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Morphology and Mitosis of *Teranympha.*

By

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With plate 11.

The genus *Teratonympha* was erected by Koidzumi in 1921 for a hypermastigote flagellate from the termites *Reticulitermes speratus* Kolbe and *R. flaviceps* Oshima. In an earlier paper, published in Japanese, Koidzumi (1916—17), by a mistake which he corrected in his 1921 paper, wrote the generic name of this organism as *Teranympha.* It is doubtful if Koidzumi had the right to correct this error in spelling, and the proper generic name for this flagellate is further complicated by the fact that Dogiel in 1917 gave a brief description of what is probably the same organism and placed it in the genus *Cyclonympha.* Although Dogiel states that the flagellate which he observed came from *Coptotermes* sp., we are now practically certain, from our knowledge of termites and their protozoa, that the termite Dogiel examined was not a species of *Coptotermes.* (All species of *Reticulitermes* contain *Pyrsonympha* and *Dinenympha*, genera restricted to *Reticulitermes*; and all of the many species of *Coptoermes* that have been examined have not only been free of *Teranympha* but also of any related form. Then, since Dogiel states that *Pyrsonympha* and *Dinenympha* were present in the termite he examined, it is probable that he examined *Reticulitermes speratus*, because this termite occurs in the region of Japan where the one he studied was collected.) If Koidzumi did not have the right to correct his error in spelling, the generic name of this flagellate is

either *Teranympha* or *Cyclonympha*, depending on whether KOIDZUMI's or DOGIEL's paper was published earlier in 1917.

DOGIEL states that he received the termites containing his *Cyclonympha* from Japan in December, 1916. His paper was published August 2, 1917. The second part of KOIDZUMI's 1916—17 paper, containing a description of *Teranympha*, was published in March, 1917. Then, *Teranympha* KOIDZUMI, 1917 has priority over *Cyclonympha* DOGIEL, 1917. It should also be noted that KOIDZUMI, in the first part of his 1916—17 paper, mentions *Metameronympha* gen. nov. in one instance and in another instance *Metameronympha monstrosa*, but he gives no description, either of the genus or the species; and it is impossible, from this paper, to tell what organism is referred to. However, in a 1922 paper, published in Japanese, KOIDZUMI states that *Metameronympha*, used in the first part of his 1916—17 paper, refers to *Teranympha*. *Metameronympha* is not a valid generic name since it is unaccompanied by a description.

I am indebted to Dr. MASATAKE YAMASAKI for the *Reticulitermes speratus* used in this study, and to the Penrose Fund of the American Philosophical Society for financial assistance.

Morphology.

In twenty individuals the body ranged in length from 90 to 275 microns with an average of 136. It is widest about one-fourth of the distance from the anterior end, ranging in twenty individuals from 31 to 158 microns with an average of 74. From this portion it tapers gradually posteriorly (Pl. 11 Fig. 1). The body is divided into two regions, the rostral and post-rostral, which are more distinct than in most hypermastigotes (Pl. 11 Figs. 1, 2, 7). In individuals attached to the chitinous intima of the termite's hind-gut — a condition frequently seen in *Teranympha* — the rostrum is usually elongated and in such individuals the width is reduced somewhat as a result of stretching.

Within the rostrum lies the broad rostral tube, a striking feature of this flagellate (Pl. 11 Figs. 1, 2, 3). It varies in length from 9 to 11 microns and in width from 8 to 9. Anteriorly the rostral tube ends in a depression of the ectoplasm, and there is no outer cap covering the rostrum as in *Trichonympha*, *Pseudotrichonympha*, *Eucomonympha*, and *Leptospironympha*; but in certain preparations a small, faint inner cap may be seen covering the rostral tube (Pl. 11 Fig. 7). In these respects *Teranympha* resembles *Macrospironympha* closely. The basal granules, from which the rostral flagella arise, lie at the

periphery of the rostral tube and encircle it (Pl. 11, Figs. 2, 6). These granules and the flagella arising from them are arranged in longitudinal rows (Pl. 11 Fig. 8), of which there are approximately forty. In this respect the rostral tube of *Teranympha* resembles that of *Trichonympha*, *Pseudotrichonympha*, and *Eucomonympha*, but is more like the latter in that it is broad, lies far from the surface of the body, and is filled with a large amount of hyaline material. In fact, the flagella arising from the posterior portion traverse even more ectoplasm before reaching the surface of the body than those of *Eucomonympha* because they slant posteriorly (Pl. 11 Figs. 1, 2, 7). In other words, these flagella leave the body a considerable distance posterior to their point of origin, and because of this the entire body appears to be flagellated when viewed from the surface (Pl. 11, Fig. 5), but there is actually a considerable space between the rostral tube and the first flagellar band or ring that is free of basal granules (Pl. 11, Figs. 1, 2, 5, 7).

