STUDIES ON PARASITIC PROTOZOA IN JAPAN III. ON PROTOZOA PARASITIC IN THE NEWT, *TRITURUS PYRRHOGASTER*

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TWO PLATES

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INTRODUCTION

As regards the protozoa parasitic in the Japanese newt *Triturus pyrrhogaster*, we have only one report dealing with *Trypanosoma tritonis* by Ogawa (1913). I have found the *Trypanosoma* in 53 out of 90 newts examined which were caught in Tokyo and Saitama Prefectures. Besides this, Flagellata (*Hexamita*), Coccidia and Balantidia were found in the intestine. *Trichomonas* was also found in a single case. Unfortunately, however, the material was so poor that morphological studies were not possible.

For the study of the living protozoa, the intestinal contents were examined without dilution and the vital staining was tried. Schaudinn's, Flemming's and Mac Clung's fluid (saturated solution of picric acid 75 c.c., 40% formol 20 c.c., glacial acetic acid 5 c.c., urea 1.0 gr.) and osmic acid vapour were used for fixation, and Heidenhain's iron-haematoxylin, safranin-light green for staining.

To obtain the section-preparations of *Balantidium*, the heavily infested intestinal tract was fixed by Bouin's fluid.

I. *Hexamita*

The locality of infection is limited to the extreme end of the intestinal canal just above the cloaca, about 5.0 mm in length. The intestine swells out a little in that part and *Hexamita* is found in the contents. The number of the parasite is very variable: in some cases they were very small in number, merely 1 or 2 specimens being found after close examination, while in other cases they were so numerous that the entire microscopic field is filled by them. The incidence of infection is very high, 83 being found infected out of the 90 specimens examined.

In the living state the body is club-like in shape, rounded anteriorly and tapering gradually towards the posterior end which is more or less distinctly truncated. Six flagella arise from the anterior end and turn posteriorly; they display rhythmic beating, the body being pushed quickly forward by this movement. From the posterior end arise two posterior flagella which are not used for the locomotion of the body, but are used sometimes for anchoring, and water-current is provoked in such occasions by the beating of the anterior flagella. The blepharoplast, nucleus and axostyle are not recognizable; many granules, which are stainable by dahlia or neutral red, are seen scattered irregularly or arranged in two rows in the posterior part. Besides the club-shaped specimens described above, pear-shaped or ellipsoidal ones were sometimes observed. They show, however, no difference from the former in all the features other than the external shape.

Stained specimens. The majority of the individuals are club-like in shape (Figs. 1, 2, 4), while the pear-shaped or ellipsoidal ones (Figs. 7, 8, 9) are also observable. Measurements of 500 specimens, 100 each from 5 different hosts, gave a range of length from $6.2\,\mu$ to $12.3\,\mu$, with an average of $8.72\,\mu \pm 0.05\,\mu$ and a range in width from $1.5\,\mu$ to $5.4\,\mu$, with an average of $2.4\,\mu \pm 0.03\,\mu$. As is easily conjectured from the variation in the shape of body, the variation coefficient of width (32.5%) is far greater than that of length (11.5%). *Hexamita* described by Dobell (1909) as *Octomitus dujardini* from Anura corresponds to the slender form and *Hexamita* described by Swezy as *Hexamitus intestinalis* from American Urodela and Anura corresponds to the broader form of the present species.

The nuclei are two in number; each of them is club-like with a slender anterior and a broader posterior end, and slightly convex outwards (Figs. 1, 3, 5). The slender anterior end touches the blepharoplast which is usually a spherical granule situated at the anterior extremity of the body. The two nuclei and the blepharoplast are arranged in the shape of a horse-shoe as a whole (Figs. 1. 4, 5). The shape of the nucleus is somewhat variable: in some specimens the anterior end is broader than the posterior (Fig. 8), in others both ends gave the same breadth (Fig. 7). Still in others the nuclei do not touch the blepharoplast (Fig. 8). The nucleus is destitute of the nuclear membrane and is stained dark by haematoxylin.

The blepharoplast is represented usually by a spherical granule as stated above (Figs. 1, 3, 5, 8). In some good specimens, however, fixed by Schaudinn's fluid, it is found to be composed of 4 small granules arranged to take position of the four corners of a minute square area free from chromatin as described by Dobell (Fig. 7). But the delicate filament connecting each granule was not recognizable.

