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[Communication]

Trematode *Centrocestus formosanus* Infection and Distribution in Ornamental Fishes in Mexico

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Abstract.—The aim of this study was to determine the ornamental fish species affected by the metacercariae of the digenean trematode *Centrocestus formosanus* and its distribution in 48 fish farms in Morelos, central Mexico. The parasite was found to form various numbers of branchial cysts in 11 of the 25 species analyzed. Goldfish *Carassius auratus* was the most commonly affected species; 20 of 30 farms were positive for this parasite, the fish showing severe clinical signs and having a high mortality rate. For the first time in the region, koi (a variant of common carp *Cyprinus carpio*), zebrafish *Danio rerio*, suckermouth catfish *Hypostomus plecostomus* and blue gourami *Trichogaster trichopterus* (also known as the threespot gourami) were positive; meanwhile, Mexican tetra *Astyanax mexicanus*, which had previously been reported positive in other studies in this region, was found to be negative. The parasite was observed in fish from 27 of the 48 farms studied. Ten of 15 municipalities had farms that were positive for the parasite. These results suggest that the distribution of *C. formosanus* is closely related to the use of water that had been contaminated with the parasite and to the presence of a snail, the red-rimmed melania *Melanoides tuberculata*.

In Mexico, the presence of the digenean trematode *Centrocestus formosanus* has been reported in wild and captive fish. According to López-Jiménez (1987), the parasite was introduced into the country in 1979 when the black carp *Mylopharyngodon piceus* was imported from China; in contrast, Amaya-Huerta and Almeida-Artigas (1994) suggested that the presence of the parasite was a consequence of the introduction of the sporocysts or rediae carried by a snail, the red-rimmed melania *Melanoides tuberculata*, which was imported as fish feed. However, the first report of its presence in Mexico was in 1985 (López-Jiménez 1987); since then, the parasite has spread into several regions of the country (Scholtz and Salgado-Maldonado 2000; Salgado-Maldonado et al. 2001b), including Morelos State, which is the main ornamental fish production area and one in which the cohabitation of species in soil ponds and irrigation canals is common (Oficina de Pesca, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación–Morelos, unpublished data). We carried out this study in Morelos to document what species were being affected by *C. formosanus*, the distribution those species, and the factors facilitating its presence in the region.

Methods

Sampling.—Between June and December 2003, sampling was carried out on 48 ornamental fish farms belonging to the Asociación de Productores de Peces de Ornato of Morelos State, a society of ornamental fish producers. Assuming a prevalence of 10% (OIE 2004), we conducted directed sampling by collecting 30 young and adult fish from each species present at a particular farm, beginning with animals showing some sign of disease; the remaining fish were sampled randomly. The fish were placed in plastic bags containing water and oxygen and transported to the Department of Aquatic Animal Health at the Centre of Research and Studies in Animal Health at the Universidad Autónoma del Estado de México in Toluca.

Necropsy.—At the laboratory, the fishes were sacrificed by an overdose of anesthetic (*p*-aminobenzoate [100 mg/L]; Veterquimica, Santiago, Chile) and underwent external and internal examination to discover any damage or abnormalities. The gills were removed and wet mounts examined under a stereoscopic microscope to determine the presence or absence of *C. formosanus* cysts (Vélez-Hernández et al. 1998).

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TABLE 1.—Prevalence of branchial infection with *C. formosanus* metacercariae among analyzed species of ornamental fish from Morelos State, Mexico.

