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ON A SPECIES OF TRICHOMONAS, PARASITIC ON PIGEON SQUABS¹⁾

BY

Kan OGUMA

(With One Plate)

Introduction

Two species of trichomonads have hitherto been recorded by RIVOLTA (1880) from two different organs of the domestic pigeon. One is known as *Trichomonas columbæ* found in the small intestine, while the other was discovered in the liver and named *Cercomonas hepatica*. It is the latter species which produces the peculiar necrosis of cheese-like appearance upon the hepatic tissue and causes ultimately the death of the squabs infected. Similar occurrences thereafter were reported by JOWETT (1907) from the liver of dead squabs two weeks of age, reared at Cape Town. In this investigation he discovered *Cercomonas hepatica* in the necrotic part of the organ. RÁTZ noticed in 1908 and 1911 that the squabs that he had at that time also suffered from a liver inflammation of similar fashion and he could detect the parasitic protists from the pathological tissue. In his case, however, he determined that the protists should not be *Cercomonas* but *Trichomonas columbæ*, in spite of the fact that it was found in the liver. Furthermore, he came to the conclusion, after close comparison of organelles, that *Cercomonas hepatica* RIVOLTA recorded from the liver is, with all probabilities, the same as *Trichomonas columbæ*, named by the same author. This opinion of RÁTZ (1913) seems to have been generally adopted by authors who dealt with this kind of protists, as seen elsewhere in text books recently published.

1) Contribution No. 18 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

WATERMAN (1918)¹⁾ reported, after RÁTZ, a trichomonad parasite of the same kind, parasitic on the pigeon reared at Curacao (West India). In this case, not only the liver but also the pancreas was attacked by a trichomonad which had two flagella of unequal length.

In May of this year (1931) I received information from TAKESHITA, a veterinary surgeon of the Japanese military pigeon house at Nakano, near Tokyo, that he had found a species of trichomonads which infected the liver and the pancreas and obviously causes the death of squabs. When I visited there, shortly afterward, I examined myself what facts he had observed concerning this protist. On returning to Sapporo, I discovered that the squabs reared in my experimental dove-cot had been suffering too from just the same kind of protists. In both cases, at Nakano and Sapporo, both the liver and the pancreas were infected and showed the necrotic changes of yellow color taking place as previous authors have observed. In case of heavy attack almost the entire part of these organs became necrotic, representing a characteristic cheese colour.

Since these findings, I have undertaken to study the morphology of this animal, while TAKESHITA has been making clear the mode of infection, cultivation and therapeutics. My study is now completed and described in the following pages. The results involve some different data from those known previously, which are important enough to extend our knowledge concerning the structure of trichomonads in general.

In closing this preface I express my sincerest thanks to Mr. K. TAKESHITA from whom I received continuously his findings and preparations under my disposal.

Material and Methods

The material of the present study was obtained from the pathological tissue of the liver and the pancreas, as well as the lymphatic

1) The data referred to are not cited from his original, but from the resumé appeared in "Jahresberichte über die Leistungen auf dem Gebiete der Veterinär-Medicin 1923."

fluid accumulated in the abdominal cavity of the dead squabs, immediately after their death, while the protists were still living and moving quite actively. The infected squabs seem to be confined to those of younger than two weeks of age. They always show some definite symptoms a few days prior to their death. If the infection occurs in a minor way the liver or the pancreas shows a number of small yellow patches on the surface of the organ, but when the disease advances the patches grow deep into the healthy tissue forming so-called nodules. The size of the nodules varies of course in great degree according to the grade of advancement of the disease until the entire organ becomes necrotic. The protists in question are always found not amidst such necrotic nodules but in tissue surrounding the latter.

A drop of tissue fluid taken from the infected organ or the lymphatics accumulated in the body cavity was spread into a thin film on slide and then fixed. In addition to the SCHAUDINN's fluid as being used for protists, osmic acid vapor was applied. Although not adequate in a strict sense, the dry smear preparations occasionally showed good results in demonstrating some organelles. The first mentioned fixative brought good results as general protozoologists prefer, but the animal body becomes a little small in magnitude, owing to shrinkage of protoplasm, compared with the live animal or those fixed by osmic acid vapor.

