A NEW SPECIES OF CILIATE, *TRICHODINA BRANCHICOLA*, FROM SOME FISHES AT PLYMOUTH

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(Text-figs. 1, 2)

While examining fishes at Plymouth for parasites I found the gills of eight species of Teleosts infected with a new urceolarid peritrich ciliate which I describe here as *Trichodina branchicola* n.sp. I also found the same species on rocklings (*Ciliata mustela* and *Gaidopsaurus tricirratus*) at Roscoff in March 1947.

All the fishes were caught at Plymouth and examined fresh, studies being made on living as well as fixed material. The living ciliates were stained with neutral red and methylene blue (1 : 5000 in sea water). The fixatives used were Bouin-Duboscq's fluid, Zenker's fluid (with formalin) and 2% aqueous osmium tetroxide. The slides were stained in iron-alum-haematoxylin with eosine, or in Delafield's haematoxylin with or without eosine.

The host species and the incidence of infection are shown in Table I.

TABLE I

Host species	No. examined	No. infected
Ciliata mustela (L.)	26	21
Gaidopsaurus tricirratus (Bloch)	8	6
Cottus bubalis Euphrasen	4	4
Spinachia spinachia (L.)	4	3
Blennius pholis L.	2	2
B. gattorugine Bloch	1	1
Trigla lucerna L.	1	1
Pleuronectes platessa L.	1	1

The intensity of infection was generally high in the first five and low in the last three hosts, but no exact quantitative studies of the parasitic population were made. *Ciliata mustela*, which is normally heavily parasitized, had a low grade of infection by *Trichodina branchicola* when caught from the brackish water of the Laira estuary near Plymouth. Four specimens of this fish taken from the estuary after a heavy rainfall had an appreciably low infection.

The following experiment supports the view that reduction in salinity of the water was the operative factor in reducing the parasitic population. The infected gills of *Ciliata mustela* were placed in diluted sea water (75 c.c. sea water and 25 c.c. distilled water) and it was found that after 3 hr. the ciliates were dead. The experiments were conducted in a bath of circulating sea water to ensure the requisite low temperature. Casual observations have repeatedly shown that *Trichodina branchicola* n.sp. is far more sensitive to changes of temperature and salinity than its hosts. This evidence tends to show that this species of *Trichodina* is a stenohaline organism and will not be found on fresh-water fishes.

After removal from its fish host *T. branchicola* does not survive in sea water for more than 2–3 hr.: it may live much longer on the dead host provided that it is kept at a low enough temperature. I have observed the ciliates still moving 32 hr. after the infected gills were removed from *Ciliata mustela* and had been placed in a refrigerator, although the sea water covering them had a thin film of ice. Richardson (1938) has done some experiments to show the viability of *Cyclochaeta domerguei* Wallengren, under different temperatures and salinity. Fresh-water fishes when infected with *Trichodina* spp. in the hatcheries are cured of the infection by a bath in saline water, or by putting them 'for 1 hour in a solution composed of 1 part of formalin (40%) to 4000 parts water' (Davis, 1947).

In *Ciliata mustela* and *Gaidopsaurus tricirratus* the only other gill parasite was a species of *Gyrodactylus*. This monogenean occurred as a moderate infection irrespective of the presence of *Trichodina branchicola* and there is no reason to think that they are incompatible.

In one *Spinachia spinachia* examined on 9 July 1946 the gills were infected with a species of *Amoeba* (6–11 μ in diameter) and hardly a dozen *Trichodina branchicola* were present. None was found to be infected by the *Amoeba*. The high infection of *Amoeba* was associated with hyper-secretion of mucus. *Trichodina branchicola*, normally a very actively moving animal, was hampered in its movements by the presence of the viscous mucus on the gills, and this may account for the unusually low infection. Chatton (1910) has described *Amoeba mucicola* infecting *Trichodina labrorum* Chatton 1910, from the gills of *Symphodus tinca*, but does not mention any pathological effect on the ciliate.

MORPHOLOGY

The shape of the organism is surprisingly variable. The oral or anterior end is strongly arched and the saucer shape of the posterior or aboral end is maintained by the 'skeletal complex', which in life is applied to the gill surface of the host (Figs. 1*b*; 2*a*, *b*). The general shape may be hemispherical or sub-spherical. When viewed from above the organism is round (30–53 μ in diameter) (Fig. 1*a*, *b*). Its height (antero-posterior) is 22–36 μ.

