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Trichodinid ciliates in two economically important fishes, *Rastrelliger kanagurta* (Perciformes: Scombridae) and *Oreochromis mossambicus* (Perciformes: Cichlidae) from Kerala, India.

Trichodinid ciliates in two economically important fishes, *Rastrelliger kanagurta* (Perciformes: Scombridae) and *Oreochromis mossambicus* (Perciformes: Cichlidae) from Kerala, India

Amrutha Shya Suresh[1], Balamurali Rakhavan Pillai Sreekumaran Nair[1]*, Arya Unni[1], Binumon Thankachan Mangalathettu[2]

[1]Postgraduate Department of Zoology and Research Centre, Mahatma Gandhi College, Thiruvananthapuram-695004, Kerala, India

[2]Department of Zoology, University of Kerala, Karyavattom, Thiruvananthapuram-695034, Kerala, India

* Author for Correspondence: drbala@mgcollegetvm.ac.in

ABSTRACT

Trichodinid ciliophorans are one of the main groups of fish parasites causing damage to skin and gills, often leading to the death of the infested fish and are still a poorly studied group in India. A study was undertaken to assess the infestation and distribution of trichodinids from the skin and gills of two economically important fishes in India, *Rastrelliger kanagurta* Cuvier, 1816 and *Oreochromis mossambicus* Peters, 1852. Dried slides were prepared from the smears and impregnated with silver nitrate (2%) and morphometric characteristics were made using photomicrographs produced from the slides. *O. mossambicus* was found positive for two species, viz., *Trichodina magna* Van As and Basson, 1989 and *Paratrichodina africana* Kazubski and El-Tantawy, 1986. *R. kanagurta* was found to be infested with *Paratrichodina* sp. Lom, 1963. Seasonal variations in the rate of parasite infestation were observed in *T. magna* and *P. africana* from *O. mossambicus* and these parasites showed an increase during post-monsoon while a decrease during monsoon. No seasonal variation was observed in

Paratrichodina infestation in *R. kanagurta*. The infection induced excessive mucus secretion, paleness in gills, and multifocal whitish areas and lesions that probably permitted entry of opportunistic bacteria. The present study reports the first record of a Paratrichodina sp. on *R. kanagurta* and *T. magna* on *O. mossambicus*.

Keywords: Trichodinids, *T. magna, P. africana*, *O. mossambicus, R. kanagurta*

RESUMEN

Los tricodinidos ciliophorans son uno de los principales grupos de parásitos de los peces que causan daños en la piel y las branquias, lo que a menudo conduce a la muerte de los peces infestados y siguen siendo un grupo poco estudiado en la India. Se realizó un estudio para evaluar la infestación y distribución de tricodínidos de la piel y branquias de dos peces económicamente importantes en la India, *Rastrelliger kanagurta* Cuvier, 1816 y *Oreochromis mossambicus* Peters, 1852. Se prepararon portaobjetos secos a partir de los frotis y se impregnaron con nitrato de plata (2%) y las características morfométricas se realizaron mediante microfotografías producidas a partir de los portaobjetos. *O. mossambicus* resultó positivo para dos especies, a saber, *Trichodina magna* Van As y Basson, 1989 y *Paratrichodina africana* Kazubski y El-Tantawy, 1986. Se encontró que *R. kanagurta* estaba infestada con *Paratrichodina* sp. Lom, 1963. Se observaron variaciones estacionales en la tasa de infestación de parásitos en *T. magna* y *P. africana* de *O. mossambicus* y estos parásitos mostraron un aumento durante el período posterior al monzón y una disminución durante el mismo. No se observó variación estacional en la infestación de *Paratrichodina* en *R. kanagurta*. La infección indujo una secreción excesiva de moco, palidez en las branquias y áreas y lesiones blanquecinas multifocales que probablemente permitieron la entrada de bacterias oportunistas. El presente estudio reporta el primer registro de una *Paratrichodina* sp. sobre *R. kanagurta* y *T. magna* sobre *O. mossambicus*.