Species	Farms		Fish		
	Number	Number positive	Number analyzed	Number positive	General prevalence
Goldfish Carassius *spp.*	30	20	900	434	48.2
Mexican molly *Poecilia sphenops*	27	1	810	2	0.23
Southern platyfish *Xiphophorus maculatus*	23	12	690	278	40.3
Guppy *Poecilia reticulata*	19	5	570	134	23.5
Koi *Cyprinus carpio*[a]	12	7	360	117	32.5
Green swordtail *Xiphophorus helleri*	12	5	360	121	33.6
Zebrafish *Danio rerio*[a]	11	7	330	142	43
Suckermouth catfish *Hypostomus plecostomus*[a]	10	3	300	11	3.7
Blue gourami *Trichogaster trichopterus*[ab]	6	2	180	5	2.8
Convict cichlid *Cichlasoma nigrofasciatum*	4	1	120	2	1.7
Giraffe cichlid *Nimbochromis venustus*[a]	5	1	150	3	2
Freshwater angelfish *Pterophylum scalare*	6	0	180	0	0
Zebra Malawi cichlid *Metriaclima zebra*	5	0	150	0	0
Siamese fightingfish *Betta splendens*	4	0	120	0	0
Midas cichlid *Cichlasoma citrinellum*	4	0	120	0	0
Bluegray mbuna *Melanochromis johanni*	3	0	90	0	0
Pleasant cichlid *Apistograma*[c]	2	0	60	0	0
Goldfinned barb *Puntius sachsi*	2	0	60	0	0
African jewelfish *Hemichromis bimaculatus*	1	0	30	0	0
Sunset dwarf gourami *Trichogaster lalius*	1	0	30	0	0
Mexican tetra *Astyanax mexicanus*	1	0	30	0	0
Oscar *Astronotus ocellatus*	1	0	30	0	0
Corydorases *Corydora* spp.	1	0	30	0	0
Sarape tetra *Hyphessobrycon eques*	1	0	30	0	0
Tiger barb *Capoeta tetrazona*[d]	1	0	30	0	0

[a] First report of *C. formosanus* in this species in Morelos.
[b] Also known as threespot gourami.
[c] Also known as *A. agassizi*.
[d] Also known as *Puntius tetrazona*.

Positive fish were defined as those with at least one branchial cyst. Positive farms were defined as those at which at least one fish of any species was diagnosed with *C. formosanus* branchial infection.

Identification of C. formosanus.—Branchial cysts were mechanically broken to obtain the metacercariae, which were treated with Berland's liquid, fixed in Bouin, and then stained with Gomory trichrome, Delafield's hematoxylin, and Mayer's paracarmine (Vidal-Martinez et al. 2001). Permanent preparations were made in Canada balsam, which allowed us to check the characteristics that lead to identification in accordance with the definition set out by Salgado-Maldonado et al. (1995).

A database containing information on the species and facilities in each farm, the water source, and fish movements was established to identify the factors contributing to the presence and distribution of *C. formosanus* at the farms and production areas of the state.

Results and Discussion

Species Distribution

In this study we examined 192 groups of ornamental fish from 25 different species. Each group consisted of 30 animals, yielding a total 5,760 fish. We found 11 of 25 species to be positive for branchial infection; the proportionally most affected species started with goldfish *Carassius auratus*, followed in decreasing order by zebrafish *Danio rerio*, southern platyfish *Xiphophorus maculatus*, green swordtail *X. hellerii*, and koi (a variant of common carp *Cyprinus carpio*) (Table 1). The parasite had previously been documented in Morelos in green swordtail (Salgado-Maldonado et al. 1995); goldfish, Mexican molly, green swordtail, southern platyfish, and convict cichlid (Scholz and Salgado-Maldonado 2000); Mexican tetra, guppy, Mexican molly, and convict cichlid (Salgado-Maldonado et al. 2001a). Koi, zebrafish, suckermouth catfish, giraffe cichlid, and blue gourami found to be positive in this study had not previously been reported as infected in the state. Although Salgado-Maldonado et al. (2001a) reported Mexican tetra to be positive, we found them to be negative. This difference may be explained by the fact that our sample was obtained from a farm where fish were kept in concrete ponds, whereas the samples of Salgado-Maldonado et al. (2001a) were collected in soil ponds.

Centrocestus formosanus shows low species speci-

TABLE 2.—Number of farms analyzed in Morelos municipalities and species testing positive for infection with *C. formosanus* metacercariae.

