Research Article

Trichodina heterodentata (Ciliophora: Trichodinidae) Parasitizing *Piaractus brachypomus* Cultivated in Ecuadorian Amazon

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Abstract | Ectoparasitic protozoa of the genus *Trichodina* are considered one of the main pathogens affecting cultured fishes, mainly in small organisms or early ages. However, its incidence and effects on Amazon traditional aquaculture species such as *Piaractus brachypomus* has been little studied. The objective of the present investigation was to establish the presence of *Trichodina heterodentata*, its parameters of infection, and associated damages in three aquaculture farms of *P. brachypomus* located in Puyo, El Coca and in Joya de los Sachas, Ecuadorian Amazon. In each farm, 15 fish were collected randomly. The size and weight of fishes were recorded, and smears were made from the surface of the skin, gills and fins for the detection of trichodinid parasites. Gill fragments of parasitized fish were fixed in 10% buffered formalin and processed by the paraffin inclusion technique, to evaluate the damages associated with parasites. *Trichodina heterodentata* was detected in specimens of *P. brachypomus* from the farm located in El Coca, with prevalence of 100%, and mean intensity of 0.21 ± 4.7 parasites per host. Histopathological analysis showed the presence of slight hyperplasia and thickening of the lamellae of parasitized fish. The fish from El Coca farm showed significantly lower average sizes and weights than the fish from the other two farms, confirming the susceptibility of the smaller fish to these parasites. This is the first record of *T. heterodentata* for *P. brachypomus* in its entire range of distribution, and the first report of *Trichodina* in aquaculture systems of Ecuador.

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Introduction

The genus *Trichodina* is composed of ciliated protozoa widely known as fish parasites. Although some species have been reported in fish internal organs (Dang *et al*., 2019), they are mainly ectoparasites that can cause abrasive lesions in the integument and gills of the hosts due to their characteristic circular movements (Khan, 2004; Pádua *et al*., 2012b). The amount and severity of tissue alterations in the hosts depends, among other factors, of infection intensity (Khan, 2004; Steckert *et al*., 2018) and the cultivation environmental variables (Hossain *et al*., 2008). The most serious tissue alterations are inflammation of the arch and branchial filaments, loss of lamellae, and hyperplasia of the interlamellar epithelium (Alcántara-Bocanegra *et al*., 2015; Verján *et al*., 2001a, b) which limits the ability of gill breathing in the infected hosts. Another important factor in the severity of lesions is the age of the host, as juvenile organisms are usually more susceptible to infestations by *Trichodina* and tend to have higher mortality rates (Basson and Van As, 2006).

Piaractus brachypomus, known as common name cachama, is a species that adapts well to captive conditions, mainly to artificial feeding, achieving high feed conversion rates (Vicuña, 2010). These characteristics, together with a good acceptance of its meat in international markets (Mesa-Granda and Botero-Aguirre, 2007), has favored increases in cachama productions in several South American countries (Vicuña, 2010), including Ecuador (Valladão *et al*., 2016b). However, as in other aquaculture species, intensifying the cultivation increases the probability of infections by pathogens that can be detrimental to the health of the fish (Verján *et al*., 2001b) due to the increase in stocking density and the consequent deterioration of physical conditions and water chemistry. Currently, there are several records of infestation by *Trichodina* sp. in culture systems of *P. brachypomus* in South America such as in the Colombian Eastern Plains (Verján *et al*., 2001b), Delta Amacuro, Venezuela (Centeno *et al*., 2004), farms of cachama broodstock in the Peruvian Amazon (Alcántara-Bocanegra *et al*., 2015), and cachama cultivation systems located in the southeast and central region of Brazil (Pádua *et al*., 2012a; Tomas *et al*., 2012; Junior *et al*., 2018).

Piaractus brachypomus is one of the main species of fish cultivated in the Ecuadorian Amazon, with export records of 800 to 1000 t between 2008 and 2015, which have decreased to 58 t in 2019 (FAO, 2020). However, despite its importance, there are still no reports of ectoparasites affecting this aquaculture resource in the country. In this sense, the objectives of this work were to report a new geographic location of *Trichodina heterodentata*; determine their prevalence and mean intensity; and describe the lesion that *T. heterodentata* cause in white cachama *P. brachypomus*.