Palabras clave: Trichodinids, *T. magna, P. africana, O. mossambicus, R. kanagurta*

INTRODUCTION

Trichodinids are among the most common fish ectoparasites in aquatic habitats (Basson and Van As 2006). More than 300 species of trichodinids have been recognized as parasites or symbionts of aquatic organisms (Maciel *et al*. 2018). These ciliates are

most frequent protozoan group invading the surface of fish and cause severe disease and mortality in various parts of the world (Valladao *et al*. 2014). They survive on and infect the external body surface like skin, fins and gills of both edible as well as ornamental fishes and are found in many zoogeographical localities (Lom and Dykova 1992). They are regarded as one of the major cause of fish mortality (Abdel-Meguid 2001).

Trichodinids have direct development on the host with both asexual and sexual reproduction. Thus, population levels can increase rapidly during crowding and poor environmental conditions for the fish hosts (Khan 2004). Parasites can then cover gill lamellae and cause gill irritation resulting in death or stunting of fishes. Proliferation of the parasite is induced by changes in the relationship among host, parasite, and environment caused by nutritional deficiency, poor water quality, and infectious or parasitic diseases, causing severe epidermal lesions and disease outbreaks (Martins *et al.* 2010). Correct identification depends mainly on the quality of the impregnation and staining techniques that are used to evaluate these characteristics. Nevertheless, some trichodinids become impregnated less than others do, thus making it difficult to describe the species. Difficulties in evaluating the results from impregnation are commonly encountered in relation to *Trichodinella* in which the correct picture is sometimes misread as artefacts because of the insignificant dimensions of the denticles, which can easily become damaged when the smear preparation dries out (Lom 1963).

Trichodinids are usually identified through the morphology of the denticles in the adhesive disc and the development of the adoral ciliary spiral, and the denticles have very high systematic value (Gong *et al*. 2005). Although some species have a wide variety of hosts and geographical distributions (Basson and Van As 1986), there are very few records of these small trichodinids in the literature.

Among the ten genera within the family Trichodinidae, four genera, i.e. *Dipartiella*, *Paratrichodina*, *Trichodina*, and *Trichodinella*, were found to occur on marine fishes (Xu *et al*. 2001). However, only about two-thirds of named trichodinids have been described following examination of silver-impregnated specimens. Trichodinid ciliophorans are still a poorly studied group (Xu *et al*. 2001). So far, studies regarding the identification of trichodinid ciliates have received little attention in India. A research in this area is essential due to the abundance of trichodinids and the great diversity of the Indian ichthyofaunal.

The tropical cichlid fish, Mozambique Tilapia (*Oreochromis mossambicus* Peters 1852) has reported to be infested with 23 species of parasites (Wilson *et al*. 2019).

Tilapia is has emerged as one of the most internationally traded food fishes in the world due to its potential and affordability (Laxmappa *et al*. 2015). The Indian mackerel (*Rastrelliger kanagurta* Cuvier 1816) constitutes an important species of commercial fisheries in countries bordered with the Indian Ocean, Indonesia, Pakistan, India, Sri Lanka, Bangladesh, Myanmar, Thailand (Jayabalan *et al*. 2014). The Indian mackerel constitutes a prominent group in the landings of both the Arabian Sea as well as in the Bay of Bengal (Goutham and Mohanraju 2015) and the available information deals with reports of parasite species in this fish belonging to Monogenea, Digenea, and Crustacea (Madhavi and Lakshmi (2011). An average annual catch in the country was estimated to be 0.16 million tonnes (CMFRI 2019). Considering the socio-economic importance of *O. mossambicus* and *R. kanagurta* in India, an attempt is made to identify trichodinid parasites in these fishes from Thiruvananthapuram, South India.

MATERIALS AND METHODS

Samples of Indian Mackerel (*Rastrelliger kanagurta)* were collected from the Vizhinjam coast (Longitude: E. 76° 59'15", Latitude: N. 8° 22' 30") and Mozambique tilapia (*Oreochromis mossambicus*) were collected from Veli Lake (Longitude: E. 76^0 52’ 30’’ to 76^0 53’ 30’’ Latitude: N. 08^0 30’ 08^0 31’) from October, 2017 to September, 2018 for parasitological analysis. The fish were collected from fishing harbours caught by local fishermen to investigate parasite infestation. Thus, the animals used in this study (fish/parasites) did not require ethical committee approval for the present work. Monthly collections of 15 fish each were done for pre-monsoon (February-May) and monsoon (June-September), and post-monsoon (October-January) seasons.

