Occurrence of two new species of the genus *Dipartiella* (Raabe, 1959) Stein 1961 (Ciliophora: Trichodinidae) isolated for the first time from ornamental fish

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Abstract During the parasitological survey of ornamental fish in India the authors identified two trichodinid ciliophorans for the first time in India, during the year 2014–16 and also revealed that goldfish, *Carassius auratus*, was the most susceptible species for trichodinid infection. Two ciliophorans have been isolated and identified on the basis of wet silver nitrate impregnation technique followed by taxonomic description, morphometric variability and comparative data with its closely related species. Considering all the important features including diameter, structure of denticles, rays and ciliatures the authors are of the opinion that the identified species are new to science. Hence, the name *Dipartiella indiana* n. sp. and *Dipartiella carassii* n. sp. are being proposed here. Till date, only two species of the genus have been described from the edible fish, but no attention has been paid to ornamental fish. Moreover, The present study confirms the first report of this genus from ornamental fish species and added two more species under the genus *Dipartiella* (Raabe, 1959) Stein 1961 along with new host, locality and prevalence rates.

Keywords *Dipartiella indiana* n. sp. · *Dipartiella carassii* n. sp. · First report · Goldfish *Carassius auratus* · India

Introduction

Ornamental fish culture plays a pivotal role in the fish industry in India, but this fish is very much susceptible to infection causing 80% mortality and decrease in growth rate of fish especially in the highly intensified systems as reported by Eissa (2002). Trichodinids are the most common ectoparasitic protozoas which can easily multiply by completing the life cycle within the host body (Hassan 1999). The first report of trichodinids from India date back to the early twentieth century. But the research arrived its peak in the late 20th century.

The work on trichodinids was restricted to freshwater fishes resulting in a good number of papers by various scientists throughout the world, most of which have so far been reported from West Bengal, India, (Mukherjee and Halder 1982; Mishra and Das 1993 Saha et al. 1995; Asmat 2002) but no records are found on ornamental fish in India. Till date only fourteen trichodinids have so far been reported from *Carassius auratus* from different parts of the world (Hu et al. 2005; Tang et al. 2005; Tao and Zhao 2006; Tang et al. 2007; Tang and Zhao 2007, 2010; Hu 2012; Saha and Bandyopadhyay 2016) excluding India.

An expedition in search of trichodinid ciliophorans in ornamental fish the author revealed for the first time in India, the occurrence of two new species of trichodinid parasites under the family Trichodinidae and genus *Dipartiella* Stein 1961. They are being named as *Dipartiella indiana* n. sp. and *Dipartiella carassii* n. sp.

Studies on *Dipartiella* (Raabe, 1959) Stein 1961, is very much important and relatively less so far, since only two species have so far been described throughout the world (Raabe 1959; Kazubski 1999; Mitra and Bandyopadhyay 2009). Genus *Dipartiella* has gone through a very complicated history. Raabe for the first time created a new

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genus *Dogielina* in 1959 to accommodate the species *D. simplex* obtained from the black goby. Stein (1961) revealed that the generic name, *Dogielina* was already assigned to another genera and proposed the new name, *Dipartiella* for the genus. Later on Lom and Dykova (1992) regarded the genus *Dipartiella* as a “possible mistaken interpretation of the genus *Trichodinella*”. However, after re-examing the original material described by Raabe, the genus *Dipartiella* was established by Kazubski in 1999 which was further confirmed and supported by Xu et al. (1999) and Mitra and Bandyopadhyay (2009) by isolating the same species from China and another new species *D. kazubski* from India respectively.

It is interesting to note that prior to our report, no record on *Dipartiella* infesting any kind of ornamental fish has been found throughout the world. The descriptions of the two species under study have been made, based on silver nitrate impregnation technique followed by the taxonomic history and prevalence along with new host and locality. Comparison of closely related species was also done before declaring the species as new to science.

