Trypanosome Diseases of Domestic Animals in Nyasaland. I.—Trypanosoma simiæ, *sp. nov.* *Part III.*

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[Plates 6–8.]

Introduction.

In previous papers* the morphology of *Trypanosoma simiæ* and its action on animals have been described. In this it is intended to give an account of its development in *Glossina morsitans.*

Before entering, however, into the details of this particular development, it may be of interest to take a general survey of the various modes of development which take place in the different groups of trypanosomes. It may then be laid down that there is a well marked separate and characteristic mode of development in each of the three main groups of trypanosomes:—

In the first group—the *T. brucei* group—which includes *T. brucei, T. gambiense, T. evansi* (?), and *T. equiperdum* (?), the parasites develop—at least in the first two named species—at first through the whole length of the intestinal tract, excluding the proboscis, and eventually reach the salivary glands, where forms resembling those found in the blood of animals are developed, and these alone constitute the infective stage.

In the second group—the *T. pecorum* group—which includes *T. pecorum* and *T. simiæ,* the development takes place in the intestinal tract, including the labial cavity of the proboscis, and afterwards the trypanosomes reach the hypopharynx, or termination of the salivary duct in the proboscis. Here they revert to the original blood form and become infective. In this group trypanosomes are never found in the salivary glands, and no blood forms or infective forms are developed until the hypopharynx is reached.

In the third group—the *T. vivax* group—which includes *T. vivax, T. uniforme,* and *T. capræ,* the initial stages of the development take place in the labial cavity of the proboscis alone; later the hypopharynx is invaded, where again blood forms are developed, which again constitute the only infective forms. Here also there is no invasion of the salivary glands, and, in addition, no development takes place in the intestinal canal.

* 'Roy. Soc. Proc.,' 1912, B, vol. 85, and 1913, B, vol. 86.

In all three groups the common factor which leads to the formation or development of the final or infective forms is the invasion of the salivary tract, and this is accompanied by a reversion to the original blood forms.

Definitions.

In this paper the word "proboscis" will mean the piercing apparatus of the "fly," made up of the labrum, labium, labellum, and hypopharynx. There are two tubes in the proboscis: one for the passage inwards of blood, made up by the coalition of the labrum and labium, the other for the passage outwards of the salivary secretion—the terminal salivary duct or hypopharynx. The term "labial cavity" in this paper will mean the former, or tube for conveyance of blood, the word "hypopharynx" the latter, or duct for conveyance of saliva. In the past the use of the word "proboscis," including both tubes, has given rise to a good deal of ambiguity.

The definition of the words "infected" and "infective" were given in a previous paper.*

The term "blood form" means a stage in the development of the trypanosomes in the "fly," when there is a reversion to the original form found in the blood of animals, and from which the cycle of development originated.

The Development of T. simiæ in G. morsitans.

Eight experiments were carried out with laboratory-bred flies. Two were positive and six were negative. The following table shows these eight experiments, the number of flies used, the number of infected flies found on dissection and the number of days which elapsed before the flies became infective:—

Table I.

Date.	Expt.	No. of flies used.	Experiment positive or negative.	No. of infected flies found.	No. of days before flies became infective.
1912.					
May 1	502	20	—		
June 27	754	31	+	2	50
Oct. 9	1477	17	—	1	
Nov. 11	1582	8	—		
„ 15	1602	20	—		
„ 21	1622	16	—		
1913.					
Feb. 5	1847	45	+	7	20?
„ 10	1856	16	—		

* 'Roy. Soc. Proc.,' B, vol. 86. ("Infectivity of *Glossina morsitans* in Nyasaland.")

It will be noted that there is a great difference between the two positive experiments as regards the time required for the flies to become infective. In the first 50 days elapsed, in the second only 20. This is due to different temperatures under which the experiments were carried out. The first positive experiment was done during the coldest time of the year on Kasu Hill, when the mean temperature was 62° F. (16·6° C.), which is much lower than on the plains, the natural habitat of *G. morsitans*. The flies in the other experiment were kept in an incubator at a temperature of 83° F. (28·3° C.), and they became infective much sooner.

Details of the Two Positive Experiments.

The following table gives the principal details of the first positive experiment :—

Experiment 754.

Table II.

Day of expt.	Procedure.	Remarks.
1–3 4 5–60	Flies fed on *T. simiæ*-infected monkey. Starved. Fed on clean monkey.	Trypanosomes first appeared on the 57th day.

It is seen that it was not until the flies had been fed on the clean monkey for 57 days that the animal showed trypanosomes in its blood. If we allow seven days for the average incubation period of the parasite in the mammalian host, then the monkey contracted the disease about the 50th day after the infecting fly had fed on trypanosome-infected blood.

The following table gives the principal details of the second positive experiment :—

Experiment 1847.

Table III.

