Lat. Am. J. Aquat. Res., 46(5): 961-968, 2018
DOI: 10.3856/vol46-issue5-fulltext-9

Research Article

An emerging infection caused by *Gyrodactylus cichlidarum* Paperna, 1968 (Monogenea: Gyrodactylidae) associated with massive mortality on farmed tilapia *Oreochromis niloticus* (L.) on the Mexican Pacific coast

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ABSTRACT. The current study aimed to describe the massive mortality of farmed Nile tilapia *Oreochromis niloticus* associated with the monogenean *Gyrodactylus cichlidarum* from the northwestern Mexican Pacific coast. The ectoparasite was identified using measurements of the hard parts and compared with previous literature. Approximately 20,000 tilapias were subjected to subsequent losses over a three day period, a total of 2% of the initial seed stocked died due to the presence of this gyrodactylid parasite. The presence of the monogenean was the principal cause for chemical treatments. There is no doubt that infections by parasites have major consequences for species of small fish in culture and must consequently be considered as a fundamental factor within any system of aquaculture. A histo-pathological examination of the gills of fish showed the presence of the monogenean attached to the fi laments was causing hyperplasia. The fish showed no signs of bacteria or virus infection. This study reports for the first time the occurrence of massive mortality caused by this monogenean in a tilapia fish farm in the Mexican Pacific and also extends their known geographical distribution.

Keywords: *Gyrodactylus*, ectoparasite, *Oreochromis*, tilapia, aquaculture, northwestern Mexico.

INTRODUCCION

Aquaculture has been defined in a number of different ways *e.g.*, the Food and Agriculture Organisation of the United Nations (FAO) in 2009 defined it as a practice which has extended globally, producing a wide variety of animal and plant species, in more recent years with species which are easier to handle and are of high-value. In the last decade, Nile tilapia *Oreochromis niloticus* Linnaeus, 1758 aquaculture has been the sector of food production that has grown more rapidly in Sinaloa, Mexico, since other aquaculture, alternatives have been developing thru shrimp aquaculture which has decreased considerably in the last years (Abdo de la Parra *et al.*, 2010). At present, it is a high-value fish due to its quality white meat and good flavor, according to the Comité Estatal de Sanidad Acuícola de Sinaloa, (CESASIN), 45 fish farms are currently developing ~300-500 t yr^{-1} around the Sinaloa State. In aquaculture facilities, fish are reared in tanks, ponds, or land-based tanks and such systems provide an opportunity for diseases or chronic infections associated with the development of different pathogens (Kearn *et al.*, 2004). Such is the case of members of the class Monogenea which are ectoparasites of aquatic vertebrates, generally fish. The genus *Gyrodactylus* Nordmann, 1832 is an ectoparasitic monogenean fluke that is widely distributed and affect many species of fish (Bakke *et al.*, 2007). Gyrodactylids have direct life-cycles, lack a free-swimming larval stage or oncomira-

Corresponding editor: Mauricio Laterça

cidium which is present in egg-laying monogeneans, but instead have developed other highly successful reproductive strategies: are viviparous (give birth to full-sized living individuals) and can increase rapidly in number (Harris *et al.*, 2004; Bakke *et al.*, 2007). *Gyrodactylus cichlidarum* was initially described from *Sarotherodon galilaeus galilaeus* from Ghana in Africa by Paperna (1968). Many *Gyrodactylus* spp. are responsible for severe impairment of culture fish health and welfare in this area (García-Vásquez *et al.*, 2007; Rokicka *et al.*, 2007; Přikrylová *et al.*, 2009; Grano-Maldonado *et al.*, 2011; Mousavi *et al.*, 2013). This study provides information to support that *G. cichlidarum* may be the primary cause of high mortality on farmed tilapias in Sinaloa, Mexico, and its identification was confirmed based on the morphological comparison with other gyrodactylids previously reported (Paperna, 1968; García-Vásquez *et al.*, 2007; Mousavi *et al.*, 2013). In Mexico, *G. cichlidarum* has been reported in Tabasco which is situated in the Gulf of Mexico (Kohn *et al.*, 2006) and Veracruz State (Rubio-Godoy *et al.*, 2012).