The anterior flagella are arranged in two sets, three of the flagella of each side arising as follows: one flagellum arises from the anterior, and two from the posterior granule. Dobell stated that 2 arise from the anterior and one from the posterior. According to Swezy, these 4 granules are arranged in a single row running transversely to the body axis and the two inside granules are apt to fuse together into one. She stated also that two flagella arise from the inside granule and one from the outside granule on each side.

From the blepharoplast two axostyles extend backwards and terminate at the posterior end of the body. At the extreme end of each axostyle, there is a minute granule from which the posterior flagellum arises (Figs. 3, 6). All of the flagella are almost equal in length and are one and half times as long as the length of the body. In rare cases, however, one of the anterior flagella of each side is much longer than the others (Fig. 4). Dobell described the axostyle as a rod-like structure and Swezy considered it to be "hyaline". In my specimens it is a delicate fibre, and it seems to be correct to consider it as the intracytoplasmic portion of the posterior flagellum, contrary to Dobell's opinion that they are homologous with the axostyle of Trichomonads.

Cytoplasm is either homogeneous or granulated, vacuolated in some cases (Figs. 6, 8). The granules and vacuoles are situated usually between the two axostyles and, in some specimens, the granules are arranged in a longitudinal row along the axostyle (Fig. 7). Besides these a large vacuole is often observed between the two nuclei just behind the blepharoplast; sometimes the vaculole is separated into two by a septum (Figs. 7, 8). Swezy also recognized this vacuole, and, from her observation that it remained still in a degenerated form, she conjectured that it might not be an ordinary protoplasmic vacuole.

The first investigator of the flagellate under consideration is Dujardin who established the genus *Hexamita*. Detailed accounts were published by Dobell (1909), Alexeieff (1911) and Swezy (1915) on

Hexamita parasitic in Amphibia. Dobell found a form bearing close resemblances to the present form in *Rana temporaria, R. esculenta* and *Bufo vulgaris*, and named it *Octomitus dujardini.* Alexeieff found *Hexamita* in several species of newt and axolotl, and named it *Octomitus intestinalis.* But it is different from *H. intestinalis* in several important features such as the structure of the nucleus and the axostyle and the position of the blepharoplast. Swezy reported octoflagellated parasites from several species of Anura and Urodela under the names of *Hexamitus intestinalis, H. ovatus* and *H. batrachorum.* But she described only the broad form, and did not find the slender form such as I have described above. My investigations convinced me that the host in which the slender form is found, harbours chiefly the slender form, and in the host in which the broad form is found in large numbers, the slender form is scarcely detected. But examinations of a large number of the host reveal us the existence of individuals intermediate between the broad and slender forms (Figs. 5, 6). So that the octo-flagellates parasites dealt with in this paper all belong to one and the same species, viz: *Hexamita intestinalis.* I am in favour of Wenyon's opinion to employ *Hexamita* as the generic name of this flagellate.

II. *Coccidia*

Out of 90 newts examined, 10 were found infested by coccidians. Oöcysts and merozoites were found in 5 and 6 specimens respectively, the specimen in which both the oöcysts and the merozoites were found together was only one.

Formation of the sporozoite in the oöcyst are completed in the intestine of the host. The oöcyst contains 4 sporocysts, each including two sporozoites, indicating the characteristics of the genus *Eimeria.* Three types may be distinguished in the oöcyst. The first type is spherical in shape, measuring from 23 μ to 26 μ in diameter and the wall is colourless, without the micropyle (Fig. 10). In the sporulated oöcyst, the sporocyst is spindle-shaped, measuring 15.4 μ in length and 6.1 μ in breadth, and contains 2 sporozoites which are almost as long as the sporocyst. Sporocystic residue is recognizable, consisting of several scattered granules. The oöcystic residue is a spherical body containing a number of granules in the periphery. The diameter of this residue is a little less than the length of the sporocyst.

The oöcysts of the first type were found in two cases. In one case they were found in fairly large numbers and a few in the other.

Desiring to trace schizogony of this type, experimental administration of the sporulated oöcyst to five newts were tried. One of them was killed after 2 days and another after 3 days; no trace of infection was revealed. In the third newt sacrificed after 5 days a single oöcyst identical with the administered one was found in the intestine, and the fourth newt sacrificed after 6 days was found harbouring a number of oöcysts of the same type. The last one were sacrificed after 8 days and a small number of gametocyte-like forms were recognized, but these were too small in number to be examined in stained preparation. Thus the experimental infection resulted in a failure. Unfortunately, any further case of natural infestation of this type was not met with in spite of my eager search.

