XL.—CHROMATOPHAGUS PARASITICUS—A CONTRIBUTION TO THE NATURAL HISTORY OF PARASITES.*

By Dr. C. Kerbert.

During the last few months my attention has been directed to a skin disease, which at first threatened to become epidemic, and which affected certain freshwater fishes in the Amsterdam Aquarium. Our representatives of *Tinca vulgaris* Cuv. (in fact *Tinca vulgaris* var. *aurata*, or *Tinca chrysitis* Agass.), *Abramis brama* Linn., *Blicca björkna* Siebold, *Cyprinus carpio* Linn., with the two varieties *Cyprinus rex cyprinorum* and *Cyprinus nudus*, *Carassius vulgaris* Kröyer, *Idus melanotus* var. *miniatus* Heck. and Knerr, *Trutta salar* Linn., *Trutta fario* Linn., and finally *Salvelinus fontinalis* Mitchill, showed all over their skin, but principally about the fins and the head and occasionally about the eyes, small but very distinct milk-white round spots the diameter of which varied from 0.25 to 0.6 millimeter. When these spots were examined microscopically, it soon became apparent that they were caused by infusoria, distinguished by their enormous size. The cause of the disease, therefore, was evident. The disease with which we have to deal here is generally termed "spot-disease" (*Fleckenkrankheit*) by ichthyologists and fish-culturists.

In my opinion this so-called "spot-disease" must not be confounded with phenomena observed in various other different cyprinoids by Wittmack[1] and other naturalists, where, on the surface of the skin, there appear bluish-gray spots of a slimy, fungus-like character, which spread more or less over the entire body, and extend to the eyes, fins, &c. This disease is termed *Pocken-Krankheit*, or "pox," by Wittmack. In his excellent work Wittmack says that possibly the real cause of the disease might be traced to infusoria. I must state, however, that in my examinations of fish afflicted with this disease I have so far not discovered any infusoria. When I examined a *Leuciscus erythrophthalmus* Linn., caught in one of the Amsterdam docks on March 31, which was

* "*Chromatophagus Parasiticus. Ein Beitrag zur Parasitenlehre.*" Von *Dr. C. Kerbert.* Amsterdam, April 9, 1884. Translated from the German by Herman Jacobson.

[1] Dr. L. Wittmack: "*Beiträge zur Fischereistatistik des Deutschen Reichs,*" IV, Diseases of Fish, in Circular of the German Fishery Association, 1875, p. 187.

suffering from this so-called "pox," I found that the latter was a local thickening of the epidermis, a condition of things to which Von Siebold[2] had first called attention. During the spawning season such thickenings of the skin are frequently observed in male cyprinoids, particularly on the head, the cheeks, and the gills, resembling whitish wart-like protuberances. This, of course, does not exclude the possibility that such thickenings of the epidermis, under certain hitherto unknown circumstances, may develop as actual pathological growths.

On the other hand the "spot-disease," *i. e.*, the appearance of white or bright spots on the epidermis of fishes, is invariably caused by animal parasites, in fact by infusoria. This "spot-disease" will make its appearance not only among freshwater fishes, but also among those living in salt water. When I examined a *Mustelus vulgaris* Müll. and Henle, and an *Acanthias vulgaris* Risso, both afflicted with this disease, I found the cause of the spots to be infusoria which, living in the pulp-cavity of numerous placoid scales, had absorbed their contents, pigment cells, &c., as nutriment, and had consequently produced colorless patches on the skin. The rapid decay of the fish unfortunately prevented me from making a more thorough examination of this species of infusorians.

