Rev Fish Biol Fisheries (2018) 28:33–56
https://doi.org/10.1007/s11160-017-9490-1

REVIEWS

Trichodinidae in commercial fish in South America

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Received: 30 August 2016 / Accepted: 28 June 2017 / Published online: 5 July 2017

Abstract Ciliates of the family Trichodinidae are protozoan parasites of importance for fish farming in South America, given that at high infestation levels, they cause significant mortality among farmed fish. Although data on economic losses due to parasitosis are not available for South America, mortality outbreaks correlated to trichodinids are very common in the tilapia production chain, especially in Brazil, the largest aquaculture chain in the country. In Brazil in the past, trichodinids were considered only as *Trichodina* sp. Today, they have been better studied and identified taxonomically in wild and farmed fish. However, in other countries in South America, trichodinids continue to be described only as *Trichodina* sp. This review presents the history of occurrences of trichodinids in fish of interest in South America, highlighting 15 new species that have been described in three genera in Brazil, along with information on parasite-host-environment relationships, diagnostic methods and treatments. The occurrence of parasitic ciliates must be correlated with farming conditions such as stress factors, water quality, seasonality, age and host immunity to elucidate the critical points of each production system. Furthermore, for tropical fish, studies on treatment against trichodinid species are needed to provide support for approval of antiparasitic medications for use in fish farming. However, it is recommended that the production sector use intensive production systems that are more sustainable, with biosafety protocols, to increase production and productivity.

Keywords Aquaculture · Fish Disease · Parasites · Protozoan · Trichodina

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Introduction

The South American continent is known for its high production and exports in fisheries and aquaculture. Aquaculture plays a particularly important role in food production and in the local economy, in addition to having a large international presence with an impact on the global economy. The production of native fish is currently overtaking the production of non-native species in some countries, which is considered a milestone for South American aquaculture (Valladão et al. 2016b). South America has recorded increases in fish production. In terms of production of freshwater water fish, Brazil is the largest power in the Americas (611.343 tons), and its production in this area is approximately 10 times greater than that of Chile (59.527 tons). The main pathogens of South American fish are similar to those observed throughout the world, such as the bacteria *Aeromonas hydrophila*, *Ichthyophthirius multifiliis*, monogeneans and trichodinids (Valladão et al. 2016b).

Organisms of the family Trichodinidae are commonly found in aquatic environments as symbionts. However, they can behave as commensals or parasites of a variety of aquatic vertebrates and invertebrates, such as ciliates, coelenterates planctophagous crustaceans, mollusks, echinoderms, amphibians and fish (Van As and Basson 1987; Basson and Van As 1989; Sabry and Magalhães 2005; Pinto et al. 2006; Dias et al. 2009; Silva-Briano et al. 2011; Martins et al. 2016). Trichodinid species are therefore of great importance for fish farming, given that they parasitize farmed fish. When parasitism levels are high, they can cause epizooty and considerable economic losses in intensive production systems.

Most trichodinids have a conical, rounded or hemispherical body shape (e.g., *Tripartiella*) that is completely covered with a thin membrane (pellicle) and have a typical structure in the aboral region known as the adhesive disk. The presence of cilia, in both the aboral and adoral regions, provides them with motility in free-living environments and on their hosts and aids in feeding (Basson and Van As 2006). They range in size from 20 to 100 µm and can be classified as small, medium or large (Van As and Basson 1987; Basson and Van As 1989; Valladão et al. 2013).

In fish, trichodinids mainly parasitize the skin, fins and gills (Van As and Basson 1987), and less frequently the eyes, mouth, gastrointestinal tract, urinary tract and gonads (Noga 2010; Valladão et al. 2014). In these hosts, they feed on cells, bacteria and detritus from the body surface and are carried by the cilia to the cytostome, which is in the adoral region of the parasite (Basson and Van As 2006; Bruno et al. 2006).

The life cycle of these parasites is monoxenic, and reproduction occurs by means of binary division and, under certain conditions, conjugation (Van As and Basson 1987; Martins et al. 2015). In *Trichodina pseudoheterodentata* from *I. punctatus*, during asexual reproduction, the youngest individuals that form immediately are smaller than the older individuals and only possess 50% as many denticles in their adhesive discs. At subsequent developmental stages, the old ring with 50% of the usual number of denticles underwent gradual resorption, while a new ring was formed with the usual number of denticles around the old one around the perimeter of the disc (Tang et al. 2017). Wild larvae of amphibians (tadpoles) may be reservoirs of trichodinids for some fish (Lom and Dykova 1992). Trichodinids are present in natural environments but do not occur at high levels of infection in healthy animals (Basson and Van As 2006).

The mechanism of pathogenic action of trichodinids is related to the manner in which they infect their hosts, since when the parasite is fixed firmly onto its host, the border of the aboral membrane creates a suction movement on the surface of the epithelial cells, which likely causes irritation to the tissues of the fish (Basson and Van As 2006). Thus, a high abundance of these parasites and their constant circular movements may seriously damage the epithelium of their hosts, thereby triggering physiological alterations (Van As and Basson 1987).

In the past, trichodinids in Brazil were generally considered only as *Trichodina* Ehrenberg, 1830 (Martins and Ghiraldelli 2008; Marchiori and Martins 2012), since this is the largest genus of the Trichodinidae, with more than 150 species that parasitize fish (Asmat et al. 2003). In addition to this genus, the family Trichodinidae includes seven of the ten associated genera (Basson and Van As 2006). However, *Trichodina* Ehrenberg, 1830, *Paratrichodina* Lom, 1963, *Trichodinella* (Raabe 1950) Šrámekhušek, 1953, and *Tripartiella* Lom, 1959, are the genera that have been described as parasitizing aquatic animals in Brazil (Martins et al. 2015). Therefore, it is possible

that some trichodinids that were previously classified as *Trichodina* sp. are members of the genera *Trichodinella* and, especially, *Tripartiella* (Marchiori and Martins 2012).

The genus *Paratrichodina* comprises more than eleven species that are known in Europe, Asia, Africa and the United States, of which eight parasitize the gills, and three the urinary tract of fish (Basson and Van As 2006). The few known species of *Trichodinella* parasitize the gills and are distributed in Europe, Asia, Africa, Middle East, Mexico, United States and Philippines. *Tripartiella* species have well-known distribution in Europe, Asia, United States and the Far East (Basson and Van As 2006; Kent and Fournie 2007) but are not well known in South America. In Brazil, only two species of *Tripartiella* are known (Martins et al. 2015).

In recent years, there has been considerable evolution in taxonomic studies of the diversity of trichodinids among different species of fish in the wild and in farms, especially in Brazil (Fig. 1). The objective of this review was to present a history of the descriptions of occurrence of trichodinids in farmed fish in South America, highlighting new described species and including information about parasite-host relationships, the pathology of infection and methods for diagnosis and treatment.

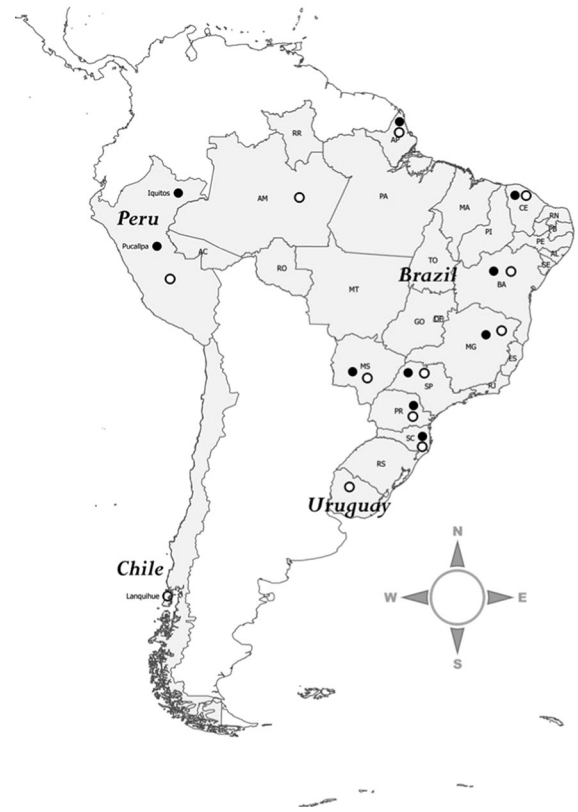

Fig. 1 Distribution of the records of trichodinids in South America (*gray* countries), with emphasis on Brazil and on new species that have been described over the last ten years. *Open circles* denote descriptions of *Trichodina* sp. and *solid circles* denote the new species that have been described

