AQUATIC ANIMAL REPORTS 3(2) (2025) : 1-8
DOI: 10.5281/zenodo.15542999
aqar@scopesscience.com

AQUATIC ANIMAL REPORTS

Journal homepage: https://scopesscience.com/index.php/marep/

Received: 10 March 2025; Received in revised form: 02 May 2025
Accepted: 17 May 2025; Available online: 31 August 2025
RESEARCH PAPER

Citation: Zamora-Roa, E., Castelán-Rosas, L.R., Vega-Sánchez, V., Cordero-López, A.P., Gómez de Anda, F.R. & Acosta Pérez, V.J. (2025). *Centrocestus formosanus* (Heterophydae) infection in ornamental fish *Cyprinus carpio var. koi* (Cyprinidae): in Mexico center. *Aquatic Animal Reports*, 3(2), 1-8. https://doi.org/10.5281/zenodo.15542999

Centrocestus formosanus (Heterophydae) INFECTION IN ORNAMENTAL FISH *Cyprinus carpio var. koi* (Cyprinidae): IN MEXICO CENTER.

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Abstract

Parasite problems in ornamental fish represent a health risk that results in economic losses in the sector, including infections by *Centrocestus formosanus*, which have been identified as diseases that affect fish health and have zoonotic potential. Due to the sanitary importance of this trematode, this study aimed to report the presence of *C. formosanus* in ornamental fish in Tulancingo de Bravo, Hidalgo. 36 aquarium koi carp (*Cyprinus carpio var. koi*) were acquired for parasitological analysis in search of ecto- and endoparasites. A water sample was also collected to determine water quality parameters using a Hanna® colorimetric kit (Hannapro, SA de CV © 2022, CDMX, Mexico). The fish analyzed showed metacercariae cysts in the gill filaments, corresponding to *C. formosanus* with a prevalence of 5.5 %, while the water quality showed pH (10.84± 0.05), nitrite (28± 0.00), nitrate (17.72± 0.00) and CO_2 (23± 2.64) values indicating poor water quality. These findings suggest that the occurrence of *C. formosanus* may be related to poor water quality. This study presents the first report of *C. formosanus* in ornamental fish in the municipality of Tulancingo de Bravo.

Keywords: Ornamental fish, Parasites, Trematodes, Metacercaria, Gills.

Introduction

The ornamental fish industry is a significant source of employment globally, with an annual market value of $30 billion and an increase in demand due to globalization and market expansion (Peh & Azra, 2025). In Mexico, about 40 million ornamental fish are marketed each year which represents 1,650 million pesos, among the fish marketed are cichlids, poecilids and cyprinids such as koi carp (*Cyprinus carpio var. koi*), which are produced in 20 states of the republic including the State of Hidalgo (Instituto Mexicano de Investigación en Pesca y Acuacultura Sustentables [IMIPAS], 2018). A high production is not exempt from problems, among them those of sanitary type, in this context, *Centrocestus formosanus* (Digenea: Heterophyidae) is a trematode parasite that presents occurrence in fish at the gill level, where encysted metacercariae have been reported (Ratchadaporn et al., 2019). This parasitic organism has a life cycle involving several hosts. The first is a snail, where only *Melanoides tuberculata* is recognized as its only snail host worldwide. The larvae released by the snail are encapsulated in the gills of fish, which act as second hosts. Finally, fish-eating birds and mammals ingest the parasitic larvae and the adult parasite develops in their gut (Yousif et al., 2016).

In Mexico, the introduction of *C. formosanus* is related to the importation of exotic fish and the co-introduction of associated helminths; the first record in Mexico is reported by López-Jiménez in 1987 in carp analyzed in Tezontepec de Aldama, Hidalgo (López-Jiménez, 1987; Scholz & Salgado-Maldonado, 2000). *C. formosanus* infections in ornamental fish have been previously reported in Mexico in species such as *Poecilia sphenops*, *P. reticulata*, *Xiphophorus maculatus*, *X. helleri*, *Danio rerio*, *Hypostomus plecostomus*, *Trichogaster trichopterus*, *Cichlasoma nigrofasciatum*, *Nimbochromis venustus*, *Carassius* spp. as well as *C. carpio var. koi* with prevalences between 0.23 to 48.2 % (Ortega et al., 2009). Its presence is global, although it is mainly recorded in Southeast Asia. The presence of this disease causes severe damage to the gills of fish, leading to respiratory difficulties, decreased reproduction and, finally, the death of juvenile organisms (Eissa et al., 2021; Sumuduni et al., 2020). In addition, it maintains a public health relevance since it is a zoonotic trematode parasite associated with intestinal infections in humans, caused by the consumption of raw or undercooked fish meat (Eissa et al., 2021; Pinto & de Melo, 2012). The relevance of this parasite and its impact on various hosts justifies the need to continue monitoring its presence in different fish species. For this reason, this study focuses on reporting the presence of *C. formosanus* in ornamental fish of the species *C. carpio var. koi* as part of the monitoring of its occurrence in Mexico.