Each fish was examined thoroughly and carefully for the presence of parasites. The methods suggested by Kennedy (1979) were adopted for parasitological studies. Skin scrapings from different parts of the body were examined under the high power of Transmission Light Microscope (TLM) (Optika Microscope; Optikam B5 Digital Camera) for the possible presence of ectoparasites. Buccal cavity was also subjected to thorough microscopical examination. After completing the external examination, the operculum on either side was taken out and their inner sides were thoroughly examined. Gills from the blind and ocular sides were excised and transferred to separate petri dishes containing 0.65% saline.

Thin slides were prepared for microscopic ciliate protozoan parasites from the skin and gill scrapings of the fish. The slides were stained according to Klein's dry silver

impregnation method (Klein, 1958). The slides were air-dried, covered with a 2% aqueous solution of silver nitrate ($AgNO_3$), rinse the slides thoroughly in distilled water, exposed to direct sunlight and mount with DPX mountant. Examinations of preparations were made under TLM at 100X magnifications with an oil immersion lens. Photomicrographs were taken with a dedicated digital camera fitted on to TLM. All measurements are in micrometres (µm). The identification of collected parasites was made according to the key suggested by Lom (1958) and Van As and Basson (1989 and 1992).

The data collected on the nature of infestation of parasites for *O. mossambicus* and *R. kanagurta* were processed for finding out prevalence (percentage of fish infected in the sample) and mean intensity (mean number of parasites per infected fish in the sample) of infections in relation to month of collection (Margolis *et al*., 1982) using ANOVA.

RESULTS

The examined fishes showed trichodinid infestation on the body surface, fins and gills. Two species of trichodinids were identified from the host fish, *O. mossambicus*-*Trichodina magna* and *Paratrichodina africana*. The trichodinid, *Paratrichodina* sp. was identified from *R. kanagurta.* The skin of infested fish appeared to be coated with an opaque film of heavy mucus, and in some of the fishes, the skin was eroded, and open haemorrhagic lesions were apparent. Highly parasitized fish gill exhibited excessive mucus secretion, paleness, and multifocal whitish areas (Fig. 2A).

Trichodina magna Van As and Basson 1989

Description: (based on eight specimens): Large trichodinid with a flattened disc-shaped body, 50.5 µm (37-55) in diameter. The adhesive disc is concave, 38.7 µm (34-48) in diameter and surrounded by a finely striated border membrane of 4.7 µm (4-6) wide. The Centre of the adhesive disc is finely granular. The denticulate ring is 35.8 µm (25-40) in diameter. The denticles are inserted into one another and are 26 µm (25-28) in number. Denticle 9.2 µm (8-11) long. The blade is narrow, sickle-shaped and 4.6 µm (3-6) long. The distal surface of the blade is curved, and the tangent point is slightly lower than the distal surface. The posterior margin forms a deep semilunar curve, and the deepest point lies in the middle of this curve. The anterior margin of the blade is rounded with a slightly flattened apex at the same level as the deepest point of the posterior margin.

The central part is 2.54 µm (2-4) wide and robust. The point of the central part is rounded and lies in close association with the following denticle. The posterior projection and the indentation in the lower central part absent. The connection between the blade and the central part is delicate. Ray is thin, 7 µm (5-8) long, tapering slightly to a sharp, rounded point that directed anteriorly. Apophysis of the ray is prominent. The central part connection to the ray is thin. All these features are presented in Fig. 1A & B.

Daughter cells of *T. magna* were also observed. It is in the stage after binary fission and has 23 denticles, half of the adult ones (Fig. 1C).

Taxonomic summary

Host: *Oreochromis mossambicus* (Peters) (Perciformes: Cichlidae)
Locality: Veli Lake, Kerala ($8^0$51'04" N; $76^0$88'83" E.)
Infestation site: Skin and Gills

Nature of infestation: Intensity of infestation signifies the degree of survival of parasite on the host species. The prevalence of *T. magna* fluctuated according to the season (Table 1). An increase was observed during Post-monsoon (98) and showed a decrease during monsoon (73).

