Studies on Parasitic Protozoa.

I. The Flagellate Polymastix and its Affinities with the Trichonymphida.

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With Plate 18 and 1 Text-figure.

Introduction.

The Protozoa known as Trichonymphidæ, parasites of the gut of certain orthopterous insects, have for long presented difficulties to the systematist.

The early observers of the Trichonymphids, Leidy and Grassi, worked chiefly on the more highly specialised genera, such as Pyrsonympha and Dinennympha, and not unnaturally they placed these forms among the Ciliata, to which they bear a strong superficial resemblance. Other authors, dissenting from this view, preferred to regard the Gregarinida as the nearest allies of this perplexing group.

Bütschli's observations on Lophomonas (1878), from the cockroach, put a different aspect on the case. This form, in most respects simpler, and probably more primitive, than the trichonymphids from termites, had obvious affinities with the Flagellata. Kent (1880) formed for it a special family, the Lophomonadidæ, which he placed in the neighbourhood of such flagellates as Trichomonas, Tetramitus, and Hexamitus. Grassi discovered Joenia annectens (1885) which is a link between Lophomonas and the more

elaborate forms, and strengthened the view that the trichonymphids had sprung from a flagellate stock, a view that the recent work of Janicki (1910) and others has still further confirmed.

The object of the present paper is to point out that, while the affinities of Lophomonas are with the Polymastigina, of Doflein, as Kent long ago suggested, yet, within that order, it is to the hitherto little-known genus Polymastix that we must look for further data of phylogenetic interest. (See Mackinnon, 1912).

Polymastix, Bütschli.

Habitat and Previous Records.

Polymastix is a small flagellate which has been found parasitic in the alimentary canal of certain larval insects, such as Melolontha, Cetonia, Orycytes,[1] and Tipula.

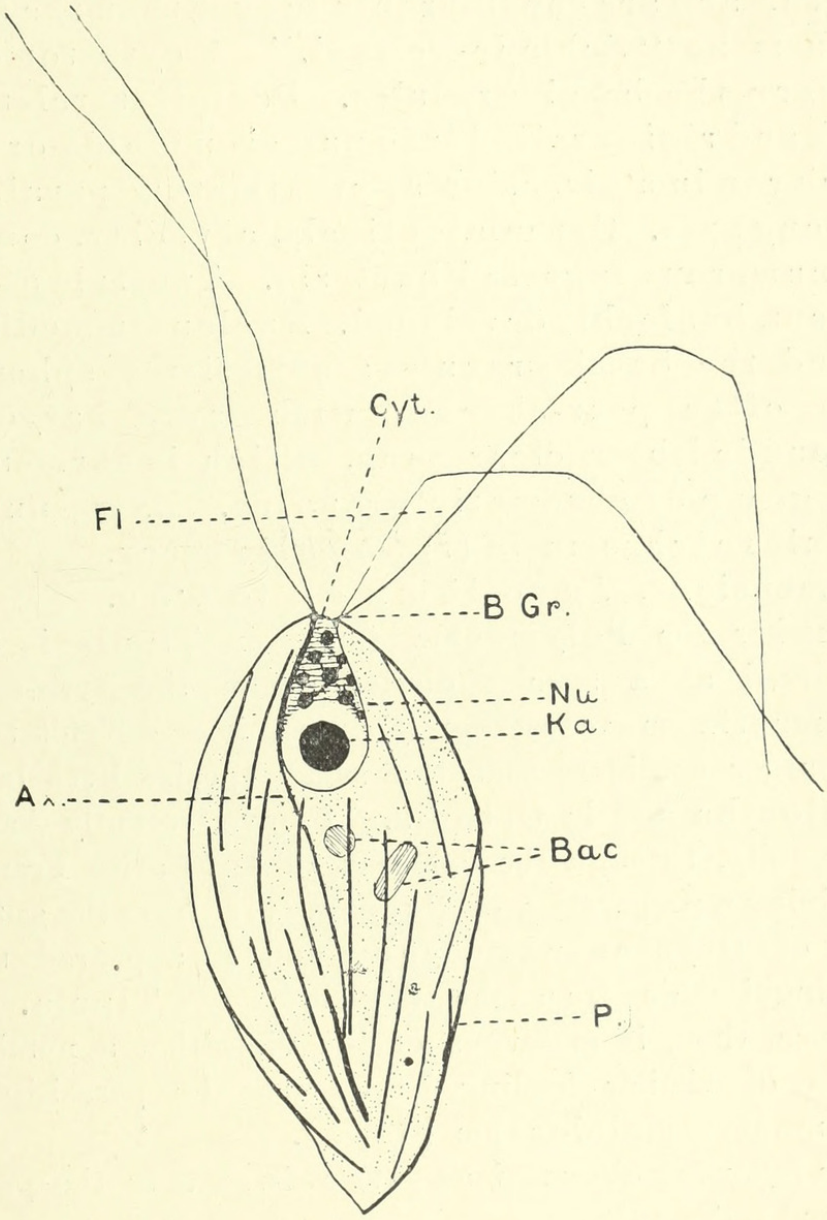
It was first described by Grassi (1882), as Trichomonas melolonthæ. Bütschli (1884) removed it from the genus Trichomonas, and formed for it a new genus Polymastix, characterised by four equal, forwardly directed flagella, and a firm striated periplast. Künstler's account (1882) brought in some confusion by his stating that there might be as many as six flagella.[2] This author also suggested that the striations on the periplast might be adherent bacteria.