Trichodinids in farmed fish in Brazil

In the 1990s, the first studies of the occurrence of trichodinids in teleost fish reared in Brazil appeared, using data from parasitological surveys. In the state of São Paulo, in southeastern Brazil, the first records of the seasonal occurrence of fish mortality associated with parasites and water temperature were obtained between 1983 and 1990 (Ceccarelli et al. 1990). These authors registered mortality rates of 83% for *Colossoma macropomum* (tambaqui) and *Piaractus mesopotamicus* (pacu), 17% for the hybrids paqui (♀*P. mesopotamicus* × ♂*C. macropomum*) and tambacu (♀*C. macropomum x* ♂*P. mesopotamicus*), and 17% for *Ctenopharyngodon idella* (grass carp), all associated with infestation by *Trichodina* sp. Between 1990 and 1991, Figueira and Ceccarelli (1991) identified *Trichodina* sp. as the most common parasite in fish farms in the northeastern region of the state of São Paulo. In the same region of this state, a survey on parasites in fish farms conducted between 1993 and 1998 recorded high mortality among both native and non-native fish, such as pacu, tambaqui, tambacu, *Leporinus macrocephalus* (piauçu), *Brycon cephalus* (matrinxã), *Oreochromis niloticus* (Nile tilapia), *Cyprinus carpio* (common carp) and *Clarias gariepinus* (African sharptooth catfish). The stocking density used by the fish farmers ranged from 1 to 8 fish/m^2, and the occurrence rate of trichodinids was 9.9% for these eight fish species evaluated (Martins et al. 2000). Also in the 1990s, the occurrence of *Trichodina* sp. in tambaquis and common carp in northeastern Brazil was reported (Békési 1992). Additionally, the parasite fauna of fish in two catch-and-pay ponds in the northeastern region of the state of São Paulo, Brazil were reported between April 1997 and March 1999 (Tavares-Dias et al. 2001). They examined *B. amazonicus*, *L. macrocephalus*, *P. mesopotamicus*, tambacus, *C. carpio*, *O. niloticus* and *Tilapia rendalli* and found infection due to *Trichodina*

sp. in *P. mesopotamicus, L. macrocephalus,* tambacus and *O. niloticus*. The highest prevalence of infection was in the first two species. In the 1990s, the southern and southeastern regions of Brazil experienced significant development of fish farming, and catch-and-pay enterprises became a common (Tavares-Dias et al. 2001).

Beginning in the 2000s the first studies were published on parasites in native species farmed in the northern and central-western regions of Brazil, along with the first records of species of trichodinids (Table 1). A low prevalence (0.8%) of trichodinids was reported in tambatingas (♀*C. macropomum* × ♂*Piaractus brachypomus*) in ten fish farms in the state of Amapá, in the Amazon region (Dias et al. 2015); in larvae, post-larvae and fry of cachapinta (♀*P. reticulatum* × ♂*Pseudoplatystoma corruscans*) and jundiara (♀*Leiarius marmoratus* × ♂*P. reticulatum*) in fish farms in the state of Mato Grosso do Sul (Pádua et al. 2012a; Ventura et al. 2013); in *P. mesopotamicus* and patingas (♀*P. mesopotamicus* × ♂*P. brachypomus*) in three fish farms in the northwestern region of the state of São Paulo (Franceschini et al. 2013); and in fish in catch-and-pay enterprises in Guariba, state of São Paulo (Schalch and Moraes 2005). A new species of *Tripartiella, Tripartiella pseudoplatystomae*, was described on the gills and skin of *P. corruscans*, farmed in Mato Grosso do Sul (Pinto et al. 2009); in addition, occurrences of *Trichodina colisae* in *P. mesopotamicus* and the hybrid patinga farmed in the central and southeastern regions of Brazil were recorded (Jerônimo et al. 2012). *Trichodina heterodentata* was recorded in *P. mesopotamicus* (Pádua et al. 2012b) and in *Prochilodus lineatus* (curimatãs) (Valladão et al. 2013) (Table 1).

In semi-intensive farming, the prevalence of *Trichodina* sp. on skin and gills of *Arapaima gigas* (pirarucu) was 69 and 39%, respectively, and these infestations were accompanied by *Dawestrema* sp. (Araújo et al. 2009b). Although the species of trichodinid was not described in these last two studies, *T. fariai* and *T. heterodentata* have been recorded in pirarucu farmed in Peru (Delgado et al. 2007; Miranda et al. 2012). In the report of *T. fariai* by Delgado et al. (2007), the morphological characteristics of the parasite were not demonstrated to confirm the description of the species.

For farmed Nile tilapias, studies show that the prevalence of these trichodinids is greater in fish kept in net-cages than in fish kept in excavated ponds. Tilapia, mainly *O. niloticus*, are currently responsible for approximately 60% of fish-farming production in Brazil (IBGE 2014; Valladão et al. 2016b). This level is due to consolidation of technologies and intensification of production, particularly in net-cages installed in reservoirs of hydroelectric power plants (Pádua and Cruz 2014). Tilapia farming in this system is currently characterized by the use of high stocking densities for the grow-out phase, ranging from 80 to 150 kg/m^3 (600 to more than 900 fish/m^2, depending on the phase of cultivation) (Garcia et al. 2013).

In tilapia farmed in ponds in the state of Santa Catarina, southern Brazil, the infection rates due to *Trichodina* sp. were low, with a prevalence of 5.5 to 22.5% (Azevedo et al. 2006; Ghiraldelli et al. 2006; Martins and Ghiraldelli 2008), due to mild temperatures. Similarly, for fish in the state of São Paulo, there was low prevalence, ranging from 2.7 to 4.0% (Tavares-Dias et al. 2001). For fertile Nile tilapia of Thai origin farmed in Maringá, state of Paraná, the prevalence was 17% (Vargas et al. 2000). A low prevalence of trichodinids was also reported in tilapia farmed with feed consisting of pig waste (1.7%) or commercial feed (0.6%) (Martins et al. 2010a). In farmed tilapia in the Brazilian Amazon region, the prevalence of *Trichodina* sp. ranged from 3.0 to 4.0% and for *Paratrichodina africana,* 7.9% (Pantoja et al. 2012). However, tilapia farmed in ponds showed prevalences from 10 to 95% (Jerônimo et al. 2011) (Table 1).

According to historical data, the increased biomass of tilapia stocked in net-cages seems to have contributed to increased proliferation and transmission of trichodinids. At the end of the 1990s, an analysis of ectoparasites on *O. niloticus* kept in net-cages at a density of 10 fish/m^3 in the Guarapiranga reservoir, state of São Paulo, showed a low prevalence of *Trichodina* sp. of 22.3% on the skin and 6.5% on the gills (Ranzani-Paiva et al. 2005). However, in a survey conducted more recently on tilapia farmed in net-cages at higher densities (133 fish/m^3), in the middle stretch of the Paranapanema River on the boundary between the states of São Paulo and Paraná, the prevalence of trichodinids on the skin of fish ranged from 0 to 100%, and findings were more frequent during the winter months (Ayroza et al. 2014). Corroborating these results, Zago et al. (2014) reported higher rates of parasitism due to *T. compacta*

Table 1 Species of Trichodinidae parasitizing fish for consumption and ornamental fish that are farmed in South America

Species of Trichodinidae	Host	Host's phase of life	Infection site	Production system (density)	Locality of occurrence	References
Trichodina compacta	*Oreochromis niloticus*	–	Skin and gills	Excavated fish ponds	Municipalities of Blumenau, Joinville and Ituporanga, State of Santa Catarina	Ghiraldelli et al. (2006); Jerônimo et al. (2011)
		–	Skin and gills	Excavated fish ponds (0.75–4 fish/m^2)		
		Fry	Gills	Excavated fish ponds with pig waste (3 fish/m^2) and with feed (4 fish/m^2)	Municipality of Nova Trento, State of Santa Catarina	Martins et al. (2010a)
		Fry and adults	Skin and gills	Net-cages of 6 m^3 (80 kg/m^3; final weight 800 g)	Água Vermelha Reserve, State of São Paulo	Zago et al. (2014)
		Larvae and fry	Body surface, skin and gills	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a)
Trichodina truncata (synonym *T. compacta*)	*Oreochromis niloticus*	–	Skin and gills	Excavated fish ponds	Municipalities of Blumenau, Joinville and Ituporanga, State of Santa Catarina	Ghiraldelli et al. (2006)
Trichodina magna	*Oreochromis niloticus*	–	Skin and gills	Excavated fish ponds	Municipalities of Blumenau, Joinville and Ituporanga, State of Santa Catarina	Martins e Ghiraldelli (2008); Jerônimo et al. (2011)
		–	Skin and gills	Excavated fish ponds (0,75 a 4 fish/m^2)		
		Fry	Gills	Excavated fish ponds with pig waste (3 fish/m^2) and with feed (4 fish/m^2)	Municipality of Nova Trento, State of Santa Catarina	Martins et al. (2010a)
		Fry and adults	Skin and gills	Net-cages of 6 m^3 (80 kg/m^3; final weight 800 g)	Água Vermelha Reserve, State of São Paulo	Zago et al. (2014)
Trichodina centrostrigata	*Oreochromis niloticus*	Larvae and fry	Body surface, skin and gills	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a)
		Fry	Gills	Natural environment, escape from fish farming	Fortaleza Creek, State of Amapá	Bittencourt et al. (2014)
		–	Gills	Excavated fish ponds, net-cages and raceways	States of Ceará, Bahia, Minas Gerais, São Paulo and Mato Grosso do Sul	Pádua et al. (2015)
Trichodina heterodentata	*Prochilodus lineatus*	Larvae	Body surface, gills, eyes, oral cavity and intestine	Excavated fish ponds	Municipality of Jaboticabal, State of São Paulo	Valladão et al. (2014)
	Piaractus mesopotamicus	Fry	Skin, fins and gills	Tanks of 400 L (0.75 fish/L)		Pádua et al. (2012b)
	Arapaima gigas	Fry	Skin and gills	Concrete tanks	Ucayali, Peru*	Miranda et al. (2012)
	Oreochromis niloticus	Larvae and fry	Preference for body	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a, b)
	Ictalurus punctatus	Fry	Skin and gills	Excavated fish ponds (1,4 fish/m^2)	Municipality of Porto União, State of Santa Catarina	Martins et al. (2010a, b)
Trichodina colisae	*Piaractus mesopotamicus*	–	Skin, fins and gills	Excavated fish ponds	Central and southeastern regions of Brazil	Jerônimo et al. (2012)
	Hybrid patinga	–	Skin, fins and gills	Excavated fish ponds		
Trichodina migala	*Oreochromis niloticus*	Larvae and fry	Body surface, skin and gills	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a)