Materials and Methods

Study area:

The fish and water samples were obtained from a commercial aquarium, located in the municipality of Tulancingo de Bravo, Hidalgo with coordinates 20°05’13.4” N 98°22’39.1” W, in the center colony (Fig. 1), the sampling point is located at 2,180 meters above sea level and in a temperate climate region with rainfall between May and October, where annual precipitation is between 500 and 553 mm, the municipality has a mean annual temperature of 14 °C (Salinas-Martínez et al., 2020). This is one of the municipalities with the largest human population in the State of Hidalgo, with 158,189 inhabitants (Badillo Flores, 2021) There is a broad culture of acquiring pets, although not with the necessary sanitary care (Ruiz-ortega et al., 2022).

Figure 1. Location of sample collection (red arrow), showing the delimitation of the municipality of Tulancingo de Bravo, Hidalgo in orange. And its location with respect to the center of Mexico in the upper right part.

Determination of water quality:
Water samples were transferred to the parasitology research laboratory of the Institute of Agricultural Sciences of the Autonomous University of the State of Hidalgo. The transfer time was 20 minutes at room temperature (22± 1 °C). In the laboratory 200 mL water sample was collected from the fish transport bag, then processed in triplicate for the determination of water quality, where the parameters of pH, nitrites (mg L^{-1} NO^{2-}), nitrates (mg L^{-1} NO^{3-}), hardness (mg L^{-1} $CaCO_3$), alkalinity and CO_2 (mg L^{-1} CO_2) were included. The values were obtained using a Hanna® brand colorimetric kit (Hannapro, SA de CV © 2022, CDMX, Mexico), with the products HI98107 (pH), HI3873 (nitrites), HI3874 (nitrates), HI3812 (hardness), HI3811 (alkalinity) and HI3818 (CO_2) (Acosta-Pérez et al., 2022; Wanja et al., 2020).

Parasitic determination:
A total of 36 ornamental carps (*C. carpio var. koi*) were obtained. The fish were weighed and measured. After they euthanized by cranial puncture for ectoparasitic and endoparasitic examinations. The examinations began with skin, gill mucus and gill samples. Later, the fish were dissected and searched endoparasites in different organs such as eyes, heart, liver, kidney, and the digestive system parts (esophagus, stomach, mid and distal intestines, and the colon and rectum) (Scott & Govett, 2009; Sepulveda & Kinsella, 2013). Observations were performed by brightfield microscopy with a Zeigen WF10x microscope (nopCommerceCopyright © 2022 Zeigen Microscopios, CDMX, Mexico) with 4x and 10x objectives and with a 10x eyepiece and a total magnification of 40x and 100x. Finally, the results were expressed as the prevalence, abundance and intensity of the infection on the 36 fish analyzed (Acosta-Pérez et al., 2022).

Results

Water quality parameters included the calculation for pH (10.83± 0.05), nitrites (3.28 ± 0.05 mg L^{-1} NO^{2-}), nitrates (17.72± 0.00 mg L^{-1} NO^{3-}), hardness (110.00± 8.66 mg L^{-1} $CaCO_3$), alkalinity (156± 10.39) and CO_2 (23.00± 2.64 mg L^{-1} CO_2). The values obtained showed that the pH values were outside the recommended range for raising ornamental fish (IMIPAS,

2018). Likewise, it is observed that the values of nitrites, nitrates and CO_2 were elevated at the time of the determinations.

The identification and prevalence of *C. formosanus* was investigated in the fish with 11.20± 1.48 cm and an average weight of 9.13± 0.75 g. Two of the 36 fish analyzed presented parasitic load by *C. formosanus*, the stage observed corresponded to metacercariae of the trematode, characterized by the shape of the excretory vesicle in the form of an X (Fig. 2a and 2b). The encysted metacercariae were located at anatomical level in the gills of the fish, specifically in the gill filaments of the left lamella. The prevalence of the parasite corresponded to 5.5 %, a total of eight cysts of *C. formosanus* were identified, showing an abundance of 0.22 in the analyzed sample and an infection intensity of 4.

