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TRICHOPHRYA PISCIUM: A PATHOGEN OR AN ECTOCOMMENSAL? AN ULTRASTRUCTURAL STUDY

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Abstract. The present paper describes the fine structure of *Trichophrya piscium* Bütschli, 1889 as far as the relation to the fish host is concerned. *Trichophrya* feeds by means of typical suctorial tentacles on ciliates from the surrounding water. The well-known, large inclusions in its cytoplasm are ingested parts of the prey at various stages of digestion. The attached face of the cell is equipped with tiny suctorial organelles which were never observed to contact the host cell. Their function is not known. A new name, scopuloid, is proposed to designate the adhesive (and often stalk producing) area on the surface of suctorian ciliates. In *Trichophrya*, pellicular pores of the scopuloid secrete a fibrous layer which secures a firm attachment of the ciliate to the surface of the gills. In addition to fine microfibrils, it contains unique structures, "attachment spirals", extending between cell membranes of *Trichophrya* and epithelial cells. The cells invaded by *Trichophrya* do not reveal any signs of damage. The ultrastructural evidence is in favour of ectocommensal nature of this protozoon.

The surface of fishes is inhabited by a large variety of protozoan parasites. Some of them, e.g., *Ichthyophthirius multifiliis*, are well-known as pathogens, in others the question of their pathogenicity continues to be a controversial issue. Along with some other species (representatives of the genera *Apiosoma*, *Epistylis*, *Cryptobia* etc.), *Trichophrya piscium* Bütschli 1889 has repeatedly been reported as the cause of fish diseases or even fish kills.

The first author to comment on the action of *Trichophrya* on its host was Mazzarelli (1906). He erroneously treated this ciliate as *Caprinia aurantiaca*, n.g., n.sp., but his illustration as well as description clearly show that he was dealing with *Trichophrya piscium*. According to his observation a massive infection on the gills provoked an abundant mucous secretion and desquamation of epithelium. The ciliates were allegedly feeding on this material. The infection resulted in a chronic or subchronic catarrh of the gill epithelium. An opposing view was held by Parisi (1920), who considered *Trichophrya* to be a harmless or perhaps even beneficial ectocommensal on the surface of *Alosa finta*, feeding on the ectocommensal fish ciliates or on those driven by in water currents. All following reports with the exception of Culbertson and Hull (1962), however, were in favour of the truly parasitic nature of *Trichophrya*. In 1937, Davis recorded a mass mortality of *Micropterus dolomieu* in a West Virginia fish farm which he attributed to the action of *Trichophrya micropteri*, present in masses on the gills of the fish. In a later paper (1947) describing another new species of the genus, he mentioned the way in which the ciliates are closely attached to the gill surface. Very often the epithelial cells are destroyed or shifted aside so that the parasite achieves a direct contact with the capillary system. Later (1956), he quotes *Trichophrya* among established protozoan pathogens of fish, describing a case of heavy losses of fingerling and adult *Micropterus dolomieu*: "... the parasites were present in large numbers... and were the primary cause of mortality, although secondary infection of fungus was undoubtedly a contributing factor". According to Davis,

Trichophrya may ingest liquids from the gills of the host through the tentacles, but its food is primarily derived from the organisms in the plankton; the parasites injure the gill chiefly through their presence on the lamellae-irritation, interference with respiration. In heavily infected specimens the distal ends of the gill filaments become enlarged and club-shaped owing to epithelial hyperplasia. Later, having become necrotic, the gills are attacked by fungi.

