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Description of *Trichodina hippoglossi* n. sp. from farmed Atlantic halibut larvae *Hippoglossus hippoglossus*

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ABSTRACT: A new species of *Trichodina*, *T. hippoglossi* n. sp., collected from skin and fins of farmed Atlantic halibut *Hippoglossus hippoglossus* (L.), is described. Heavy infections were first evident after a temperature rise from 12 to 18°C. The most infected specimens were easy to detect due to increased mucus production, which gave the larvae a greyish skin colour. *T. hippoglossi* is relatively large, 78.5 ± 6.9 µm (mean ± SD; range 65.9 to 89.6 µm), with a denticle ring comprising 28 (mode; range 26 to 30) denticles and with a denticle span of 17.6 ± 1.1 µm (mean ± SD; range 15.7 to 19.2 µm). In silver-impregnated fully grown specimens, a prominent clear central disc is present.

KEY WORDS: Atlantic halibut · *Trichodina hippoglossi* · New species · Description · Morphology

INTRODUCTION

Trichodinid ciliates are usually considered as harmless ectocommensals on fishes (cf. Lom & Dyková 1992). However, in aquaculture, with high host densities and abnormal environments, these protozoans may become pathogens and cause mortality. Since the beginning of marine aquaculture there have been several reports of infections with trichodinids. Brøderud & Poppe (1986) and Sanmartin Duran et al. (1991) have reported infections from turbot *Scophthalmus maximus* (L.), Jensen (1986) from cod *Gadus morhua* L. and Moksness et al. (1989) and Moksness (1990) from wolffish *Anarhicas lupus* L. In none of these reports are the trichodinids involved identified to species.

Lom & Laird (1969) gave a list of marine trichodinids reported prior to 1969, with an evaluation of their status, i.e. whether they were sufficiently described, poorly described or impossible to recognise ('nomen dubium'). The list contained 5 trichodinid species described from pleuronectiform hosts. Of these, only 2 species were characterised as well established: *Trichodina borealis* Shulman & Shulman-Albova, 1953, which has been reported from several flatfish species (e.g. *Platichthys flesus* L., *Hippoglossoides platessoides* Fabr., *Pleuronectes platessa* L.) (Raabe 1958, Lom & Laird 1969, MacKenzie 1969, Calenius 1980), and *T. raabei* Lom, 1962, which has been described from *P. flesus* in the Black Sea (Lom 1962). Additionally, Pearse (1972) has presented morphometric data for *Trichodina* sp. from *P. platessa* from Port Erin, UK.

The aim of the present paper is to describe a new species of *Trichodina* found on farmed Atlantic halibut *Hippoglossus hippoglossus* larvae. The species will be compared with previously described *Trichodina* spp.

MATERIALS AND METHODS

The halibut larvae originated from a hatchery outside Bergen, Norway. They were kept in tanks with running sea water (salinity 3.4 %) at 12°C for 35 d after which the temperature was gradually raised to 18°C. The larvae were used in a challenge experiment with infectious pancreatic necrosis virus (IPNV). Upon arrival at the laboratory the larvae had an average weight of 1.0 g; at the end of the experiment the average weight had increased to 3.0 g.

Air-dried fixed smears, collected by scrapings from fins and skin of the larvae, were treated using Klein's

dry silver technique (Klein 1958) to reveal the morphology of the adhesive disc. Satisfactory impregnation was difficult to obtain due to the high amount of chloride ions in sea water, which precipitate silver. A modification of the method described by Lom & Laird (1969) was used. Wet smears were placed on a slide and fixed for 2 min over a flask containing a 5% solution of boiling formalin. The slide was then placed in a petri dish (15 cm diameter), which was carefully filled with distilled water. After approximately 1 h the dish was emptied. The preparation was air-dried and impregnated for 8 min in a 2% aqueous $AgNO_3$ solution, washed once in distilled water, and exposed to ultraviolet light for 20 to 25 min.

To reveal the nuclear apparatus, air-dried smears were stained in toluidine blue for about 5 min, and then destained in acid-ethanol (1 part 0.1 M HCl: 9 parts 70% ethanol) for higher differentiation.

The stained, mounted trichodinids were examined with a stereomicroscope (Leitz, Aristoplan), while the oral ciliation on the body surface was observed in a phase contrast microscope.