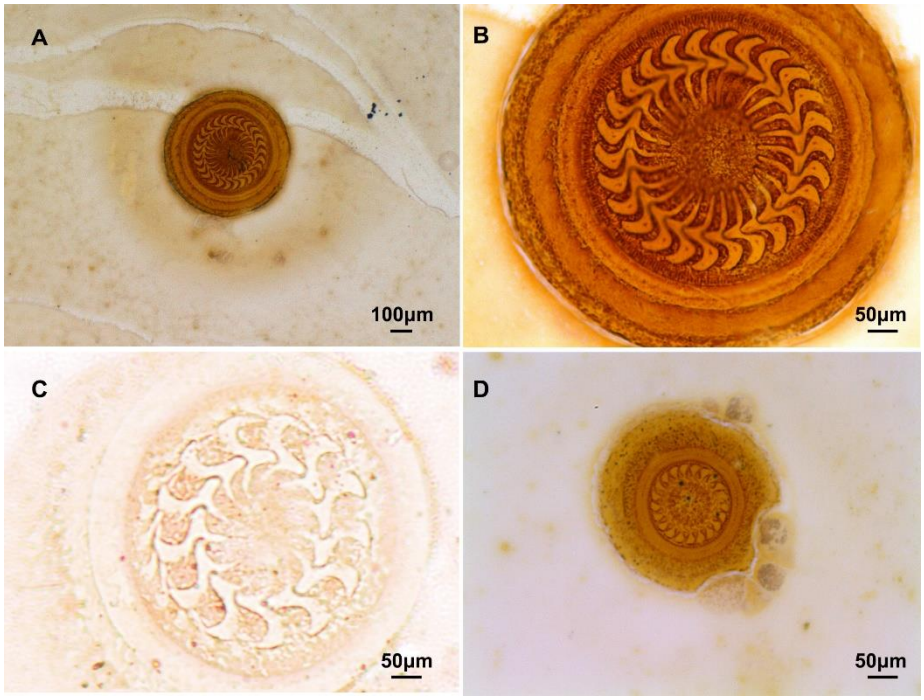

Fig. 1. Photomicrographs of silver nitrate impregnated adhesive discs of trichodinid ciliophorans from *O. mossambicus*. A&B. *T. magna* adult, X10 and X100 magnification respectively. B. *T. magna* immature stage (X100 Magnification). C. *Paratrichodina africana* (X100 magnification).

Table 1: Seasonal distribution of T. magna in O. mossambicus during the study period. Chi square = 0.204 (p>0.05).

Post-monsoon	October	15	15	100	
	November	15	14	93	98%
	December	15	15	100	
	January	15	15	100	
Pre-monsoon	February	15	14	93	
	March	15	14	93	92%
	April	15	13	87	
	May	15	14	93	
Monsoon	June	15	10	66	
	July	15	11	73	73%
	August	15	11	73	
	September	15	12	80	

Paratrichodina africana Kazubski and El-Tantawy 1986

Description (based on eight specimens): Small trichodinids with a bell-shaped body, 19 µm (18.9-24.1) in diameter. The adhesive disc, 15.71 µm (14.6-17.2) in diameter, surrounded by a finely striated border membrane with 1.2 µm (1.1-1.7) width. Denticular ring with diameter 12.03 µm (10.6-12.3). Denticles, 3.3 µm (3.1-3.3) long. Denticles have spatulate-shaped blades, 1.836 µm (1.9-2.4) length. The shape of the blade similar to an equilateral triangle, with the top directed towards the adhesive Centre. Posterior margin of blade slightly concave and anterior margin convex when seen from adhesive disc centre. The distal surface of the blade flat with a discrete curve, running parallel with the border membrane. The tangent point is rounded and situated below the distal point of the blade margin. Anterior margin makes a slightly angular turn to form an inconspicuous apex. Prominent blade apophysis present. Posterior margin of the blade with a moderately deep semilunar curve. The connection between the blade and central part elongated, slender, with a posterior projection. Posterior projection not visible.