Materials and methods

Sampling

During the period of March 2014–April 2016, around thirty fish farms, belonging to five districts of West Bengal, India, namely Nadia (23.4710°N, 88.5565°E), Hooghly (22.8963°N, 88.2461°E), Howrah (22.5958°N, 88.2636°E), South 24-parganas (22.1352°N, 88.4016°E) and North 24-parganas (22.6168°N, 88.4029°E) were surveyed and different fish specimens (namely, Goldfish, *Carassius auratus* L; Molly, *Poecilia spheonops*; Guppy, *Poecilia reticulata*) were collected and brought alive to the laboratory. All fish were kept in several aerated glass aquaria of 20 lit capacities at 26.6 ± 2.3 °C, pH 6.9 ± 0.1 with dissolved oxygen 6.0–7.8 mg/L at the Parasitology laboratory, University of Kalyani, Kalyani, West Bengal, India.

Isolation of pathogens

Length, weight, (4.38 ± 0.28 gm, $3-9 \pm 0.89$ cm) external symptoms and general health conditions of each fish were recorded immediately after transportation to Parasitology laboratory and fed once at 1% body weight daily with commercially available fish pellet.

Light microscopic study

The infected fishes were collected and examined in every month of the year. Gill, body, and tail fin smear were

prepared on grease free clean slides with the help of a drop of 0.5% NaCl solution and air-dried. Wet smears of gills were made fresh and screened for the presence of trichodinids parasites. The positive slides were impregnated with 2% silver nitrate (Klein 1958) for 8 min and washed in distilled water. The slide containings specimens were exposed to ultraviolet light for 20–25 min to study the details of the adhesive disc. Prepared slides were examined and photographed under Olympus CX 41 model (100 X) phase-contrast microscope. Numerous photomicrographs were taken in order to make comprehensive morphological analyses of the ciliates. 15–20 fresh specimens, were measured in micrometers (μm) with Olympus calibrated ocular micrometer, and the terminologies are according to Lom (1958) and presented as minimum and maximum values. The mean value and standard deviation are provided in parentheses. The span of the denticle were measured from the tip of the blade to the tip of the ray. Body diameter is measured as the adhesive disc plus border membrane. The description of denticle elements follow the guidelines of Van As and Basson (1989).

Statistical analysis

The prevalence of parasites were calculated according to Bush et al. (1997) and Yemmen et al. (2011).

Results and discussion

Two new species of trichodinid ciliate belonging to the genus *Dipartiella* (Raabe, 1959) Stein 1961 were identified from the gills of *Carassius auratus* for the first time in India. Descriptions are presented below.

Dipartiella indiana n. sp. (Fig. 1 and Table 1)

Body small, round, measures $30.5-39.1(34.8 \pm 3.6)$ μm in diameter and significantly wider than both the peristomial and adhesive discs. Adhesive disc appeared uniformly being $23.2-31.5(26.025 \pm 2.9)$ μm in diameter. Blade elongated with almost parallel margins and tapering to the portion of the anterior projection with a denticular ring measuring $11.7-14.1(12.85 \pm 1.1)$ μm . Blade slender and occupies most areas between y-axes (Fig. 1e). Tangent point is blunt with rectangular end (Fig. 1f). Distal margin of the blade runs parallel to the wide border membrane measuring $1.9-3.0(2.5 \pm 1.3)$ μm in width. The number of denticles are 21. Number of radial pins per denticle usually 3–6 (4.5 ± 1.2). Span of denticle $3.9-4.7(4.32 \pm 0.3)$. Length of denticle $3.4-4.9(4.13 \pm 0.6)$ μm . Ray absent. Central part reduced and without any projection.

Fig. 1 Photomicrographs of *Dipartiella indiana* n. sp. (a–d) silver nitrate impregnated adhesive discs and (e, f) diagrammatic drawings of the denticles of *Dipartiella indiana* n. sp. identified from *Carassius auratus* (L.). Scale Bar 10 μ m

Taxonomic summary

Host: *Carassius auratus* (L.)

Locality: Haringhata, Nadia, West Bengal, India (23.4710°N, 88.5565°E).

Location: Gills.

Prevalence: 07 out of 20 host fishes (35%).

Etymology: The specific epithet “indiana” is given after the name of the country India from where the parasite has been found.