The rostral tube of *Teranympha*, like that of other hypermastigotes where this organelle is prominent, is composed of an outer core or lamella which lies adjacent to the rows of basal granules. This structure may be seen clearly in living organisms and in fixed and stained dividing ones where it is being duplicated (Pl. 11 Figs. 13, 16, 17). The interphase or parent rostrum splits in two, thus forming two semicircular daughter rostra, which, as they grow, become more and more circular, finally becoming circular, like the parent when growth is completed.

Within the rostral tube lies the anterior portion of the nuclear sleeve, an organelle present in several other hypermastigotes such as *Urinympha*, *Rhynchonympha*, *Barbulanympha*, and *Trichonympha* (only the species in *Cryptocercus punctulatus*), being more prominent in *Rhynchonympha* than the others. In *Teranympha* this organelle is so highly developed that it is one of the first things one notices when studying living material. It extends posteriorly around the nucleus and anteriorly to the end of the rostral tube where it broadens and becomes almost as wide as the tube, being fastened to the lamella at this portion of the tube (Pl. 11 Figs. 2, 13, 16). Posterior to its point of attachment, it narrows rather sharply, soon becomes one-third to one-fourth the width of the rostral tube and lies in the centre of the tube; then it gradually broadens posteriorly and becomes as wide as the nucleus which it surrounds (Pl. 11 Fig. 7). It is sufficiently rigid that it swings back and forth like a pendulum, carrying the nucleus with it, when the body bends.

Just outside the nuclear sleeve, and adhering to it from the base of the rostrum to the mid-portion of the nucleus, lie the nuclear supporting strands, organelles, so far as known, present only in *Teranympha* (Pl. 11 Fig. 2). These strands appear to arise from a ring-like structure surrounding the nuclear sleeve at the posterior end of the rostral tube, although it may be that they form the ring (since they are so close together here) and follow the nuclear sleeve through the rostral tube to its anterior end. However, if they are within the rostral tube, it is impossible to see them. They vary in number from 14 to 16 and most of them bifurcate near the point where they leave the nuclear sleeve in the mid-portion of the nucleus. At the point where they cease to adhere to the nuclear sleeve, they turn almost at right angles (Pl. 11 Fig. 2) and extend to the first flagellar band or ring which they join (Pl. 11 Fig. 3). From this point, they continue as fine strands posteriorly just underneath or within the flagellar bands, some of them extending almost to the posterior end of the body (Pl. 11 Fig. 1). It is difficult to determine whether they are fastened to any of the bands except the first one, because they soon become very fine and lie close to the bands. The fact that they extend for such a great distance adjacent to the bands suggests a connection. When the rostral tube splits preparatory to cytoplasmic division, they, together with the anterior or rostral portion of the nuclear sleeve, are distributed equally between the daughter rostral tubes and pass over into the daughter cells (Pl. 11 Fig. 13). As the daughter nuclear sleeves develop and extend posteriorly to surround the nucleus and hold it in position after the achromatic figure is no longer able to perform this function, they gather up, so to speak, the persisting daughter nuclear supporting strands and adhere to them (Pl. 11 Figs. 16, 17). At the same time, new nuclear supporting strands grow out posteriorly and adhere to the nuclear sleeve; and thus the number of strands in each daughter becomes the same as in the parent cell. These strands stain rather heavily with haematoxylin following most fixatives. They are perhaps somewhat more closely related to axostyles than to any other known protozoan organelles, because, even though they are grouped around the nucleus much like the parabasals of certain hypermastigotes, they can scarcely be regarded as parabasals when their staining reactions differ so greatly from those of parabasals. For example, they stain heavily with iron-alum haematoxylin following SCHAUDINN's fixative.