According to Prost (1952), *T. intermedia* adheres to deep depressions in the surface of the gill epithelium and the suctorial tentacles are very often in contact with the epithelial cells in such depressions. Therefore, she supposed that the ciliates might feed on the host cells, especially when she did not encounter in her preparations any trichodinids or other possible prey. Moreover, the author suggested that *Trichophrya* reduces the respiratory function of the gills by covering large areas of its surface. Nie Da-Shu (1956, 1957—according to Bogdanova 1962) reported serious diseases in *Ctenopharyngodon idella* caused by the infection of *T. sinensis*. He did not elaborate, however, on the precise way in which the protozoan hurts the host. Chen Chih Leu (1955) suggested that *Trichophrya* may feed not only by means of suctorial tentacles, but also through the adhering surface of the body. Bogdanova (1962) observed a mass kill of *Coregonus lavaretus* fingerlings. She noticed that the colour of gil filaments was pale pink with a yellowish hue, due to cell inclusions in massively attached *T. piscium*. From her observations and previous records she concluded that the heaviest infections occur in summer, while in other periods of year the invasion was less intensive. In a very thorough paper, Culbertson and Hull (1962) analysing the taxonomic situation in the genus gave a good evidence that there was a single species, *T. piscium*, a senior synonymum to all later described species. They investigated the pigment found in *Trichophrya* populations from *Salmo* and *Oncorhynchus*. Judging from its solubility, they identified it as melanin. Discussing the probable origin of the pigment, they excluded the possibility that it might come from the fish host melanophores, since among other reasons they could never observe any necrotic spots associated with the presence of the suctorians. Observations of Kozicka (1966) on *T. piscium* from *Coregonus albula* and *Perca fluviatilis* were strongly in favour of the pathogenicity of the suctorian. She believed that the ciliates attached in numbers to the gill filaments exert a constant pressure upon the gill cells which results in their destruction. The epithelial cells are allegedly flattened, loosened and finally peel off. The same pressure damages also the blood capillaries of the gill filaments. She could not disclose the source of nutrition. Without a really persuasive evidence, she assumes that the ciliates feed on red blood corpuscles of the host which first have to escape in some way from the blood vessels into the epithelial layer.

This account of contradicting observations on the feeding of *Trichophrya* reflects the confusion in views on the pathogenesis of this parasite and documents at the same time the general belief in its pathogenic action. To corroborate or to disprove such assumption we undertook the present study.

METHODS

The ciliates were found on the gills of *Etheostoma flabellare* collected in September 1969 in streams in the vicinity of Coal City south-east of Joliet, Illinois. The invasion being heavy, it could be estimated that about 50% of the gill filament surface was covered by the attached suctorians. However, the fish were quite symptomless and of a healthy appearance. The whole gill arcs were fixed in toto in various fixatives and small pieces were then cut out. Good results were obtained with 2 % osmic acid in Palade's buffer (30 minutes to 2 hours); glutaraldehyde fixation with postosmification was superior only in a better preservation of fibrils associated with kinetosomes of the larval stages. The material was embedded in epon-araldite (Mollenhauer 1964—mixture No. 1), sections were cut on the Sorvall Ultratome II., mounted on parlodion—coated and on uncoated grids and doublestained with uranyl acetate and lead citrate. They were examined with a Hitachi 11 B electron microscope operated at 75 kV accelerating voltage. For complementary observations additional material from other species of fish was kindly supplied by Dr. T. L. Wellborn, to whom I wish to express my sincere gratitude. This material yielded exactly the same results as my material from *Etheosoma flabellare*.

RESULTS

Two questions were in the focus of our attention. First, how does the *Trichophrya* attach itself to the epithelial cells and to what extent does this attachment affect the occupied cells, i.e., the trichophryan—host cell interface; second, what does the *Trichophrya* actually feed on.

A. THE ATTACHMENT OF *TRICHOPHRYA*

As evident already from numerous microscopic observations (e.g. Davis 1947, Chen Chin Leu 1955, Lom 1960), *Trichophrya* is either wedged firmly between the base of two neighbouring secondary gill platelets, or adheres to the surface of the latter. Sometimes, the attachment area is limited to the basal part of the cell, in other cases the whole flattened cell of the ciliate adheres to the epithelial surface. The pellicle has essentially the same structure all over the body, including the attached face, except for the frequency of pellicular pores and the suctorial organelles. In *Trichophrya* the structure of pellicle is of the type widely distributed among suctoria and clearly depicted by Jurand (1966) in *Podophrya* (Fig. 1 A.) The pellicular alveoli, flat and without any inclusions, are 120 to 600 Å thick on the unattached face of the cell, their thickness may reach 0.4 μ. The inner face of pellicular alveoli is subtended by an epiplasmic layer about 280 Å thick; on the attached face, the pellicular alveoli are of a much smaller square area than those on the free surface. The latter may have the diameter (as seen on cross sections) of up to 25 μ, so that the septa separating individual alveoli are almost absent on some areas of the surface. The only joints of the membranes are the discharge openings of the pellicular pores and the suctorial organelles.