Polymastix was not further investigated until 1911, when Hamburger briefly described the flagellate as it is found in Melolontha and Cetonia. Contemporaneously with Fräulein Hamburger, I had been working on Polymastix from the hind-gut of the larva of Tipula, sp. I published a short account in a preliminary note in 'Parasi-

[1] I am indebted to Dr. Carlos França for this record of another coleopterous host of Polymastix.

[2] This statement led Alexeieff (1911) to place in the genus Polymastix as P. batrachorum, a flagellate with six flagella from Triton tæniatus, for which he has since (1912) been obliged to erect a new genus, Hexamastix.

tology' (1912), an account which I am now able to amplify in certain particulars that seem to me to bear on the probable affinities of Polymastix with Lophomonas.



Polymastix from Tipula. Characteristic flagellate individual, showing four flagella, ribbed periplast, pear-shaped nucleus, with karyosome, cytostome, basal granules, axostyle, and ingested bacteria.

Description.

Body pear-shaped to spindle-shaped; the anterior end rounded, the posterior end tapering, or often

forked and otherwise "deformed." Four flagella[1] arise from two basal granules at the extreme anterior end in two groups of two; they are sub-equal in length,[2] and are considerably longer than the body; there is no "Schlepp-geissel." A cytostome lies between the basal granules. Periplast relatively thick and rigid, raised into numerous folds or ribs, which run in a direction approximately parallel to the long axis. Cytoplasm finely alveolar, containing numerous ingested bacteria. Axostyle usually present, but feebly developed. Nucleus immediately behind the basal granules; sometimes spherical, more often pear-shaped, with large karyosome surrounded by a clear zone, which is surmounted by a group of chromatin granules. Extra-nuclear granules of chromatin (?) may also occur.

Dimensions, 7 μ to 15 μ × 4 μ to 6·5 μ.

Whether the Polymastix found in Tipula is to be considered as a species distinct from the type-species, Polymastix melolonthæ (Grassi), it is difficult to say. Hamburger considers that the same species lives both in Melolontha and in Cetonia. I have recently had the opportunity, through the courtesy of Dr. Carlos França, of examining a preparation of Polymastix from the gut of a larva of Oryctes nasicornis, and it appeared to me indistinguishable from the flagellate of Tipula. The evidence, then, is in favour of the view that a number of insects of similar feeding habits may be parasitised by Polymastix melolonthæ (Grassi).

The following observations have reference to the parasite as found in Tipula.