Table 1 continued

Species of Trichodinidae	Host	Host's phase of life	Infection site	Production system (density)	Locality of occurrence	References
Trichodina nobilis	*Carassius auratus*	Adults	Pele	Aquariums	Municipality of Florianópolis, State of Santa Catarina	Martins et al. (2012)
	Poecilia reticulata					
	Xiphophorus maculatus					
	Aequidens tetramerus	Adults	Gills	Natural environment	Fortaleza Creek, State of Amapá	Bittencourt et al. (2014)
Trichodina reticulata	*Carassius auratus*	Adults	Pele	Aquariums	Municipality of Florianópolis, State of Santa Catarina	Martins et al. (2012)
Trichodina modesta	*Betta splendens*	Fry	Skin and gills	Aquariums (1 fish/2 L)	Municipality of Muriaé, State of Minas Gerais	Valladão et al. (2015)
					Municipality of Ribeirão Preto, State of São Paulo	
					Municipality of Cascavel, State of Paraná	
Trichodina acuta	*Xiphophorus maculatus*	–	Gills	Aquariums (shop)	Municipality of Florianópolis, State of Santa Catarina	Piazza et al. (2006)
	Xiphophorus helleri					
	Poecilia sphenops					
	Beta splendens					
	Carassius auratus					
Trichodina fariai	*Arapaima gigas*	Fry	Pele	Concrete tanks	Iquitos, Peru*	Delgado et al. (2007)
Paratrichodina africana	*Oreochromis niloticus*	–	Gills	Net-cage and excavated fish ponds (only in Bahia)	Rio das Velas Reserve, Municipality of Nova Ponte, State of Minas Gerais	Valladão et al. (2013)
					Rio Paraná Reserve, Municipality of Santa Fé do Sul, State of São Paulo	
					Vale do Juliana Reserve, Municipality of Ituberá, State of Bahia	
		Fry	Gills	Excavated fish ponds	Macapá, State of Amapá	Pantoja et al. (2012)
		Larvae and juveniles	Preference for gills	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a, b)
		Fry	Gills	Natural environment, escape from fish-farming	Fortaleza Creek, State of Amapá	Bittencourt et al. (2014)
		–	Gills	Excavated fish ponds, net-cages and raceways	States of Ceará, Bahia, Minas Gerais, São Paulo and Mato Grosso do Sul	Pádua et al. (2015)
		Fry	Gills	Fish-farming Natural environment	State of Amapá	Tavares-Dias et al. (2013)

Table 1 continued

Species of Trichodinidae	Host	Host's phase of life	Infection site	Production system (density)	Locality of occurrence	References
Tripartiella pseudoplatystomae	*Pseudoplatystoma corruscans*	Juveniles and adults	Skin and gills	Excavated fish ponds	Municipality of Dourados, State of Mato Grosso do Sul	Pinto et al. (2009)
Tripartiella orthodens	*Oreochromis niloticus*	Larvae and fry	Gills	Excavated fish ponds	States of São Paulo and Minas Gerais	Valladão et al. (2016a)
Tripartiella tetramerii	*Aequidens tetramerus*	Adults	Gills	Natural environment	Fortaleza Creek, State of Amapá	Bittencourt et al. (2014); Martins et al. (2016)

No reports of parasitism due to *Trichodina* sp. have been inserted in this table (except for localities of occurrence indicated by *; the remainder are records from Brazil)

– Not stated in the article

and *T. magna* (prevalence of 76.2% to 100%; mean intensity of 23.9 ± 7.1 to 603 ± 405.3 and mean abundance of 18.2 ± 5.8 to 545.5 ± 367.9, respectively) among tilapia reared in net-cages in a reservoir in the state of São Paulo. Furthermore, these trichodinids presented the highest frequencies (dominance) among all parasites found (*Ichthyophthirius multifiliis*, *Piscinoodinium pillulare*, *Epistylis* sp. and monogeneans).

In tilapia farmed in net-cages in reservoirs in northeastern Brazil (Bahia) and southeastern Brazil (São Paulo and Minas), the prevalence of *P. africana* on the gills was 65.9, 87.5 and 100%, respectively, considering both sick and healthy fish (Valladão et al. 2013). Garcia et al. (2013) correlated mortality events among tilapias reared in tanks with different stocking densities, with a high prevalence (66–100%) of *Trichodina* sp. on the skin and gills. However, the highest rates of *Trichodina* sp. were recorded at the highest densities, from which *Streptococcus agalactiae* (33.3 and 66.7%) was also isolated. Associations between trichodinids and secondary bacteriosis are known (Basson and Van As 2006), not only due to losses in Brazilian tilapia farming due to parasitic and bacterial diseases (Pádua and Cruz 2014) but also because the response to vaccination against *S. agalactiae* is reduced in fish that are parasitized by *T. heterodentata*, *Gyrodactylus cichlidarum* and *I. multifiliis* (Martins et al. 2011). Thus, these authors highlighted the importance of monitoring and controlling parasitism levels before conducting vaccination programs among farmed fish. However, at the reproduction and fry phases of farming of *O. niloticus* in net-cages, sanitary conditions influence the grow-out phase (Pádua and Cruz 2014), since Valladão et al. (2016a) reported that in farms in the southeastern region of Brazil, these fish presented three genera of trichodinids and histories of parasitosis and mortality among larvae and fry (Table 1). Thus, production of *O. niloticus* is a major challenge for Brazilian fish farmers, since the production process for *O. niloticus* should start with production of good-quality larvae and fry, with production protocols of greater efficiency, to attain the main objective, which is to produce fish under hygienic, healthy conditions that are acceptable to consumers. However, success in these production methods depends on the technologies available and accepted by the producers.

In the rearing of ornamental fish, trichodinids have been recorded both in fish for sale in aquarium shops and in fish in the wild or in the stocks of traders who catch and maintain wild fish for sale. A low prevalence of *Trichodina* sp. was recorded in *Xiphophorus helleri* (green swordtail) and *Xiphophorus maculatus* (platy) that were farmed both in tanks (13%) and in excavated ponds (54%) in the state of São Paulo (Garcia et al. 2009). Recently, Santos et al. (2017) reported low prevalence rates of *T. heterodentata* (23%) *Xiphophorus maculatus* as well as *Trichodina* sp. (10%) in *Xiphophorus helleri* of three facilities of ornamental fish from the state of Santa Catarina. *Trichodina acuta* was found at a low prevalence (4.7%) and medium intensity (1–31) in five species of ornamental fish in shops (Piazza et al. 2006), while *T. nobilis* and *T. reticulata* sp. infected half of the 95 fish analyzed in a shop in Florianópolis, Santa Catarina (Martins et al. 2012) (Table 1). By evaluating fish caught in a creek that forms a tributary of the Negro River in the state of Amazonas, Tavares-Dias et al. (2010) found *Trichodina* spp. only in the gills of *Carnegiella strigata* (14.3%), *Carnegiella marthae* (7.9%) and *Nannostomus eques* (9.7%), with the highest mean intensity in *C. strigata* (6.5 ± 3.5 parasites/fish). *Trichodina modesta,* which was described only in Eurasia and as a specific parasite of Cypriniformes, was also found by Valladão et al. (2015) for the first time, infecting *B. splendens* in Brazil. This is therefore the first report of this trichodinid in the Americas (Table 1).