And perhaps a more valid reason for not regarding them as parabasals is the presence of other structures in this organism which

vary in number from 6 to 8 and which correspond more closely to flagellate parabasals. In fact, the only way they differ from the usual hypermastigote parabasal is that they appear to lack the thread portion. This, however, may not be true since the thread portion, if present, lies within the rostral tube where it may be rendered invisible due to the nature of the contents of this organelle. The body portions begin at the base of the rostral tube and probably extend posteriorly through half or more of the body (Pl. 11 Fig. 1). Doubt is expressed regarding the length of the body portions of these organelles because they become finer shortly after passing the nucleus, and it is possible that this fine portion should be regarded as the parabasal thread or filament (Pl. 11 Fig. 7). If so, it follows the parabasal body and extends beyond it posteriorly for a considerable distance. Certain parabasals, particularly those of polymastigote flagellates, have a thread following the body portion, but no hypermastigote is known where the thread extends beyond the body posteriorly. I therefore think it more likely that the parabasals of *Teranympha* differ from those of other hypermastigotes in that they gradually become more slender posteriorly instead of rounding off rather abruptly.

Teranympha differs markedly from all known hypermastigotes in that the post-rostral portion of the body is encircled by flagellar bands or rings which, so far as can be ascertained, have no connections. In twenty individuals these bands varied in number from 16 to 24 with an average of 20 (Pl. 11 Fig. 1). Each band is traversed crosswise by rows of basal granules (from which flagella arise) just as are the spiral flagellar bands of the Spirotrichonymphidae, but in the Spirotrichonymphidae the bands are continuous spirals and range in number, in different genera, from two to twelve. Each band is about two microns wide and the distance between the bands is seen to be remarkably constant when undistorted organisms are studied. The flagella, which extend for about 20 microns beyond the surface of the body, arise from the bands in the same manner as those of *Macrospironympha* and *Leptospironympha* arise from the spiral flagellar bands of these genera; but, since this process has been described (Cleveland, Hall, Sanders, and Collier, 1934), it need not be considered. The rows of basal granules are much closer together than those of *Macrospironympha* and *Leptospironympha*; and the basal granules also lie closer together in a row than in *Macrospironympha* and *Leptospironympha*; so close, in fact, that the number has not been determined (Pl. 11 Figs. 1, 4, 7).

Another feature of *Teranympha* found in no other known hypermastigote, is the segmented appearance of the body (Pl. 11 Figs. 1, 4, 5). It is difficult to explain how this is brought about since the manner in which the bands are duplicated or renewed in daughter individuals at the time of cytoplasmic division is not known. The body is depressed at each band and is widest halfway between the bands (Pl. 11 Figs. 1, 4). There is an overlapping of the cuticular or pellicular layer of the ectoplasm for a short distance posterior to each band and this outer portion is free from its posterior border anteriorly to the band or thereabouts; that is, it is not continuous with or connected to the layer immediately beneath it (Pl. 11 Fig. 4). The flagella from each row of basal granules on a band adhere to form a bundle. As each bundle leaves the band, it extends posteriorly, slants toward the surface of the body, and is fastened to the outer margin of the lower (not overlapping) portion of the pellicle; and of course, it reaches the surface at the posterior border of the free or overlapping portion of the pellicle. From this point, it is directed posteriorly in almost a straight line, but continues to adhere to the pellicle until it reaches the point where the next depression begins (Pl. 11 Fig. 4). Thus, the flagella are attached to the body from their point of origin on one band almost to the next band, and in this respect closely resemble those of several genera of the Spirotrichonymphidae. From the point where the flagella cease to adhere to the pellicle, they extend outwardly and posteriorly for a distance of approximately 20 microns, each bundle breaking up to form individual flagella as it leaves the pellicle.

The nucleus is situated near the first flagellar band, frequently lying almost in the centre of the circle made by this band. It is about 10 microns posterior to the base of the rostral tube and, as already noted, lies within the nuclear sleeve, nuclear supporting strands and parabasals.

