First Record of the Parasitic Ciliate *Trichodina truttae* Mueller, 1937 on Chum Salmon Fry (*Oncorhynchus keta*) from Japan

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The parasitic ciliate *Trichodina truttae* Mueller, 1937 is redescribed from silver-impregnated specimens collected from the body surface of chum salmon fry (*Oncorhynchus keta*) reared in five hatcheries of Hokkaido and northern Honshu, Japan. The description is supplemented by scanning electron microscopic observations. The distinguishing morphological features include its large size (body diameter 115–178 μm), high number (10–23) of radial pins per denticle, and the presence of two markedly different lengths of cilia within the adoral ciliary spiral. Geographical variation was observed in the body size and number of denticles. This is the first record of *T. truttae* from Japan.

Ciliates belonging to the family Trichodinidae are common and occasionally pathogenic parasites of freshwater and marine fishes (Lom and Hoffman, 1964; Lom and Laird, 1969; Hoffman, 1978). In Japan, trichodinid infections frequently occur on cultured freshwater and marine fishes, but taxonomic and pathological studies of this group have been very few (Egusa, 1978). Although Ariake (1929) and Suzuki (1950) reported several new species of trichodinids from Japan, these descriptions were considered by Albaladejo and Arthur (1989) to be too inadequate to permit positive reidentification. The only other confirmed identifications of *Trichodina* from Japanese fishes are those of Ahmed (1976, 1977), who reported *T. reticulata* Hirschmann and Partsch, 1955 on goldfish (*Carrasius auratus*), crucian carp (*C. auratus*) and color carp (*Cyprinus carpio*).

In 1968, high mortalities occurred among chum salmon (*Oncorhynchus keta*) fry reared in Ichani Hatchery, eastern Hokkaido, due to trichodinid infections (Takeda *et al.*, 1969). Although the parasite was reported as *T. domerguei* (Wallengren, 1897), its identification is suspect, since it was not based on detailed morphological studies (Nagasawa *et al.*, 1987). Several other papers also reported trichodinid

ciliates from farmed salmonids in Honshu (see Nagasawa *et al.*, 1987). Recently, we also encountered many cases of *Trichodina* infections among hatchery-reared salmon fry. Experimentally, the ciliate caused severe mortalities in chum salmon fry (Urawa, unpublished data).

The present study was conducted to clarify the species of trichodinid ciliate occurring on the skin of chum salmon fry in northern Japan, and consequently the ciliate was identified as *Trichodina truttae* Mueller, 1937. This species was originally described from the gills of hatchery-reared cutthroat trout (*Oncorhynchus clarki*) in Oregon (Mueller, 1937), and has been subsequently reported from various salmonids in western North America and the USSR (Davis, 1947, 1953; Bogdanova, 1963, 1967, 1977; Bogdanova and Shtein, 1963; Arthur and Margolis, 1984). Because this finding constitutes the first record of *T. truttae* from Japan, the parasite is redescribed according to Klein's (1958) dry silver-impregnation technique and scanning electron microscopy (SEM), and some aspects of its geographical variability are noted.

Materials and Methods

Trichodinids were collected from the body

Fig. 1. Map of collection localities for *Trichodina truttae* infecting chum salmon fry in northern Japan. Numbers refer to hatcheries given in the legend of Fig. 10.

surface of chum salmon fry from five hatcheries in Hokkaido and northern Honshu between 1981 and 1990 (Fig. 1). Air-dried smears of living ciliates taken from the skin were prepared for light microscopical observations. Smears were immersed in a 2% solution of silver nitrate (AgNO_3) for 10 min, and exposed to ultraviolet light for 15–20 min (Klein's dry silver-impregnation technique). In addition, some formalin preserved specimens were impregnated with silver according to Wellborn (1967). For SEM, ciliates were fixed by immersion of whole living chum salmon fry in 1% glutaraldehyde in 0.1 M phosphate buffer. Parasites were centrifuged and dehydrated through a graded alcohol series. The alcohol was replaced with isoamyl acetone, and the samples were dried in a Hitachi critical point drying unit, gold-coated in a sputter-coater

(Eiko), and then observed using an Akashi scanning electron microscope. Terminology and methods of measurement follow the uniform specific characteristics proposed by Lom (1958) and Arthur and Lom (1984). All measurements are given in micrometers.

Results

Trichodina truttae Mueller, 1937 (Figs. 2–9)

Host: chum salmon fry (*Oncorhynchus keta*).

Site of infection: Body surface.