The first type of the oöcyst closely resembles the figures given in Doflein-Reichenow's Lehrbuch as *Eimeria propria* Schn. According to Phisalix, however, Schneider found two types of oöcyst in newts, and named the spherical oöcyst *Coccidium sphericum*, the ellipsoidal one *C. proprium*. Phisalix gave 36 μ as the diameter of *E. spherica* which is a little larger than in the present form. It will be not unreasonable, however, to identify the first type as *E. spherica* Schn.

The second type (Fig. 11) is ellipsoidal in shape, measuring 44.5–55.2 μ in length, with an average of 50.2 μ and 31.1–38.0 μ in breadth, with an average of 34.5 μ. As stated above, Schneider named the ellipsoidal oöcyst *C. proprium*, so that this type may be considered to be *E. propria*. The diameter, however, differs considerably: according to Schneider, *E. propria* measures 30–36 μ in length and 20–30 μ in breadth.

The oöcyst is colourless, with no micropyle, and filled with granules in the early stage. These granules become condensed gradually towards the center, leaving large spaces at the poles. According to Siedlecki the microgamete enters into the oöcyst to fertilize the macrogamete in this stage. In the oöcyst the zygote divides into 4 sporocysts, in which 2 sporozoites and a granular residual body are differenciated. The sporozoite is shorter than the sporocyst, being two-thirds the length of the latter. The sporocystic residue is irregular in shape and consists of many granules tightly aggregated together. The oöcystic residue is a spherical body with a thin wall and contains many granules in the periphery. It varies between 20.7 μ and 31.1 μ in diameter, the average being 25.1 μ.

Schneider reported in 1881 a coccidian from newts (*Triton cristatus*, *T. palmatus*, *T. punctatus*) under the name of *Orthospora propria*. It resembles my second type in the shape of the oöcyst as well as in the formation of the large residual body in it, although his description is somewhat unsatisfactory and inadequate as regards the process of sporogony. It was imposible for me to obtain Schneider's further papers published later (Tablettes Zoologiques, 1887–1892), so that the identification of this type is difficult at present. But it may not be unreasonable to consider my form as identical with *E. propria,* although in dimensions they do not coincide with each other.

The second type of the oöcyst was found only in two cases out of 90 newts examined. In one of these cases many oöcysts were found, but in the other there were only a few of them. The material containing many mature oöcysts was administered to two newts which were sacrificed after 6 and 7 days respectively. In the former the result was negative and in the latter only a few merozoites were found. Thus the feeding experiment was not successful.

The third type of the oöcyst (Fig. 12) is subspherical or ovoid in shape, measuring 38.0–44.9 μ (on the average 42.8 μ) in length and 34.5–44.9 μ (on the average 39.9 μ) in breadth. The internal feature is almost the same as in the second type. The sporocyst is spindle-like as that of measuring 22,2 μ in length and 8.2 μ in breadth. The sporocyst always surrounds the large oöcystic residual body, lying on its surface. The sporozoite is shorter than the sporocyst, and the sporocystic residue is composed of granules loosely grouped. The oöcystic residue is a very large spherical body measuring 24.2–31.1 μ, with an average of 28.3 μ in diameter, and is provided with a thin wall and many granules within, scattered chiefly in the periphery. This type of oöcyst is found only in a single case out of 90 newts examined. It is distinguished from the second type by the shape of the oöcyst, the dimension of the sporocyst and by the structure of the sporocystic residue. It is hardly possible to determine for the present whether this type belongs to either *E. spherica* or *E. propria,* or to a new species. I am inclined, however, to consider this type as a variety of the second type, as the oöcystic residue, the most remarkable feature of *Eimeria* of the newt, closely resembles that of the second type.

As regards the endogenous cycle of coccidia of the newt, Steinhaus reported as early as 1891 on the schizogony of a coccidian and he named it *Cytophagus tritonis,* considering it to be a "zystenlos" coccidian. Phisalix (1933) described the endogenous cycle of *E. spherica* in detail.