The ornamental fish market has grown over the last ten years. Among the fish placed on the market, 90% were produced through farming, and only 10% were caught in the wild, mostly in the Amazon region for export (Tavares-Dias et al. 2009). Trichodinid species have a worldwide distribution that has increased because of transcontinental shipments of ornamental fish (Basson and Van As 2006). Introduction of cichlids from Africa to countries such as Brazil has resulted in the introduction of trichodinids into this country. *Trichodina nobilis* can access the host *Aequidens tetramerus*, which is an ornamental fish native to the Amazon region, possibly transmitted by specimens of *O. niloticus* that escaped from fish farms in the region of Macapá, state of Amapá (Bittencourt et al. 2014). In this region, *A. tetramerus* was also found to be parasitized by *Tripartiella tetramerii* (Fig. 2), which was the first trichodinid described in the Amazon region (Martins et al. 2016). It was observed that *A. tetramerus* acquired only this species of trichodinid, among the diversity of parasites found in its cohabitant *O. niloticus*, possibly because of difficulty in accessing a community of ectoparasites that already existed in native species. *Paratrichodina africana* is another exotic parasite that has been introduced into Brazil, likely through importation of

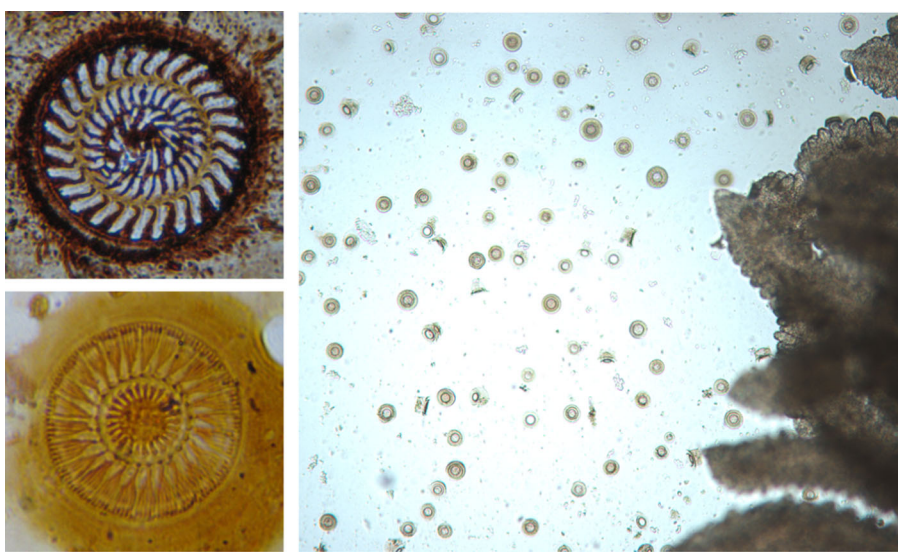

Fig. 2 *Trichodina* sp. in gills of pirarucu fingerlings (*larger image*); *Trichodina centrostrigata* ectoparasite of Nile tilapias (*above*) and *Tripartiella tetramerii* described in a Brazilian cichlid (*below*)

O. niloticus for aquaculture (Valladão et al. 2013). Because of these results and evaluation of recent studies on taxonomic identification of trichodinids, it can be supposed that transport of other species of trichodinids to Brazil may have also occurred.

Trichodinids in fish farmed in Peru, Argentina, Chile, Colombia, Venezuela and Uruguay

In South America, parasites such as *Trichodina* sp. (Rojas and Wadsworth 2007) are major health problems in net-cage fish farming, especially in tilapias; this is because during the reproductive period, parasites invade the mouths of fertile fish and transmit the infection to the larvae (Conroy 2001).

In Peru, infection due to *Trichodina* sp. has been reported to cause problems in rearing the fry of *Oreochromis* spp. (Gonzales-Fernández 2012), *Osteoglossum bicirrhosum* (Vázquez et al. 2007), *Piaractus brachypomus* (Alcántara-Bocanegra et al. 2015), and *Arapaima gigas* (Delgado et al. 2013; Serrano-Martínez et al. 2015). However, *Trichodina fariai* (Delgado et al. 2007) and *Trichodina heterodentata* (Miranda et al. 2012) are the species that are known to parasitize *A. gigas* (Table 1).

In Argentina, although several native species (*Odontesthes bonariensis*, *Odontesthes hatcheri*, *Leporinus obtusidens*, *P. mesopotamicus*, *Rhamdia quelen*, *P. corruscans* and *P. lineatus*) and non-native species (*Salminus brasiliensis, Salmo salar*, *Oncorhynchus mykiss*, *Ctenopharyngodon idella*, *C. carpio, Oreochromis mossambicus* and *Acipenser baerii*) are farmed, there are no studies on infection due to trichodinids. However, such studies have been conducted on populations of wild fish, and the occurrence of *Trichodina* sp. in *R. quelen*, *Astyanax* spp. and *O. bonariensis* has been recorded (Vanotti and Tanzola 2005; Tanzola et al. 2009), while *Trichodina puytoraci* Lom, 1962, *Trichodina lepsii* Lom, 1962, and *Trichodina scalensis* Marcotegui; Martorelli, 2009 have been reported in *Mugil platanus* and *Trichodina murmanica* Poljansky, 1955 in *Micropogonias furnieri* in marine environments (Marcotegui and Martorelli 2009).

In Chile, in freshwater salmonids (*Oncorhynchus kisutch*, *O. tshawytscha* and *O. mykiss*) farmed in net-cages, trichodinids have only been described as *Trichodina* sp. (Wood 1970; Bravo 2007). However, *Trichodina lascrucensis* is known and has been described as a parasite of the marine fish *Scartichthys viridis* (Khan et al. 2008).

In Colombia, only *Trichodina* sp. has been recorded, parasitizing farmed fish such as *P. brachypomus* (Verján et al. 2001; Carrillo et al. 2007; Pulido and Iregui 2008), *C. macropomum* (Pulido and Iregui 2008/2009), and its hybrids, *Prochilodus magdalenae* (Calderon et al. 2003) and *Oreochromis* spp. (Carrillo et al. 2007). Although *C. carpio*, *Oncorhynchus mykiss, Pseudoplatystoma fasciatum* and *Brycon moorei* (Sanabria 2012) are also farmed in Colombia, infection by trichodinids has not been investigated in these fish.

In Uruguay, infection due to *Trichodina* sp. has been found to occur in *Mugil platanus*, *O. bonariensis*, *R. quelen*, *C. carpio*, *C. idella*, *Acipenser baerii*, *Acipenser gueldenstaedtii*, *O. niloticus* (Carnevia et al. 2010), *Xiphophorus helleri* (Carnevia and Speranza 2003a), *Carassius auratus* and 19 other species of farmed ornamental fish (Carnevia and Speranza 2003b).

Among fish farmed in Venezuela, infection due to *Trichodina* sp. has been studied in *C. macropomum* and its hybrid (♀ *C. macropomum* × ♂ *P. brachypomus*) (Mujica and Armas 1985; Centeno et al. 2004). However, parasitism due to trichodinids has not been investigated in other farmed species, such as *O. mykiss*, hybrids of *Oreochromis mossambicus* × *O. urolepis* × *O. niloticus* × *O. aureus*, *C. macropomum*, *P. brachypomus*, *Mylossoma duriventre*, *Phractocephalus hemioliopterus*, *Astronotus ocellatus, Prochilodus mariae*, *Cichla orinocensis*, *P. fasciatum* or *Leiarius marmoratus*. Van As and Basson (1989) reported the occurrence of *T. heterodentata* in *O. mossambicus* in Lake Valência.

In Paraguay, studies of infection due to *Trichodina* sp. have been conducted only in fish of commercial interest in Lake Yparacaraí (Insaurralde and Romero 2013). No studies on the occurrence of trichodinids in farmed fish in Ecuador, Paraguay, French Guiana or Guyana were found. In all countries cited, the species of trichodinids in farmed fish are still unknown.

Host specificity

Trichodinids have become specialized for various niches in hosts. Larger species have a broader

spectrum of preferences among the microenvironments of their hosts, and smaller species generally prefer the gills (Van As and Basson 1987). To formulate these hypotheses, Van As and Basson (1987) studied more than 100 species of fish in 70 localities in South Africa, Taiwan and Israel, from which they classified trichodinids into four groups of species. One group comprised *T. acuta* and *T. pedicula*, which both have wide geographical distribution, are opportunistic and are located only on the skin and never on the gills. The second group comprised *T. heterodentata*, *T. minuta*, *T. nigra* and *T. reticulata*, which also had a wide geographic distribution and are predominantly parasites of the skin but also infest the gills of fish. The third comprised *T. centrostrigata* and *T. mutabilis,* which predominantly parasitize the gills of fish and occasionally the skin. *Trichodina centrostrigata* (Fig. 2) was associated with species of cichlids, but when this parasite was present in fish farms, it infected and caused the death of cyprinids that inhabited the same production system (Van As and Basson 1987). Thus, *T. centrostrigata* was pathogenic to species other than cichlids and presented host specificity in addition to preference for a given micro-habitat. Marchiori and Martins (2012) stated that this host specificity varied according to the environmental quality and fish species present. However, *T. mutabilis* was found to be a parasite of cyprinids that might occasionally parasitize some species of cichlids if they were farmed together (Van As and Basson 1987). The last of these four groups of trichodinids comprised ten species, of which two were in the genus *Trichodinella* and eight were in the genus *Tripartiella*. All species were essentially parasites of the gills and presented a degree of host specificity. The exception was *Trichodinella epizootica*, which may comprise a pool of species, since it presented a variety of hosts (Van As and Basson 1987). In Brazil, Valladão et al. (2016a) indicated that *T. heterodentata* predominated on the body surface of *O. niloticus* and *P. africana* on the gills of larvae and juveniles of this species, among another four species that were described as cohabiting on this host.