A recent infestation of this parasite in the coast of the Mexican Pacific coast has occasioned high mortalities in tilapia´s aquaculture; this particular situation also provided a chance to study this ectoparasite species. Partial identification was elaborated using literature previously published (Paperna, 1968; García-Vásquez *et al.*, 2007; Mousavi *et al.*, 2013) in order to confirm this gyrodactylid species. In the present study, we report for the first time a new geographical distribution in the East Pacific, a Neotropical region for the cultured tilapia (*Oreochromis niloticus*). This study aimed to identify the ectoparasites specimens accurately causing massive mortalities in tilapias and considering industry's best interest to identify health problems that cause great economic losses is essential, provide an accurate description of the pathogen and effective and prompt treatment in a controlled fish farm are needed.

MATERIALS AND METHODS

Collection and maintenance of fish host

During August 2016, ~1 million Nile tilapias (weight range 2 mg, mean total body length range 1.16 ± 0.04 cm) were farmed in Sinaloa State in the Pacific coast, northwest Mexico (Fig. 1).

The Nile tilapia were held in ten flow-through freshwater fiberglass tanks of 15 m^3, and eight cages of 15 m^3 which can hold 3000-5000 fish m^{-3}.

The total production at the fish farm is estimated at ~1million fish $month^{-1}$. Fish were fed daily in the facilities where they were acclimatized for growing purposes. The first face, holds up to 100,000 and second face, up to 50,000 fish during the reversion period or sex reversal process ~30 days. Gradual mortalities were observed during the reversion period which increased time and effort concerning fish farm welfare personnel when water temperature was incremented by 27ºC up to 32ºC (August). During the medication treatment, for the presence of ectoparasites, the fish undergo high levels of stress, exacerbated by high water temperature (30 ± 2°C). Circular tanks forming part of a recirculation system with constant aeration and daily water renewal of 500% per day (L h^{-1}), water temperature (30 ± 2°C), pH 7.8 ± 0.5 and dissolved oxygen for the entire rearing period was 4-5 mg L^{-1}, total ammonia (TAN) 1 mg L^{-1}, and photo-period was 12 L:12 D (Light:Dark). All experiments followed the Mexican laws for animals and scientific procedures (NOM-033-ZOO-1995).

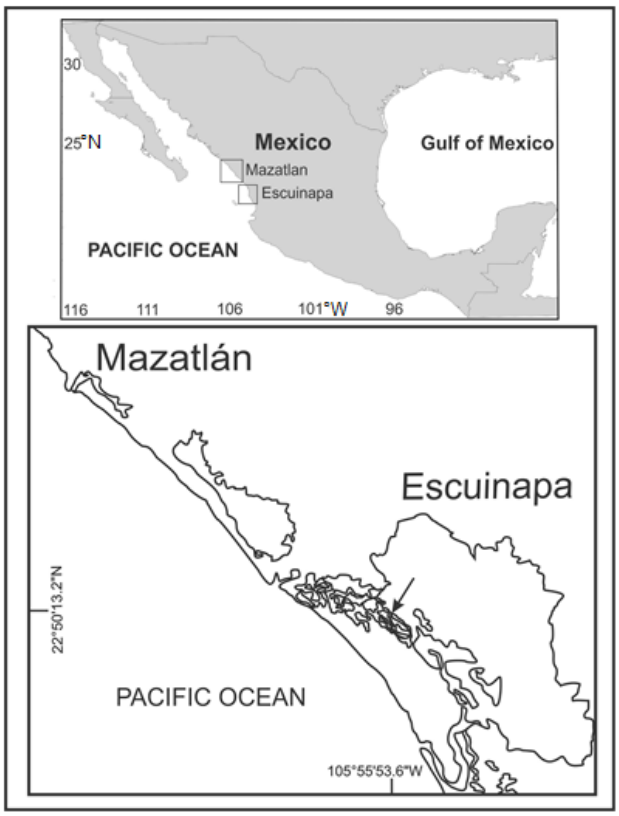

Figure 1. Location of the study area in Escuinapa, Sinaloa, northwestern Mexico.