There are no prominent centrioles and centrosomes in the interphase of *Teranympha*, such as occur in *Barbulanympha*. It is difficult to determine whether the centrioles are short or long in the interphase because there are so many darkly staining strands which lie where the centrioles if elongate must lie (Pl. 11 Figs. 1, 2). These cannot be differentiated from centrioles with certainty. An attempt was made to get around this difficulty in part by studying longitudinal sections of the rostrum, but it was difficult to obtain many rostra in perfect longitudinal sections for study. In a few of these what may be one elongate and one short centriole has been seen

lying within the nuclear sleeve (Pl. 11 Fig. 7), but I do not want to say definitely that the structures seen are centrioles, even though they lie exactly where the functioning and unquestionable fairly late telophase centriole lies (Pl. 11 Fig. 16). However, the dark line shown within the nuclear sleeve of Pl. 11 Fig. 17 is a centriole and in this organism the nucleus has almost returned to the interphase. If there is one long and one short centriole in the interphase, as suggested by this illustration and by Pl. 11 Fig. 7, *Teranympha*, in this respect, resembles *Trichonympha*, except that it has centrosomes surrounding the distal ends of the centrioles during mitosis. But the possibility cannot be excluded altogether at present of the disintegration in the very late telophase of the distal ends of the centrioles of *Teranympha* as in *Pseudotrichonympha* (Cleveland, 1935); and it may be, as Pl. 11 Figs. 7 and 17 indicate, that the disintegrating portion of the centriole is smaller than in *Pseudotrichonympha* (compare the length of the anaphase centrioles in Pl. 11 Fig. 13 with that of the telophase centriole in Pl. 11 Figs. 16 and 17).

Mitosis.

The chromatin of the interphase nucleus is arranged in both straight and curved granular prochromosomal rods (Pl. 11 Fig. 9), which are really chromosomes since all they have to do to become chromosomes is to elongate (Pl. 11 Figs. 10—12). They are all probably double in the interphase and merely move apart as the chromosomes elongate (Pl. 11 Fig. 11).

As the chromosomes elongate and move apart, astral rays arise from the distal ends of the two darkly staining, elongate centrioles and pass through the centrosomes surrounding the distal ends of the centrioles; some of those arising from one centriole soon meet those arising from the other; then they join, overlap, and grow along one another to form the early central spindle, which, at this time, lies anterior to the nucleus (Pl. 11 Fig. 11). Other astral rays do not overlap, but extend individually from the distal ends of the centrioles, many of them extending toward the nucleus. As the overlapping astral rays composing the central spindle portion of the achromatic figure increase in length, the central spindle becomes longer; and with an increase in the length of the centrioles, which occurs at this time, the central spindle depresses the ever intact nuclear membrane, thus bringing the distal ends of the centrioles to the periphery of the nuclear membrane and hence nearer the chromosomes (Pl. 11 Fig. 12). At the same time, some of the

astral rays become extranuclear chromosomal fibres by becoming attached to the intranuclear chromosomal fibres which extend from the chromosomes to the nuclear membrane. Then, as the central spindle increases in length due to the growth of the overlapping astral rays forming it and in width due to the overlapping of more and more astral rays arising from the centrioles, the daughter chromosomes gradually move apart, the nuclear membrane becoming elongate as the process continues and finally pulling in two (Pl. 11 Figs. 12—15). As the centrioles, which are fastened anteriorly to the daughter rostral flagellated areas (Pl. 11 Fig. 13), move in opposite directions, the central spindle is gradually pulled apart, and then the astral rays composing it, together with those which did not overlap, begin to disintegrate; but they do not all disappear until the daughter nuclei and extranuclear organelles have moved to opposite ends of the cell, and sometimes not until after cytoplasmic division has occurred. The last astral rays to disappear are usually those holding the chromosomes — the extranuclear chromosomal fibres (Pl. 11 Figs. 16, 17). These fibres now serve to keep the daughter nucleus in place until the new nuclear sleeve grows out and surrounds it. Shortly after the nuclear membrane pulls in two, the daughter chromosomes begin to shorten and soon become less than half their former length (Pl. 11 Figs. 15, 16). In the late telophase they elongate somewhat (Pl. 11 Fig. 17) and probably split before becoming the interphase prochromosomal rods.