Reference Material: Holotype, slide no. GF-DSI-7/2015, and paratype slides no. GF-DSI-13/2015, GF-DSI-14/2015, GF-DSI-18/2015, GF-DSI-1/2016, GF-DSI-7/2016, GF-DSI-9/2016 have been deposited in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Remarks: The overall morphology of the specimen is identified as *Dipartiella* sp. Comparative study of morphological and morphometric characteristics of the present specimen with the other closely related species of this genus showed that the present species has a slight affinity to *Dipartiella kazubski* described by Mitra and Bandyopadhyay (2009). But the present specimen differs from any other species belonging to this genus in all aspects in relation to body dimension, denticle morphology, denticle number, host and habitat preference. It has a much larger body than *D. simplex* and *D. kazubski*. The

denticles of the species show extreme variability in size and shape, almost sharply rectangular whereas the denticles of *D. simplex* are triangular (Xu et al. 1999) and the denticles of *D. kazubski* appeared bluntly rectangular (Mitra and Bandyopadhyay 2009). The distal blade margin in the species under study is extremely flat and truncated in comparison to others. Moreover, the specimen consist of a much larger body than others and particularly, the number of denticles are much less in number compaed to other species.

Furthermore, the host of all the previously described species differs clearly from the present species. Also, throughout the world, no *Dipartiella* infection has been noticed in the literature in the present host.

Therefore, taking into account all the differences with its closely related species the present species has been designated as a new species, hence it is new to science and the name *Dipartiella indiana* n. sp is being proposed in the present communication.

Dipartiella carassii n. sp. (Fig. 2 and Table 1)

A small trichodinid with disc-shaped body; 18.4–24.1(20.86 $\pm$ 2.1) μ m in diameter; Adhesive disc concave and 14.9–21.6 (18.23 $\pm$ 2.6) μ m in diameter, Border membrane slightly wide, but distinct with fine striations with 1.4–1.9 1.5–2.9 (2.16 $\pm$ 0.6) μ m wide. Span of denticle

Table 1 Morphometric comparison of *Dipartitella indiana* n. sp and *Dipartitella carassii* n. sp. obtained in the present study with other closely related species

Species	<i>Dipartitella indiana</i> n. sp.	<i>Dipartitella carassii</i> n. sp.	<i>Dipartitella kazubski</i>	<i>Dipartitella simplex</i>	<i>Dipartitella simplex</i>
Host	<i>Carassius auratus</i>	<i>Carassius auratus</i>	<i>Wallago attu</i>	<i>Lateolabrax japonicus</i>	<i>Gobius niger</i>
Locality	Haringhata, West Bengal, India	Haringhata, West Bengal, India	India	China	Baltic Sea
Site of infection	Gills	Gills	Gills	Gills	Gills
Reference	Present study	Present study	Mitra and Bandyopadhyay	Xu et al. (1999)	Kazubski (1999)
Diameter of Body	30.5–39.1 (34.8 ± 3.6)	18.4–24.1 (20.86 ± 2.1)	15.5–20.7 (18.2 ± 1.7)	25–34 (30.5 ± 2.5)	15–38 (24.4 ± 3.47)
Adhesive disc	23.2–31.5 (26.025 ± 2.9)	14.9–21.6 (18.23 ± 2.6)	14.0–19.2 (15.1 ± 1.1)	21–30 (26.3 ± 2.3)	15.5–22.0 (18.0 ± 1.51)
Dimension of body					
Denticular ring	11.7–14.1 (12.85 ± 1.1)	4.6–8.9 (6.45 ± 1.6)	9.0–11.0 (9.8 ± 0.6)	9–13 (11.0 ± 1.2)	4.4–8.8 (6.3 ± 0.84)
Central area	6.1–9.1 (7.06 ± 1.2)	4.4–7.1 (5.92 ± 0.8)	5.5–8.0 (7.2 ± 0.7)	–	–
Width of broader membrane	1.9–3.0 (2.5 ± 1.3)	1.5–2.9 (2.16 ± 0.6)	1.0–1.5 (1.3 ± 1.1)	1.5–3.0 (2.0 ± 0.4)	–
Number of denticles	20–21	20	23–33 (31)	23–28 (25.1 ± 1.3)	17–20 (18)
Number of radial pins/Denticle	3–6 (4.5 ± 1.2)	3–5 (4 ± 0.9)	3–5 (4)	4–5	–
Dimension of denticle					
Span (large denticle)	3.9–4.7 (4.32 ± 0.3)	3.5–4.3 (3.95 ± 0.5)	3.7–4.5 (4.2 ± 0.8)	4–6 (5.3 ± 0.6)	–
Span (small denticle)	–	–	1.6–2.4 (1.8 ± 3.0)	–	–
Length	2.1–3.7 (2.92 ± 0.5)	1.3–2.21 (1.9 ± 0.3)	1.9–2.8 (2.3 ± 1.1)	1.5–2.5 (2.0 ± 0.3)	2.2–4.0 (3.2 ± 0.32)