Fish mortalities

No data on Nile tilapia mortalities during their initial maintenance or growth (in spring 2016) were observed at the facilities. The Nile tilapia began dying shortly after the temperature increased from 30 to 32°C in August at the growth facility. In the first three days, daily losses of stock were approximately 8,000 d^{-1}.

Macroscopic examination of approximately ~30 fish *in situ* revealed the presence of monogeneans on the skin. The fish farm personnel employed firstly a series of formaldehyde treatments (100 ppm L^{-1}, and water change after one hour) with constant aeration were conducted in each rearing tank at days 1 and 2 respectively to control the worms. In addition, a 1 mg L^{-1} mebendazole (Sigma) (Buchmann *et al.*, 1987), and water change after 12 h treatment was given to control worms on day 2 and 3. Despite these chemical interventions, the treatment administration did not reduce the rate of fish mortality in the first day. Since the infection was detected, a total estimation of ~20 000 fishes was lost during three days, representing 2% of the entire stock in the fish farm. Water chemistry parameters were not affected by chemical treatments. On day 2, post-massive mortality following the formaldehyde treatment, a random sample of 50 dead fish was fixed in 10% formalin and revised in the laboratory for evaluation

Morphometric identification

Each fish was examined macroscopically, monogenean attaching to the external surfaces being counted and carefully removed using mounted triangular surgical needles (size 16, Barber of Sheffield, UK). The skin, gills, and the mouth cavity of the remaining fish were examined for ectoparasites under an Olympus SZ30 stereomicroscope. Twenty monogeneans were prepared as whole mounts using ammonium picrate glycerine (APG) according to the method of (Malberg, 1970). Air-dried haptors were then subjected to partial digestion using a proteinase K-base method. Each haptor was digested was used (see the detailed method in Harris & Cable, 2000). The digestion of each specimen was monitored on a Leica MZ 9.5 dissecting microscope using the 4x objective (total magnification of 40x). Each specimen was mounted with a square 18×18 mm "0" thickness (VWR International®) coverslip and sealed with commercial nail varnish. The haptoral armature and copulatory complex were studied using an immersion oil objective on a Leica DMLB 10 compound microscope. Images of the haptoral attachment hooks and copulatory complex were captured using a camera Sony CCD Iris fitted to the compound microscope using 100x oil immersion lens. Each specimen was subjected to morphometric analysis. Measurements made on the attachment hooks of each specimen follow those described (Paperna, 1968, García-Vásquez *et al.*, 2007; Mousavi *et al.*, 2013) but also include the length and width of the dorsal bar. The measurements are expressed as the mean ± standard deviation (SD), followed by the range in parentheses in micrometers.

Histological analysis

Gills from 15 fish were fixed in alcoholic Bouin solution during 24 h and posteriorly were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Transverse sections were cut at 5 microns and stained with hematoxylin and eosin (H&E). Finally, the slides were microscopically examined and photographed using a camera mounted on a light microscope.

RESULTS

Macroscopic examination

Each fish (n = 30) (Fig. 1) was found to be parasitized by a single monogenean species, *Gyrodactylus cichlidarum* (prevalence = 100%), on the skin, gills and the mouth (~40-100 parasites per host) (Fig. 2).

The main morphological characters used to identify this species of monogenean was based on morphometrical features of the hard parts of *Gyrodactylus* spp., collected in the present study that was very similar to that of *G. cichlidarum* (Fig. 3) (Table 1).

This number of recovered parasites may represent an undervalue, as the fish sampled had already been exposed to several treatments before the time of recovering samples. No virology and bacteriology based examinations were conducted since the personnel did not found evidence of diseases related to this pathogens.