Terminology and methods of measurements are as given by Lom (1958) and Van As & Basson (1989, 1992).

Measurements of the trichodinids were compared using Student's *t*-test and were considered significant if $p < 0.05$.

RESULTS

Extent of infection

The first indication of trichodinid infection was evident after the temperature was raised to between 16 and 18°C. At this temperature, heavy infections were prevalent and more than 1000 specimens on individual hosts were registered. While no trichodinids were found prior to the increase in temperature, heavy infections (more than 500 specimens per individual host) were found on 21.7% of the halibuts after the increase. Infections occurred on skin and fins only, and heavily infected halibut larvae were easy to detect due to their greyish skin colour. These specimens also had skin haemorrhages.

Description of *Trichodina hippoglossi* n. sp. (Figs. 1 to 6)

Host: Farmed fish, *Hippoglossus hippoglossus* (L.).
Locality: Bergen, Norway.
Site of infection: Skin and fins.

Judging from the large number of post-dividing stages of different age, the infections on the halibut had a high growth rate. The various developmental stages differed in the size and shape of their adhesive disc components (see Figs. 2 & 4). To avoid confusion, only specimens with a prominent clear central disc (when silver impregnated) were considered as fully grown and used in the description (see Figs. 1 & 3).

Table 1. *Trichodina hippoglossi* n. sp. from Atlantic halibut *Hippoglossus hippoglossus*. Morphometric data; all measurements are given in µm and are presented as mean ± SD (with range in parentheses), except where otherwise noted

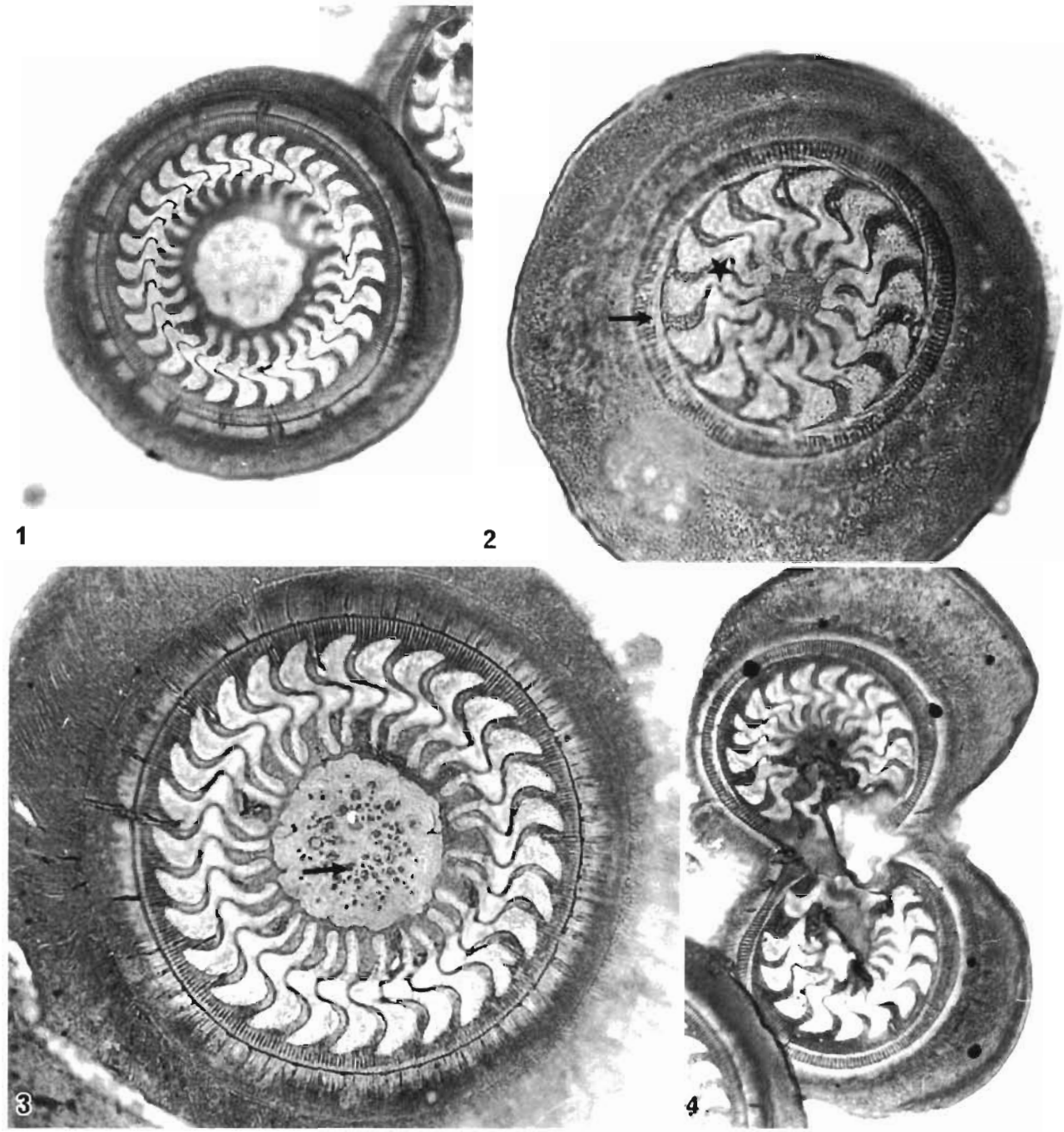
Location on host	Skin and fins
Specimens measured (n)	30
Body shape	Disc
Body diameter	78.5 ± 6.9 (65.9–89.6)
Diameter, adhesive disc	68.7 ± 6.0 (57.8–80.0)
Diameter, denticle ring	43.0 ± 4.5 (35.2–51.7)
Width of border membrane	4.9 ± 1.1 (2.5–7.2)
Denticle number	28[a] (26–30)
Radial pins denticle^{-1}	12[a] (10–14)
Denticle length	10.8 ± 0.8 (9.6–12.3)
Blade length	7.5 ± 0.7 (6.4–8.3)
Ray length	7.3 ± 0.7 (6.4–8.6)
Central part length	2.9 ± 0.8 (1.6–4.3)
Span of denticle	17.6 ± 1.1 (15.7–19.2)
Macronucleus shape	U-shape
Macronucleus external diameter	68.1 ± 4.2 (56.0–72.0)
Macronucleus thickness	11.8 ± 1.2 (9.6–13.6)
Macronucleus X[b] distance	19.0 ± 3.5 (14.4–27.2)
Micronucleus position	+*y*
Adoral spiral	360–400°