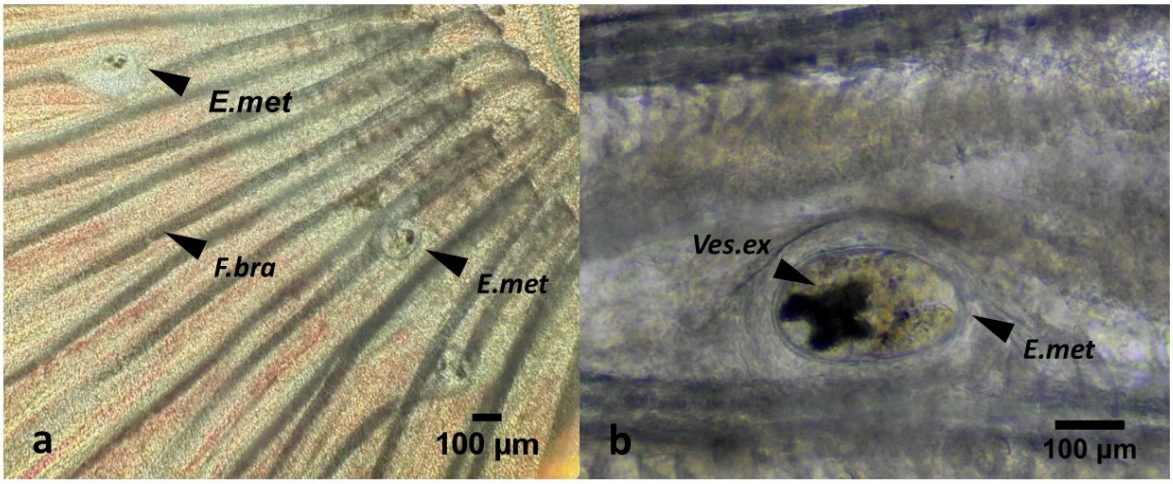

Figure 2. The cysts corresponding to metacercariae of *C. formosanus* are observed, the observations were made at 40x (a) and 100x (b). *E.met= metacercarial cyst; F. bra= gill filament and Ves.ex= excretory vesicle.*

Discussion

The prevalence of *C. formosanus* in this study (5.5 %), is in agreement with the wide ranges of occurrence that have been reported in ornamental fish, ranging from 0.23 to 48.2 % (Ortega et al., 2009). Previously, *C. formosanus* has been reported in cyprinids such as grass carp and common carp, where *C. formosanus* infection was associated with six-gram fry mortalities (Rezaie et al., 2017).

In common carp (*C. carpio*) analyzed in Colima, Mexico, the presence in gills of *C. formosanus* was reported with 93 % prevalence, as well as the presence of 377 cysts in 30 fish sampled (Vélez-Hernández et al., 1998), abundance values higher than those observed in the koi carp of this study. The stage of *C. formosanus* identified in the analyzed carp corresponds to the metacercaria, spherical cysts were observed in the gill filaments, the parasites presented rapid movements and the distinctive "X" shape in the excretion vesicle could be identified in them (Rezaie et al., 2017). Although, the anatomical distribution of metacercariae is usually in the gills, it has been reported that upon high cercariae exposure, the establishment of metacercariae in the fish can reach anatomical regions such as the head and fins (Sumuduni et al., 2017).

Koi carp in particular have shown high resistance to *C. formosanus* infection, Sumuduni et al. reported in 2017 a mean lethal of 1 467 cercariae in fry exposed in an infection model (Sumuduni et al., 2017). This is related to the fact that cyprinids are highly traditional fish in culture (Savaya et al., 2020) which stand out for their high resistance to diseases, however, this

characteristic places them as frequent carriers of parasitic loads of high importance in animal and human health (Dao et al., 2017; Wang et al., 2017). Moreover, cyprinids have been reported as the main freshwater fish that can transmit trematode metacercariae, evidencing the ecological importance of these fishes (Lima dos Santos & Howgate, 2011). In addition to the above, it could be evidenced that the water quality characteristics in this study presented deficiencies, parameters such as pH (10.83), nitrites (3.28) and nitrates (17.72), were found outside the recommended parameters for the culture of ornamental fish, where a pH of 5-8, nitrites <0.1 and nitrates between 0.4-0.8 are suggested (IMIPAS, 2018). The values exposed suggest that poor water quality is a determinant for the occurrence of parasites in different fish populations (Ojwala et al., 2018). The above denotes the influence of water quality, in addition the relationship of this trematode with snail populations, pose its control through prophylactic measures that may include cleaning and disinfection of facilities, as well as the removal of vectors associated with the life cycle of *C. formosanus* (Rezaie et al., 2017).

On the other hand, the presence of *C. formosanus* in the fish analyzed is of public health relevance due to the risk of infection in humans which derive in intestinal pain, diarrhea and chronic enterocolitis (Rezaie et al., 2017). In this context, ornamental fish may play an ecological role associated with the dispersal of *C. formosanus* (Scholz & Salgado-Maldonado, 2000). Given this scenario, it is relevant to continue monitoring *C. formosanus*, a pathogen that negatively affects the health of animals and humans, in search of better management for the control of parasitosis.

Conclusion

The ornamental fish (*C. capio var koi*) analyzed showed the presence of metacercarial cysts of *C. formosanus*. This study is the first report of *C. formosanus* in ornamental fish marketed in Tulancingo de Bravo, Hidalgo. This finding is relevant for future epidemiological monitoring of metacercariae in ornamental or consumer fish, as it points to the need to establish prophylactic measures that contribute to reducing health risks in both fish and human populations.

Ethical approval

Ethical review and approval were waived for this study.

Informed consent

Not available.

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest

There is no conflict of interests for publishing this study.

Funding organizations

No funding available for this study.

Contribution of authors

Zamora-Roa Elideth: Methodology, Writing original draft.
Castelán-Rosas Luis Rene: Methodology, Writing original draft.
Vega-Sánchez Vicente: Supervision, Validation, Review, Editing.
Cordero-López Ana Paola: Validation, Review, Editing.
Gómez de Anda Fabián Ricardo: Project administration, Resources, Supervision, Validation.

Acosta Pérez Víctor Johan: Conceptualization, Data curation, Formal analysis, Writing original draft, Review, Editing.

All authors have read and agreed to the published version of the manuscript.

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