Hilgendorf and Paulicki, of Hamburg, were the first to report the existence of infusoria as parasites on the skin of freshwater fishes.[3] In the aquarium of the Hamburg Zoological Garden the "appearance of slimy excrescences, finally assuming a fungus-like appearance, and causing the death of the fish," was observed in 1868 in many different kinds of fishes in the freshwater tanks. The above-mentioned naturalists discovered in the epidermis, which had become thickened owing to the fungus-like growth, small infusoria, measuring about 0.5 millimeter in diameter, which at first were only considered as occurring casually, but which, on closer examination, were found to be the cause of the disease. Hilgendorf and Paulicki could not discover in these infusoria either a mouth or cilia of any considerable size, or any characteristic shape of the body. The cuticle was covered with very fine evenly-developed cilia, arranged in long, gently-curved lines standing close together. All that could be recognized in the body was a nucleus (in large specimens having the shape of a horseshoe), the contractile vesicle, vacuoles, and granules. According to Hilgendorf and Paulicki, these infusoria appertain to a genus termed by Ehrenberg *Pantotrichum*. In some of them, which had gathered at the bottom of a glass vessel, the process of fission was observed. In my opinion Hilgendorf and Paulicki very correctly consider the appearance of the infusoria as the primary, and the development of fungus as the secondary, process.

This entire process resembles very strongly another phenomenon ob-

[2] Von Siebold: *"Die Süsswasserfische von Mittel-Europa."* Leipsic, 1863, p. 89.

[3] Dr. F. Hilgendorf and Dr. A. Paulicki: *"Infusionsthiere als Hautparasiten bei Süsswasserfischen,"* in *Centralblatt für die Medicinischen Wissenschaften*, 1869, No. 3, p. 33.

served in 1876 by D. Fouquet[4] in trout fry in the fish-cultural tanks of the College of France. For about ten years an epidemic was raging among the young individuals of *Trutta fario* L. contained in said tanks, "caused by the presence of infusoria," described in a treatise by Fouquet. It will be necessary to enter more fully into this investigation which Fouquet made, under the direction of Balbiani, because many of the phenomena described by him are closely analogous to those observed by me, while on the other hand there are many points in Fouquet's treatise which can in no way be harmonized with my observations.

The symptoms of the disease in the case observed by Fouquet doubtless agree with those described by Hilgendorf and Paulicki, as observed by them in the Hamburg Aquarium, and those recently observed by me.

Fouquet likewise observed on the skin, fins, eyes, and gills very distinct round milk-white spots, having a diameter of from 0.3 to 0.8 millimeter. A microscopical examination showed that each spot had been caused by the presence of a ciliated infusorian. While Hilgendorf and Paulicki state distinctly that "the epithelium, without exhibiting any other changes, forms a considerable protuberance over the parasite," Fouquet[5] has observed that "the skin is covered with a viscous layer, and that the cells of the epithelium have changed." Sometimes Fouquet finds two or three infusoria of the same or different size in the same cyst, and states distinctly that the form of the young individuals of these infusoria differs from that of the grown individuals.

The former, he says, are "longer," and the latter "more globular." The cuticle is very elastic, transparent, tolerably tough, and covered with very fine cilia, "presenting everywhere the same length, and running in lines which are twisted in spirals and cross each other." The cortical layer [ectosarc] is pale, forms "a white band," and contains scarcely visible trichocysts and numerous contractile vacuoles. The endoplasm is granular and is not stained by carmine. It contains "blackish pigment spots of irregular shape," which, however, do not occur in all cases. At the anterior pole Fouquet observed a small round opening, measuring 0.04 millimeter in diameter, surrounded by much larger and thicker cilia than are found on other parts of the surface of the body, radiating from the periphery toward the center. Below this there is a small cavity terminating in a cul-de-sac, and formed by a transparent membrane. Fouquet states further in a very emphatic manner that this orifice is not the mouth opening, but that it is rather "a modified mouth, transformed into a sucking-disk." In proof of this strange assertion, he states that he never found solid food inside

[4] D. Fouquet: "*Note sur une espèce d'infusoires parasites des poissons d'eau douce*," in *Archives de Zoologie expérimentale*, published by Henri de Lacaze-Duthiers, vol. v, 1876, p. 159.

