Trichomastix serpentis, n.sp.

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With Plate 27, and 2 Text-figures.

The subject of this paper is a parasitic flagellate which I have obtained from the rectum of Boa constrictor, L. Owing to the kindness of Mr. W. A. Harding, of Histon, Cambridge, I was enabled to examine one of these snakes shortly after it had died of canker of the mouth. In the rectum there was about 30 c.c. of a brownish, almost odourless, alkaline fluid, containing many organic particles in suspension. This fluid was transferred to a glass dish, and prevented as far as possible from evaporating by covering with a thick glass plate fixed down by vaseline. It was by examination of this culture from time to time that the observations here recorded were made.

The first examination was made upon October 30th, 1906, one day after the death of the snake. Trichomastix was found to be present in small numbers. The parasites increased in numbers in the culture, and reached a maximum at the beginning of December, 1906. A decline in the number of the organisms present in the fluid then followed, accompanied by several curious changes in the animals themselves. At the end of February, 1907, there were very few specimens to be found after a very careful search, and by March 2nd, 1907, all the parasites had died out. Alto-

gether I was able to keep the organisms alive in this manner for a period of 120 days. All attempts to grow the organisms in other fluids (solutions of peptone, albumen, etc.) were unsuccessful. The death of the culture was not due to increase in the number of bacteria or to a change in the reaction of the medium, both of which remained very much as they were in the beginning. The numbers of the bacteria were somewhat reduced however. Death resulted, I believe, from a too great increase in the amount of katabolic products.

The method of examination was as follows:—A few drops of the culture were drawn up in a fine pipette (used exclusively for this purpose), and examined fresh either in a hanging drop preparation or under a coverslip with wax feet, and waxed round the edges. In this latter anærobic condition I have been able to keep the animals alive and active for thirteen days. For examining the living animals I used almost exclusively a 2·5 mm. apochromatic water immersion objective by Zeiss, with compensating oculars 2, 6, 12, and 18. Most of the observations here recorded were made from the living animal. Good permanent preparations were exceedingly hard to obtain owing to the small numbers of the parasites and the large amount of gritty foreign matter in the fluid. However, a few successful preparations were made, the stains employed being Delafield's hæmatoxylin, Heidenhain's iron hæmatoxylin, and Giemsa's stain. Observation of the living animal was often facilitated by intravitam staining with neutral red. Brillantcresylblau (Grübler) and methylene blue were also tried, but proved to be of but little use.

From the morphological characters of this parasite there can be no doubt that it is properly referable to the genus Trichomastix, Blochmann. Hitherto this genus has been represented by but a single species, T. lacertæ, Bütschli. The life-history of this form has been fully worked out by Prowazek. It is, I think, highly probable that the form which I am about to describe will prove to be quite common

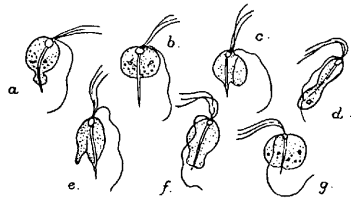
in Ophidia generally. Unfortunately I have not been able to obtain suitable material for deciding this point up to the present. I venture, however, to give the specific name serpentis to this parasite.

It is possible that the form under consideration is identical with that described by Grassi under the name Monocercomonas coronellæ from the gut of Coronella austriaca. The description given is, however, not sufficiently accurate for the identity to be established. The same may be said of an organism described by Hammerschmidt in the cloaca of Tropidonotus natrix, and named by him Cercomonas colubrorum. This form appears to be the same as Monocercomonas colubrorum (Hammerschmidt), Doflein, and Bodo colubrorum (Hammerschmidt), Saville-Kent.

Structure, etc.

Trichomastix serpentis is usually of a more or less oval or pyriform shape. It is subject to considerable variations however. Some of the more commonly occurring modifications of its form are seen in text-fig. 1 *a*—*g*.

Text-fig. 1.



Most of these shapes are only temporary, the animal usually returning after a time to a condition more or less

closely corresponding with that depicted in Pl. 27, fig. 1. This shape may therefore be considered as "normal." The length varies from about 8 μ to 17 μ, an average-sized animal being about 14 μ. At the anterior end [see Pl. 27, fig. 1] are inserted three anteriorly directed flagella, in length about one and a quarter times that of the body. From the same basis arises another and longer flagellum, which is directed backwards (Schleppgeissel). The spot from which these flagella arise appears in the living animal as a refractive granule, situated immediately above the nucleus, which is placed anteriorly. In stained preparations this basal granule is seen to be composed of a chromatic substance which stains like the nucleus. Running through the whole length of the body of the animal is a somewhat flexible axial rod (Achsenstab), which terminates anteriorly in the basal granule of the flagella, and posteriorly is drawn out into the caudal process of the body. The axial rod either traverses the nucleus, in order to reach the base of the flagella, or else lies in close contact with it. It is impossible to be quite certain of the exact relationships of these structures. The rod is, I believe, skeletal in function. Its general aspect and relations to the nucleus recall the axial rods of the pseudopodia of the Heliozoa. Near the base of the flagella is to be seen a well-marked cell mouth or cytostome.