In the genus *Trichodina* there are two rings of cilia, an anterior oral and a posterior aboral ring—the latter is the chief organ of locomotion. In between these two rings of cilia is the velum—a fold of protoplasmic pellicle, capable of great extensions and contractions (Fig. 2*a*, *b*). It attains its greatest size when the animal is crawling on the gills or moving freely in the water (as seen on the slide under the microscope), and almost disappears when the animal is motionless or nearing death.

On the posterior surface, internal to the aboral ring of cilia, lies the 'skeletal complex' composed of the outer striated and the inner denticulate rings (Figs. 1*b*; 2*b*, *c*).

The skeletal complex and the centre of the ring are covered by a thin protoplasmic pellicle (Fig. 2*b*). The denticulate ring (10–19 μ in diameter) consists of 20–26 denticles. The following table shows the percentage of individuals with different numbers of denticles and the average diameter of the ring. These measurements are from 142 individuals from a single *Ciliata mustela*.

No. of denticles ...	20	21	22	23	24	25	26
Percentage of organisms ...	5	26·4	29·5	17·2	12·7	5	4·3
Average diameter of the ring (μ) ...	12·8	13	13·5	14·8	15·2	15·6	16

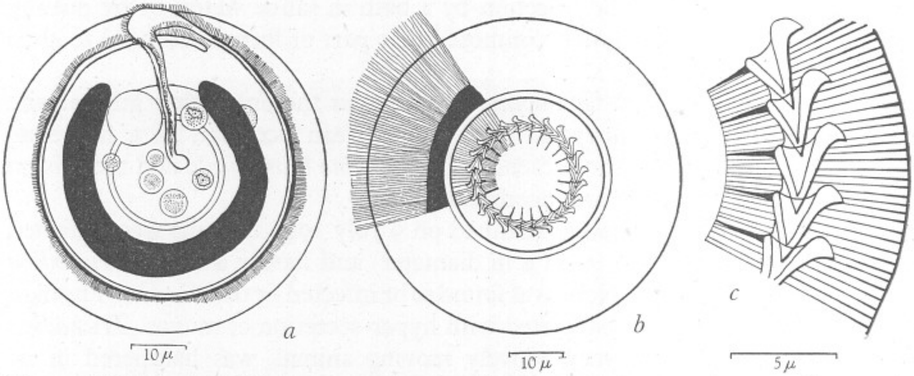

Fig. 1. *Trichodina branchicola*: *a*, anterior view showing oral groove and contractile vacuole and macronucleus; *b*, posterior view showing the skeletal complex and aboral cilia; *c*, portion of skeletal complex (further magnified).

The highest percentage of individuals is with 22 denticles; individuals with 21 and 23 denticles coming second and third. The size of the ring increases with the number of the denticles. Each denticle (Fig. 1*c*) is cone-shaped, 1·5–2·0 μ thick and 3·5 μ long, with a broad curved hook (2·3 μ long) on the outer side. The concave side of the hook is slightly thicker than the convex side. Each hook is attached to the cone at an angle like an oar. On the inner side of the cone there is a ray (3·2 μ long) pointing centrally and slightly anteriorly. It is thicker at the base, tapering towards the centre. In young *Trichodina branchicola* which has just undergone fission the number of denticles is halved, being 10–13 only. The outer hook is a little in front of the inner ray, as in *T. truttae* Mueller, and not exactly opposite, as in *T. pediculus* Ehrb. as described by Mueller (1937). The denticulate ring stains beautifully red with eosine and this helps very much in counting the number of denticles under the oil immersion lens.

External and somewhat anterior to the denticulate ring is a striated ring band 19–33 μ in diameter (Figs. 1*b*, *c*; 2*c*). There are six striae to each denticle. Each stria extends inwards as far as the inner ray. Each also is connected with the ciliary girdle by a strand of material which takes up the eosine stain less readily than the stria. Mueller (1938) supposed these structures to be myonemes. The outer borders of the striae fuse to form the edges of the adhesive disk. Each stria is thicker at the outer ends and tapers towards the centre. The ciliate secures itself to the gill surface with the adhesive disk. The skeletal complex is capable of movement as a whole in the horizontal plane but whether it can move in the antero-posterior direction as well is not clear.

The aboral cilia, 15–20 μ long, are fused at their base to form a thin membranelle, their distal ends being free (Figs. 1*b*; 2*c*). In *T. pediculus* Ehrb., *T. steinii* (Clar. & Lach.) and *T. renicola* (Mueller) the cilia are fused to form a series of membranelles. In the present species there is one continuous membranelle like that in *T. spheroidesi* Padnos & Nigrelli. The cilia do not fuse to form any membranelle in *T. halli* Padnos & Nigrelli and *T. urinicola* Fulton.