[5] Fouquet, I, p. 34.

the body, and that he had never succeeded in making these infusoria absorb carmine. Fouquet therefore supposes that this apparent opening is an apparatus used by the infusoria for adhering to other objects, and that food is absorbed only by an endosmotic process, as in *Ophalina*. So far, however, it has remained utterly incomprehensible to me how a parasite living between the epidermis cells of a fish, which has therefore no immediate access to liquid food, could absorb food by an endosmotic process.

After Fouquet has mentioned the horseshoe-shaped nucleus, he gives a full description of the mode of reproduction of these infusoria. The fully developed individuals go through a process of encystation, and ultimately of fission, which, after three or four days, results in the production of numerous small infusoria (0.046 millimeter long and 0.028 millimeter broad). The sarcode of these young infusoria is not of a granular character, and they possess no sucking-disk. Whether instead of this apparatus these young individuals have a mouth opening, or what changes the anterior pole of the body undergoes during its further development—in what way, that is, the sucking disk develops in the grown animals—all these are important questions which present themselves to the reader of Fouquet's treatise, but which he leaves entirely unanswered.

Fouquet states, in conclusion, that in the fish-cultural tanks of the College of France this infusorial disease affects the young trout only during three months of the year, from the end of May till some time in August. The young trout became completely emaciated, the epidermis formed became thickened at certain points in which the infusoria were gathered; finally, fungi began to form, and soon death came. Fouquet thinks that a higher temperature of the water, and an increased supply of the same, favor the development of the infusoria. When, in 1876, the hatching troughs received their water from another source, the disease, and with it the infusoria, disappeared.

Fouquet finally discusses the question as to the place to be assigned in the system to these infusoria. In view of their structure and shape, manner of propagation, and anatomical differences between the young and the grown individuals—all of which differ greatly from anything observed in other infusoria—Fouquet assigns to this infusorian a special place, and calls it *Ichthyophthirius multifiliis*, grouping it with the heterotrichous infusoria.

As a third communication relative to the occurrence of infusoria on the skin of fishes, we must mention a statement made by Livingston Stone,[6] who, in the work referred to, takes great pains to describe a parasite on the skin of *Trutta fario* L., which, in the spring of 1872, made its appearance in his hatcheries in vast numbers. The description, however, is so superficial, so vague in every respect, that I find it

[6]Livingston Stone: "Domesticated Trout: How to breed and grow them." 3d edition. Charlestown, N. H., 1877, p. 277. (Appendix I. A new discovery.)

utterly impossible to determine whether the parasites in question are really infusoria. To judge from the very primitive wood-cuts accompanying the article, the parasites may have been *Rotifera*, and in all probability were merely worm-shaped atrochous rotifers, belonging to the genus *Albertia* Duj.,[7] or *Balatro* Olap.,[8] or perhaps to *Dictyophora* Leidy.[9]

Von La Valette St. George[10] mentions the articles by Hilgendorf and Paulicki, and by Livingston Stone, but makes no further statement relative to the occurrence of infusoria as skin parasites.

In the most recent and very extensive work on infusoria, by Saville Kent,[11] this naturalist in speaking of the distribution of the infusoria (vol. i, p. 109) mentions also those forms which so far have been known to zoologists as endo parasites, or ecto-parasites; among the rest, the *Ichthyophthirius multifiliis* described by Fouquet in his article referred to above.

In the "Systematic description of the Infusoria-ciliata"[12] Saville Kent mentions this infusorian, and on the basis of Fouquet's observations establishes among the infusoria-ciliata a new family for the *Ichthyophthirius multifiliis*, which he calls the *Ichthyophthiriidæ* Saville Kent, distinguishing this from the other families by the following:

"*Fam. VIII. Ichthyophthiriidæ* S. K.—Animalcules adherent, more or less ovate, ciliate throughout, oral cilia of larger size than those of the general cuticular surface, oral region adhesive, acetabuliform."

Saville Kent, however, establishes this new family of ciliates with an expressed reservation, which I can very well understand. He very justly remarks[13] that the presence of a sucking-disk and the absence of a mouth opening in these infusoria are points by no means certainly determined. For physiological reasons the absence of a mouth opening is characteristic only of endo-parasites, as is very distinctly shown by the different species of *Opalina*. The occurrence of spots of a blackish color[14] in the body substance sufficiently opposes the opinion entertained by Fouquet, that there is no oral opening. In all probability these black spots were nothing but granules of figment from the epidermis of the fish.