The merozoites were found in 6 cases out of 90 newts examined, and also in a newt experimentally infected by the oöcysts of the second type and sacrificed after 8 days. The merozoites were found generally in a small number, except in a single case in which fairly a large number of merozoites were observed. The intestine of this specimen was fixed by Bouin's fluid and the section preparations were studied. In this case, however, the oöcysts were not recognizable, so that it was impossible to determine to which species these merozoites belonged. Schizogony was observed between the cuticular layer and the nucleus of the epitherial cells. The mature schizont is ellipsoid in shape and contains about 20 merozoites. According to Stienhaus, they are 16 in number. The merozoite is crescent-like in shape, being pointed at the ends. The posterior half is narrower than the anterior, where a round nucleus with a definite large karyosome is situated. The merozoite measures 9.2 μ in length and 1.5 μ in breadth on an average. All these features coincide with those of *E. spherica* of which the endogenous cycle was studied by Phisalix.

Gametogony was observed only in a single case, in which a small number of gametocyte-like bodies were found. It was impossible for me to stain and examine these bodies in detail, owing to the difficulty in getting them in sufficient numbers.

III. *Balantidium*

Accompanying *Hexamita*, *Balantidium* was found in 56 cases out of 90 newts examined. Measurements of the specimens made me consider all of them to belong to a single species.

The body is oval or egg-shaped in the side view, with a narrow anterior end which is bluntly pointed or obliquely truncated, and circular in the cross section (Figs. 14, 15). The peristome is an elongated and reversed triangular groove, with its broader base lying at the anterior end of the body, becoming narrower and deeper as it passes backward, and ends in a narrow pocket-like cavity, the cytostome, which lies a little above the middle of the body. But in fixed and stained specimens the antero-posterior difference in breadth is indistinct. The left inner margin of the peristome is beset with a broad membranella extending basally along its whole length (Fig. 15). The membranella extends ventrally in its anterior part where the peristome is broad and shallow, while it gradually tends towards the right side as the preoral groove becomes narrower and deeper posteriorly, and finally the posterior part of the membranella extends transversally, springing from its left wall towards the right side, lying entirely in the groove. Still another conspicuous structure can be seen in fixed and stained specimens. There are many distinct, and more or less parallel, transverse striations, stained deeply by haematoxylin, lying just beneath the floor of the peristomeal groove (Fig. 14).

The cytoplasm is clearly differentiated into hyaline anterior and granulated posterior regions, the former occupying the anterior one-fourth and the latter the remaining and greater portion of the body. This differentiation can be seen also in some of the fixed and stained specimens, and the latter seems to be more coarsely granulated in the smaller specimens. Two contractile vacuoles were observed in the posterior region and the cytopyge or anal pore was not recognized. The macronucleus is kidney-shaped or ellipsoid, the micronuleus is spindle-shaped, lying usually in the hollow of the former. Position of the nucleus in the body is variable to some extent, though lying exclusively within the posterior half of the body. Once in May, a newt was found harbouring prenty of conjugating individuals. The macronucleus of the conjugating individual was spherical and the micronucleus was found swollen to a large spherical ball with a distinct nuclear membrane, showing a loose central cluster of minute chromatin granules. Frequently a number of rod-like bodies were found in place of the granules, which may be regarded as chromosomes. But unfortunately it was impossible to count their exact number.

There can be found fine fibrillae, lying longitudinally in the anterior part of the body just at the dextro-dorsal region of the peristome (Figs. 14, 15), They are traced anteriorly to the layer just beneath the pellicule, and posteriorly they gradually converge or centralize into a bundle. In preparations in extremely good conditions, they are traced extending to the posterior part of the body, where it seems to end freely in the cytoplasm. In most cases, this bundle of fibrillae fades away or becomes indistinct at various distances from the anterior end of the body, most frequently above or below the level of the fundus of the cystome. The fibrillae often extend posterioly, without forming or converging into a distinct bundle, and in a few cases the bundle was perceived to be in the antero-dorsal region of the body as if drifted together as a result of the cyclosis. There is another small bundle of fibrillae surrounding the cytostome, which extends into the body in the dorso-posterior direction. Besides these, in some cases, an additional small bundle can be seen lying in the right side of the posterior part of the peristome.