[1] It is to be noted that certain undoubted Trichonymphids, such as Devescovina striata, have only four flagella.

[2] Authors—Hamburger, Jollos, Alexeieff—state that the flagella are unequal in length. I think that this is so only in individuals that have recently undergone division; in such a case two flagella may be regrown, and for some time these will appear shorter than the rest (vide infra).

The Pellicula.

One of the most characteristic features of Polymastix is the thickened periplast or pellicula, which gives the body a certain rigidity and stiffness, and prevents any "metabolic" altering of shape. Sometimes the empty periplast is found persisting after the disappearance of its softer contents. A distinct gap in its continuity may be observed at the extreme anterior end; this gap marks the position of the cytostome. The surface of the periplast is raised up into a number of thickened ribs or ridges ("côtes" of French authors), which stain deeply with iron-hæmatoxylin. These are not continuous from end to end of the body, but, while approximately parallel to the long axis, they fall into little groups of two or three, often inclined at considerable angles. Grassi suggested that these structures, which appear as dark striations in stained preparations, might be trichocysts; Künstler held them for adherent bacteria. Sometimes the periplast may be seen fraying off, so that the "ribs" project from the surface, when they look very like adherent bacilli (Pl. 18, figs. 12 and 14).

Hamburger compares these striations with thickenings in the periplast of Euglena.

Now, it is of interest to find that such pellicular thickenings are eminently characteristic of the Trichonymphid. "Für die meisten Gattungen ist das Auftreten von Stäbchenförmigen Bildüngen in der Pellicula charakteristisch" (Doflein, 1911). Lophomonas striata, Bütschli, in particular, is provided with a thick, striated periplast, and Janicki's excellent account and figures (1910) leave no doubt that this structure presents the same general characters as in Polymastix.[1]

[1] Alexeieff shares my opinion as to the affinities between Polymastix and the Lophomonadidæ—"Le genre Polymastix pourra être placé dans la famille des Lophomonadidæ, ou tout au moins considéré comme une forme de transition entre les Polymastigines et les Lophomonadidæ (Trichonymphines) (1912)."

The Nucleus.

The nucleus presents very different appearances according to the intensity of the staining. In badly differentiated individuals stained with iron-hæmatoxylin it has the aspect figured and described by Hamburger, i. e. that of a pear-shaped body at the anterior end of the organism, containing a voluminous karyosome. Better staining reveals a clear zone surrounding the karyosome, and sometimes containing a few grains of chromatin. Above this is a dark-staining cone-shaped area, in which lie a number of chromatin granules supported in a delicate reticulum. These structures are enclosed within a definite pear-shaped membrane, the upper borders of which touch the basal granules. This is the most usual aspect. Sometimes, however, the nucleus wanders from its anterior attachment, and appears as a sphere containing a karyosome, and surmounted by a group of extra-nuclear granules; in this condition it is strikingly like the nucleus of the Monocercomonas that occurs abundantly alongside Polymastix.

The pear-shaped nuclear apparatus presents a certain resemblance to the "calyx" and contained nucleus of Lophomonas. Certain of Janicki's figures of Lophomonas blattarum, Stein, are very suggestive in this connection—I refer more particularly to figs. 2B, D and E on pl. vi of "Untersuchungen an Parasitischen Flagellaten," I ('Zeitschrift f. Wissenschaft Zool.,' 1910). There, within a cup-shaped area, limited by a membrane, lies a spherical nucleus, which contains an eccentric karyosome surrounded by a clear zone and surmounted by a dark-staining mass containing scattered chromatin granules. In Polymastix there is no trace of the "collar" and other circumnuclear structures, though the cytostome may bring in another complication.

The Axostyle.

My preparations of Polymastix show the presence of a feebly developed axostyle, which had been overlooked by

previous observers. The axostyle arises from the neighbourhood of the basal granules, passes down one side of the nucleus and pursues a curved course for some distance below; it seldom extends into the posterior third of the body, and is often completely absent.