[a]Modal value; [b]as defined by Li & Desser (1983)

Morphometric data for *Trichodina hippoglossi* n. sp. are summarised in Table 1. The species is relatively large with a disc-shaped body in top view.

Denticle description

In typical individuals the blade of the denticle fills most of the sector between *y* and *y*+1 axes. The distal surface is slightly rounded with the tangent point below the distal surface. The blade apex is triangular and typically extends across the *y*+1 axis, with a rounded anterior surface. There is no blade apophysis present. The posterior blade surface is rounded and semilunar, with the deepest point corresponding to the blade apex. The blade connection (i.e. the region where the blade is joined to the central part) is wide, and no posterior projection is present. The central part is rounded, reaching more than halfway to the *y*–1 axis (but never touching it), closely fitting the preceding denticle. The sections above and below the *x*-axis are of approximately equal shape and size. There is no indentation in the proximal central part of the denticle. The sector of the central part connected to the ray slants in an anterior proximal di-



Figs. 1 to 4. *Trichodina hippoglossi* n. sp. Silver-impregnated adhesive discs. Fig. 1. Mature specimen showing adhesive disc components and a prominent central circle. ×524. Fig. 2. Post-dividing stage displaying half the denticle number as in mature specimens and an adhesive disc with a dark centre. Arrow indicates the new denticle ring, asterisk shows the old denticle ring. ×918. Fig. 3. Mature specimen showing argentophile granules in the central circle (arrow). ×1084. Fig. 4. Specimens in binary division generating individuals of similar size and shape to that in Fig 2. ×583

rection, making an angle larger than 30° with the *x*-axis, with a robust and thick connection to the ray. Rays are slightly curved, thick and of almost equal thickness throughout, filling a large part of the sector between the *y* and *y*+1 axes. The ray apophysis is triangular, pointing anteriorly and situated distally on the ray. The ray point is rounded and does not touch the central circle (see Fig. 5 for drawing of the denticle). There was no significant difference in the span of the ray and blade (Student's *t*-test, $p > 0.05$).