The mitotic processes in *Teranympha* are similar to those of *Barbulanympha* and *Trichonympha*, the principal difference being in the size of some of the organelles concerned, those in *Teranympha*, for the most part, being smaller. It would be difficult, however, if the processes in *Barbulanympha* and *Trichonympha* were not known and were not so very plain, to interpret correctly the processes in *Teranympha*. *Teranympha* in its mitotic processes lies in an intermediate position between *Barbulanympha* and *Trichonympha* on one hand and certain metazoan cells on the other where it is impossible to follow clearly all the mitotic processes. With more study of hypermastigotes and certain metazoan cells where the details of mitosis may be followed fairly well, it will perhaps be possible in time to determine beyond question whether the mitotic processes of all cells are fundamentally the same.

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Explanation of plate.

Plate 11.

Most of the commonly used fixatives and stains were employed. The drawings were made with the aid of a camera lucida from material fixed either in Schaudinn's, Flemming's, or Hollande's fluids and stained with haematoxylin.

Fig. 1. Entire organism. Note the nucleus; the rostral and post-rostral regions of the body; rostral flagella, arising from the rostrum and slanting posteriorly; the post-rostral flagella, arising from the flagellar bands or rings, extending posteriorly as a bundle from one band almost to the next, adhering to the pellicle for this distance, and then slanting after leaving the body as individual flagella; the rostral tube containing the nuclear sleeve, which extends from the anterior tip of the rostral tube posteriorly to the nucleus which it surrounds and holds in position; the nuclear supporting strands which extend from the base of the rostrum posteriorly to the nucleus, adhering to the nuclear sleeve until they reach the mid-portion of the nucleus or thereabouts, then turning at almost a right angle and extending to the first flagellar band which they join, bifurcating shortly after leaving the nucleus, and after leaving the first flagellar band extending posteriorly just beneath the bands through most of the body as fine lines; the granular parabasals, arising from the base of the rostrum just outside the nuclear supporting strands and extending posteriorly outside the nucleus and within the flagellar bands through half or more of the body; the segmented appearance of the body produced by the flagellar bands encircling it, together with the free portion of the pellicle covering each band and extending for a short distance beyond it posteriorly; and the rows of basal granules (from which the post-rostral flagella arise) extending antero-posteriorly across the flagellar bands. $\times$ 750.

Fig. 2. Detail of anterior portion of body showing rostrum, rostral flagella, rostral tube, nucleus, nuclear sleeve, and nuclear supporting strands. Flagellar band and parabasals omitted. $\times$ 1200.

Fig. 3. Transverse section of body just anterior to nucleus showing the manner in which the nuclear supporting strands are grouped around the nuclear sleeve and nucleus and how they extend to the first flagellar band. Parabasals omitted. $\times$ 1200.

Fig. 4. Details of a portion of body to show how the flagella arising from a row of basal granules on a flagellar band, leave the band as a bundle, and adhere to the body from their point of origin almost to the next band. Note the free portion of the pellicle overlapping the bands. The continuous lines antero-posteriorly directed are the nuclear supporting strands. × 2400.

Fig. 5. Surface view of body showing flagella. Owing to the slanting direction of the flagella arising from the rostrum and those arising from the flagellar bands, the entire surface of the body appears to be flagellated. × 400.

Fig. 6. Transverse section of the rostrum showing the rostral tube, the nuclear sleeve, and the manner in which the flagella arise from the rostrum. × 1200.

Fig. 7. Detail of anterior portion of body showing rostrum, rostral tube, nucleus, nuclear sleeve, two of the nuclear supporting strands, two of the granular parabasals, one flagellar band, and probably one short and one elongate centriole within the rostral portion of the nuclear sleeve. × 1200.

Fig. 8. Surface view of the rows of flagella on the rostrum (the basal granules from which the flagella arise lie a considerable distance beneath at the border of the rostral tube). × 1200.

Fig. 9. Interphase nucleus and chromosomes. × 1600.

Fig. 10. Early prophase nucleus. × 1600.

Fig. 11. So-called metaphase nucleus with the rod-shaped chromosomes moving apart. Note anterior to the nucleus the elongate centrioles with centrosomes surrounding their distal ends and the early achromatic figure. Some of the astral rays arising from the distal end of one centriole have met and overlapped those arising from the other contriole to form the early central spindle. × 1600.

Fig. 12. The central spindle has become longer and broader and is depressing the nuclear membrane, and the long daughter chromosomes are beginning to move towards the poles (the distal ends of the centrioles). × 1600.