Municipality	Farms		Positive species
	Number analyzed	Number positive	
Zacatepec[a]	14	8	Goldfish, southern platyfish, green swordtail, guppy, zebrafish, koi, suckermouth catfish
Tlaltizapán	7	5	Guppy, koi, goldfish, suckermouth catfish, zebrafish
Jojutla	8	4	Goldfish, koi, southern platyfish, green swordtail, zebrafish, Mexican molly
Ayala[a]	4	3	Goldfish, koi, southern platyfish
Xochitepec[a]	3	1	Guppy
Tetecala	2	2	Southern platyfish, goldfish, guppy, suckermouth catfish
Jiutepec[a]	2	1	Goldfish
Cuautla[a]	1	1	Goldfish, koi, southern platyfish, blue gourami
Tlaquiltenango	1	1	Koi
Coatlán del Río[a]	1	1	Goldfish, koi, southern platyfish, zebrafish, convict cichlid, giraffe cichlid, blue gourami
Yautepec	1	0	
Cuernavaca	1	0	
Miacatlan	1	0	
Puente de Ixtla	1	0	
Emiliano Zapata	1	0	
Total	48	27	

[a] First report of *C. formosanus* in this municipality.

ficity, affecting a great variety of fish to different degrees of prevalence and severity (Scholz and Salgado-Maldonado 2000; Vidal-Martínez et al. 2001). However, the reasons behind the variable resistance of some fish to the parasite have not been clearly established. Mitchell et al. (2002) reported a lower number of cysts in channel catfish *Ictalurus punctatus* and golden shiner *Notemigonus crysoleucas* after experimental infection than in fathead minnow *Pimephales promelas* and sunshine bass (white bass *Morone chrysops* × striped bass *M. saxatilis*), suggesting that certain intrinsic conditions contribute to resistance. In our study we also observed different prevalences between species; moreover, not all species from a particular farm were positive, few cases being observed in cichlids cohabitating with more severely affected species. We found only one case of infection in Mexican molly from a total of 27 farms (i.e., in 2 of 810 fish). However, in other studies Mexican molly has been heavily infected, with up to 1,277 cysts (Scholz and Salgado-Maldonado 2000). Further investigation is needed to determine the cause of these variations.

Fish Farm Distribution

Infection of fish with *C. formosanus* has previously been reported in rivers and natural water bodies in several municipalities in Morelos (Salgado-Maldonado et al. 1995, 2001a; Scholz and Salgado-Maldonado 2000), but its distribution in ornamental fish farms was not known. Twenty-seven of 48 farms (56.2%) in 10 of the 15 municipalities we sampled were positive for the infection (Table 2; Figure 1). The parasite showed a wide distribution throughout the state, both in municipalities with a high number of farms and in those with only one fish farm.

The observed distribution could be related to the use of soil ponds in the region, an environment favorable for the development of the intermediate host *Melanoides tuberculata* and for the arrival of piscivorous birds participating in the life cycle of *C. formosanus*. Also, most of the farms receive water from the Las Estacas and Amacuzac rivers or related channels, which have previously been identified as reservoirs of the parasite (Salgado-Maldonado et al. 1995) and *M. tuberculata* (Scholz and Salgado-Maldonado 2000; P. Trujillo-Jiménez, Universidad Autónoma del Estado de Morelos, unpublished data).

Interchange of fish without a prior health check and the introduction of wild fish and snails are frequent practices in the region (Oficina de Pesca, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación–Morelos, unpublished data), presenting a risk of spreading the parasite in any of its life forms (Evans and Lester 2001). The samples collected at the farm located in the Puente de Ixtla municipality were negative, in contrast to those of P. Trujillo-Jiménez (unpublished data), which caught in the Amacuzac river. Again, this difference could be attributable to the use of concrete ponds, in which there are no snails. The samples collected in fiberglass and concrete ponds in Yautepec, Cuernavaca, Miacatlan, Puente de Ixtla, and Emiliano Zapata were also negative.