The creatures exhibit great activity of movement, which does not appear to be affected in the very least by light, as is the case with some flagellates. Warming appears to increase their activity, but no critical experiments on the effects of temperature were made. The food consists of the small micro-organisms abounding in the medium.

Division.—Division is, as in most flagellates, longitudinal, and presents certain features of interest. Briefly, the process is as follows [see Pl. 27, figs. 2—10] :—An ordinary individual [fig. 2] becomes more or less globular [fig. 3]. At the same time the axial rod is absorbed, that is to say, it disappears. The flagella are now seen as a very rapidly vibrating bunch, springing from a refringent spot—the

basal granule. This latter loses its connection with the nucleus, and divides, so that two bunches of flagella are formed. It has been found impossible to count the flagella at this stage, or to determine how the new ones arise. Concomitantly the nucleus becomes dumb-bell shaped [fig. 4]. The organism then assumes a roughly triangular appearance, but is rapidly drawn out, and becomes somewhat sausage shaped. A constriction appears in the middle [fig. 6], and it is now possible to make out that there are four flagella at either end, one in each case directed away from the other three. The nucleus is still dumb-bell shaped, and situated mesially. The daughter-cells now rapidly draw apart [fig. 7], and remain connected by only a small bridge of protoplasm, at either end of which is a nucleus, the daughter nuclei being still connected by a very fine protoplasmic strand. Further separation now takes place, finally resulting in the snapping of the connecting strand of protoplasm [figs. 8, 9]. In the daughter individuals which are thus separated, it will be seen that the nuclei lie at the extreme posterior end of the body, and there is no axial rod. Up to this stage the process usually occupies about twenty minutes. If one of these daughter cells be carefully watched, it will be seen that the nucleus travels forwards, and as it does so the axial rod is seen to be developed in the region immediately posterior to it. The rod appears to be formed by the nucleus in its track as it passes forward [figs. 9, 10]. After some time—usually one to two hours—the nucleus reaches the anterior end of the body, and enters once more into relation with the basal granule of the flagella. In some way the axial rod becomes connected with the basal granule, but it is impossible to see how this is effected. The cytostome appears to be lost prior to division, and to be re-formed in each of the daughter cells, but I am not quite clear on this point.

This account of the division of T. serpentis differs from that of T. lacertæ as described by Prowazek. If I understand this author correctly, the axial rod would appear to function as a kind of division centre; for it retires towards

the nucleus, arranges itself there at right angles to its original position, and the chromatin passes along it forming a nucleus at each end.

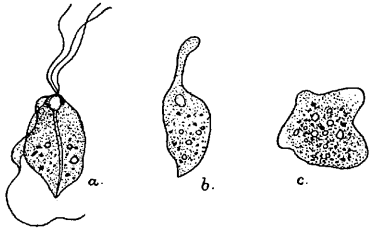
Unfortunately, in spite of much careful observation, I was unable to ascertain whether conjugation occurred or not. In T. lacertæ only autogamy occurs, and has been very carefully worked out by Prowazek in his excellent paper on parasitic flagellates. I have frequently seen two individuals apply themselves to one another for a short time, but they always separated without any conjugation taking place. In some cases a very delicate cyst wall was formed, the animal becoming globular, and a number of refractive bodies were developed inside. In the course of a few hours, however, these forms always disintegrated.

Degeneration and Death.

As already mentioned, it was found possible to keep the organisms in cultures for 120 days, but no longer. The animals all underwent degenerative changes, and finally died. A very great variety of forms occurred among those animals which were degenerating. The following is the course of events which usually took place, though many variations were seen. First, the animals cease to move from place to place. Movements of the flagella continue, but at a slower rate, the animal remaining approximately in the same position. At this period the posterior flagellum frequently shows a tendency to become adherent to the body, a delicate membrane uniting them together [see text-fig. 2, *a*]. In this condition the creature is almost indistinguishable from a Trichomonas — a genus which differs from Trichomastix in possessing an undulating membrane (often with a short free flagellum) in place of the posterior flagellum. This adhesion of the posterior flagellum soon becomes more extensive, and finally the flagellum is completely merged in the protoplasm of the body. The move-

ments of the anterior flagella are meanwhile becoming slower, and they very frequently become completely fused into a single finger-like process [text.-fig. 2, *b*], which continues to contract in the same direction as did the flagella. This state may continue for hours, or even days. The axial rod disappears before long, and finally the whole organism becomes an amœboid mass of protoplasm [text.-fig. 2, *c*]. In this condition no movement away from the original position takes place, the protoplasm being simply thrust out and then rapidly withdrawn. Undulating movements, involving the

TEXT-FIG. 2.



whole amœba, frequently and rapidly occur. The creature remains in this condition sometimes for days. Before death the movements become slower, and finally cease. The animal then becomes rounded off, and, after a varying period of time, disintegrates. Not uncommonly two or more creatures, degenerating side by side, run together and fuse before death. This is even more remarkable in the case of the degenerate forms which attempt to divide. All the stages of division up to that corresponding with fig. 7, Pl. 27, may be gone through, when suddenly both individuals, instead of separating, become amœboid and fuse. Death follows, as in the case of the ordinary amœboid forms. In all cases disintegration of the nucleus appears to occur before that of the cytoplasm.