There is good agreement between the measurements made on farmed Nile tilapia in the current study with those provided the latest research. The number of these parasites per fish, however, can only be approximated

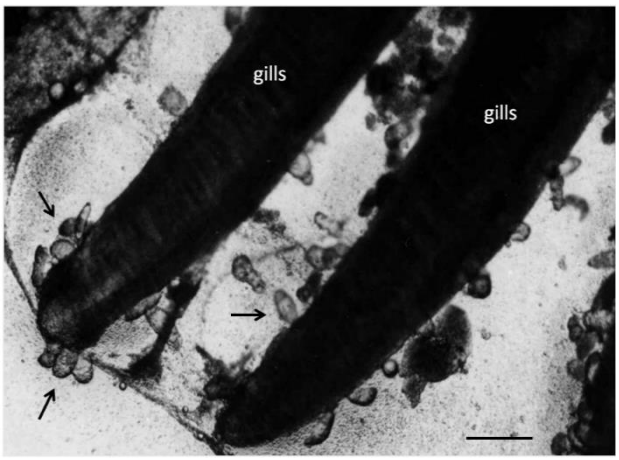

Figure 2. Light microphotography of *Gyrodactylus cichlidarum* (arrow) parasitizing the gills of Nile tilapia (scale bar = 200 µm).

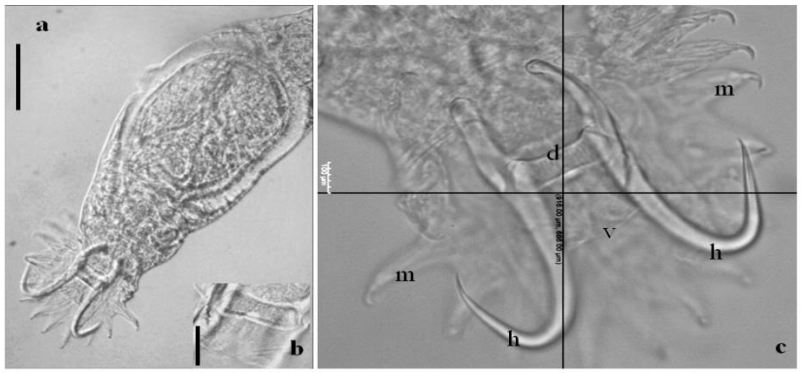

Figure 3. a) Light microphotography of *Gyrodactylus cichlidarum* parasitizing the Nile tilapia (scale bar = 50 µm), b) ventral bar (scale bar = 10 µm), c) the opisthaptor consists of two centrally positioned large hooks or hamuli (h) joined by two connecting bars, a simple dorsal bar (d) and an approximately triangular shaped ventral bar (v). There are 16 marginal hooks (m) positioned around the periphery of the opisthaptor.

Table 1. Morphological measurements of *Gyrodactylus cichlidarum* collected from Nile tilapia (mean ± SD followed by the range in parentheses, in micrometers) and compared to the morphometric measurements published in the literature.