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[7] Dujardin: "*Histoire naturelle des Infusoires.*" Paris, 1841, p. 653.

[8] Claparède: "*Miscellanées zoologiques,*" in *Annales des Sciences Naturelles*, vol. viii, 1867.

[9] Leidy: "Retifora without rotatory organ," in Proceedings Acad. Phil., 1882, pp. 243–250.

[10] V. La Valette St. George: "*Ueber die Feinde der Fische,*" in Circular of the German Fishery Association, 1879, p. 77.

[11] W. Saville Kent: "A Manual of the Infusoria, including a description of all known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and foreign." London, 1880–'82. Vols. i, ii (text), and iii (plates).

[12] Saville Kent, vol. ii, p. 530.

[13] Saville Kent, p. 531.

[14] Fouquet, p. 169.

Farther on we shall have occasion to refer again to Fouquet's investigations and the new family of *Ciliata* established by Saville Kent, and we now return to the infusorian discovered by me. As has already been mentioned, we observed on the skin of various freshwater fishes in our aquarium—principally on the fins and the head, sometimes also on the eyes and on the opercula—very distinct milky-white round spots, whose diameter varied from 0.25 to 0.6 millimeter. Microscopic investigation of these spots showed that they were due to the presence of large infusoria, whose form and structure I have endeavored to represent in Fig. 1, drawn from life. These infusoria were found on or in the epidermis, and showed between the epidermic cells a very distinct rotating motion, either from right to left or from left to right. Although in most cases a single white spot on the skin of the fish indicated the presence of a single infusorian, I observed in some rare cases two, three, or more infusoria imbedded in the epidermis close by each other. The shape of the infusoria was in most cases oval, with a long diameter of 0.615 millimeter, and a short diameter of 0.408 millimeter. The globular individuals, which were much rarer, had a diameter of 0.514 millimeter. I must state, however, that I also observed a number of much smaller individuals.

The body is inclosed in a thin, tender, elastic cuticle, covered all over with very fine cilia, about 0.0046 millimeter in length. The layer below the cuticle is finely granular, with a large number of contractile vacuoles of different sizes. Even with the aid of the highest powers of the microscope I have not been able to discover any trichocysts. Even in the live animal the strangely bent nucleus is seen very distinctly shimmering through the plasma of the body (Figs. 1, 2, 3); but when the infusoria have been killed by osmic acid (½ per cent), and are then treated according to the well-known method of Certes,[15] the horseshoe-shaped nucleus in the largest individuals shows a beautiful red color, and a transverse diameter of 0.139 millimeter (Fig. 4). This nucleus consists of a coarsely granular substance and is enveloped in a very delicate membrane, which appears very distinctly in individuals which for some hours have been kept in a glass cell. I have not noticed a nucleolus in any of the individuals examined by me.

The endoplasm proper contains a large number of granules and particles of different size; and in most individuals—and this is important—there may be observed very distinctly larger or smaller heaps of pigment granules which show a very striking resemblance to the granules found in the pigment cells and chromatophores of the epidermis of fish. The plasmic contents of these infusoria at any rate contained many small particles, which, as I suppose, can only have entered the endoplasm from without. This supposition was confirmed by the discovery of a very distinct oral opening with a well-developed gullet, which was

[15] A. Certes: "*Sur une méthode de conservation des Infusoires*," in *Compt. Rend. Ac. de Sc.*, Paris, 1879, vol. 88, pp. 433–436.

always found in a somewhat lateral position at the anterior pole of the body. This oral opening (Fig. 1) is circular, surrounded by an annular thickening of the cuticle, and has, according to the size of the individual, a diameter of 0.025 to 0.04 millimeter. The annular thickening of the cuticle which surrounds the oral opening proper bears a wreath of somewhat longer and stiffer cilia than are found on the rest of the surface of the cuticle.