Irregularly situated below the epiplasm, the single ectoplasmic microtubuli of the outer diameter of about 270—290 Å observed a more or less parallel course. They are never found in two or more layers. The outer cell membrane covers the pellicular alveoli all over the body. Pellicular pores (Fig. 1A; Pl. II—1, 2,; Pl. III—3) of an average depth of 0.24—0.38 μ are lined with the outer cell membrane. Their aperture has the diameter of 410 Å to 1,000 Å, evidently in relation with the state of its contraction. Basically, these pores are distributed over the whole body surface. However, on the free surface of the suctorian, they are extremely scarce, being abundant on the attached face. In some parts of the attached surface, there are more than 10 pores per 1 μ². The pores seem to be responsible for the production of the layer of the substance gluing the parasites to the host cells. This layer, 700 to 8,000 Å thick, was rarely adequately preserved in our micrographs. It was due to preparation-induced shrinkage. In most cases there was a wider gap between the boundaries of the host and suctorian cells. In well preserved specimens (Pl. II, 1) the cementing layer appears to be made up of quite homogeneous, agranular material of low electron density. Under close inspection, densely dispersed microfibrils and special coiled structures can be distinguished. The homogeneous substance is obviously secreted within the pellicular pores which appear full of this substance. Afterwards it is discharged to form the cementing layer. The coiled structures extend obliquely in the space between the host cell membrane and the suctorial pellicle.

In cases where the fixation procedures produced a shrinkage, the suctorial pellicle is separated by a wide gap from the epithelial surface and the cementing substance is reduced to clumps of amorphous appearance; however, the coiled, helical structures can be clearly distinguished. They can be compared to long, flexible bed-springs, each with up to about 18 threads. The diameter of the coils is about 380 to 440 Å, while their spacing depends on the state of the extension of these pellicle structures. At higher magnification the helices seem to consist of a double thread or, more likely, to be a hollow structure of a diameter of about 80 Å. As Fig. 1, 2, Pl. III shows, the helices are solidly attached both to the host cell membrane and to the pellicle of the suctorian, leaving the impression of a spring-padded attachment. Thusfar, there is no evidence that these attachment spirals originate in the pellicular pores. It seems necessary to assume that they are assembled from the secreted material after it had been discharged through the pores. None of these structures, neither microfibrils nor the

attachment spirals, do affect the host cell membrane except for being attached to it. At no place does the cell membrane of the epithelial cell seem to be penetrated or disrupted. The cytoplasm of the cells bearing attached suctorians did not reveal any significant changes testifying an adverse effect of the attachment (see Pl. I).