For staining, GIEMSA and HEIDENHAIN's iron-hæmatoxylin have been adopted. Some organelles are only observed when the former is applied, while some other ones by both dyes or merely by the latter.

Results of Observation

Concerning the morphology of *Trichomonas* in general the range of our knowledge has been greatly extended through investigations by BLOCKMANN (1884) MARCHAND (1894) BENSEN (1909), KUCZYNSKI (1914), KOFOID and SWEZY (1915), WENRICH (1921), REULING (1921), HEGNER (1924), TANABE (1925) and GRASSÉ (1926), although there remain some questionable points. The present results of observation

on the structure of the trichomonad, as described as follows, shows that the present species possesses no particular structure which is impossible of being found in other species. At least in some trichomonad I find the same structures as those observable in the present species. Still I believe, it is necessary to try here to describe what was observed in the present species in detail, for the further extension of our knowledge of trichomonads, which still have some doubtful structures in exactly described.

Body Form and Size

The body does not show a constant form while it is living and moving, because of the soft consistency of the cytoplasm of which the entire body is composed. Consequently I found various forms in the fixed specimens too. The most common and typical form is citron shape or a thick spindle with the anterior end being rounded and the posterior rather pointed (Figs. 1, 3, 4, 6 and 7). But the body form may become much narrower, on one hand, as shown in Fig. 2, and may grow thicker, on the other hand, as to assume an apparently spherical form as seen in Figs. 8 and 9. In most of the individuals of the latter form, one can hardly observe the end of the axostyle which is long and projected out of the body in normal form. Thus in Fig. 9, the position where the axostyle ends is still indicated by a short cone, but in Fig. 8 it is by no means clear to demonstrate.

From measuring one hundred individuals, taken at random in microspical fields, I obtained the following results. The body length, when measured from the blepharoplast to the posterior pointed part of cytosome, from where the axostyle emerges out of the body, shows a great fluctuation between 5μ and 16μ . But the individual of 6μ - 10μ in length are most abundant. Similar fluctuation also occurs in the body breadth although in less degree than in the length. The diameter of the thickest portion of a citron-shape, varies from 3μ to 10μ , showing 5 - 7μ as the medium breadth. A size variation is also found in individuals of spherical form, in which the diameter ranges between 5μ and 12μ . From the existence of such a great variation of body size,

we must obviously conclude that any data obtained from measurement on any species of trichomonads of pigeon will be no use to taxonomy. Thus, the case of RIVOLTA (1880) should be of the minimal size ($3\mu \times 6-7\mu$) and that of WATERMAN (1918) the maximal size ($10\mu \times 16\mu$) of one and the same species.

Although quite unknown at present, by what cause the variation has been brought forth in great degree as mentioned above, it is evident that a similar variation has been observed by many investigators in several species of trichomonads. So the present case seems by no means to be of particular occurrence.

Cytosome and Nucleus

The cytoplasm is finely granulated and stains intensely purple by GIEMSA, except a part directly surrounding the nucleus. This part usually presents a clear blue color in GIEMSA-preparation (Figs. 2, 3, 4 and 5), while transparent in specimens on which hæmatoxylin is applied (Figs. 7, 8 and 9). The remaining part of the cytosome contains vacuoles and chromatic granules of various size in more or less degree, and they are very clearly demonstrated in hæmatoxylin-preparations (Figs. 7, 8 and 9). The vacuoles are found distributed rather peripherally in the cytosome, while the chromatic granules take their position rather axially. The latter occasionally arrange themselves along the axostyle (Fig. 6), but are never found in linear arrangement near the base of the undulating membrane as in the case of *Trichomonas gallinarum* M. et R., *T. caviae* DAVAINÉ or *T. muris* HARTMANN. The nucleus lies not at the center of the cytosome but near the anterior end as usual, and it generally takes a spherical form, although in an individual of extremely narrow body as shown in Fig. 2, it appears correspondingly narrow. The nuclear contents are stained intensely by any of the basic dyes. Chromosomes were not observed.

Flagella

As a large spherical cytoplasmic element, the blepharoplast is always observable at the anterior end of body in front of the nucleus.