120 specimens from 6 hosts were measured in reference to length and breadth of the body and the macronucleus, and the ratio L/B was calculated. The length of the peristome was also measured in each specimen and the ratio of length of the body to that of the peristome was likewise calculated. The results are given in the table. Among the dimensions and the ratios here considered, the ratio of the body length to breadth proved to be the least variable, and to be of an important datum for the taxonomy of *Balantidium.*

Table

		Maximum	Minimum	Average	Standard deviation	Coefficient of variation
Body	Length	138.0 μ	65.5 μ	100.4±1.45 μ	±15.7 μ	±16.45%
	Breadth	93.0 μ	44.8 μ	62.4±0.86 μ	±9.4 μ	±15.16
	Ratio (L/B)	1.95	1.30	1.6±0.011	±0.12	±7.75
Macronucleus	Length	34.5 μ	17.2 μ	26.6±0.27 μ	±2.96 μ	±12.02
	Breadth	24.1 μ	10.3 μ	13.2±0.19 μ	±2.06 μ	±15.16
	Ratio (L/B)	2.67	1.25	1.82±0.027	±0.30	±16.37
Peristome	Length	62.1 μ	27.6 μ	40.5±0.62 μ	±6.79 μ	±16.74
	Ratio $\frac{\text{(Body L.)}}{\text{(Perist. L.)}}$	3.44	1.78	2.46±0.033	±0.36	±14.58

Two species of *Balantidium* have been reported from the newt: *B. entozoon* and *B. elongatum.* *Balantidium* under consideration resembles the former in the shape of body and of macronucleus, and in dimensions also. They differ from each other, however, in some important features, such as the number of the contractile vacuole, and the ratio of length of the body to that of the peristome. In *B. entozoon* the former counts 4, and the length of the body does not reach twice of the peristome, the peristome surpassing usually the middle of the body.

It is rather difficult for the present to determine whether the *Balantidium* under consideration is *B. entozoon* or not, although I am inclined to consider it as a new species.

SUMMARY

(1) *Hexamita, Eimeria* and *Balantidium* were found parasitic in the Japanese newt, *Triturus pyrrhogaster.*

(2) The *Hexamita* is identical with *H. intestinalis*; both the broad and slender forms are found.

(3) Three types are distinguishable in the oöcyst of the *Eimeria*. The first type is spherical and is to be indentified with *E. spherica* Schn. The second type is ellipsoid and may be identified as *E. propria*, although the dimension is far greater in my form. The third type is subspherical or ovoid and may be considered as a variety of *E. propria*.

(4) The *Balantidium* seems to be a new species.

ADDENDUM

After finishing this paper, Lavier's interesting paper on the coccidia of newts came to my hand. Conditions, however, do not alow me to review my observations, basing upon the knowledge contributed by him. So some remarks upon his findings will be added.

He has described four distinct species, two of them being new species, i. g., *Eimeria propria*, *E. spherica*, *E. tertia* n. sp., and *E. canaliculata* n. sp. Another important finding is that the size of the oöcyst increases as the process of sporulation advances. The difference in size of immature and mature oöcysts, according to him, exceeds 5 μ.

The diameter of oöcyst of the first type of my case (*E. spherica*) is slightly smaller than that measured by Lavier, but is still within the range of variation of his measurements. On the size and appearance of the oöcystic residue some differences are recognizable.

The second type of my case resembles very closely *E. propria* of him in every respect, except the dimension of oöcyst which is smaller in his case. The above mentioned difference may not be large, if his statement on the augmentation in the size of oöcysts accompanying the progress of sporulation is taken into consideration, for I have measured only the sporulated oöcysts while his measurements were limited to the unsporulated oöcysts.

Existence of minute canals in the wall of oöcystand the appearance of the oöcystic residue consisting of several large globules irregularly grouped are given by Lavier as distinguishing characteristics of *E. canaliculata*. And as regards the other new species, *E. tertia*, the smaller size and the shape of the oöcyst, being ovoid and measuring from 22 μ to 33 μ in length and from 18 μ to 25 μ in breadth, are mentioned to be of specific characters. No oöcysts showing the characteristics given above were met with in my material; futhermore, the third type of my case shows resemblance with them.

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EXPLANATION OF THE PLATES

Figs. 1–9. *Hexamita intestinalis.*

Fig. 1. The most common type.

Fig. 2. An individual with the nuclei projecting from the body surface.

Fig. 3. A specimen having a minute granule at the posterior end of each axostyle.