Localities: Asahi Hatchery, Rishiri Island, northern Hokkaido; Chitose Hatchery, western Hokkaido; Ichani Hatchery, eastern Hokkaido; Ohkawa Hatchery, Miyagi Prefecture, Pacific coast of northern Honshu; Gakko Hatchery, Yamagata Prefecture, Japan Sea coast of northern Honshu.

Deposition of specimens: One slide including silver-impregnated specimens from chum salmon fry from Asahi Hatchery has been deposited in the National Science Museum, Tokyo (NSMT-Pr. 165).

Description

The following description is based mainly on silver-impregnated ciliates collected from Asahi and Chitose Hatcheries (Table 1), supplemented by SEM observations.

A relatively large trichodinid with a disc-shaped body (Fig. 6), 114.7–177.5 in diameter. Adhesive disc cup-shaped, 81.1–125.0 in diameter, surrounded by a wide border membrane, 6.5–12.0 in width. Adoral ciliary complex consisting of three distinct ciliary bands: basal ciliary ring, locomotor ciliary wreath, and marginal ciliary ring (Figs. 6–8). Center of adhesive disc dark and uniformly granular in silver-impregnated specimens. Denticulate ring 45.3–75.0 in diameter, consisting of 24–31 denticles (Figs. 2 and 3). Span of denticle 24.5–35.5; length 12.8–20.0. Blade of denticle acutely falciform with highly concave anterior border and sharply angled posterior margin (Fig. 4). Blade joining central portion of denticle posterior to median axis of thorn. Length of blade 8.8–14.0. Central part thick, 4.5–8.5 in width, its external portion

Figs. 2-5. Photomicrographs of *Trichodina truttae* from chum salmon fry. **2**, Adhesive disc of silver-impregnated specimen collected from Asahi Hatchery. Bar=20 μ m. **3**, Adhesive disc of silver-impregnated specimen from Chitose Hatchery. Bar=25 μ m. **4**, Detail of adhesive disc in Fig. 3, showing radial pins, which appear as pairs in this specimen. Bar=10 μ m. **5**, Oral view of fresh specimen from Chitose Hatchery, showing adoral ciliary spiral. Bar=40 μ m. LAC, long adoral cilia; SAC, short adoral cilia.

Figs. 6-9. SEM photomicrographs of *Trichodina truttae* from chum salmon fry reared in Chitose Hatchery. **6**, Oral view of entire specimen. Bar=35 μ m. **7**, Enlargement of oral surface showing short adoral cilia. Bar=7 μ m. **8**, Enlargement of oral surface showing long adoral cilia and buccal cavity. Bar=8 μ m. **9**, Adhesive disc showing radial pins and denticles. Note the low number of radial pins per denticle (about 12) indicative of a recently divided specimen. Bar=5 μ m. ACS, adoral ciliary spiral; BC, buccal cavity; BM, border membrane; D, denticle; LAC, long adoral cilia; LCW, locomotor ciliary wreath; MCR, marginal ciliary ring; RP, radial pins; SAC, short adoral cilia.

cylindrical. Thorn thickened at base, tapering gradually to a sharp point. Length of thorn 10.4-16.0. Number of radial pins per denticle 10-23 (Figs. 4 and 9). Adoral ciliature forming a spiral of about 450° (Fig. 5). The majority of cilia comprising the adoral ciliary spiral short

(Fig. 7), only final area near buccal cavity comprised of long cilia (Fig. 8). Macronucleus U-shaped, about 80 in diameter.

Geographical Comparison

Fig. 10 compares variation in parasite size

Table 1. Comparison of morphometrics of *Trichodina truttae* from salmonids in Japan and Canada. Measurements are given in micrometers, and are based on silver-impregnated specimens