In connection with this organelle all the fibrillar element, the flagella and the fibrillae constituting the upper and lower border of the undulating membrane, are to be found. The number of flagella is unexpectedly four, in contrast to cases observed by previous authors. They are nearly as long as the axostyle which project out of the cytosome, all having the same thickness. Frequently two or three of the four are to be united together, representing a less number in appearance. Such has certainly been the case where only one, two or three flagella have been reported. The united flagella are of course thicker than a separated single flagellum. Looking through the previous works of *Trichomonas* of pigeon, RIVOLTA (1880) described a single flagellum from *Cercomonas* as he believed, while JOWETT (1907) reported one or two in the supposed species. Thereafter WATERMAN (1918) reported merely two flagella. RÁTZ (1913) observed, on the other hand, three flagella in *Trichomonas columbæ*, and found, at the same time, cases in which coalescence of flagella had taken place so as to reduce their number, and concluded at last that *Cercomonas* reported by RIVOLTA would be the same species as the former in which the coalescence of flagella had occurred. It is very important to notice that RIVOLTA described 4-8 flagella in *Trichomonas columbæ*¹⁾. From this evidence we can see at least that this species possesses more flagella than three in number in contrast to the species described by RÁTZ as *T. columbæ*.

This fact reminds us at the same time, that RIVOLTA observed the actual number of the flagella of the protist when he counted four. But it is still obscure as to the cases in which the flagella count was more than four. It is probable to suppose that if conjugation occurred, an individual would arise, which could be represented with eight flagella.

All four of the flagella found in the present case are quite similar in their length and thickness, and they are usually separated from one another in fixed specimens, while very often united into less number while they are living.

1) Original paper was not accessible to me; this datum is cited from RÁTZ.

Undulating Membrane

Along the dorsal surface of the body a long undulating membrane develops, constituting a dorsal fin, as usual case of *Trichomonas*. While the animal is living the membrane undulates very actively, and even in dead condition it retains its wave-form. By a fiber of more rigid nature than the cytoplasm and considerably thicker than the flagellum, this undulating membrane is framed along its marginal border. It is this fiber that is called Randfaden by German authors, and corresponds to the posterior flagellum, which is stretched out of the body, constituting a freely moving flagellum as in the case of *T. muris* or *T. caviae* etc. In the present case, however, this marginal fiber lasts up to a point where the membrane ends, but grows out no more, thus being destitute of flagellar structure. In this respect the present species coincides with *Trichomonas vaginalis*, although it is sharply distinguished from the latter by having a longer extension of that membrane. The marginal fiber, as well as the undulating membrane, has direct connection with the blepharoplast anteriorly, and extends posteriorly about four fifths of total course between blepharoplast and the pointed end of the body where the axostyle is projected freely. All the literature show the undulating membrane to be by no means so long in *T. vaginalis*, as in *T. columbae*, merely extending less than half the length of body (c.f. HEGNER 1925).

Opposite to the marginal fiber in position, a fine fibril, the basal fibril, is always found along the basal line of the membrane, showing no undulation, connecting the blepharoplast and the extremity of the membrane. A similar structure has been known for nearly every kind of *Trichomonas*, and is termed the chromatic basal rod. Both this fibril and the marginal fiber show the same affinity to dyes. This color reaction shows, at the same time, not the slightest difference between these two fibrillar elements and the four anterior flagella, which similarly develop out of the blepharoplast.

Blepharoplast

Now we come back again to consider the blepharoplast which appears at a glance as a single spherical body. In one individual I observed two blepharoplasts of smaller size arranged on the marginal fiber as shown in Fig. 7. This fact suggests to us that the blepharoplast is never of simple structure but at least duplicated. The blepharoplast of duplicate nature has already been recorded by WENYON (1907), KOFOID and SWEZY (1915) and WENRICH (1921) in different species of *Trichomonas*. With a purpose to discover its true constitution I tried to press the animal under the cover glass with the point of a needle, expecting the separation of the morphological elements, of which it is constituted. Under light pressure the animal of course flattens, and the single blepharoplast very often disintegrates into three small granules, of which it is apparently composed. Two of these granules are accompanied by two flagella in each, while the remaining one is accompanied by both the marginal fiber (corresponding to posterior flagellum in other species) and the basal fibril (corresponding to basal chromatic rod in others) of the undulating membrane. It is difficult to determine whether such a constitution will be found in general with every kind of trichomonads.