The length of the gullet is about 0.02 millimeter, and its diameter about 0.01 millimeter. The inner end of the gullet, which is directed toward the endoplasm of the body, was slightly bent. Its entire inner surface is covered with long, active cilia, the free ends of which are directed toward the oral opening and protrude a little beyond it. These infusoria have no well-defined opening serving as an anus, and, as I have very distinctly observed, the feces are expelled from various parts of parts of the body.

After having given my observations on the structure of these infusoria, I must call attention to the shape of the body, or rather to the extreme variability of its form, in the individuals which have been removed from the epidermis of the fish. If the infusoria are put in a glass vessel, the shape of the body of the different individuals shows such great differences that at first it seems hardly possible that they belong to one and the same species. The body appears to be extremely metabolic (Fig. 2). Folds, emarginations, and projections appear in one place and disappear in another; so much so that one feels inclined to think that the organism is not an infusorian but an amœba. In fact, I once observed an individual (Fig. 3) that was retort-shaped and had a neck, at the free end of which the oral opening and the gullet could be distinctly recognized.

In conclusion, I must discuss more fully the propagation of these infusoria. From the beginning of my observations it struck me that the individuals that, for the purpose of closer examination, were removed from the epidermis of the fish never showed the slightest indication of the process of fission; and any other mode of reproduction of these infusoria living in the epidermis seems hardly possible. During my observations conducted in the daytime I have never noticed the phenomenon of fission. I consequently arrived at the supposition that possibly propagation by fission might take place during the evening, or even during the night, on the epidermis of the fish. I therefore examined the infusoria removed from the epidermis for several hours after sundown, constantly taking new individuals from the epidermis and subjecting them to a very thorough examination under artificial light in Engelmann's live-box. The result, however, was a negative one, and no sign of a process of fission could be detected. In order to convince myself whether such a process actually took place by night, on March 16 and 17 I took from a *Tinca vulgaris* Cuv., to which a large number of infusoria adhered, some infusoria every hour of the night,

and immediately fixed them in the picro-carmine solution, as given by Certes,[16] with a very weak solution of osmic acid ($\frac{1}{8}$ per cent). The individuals which in this manner had been collected all through the night were examined on the following day, but with the same result. Even in these individuals no process of fission had taken place.

These results were all the more astonishing, as invariably individuals of greatly differing dimensions were found on the epidermis, which circumstance could be explained only by the supposition that the smallest individuals had reached the epidermis from the outside. I now examined very carefully the bottoms of the small aquariums which had been provided for the fish experimented with. The bottoms and the glass walls of the aquariums were thickly covered with these infusoria, but even among these I could not, at least by daylight, find any indications of a process of reproduction by fission. The case was different, however, with these infusoria after sundown. During four evenings I examined the infusoria at the bottom of the aquarium with my microscope by artificial light, and obtained positive results, which were the same every evening. I take the liberty to give below the results of these investigations:

7 p. m. The infusoria became encysted on the bottom of the aquariums. Some of these encysted forms lay entirely motionless in the cyst, while others showed very distinct cilia and were observed to be rotating actively (Fig. 5). Temperature of the water, 8°.5 C. [=47°.3 Fahr.].

7.45 p. m. Most of the encysted forms were in process of fission or division; or the fission had already taken place (Fig. 6). No division of the nucleus could be observed; only once I saw a long nucleus-like body lying perpendicularly to the plane of cleavage of the two individuals.

8.45 p. m. The encysted individuals were segmenting into four parts (Fig. 7).

9.35 p. m. In most of the cysts eight individuals were observed, which, as in the previous stages, were swimming about in the cyst in a lively manner (Fig. 8).

10.15 p. m. The process of cleavage, or fission, had progressed so far that cysts with 16, 32, and even more individuals were very frequent.

11.15 p. m. The different cysts were filled with a large number of very small active individuals. Generally after about ten minutes the cyst burst, and the young infusoria were set free from it. The size of these small individuals was as follows: Long diameter 0.0816, and short diameter 0.0612 millimeter. Temperature of the water, 8.5 degrees C.