Source	Present study	Present study	Arthur & Margolis (1984)
Host	<i>Oncorhynchus keta</i>	<i>O. keta</i>	<i>O. kisutch</i>
Site	Body surface	Body surface	Body surface
Locality	Asahi Hatchery, Rishiri Island, Hokkaido, Japan (n=25)	Chitose Hatchery, Hokkaido, Japan (n=21)	Quinsam Hatchery, Vancouver Island, B.C., Canada (n=25)
Diameter of:			
body	125.0–177.5 (144.7±14.2*)	114.7–143.9 (125.5±9.4)	114.0–170.5 (148.0±12.2)
adhesive disc	100.0–125.0 (108.8±7.2)	81.1–104.8 (90.0±6.9)	95.0–136.8 (121.1±10.1)
denticulate ring	56.5–75.0 (63.7±5.2)	45.3–62.1 (51.0±4.4)	45.6–69.4 (58.5±5.6)
Number of:			
denticles	26–31 (27.2±0.9)	24–26 (24.8±0.7)	27–31 (29.5±1.0)
radial pins per denticle	12–23	10–20	14–22
Dimensions of denticles:			
length	14.3–19.0 (16.5±1.4)	12.8–20.0 (15.4±2.2)	14.2–22.8 (19.6±1.7)
blade	11.0–14.0 (12.1±0.9)	8.8–11.2 (9.7±0.9)	8.6–14.7 (11.7±1.4)
thorn	12.3–16.0 (14.3±1.0)	10.4–15.2 (13.4±1.6)	10.4–18.0 (14.5±1.9)
center	4.5–6.2 (5.5±0.5)	6.1–8.5 (7.0±0.6)	4.9–8.6 (6.8±1.0)
span	28.5–35.5 (32.0±1.8)	24.5–32.2 (28.5±2.0)	24.7–37.0 (32.9±2.7)
Width of border	6.5–10.0	7.2–12.0	7.6–12.4
membrane	(8.4±1.0)	(9.4±1.7)	(10.4±1.4)
Adoral ciliature	about 450°	about 450°	440–450°

* Mean ± SD.

(denticulate ring) and number of denticles among *T. truttae* populations collected from five hatcheries in northern Japan and one in Canada. The ciliates from Asahi (location 1) and Ichani (2) Hatcheries in Hokkaido are significantly larger than those from other hatcheries ($P<0.01$). The number of denticles appears to increase with decreasing latitude in northern Japan except for specimens from Chitose Hatchery (3) where the denticle number is very small (24–26).

Discussion

Arthur and Margolis (1984) stated that the diagnostic features of *T. truttae* are its large size and large number of radial pins per denticle. This species is the largest ciliate among

the more than 100 species of *Trichodina* described from freshwater fishes, and the number of radial pins per denticle (14–26) is the highest number reported for any member of the genus (Arthur and Margolis, 1984). In addition, their SEM observations, confirmed by the present study, note that the presence of two markedly different lengths of cilia within the adoral ciliary spiral may also be unique to *T. truttae*. Morphological features of our specimens are comparable with those of *T. truttae* as given by Arthur and Margolis (1984) (Table 1).

Many of our Japanese specimens have low numbers (8–12) of radial pins per denticle (Fig. 9). These individuals are smaller than ciliates with high denticle number (18–23). These smaller ciliates have recently undergone mi-

Fig. 10. Geographical variation in the diameter of the denticulate ring and number of denticles for *Trichodina truttae*. Each open circle represents the mean, the vertical bar represents the range. The number above or below each point is the sampling locality. 1, Asahi Hatchery ($n=25$); 2, Ichani Hatchery ($n=20$); 3, Chitose Hatchery ($n=21$); 4, Ohkawa Hatchery ($n=20$); 5, Gakko Hatchery ($n=20$); 6, Quinsam Hatchery (49.3°N, 124.2°W) on Vancouver Island, B. C. ($n=25$; from Arthur and Margolis, 1984).

tosis. They have completed production of a new ring of denticles but have not yet replicated the number of radial pins.

Trichodina truttae shows geographical variability in body size and number of denticles within northern Japan (Fig. 10). The ciliates had a larger body size in Asahi and Ichani Hatcheries than in the other three hatcheries. When the host fish were collected, the rearing water temperature was about 5°C in the former two hatcheries, but between 8° and 10°C in the

other hatcheries. Then, these differences in the body size may be due to differences in environment (e.g., water temperature) and/or differences in the number of recently divided ciliates among the populations. Kazubski and Pilecka-Rapacz (1981) similarly reported seasonal and interpopulational variability in the body dimensions of *T. nigra* Lom, 1961.

Takeda *et al.* (1969) recorded *T. domerguei* from chum salmon fry reared in Ichani Hatchery without providing any morphological description. Takeda (1971) also reported a heavy infection of *Cyclochaeta* (= *Trichodina*) *domerguei* on chum salmon alevins in Nemuro Hatchery, eastern Hokkaido, and listed Sakhalin taimen (*Hucho perryi*) and masu salmon (*Oncorhynchus masou*) as experimental hosts. Although *T. domerguei* has, on one occasion (see Bogdanova and Shtein, 1963), been reported from chum salmon fry, this species is primarily a parasite of gasterosteid fishes (see Lom and Stein, 1966). Our finding of only *T. truttae* from the same hatcheries as those from which *T. domerguei* was reported by Takeda *et al.* (1969) and Takeda (1971) and the lack of supporting evidence to validate their identifications leads us to believe that these reports are probably referable to *T. truttae*.

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