The central part 0.53 µm (0.5-0.9) wide, delicate, triangular, and oblong-pointed. Centripetal rays 1.3 µm (1-1.8) length straight, narrow, finger-shaped and with the same thickness along their length. Tip of ray rounded. Rays are directed towards the geometric Centre of the adhesive disc. All these features are presented in Fig. 1D.

Taxonomic summary

Host: *Oreochromis mossambicus* (Peters) (Perciformes: Cichlidae)
Locality: Veli Lake, Kerala ($8^0$51'04" N; $76^0$88'83" E.)
Infestation site: Skin and Gills

Nature of infestation: Seasonal variations in the rate of parasite infestation were observed (Table 2). An increase in parasite prevalence was observed during Post-monsoon (73) and showed a decrease during monsoon (57).

Table 2: Seasonal distribution of P. africana in O. mossambicus during the study period. Chi square = 0.0304 (p>0.05).

Season	Months	No. of fish examined	No. of fish infected	Prevalence (%)	Average
Post-monsoon	October	15	12	80	
	November	15	10	66	73%
	December	15	10	66	
	January	15	12	80	
Pre-monsoon	February	15	11	73	
	March	15	10	66	70%
	April	15	11	73	
	May	15	10	66	
Monsoon	June	15	10	66	
	July	15	08	53	57%
	August	15	09	60	
	September	15	07	47	

Paratrichodina sp. Lom 1963

Description (based on eight specimens) (Fig. 2 B, C & D): Small trichodinids with a flat disc-shaped body having a diameter of 275.76µm with rows of cilia at the circular periphery. The border membrane is 19.37µm long. The adhesive disc concave and surrounded by a fine striated border membrane. A lightly stained central area of the adhesive disc present, which helps attach firmly to the fish's skin or gills. The adhesive disc 148.80µm in diameter. Denticular ring present with denticles. Denticular ring, 98.10µm in diameter. The denticle spans 31.18µm in width. Denticle blade has a length of 11.55µm, Central area and ray are visible. The central part is 37.92µm wide, and Ray is short, 9.49µm long. All these features are presented in Fig. 2D.

Taxonomic summary

Host: *Rastrelliger kanagurta* (Cuvier) (Perciformes: Scombridae)
Locality: Vizhinjam, Kerala ($8^0$39'32" N; $77^0$00'46" E.)
Infestation site: Skin and Gills

Nature of infestation: Seasonal variations in the rate of parasite infestation were observed (Table 3). An increase in parasite prevalence was observed during Post-monsoon (100%) and showed a decrease during monsoon (87%).

Trichodinid parasitized gills of fish showed increased mucus production, paleness in the gills, and multifocal whitish areas (Fig. 2A). The parasite is in permanent rotation while attached to a host, which has an irritating effect on the epithelial cells.