Axostyle

The axostyle arises from the blepharoplast. At the arising point it seems to have nearly an equal breadth to the blepharoplast, but soon grows narrower and then curves ventrally (opposite to the underating membrane) in such a way as if avoiding direct contact with the nucleus. When arriving at an opposite point against the nucleus, to the blepharoplast, or when upon reaching nearly to the center of the animal body in other words, it runs along the middle axis, and at last grows out of the body. Throughout the entire length, the axostyle gradually tapers up to its terminal pointed end. This terminal projected part strongly varies in its length. In the most common examples it mea-

sures from one eighth to one third of the whole length. In an extraordinary case I found one in which about one half or more than the half length of the axostyle is projected freely out of the body. (Figs. 10 and 11). This variation of the length is not only due to the relative prolongation of the body, but also to its absolute length.

The axostyle is observable in striking clearness when stained by GIEMSA (Figs. 1, 2, 3 and 4), while taking color in least degree in hæmatoxylin preparations fixed by SCHAUDINN'S method (Fig. 6). When treated with hæmatoxylin, the preparations fixed with osmic vapour are scarcely of use for its demonstration (Figs. 7, 8 and 9). Comparing with the other species of this genus, the axostyle of this species shows the thinnest type of axostyle, having neither chromatic granules in the composing substance as in *T. augugsta* and *T. eberthi*, nor any chromatic ring at the emerging point from the cytosome as in *T. muris*.

Now I come to describe an interesting structure which stands in an intimate relation to the axostyle. It is a fiber with its origin in the blepharoplast too. In Fig. 3, one can not overlook a peculiar fiber which curves dorsally (in contrast to the axostyle) against the nucleus while its distal extremity fades in the axostyle. But frequently this extremity does not lie upon the axostyle but is crossed over the latter and ends freely in the cytoplasm as shown in Fig. 4. In the latter case we can easily measure the entire length of the fiber in question. It is about three fourths the length of the axostyle, and close observation reveals that it is never a fiber of simple structure. Because, if we observe it in detail under high magnification it will be quite clear that the fiber is also thick at the arising point from the blepharoplast and tapers gradually towards its tip, thus representing the same kind of structure as the axostyle. Fig. 5 shows an adequate example in which the fiber detaches completely from the axostyle, and shows, moreover, very like structure with the latter.

Considered from the color affinity, this fiber is regarded also to be of the same nature as the axostyle, but different from the flagella. All four flagella, marginal and basal fiber of the undulating membrane

always stain a similar color even when the axostyle hardly takes the same dye, and the fiber in question shows quite similar property to the axostyle in this color reaction. From the reason above mentioned the following conclusion may be accepted without difficulty: the fiber-like body in question is by no means a true fiber, but an organelle similar to the axostyle, with which it embraces the nucleus, and will be called the paraxostyle in the present paper.

The paraxostyle corresponds with the problematical fiber which is described for the structure of *Trichomonas vaginalis*. The presence of this fiber was first suggest by MARCHAND (1894), whose description tells us that the axostyle is longitudinally separated into two parts and represents a spindle form with the nucleus being embraced between two separated fibers. One of the latter obviously corresponds with the axostyle, while the other with the paraxostyle in the present species. REULING (1921) considers it as a distinct fiber which belongs to the axostyle, as illustrated in his Fig. 1 a. A similar conception has been adopted by HEGNER (1924) who takes the view that this fiber probably represents the lip of the cytostome.¹⁾ REICHENOW (1928) maintains, on the other hand, in the DOFLEIN's Handbuch, that this fiber is rather to be considered as a component of the parabasal body. WENRICH (1913) also takes the same view, with his collaborators, BLAND and GOLDSTEIN. Position of this fiber never coincides with that of the cytostome, but with the parabasal body. So far as the position is concerned, however, the view of REICHENOW seems to be more probable than that of HEGNER.

In the present species, neither cytostome nor parabasal body develops enough to constitute a definite feature of the animal, and thereby both opinions of HEGNER and REICHENOW will be unable to account for the existence of this fiber.

Quite recently one more example of trichomonad with similar organelle in addition to axostyle became known to us. It is the case of

1) According to HEGNER's description and figure this fiber takes a position, in just ventral to the nucleus, where the undulating membrane is not found, but in the figure found in DOFLEIN's Handbuch it lies dorsally just like in my case.