Lastly, the data on relationships between trichodinids and their hosts are generally very inconclusive, given that in many Brazilian studies, all species are placed in the genus *Trichodina*. Similar problems were described by Van As and Basson (1987). Current methods to identify trichodinid species in fish in Brazil are better than those in the older studies included here.

Pathology of trichodinids

The pathology of trichodinid infection and the occurrence of host mortality events depend on factors such as the intensity of trichodinids, infection site, host age, pathogenicity of the etiological agent, and relationship to other infections or infestations by other organisms, in addition to the environmental conditions that can stimulate the infection and immunological conditions of the host.

Seasonality has an influence on the occurrence of trichodinids in fish in countries with temperate and cold climates because spring and winter are the seasons that are more favorable for multiplication of these ciliates (Hossain et al. 2008; Abdel-Baki et al. 2011; Yemmen et al. 2011; Özer et al. 2015). In Brazil, a large country, there is no pattern of seasonal occurrences given the regional differences in temperature and rainfall levels. In the northeastern region of the state of São Paulo, the prevalence of *Trichodina* sp. was found to increase in the spring and summer due to the increasing temperature and consequent stimulation of parasite reproduction and not because of the seasonality of rainfall levels (Schalch and Moraes 2005). In contrast, similar studies on fish in the same region found higher rates of parasitism in the winter (Tavares-Dias et al. 2001; Valladão et al. 2013). In the southern region of the country, Martins et al. (2010a) found *Trichodina* sp. in *O. niloticus* only in winter and spring, and the intensity and prevalence of parasites did not show any correlation to the seasonality of rainfall or water temperature. In the Amazon region, which has the climate of tropical forest, the dry and rainy seasons have a strong influence on the dynamics of parasite communities. Thus, Neves et al. (2013) reported that in *A. ocellatus* in Lake Pracuúba, the prevalence and intensity of *Trichodina* sp. was greater during the rainy season. However, the occurrence of trichodinids and the outbreak of diseases has a close relationship to the physiological and immunological conditions of the host, the management used, water quality conditions and the ecology of the specific species of parasite. These factors may provide conditions that favor multiplication of parasites to pathogenic levels (Ceccarelli et al. 1990; Palm and

Dobberstein 1999; Garcia et al. 2009; Martins et al. 2010a, b; Valladão et al. 2014; Valladão et al. 2016a).

A water temperature of 29 °C stimulated growth of *Trichodina puytoraci*, while a temperature of 18 °C inhibited growth. These antagonistic effects influenced the prevalence and intensity of infection by this trichodinid on *Mugil cephalus* from Tunisia (Yemmen et al. 2011). During the winter, higher rates of infection due to *Trichodina* spp. in *Platichthys flesus* were correlated with increased bacterial biomass, which in turn provided food for these parasites and supported their multiplication (Palm and Dobberstein 1999). Reduction of dissolved oxygen levels and addition of organic fertilizer favored reproduction of *Trichodina* sp. (Garcia et al. 2009), demonstrating that eutrophication levels, which can be measured from the degree of transparency of the water, may influence the levels of parasitism of these parasites.

In the coastal ecosystem of the Black Sea in Turkey, primary production peaks were a factor for the increased prevalence of *Trichodina* spp. in *Merlangius merlangus*. Moreover, nitrate, nitrite and phosphorus levels were shown to have synergistic effects on ciliate density (Ogut and Palm 2005). The growth of algae and bacteria in organically polluted (eutrophized) environments stimulated planktonic growth because of the availability of food, and these conditions stimulated the growth of parasites on the surface of their hosts (Ogut and Palm 2005). This study aimed to validate trichodinids as bioindicators, and thus, the field results can be extrapolated to fish farming.

In relation to the level of infestation, mild to moderate infestation by trichodinids may be asymptomatic and not cause macroscopic alterations (Valladão et al. 2014). In other cases, mortality and clinical signs may be observed; however, these signs may be nonspecific and common to other diseases (Jerônimo et al. 2011; Garcia et al. 2013; Valladão et al. 2013, 2014). Clinical signs among fish, such as swimming on the water surface, slowness, anorexia, darkening or alteration of skin pigmentation to bluish-gray due to excessive mucus production, and peeling of the epithelium can be observed (Basson and Van As 2006; Abd El-Galil and Aboelhadidb 2012; Valladão et al. 2013). Thus, *Schizothorax niger*, with its skin and gills parasitized by *T. heterodentata*, showed lethargy and body pallor, along with excessive mucus secretion and hemorrhagic areas on the body (Dar et al. 2016). In *P. lineatus*, erratic swimming was correlated with impaired vision due to corneal lesions caused by *T. heterodentata* (Valladão et al. 2014). In *O. niloticus*, macroscopic signs of parasitism due to *Paratrichodina africana* consisted of pallid gills with multifocal whitish areas suggesting necrosis and the presence of a substance with a milky appearance (Valladão et al. 2013). These events may have been associated with secondary bacterial infection due to the abrasive action of the ciliate that favored penetration and multiplication of bacteria (Valladão et al. 2013).

In farmed cyprinid species (Figueira and Ceccarelli 1991), as well as *Astronotus ocellatus* and *Symphysodon discus* (Mohammadi et al. 2012), concurrent infections by monogeneans, *I. multifillis* and *Trichodina* sp. and by *Argulus* sp., monogeneans and *Trichodina* sp. (Tomec et al. 1995) were reported. Concurrent infection of trichodinids and monogeneans is common in farmed fish, where they may act with synergistic deleterious effects (Colorni and Diamant 2005). In *Arapaima gigas*, association of infection by *Trichodina* sp. (Fig. 2) and monogeneas *Dawestrema* sp. was responsible for mortality of 37% in a batch of fry (Araújo et al. 2009a). The relationship between trichodinids and other infections or infestations by other organisms has also been demonstrated, showing that coinfection by protozoans and bacteria increases the mortality rate among farmed fish. Infection by parasites increases the capacity for invasion by bacteria, may make hosts more susceptible to bacteriosis and may compromise humoral stimulation (Martins et al. 2011; Xu et al. 2012; Valladão et al. 2014). Hyperparasitism by trichodinid ciliates on the monogeneans *Diplectanum aequans* in the gills of *Dicentrarchus labrax* from southern Israel was also reported (Colorni and Diamant 2005).

A correlational study between diseases caused by parasites and host age was conducted on larvae and juveniles of *O. niloticus* in three fish farms in São Paulo and Minas Gerais (Valladão et al. 2016a). Among the larvae, low to moderate mean intensities of infection (31.8 ± 19.07 parasites/fish and 197.67 ± 202.24 parasites/fish, respectively) were observed, compared to high infection among the juveniles (1132.60 ± 949.71 parasites/fish), which presented a parasite intensity tenfold greater than that of the larvae. However, only the larvae presented lesions on the fins and eyes, which were characterized as areas of parasite suction, skin peeling and

ulceration. The juveniles rarely presented lesions, and these primarily appeared in areas without scales, such as the head (Valladão et al. 2016a). Mortality among fish larvae was also reported by Eisa et al. (1985) and Valladão et al. (2014). In younger fish and those that had become debilitated by environmental factors, for example, the natural protection of the body surface is impaired and trichodinids find it easier to proliferate (Basson and Van As 2006). The high parasitism among juveniles (but with rare lesions) may be related to the influence of host age. However, the harmful effects on larvae, even when parasitism levels are low, are due to the absence of completely formed scales, which function as the first defense barrier against the entry of parasites.

Regarding the extent of damage caused by trichodinids, Basson and Van As (2006) stated that the different attachment structures and feeding habits of these parasites may give rise to different lesions. Recent studies have confirmed that the pathogenicity of these parasites varies between species and site of infection. Among the larvae of *O. niloticus*, lesions of different sizes and intensities of damage were found because of the presence of more than one species of trichodinid was involved in the infection (Valladão et al. 2016a). Although little is known about the genus *Paratrichodina*, Valladão et al. (2013) stated that pathological alterations caused by *P. africana* in *O. niloticus* showed that this species has significant pathogenic potential in intensive farming systems in net-cages.