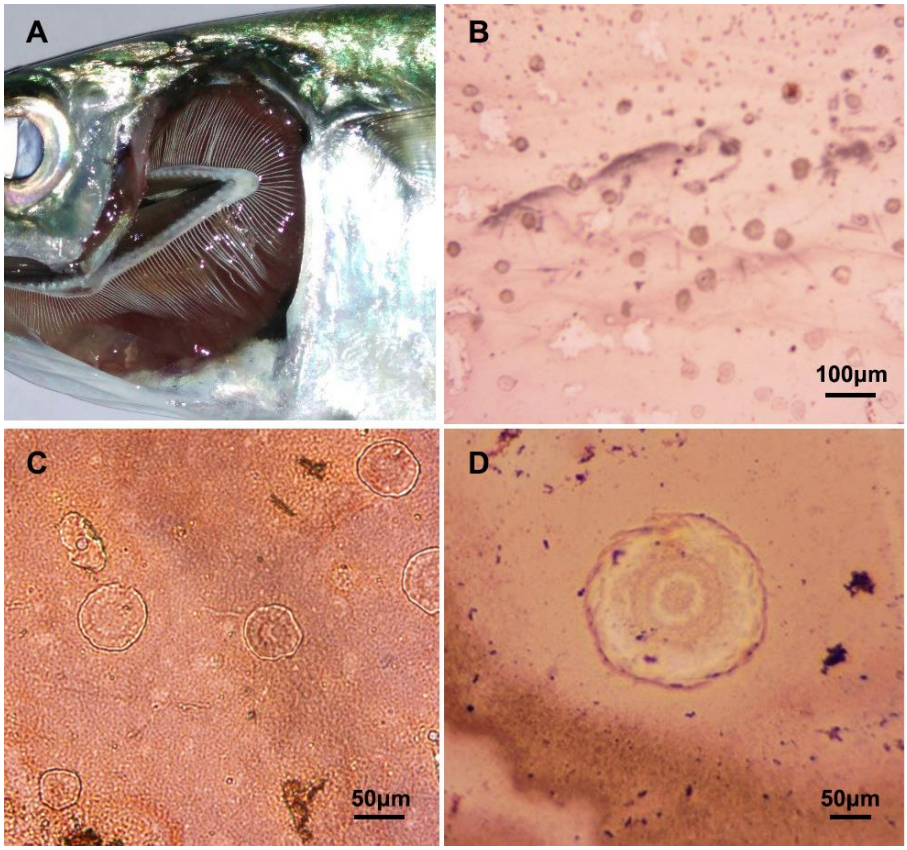

Fig. 2. Trichodinid infestation on *R. kanagurta*. A. Gills of fish parasitized by Trichoinids showing the whitish multifocal area, increased mucus production and paleness. B-D. Photomicrographs of silver nitrate impregnated adhesive discs of *Paratrichodina* sp. X4, X10 and X 100 respectively.

Table 3: Seasonal distribution of *Paratrichodina* sp. in *R. kanagurta* during the study period Chi square= 0.074 (P>0.05).

Season	Months	No. of fish examined	No. of fish infected	Prevalence (%)	Average
Post-monsoon	October	15	15	100	
	November	15	15	100	100%
	December	15	15	100	
	January	15	15	100	
Pre-monsoon	February	15	14	93	
	March	15	15	100	95%
	April	15	14	93	
	May	15	14	93	
Monsoon	June	15	14	93	
	July	15	13	87	87%
	August	15	12	80	
	September	15	13	87	

DISCUSSION

The study presented new records of *T. magna* in India. *T. magna* was firstly described by Basson *et al*. (1983) as *T. pedicuIus.* Van As and Basson (1989) classified these trichodinids into a new species, *T. magna.* The same authors also reported the parasite on the skin, fins and gills of *M. macrolepidotus, H. odoe, S. mystus, O. andersoni, S. angusticeps* and *T. rendalli* (Van As and Basson 1992).

T. magna resembles the species described by Martins and Ghiraldelli (2008) and Basson and Van As (1994). The species differs morphologically from Van As and Basson (1989), Van As and Basson (1992), Abdel Ghaffar *et al.* (1996), Koura *et al*. (1997), Ghoneim (1998), Ahmed *et al.* (2000), El-tantawy and El-Sherbiny (2010). A similar diameter of the adhesive disc and denticulate ring is reported by Arthur and Lom (1984). The specimens of *T. magna* differ from other species in having different shape and length of denticles and characteristics of blade and ray. Adhesive disc and denticulate ring of *T. heterodentata*, described by Duncan (1997), has a smaller number of denticles compared to the present specimens despite the similarity in the body diameter. In addition, *T. heterodentata* differs from the samples of *T. magna* reported from Santa Catarina in having considerable variation in the shape of the denticle. The description of

T. magna in the original description (Van As and Basson, 1989) is similar to the present specimens. Specimens from Santa Catarina have smaller macronucleus diameter when compared to the samples described by Van As and Basson (1989). The apophysis morphology of the prominent ray of the denticle had a similar structure, although Van As and Basson (1989) did not give its measurements. There is a variation in the length of the apophysis of the ray. Despite the lack of significant difference in morphology, these specimens must be considered members of *T. magna*.