Fig. 4. An individual with one of the anterior flagella on each side longer than the others.

Figs. 5, 6. Two individuals intermediate between slender and broad types.

Figs. 6, 7. A specimen having the special vacuole between the two nuclei.

Fig. 8. An ellipsoidal form.

Fig. 9. A broad form.

Fig. 10. The first type, *Eimeria spherica.*

Fig. 11. The second type, *Eimeria propia.*

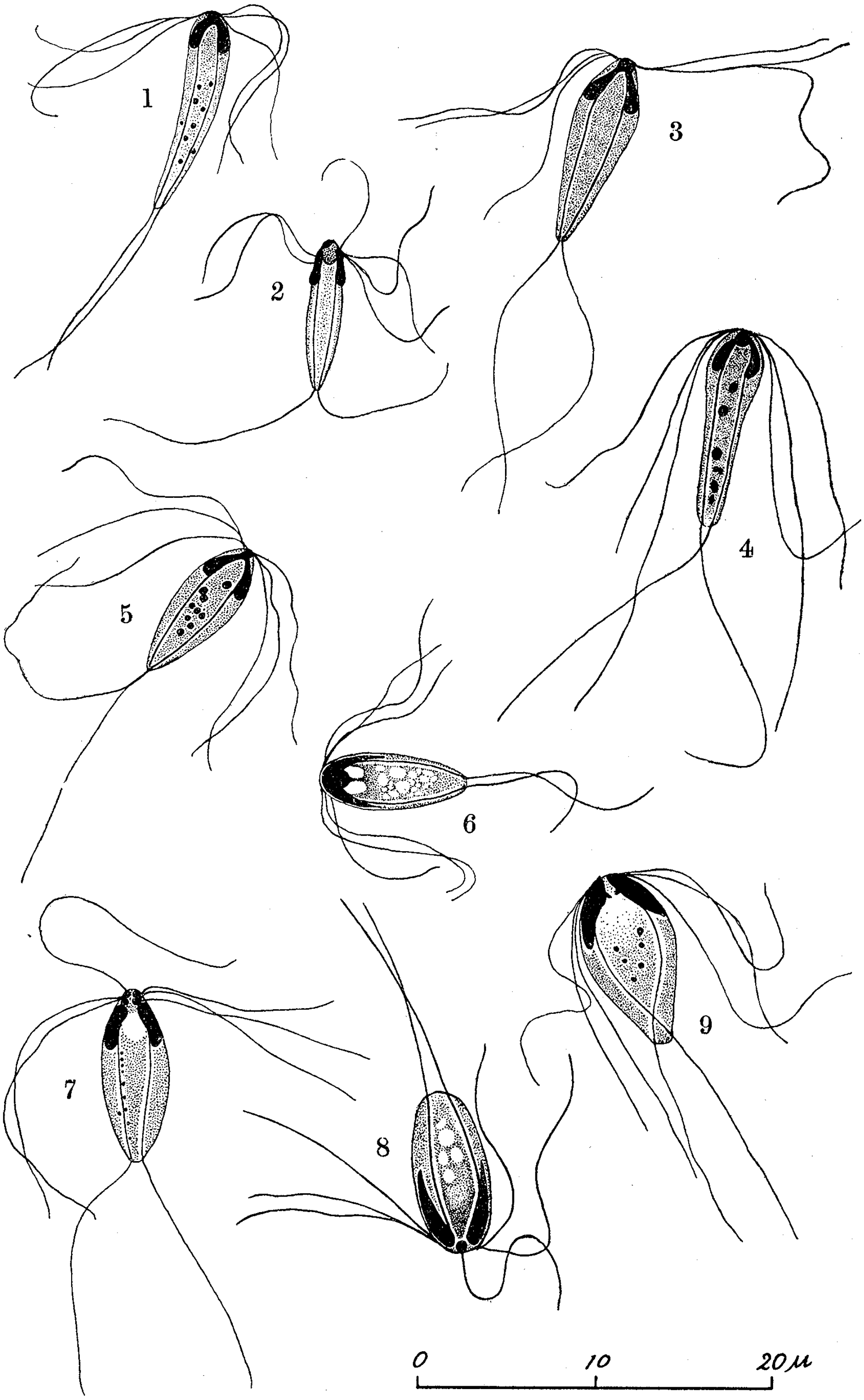
Fig. 12. The third type, variety of *Eimeria propria.*

Figs. 13–15. *Balantidium* sp.