Histopathological alterations may indicate that host exposure to trichodinids is either acute or chronic. Chronic alterations to the gills are characterized by hypertrophy and hyperplasia of epithelial cells, partial or total fusion of the secondary lamellae, hyperplasia of mucosal cells, inflammatory infiltration of eosinophils and mononuclear cells, interstitial hemorrhage, congestion and necrosis (Valladão et al. 2013; Dar et al. 2016). However, lesser alterations such as subepithelial edema and mild inflammatory reactions were recorded in acute infestations due to *T. heterodentata*, which caused mortality among *P. lineatus* larvae (Valladão et al. 2014).

Methodologies for identification and quantitative and qualitative evaluation

Taxonomic identification of trichodinids is achieved through a set of characteristics, including the skeletal, nuclear and cilia structures of the parasites, after subjecting the material to specific preparations and stains. Within the Trichodinidae, differentiation between genera is achieved using the morphological characteristics of the denticles of the adhesive disk and the adoral spiral ciliature, which may comprise spirals of cilia with complete turns, more than or less than 360°, as shown in Table 2 (Basson and Van As 1989).

A variety of characteristics have been evaluated to identify species of trichodinids. However, with time and in accordance with more recent studies, the structure of the adhesive disk and the morphology of the denticles have become the reference structures (Gaze and Wootten 1998; Basson and Van As 2006). According to Van As and Basson (1987), the revolution in the taxonomy of the trichodinids occurred in 1958, when Lom proposed using impregnation with silver, adapted from Klein's previously established method.

The ring of denticles that forms the adhesive disk is the only example of a support system in single-cell

Table 2 Main characteristics that differentiate the genera within the family Trichodinidae, for the species that occur in freshwater and saltwater fish, in accordance with Basson and Van As (1989)

Genders	General morphological features	
	Adoral ciliary spiral	Denticles in the adhesive disk
Trichodina	Lengths ranging from 360°–540°	Denticles consisting of blades, central parts and rays
Paratrichodina	Arch makes a turn of 150°–280°	Well-developed rays and wedged only by central part
Tripartiella	Arch makes a turn of 180°–290°	Denticles with a delicate central part
Trichodinella	Arch of 180°–270°	Denticles with a delicate central part; ray forms a delicate hook curved along central part
Dipartiella	Arch of about 270°	Consisting only of blades and weakly developed central parts

organisms that is analogous to the spinal column. It is responsible for lesions in the host and functions as a suction structure (Basson and Van As 2006). These structures are evaluated after sample preparation using silver nitrate ($AgNO_3$) by means of Klein's technique (Klein 1958; Lom and Dykova 1992). Smears that have been dried at room temperature are impregnated with an aqueous solution of 2% silver nitrate for 8 min, followed by exposure to ultraviolet light for 1–2 h (Eiras et al. 2006).

The following characteristics of the trichodinid parasites are evaluated: body diameter, diameter of the adhesive disk, width of the membrane border, diameter of the denticle ring, number of denticles, number of radial pins per denticle, denticle length, lamina length, width of the central portion, radial length and palm length. The denticles are solid and composed of three regions: the distal lamina, central part and proximal radius (Arthur and Margolis 1984; Basson and Van As 2006).

Body diameter measurement comprises the diameter of the adhesive disk plus the thickness of the membrane border (Basson and Van As 1989). However, this measurement should be made cautiously, as there is a certain degree of variability and deformation of the structure of the adhesive disk (Van As and Basson 1989). Gaze and Wootten (1998) reviewed the species of *Trichodina* among freshwater fish in England and warned about errors in the measured values of the adhesive disk structures. Moreover, since skeletal growth is a means of determining the age of trichodinids, a given population will include individuals with half the number of skeletal structures. Thus, these authors recommend that these should be excluded from species evaluations.

Measurements of parasites are generally made by means of images obtained under an ordinary optical microscope. It is recommended that schematic drawings of denticles are presented in each published study. Electron microscopy may aid in identifying and making finer morphological descriptions of the structures of trichodinids (Basson and Van As 2006).

The characteristics of the nuclear apparatus are evaluated using mucus smears or gills containing the parasites that have previously been fixed in methanol, using Giemsa solution (one drop of Giemsa for 1 mL of distilled water, with exposure for 2–3 h) (Ghiraldelli et al. 2006; Jerônimo et al. 2012; Pádua et al. 2012b), or by means of smears stained with Gömöri's trichrome (Miranda et al. 2012). The nuclear apparatus consists of a macronucleus that usually has an ellipsoid or horse-saddle format and a micronucleus that is only visible in some species (Basson and Van As 2006). The macronucleus shape, external diameter, thickness and length of sections between the ends are measured (Van As and Basson 1989).

The intensity of parasitism is determined from the number of parasites in each infected host (Bush et al. 1997). The methods for quantifying trichodinids are based on direct or indirect counting. In direct evaluations, the total number of parasites is counted, either from mucus smears from the body surface of each host, prepared on glass slides (Jerônimo et al. 2012; Zago et al. 2014; Ikefuti et al. 2015; Özer et al. 2015) or the number on one side of the host's body (Madsen et al. 2000). To analyze the gills, the arches are collected and placed between a slide and cover slip (Jerônimo et al. 2012; Ikefuti et al. 2015). The fresh material can be analyzed under an ordinary optical microscope after drying at room temperature (Valladão et al. 2013; Valladão et al. 2016a) or after fixing in 70% alcohol (Zago et al. 2014). In fresh preparations, the high mobility of the parasites, the cilia and the bell or circular shape can be observed, depending on the position of the parasite (Noga 2010; Özer et al. 2015).

Another method of quantifying trichodinids is to directly use the total count of parasites present on the body surface of the hosts, as recommended by Valladão et al. (2016a) for the larvae of *O. niloticus*, and by Fernandes et al. (2011) for tadpoles of *Rhinella pombali*. However, to count trichodinids on the body surface of larger fish, a standard sampling area must be defined, or a single gill arch must be selected for analysis (Sommerville et al. 2016).

Parasites can be quantified indirectly using a Sedgwick-Rafter or MacMaster chamber or by means of a hemocytometer from mucus or gill samples conserved in formalin (Fernandes et al. 2011; Jerônimo et al. 2011; Basson et al. 1983). Each fish can also be placed in an individual flask with an appropriate amount of water and shaken to detach the trichodinids (Sommerville et al. 2016). In this method, the sample should be homogenized to remove aliquots of 1.0 mL for counting in a Sedgewick-Rafter chamber, which is the method that is currently used most often. After the total volume of stored solution has been determined with a measuring beaker, the total

number of parasites in the sample is calculated (Fernandes et al. 2011; Jerônimo et al. 2011). It is recommended that several subsamples be counted (at least three) to provide a more appropriate estimated value (Sommerville et al. 2016). In counting the three aliquots in a Sedgwick-Rafter chamber, the volume is 3.0 mL. Thus, the average from the number of trichodinids in the three aliquots should be obtained by multiplying by the total number of parasites quantified.

Another method was proposed by Garcia et al. (2009), in which slides prepared from scrapings of mucus are examined and the number of parasites found per field at a magnification of 10× under an ordinary optical microscope is estimated from zero to five parasites (scores). Indirect quantification can also be performed by counting the mean number of parasites in five microscope fields of fresh samples (Abd El-Galil and Aboelhadidb 2012) or determined according to categories and levels of infection, as suggested by Madsen et al. (2000): 0 parasites, category 0; 1–10 parasites, category 1; 11–100 parasites, category 2; 100–1000 parasites, category 3; and 1000–10,000 parasites, category 4; or as suggested by Rach et al. (2000): 0 (parasite does not exist); 1–10 (low); 11–20 (moderate); ≥21 (high).

For studies of the efficacy of medications, it has been recommended that quantification methods should be predetermined and followed rigorously during the trial. In addition, especially in these cases, counts should be made by a single trained handler. In in vivo tests, when the samples are not fixed and counted immediately, quantified trichodinids should be classified as alive, moribund or dead (Sommerville et al. 2016).

Another method for identifying trichodinids is histopathological analysis using ordinary optical microscopy and electron microscopy, which also contributes to greater comprehension of the aggression caused by trichodinids at different attachment sites and of host responses (Palm and Dobberstein 1999; Valladão et al. 2014; Özer et al. 2015). In histological sections, depending on the orientation of the sections through the parasites, trichodinids may appear hemispherical, bag-shaped or flattened-cylindrical. The macronucleus may be visible as a horseshoe shape, along with some elements of the adhesive disk and cilia (Bruno et al. 2006).

One problem in quantifying trichodinids is the method used for sacrificing fish prior to sample collection. This involves the use of an overdose of anesthetic and should be evaluated or tested beforehand to ascertain whether the anesthetic or solvent used might destroy parasites in the host (Sommerville et al. 2016).