The feeble development of the axostyle is no doubt associated with the presence of the rigid pellicula, which makes internal skeletal support superfluous (cf. the different degree of development of the axostyle in the plastic Lophomonas blattarum and the rigid L. striata).

The relations of the axostyle to the nuclear apparatus seem to be different in Lophomonas. There Janicki describes the central fibrils of the axostyle as traversing the calyx and nucleus and ending beneath the circle of basal granules: "Irgend welche direkten Beziehungen zwischen Basalkörnern und Kern konnten in Lophomonas nicht beobachtet werden." Furthermore, the axostyle of Lophomonas is re-formed by the division spindle, as in Trichomonas batrachorum, Perty, and other allied flagellates. In Polymastix I could find no sign of this.

Division.

Early observers of Polymastix noted something unusual in the mode of division. "Wahrscheinlich erfolgt die Vermehrung durch Querteilung" (Doflein, 1911). Hamburger failed to find any evidence on this point.

My own experience has been that, though the flagellate may be present in enormous numbers, it is very hard to find individuals in division. Consequently I am able to supply only the outline of the process, but I have been so much struck by the general similarity between this and the division in Lophomonas that I feel justified in publishing the figures.

It should perhaps be noted first that the individuals with forked posterior ends, so commonly met with, are not dividing forms. I have watched the living organisms for long periods to satisfy myself of this, nor is there any indication of nuclear

division in such forked forms as I have found stained (Pl. 18, fig. 11).

What happens in division is as follows. The spherical karyosome elongates, becomes dumbbell-shaped (Pl. 18, fig. 1), and finally divides into two (Pl. 18, fig. 2). Each daughter-karyosome is surrounded by a clear zone, and takes over with it a certain number of the supra-nuclear chromatin granules (Pl. 18, fig. 3); the reticulum supporting these chromatin grains has previously become much fainter, and they show a tendency to wander beyond the limits of the nuclear membrane.

One of the daughter-nuclei so formed remains in the original position at the extreme anterior end of the body; the other wanders back just below the periplast until it comes to lie at the posterior end (Pl. 18, figs. 4 and 5). Meanwhile the body has shown signs of elongation, a constriction appears halfway, and the two halves, after hanging together for some time, gradually separate (Pl. 18, figs. 6 and 7). I have not been able to find the last stage of separation in the stained material, but I saw it once in the living organism.

The fate of the flagella and the basal granules is rather curious. Generally one basal granule and two flagella go over with the travelling nucleus; sometimes a fine fibril is seen to extend from the basal granule to the nucleus (Pl. 18, fig. 4). In such a case each daughter-nucleus must re-grow two flagella; in this way I explain the occurrence of individuals with exceedingly unequal flagella. The sprouting of the new flagella is preceded by division of the basal granule into two (Pl. 18, figs. 4 and 5). But sometimes all four flagella remain behind with the stationary nucleus (Pl. 18, figs. 8 and 9), while the wandering nucleus moves off without any, and presumably must re-grow all four when it reaches its destination. If the flagella grow out from the basal granules, as seems most probable, we should expect that, in this case, the wandering nucleus would receive its share of these, but I have not been able to observe this.

The axostyle may persist for some way through the division

process (Pl. 18, fig. 8); then it seems to be absorbed, and I have seen no indication as to the way in which it may be re-grown.

Now, while the process described above is much simpler than what takes place in Lophomonas, there are some striking resemblances, which a comparison of my figures with those of Janicki will at once reveal. This is particularly well seen in the case of Lophomonas striata, where the daughter-nuclei, separating from a division-spindle which is parallel with the long axis, migrate, the one to a position alongside that occupied by the mother-nucleus, the other to the posterior end of the body. The flagella are then re-grown, a constriction appears in the middle of the body, and the two halves separate. As Janicki shrewdly points out, "Es konnte ja diese Art der Körperteilung auf den ersten Blick als eine Querteilung gedeutet werden; das ist sicher nicht der Fall, sondern die wachsenden Plasmamassen, anstatt durch die Längsteilungebene einfach nach rechts und links auseinandergeklappt zu werden, gleiten sozusagen an derselben polarwärts, bis sie von einander lostrennen" (Janicki, 1910, p. 306).