Materials and Methods

The fishes were collected in three production farms of white "cachama" *P. brachypomus*, two farms located in the province of Orellana and one in the province of Pastaza, all in the Ecuadorian Amazon. The names of the farms are confidential; but we will call them as farm 1: El Puyo (-1.500801, -77.981437), farm 2: El Coca (-0.443606, -77.019430) and farm 3: Joya de los Sachas (-0.322723, -76.864826) (Table 1). Fifteen fish were randomly collected from each farm (n = 45) and sacrificed by decapitation with a cut in the spinal cord behind the head. The total length (TL) in cm and weight (W) in g were recorded for each fish. Each organism underwent an *in situ* review of the body surface, oral cavity, eyes, fins, operculum cavity, and gills using a stereoscope, in order to detect other ectoparasites. Wet smears were prepared in the field and examined under microscope. When parasites were present the smears were air-dried and impregnated with Klein´s dry silver method for observation of the adhesive disc as suggested by Lom (1958). Fish gill fragments were fixed in 10% neutral formalin for histopathological analysis. All the fixed samples were transferred to the Centre for Aquaculture Health at the Technical University of Manabí (Centro de Sanidad Acuícola - Universidad Técnica de Manabí), for processing. The infection parameters (prevalence and mean intensity) were calculated according to Bush *et al*. (1997). Gill fragments were fixed for histopathological analysis and processed by the histological paraffin embedding technique, cut into 5 µm thin sections, stained with the routine stain's hematoxylin and eosin, and permanently mounted with Entellan® resin (Ewijayanti *et al*., 2017). All slides were analyzed with a BX53 Olympus microscope and images were captured with an AmScope 18 MP camera.

Table 1: *Mean values ± standard deviation of the total length (TL) and weight (W) of cachama Piaractus brachypomus per farm analyzed.*

Locality	Farm number	TL (cm)	W (g)
El Puyo	1	20.00±7.03	126.14±66.51
El Coca	2	10.75±5.70	54.00±22.55
Joya de los Sachas	3	39.00±10.10	966.60±159.62

Results and Discussion

Piaractus brachypomus specimens from farm 2 showed significantly lower values in TL ($F_{(2,42)} = 273, P < 0.05$) and W ($F_{(2,42)} = 443, P < 0.05$) than the specimens from farms 1 and 3 (Table 1).

Trichodina heterodentata Duncan, 1977 was detected infesting gill tissue and fins (Figure 1A-B) of *P. brachypomus* from farm 2, with a prevalence of 100% and intensity of infection of 0.21±4.69 parasites per host. The measurements of the specimens in this study compared to those described by Duncan (1977) and reported in other studies around South America, are shown in Table 2.

Trichodina heterodentata was characterized by presenting a medium-sized disc-shaped body. The centre of adhesive disc shows small granules in silver-impregnated specimens (Figure 1C). Macronucleus

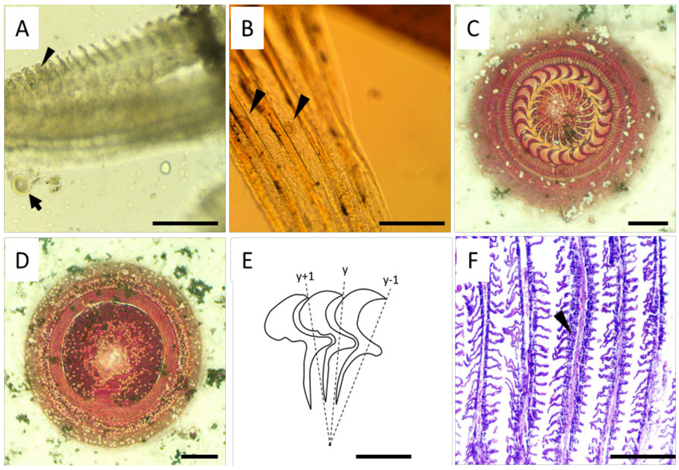

Figure 1: *Trichodina heterodentata parasitizing Piaractus brachypomus. (A) Fresh-mounted smear from the gill arches showing light hyperplasia (arrowhead) and Trichodina (arrow); (B) Trichodinids (arrowheads) in a fresh-mounted smear from the fins; (C) Silver impregnated adhesive disc; (D) Nuclear apparatus; (E) Schematic drawing of the denticles; (F) Histological section of gill from parasitized fish showing light hyperplasia (arrowhead) and thickening of gill filaments. H-E. Scale bars: A, B, F = 200 μm; C, D = 10 μm; E = 5 μm.*