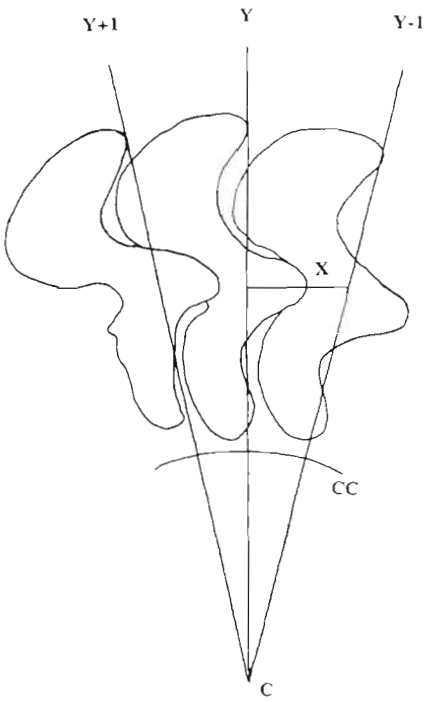


Fig. 5. *Trichodina hippoglossi* n. sp. Drawings of denticles using the method introduced by Van As & Basson (1989, 1992). C: centre of the adhesive disc; CC: central circle: X: *x*-axis, Y: *y*-axis

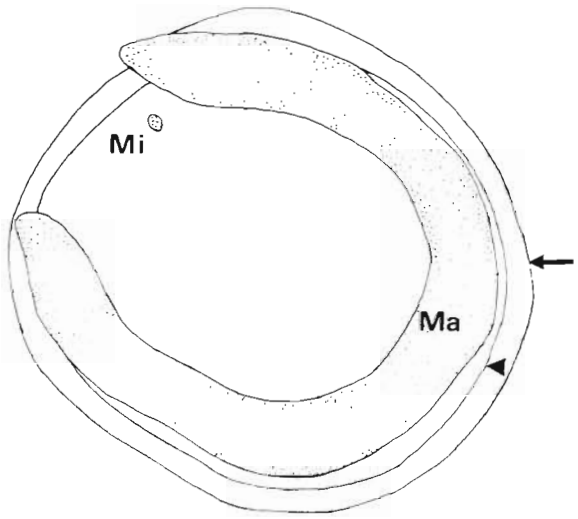


Fig. 6. *Trichodina hippoglossi* n. sp. Drawing of macro- and micronucleus. The macronucleus is U-shaped and follows the circle of radial pins, with the exception of the outer part of the arms where it extends across the lining of the adhesive disc. The micronucleus is in the *+y* position. Ma: macronucleus; Mi: micronucleus; arrow indicates the lining of the adhesive disc; arrowhead indicates the lining of the circle with radial pins. ×905

Nuclei

Measurements and drawings of the macro- and micronucleus are given in Table 1 and in Fig. 6 respectively. The macronucleus is U-shaped and follows the circle of the radial pins throughout, with the exception of the outer part of the arms. In this region the macronucleus extends across the lining of the adhesive disc. The micronucleus is located in the *+y* position (see Fig. 6).

Developmental stages

Several developmental stages of *Trichodina hippoglossi* were observed in silver-impregnated smears. The most common were recently divided specimens containing half the number of denticles compared with mature individuals (see Fig. 2). These specimens display an adhesive disc with a darkly stained centre, and a body diameter approximately half that of mature specimens. In dividing specimens the new denticle ring is visible prior to complete division (see Fig. 4).

DISCUSSION

This is this the first description of a trichodinid from either farmed or naturally occurring Atlantic halibut. Whether the species reported from the other farmed marine fishes are similar to *Trichodina hippoglossi* n. sp. is at present unknown. However, 3 different species of *Trichodina* have been described from wild cod (Poynton & Lom 1989) but none of these is similar to the present species.