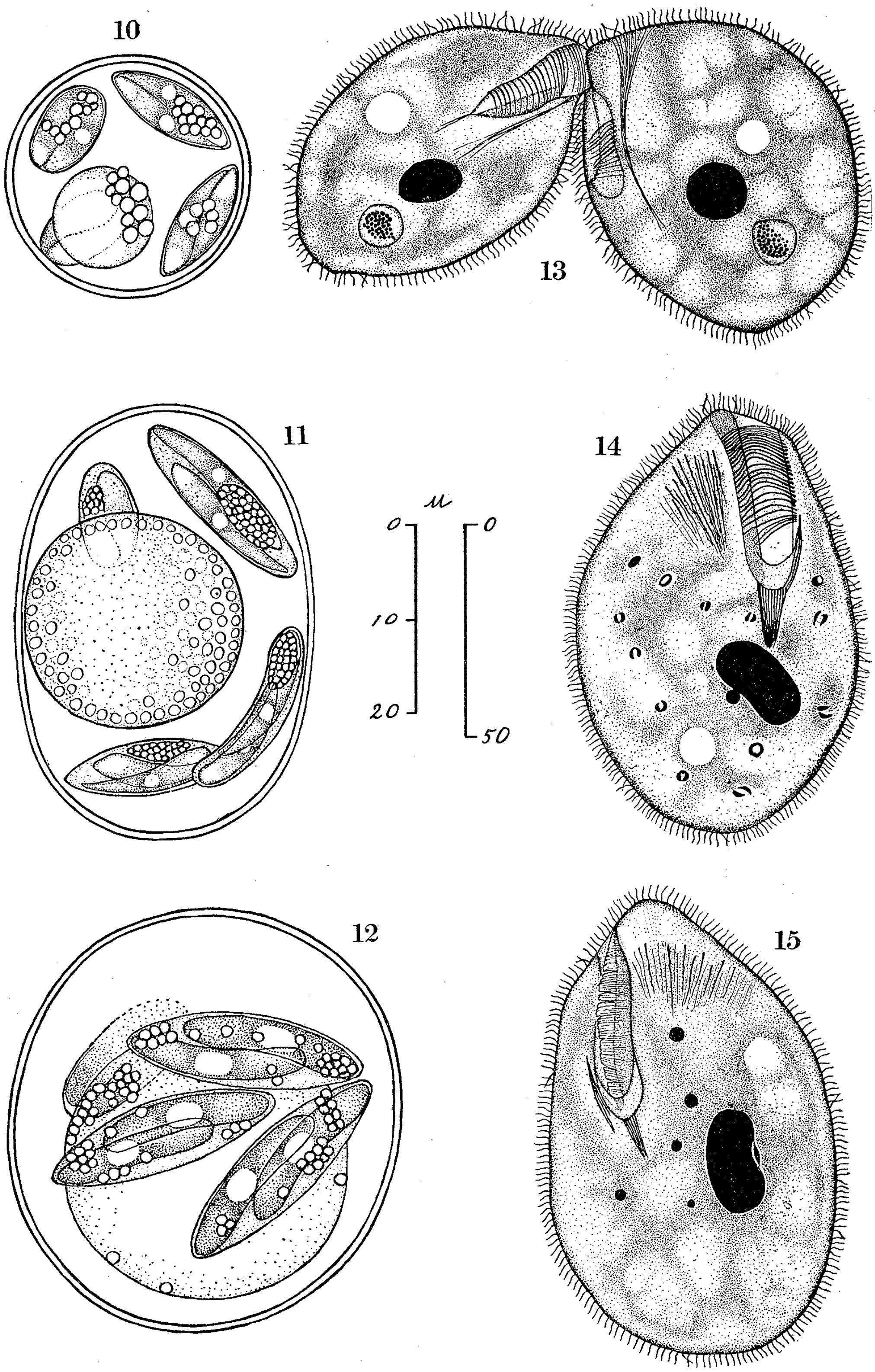
Fig. 13. A conjugated pair.

Fig. 14. A specimen seen dorsally.

Fig. 15. A specimen seen ventrally.



H. MATUBAYASI: STUDIES ON PARASITIC PROTOZOA IN JAPAN III.



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