The oral groove runs round the anterior surface of the organism and makes little more than one anti-clockwise turn and then descends into the vestibulum which is connected with the gullet at its posterior end (Figs. 1*a*; 2*b*). The vestibulum descends obliquely and makes an angle of nearly 45° with the vertical axis of the organism. The base of the groove is slightly thickened and from its edges two parallel rows of cilia arise which are fused at their bases to form two parallel membranelles. The outer cilia are slightly longer than the inner. The inner membranelle ends at the entrance of the vestibulum. In the anterior three-quarters of the vestibulum the cilia are curved at their free ends and fused at their bases. The wave of movement starts at the anterior end and passes towards the gullet. In the last quarter of the vestibulum the cilia are longer and beat quickly towards the gullet. The undulatory motion of the cilia of the oral groove appears to form four waves in the whole circumference. The gullet is devoid of any cilia. The food vacuoles arise near the fundus of the gullet and then move peripherally in spirals. When stained *intra vitam* with neutral red (1 : 5000 in distilled water) the food vacuoles take the stain in 5 min. The young vacuoles stain yellowish and later on become pinkish. This shows a change in the pH content of the food vacuoles from basic to acidic, i.e. at a rough estimate initially at pH 8 or more to pH 7·5 or thereabout. The food vacuoles are mostly in the anterior half of the body. The food of the ciliate consists of desquamated epithelial cells and erythrocytes of the host fish. Diller (1928) states that *Trichodina* on the gills of tadpoles feed only on bacteria.

The contractile vacuole is 6·0–11·5 μ in diameter and situated near the vestibule (Fig. 1*a*). It pulsates every 30–35 sec. In *T. urinicola*, Fulton (1923) records that the pulsation is very sluggish, while in *T. pediculus* Ehrb., Mueller (1937) records the pulsation at the interval of 10–12 sec. The opening of the vacuole is not clear in the living or fixed specimens, but in sections it can be seen as a very thin short duct opening in the anterior part of the vestibulum. When the organism is stained *intra vitam* the vacuole does not take any stain.

No accessory vacuoles are present in any species of *Trichodina* such as have been reported for *Cyclochaeta domerguei* (Wallengren) by MacLennan (1939) and in various vorticellids by Fauré-Fremiet (1925).

The macronucleus is situated in the posterior half of the body, is horseshoe-shaped, and lies parallel to the plane of the 'skeletal complex' (Figs. 1*a*; 2*a*). It is 6·0–10·5 μ thick in the centre and somewhat thinner at the ends. The two ends of the nucleus are separated by the vestibulum and the contractile vacuole. The micronucleus is situated in a pocket in the macronucleus on its outer edge and can be seen only in the sections. During vegetative life the macronucleus is uniformly granular.

The body of the ciliate is covered by a thin protoplasmic pellicle. Within this is the dense granular layer of the protoplasm. The endoplasm is less granular and has many food vacuoles and the contractile vacuole.

LOCOMOTION

The organism moves by the help of the posterior cilia. Each cilium moves with a gyratory motion while the resultant movement of the whole ring appears as a wave motion. The animal rotates in a clockwise or anti-clockwise direction and moves very quickly with the posterior end forwards when swimming freely in water. There does not seem to be any relation between gyratory and rotatory motion. During movement the body is very much constricted antero-posteriorly and the velum increases in size. When the animal is 'browsing' on the gill with its adhesive disk in contact with the gill surface, it does not move very quickly.

PATHOGENIC EFFECT

When *Trichodina* settles on the gills and makes itself secure by the application of the adhesive disk then considerable local irritation must be caused, and this seems to bring about the desquamation of the epithelial cells of the gills. No very marked pathogenic effect was observed on the gills of the fishes examined during the course of the present studies, though there is no doubt that considerable harm could be done to the host if the ciliates were present in large numbers. At times, in fresh-water fish hatcheries, some species of *Trichodina* cause epidemic mortality.

REPRODUCTION

As a rule in the family Urceolaridae reproduction is by binary fission, though conjugation and endomixis also take place. Some stages of fission have been observed in *T. branchicola*. Preparatory to the fission the macronucleus becomes round and then oblong and stains deeply with haematoxylin. The shape of the organism changes and it too becomes oblong and later on dumb-bell-shaped. The micronucleus divides into two and can be seen lying on the outside of the macronucleus (Fig. 2*d*). There is a constriction in the adhesive disk, and then in the denticulate ring. The macronucleus and the whole body then divide into two daughter individuals.