Infections with *Trichodina hippoglossi* were first evident at high temperature (approximately 18°C). This may indicate that *T. hippoglossi* has a relatively high optimum growth temperature, or that this temperature is suboptimal for Atlantic halibut, enabling the parasite to proliferate. Similar observations have been reported for other trichodinids. Lom (1961) and Calenius (1980) observed highest infection rates with trichodinids on freshwater fishes when water temperature was low. Lom (1961) suggested that this could be due to suboptimal conditions for the fish, enabling the parasite to proliferate. In a study of IPNV in Atlantic halibut larvae and fry, Biering et al. (1994) observed significantly higher mortality in fish challenged at 15°C than fish challenged at 12°C. This finding supports the view that larvae and fry of Atlantic halibut are more susceptible to infectious agents at high temperatures (i.e. 15°C and up).

A wide variety of *Trichodina* species with a light central circle have been described from both marine and freshwater fish hosts. From marine fishes there

are at least 13 known species (see Lom 1962, 1970, Stein 1973, 1976, 1979, Grupcheva et al. 1989) and some of the previously described species are of similar size to the present species. *T. lairdi* Lom, 1970, which inhabit the gills of *Oligocottus maculosus*, possess a light central circle in the adhesive disc and have a similar body diameter. However, there are several features which separate *T. lairdi* from *T hippoglossi* n. sp. The former has a significantly different denticle shape, i.e. the ray is approximately twice the length of the blade (in the present species no significant difference was observed in the span of the ray and blade). Furthermore, *T. lairdi* have 33 (mode; range 30 to 35) denticles in contrast to 28 (26 to 30) in *T. hippoglossi* (cf. Lom 1970).

Trichodina sp. 2 (Grupcheva et al. 1989) possesses a central circle and is of similar size to the present species, but this species has significantly smaller denticles (mean span 12.7 µm, range 11.1 to 14.4 µm) than *T. hippoglossi* n. sp. (see Table 1). In addition, *Trichodina* sp. 2 has a smaller adhesive disc and denticle ring diameter, and fewer radial pins per denticle.

Trichodina borealis Shulman et Shulman-Albova, 1953 which occurs on the gills of *Pleuronectes platessa* (and some other species) is considerably smaller than the present species (cf. Lom 1970). Another flatfish-inhabiting species, *T. raabei* Lom, 1962 (from the gills of *Pleuronectes flesus luscus*), is also smaller and possesses a dark-staining adhesive disc centre (see Lom 1962). Pearse (1972) reported on *Trichodina* sp. from the skin of captive *P. platessa* from Port Erin; this species possessed a dark-staining adhesive disc centre and was larger than the present species.

From marine fish hosts, several subspecies of the freshwater species *Trichodina domerguei* (from skin and fins of stickleback) have previously been described (e.g. Lom 1962). These species all have an adhesive disc with a distinct central circle. *T. domerguei* f. *marisnegri* Lom, 1962 from the skin of *Gaidropsis mediterranaeus* L. possess a light central circle and have an adhesive disc and denticle ring of similar size to the present species. However, there are some distinct differences in the denticle morphology. The former has a larger denticle, and the blade span is larger than the ray. These features, and the larger body diameter of the former, separate these 2 species.

Trichodina murmanica Polyanskii, 1955, a species reported from the skin, fins and gills of several different marine fishes (cf. Poynton & Lom 1989), possesses a light central circle in the adhesive disc. However, this species can be distinguished from the present species by the notably smaller size of all the adhesive disc components. Additionally, typical specimens of *T. murmanica* have a significantly longer ray than blade (Nilsen 1993).

Trichodina elegini Shulman-Albova, 1950 from the gills of *Eleginus navaga* Pallas and *T. micromaculata* Stein, 1975 from *Mugil cephalus* L. and *Opisthocentrus dybowskii* differ from the present species by their considerably smaller size (Stein 1976). In addition, *T. domerguei* f. *partidisci* Dogiel, 1940, *T. domerguei* f. indet. Lom, 1962 and *T. domerguei* f. *gobii* Raabe, 1959, which all have a distinct central circle (Raabe 1958, Lom 1962), are notably smaller than the present species.

To conclude, the present species, *Trichodina hippoglossi* n. sp., can be differentiated from all previously described *Trichodina* spp., and is the first species described from Norwegian farmed fish.

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