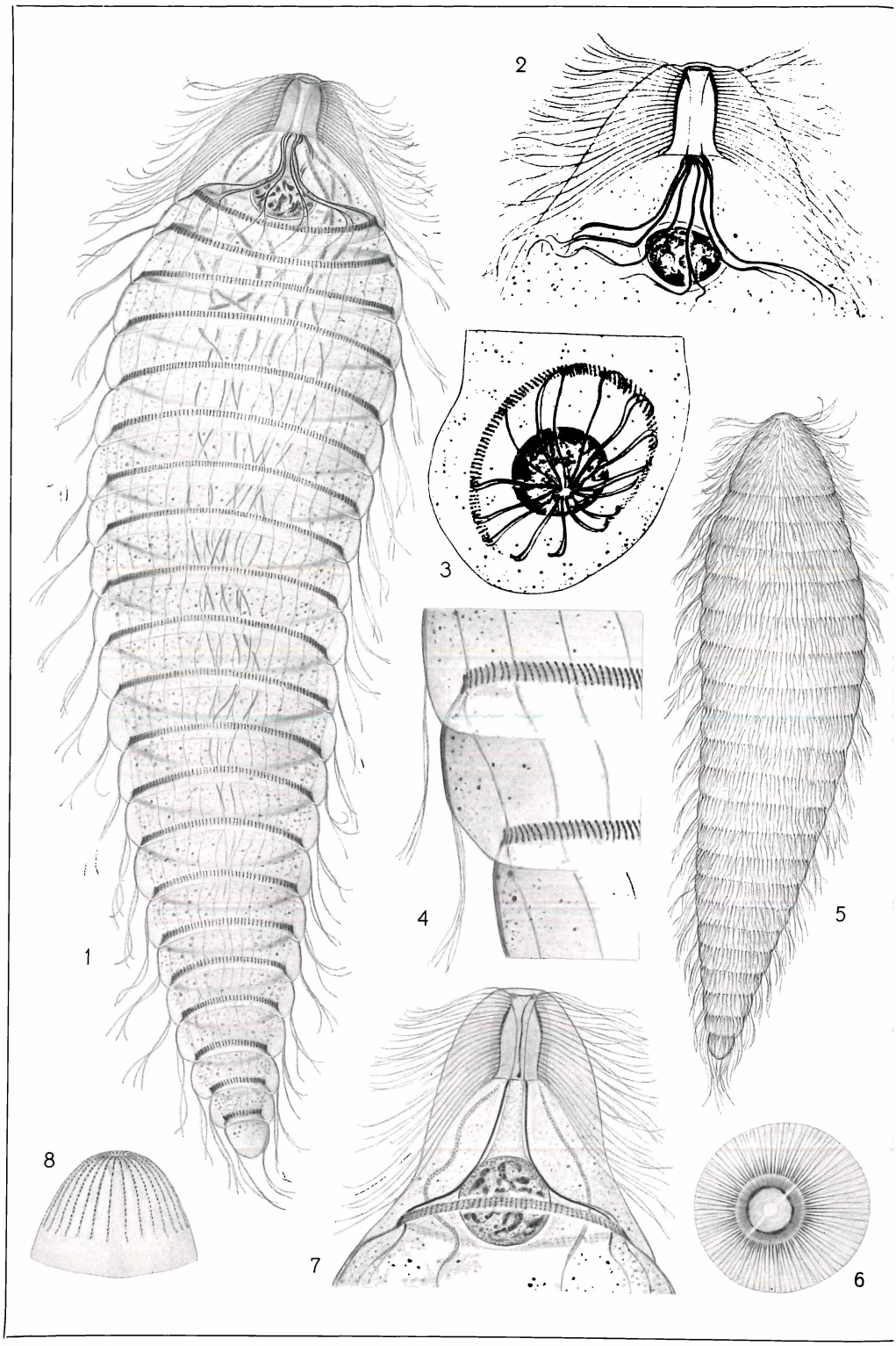
Fig. 13. A later stage in mitosis. Note the length of the contrioles. Anteriorly each centriole is connected with the base of a daughter rostrum and posteriorly to the achromatic figure which has arisen from the centrioles. Portions of the daughter parabasals, nuclear supporting strands, and nuclear sleeves are shown. × 1600.

Fig. 14. Elongate telophase nucleus. × 1600.

Fig. 15. Telophase nuclei with a broad central spindle between them. The position of the daughter organelles is indicated by the direction of the centrioles. Central spindle is on the verge of pulling apart. × 1600.