Table 2: *Measurement data of Trichodina heterodentata Duncan (1977) reported in fishes from Philippines (original description) and South America, including this study.*

Characters	**Present study**	**Duncan (1977) Population B**	**Van As and Basson (1989)**	**Martins *et al.* (2010)**	**Miranda *et al.* (2012)**	**Pádua *et al.* (2012b)**	**Valladão *et al.* (2014)**	**Valladão *et al.* (2016b)**	**Sousa-Filho *et al.* (2021)**
Country	**Ecuador**	**Philippines**	**Venezuela**	**Brazil**	**Peru**	**Brazil**	**Brazil**	**Brazil**	**Brazil**
Host	***Piaractus brachypomus***	***Tilapia zilii Oreochromis mossambicus Trichogaster trichopterus***	***Oreochromis mossambicus***	***Ictalurus punctatus***	***Arapaima gigas***	***Piaractus mesopotamicus***	***Prochilodus lineatus***	***Oreochromis niloticus***	***Gymnotus carapo***
Body^{D}	50.0 ± 6.6 (40.9-57.6)	80 (58.0-108.0)	65.6 ± 5.3 (52.4-78.0)	59.4 ± 8.5 (27.0-77.0)	56 ± 5.3 (47.3-76.0)	50.5 ± 3.7 (45.0-58.8)	56.9 ± 3.6 (48.4-65.9)	50.5 ± 4.1 (38.4-59.2)	52.8 ± 0.9 (51.9-53.7)
Border membrane^{W}	4.7 ± 0.7 (3.8-6.0)	4.7 (3.4-5.5)	4.9 ± 0.5 (3.9-5.9)	5.1 ± 1.7 (3.0-7.0)	ND	4.3 ± 0.4 (3.5-5.5)	4.5 ± 0.4 (2.8-5.7)	4.2 ± 0.4 (3.2-5.2)	4.3 ± 0.2 (4.1-4.5)
Adhesive disc^{D}	38.7 ± 5.0 (33.0-52.0)	57.0 (45.0-74.0)	56.2 ± 4.9 (44.3-67.0)	60.2 ± 6.7 (40.0-72.0)	45.7 ± 3.8 (37.1-51.0)	41.2 ± 3.3 (35.1-49.7)	47.7 ± 3.6 (39.4-55.3)	42.3 ± 4.0 (31.3-50.3)	45.0 ± 3.8 (41.2-48.8)
Denticle ring^{D}	23.2 ± 2.7 (20.0-30.0)	36.0 (29.0-45.0)	34.9 ± 3.4 (23.9-35.6)	38.5 ± 4.5 (27.0-47.0)	28.2 ± 2.7 (20.0-34.7)	24.4 ± 2.4 (19.9-30.3)	29.4 ± 2.6 (23.0-37.6)	25.4 ± 3.1 (12.2-32.4)	30.8 ± 0.2 (30.6-31.0)
Denticle number	25.1 ± 0.6 (24-26)	26.0 (20-31)	24 (20-30)	24.4 ± 1.6 (23-28)	20.7 ± 2.6 (12-24)	20 (16-22)	23 ± 0.9 (20-26)	23.7 ± 2.2 (11-27)	20.0 ± 0.0
Denticle^{L}	6.8 ± 0.8 (5.8-8.0)	9.2 (7.5-11.0)	8.6 ± 1.0 (6.4-11.2)	10.3 ± 1.2 (7.0-13.0)	7.5 ± 0.6 (6.3-8.6)	7.7 ± 0.7 (6.1-9.1)	7.8 ± 0.7 (5.8-9.3)	6.9 ± 0.6 (5.6-9.7)	8.4 ± 0.4 (8.0-8.4)
Blade^{L}	4.4 ± 0.9 (3.5-7.3)	5.7 (4.7-7.1)	4.3 ± 0.4 (4.6-8.1)	6.2 ± 0.8 (4.0-8.0)	4.5± 0.6 (3.9-5.5)	4.6 ± 0.4 (3.7-5.5)	4.6 ± 0.4 (3.8-5.7)	4.2 ± 0.4 (2.9-5.2)	3.5 ± 0.2 (3.3-3.7)
Ray^{L}	4.9 ± 0.7 (3.9-6.2)	ND	8.8 ± 1.3 (5.8-11.8)	8.5 ± 1.7 (3.0-12.0)	6.7 ± 0.8 (3.9-7.8)	7.2 ± 0.8 (5.1-9.2)	7.7 ± 0.8 (6.0-9.9)	6.1 ± 1.0 (3.1-8.1)	-
Central part^{W}	1.9 ± 0.2 (1.8-2.5)	2.6 (1.4-3.4)	3.3 ± 0.5 (1.8-4.7)	3.8 ± 0.7 (2.0-6.0)	2.4 ± 0.3 (1.5-3.1)	1.9 ± 0.4 (1.2-2.7)	3 ± 0.5 (2.1-4.4)	2.3 ± 0.4 (1.4-3.2)	2.0 ± 0.0
Denticle span	10.1 ± 1.0 (8.8-13.0)	ND	ND	18.4 ± 2.2 (12.0-22.0)	13.2 ± 1.7 (7.1-15.7)	13.7 ± 0.9 (11.6-15.5)	15.4 ± 1.0 (13.0-17.6)	12.5 ± 1.4 (7.8-15.5)	14.1 ± 0.3 (13.8-14.4)
Radial pins/ denticle	10.5 ± 0.9 (8-12)	10.0 (6-14)	11 (10-12)	11.8 ± 2.1 (5-15)	11.8 ± 1.6 (7-13)	10 (8-10)	9.8 ± 1.2 (6-12)	11.3 ± 1.9 (7-16)	ND