	Mousavi *et al.* (2013) n = 20	García-Vásquez *et al.* (2007) n = 20	Paperna (1968)	Present study n = 20
Total body length	319.28 ± 79.42 (239.86 - 398.7)	313.5 ± 49.9 (237.5 - 375)	(250-350)	315.35 ± 44.52 (239.6 - 392.25)
Total body width	73.24 ± 22.66 (50.58 - 95.9)	72.7 ± 19.7 (24.5 - 90)	(60-100)	76.08 ± 26.3 (55.58 - 92.3)
Haptor length x width	75.55 ± 12.78 (62.77 - 88.34) x65.48 ± 14.97 (50.51 - 80.46)	75.8 ± 7.4 (67.5 - 87.5) x89.3 ± 7.9 (75 - 100)	(50-60)	71.53 ± 6.5 (69.3 - 89.2) x69.6 ± 12.3 (52.9 - 86.2)
Total length of anchor	55.46 ± 3.93 (51.53 - 59.39)	54.3 ± 3.4 (46.6 - 59.9)	(10-80)	53.98 ± 3.78 (48.5 - 58.95)
Length of anchor root	20.04 ± 1.7 (18.34 - 21.74)	19.8 ± 1.8 (15.6 - 22.2)	(15-20)	20.27 ± 1.3 (16.4 - 21.89)
Length of the anchor shaft	40.97 ± 1.32 (39.65 - 42.3)	32.7 ± 2.2 (26.9 - 35.1)	-	33.76 ± 2.9 (28.4 - 38.64)
Length of anchor point	22.31 ± 2.66 (19.65 - 24.97)	25.9 ± 1.3 (23.3 - 27.9)	-	21.79 ± 2.45 (18.96 - 26.54)
Total length of marginal hook	27.9 ± 1.7 (26.2 - 29.6)	28.2 ± 1.4 (24.3 - 30.3)	(15-30)	27.13 ± 1.9 (25.7 - 29.1)
Marginal hook sickle length	7.8 ± 0.6 (7.2 - 8.4)	7.5 ± 0.4 (6.9 - 8.4)	-	7.6 ± 0.3 (7.4 - 8.2)
Marginal hook handle length	20.56 ± 1.36 (19.2 - 21.93)	-	-	22.69 ± 1.74 (18.3 - 21.98)
Marginal hook sickle width (distal)	4.29 ± 0.45 (3.84 - 4.75)	4.9 ± 0.5 (4.1 - 6.3)	-	4.3 ± 0.63 (3.75 - 6.1)
Marginal hook sickle width (proximal)	3.75 ± 0.37 (3.38 - 4.13)	4.4 ± 0.3 (3.9 - 5.2)	-	4.2 ± 0.2 (3.1 - 4.8)
Length of ventral bar	22.44 ± 0.32 (22.12 - 22.77)	22.5 ± 1.5 (19.4 - 25.7)	(20)	22.1 ± 0.23 (20.6 - 22.8)
Median width of ventral bar	6.7 ± 0.8 (5.9 - 7.45)	7.6 ± 0.9 (5.9 - 9.5)	(5-8)	6.9 ± 0.6 (5.7 - 8.1)
Total length of dorsal bar	22.55 ± 2.08 (20.47 - 24.64)	21.5 ± 3.0 (18.2 - 25.3)	(16-22)	21.31 ± 2.3 (21.1 - 24.46)
Median width of dorsal bar	1.71 ± 0.16 (1.55 - 1.88)	1.5 ± 0.2 (1.2 - 1.8)	(1-2)	1.6 ± 0.12 (1.45 - 1.79)

at ~40, based on the number observed on individual fish *in situ*.

Histological examination

Histology of the gills infected with *G. cichlidarum* from the farmed Nile tilapia showing swelling of the apex of the primary and secondary lamellae with hyperplasia. Presence of a large number of undifferentiated proliferating cells, hypertrophy and inter-lamellar necrosis was found (Fig. 4).

The presence of *G. cichlidarum* in the gills and on the skin although these pathogen infections may themselves be a consequence of earlier stressors (high temperatures, low oxygen concentration, medication treatment). The descriptions of *G. cichlidarum* are according to existing current descriptive practices previously described (Table 1).

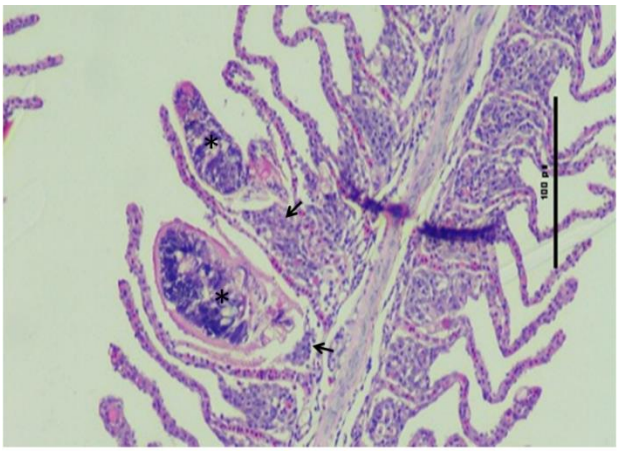

Figure 4. Histology of the gills from the farmed Nile tilapia infected with *Gyrodactylus cichlidarum* (*) showing swelling of the apex of the primary and secondary lamellae with hyperplasia. Note the presence of a large number of undifferentiated proliferating cells, hypertro-phy and inter-lamellar necrosis (arrow).