The young individual has about half the adult number of the denticles in the denticulate ring. The old vestibulum persists in one of the daughter individuals while a new one seems to develop in the other. Peshkowsky (1923) states that the oral cilia, gullet and contractile vacuole are absorbed during division in *T. mitra* and *T. steinii*. Padnos & Nigrelli (1942) report that these organs definitely remain in *T. spheroidesi* during the fission, as occurs in the present species. The fate of the contractile vacuole could not be followed, though according to Padnos & Nigrelli it cleaves at the same time as the macronucleus in *T. spheroidesi*. Diller (1928) states that the contractile vacuole probably persists in one of the daughter individuals in *Trichodina* sp. on the gills of tadpoles.

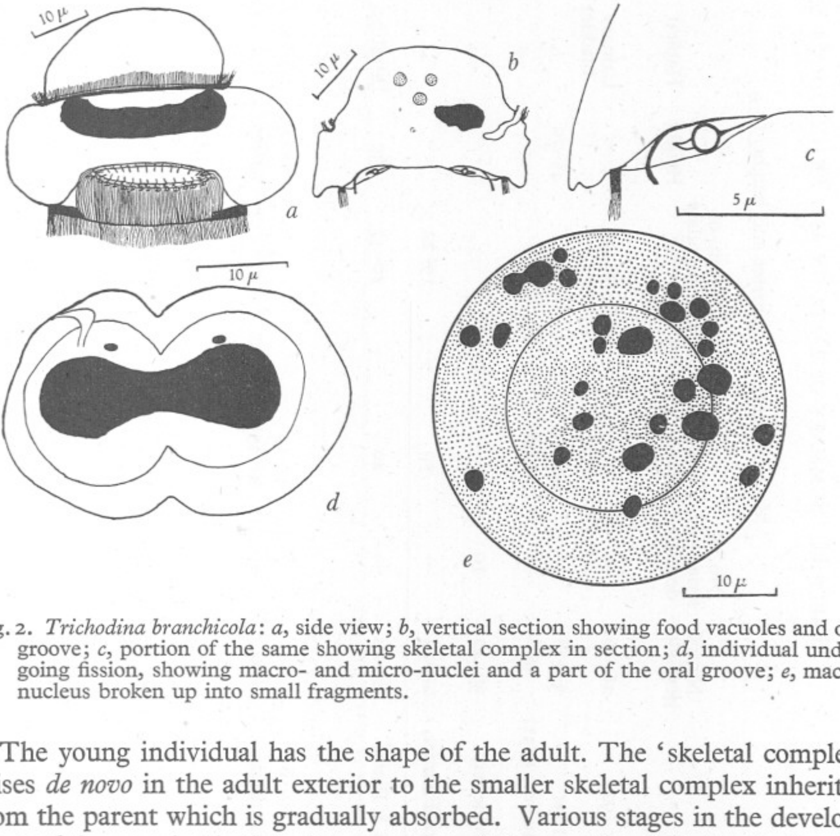

Fig. 2. *Trichodina branchicola*: *a*, side view; *b*, vertical section showing food vacuoles and oral groove; *c*, portion of the same showing skeletal complex in section; *d*, individual undergoing fission, showing macro- and micro-nuclei and a part of the oral groove; *e*, macronucleus broken up into small fragments.

The young individual has the shape of the adult. The 'skeletal complex' arises *de novo* in the adult exterior to the smaller skeletal complex inherited from the parent which is gradually absorbed. Various stages in the development of the new denticulate ring are seen and they confirm fully the findings of Fauré-Fremiet & Thareaux (1944, pl. 1, figs. 1–5). At first the central cone is formed, then the outer hook and lastly the inner ray. At first the denticle is thin but gradually it becomes thick. The young individuals, with one ring only, measure 25–33 μ in diameter, the diameter of the denticulate ring being 6·5–10 μ. The number of denticles is 10–13. The macronucleus is round or