Due to the difficulty of differentiating similar trichodinid species solely based on morphological data, molecular identification methods are necessary for identification (Tang et al. 2017). Gong et al. (2006) performed the first investigation on phylogenetic relationships using small subunit ribosomal RNA (SSU-rDNA) sequences for Trichodinidae species using *T. nobilis*, *T. heterodentata*, *T. reticulata* and *Trichodinella myakkae*, showing that *Trichodinella* were nested within the *Trichodina*. Furthermore, *T. reticulata*, a *Trichodina* species with granules in the center of the adhesive disc, branched separately from its congeners *T. nobilis* and *T. heterodentata*, which are trichodinids without such granules (Gong et al. 2006). Utz and Eizirik (2007) and Zhan et al. (2009) questioned the results of Gong et al. (2006) in *Trichodinella*. Recent studies using SSU-rDNA for *Trichodinella* sp. and *Trichodina pectenis* reported that *Trichodinella* is nested within *Trichodina* (Zhan et al. 2013). However, according to Tang et al. (2013) the genus *Trichodina* is paraphyletic when species of *Trichodinella* are included in the analyses. Very recently, Tang et al. (2017) sequenced, for the first time, the SSU rDNA of a new species of trichodinid: *Trichodina pseudoheterodentata* of the channel catfish *Ictalurus punctatus*. Phylogenetic analysis revealed that the genetic distances among the new species and similar species reached interspecific levels, validating the identification of this new species and its placement in the genus *Trichodina*. There are limited molecular data for trichodinids because many species have not been sequenced, and the effectiveness of DNA-based identification of species relies on the availability of sequences in public databases for comparison (Zhan et al. 2013; Tang et al. 2017); therefore, there is not yet a consensus about the relationships based on molecular phylogenetics.

Methods for prevention and control of trichodinids

Trichodinids have the capacity for rapid multiplication. Diagnosis must be made quickly so that appropriate management can be instituted, or, as a second

course of action, emergency treatment can be administered before these parasites can cause high mortality (Basson and Van As 2006). Since outbreaks of infection due to trichodinids indicate environmental imbalance and the incapacity of the host to respond, treatment alone is insufficient to solve the problem. Improvements to management and water quality conditions, along with use of preventive measures, are of prime importance. Reduction of the concentration of organic matter is an effective preventive method against these ciliates (Basson and Van As 2006; Buchmann 2013).

Another preventive strategy is to supply feed with vitamin supplementation (consisting of 300–500 mg of vitamin C/kg of dry feed) before handling the fish and before the annual cold period, as supplementation may contribute to increasing the immune response of fish against parasites (Martins et al. 2002). Among the larvae of *O. niloticus* that were given feed containing 300 mg of vitamin E/kg, there was also a reduction in the intensity of trichodinids (Cavichiolo et al. 2002).

Various products for combating trichodinids have already been evaluated, including salts such as copper sulfate and potassium permanganate; glacial acetic acid; coccidiostatic agents such as toltrazuril; anthelmintics such as bithionol; insecticides such as teflubenzuron; commercial disinfectants (Madsen et al. 2000); and aldehydes such as formol and sodium chloride (NaCl). The concentrations used and the exposure times are very different and depend on the sensitivity of the fish species, water quality, dose, length of exposure and product used (Table 3).

Copper sulfate is administered only by means of immersion and is actively absorbed throughout the exposure period. Increased salinity and diminished pH of the water and vehicle result in concentrations of greater toxicity, e.g., freshwater fish are more sensitive to copper sulfate than are saltwater fish (Harms 1996). This form of chemotherapy presents proven action against protozoa, since it was effective for achieving a 100% reduction of *Trichodina* spp. in freshwater trout (Balta et al. 2008) but not in the fry of the saltwater turbot *Colistium nudipinnis* (Diggles 2000).

Acetic acid is the main component of vinegar, but the concentrations used in aquaculture are based on commercial glacial acetic acid, which is indicated against ectoparasites (Harms 1996). Toltrazuril is a coccidiostatic compound with wide-ranging action against protozoa. Because it is commercially available in a water-soluble form and it has ready action against host cells, it was tested against the ectoparasites of fish (Mehlhorn et al. 1988). Bithionol is an anthelmintic that is directed towards trematodes and cestodes, but because of its good action on recirculation systems, it can be used on species of protozoa (Madsen et al. 2000). However, Madsen et al. (2000) emphasized that bithionol has a narrow safety margin and that the dynamics of its residues and metabolites in the musculature of fish exposed to it are unknown.

Potassium permanganate is an oxidizing agent that reacts with organic matter. Thus, if applied to fish-farm ponds, it reacts with bacteria, algae, organisms in sediments, suspended particles and fish. For this reason, it is used as a treatment for ectoparasitic and fungal diseases (Lay 1971). Teflubenzuron is an insecticide that inhibits the production of chitin and thus selectively controls arthropod and crustacean crop pests. For this reason, its toxicity towards aquatic invertebrates is high (Harms 1996). Therefore, because of its low toxicity towards vertebrates and because it acts on chitin, which is also present in the structure of the adhesive disk of trichodinids, it was tested against this ciliate (Ikefuti et al. 2015) (Table 3). In another study, hydrogen peroxide did not show any positive results against trichodinids (Rach et al. 2000).

Disinfectants such as formaldehyde are commercially available in the form of a 37–40% aqueous solution known as formalin. It has broad-spectrum action against ectoparasites (Burka et al. 1997; Fajer-Ávila et al. 2003) and a good cost-benefit relationship if correctly applied, but it may be toxic or compromise the integrity of the epithelium and production of mucus in exposed fish (Buchmann et al. 2004). Recently, Valladão et al. (2016a) demonstrated that lower concentrations of formalin (0.25 mL/L), which are commonly used on farmed tilapias, are sufficient to eliminate *Trichodina* spp. in larvae. Thus, high concentrations cannot be justified from health and environmental standpoints. Furthermore, in these baths, salts were combined with formalin to stimulate mucus production (Valladão et al. 2016a).

To reduce the use of chemotherapeutic agents within aquaculture, alternative methods such as the use of phytotherapeutic agents are being tested. Plants with bioactive properties form an important source of new biologically active compounds for treating fish diseases, especially parasitic diseases (Kumar et al.

Table 3 Chemotherapeutic agents used for controlling Trichodinidae in fish of zootechnical interest

Product	Species (size and weight[a])	Dosage (dose and length of exposure)	References
Salt (NaCl)	*Ctenopharyngodon idella*	0.7%—bath for 21–24 h	Willomitzer (1980)
	Hypophthalmichthys molitrix and Cirrhinus mrigala	30 mg/L—bath for 10 min	Singhla et al. (1986)
	Arapaima gigas (fry)	15 g/L—bath for 5 min	Guerra (2002)
	Oreochromis niloticus	3%—bath for 10 min	Vargas et al. (2003)
	Oncorhynchus mykiss, Salvelinus fontinalis, Salmo trutta fario (fry 1.0 ± 0.2 g and juveniles 40 ± 0.7 g)	20 g/L—bath for 20 min	Balta et al. (2008)
Formalin	*Ctenopharyngodon idella*	1:2500—bath for 60 s (mortality rate of 23% among fish)	Willomitzer (1980)
	Hypophthalmichthys molitrix and Cirrhinus mrigala	0.004–0.006 mg/L—bath for 10 min	Singhal et al. (1986)
	Oreochromis niloticus	250 ppm—bath for 35–40 min	Neguenga (1988)
	Astyanax bimaculatus	0.25 mL/20 L—bath for 72 h	Rocha et al. (1993)
	Anguilla anguilla (1–30 g; 70–250 g)	75 ppm—bath for 3 h	Madsen et al. (2000)
	Colistium nudipinnis (fry 4.1–8.9 cm)	200 ppm—bath for 30 min 200 ppm—bath for 1 h	Diggles (2000)
	Arapaima gigas (fry)	0.25 mL/L—bath for 20 min	Guerra (2002)
	Arapaima gigas	Eggs: 100 mg/L—bath for 30 min, 3 × day Fry: 50–100 ppm—bath for 1 h	Sebrae (2013)
	Oreochromis niloticus	250 ppm—bath for 60 min	Vargas et al. (2003)
	Oncorhynchus mykiss, Salvelinus fontinalis, Salmo trutta fario (fry 1.0 ± 0.2 g and juveniles 40 ± 0.7 g)	0.10–0.15 mL/L—bath for 60 min	Balta et al. (2008)
	Not specified	25–40 ppm—bath for indeterminate time	Abowei et al. (2011)
	Oncorhynchus mykiss	250 ppm—bath for 24 h	Khoshnood and Khoshnood (2014)
Formalin + salt (NaCl)	*Oreochromis niloticus*	0.5 mL/L + 1%—bath for 15 min	Valladão et al. (2016a)
Copper sulfate	*Oreochromis niloticus*	0.03–0.06 mg/L—bath for 48 h 0.3–0.09 mg/L—bath for 24 h	Abdel-Meguid (2001)
Potassium permanganate	*Ctenopharyngodon idella*	1:1.000 only dipping the fish into it	Willomitzer (1980)
Acetic acid	*Hypophthalmichthys molitrix and Cirrhinus mrigala*	0.001 mg/L—bath for 10 min	Singhal et al. (1986)
	Oncorhynchus mykiss, Salvelinus fontinalis, Salmo trutta fario (fry 1.0 ± 0.2 g and juveniles 40 ± 0.7 g)	10 mL/L—bath for 3 min	Balta et al. (2008)
Toltrazuril	Not specified	10 µg/ml—bath for 2–4 h, eliminated most parasites 50 µg/mL—bath for 20 min, for major infections	Mehlhorn et al. (1988)
	Piaractus mesopotamicus	3.0 mg/L—bath for 1 h, on 5 consecutive days	Carraschi et al. (2014)
Bithionol	*Anguilla anguilla* (1–30 g; 70–250 g)	0.1 ppm	Madsen et al. (2000)