DISCUSSION

In the present study, the examination of a sample of farmed Nile tilapia fish in the rearing and growing facility revealed infection by the monogenean *G. cichlidarum* in the northwestern Mexican Pacific coast may be the consequence of considerable fish mortality. There is no doubt that infections by parasites have major consequences for species of small fish in culture and must consequently be considered as a fundamental factor within any system of aquaculture. The differentiation of *Gyrodactylus* species has traditionally been based on morphological studies using consistent differences in the shape of the attachment hooks (hamuli, marginal hooks, ventral and dorsal bars) to separate species (Shinn *et al.,* 2004). In the present study, the species of *Gyrodactylus* was identified based on morphometric characterization in tilapia. Infestation of *G. cichlidarum* in aquaria system resulted in high mortalities in *O. niloticus niloticus* in the UK (García-Vásquez *et al.,* 2007), in *Hemichromis fasciatus* from Senegal (Přikrylová *et al.,* 2009), and recently in *Astronotus ocellatus* in Iran (Mousavi *et al.,* 2013). In the present study, the detailed morphometric data of this species of gyrodactylid was compared with other reports from cichlids (Table 1)**.**

In this work, some of the feature measurements of gyrodactylids species are similar in size to those reported by (García-Vásquez *et al.,* 2007; Mousavi *et al.,* 2013) but differ in having a smaller haptor, total length marginal hook and total length dorsal bar. The attachment organ morphology is known in the Parasitology field to be variable and is not absent of criticism. Factors like; temperature, host, location may influence the phenotypic haptor variation (Rokicka *et al.,* 2007) can be the reason for that differences. Confirming the morphological identification of *Gyrodactylus* specimens found in Nile tilapia was performed in the present study. Although the gyrodactylids were the evident species found and the reason for chemotherapy applied, it is unknown whether gyrodactylids found on the fish were the exclusive cause of the fish mortalities; however, the results can be consistent with the size of the fish (~1 cm) making more vulnerable to infection as reported in other fish larvae (Valladão *et al.,* 2014, 2016). The skin and oral cavity are the principal surfaces of fish that are in contact with water, offering particularly favorable conditions for the establishment and survival of parasitic animals (Grano-Maldonado, 2014). The presence of *Gyrodactylus cichlidarum* in the buccal cavity and on the skin of small fishes (1.16 ± 0.04 cm) are believed to be the most likely cause of the fish mortalities since fish started dying the first chemotherapeutic treatment previously. *Gyrodactylus* attach to fish using a terminal specialized attachment organ, the opisthaptor which is equipped with two sharp, centrally positioned hooks called hamuli and an array of 16 peripherally distributed hooks (Fig. 3). The parasite can also temporarily attach to its host by fixing its anterior extremity, the prohaptor, which consists primarily of two cephalic lobes, which produce a sticky secretion and the pharynx. When *Gyrodactylus* feeds, it inverts its pharynx through its mouth and releases a digestive solution containing proteolytic enzymes, *i.e.,* proteases and lysozyme (Buchmann & Bresciani, 1998) which act to break down the fish skin. Mucus and dissolved skin are then sucked into the gut. This feeding

activity can result in small lesions in the fish skin (Cable & Harris, 2002). A few parasites do not represent any problem for an otherwise healthy fish, but the presence of large numbers of parasites, *e.g*., up to 10,000 *G. salaris* which were found on a single *S. salar* parr (Bakke *et al.*, 2007) may cause death through disruption of normal osmoregulatory function. Previously, another massive infection of gyrodactylids causing death on fish was reported in Spain (Grano-Maldonado *et al.*, 2011), mortalities in *O. niloticus* in the United Kingdom (García-Vásquez *et al.*, 2007), on *Hemichromis fasciatus* from Senegal (Přikrylová *et al.*, 2009) and recently in *Astronotus ocellatus* in Iran (Mousavi *et al.*, 2013).

