaching no more than ten species (Salgado-Maldonado and Osorio-Sarabia 1987; Choudhry and Dick 2000; Pérez et al. 2000). Each of these communities includes very few helminth specialists: the monogenean *Octomacrum mexicanum* Lamothe-Argumedo, 1981 in the cyprinid *Algansea lacustris*, the trematode *Allocreadium mexicanum* Osorio-Sarabia, Pérez and Salgado-Maldonado, 1986 in the atherinid *Chiorostoma estor* and the nematode *Rhabdochona lichtenfelsi* Sánchez-Álvarez, García and Pérez, 1998 in the goodeid *Goodea atripinnis* (see Salgado-Maldonado et al. 2001b). The presence of 14 bird-transported allogenic species (see Esch et al. 1988) and three anthropogenically introduced helminth species has been recorded among the 43 documented helminth species in the Lerma Santiago basin of the Mexican

Mesa Central (Salgado-Maldonado et al. 2001b). Anthropogenic introduction of helminths in central Mexico and other geographic areas of Mexico has been documented by Scholz and Salgado-Maldonado (2000) and by Salgado-Maldonado and Pineda-López (2003).

Data collected during the present study show the helminth parasite community of *Girardinichthys multiradiatus* to be relatively poor when compared with helminth communities of freshwater fish from other parts of Mexico. This community includes a number of generalist helminth species, mostly transported by birds. A further component of this community consists of helminth species that have been introduced anthropogenically.

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