Arithmetic mean ± standard deviation. ND: No Data; ^{D}: diameter; ^{W}: width; ^{L}: long.

is a horseshoe-shaped; micronucleus was not observed (Figure 1D). Denticles were characterized by wide blade and sickle-shaped and filled the space between the y-axes and y+1 (Figure 1E). Apophysis of the blade is prominent and in connection with the central part. The central part robust ending rounded fitting on the next denticle filling the space between y and y - 1 axis. The ray was long, robust, slightly directed anteriorly that was situated between the axes y and y + 1, tapered at a point (Figure 1E). Parasitized fish showed slight interlamellar hyperplasia and thickening of the secondary filaments of the gills, presumably associated with the presence of *T. heterodentata* (Figure 1F).

The protozoan identified in this study has well-developed denticles that formed by sword, central part, and rays, with the latter being prominent and needle-shaped that are distinctive characteristics of the genus *Trichodina* (Basson and Van As, 2006; Wang *et al*., 2019). This genus is the richest of the Trichodinidae family, recognizing approximately 300 species, which parasitize skin, fins, gills, and urinary bladder of fish and amphibians from all continents except Antarctica (Tang *et al*., 2013; Dang *et al*., 2019). In South America, the genus *Trichodina* has been reported in native and/or cultured fish from Brazil, Peru, Argentina, Chile, Colombia, Venezuela, and Uruguay, but there are no reports in Ecuador, Paraguay, or Guyana (Oliveira *et al*., 2018). The present study not only constitutes the first report of the genus *Trichodina* in Ecuador, but also the first record of *T. heterodentata* infesting *Piaractus brachypomus*.

Trichodina identified in this study has a significant morphological similarity to *T. heterodentata* described by Duncan (1977) in *Tilapia zillii* and *T. mossambica* from the Philippines. This species is cosmopolitan, registering in more than 35 species of fish (Martins *et al*., 2010). In South America, it has been recorded in at least seven host species, both native (*Ictalurus punctatus*, *Arapaima gigas*, *Piaractus mesopotamicus*, *Prochilodus lineatus*, *Gymnotus carapo*) and exotic species (*Oreochromis mossambicus*, *O. niloticus*) (Van As and Basson, 1989; Martins *et al*., 2010; Miranda *et al*., 2012; Pádua *et al*., 2012a; Valladão *et al*., 2014, 2016a; Sousa-Filho *et al*., 2021). The organisms collected in this study are very similar in morphometry with the specimens collected in cultures of *P. mesopotamicus* (Pádua *et al*., 2012b), *O. niloticus* (Valladão *et al*., 2016a), and *G. carapo* (Sousa-Filho *et al*., 2021) from Brazil.