Centrocestus formosanus has not previously been reported in the municipalities of Zacatepec, Jiutepec, Coatlan del Río, Xochitepec, Ayala, and Cuautla. Because the parasite has been observed in Morelos

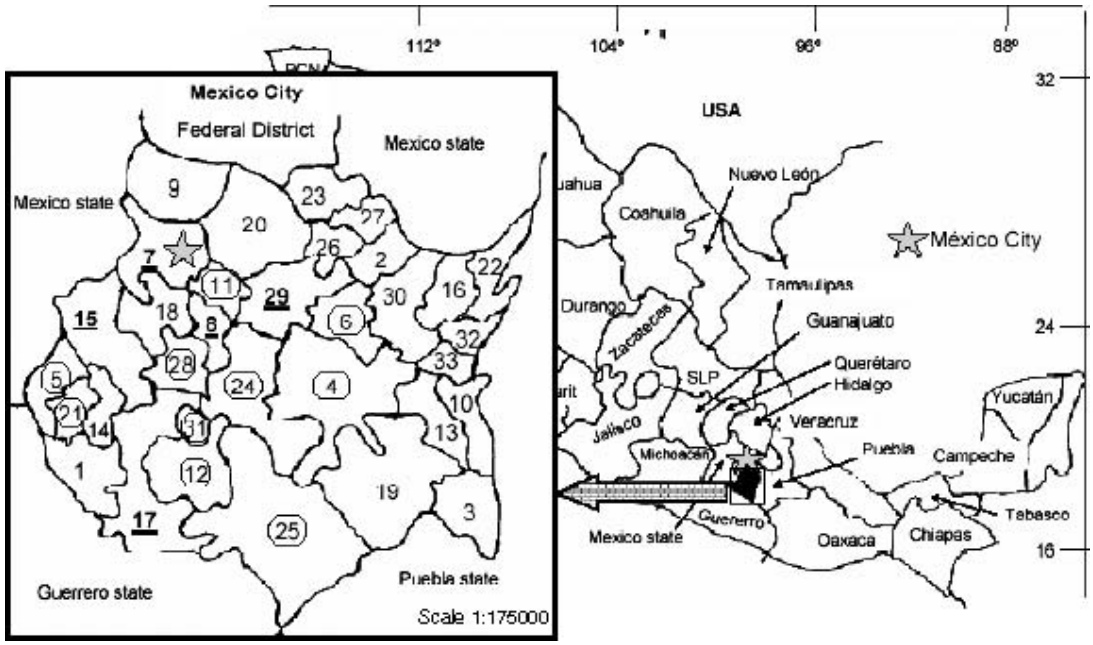

FIGURE 1.—Municipalities in Morelos State: 1, Amacuzac; 2, Atlatlahucan; 3, Axochiapan; 4, Ayala; 5, Coatlán del Río; 6, Cuautla; 7, Cuernavaca (capital); 8, Emiliano Zapata; 9, Huitzilac; 10, Jantetelco; 11, Jiutepec; 12, Jojutla; 13, Jonacatepec; 14, Mazatepec; 15, Miacatlán; 16, Ocuituco; 17, Puenta de Ixtla; 18, Temixco; 19, Tepalcingo; 20, Tepoztlán; 21, Tetecala; 22, Tetela del Volcán; 23, Tlalnepantla; 24, Tlaltizapán; 25, Tlaquiltenango; 26, Tlayacapan; 27, Totolapan; 28, Xochitepec; 29, Yautepec; 30, Yecapixtla; 31, Zacatepec; 32, Zacualpan; and 33, Temoac. Municipalities is which fish tested positive for *Centrocestus formosanus* are denoted by hexagons; those in which fish tested negative are denoted by underlining.

since 1990, its appearance could be a consequence of the lack of sanitary and disease control measures in the region's aquaculture (Scholz and Salgado-Maldonado 2000).

Although there are no official data on the impact of *C. formosanus* in the region, the parasite has been demonstrated to negatively affect native and cultured fish (Alcaraz et al. 1999; Mitchell et al. 2000). In the present study this effect was confirmed in moribund fishes that clinically manifested respiratory abnormality and histologically showed severe branchial lesions caused by the metacercariae (data not shown). In this context, measures to prevent the introduction and dispersion of *C. formosanus* in the region—as stated in Mexican legislation (Norma Oficial Mexicana NOM-011-PESC-1993 1993), which recognizes *C. formosanus* as a notifiable agent—have not been appropriately implemented, permitting its dissemination into several hydrological basins of the country (Scholz and Salgado-Maldonado 2000). After a few reports of the presence of the parasite in Morelos State, the Asociación de Productores de Peces de Ornato of Morelos commissioned this study to determine the sanitary condition of their farms and to identify the factors that influence fish health in order to implement control measures.

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