The record of *P. africana* presented the second record from India. This species resembles the specimens reported by Mitra and Bandyopadhyay (2006) and Valladão *et al*. (2013) and differs from Tavares-Dias *et al*. (2013) and Kazubski El-Tantawy (1986). *P. africana* was first described in Nile tilapia by Kazubski and El-Tantawy (1986). Later, the species was reported in *O. mossambicus* in West Bengal, India by Mitra and Bandyopadhyay (2006) and in *O. niloticus* in Northern Brazil by Pantoja *et al*. (2012). *P. incisa,* the genus type, shows remarkable similarity with *P. africana* regarding some morphological features. However, it differs from the latter by presenting smaller and less rounded spine-like processes continuous with the central part. In addition, the distal surface of the blade of *P. incisa* is straight, whereas it tends to be rounded in *P. lizae. P. lizae* also presents biometrical data similar to the present species. However, Mitra and Bandyopadhyay (2006) reported that the species contain one to eight non-impregnable round particles in the central part of the adhesive disc, which are absent in *P. africana*. *P. africana* reported fall within the ranges for *P. africana* presented in the original description by Kazubski and El-Tantawy (1986) with respect to measures of body diameter, number of denticles, denticulate ring, size of the adoral spiral, and wide border membrane. The subsequent report provided by Mitra and Bandyopadhyay (2006) also presents similar measures of the denticulate ring, number of denticles, pins per denticle, and some dimensions of denticle components, but it differs in body diameter. The present material resembles in all respects *P. africana*.

The finding of trichodinid ciliates (*Paratrichodina* sp.) from *R. kanagurta* represents its first record from this fish in the world. The presence of two important characters, i.e., the straight blade in line with the radius of the adhesive disc and no incision at the base of the blade into which the central part of the neighbouring denticle could enter, helped them allocate this trichodinid ciliophoran to the genus *Paratrichodina*.

Trichodinids are a widely dispersed group of ectoparasites in freshwater, marine and euryhaline environments. Trichodinids were increased in intensity and cause excessive mucus secretion, paleness in gills and lesions. Epithelial erosion permits the

entry of opportunistic bacteria, which ultimately cause mortality. Suppressive effect on the defence systems and subsequent injury in fish by trichodinids has been reported previously (Lom 1964). Valladao *et al*. 2013 observed hyperplasia of mucous cells and mononuclear and eosinophilic inflammatory infiltrate beyond desquamation in the spaces between the gill filaments of trichodinid infested fish. Multifocal to coalescent areas of gill necrosis was observed in severely parasitized fish (Valladao *et al*. 2013). These parasites attack the fish and cause massive destruction of the skin and gill epithelium (Sterud *et al*. 2003). The skin of trichodinid parasitized fish showed a change in body colouration and excessive production of mucus. The clinical signs most commonly observed was a mottled/grey appearance on the skin (caused by the excessive production of the mucus) (Gomes *et al*. 2017). According to Lom and Dyková, 1992 heavy trichodinasis may cause loss of up to fifty per cent of the fish stocks and inhibits fish growth.

The seasonal analysis reveals that the trichodinids show higher prevalence. This higher prevalence of this species may be due to the selection of larger sized fishes for the study. In general, the severity of most parasitic infections increases with the age of the host fish, possibly due to the greater accumulation period and the larger space for feeding and breeding of the parasite (Ozer and Erdem 1998). Other factors like pollution and stress, which lowered the immune response of the host, may also result in the proliferation of the parasite (Xu *et al*. 2001). Intensities or prevalence of ciliates increased with oil pollution, pulp mill effluent (Khan *et al.* 1993), industrial effluent (Nilsen 1995) and thermal effluent.

Season dependent variation was noticed in the prevalence of *T. magna* and *P. africana* collected from *O. mossambicus* and *Paratrichodina* sp. isolated from *R. kanagurta*. The highest infestation with *Trichodina* sp. was found in post-monsoon, while the lowest infestation rate was in monsoon. Similar results were obtained by McArdle (1984) and El-Khatib (1989). They reported that, trichodiniasis was prevalent all over the year with maximum rate of infestation during spring and winter. The present findings agreed with Hossain *et al*. (2008).

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