T. diversa reported by VOLKMAR (1930), who clearly figured it and described thereon "from near the center of the axostyle, 7 to 9 μ in length, branches off, leading to the dorsal side of the nucleus". The so-called branch of axostyle evidently corresponds to paraxostyle in my case.

As already mentioned, this questionable fiber of *T. vaginalis* and *T. diversa* comes in coincidence to the paraxostyle of the species now dealt with in the present paper, and it will serve as an accessory organelle of the axostyle, to support the body form as axial skeleton.

Remarks

1. On the scientific name of the protist.

After observation on the structure of the parasite dealt with in this paper, I have been able to come to the same conclusion, as RÁTZ (1913). Or, in other words, the protist obviously is to be classified in the genus *Trichomonas*, not *Cercomonas*, and all the trichomonad species recorded by various authors at different times and in different localities will be of the same species, so far as they infect or destroy the digestive glands belonging to the alimentary tract of pigeon squabs. As mentioned at the beginning of this paper, some authors observed only one flagellum at the anterior end of the animal on one hand, and others three (RÁTZ) or more (RIVOLTA) on the other hand. This discrepancy in number of flagella will be merely due to condition whether all the flagella were observed separated or some of them united into less number. The number of flagella is really four, one more than the case reported by RÁTZ. But this difference is seemingly due to his misobservations. Thus I can not hesitate to adopt the view that the trichomonad deals with in this paper is no other than *Trichomonas columbæ* RIVOLTA.¹⁾

Turning our attention towards allied species of *Trichomonas*, there are at least two species which show a close relation in their morphological respects. The one is *T. vaginalis* DONNÉ and the other *T. diversa*

1) WENYON (1926) noticed that *Trichomonas columbarum* PROWAZEK and ARAGÃO (1909) is possibly the same with *T. columbæ* RIVOLTA.

VOLKMAR. The former presents particularly a like feature concerning the structure of the undulating membrane, as is readily recognizable from my description in the foregoing pages, but in its relative length to the body axis these two are to be sharply distinguished from each other. *T. diversa* is a species quite recently reported by VOLKMAR (1930) as a parasite of the turkey, but in two points they are quite distinctly separated; viz. it has only three flagella and the marginal fiber of the undulating membrane develops into a posterior flagellum. *T. columbæ* seems therefore to have a less close relation to *T. diversa* than *T. vaginalis*.

2. On occurrence.

In connection with the presence of the trichomonad protist, every author observed a definite pathological change of the tissue in which they were found. It is an inflammation of particular nature; that is the organ (either liver or pancreas) undergoes a cheese-like change both in color and texture wherever the protists are found. This symptom is extraordinarily distinct if the infection occurs in the liver which is by contrast of dark red color.

During the early state of infection there is produced a number of nodules of pale yellow color as figured by JOWETT (1907), but in a late condition nearly all of such nodules are connected together by their growth until nearly the whole organ is converted into a cheese-like mass. In the abdominal cavity in such a case, turbid lymphatic fluid accumulates in great amount, in which there are so many individuals, that they can be considered as pure culture.

The squabs which suffer and die from the infection of this parasite are said to be very young, up to two weeks of age. As to this point my case shows a fair coincidence with all previous information. From this point, on the other hand, it is supposed that the adult pigeons do not receive any mortal effect from the infection of this parasite. This means that the adults have resistant power, sufficient enough to avoid serious infection; because, there remains one conceivable reason,

as to the course of infection in the squab, that is the parasites were originally living in the alimentary canal of the parents and migrated to the squabs through their mouth when they received the so-called pigeon milk produced in the parents' crops. This will explain at the same time why RIVOLTA found his *Trichomonas columbæ* in the small intestine, while *Cercomonas hepatica* in the liver of the young pigeons.

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Plate V

Explanation of Plate V

Abbreviation: *F*, Flagellum; *Mf*, Marginal fiber of the undulating membrane; *Bf*, Basal fibril of the same; *A*, Axostyle; *Pa*, Paraxostyle.

Figs. 1-3. Osmic acid vapor-Giemsa preparation. ×5000

Figs. 4-5. Giemsa dry preparation. ×5000

Notice in Fig. 5 that the marginal fiber becomes free from the body, followed by break of the membrane, and the paraxostyle gives up its terminal connection with and detached from axostyle.