Fig. 16. Late telophase after the disappearance of most of the achromatic figure. The growth of the new rostral tube is nearly complete and the nuclear sleeve is extending posteriorly to surround the nucleus. Note the nuclear supporting strands adhering to the nuclear sleeve as it extends posteriorly. Parabasals omitted. × 1600.

Fig. 17. A very late telophase. Chromatin has almost returned to the interphase, achromatic figure has disappeared, nuclear sleeve surrounds most of the nucleus, and a centriole extends within the nuclear sleeve from the nucleus to the anterior end of the rostrum. Only two nuclear supporting strands are drawn and the parabasals are omitted. × 1600.



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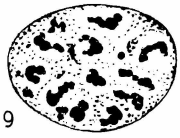

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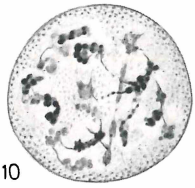

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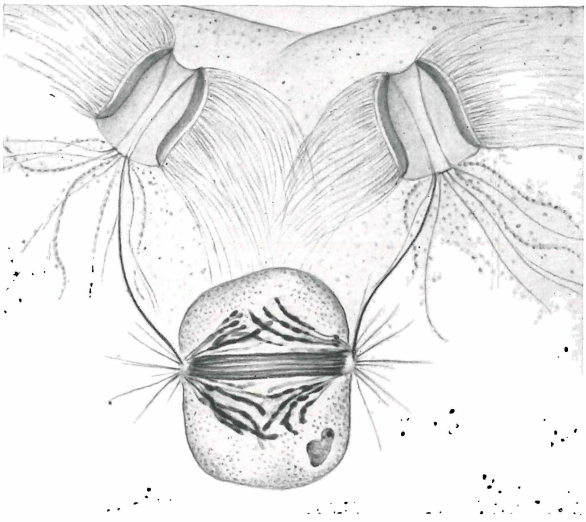

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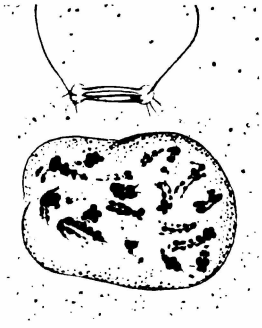

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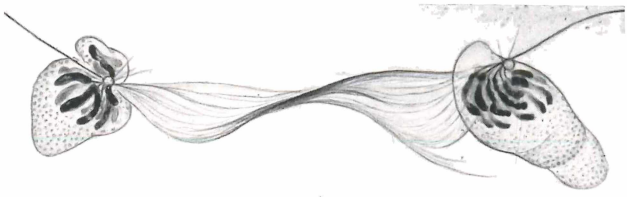

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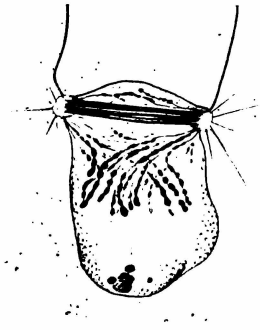

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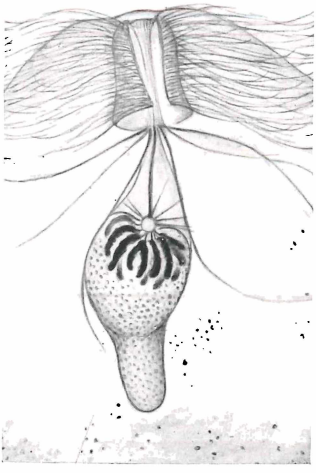

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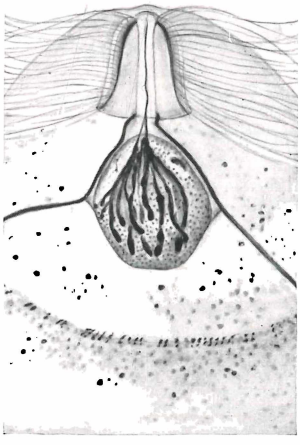

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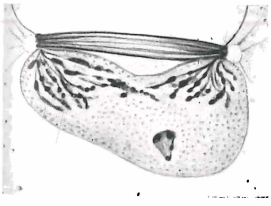

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FISCHER IN JENA