Fig. 2 Photomicrographs of *Dipartiella carassii* n. sp. (a–c) silver nitrate impregnated adhesive discs and d diagrammatic drawings of the denticles of *Dipartiella carassii* n. sp. identified from *Carassius auratus* (L.). Scale Bar 10 μ m

3.5–4.3(3.95 ± 0.5) μ m. Length of denticles 1.3–2.2(1.9 ± 0.3) μ m in 1.3–2.2(1.9 ± 0.3) μ m in diameter consisting of broad, almost identical, elongated blades occupying most areas between y-axes along with a denticular ring 4.6–8.9 (6.45 ± 1.6) μ m in diameter (Fig. 2a–c). Anterior margin slanting down backward to form indistinct apex at base of blade. Anterior margin of the blade straight, which never crosses the y+1 axis (Fig. 2d). Posterior margin of blade curved slightly at base, giving it a swelling appearance. Blade thin and poorly triangular, obliquely backwards and almost broadening towards central part. Number of denticles 20, each consisting 3–5 (4 ± 0.9) radial pins along with the absence of ray and inconspicuous central part.

Taxonomic summary

Host: *Carassius auratus* (L.)

Locality: Haringhata, West Bengal, India (23.4710°N, 88.5565°E).

Location: Gills.

Prevalence: 04 out of 20 host fishes (20%).

Etymology: The specific epithet “*carassii*” is given after the name of the genus of host fish, *Carassius auratus* from which the parasite has been isolated.

Reference Material: Holotype, slide no. GF-DSII-1/2015, and -DSII-8/2016, GF-DSII-16/2016 have been deposited in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Remarks: On analyzing the morphometric and quantitative data of the present specimen, it was readily identified as *Dipartiella* sp. Comparative study of morphological and morphometric characteristics of the present specimen with the other closely related species of this genus showed that the present species has a remarkable affinity with the *D. kazubski* described by Mitra and Bandyopadhyay in (2009) and very little with *D. simplex* reported by Xu et al. (1999). The present species differs from *D. simplex*, morphologically and contains denticles with well-developed blades, reduced

central part and no rays as that of *D. kazubski* but the dimension of the body is slightly larger than that of *D. kazubski* and the dimension of denticular ring is smaller than that of *D. kazubski*. The denticles of *D. simplex* and *D. kazubski* are purely triangular and rectangular respectively (Xu et al. 1999; Mitra and Bandyopadhyay 2009) while the blade of the present species is poorly triangular. The distal blade margin in *D. simplex* is sharp in the present species it is usually flat and truncated like *D. kazubski*. Moreover, the number of denticles of the species is 20, which is much less than in *D. kazubski* described by Mitra and Bandyopadhyay (2009) (23–33).

Furthermore, the host of all the previously described species differs from the present species. Therefore, after careful consideration of all the differences with its closely related species the present species has been designated as a new species and hence the name *Dipartiella carassii* n. sp is being proposed.

It is confidently commented that the present investigation helps us to include the fish *Carassius auratus* in addition to the list of hosts for *Dipartiella* sp. in India. The research work is also the first record of the genus *Dipartiella* obtained from goldfish collected from different ornamental fish farms of India and adds two new species under the genus *Dipartiella* to the large taxon list of trichodinids.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval The fishes were collected in fresh form different ornamental fish farms of West Bengal. It has been informed that as per CPCEA instruction's protocol for experimentation on fishes, does not require approval.

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