In Fig. 9 I give an illustration of this last stage of their development, which closely resembles the so-called "Morula" stage of the *Metazoa*, the figure being drawn from a picro-carmine preparation.

[16] Certes, p. 435.

From the foregoing observations upon the propagation of these infusoria the following conclusions may be drawn: In the first place, it should be stated that propagation never takes place as long as the infusoria are still lodged in the epidermis of the fish. After the infusoria have reached a certain size by absorbing food, which consists principally of pigment cells, they leave the epidermis, swim about in the water for some time, settle at the bottom, become encysted, and finally undergo a process of fission, which, however, takes place only in the dark. In about five hours this process of fission has been completed, and the young infusoria leave the cyst. These young infusoria now seek the epidermis of some fish in order to go through the same development which we have observed and which we have endeavored to describe above. Occasionally I found among the larger individuals on the skin of the fish very small ones, which were exactly of the same size as those which had just left the cyst. I have never been able to observe any other process of propagation in the free-swimming individuals than that by fission.

In conclusion, I must add a few remarks as to the place in our zoological system which will eventually be occupied by this infusorian. Judging from the phenomena observed by Fouquet in *Ichthyophthirius multifiliis* and described above, I think that I am not in error when I express the belief that the infusoria observed by me in our aquariums belong to the same species as those discovered by Fouquet in the tanks of the College of France on the skin of *Trutta fario* L. My reasons for this are: (1) on account of the agreement in the mode of life of the two forms; and (2) on account of the very striking similarity of their structure and mode of propagation. There is, however, still a wide gap between the *Ichthyophthirius multifiliis* Fouquet, and the infusorian described by me, namely, the absence of an oral opening, the occurrence of a sucking-disk, and the stable form of the body of the first-mentioned species; and, on the other hand, the occurrence of a distinct oral opening and the complete absence of a sucking-disk in the form observed by me, the mass of the body of which is, moreover, metabolic to a high degree.

Further investigations must show whether this supposition of mine is well-founded, and whether Fouquet, when he says that his *Ichthyophthirius multifiliis* has no oral opening, but a sucking-disk, has not perhaps been the victim of an optical delusion. As has already been stated, Saville Kent has likewise expressed a similar opinion grounded upon physiological reasons.

With the aid of all the literature on infusoria which was at my disposal, especially Saville Kent's classical work, I have in vain endeavored to find the position in the system of the infusorian observed by me. As to its structure it resembles most those infusoria which Saville Kent places in the family of the *Trachelocercidæ*[17] among the *Ciliata-Holo-*