In the same way, Malmberg & Malmberg (1993) described this for *G. salaris* on rainbow trout, *Oncorhynchus mykiss* (Busch *et al.*, 2003) working with *O. mykiss* fry (0.8-2.5 g) found that mean intensities of 26.1 ± 25.9 *G. derjavini*, *sic G. derjavinoides*/ fish resulted in the loss of 22% of the starting. Mo (1992) reported infections of up to 12,500 *G. salaris* specimens on individual salmon parr. Infections as high as 5000 individuals of *G. salaris* on a 1 ± year-old Atlantic salmon *Salmo salar*, were reported by Appleby *et al.* (1997). *Gyrodactylus* species can result in the death of little fish; it is possible that the levels of this ectoparasite reported here were responsible for a proportion of the losses. During the increasing temperature period (summer), the tilapias experienced high levels of stress through long periods of high temperature and as consequence fasting, which may also have led to difficulties in acclimating to tank conditions during the reversion period. Temperature strongly regulates population dynamics of these viviparous flatworms in farmed and wild fish populations, with most gyrodactylid species showing positive temperature-abundance associations (Rubio-Godoy *et al.*, 2012).

In Mexico, *G. cichlidarum* has not been described in causing this severe mortality in the coast of Sinaloa in the Pacific coast. However, this species has been reported in Tabasco which is situated in the Gulf of Mexico (Kohn *et al.*, 2006) and Veracruz State (Rubio-Godoy *et al.*, 2012). In the present work, substantial *Gyrodactylus* infection losses have been observed during these days. It is important to note that, because the samples collected represent only affected Nile tilapia population and because these fish were sampled the fish were moved to the nursery towards reversion and growth section, it is uncertain whether the parasite burden recorded here accurately reflects that of earlier mortalities. There are, however, many viral pathogens of tilapia that have been reported African fish are lymphocystis, in cichlids from the East African lakes (Paperna, 1973) which are manifested by readily detected, macroscopic dermal pustules (pox-like infection). Neither virological nor bacteriological assays were conducted on the samples that were received, although the fish examined did not show any clinical signs related to this pathogens according to the farm personnel. However, this is just an assumption since this parasite species may have come to this stock by other fish farm or from the share water supply. Biosecurity measurements are highly recommended as, disinfection of tanks and equipment.

The treatments administered (formaldehyde) to control the gyrodactylid infection seem to have been at least partially unsuccessful maybe as a late identification (day 2) and consequently prompt benzimidazoles treatment (mebendazole). However, for therapeutic formalin baths in Nile tilapia juveniles, Perera & Pathiratne (2005) performed same recom-mendation of 50 mg L^{-1} for 1 h. The reasons for treatment failure in this instance are unknown its proliferation might be promoted by changes in the relationship among host, parasite, and environment caused by nutritional deficiency, poor water quality, and parasitic diseases (Khan, 2004; Borji *et al.*, 2012), causing severe epidermal lesions and disease outbreaks (Martins *et al.*, 2010).

All treatments were conducted under the same conditions, further natural or plant-derived treatments should be explored in this fish size, as recommended by Valladão *et al*. (2015). In summary, this study reports for the first time the presence of *G. cichlidarum* causing mortalities in a fish farm in the Pacific coast, although it is difficult to assess the role of this parasite in the high mortality of fish experienced. The early accurate detection of the presence of these ectoparasite species provided by specialized personnel may prevent the mass mortality of Nile tilapia fish, also with some monitoring of ectoparasites on this specific size fish would be an appropriate precautionary measure during summer or customary quarantine conditions. Due to the intensification of culture conditions in this area, studies examining multiple pathogens will be invaluable to solve Nile tilapia producers' current and future problems. In this case, in particular, molecular techniques were not possible to use for the reason that the samples were preserved in formalin. However, future comparison of 18S ribosomal RNA gene (or another conserved gene) sequence between the monogenean collected from Nile tilapia should be conducted. There is no doubt that infections by parasites have major consequences for species of small fish in culture and must consequently be considered as a fundamental factor within any system of aquaculture.

ACKNOWLEDGMENTS

The authors would like to thank the personnel in the fish farm for all their invaluable help to collect and provide samples. Special thanks to Dr. Amy Kathryn Lowe who provided editorial comments. To the Comité Estatal de Sanidad Acuícola de Sinaloa (CESASIN) that provided current regional data, and the anonymous reviewers that helped us to improve the manuscript.

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Received: 7 January 2018; Accepted: 8 May 2018

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