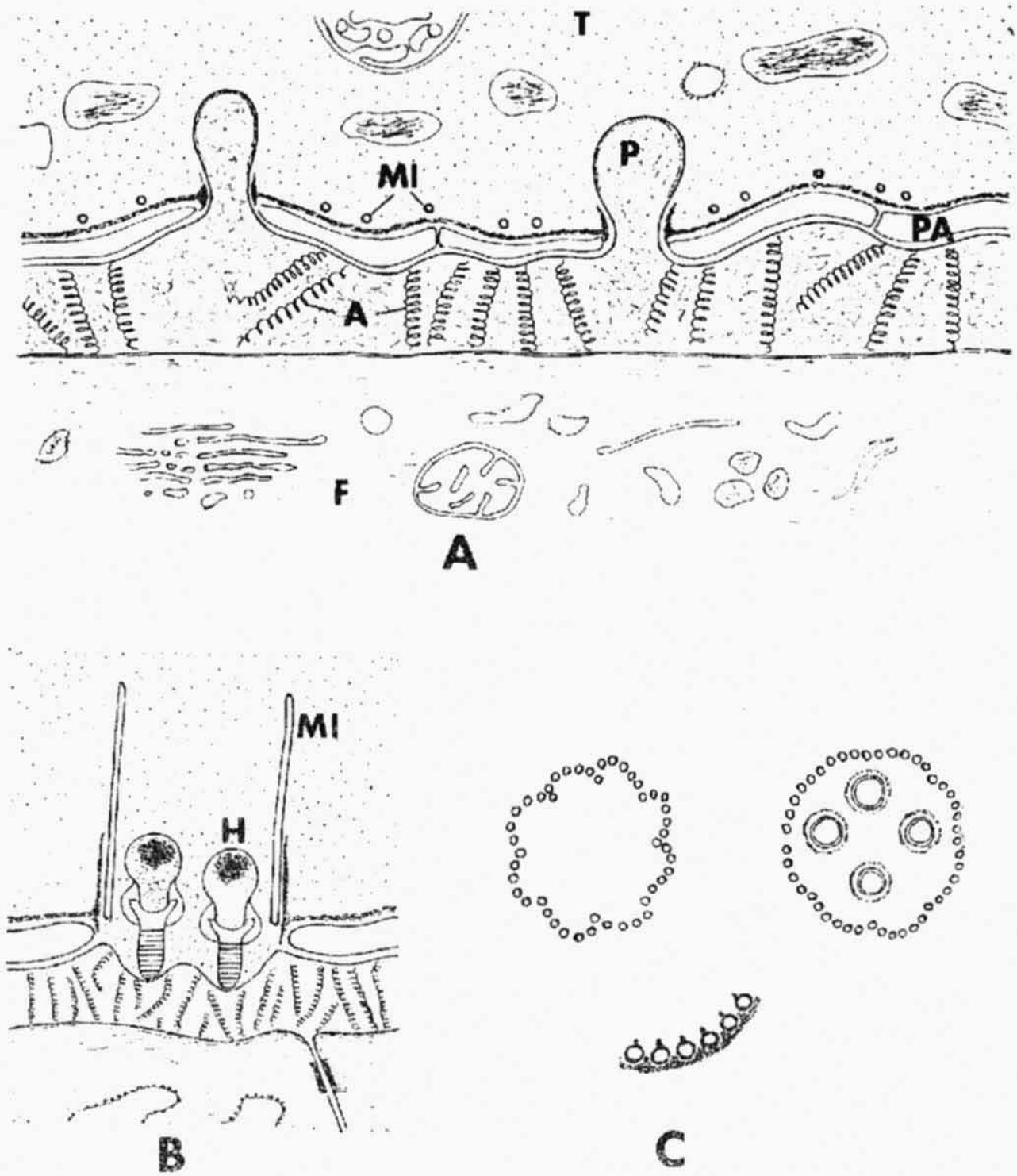

Fig. 1. A—diagramatic drawing of *Trichophrya*-host cell interface. B—longitudinal section of a suctorial organelle. C—suctorial organelle in transverse section: upper left of the proximal part, upper right of the distal part; bottom, detail of the microtubular wall.

Abbreviations: A—attachment spirals, F—cell of the fish host, H—haptocyst, MI—microtubuli, P—pellicular pore, PA—pellicular alveoli, T—*Trichophrya*

B. FEEDING OF *TRICHOPHRYA*

Like other Suctoria, *T. piscium* has well developed suctorial tentacles. They are built according to the same plan as in other suctorians. Their structure corresponds fully, *e.g.*, to those found in *Acineta tuberosa* (Bardele and Grell, 1967). Most of the tentacles are arranged in one to more bundles. They tend to extend away from the ciliate's substrate into the surrounding water where they might come in contact with the prey, a free swimming ciliate. Some light microscope preparations, also of sectioned material, might have perhaps given the false impression that some of the tentacles contact the epithelial cells of the gill. We were unable to prove this in our electron microscope preparations. Neither did the inspection of thick sections of Epon-embedded material observed in the light microscope reveal a contact of tentacles with the host tissue. The electron microscope explained the true nature of numerous large and small inclusions seen in the cytoplasm of all *T. piscium* specimens as light-breaking granules. These are digestive vacuoles containing portions of the captured free living ciliates (Pl. IV) at various stages of digestion. Mostly they contain whole tufts or single cilia with kinetosomes, intact mitochondria of a struture different from *Trichophrya*'s own, and fragments of pellicle. The more advanced the digestion of the food particles, the more electron dense their appearance; finally, it very often results in formation of myelinic figures.