TABLE II. SUMMARY OF DATA ON ALL *TRICHODINA* SPECIES

Measurements (of diameter and height) in μ

Species	No. of denticles	No. of striae per denticle	Total diam.	Diam. of striated ring	Diam. of denticulate ring	Height	Habitat	Host	Habitat of host
ENDOPARASITES:									
T. urinicola Fulton, 1923	26–36	—	—	—	34–35	—	Urinary bladder	*Triton cristatus, Bufo* sp.	Fresh water
T. okajimae Ibara, 1931	34–38	—	40–60	20–40	—	45–70	,,	*Hynobius tokyoensis*	,,
T. [*V.*] *renicola* (Mueller, 1931) n.comb.	56	—	70–96	—	—	—	,,	*Esox niger*	,,
T. [*V.*] *nephritica* (Mueller, 1938) n.comb.	36–40	10	—	62–75	—	—	,,	*Esox masquinongy*	,,
T. vesicularum Fauré-Fremiet, 1943	21–33	—	50	—	19–22	—	,,	*Triton cristatus, T. palmatus*	,,
T. fariai Da Cunha & Pinto, 1928	24–28	—	40	—	20–42	32	Intestine	*Spheroides testudineus*	Marine
ECTOPARASITES:									
T. labrorum Chatton, 1910	21	—	30–34	—	—	18–22	Gills	*Symphodus tinca* and *S. melops*	,,
T. chelidonichthys Fantham, 1930	30	—	30–45	19–32	—	19–27	,,	*Chelidonichthys cupensis*	,,
T. mugilis Fantham, 1930	32	—	33–34	23–28	—	14–20	,,	*Mugil capito*	,,
T. blennii Fantham, 1930	24–32	5	40–45	24–27	—	20–32	Gills and pericardium	*Blennius cornutus*	,,
T. clini Fantham, 1930	24	—	37	20	—	20	,,	*Clinus anguillaris*	,,
T. halli Padnos & Nigrelli, 1942	26–34	—	45–86	41–81	30–54	—	Gills and skin	*Spheroides maculatus*	,,
T. spheroidesi Padnos & Nigrelli, 1942	21–31	—	17–54	18–32	14–22	12–42	,,	,,	,,
T. branchicola n.sp.	20–26	6–8	30–54	19–33	10–20	22–36	Gills	See p. 440	,,

T. *pediculus* Ehrenberg, 1838	16–26	—	60	—	—	—	Body surface and gills	Various fresh-water fishes, tadpoles and salamanders; *Hydra fusca*, *H. viridis*	Fresh water
T. *steinii* Clarapede & Lachmann, 1858	21–26	—	40	—	—	40	Body surface	*Polycelis nigra*, *Dugesia lugubris*, *Polycelis cornuata*	,,
T. [*C.*] *spongillae* Jackson, 1875, n.comb.	37	—	*c.* 61–60	—	—	—	Inside the body	*Spongilla fluviatilis*	,,
T. [*C.*] *domerguei* (Wallengren, 1897), n.comb.	18–25	—	23–56	—	—	⅕th of diameter	Gills	On various fishes	,,
T. *truttae* Mueller, 1937	28–31	20	120–140	110–125	75–85	—	,,	*Salmo clarkii*	,,
T. *myakkae* Mueller, 1937	17–24	4–5	—	21–25	11–12	—	,,	*Aplites salmoides*, *Ictiobus bubalus*, *Carpoides carpio*, *Salvalinus fontinalis*	,,
T. [*C.*] *guberleti* MacLennan, 1939, n.comb.	28–32	—	50–100	—	—	25	Gills and skin	*Richardsonius balteatus*, *Apocope oscula carringtoni*	,,
T. *tenuidens* Fauré-Fremiet, 1943	27–37	—	—	—	—	41–55	,,	*Gasterosteus aculeatus*	,,
T. *discoidea* Davis, 1947	18–30	6–8	—	35–50	19–29	—	Gills	*Lepomis macrochirus*, *Pomoxis sparoides*, *Ambloplites rupestris*, *Ictalurus punctatus*	,,
T. *platyformis* Davis, 1947	26–35	10	—	56–75	31–50	—	,,	*Margariscus margarita*, *Rhinichthys atronasus*	,,
T. *vallata* Davis, 1947	18–21	10	—	38–48	25–30	—	,,	*Ictalurus punctatus*	,,
T. *fultoni* Davis, 1947	25–30	12–14	100	75–90	50–58	—	,,	*Huro salmoides*, *Micropterus dolomieu*, *Lepomis macrochirus*, *Ambloplites rupestris*, *Salmo irideus*	,,
T. *symmetrica* Davis, 1947	21–28	5	—	24–35	13–22	—	,,	*Ictalurus punctatus*, *Margariscus margarita*, *Rhinichthys atronasus*	,,
T. *californica* Davis, 1947	25–32	8–10	—	38–50	25–33	—	,,	*Oncorhynchus tschawytscha*	,,
T. *tumefaciens* Davis, 1947	19–26	7	—	29–30	18–23	—	,,	*Cottus bairdii*	,,
T. *bulbosa* Davis, 1947	19–24	5–6	—	22–26	10–12	—	,,	*Margariscus margarita*	,,
T. *brusiformis* Davis, 1947	24–27	5	—	25–35	14–18	—	,,	*Ambloplites rupestris*	,,