A very remarkable fact was sometimes observed in the involution forms. An individual, instead of dividing when it reached a certain size, continued to grow. In this way giant individuals arose, which reached the enormous length of 30 μ, i. e. about twice the normal length. Very few of these were observed, but quite a number reached a length of 20 μ—24 μ. In these exceptionally large forms I was able, in an unexpected manner, to confirm my previous observations on the inter-relationships of nucleus, flagella, and axial rod. Structures which had been made out with great difficulty, and after many hours' watching, were here seen very plainly after an examination lasting a few moments. These giant forms divided abnormally, commonly giving rise to three or four daughter-cells [see fig. 14, Pl. 27]. Division rarely became complete, the whole usually fusing into a large amœboid mass, which finally died. Unfortunately, owing to insufficiency of material, I was never able to obtain stained preparations of these stages. It could be seen in the living animals, however, that each of the daughter individuals possessed a nucleus, axial rod, and full complement of flagella. Sometimes the old axial rod was seen sticking out of the central mass of protoplasm [fig. 14].

A very similar atypical division has been described by Prowazek in the allied form, Trichomonas lacertæ, Prow. —apparently as a normal occurrence. I cannot think, however, that this is a normal process in Trichomastix serpentis.

Before concluding I may call attention to the resemblance between the basal granule of the flagellar apparatus and the blepharoplast of a trypanosome. As I find that a detailed comparison of these structures has been made already by Laveran and Mesnil in the case of the closely allied form Trichomonas, I refer the reader who may be interested in such speculations to their original paper.

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EXPLANATION OF PLATE 27,

Illustrating Mr. C. Clifford Dobell's paper on "Trichomastix serpentis, n. sp."

[The drawings from the living animal were all made under a Zeiss 2·5 mm. water immersion apochromatic objective (apert. 1·25). Those from stained preparations (Figs. 11, 12, 13, and 15) under a 3 mm. oil immersion (apert. 1·40) by the same maker. Compensating oculars, Nos. 2, 6, 12, and 18, were employed.]

FIG. 1.—Living Trichomastix serpentis, showing details of structure. At the upper end is seen the large vesicular nucleus, upon which lies the basal granule of the flagella. The axial rod is seen supporting the basal granule and flagella; and passing backwards, in relation to the nucleus, it traverses the whole length of the body and terminates in the caudal process.

To the left of the nucleus is seen the cystostome. Numerous food particles are seen in the posterior part of the body. This figure is drawn on a larger scale than the others.

Figs. 2—10 show division as observed in the living animal. (For the sake of clearness, the nucleus is represented as being more distinctly outlined than is really the case.)

FIG. 2.—Animal before division.

FIG. 3.—Animal becoming rounded posteriorly. Axial rod disappearing.

FIG. 4.—Nucleus has become dumb-bell shaped, and there are now two bunches of rapidly vibrating flagella, at the base of which a refringent spot is visible, no longer attached to nucleus.

FIG. 5.—Flagella separating—likewise the nuclei. These latter are still connected by a protoplasmic strand, however.

FIG. 6.—Body has become elongated, and a constriction has appeared. Nuclei still connected, and flagella now distinctly seen to consist of two groups of four each, one being in each case directed away from the other three.

FIG. 7.—Separation of daughter-cells from one another. Nuclei still connected, and lying between the two individuals.

FIG. 8.—Daughter-individuals nearly drawn apart.

FIG. 9.—One of the daughter-individuals soon after separation. The nucleus, which lay at the extreme posterior end, is now making its way anteriorly, and the axial rod is seen behind it.

FIG. 10.—Later stage of same individual. The nucleus has not yet reached the base of the flagella, and the growth of the axial rod is therefore not yet complete.

FIG. 11.—Preparation, showing nucleus, basal granule, and flagella, etc.

FIG. 12.—Stage in division, corresponding with Fig. 4. The chromatin masses at the bases of the groups of flagella are well shown.

FIG. 13.—Specimen at about the stage seen in Fig. 10.

FIG. 14.—Large individual dividing into three. Living animal.

FIG. 15.—Degenerate animal which has become amœboid. The nucleus is breaking up, and there are, in addition, many food particles in the cytoplasm.

The specimens reproduced in Figs. 11, 12, 13, and 15 were stained by Giemsa's method.

TRICHOMASTIX SERPENTIS.