In addition to the above mentioned typical suctorial tentacles, *T. piscium* is equipped with much smaller organelles, very similar to the primitive suctorial organelles of the curious ectoparasitic suctorian *Phalacroleptes verruciformis* (Lom and Kozloff 1967). The suctorial organelles in *Trichophrya* (Fig. 3, Pl. II; Fig. 1B) do not protrude above the surface of the body; only their very tips form an elevation rising about 0.1 μ above the surface of the pellicle. The fibrillar sheaths consist of a single ring of microtubular ribbons (there are 2 rings in the large tentacles of *Trichophrya*) and it has a diameter of about 0.4 to 0.54 μ (compared to 0.6 to 0.75 μ in suctorian tentacles). Its length does not surpass 0.9 μ reaching mostly about 0.7 μ, in contrast to tenths of microns in suctorial tentacles. There are 1 to 5 haptocysts located in the apex of the suctorial organelle, as opposed to many in the knob-like type of the suctorial tentacles. These peculiarly simplified organelles are found practically exclusively on the attached face of the cell. As far as we could see in the migratory larvae formed in the brood pouch of adult ciliates, the suctorial organelles are present also only on one side of the larva, corresponding to the attachement face of the adult ciliate. However, they are already developed at this stage, *i.e.*, long before the suctorial tentacles are formed. Although the suctorial organelles face the surface of the host cell across a gap of only a fraction of a micron, they have never been observed to pierce its surface or to be closely attached to the cell membrane. The haptocysts were always in the apical position without any sign of functional change. The possible, though in this case not very probable assumption, that the pellicular pores might serve as sites of pinocytosis of particles dispersed in the surrounding water, was given no support in experiments with Thorotrast. After exposures to various dilutions of Thorotrast for various periods of time, none of the pores contained any Thorotrast particles.

DISCUSSION

We could clearly observe that the ciliate feeds by means of suctorial tentacles on other ciliates. In some fish specimens used for the above observation, the gills were invaded also with mobiline peritrichs of the genus *Tripartiella*. In no instance were any recognizable parts of *Tripartiella* observed within the cell of *Trichophrya*, although the dentic-

les of the adhesive disc of the former would be a very good marker. *E.g.*, they remain visible for a long time in the cytoplasm of the holotrichous ciliate *Hemiophrys*, which feeds on trichodinids. This corresponds with the observations of trichodinids gliding over the *Trichophrya*-infested gills without becoming prey of the suctorians. *Trichophrya* evidently captures free-swimming ciliates from the surrounding water, driven constantly through the gill basket. This also explains why these ectocommensals are never found on the skin—unlike "true" pathogens as, *e.g.*, *Ichthyobodo*, *Ichthyophthirius*, *Chilodonella*, *Oodinium* or even peritrichous ectocommensals of the genus *Apiosoma*. The site on the gills is much more convenient for capture of prey. The suctorial tentacles have never been observed in contact or even to feed on the epithelial cells or tissue of the host and there is no reason to consider such a possibility.

The function of suctorial organelles however is not clear, and the explanation of their function may be controversial. While existing only on the adhering face of the ciliate, they have never been found in contact indicating possible feeding on the host cell. Since they are formed already in the larval stage, they may—perhaps—function at the very beginning of the adult sedentary phase of the life cycle after the larval stage has just settled down on the epithelium. If this is true, they could function to a limited extent by feeding on the host cell for a very short period of time before the suctorial tentacles are developed again. However, the metamorphosis of the swarmer, once it has settled on the substrate, goes on very quickly. *E.g.*, in stalk-forming suctorians of the genus *Tocophrya*, it takes only one (Noble 1932) to 4 hours (Claparède and Lachmann 1858—1861) to produce even a long complicated stalk. However, we found no evidence that the adult ciliates feed on anything but free-swimming protozoa from surrounding water. The attachment layer also did not provoke any alteration whatsoever suggesting a damage done to the host cell.