Of the three farms analyzed, we found fish infested by *T. heterodentata* only in the El Coca farm. All fishes were parasitized (100 % of prevalence) but with low infection level, which could be due to the sizes of the analyzed hosts. Parasitized fish corresponded to rearing organisms with average sizes significantly ($P < 0.05$) lower than the fish from El Puyo and Joya de los Sachas farms, which were fattening organisms (Table 1). Increased of *Trichodina* infestation in juveniles may be associated with lower immunity in this age group compared to larger fish. Several studies have reported that *Trichodina* show higher prevalence and average intensity in smaller organisms, especially in fingerlings (Meyer, 1991; Pádua *et al*., 2012b; Martins *et al*., 2015; Oliveira *et al*., 2018). In this sense, we could find higher levels of infestation in future studies that include smaller organisms such as larvae and fingerlings. The low levels of infestation could also be explained because the farms analyzed are based on extensive systems with low stocking densities, which reduces the contagion rate. The increase in fish biomass increases the proliferation and transmission of *Trichodina* (Oliveira *et al*., 2018) and this is particularly important during reproduction and hatchery, where high densities and poor water quality increase the parasitic infections (Valladão *et al*., 2016b).

The parasitized fish showed slight interlamellar hyperplasia and thickening of the secondary filaments of the gills, which could be associated with the presence of *T. heterodentata* despite the low levels of infection. These damages are consistent with those produced by the circular movements and the suction that the aboral membrane of these protozoa exert on the epithelial cells of the hosts causing irritation (Basson and Van As, 2006). These irritations could cause physiological alterations in the fish and constitute a gateway for opportunistic pathogens such as bacteria and fungi (Thoney and Hargis, 1991). We observed little histologic damage associated a low intensity of infection. The severity of the damage could range from irritation to hypoxia, loss of balance, and erratic movements (Martins *et al*., 2015; Valladão *et al*., 2013, 2014), which is also associated with the abundance of these parasites (Basson and Van As, 2006). Future histological studies will be required to demonstrate that the observed damages correspond to the levels of infection by *T. heterodentata.*

Conclusion and Recommendations

This is the first record of *T. heterodentata* in *P. brachypomus*, a native fish of the Amazon Basin with great aquaculture potential and widely cultivated in South America. This finding also represents an extension of the known geographical range for *T. heterodentata*, and contributes to the knowledge of this group of pathogens in countries of the region. The high observed prevalence indicates that this pathogen is common in *P. brachypomus* and could represents a health risk not only for *P. brachypomus*, but also for other Amazonian species cultured in Ecuador. Thus, considering that *T. heterodentata* species at high levels of infection can cause epizootics and considerable economic losses in production, it is recommended to monitor the presence of these protozoa in fish culture systems in Ecuador, including *P. brachypomus*, mainly at the most vulnerable stages, as well as implement measures for its prevention and control.

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Novelty Statement

This research provides the first record of *Trichodina heterodentata* in aquaculture of *Piaractus brachypomus* from Ecuadorian Amazons.

Author's Contribution

Yanis Cruz Quintana: Conceptualization, formal analysis, funding acquisition, investigation, methodology, supervision, validation, writing, review, and editing.
Ana María Santana-Piñeros: Conceptualization, formal analysis, investigation, supervision, validation, writing, review, and editing.
Byron Manuel Reyes-Mero: Formal analysis, methodology, writing.
Leonela Griselda Muñoz-Chumo: Formal analysis, methodology.
Lenin Cáceres-Farías: Funding acquisition, methodology.

Ethical aspects
The animal bioethics procedures of this study had the permission of the Institutional Bioethics Committee of the Universidad Técnica de Manabí, established in volume 021-5 folio 21-5-1.

Conflict of interest
The authors have declared no conflict of interest.

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