The wandering of the nucleus of Polymastix also recalls very vividly the description of the division of Lophomonas blattarum: "Die zweikernigen Formen sind äusserst lebhafte Tierchen. . . . Die Korperpartien welche den Kern mit den zugehörigen Nebenorganellen beherbergen sowie die Flagellenschöpfe, sind befähigt, über den ubrigen Körperplasma in oberflächlicher Schicht mit grosser Geschwindigkeit hin und her zu gleiten, so dass sie bald nahe aneinander liegen, bald nach den entgegengesetzen Polen sich entfernen" (Janicki, 1910, p. 273).

Encystment.

As to the mode of encystment in Polymastix I can say very little. In preparation the organism loses its flagella and the nucleus migrates to the centre of the body (Pl. 18, figs. 12, 15 and 16). Meanwhile the periplast shows signs of

disintegrating, and the "ribs" fray off, exactly as in Lophomonas striata. I have seen no completely formed cysts nor any trace of division in the encysted state.

Summary.

Whatever be the relationship of the Lophomonadidæ with other Trichonymphids, recent research has clearly indicated that their affinities on the flagellate side are with the order Polymastigina, Doflein, and in this order with the family Polymastigidæ, Bütschli.

The genus Polymastix, Bütschli, itself shows certain structural features that greatly strengthen this view. While the comparison must not be pushed into too great detail, the evidence so far collected seems strong enough to make mere coincidence of resemblance improbable. The points of similarity may be summed up as follows :

(1) The similar character of the ribbed periplast in Lophomonas striata and in Polymastix.

(2) Certain points of resemblance in the nuclei of the two.

(3) The peculiar nature of the division process, "apparently transverse," in Lophomonas striata and in Polymastix.

To this may perhaps be added the fact that Lophomonas and Polymastix, as far as the records show, are both parasites of insects only.

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EXPLANATION OF PLATE 18,

Illustrating Miss Doris L. Mackinnon's paper, "Studies on Parasitic Protozoa.—I. The Flagellate Polymastix and its Affinities with the Trichonymphida."

[All figures drawn to scale (× 4000 approx.) under Zeiss comp. oc. 12 and 2 mm. apochromat. The stain employed was in all cases Heidenhain's iron-hæmatoxylin after fixation with sublimate-alcohol.]

Fig. 1.—Polymastix from Tipula, showing dividing karyosome.

Fig. 2.—A later stage in the division process: the daughter-karyosomes have separated.

Fig. 3.—Separation of the nuclei.

Fig. 4.—One nucleus remains at anterior end; the other migrates. Note that the basal granules have divided, and one of the missing flagella has been re-grown in each case.

Fig. 5.—A slightly later stage. The body of the flagella shows signs of elongating.

Figs. 6 and 7.—The wandering nucleus has reached the posterior end of the body. In fig. 7 the elongated body is beginning to constrict in the middle.

Fig. 8.—The migrating nucleus has moved off without being accompanied by flagella. Note persistent axostyle.

[1] Fuller references to the literature on Trichonymphid may be got by consulting the bibliography at the end of this work.

Fig. 9.—A later stage of fig. 8. The migrating nucleus approaches the posterior end of the body.

Fig. 10.—"Tailed" individual of Polymastix.

Fig. 11.—"Forked" individual.

Fig. 12.—Preparation for encystment. Periplast fraying off, flagella disappearing. Nucleus has taken up a central position.

Fig. 13.—"Deformed" individual with displaced nucleus. Possibly an early stage of encystment.

Fig. 14.—"Ribs" of the periplast fraying off. In this condition they look very like adherent bacteria.

Fig. 15.—Encysting Polymastix. The flagella have disappeared, and the nucleus has moved to the centre of the body.

Fig. 16.—Encysting Polymastix. A rather later stage. The ribs of the periplast have almost all disappeared. Note the basal granules, surrounded by a clear zone.