Selecting its food from a source other than its host, inflicting no damage upon the invaded cell (except, if our conjecture is true at all, by the tiny suctorial organelles for a very limited extent and time), *Trichophrya* has not a truly parasitic nature and has to be classified rather as an ectocommensal. Reported massive occurrence of *Trichophrya* in cases of diseased fish or even fish kills is not incidental, but it is always questionable if this ciliate had been, in fact, the primary cause of the trouble, even if it was the only one detectable by superficial inspection. A fish in a good physiological condition is able to repel or control the surface faunule of, *e.g.*, peritrich ciliates of the genera *Epistylis*, *Apiosoma*, *Trichodina*, *Trichodinella* (Lom 1969), at least their ample growth. If they do proliferate massively, there is always something wrong with the host's defense system. *Trichophrya*, too, invades to a noticeable degree evidently only fishes with a lowered resistance. Having multiplied on a fish weakened for some other reasons, masses of *Trichophrya* may, of course, finally interfere with the normal state and function of the gill platelets or cause an irritation, as would any other object sticking to the gill tissue in large numbers. This, of course, is to be regarded as a secondary consequence. There is a clear difference between the true protozoan ectoparasites of fish and the ectocommensal nature of *Trichophrya*.

The small suctorial "tentacles" in *Trichophrya* represent another type of this kind of organelles in Suctoria. So far, four types of suctorial tentacles have been recorded in this group of ciliates: 1. The prehensile tentacles, serving only for food capture, not ingestion, and found thus far in *Ephelota*, *Acinetopsis*, and *Paracineta limbata*. In *Ephelota*, they are pointed and have a solid central axis of mictrotubules (Fauré-Fremiet et al. 1956, Batisse 1965). In *Paracineta* (Hauser 1970) their outer shape resembles the suctorial tentacles. 2. The "normal", most widely distributed type of suctorial tentacle, serving for capture and ingestion of prey (*e.g.*, *Tocophrya*—Ruzinska 1965, *Acineta*—Batisse 1966, Bardele 1968). 3. The aberrant type of the latter found

in *Pseudogemma* as an adaptation to special living conditions on another suctorian (Batisse 1968). It serves for permanent attachment to the host ciliate and lacks completely the otherwise generally distributed haptocysts. 4. Suctorial organelles in *Phalacroleptes verruciformis*. In this very primitive type of suctorial tentacles, the microtubular sheath is reduced to 8 pairs of short microtubules, it contains but a single haptocyst and the overall length of the organelle is about 0.4 μ. The small "tentacles" of *T. piscium* display really a much closer kinship to suctorial organelles of *Phalacroleptes* — and thus they fully deserve this term —than to typical suctorial tentacles. They are extremely short, hardly protruding over the surface, their microtubular tube consists of merely 1 ring of microtubular ribbons, they have no apical knob-like enlargment and possess but 1 to 5 haptocysts. In spite of the fact that numerous suctorian genera were examined with the electron microscope (just to quote the most important ones— Rudzinska 1954, 1965, 1967, Jurand 1966, a series of papers by Batisse 1966 to 1968, Fauré-Fremiet et al. 1956, 1967, Mignot et DePuytorac 1968, Bardele 1968, 1970 etc.) none of the genera of the family Dendrosomatidae, to which *Trichophrya* belongs, has been chosen thusfar for an electron microscope examination. Thus we may not exclude that other symphoriont genera of this family (*e.g. Lernaeophrya*, *Dendrosomides* or *Rhabdophrya*) are also equipped with suctorial organelles of this type, so that it would be a more general character in this family, rather than a special adaptation developed solely by *T. piscium*.