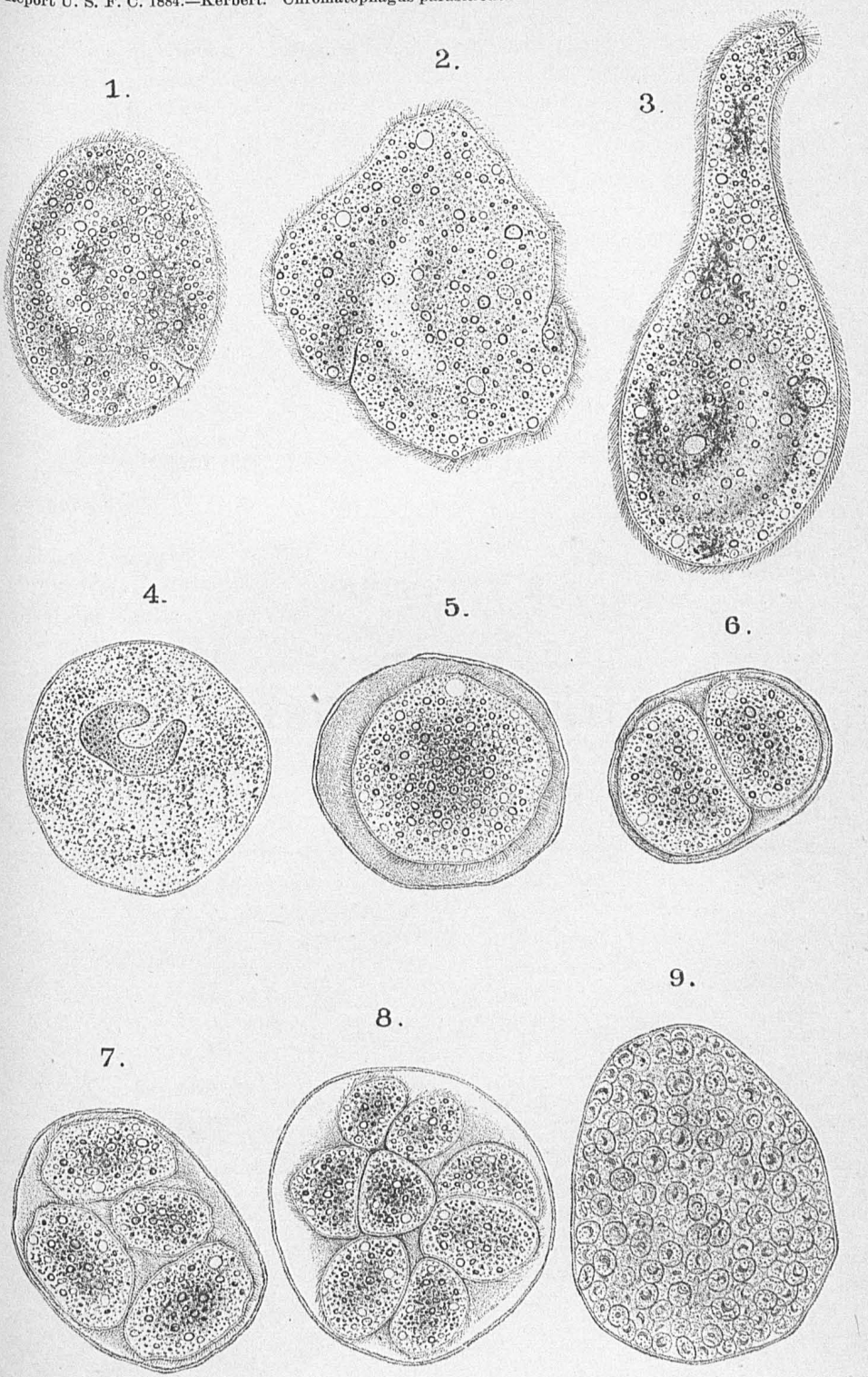
[17] Saville Kent, p. 509.

tricha. As in those, the more or less elongated or flask-shaped body is covered entirely with cilia, which around the oral opening are somewhat (but very little) longer than those found on other parts of the body; the cuticle is very delicate and flexible, the position of the oral opening is subterminal, and the anterior part of the body may at times be extended like a proboscis.

Although this infusorian may be placed in the family of the *Trachelocercidæ*, it is more difficult as to which one of the known genera of this family it belongs. While this infusorian differs essentially from *Trachelocerca* Ehr., *Lacrymaria* Ehr., *Phialina* Ehr., and *Maryna* Gruber, by the entire absence of anything like an anterior head-like differentiation, it is on the other hand impossible to place it under *Lagynnis* Quennerstedt, or *Chœnia* Quenn., because the gullet is not plicate, and because the oral opening is always distinctly visible.

I therefore feel compelled to erect a new genus for the reception of the species described by me, and propose the name *Chromatophagus*, therefore incorporating this animal into the system as *Chromatophagus parasiticus*, gen. et spec. nov.

If future investigations should show that the views expressed above by me, regarding the observations and opinions of Fouquet relative to his *Ichthyophthirius multifiliis*, are correct, it becomes evident in that event that the new family of the *Ichthyophthiriidæ* for the reception of Fouquet's species established by Saville Kent must be abandoned and eliminated from among the families of the *Ciliata-Holotricha*.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