ellipsoidal at first but gradually becomes horseshoe-shaped. No stages of conjugation were observed. I have found one individual in which the nucleus is broken up into small spherules (Fig. 2*e*) and is very much like the fig. 14, pl. II of Padnos & Nigrelli (1942) and figs. 29–36, pl. 3 of Diller (1928). In the absence of other stages it is very difficult to interpret correctly the nucleus phase of this individual.

The rate of fission during the summer increases, for it is only during the months of March to August that various individuals undergoing fission were observed. No quantitative population count was done to find the percentage of the dividing forms. Only one young individual was observed during the period September 1946–March 1947, while several have been observed from April 1947 to August 1947.

Discussion on Taxonomy

Fauré-Fremiet (1943) has recently given a very good systematic review of the family Urceolaridae. He retains only two genera, *Trichodina* Ehrb. and *Urceolaria* Stein., in this family; and he relegates the genus *Cyclochaeta* Jackson as a sub-genus of *Trichodina*, and *Leiotrocha* Fabre-Domergue as a sub-genus of *Urceolaria*.

The members of the genus *Trichodina* are generally found as ectoparasites on the gills and skin of fishes and tadpoles, and as endoparasites in the urinary bladder of fishes and Urodela and rarely in Anura. Mueller (1938) has created a new genus *Vauchomia* for endoparasitic urceolarids. The only difference between *Vauchomia* and *Trichodina*, according to Mueller, is that the former has a system of 'myonemes' and the oral groove makes more than two turns. The shape of the denticulate ring is similar in both these genera. The 'myonemes' have, however, since been described in additional species of ectoparasitic *Trichodina*. The distinction, therefore, does not strictly hold, and the name *Vauchomia* is relegated to a synonym of *Trichodina*.

In distinguishing the different species of the genus *Trichodina* (which now includes *Cyclochaeta* and *Vauchomia* as well) the size, shape and the number of the denticles are of great value. Fauré-Fremiet (1943, p. 163) and Davis (1947, p. 7) have also emphasized this point. Biometrical studies on the variation in the number of denticles in a population of *Trichodina* are of great help in finding out whether that particular population contains one or two species of *Trichodina*. Mueller (1937) pointed out that Wallengren (1897) was probably dealing with more than one species of *Trichodina* which he described as *T. pediculus*. Recently Fauré-Fremiet (1943), on the basis of his biometrical studies, has pointed out that there are really two species of *Trichodina* on the skin of *Gasterosteus aculeatus*. The one with 20–26 denticles is *T.* [*Cyclochaeta*] *domerguei* (Wallengren), and the other with 30–34 denticles he named *T. tenuidens*. The variation in the number of denticles is the same in *T.* [*Cyclochaeta*] *domerguei* and *T. branchicola* n.sp., but the size of the denticles and the shape of the body differ in the two species.

At present there are 31 species of *Trichodina* out of which 16 are freshwater, 8 marine, 6 endoparasites in the urinary bladder, and one in the intestine of *Spheroides testudineus*. In Table II the measurements, habitats and hosts of all the species of *Trichodina* are given.

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SUMMARY

The morphology and reproduction of *Trichodina branchicola* n.sp. are described. Its total diameter is 30–54 μ, and its height is 20–36 μ; the diameter of the striated ring is 19–33 μ, and the diameter of the denticulate ring 10–20 μ. There are 20–26 denticles in the ring, with 6–8 striations to each denticle. The denticles measure 1·5–2 μ in thickness, 3·5 μ in length, each with a broad outer hook 2·3 μ long and an inner ray 3·2 μ long. The aboral cilia are fused at their base to form a complete membranelle.

This organism is found on the gills of the following marine fishes at Plymouth: *Ciliata mustela*, *Gaidopsaurus tricirratus*, *Cottus bubalis*, *Spinachia spinachia*, *Blennius pholis*, *B. guttorugine*, *Trigla lucerna* and *Pleuronectes platessa*.

The genus *Vauchomia* Mueller 1938 is regarded as a synonym of *Trichodina*.

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