The basal plate secreted by the suctoria in order to attach itself firmly to the cell membrane of the host is homologous with basal platelets of stalks in stalked genera and with "basal disc" in *Dendrocometes paradoxus* and *Heliophrya erhardi* (Bardele 1970). Suctorial stalks and their basal platelets contain as the most common constituents, a mass of microfibrils (*e.g. Acineta*—Batisse 1966, *Discophrya* Mignot and DePuytorac 1968). In addition to that, stalks in some genera contain a more elaborate kind of fibrils (*e.g.*, striated fibrils in *Acineta*—Batisse 1966). The attachment platelet of *Trichophrya* consists of microfibrils and of a special kind of fibrils. The latter, the attachment spirals, are a structure unique thusfar not only among suctorians, but among other protozoan species as well. They deserve a thorough future study to elucidate their chemical composition. Linked between the host-parasite surfaces, they behave as elastic elements changing their shape with the degree of extension. Perhaps their function really contributes to the resilience of the *Trichophrya*-host cell junction. The secretion of the attachment substance through pores complies with the patterns of stalk formation in most other suctorians (*e.g. Discophrya*—Mignot and DePuytorac 1968, *Paracineta*—Hauser 1970, *Acineta*—Bardele 1970). Though in some species (*Acineta tuberosa*, *Acineta homari*, *Metacineta*), the elaborate skeletal structures of the stalk are believed to be secreted through the whole surface of some parts of the body (Batisse 1966, 1967), there is no distinct source of the secreted material inside the cell of the suctorian; it is unlike the presence of osmiophile granules found in the cytoplasm of *Paracineta limbata* which were described to be secreted through the pellicular pores.

The whole attachment surface of *Trichophrya* corresponds to what was formerly named by many authors a scopula and what the French authors call "*fosette de fixation*". This area of the pellicle, restricted in some and very large in others, is functionally analogous to scopula in peritrich ciliates by securing the firm attachment of the protozoan to the surface of the substrate. In some suctoria, it produces elaborate stalks like in peritrichs. However, it lacks the characteristic feature of peritrich scopula, *i.e.*, the scopular kinetosomes, ciliated or not. It is equipped only with pellicular pores of secretory function. In most species observed they did not differ from pellicular pores of the rest of the body surface. The term "scopula" is evidently unsuitable for the attachment surface of Suctoria; the term "fosette de fixation" could be logically

related also to other kinds of attachment surfaces in ciliates. We, therefore, propose the term "scopuloid" to indicate the functional analogy with scopula, but yet to stress the separateness of both structures.

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EXPLANATION TO THE PLATES

Plate I.

Fig. 1. *Trichophrya piscium* wedged between the basal part of two secondary gill filaments. The bundle of suctorial tentacles is located outside the field of vision of this picture. The adhering surface of the ciliate is to the left and continues for a short distance above the bottom part of the right secondary filament. Conspicuous inclusions are digestive vacuoles containing prey cytoplasm. × 7 800.

Plate II.

Fig. 1. *Trichophrya*- gill epithelium interface in well preserved state. × 55 000. **Fig. 2.** Pellicular pore (P) on the adhesive face of the ciliate. × 71 000. **Fig. 3.** The border of the adhesive face of *Trichophrya*. The ciliate host-cell interface is well preserved. The suctorial organelle is separated from the host cell by a layer of secreted substance. × 33 000.

Plate III.

Fig. 1. Transverse section of the suctorian-host cell interface. Due to slight contraction the gap is somewhat wider, but the attachment spirals (A) are clearly visible. × 52 000. **Fig. 2.** A detail of the ciliate-host cell interface; note the double outlines of the attachment spirals. × 153 000. **Fig. 3.** Tangential section of the attachment surface showing transverse section of the proximal part of one suctorial organelle and numerous pellicular pores. × 45 000.

Plate IV.

Fig. 1. Part of the *Trichophrya* cytoplasm with one large (to the left) and two smaller (to the right) digestive vacuoles containing prey cytoplasm, parts of pellicle with cilia (C) and mitochondria of much denser structure than *Trichophrya*'s own (M). × 28 000.

Abbreviations: A—attachment spirals; C—ingested cilia of the prey; E—epiplasm; F cell of the fish host; H—haptocyst; M—mitochondrium; MI—microtubuli; P—pellicular pore; Pa—pellicular alveoli; S—suctorial organelle; T—*Trichophrya*; V—digestive vacuole;