Fig. 6. Schaudinn-Hæmatoxylin preparation. ×5000

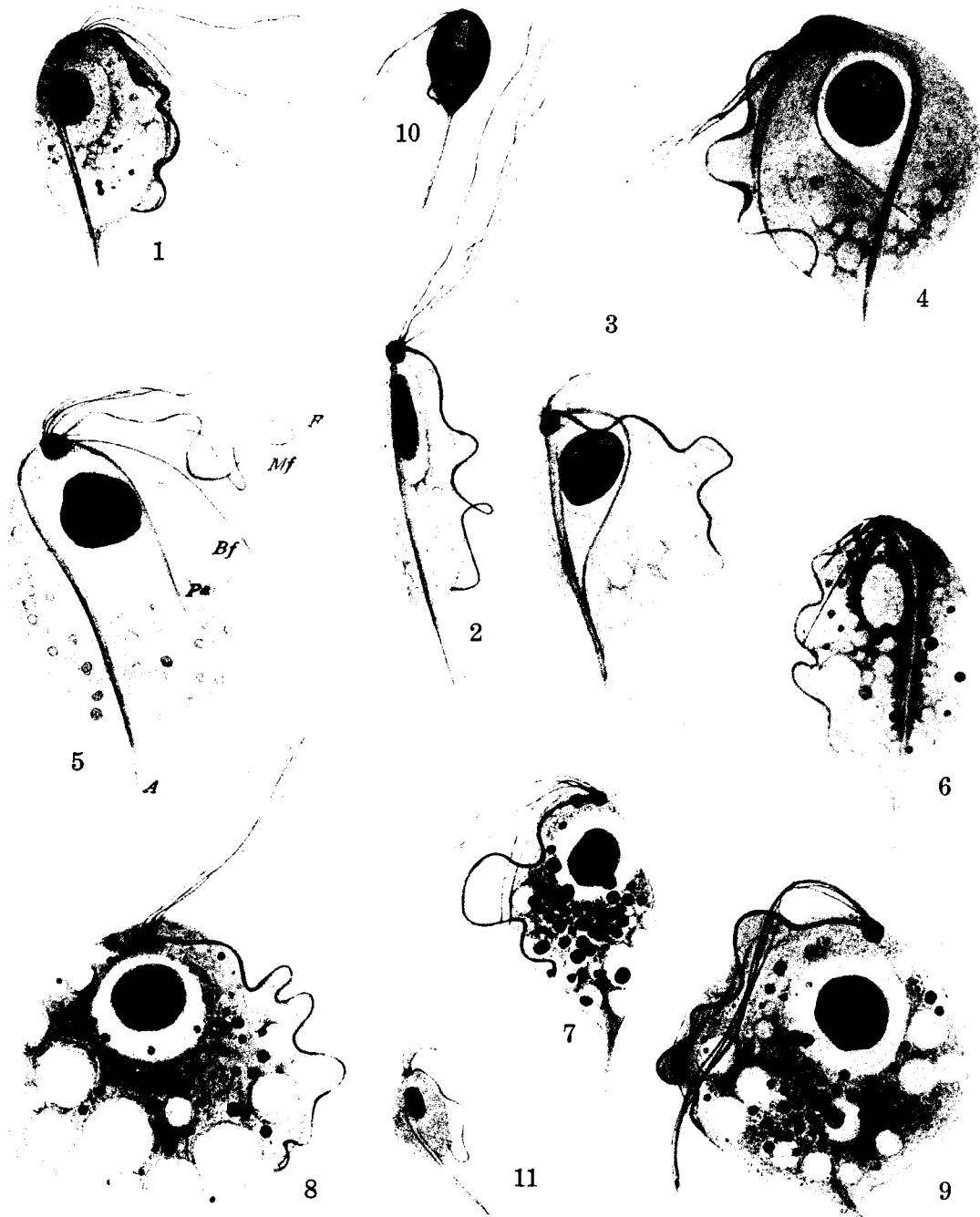
Figs. 7-9. Osmic acid vapor-hæmatoxylin preparation. ×5000

In these preparations both the axostyle and paraxostyle are obscure.

Fig. 10. Schaudinn-hæmatoxylin preparation. ×2000

Fig. 11. Schaudinn-Giemsa preparation. ×2000

Examples having an extremely long axostyle.



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