Table 3 continued

Product	Species (size and weight[a])	Dosage (dose and length of exposure)	References
Teflubenzuron	*Piaractus mesopotamicus*	50 mg/L—bath for 2 h for 5 days between 24-h intervals	Ikefuti et al. (2015)
	Oreochromis niloticus	50 mg/L—bath for 1 h for 5 days between 24-h intervals	Ikefuti et al. (2015)
Compound based on peroxide	*Anguilla anguilla* (1–30 g; 70–250 g)	25 ppm (supplemented with another 20 ppm)	Madsen et al. (2000)
	Oreochromis niloticus	2 ppm—bath for 40 min 5 ppm—bath for 20 min	Marzouk et al. (2013)

1 ppm = 0.001 mL/L; 1% = 1 g/100 mL

[a] When stated

2012; Wu et al. 2011; Zhang et al. 2013). Moreover, phytotherapeutic agents appear to have fewer harmful effects on human health and the environment (Chu et al. 2010). Thus, extracts and essential oils are used, especially against *I. multifiliis* (Yao et al. 2011; Yi et al. 2012; Fu et al. 2014), but few studies have been directed towards trichodinids, and most studies were conducted in northeastern Africa.

Administration of *Allium sativum* (garlic) in the form of immersion baths controls infestations by trichodinids in various species of fish through its main active agent, allicin. Madsen et al. (2000) showed that 200 mg/L of crude or macerated extract of *A. sativum* in 24-h baths (25 °C) reduced the intensity of infection by *Trichodina jadranica* in the eel *Anguilla anguilla*. For *O. niloticus*, the lethal concentration of garlic oil extracted directly from the bulb was 61.86 ppt, which is many orders of magnitude lower than the effective dose. Thus, this phytotherapeutic agent can be used on fish with a good safety margin (Abd Abd El-Galil and Aboelhadidb 2012). Therefore, for larvae of *O. niloticus* that were cultivated in the laboratory, prevention of infection by *T. heterodentata*, *T. compacta* and *Gyrodactylus* can be achieved with 2.0, 2.5 and 3.0 ppt *A. sativum* in baths lasting up to 4 h (Abd Abd El-Galil and Aboelhadidb 2012). Baths containing 3 ppt *A. sativum* oil applied for 60 min and containing 300 mg/L crushed *A. sativum* applied for an indeterminate time period were also shown to be effective for larvae in an incubator and for fry in excavated ponds (Abd Abd El-Galil and Aboelhadidb 2012). Extracts of *A. sativum* and *Terminalia catappa* (almond tree) administered in the form of immersion baths, both at a concentration of 800 mg/L, eliminated 100% of *Trichodina* sp. in *O. niloticus* after 2 days of application (Chitmanat et al. 2005). However, reinfection occurred 14 days after treatment, and this was attributed to deterioration of water quality because the extracts used increased the concentration of organic matter (Chitmanat et al. 2005).

In addition to 8.0 g/kg of *A. sativum* extract, 4.5 g/kg of *Artemisia vulgaris* extract in feed given to *O. niloticus* exhibited an antiparasitic effect against *Trichodina* sp. but gave rise to mortality rates of 20 and 16%, respectively (Noor El Deen and Mohamed 2010). Use of *Camellia sinensis* extract (green tea) in therapeutic baths at a concentration of 0.05% for 15 min or 0.09% for 5 min reduced the number of parasites on the skin and fins of *O. niloticus* by 80 and 95%, respectively (Noor El Deen 2010). Bioactive compounds such as chelidonin, chelerythrine and sanguinarine, isolated from the plant *Chelidonium majus*, demonstrated 100% effectiveness for elimination of *Trichodina* sp. from *Parabramis pekinensis*. These compounds are thus more effective than formalin (Yao et al. 2011). Other phytotherapeutic agents such as ginger *Zingiber officinale* (Abo-Esa 2008) and the plant sheh el-baathran have also been evaluated (Aboud 2010).

Sodium chloride (salt) is a product with many functions within aquaculture. It acts against parasites of the gills and skin but also functions as an agent for reducing the effects of nitrite poisoning, stimulating mucus production and reducing stress due to routine handling (Francis-Floyd 1995; Valladão et al. 2016a). The effects of common salt on fish, and on parasites, are determined through concentration and duration of exposure. Fish such as the tilapia *O. niloticus*, *C. macropomum* and *P. mesopotamicus* are more resistant than species of Siluriformes. For this reason, a

quick test on a few fish should be performed before using salt to treat larger numbers of fish (Neguenga 1988; Francis-Floyd 1995). For farmed *O. niloticus*, Neguenga (1988) recommended that 25 g/L of salt should be used in 15-min baths every two months in the tank to prevent reinfestations due to *Trichodina* sp. Use of 40–100 g of salt/m^3 in three treatments at 1-day intervals was also recommended for controlling infestations due to *P. pillulare*, *Trichodina* sp., monogeneans, *Argulus* sp. and *Dolops* sp. (Martins et al. 2002) (Table 3). Since controlling trichodinids using sodium chloride is relatively easy, the use of other products should be evaluated cautiously (Garcia et al. 2013), if only because most have not been approved by the Brazilian Ministry of Agriculture, Fisheries and Supply (MAPA).

Currently, in Brazil, there are 29 veterinary products that have been approved for use in aquaculture, of which six are antibiotics (e.g., florfenicol and terramycin) and four are antiparasitic agents (e.g., diflubenzuron and trichlorfon). Two other products with active agents not clearly specified have an indication for *Trichodina* sp. (see in SINDAN 2016). Despite the small number of products that are legally available for aquaculture in Brazil, a great variety of chemical products for which the lethal and clinical concentrations, length of exposure, waiting period and environmental impact have not yet been defined are currently being used there (Valladão et al. 2015). Therefore, although different studies conducted in Brazil have demonstrated that some chemotherapeutic and phytotherapeutic agents present antiparasitic effectiveness, their use has not been approved through legislation, and use of these products is limited. These products should be used sparingly.

Conclusion

Advances in studies of the biodiversity and molecular identification of Trichodinidae will contribute to knowledge of the epidemiology of these ciliate protozoa in different fish-farming systems used in South America. Studies correlating trichodinid species in hosts with specific pathological alterations observed in fish must be expanded to classify the species that have the greatest pathogenic potential for fish farmed in South America. From this knowledge, targeted control and prevention protocols of greater efficiency must be established. Likewise, new studies should correlate the occurrence of these protozoa with fish-farming conditions (stress factors, water quality, seasonality, fish age, etc.) to elucidate the critical points in each intensive production system. In addition, studies of treatments against trichodinids for farmed species in South America should be conducted to support and encourage approval of medications for use in aquaculture in Latin America.

For the production sector in South America, the focus should be on production systems that are more sustainable, with biomass stocked at tolerable limits that indicate wellbeing among the farmed fish, along with adoption of prophylactic management at all stages of farming, which will contribute to diminishing the pressure of parasites on fish. Early diagnosis and preventive actions must be implemented, rather than curative treatment, particularly regarding production of tilapia fry, for which problems relating to trichodinids are more severe and transferred to fish at the fattening stage. Therefore, there is a need to adopt biosafety protocols within fish farming. These not only require adjustment of farming densities to appropriate levels but also adjustments to feeding rates and genetic improvement of native species to increase their production and productivity. Biosafety in South America is still at an initial stage, and there is still a need for more precise information regarding disease transmission caused by trichodinid species, along with eradication of these parasites from farmed fish.

Acknowledgements We thank Daniel Chaves Webber, analyst at Embrapa Fishery and Aquaculture, for drawing up the cartogram presented in this document; National Council for Research and Technological Development (CNPq, Brazil) for its technical support given to Dr. M. Tavares-Dias; and SEBRAE (Brazilian Micro and Small Business Support Service), CNPq (Amazon Pirarucu Project) and Embrapa (Project MP2-01/2012) for financial support.

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