Day of expt.	Procedure.	Remarks.
1–10 11 12–27	Flies fed on *T. simiæ*-infected monkey. Starved. Fed on clean monkey.	Flies became infective on the 26th day after first infected feed ; 16 days after the last.

Since the flies of this experiment were fed on infected blood for a period of 10 days, the time required for the trypanosomes taken up by the "fly"

to multiply and regain their virulence cannot be accurately estimated. Allowing seven days for the incubation period it cannot be more than 20 days.

When the healthy monkey became infected, in order to separate the infective flies, those remaining alive were divided into three batches. Each batch was put into a cage and fed separately on a healthy monkey. The following table gives the details and results of feeding the three batches of flies:—

Table IV.

Expt.	Batch.	No. of flies.	No. of days fed.	Result.	No. of infected flies found.
1847	1	12	7	−	0
1847	2	10	7	+	3
1847	3	13	7	+	3

The monkeys on which Batches 2 and 3 were fed showed trypanosomes in their blood on the sixth day after the first application of the flies. It is therefore highly probable that the flies infected the monkeys on the first day of feeding.

Details of the Six Negative Experiments.

The following table shows the method of procedure in carrying out the six negative experiments:—

Table V.

Expt.	Day of expt.	Procedure.	Remarks.
502	1–2	Fed on infected monkey.	
	3–4	Starved.	
	5–42	Fed on clean monkey.	All flies negative on dissection.
1477	1–3	Fed on infected goat.	
	4–5	Starved.	One infected fly found on the 40th day; proboscis and gut infected.
	6–45	Fed on clean monkey.	
1582	1st	Fed on infected pig.	
	2nd	Starved.	
	3–30	Fed on clean monkey.	All flies negative on dissection.
1602	1st	Fed on infected pig.	
	2nd	Starved.	
	3–35	Fed on clean monkey.	All flies negative on dissection.
1622	1st	Fed on infected pig.	
	2nd	Starved.	
	3–29	Fed on clean monkey.	All flies negative on dissection.
1856	1–7	Fed on infected monkey.	
	8–26	Fed on clean monkey.	All flies negative on dissection.

In Experiment 1477 a portion of the intestine of the infected fly was inoculated subcutaneously into a pig; the pig did not become infected.

Out of a total of 173 flies used in these experiments, 10 flies (5·8 per cent.) became infected with a growth of trypanosomes in the intestines and in the probosces. It will also be seen that only 1 fly in 31 (2·7 per cent.) became infective when the flies were kept at ordinary room temperature, whereas 4 became infected in 45 (9 per cent.) when the flies were kept at a temperature of 28° C.

General Considerations regarding the Development of T. simiæ in G. morsitans.

All the flies dying during the progress of the experiments were dissected. In the two positive experiments, out of 76 flies dissected, nine infected flies were found. The following table gives the results of the dissection of these nine flies :—

Table VI.

Expt.	Time, days.	Proboscis.		Proventriculus.	Crop.	Foregut.	Midgut.	Hindgut.	Proctodæum.	Salivary glands.
		Labial cavity.	Hypopharynx.							
754	37	+ +		+ +	—	+ +	+ +	—	—	—
754	50	+ +		+ +	—	+ +	+ +	—	—	—
1847	16–26	—	—	—	—	+ +	+ +	—	—	—
1847	30–40	+	+	—	—	+ +	+ +	—	—	—
1847	31–41	+ +	+	+ +	—	+ +	+ +	+ +	—	—
1847	31–41	+ +	—	+	—	+ +	+ +	+ +	—	—
1847	31–41	+ +	+	+		+ +	+ +			
1847	32–42	+ +	+	+	—	+ +	+ +	+ +	—	—
1847	32–42	—	—	+	—	+ +	+ +	—	—	—

From this table it will be seen that in seven out of nine flies dissected the labial cavity is found to contain trypanosomes. This is very different from what is seen in the similar table relating to *T. gambiense*. There not a single case of infection of the proboscis is recorded.*

At what stage in the development of the trypanosome the proboscis takes a part is not known. It is probable that the infection commences in the intestinal tract and moves forward into the proboscis, but owing to the difficulty of obtaining sufficient laboratory-bred *G. morsitans* the Commission have not, up to the present, enough evidence to establish this detail.

In the two infected flies found in the cage of flies, Experiment 754, it is to be regretted that the contents of the hypopharynx were not noted, but

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 516.

in all the infected flies found in Experiment 1847 this was done, with the result that the hypopharynx was found invaded by trypanosomes in four out of the seven.

Plate 6 represents, at a magnification of 500 diameters, the labial cavity and hypopharynx of an infected fly. While the labial cavity contains clusters of large ribbon-like trypanosomes, the hypopharynx is swarming with small active forms resembling the original blood forms, from which the developmental cycle arose. When the plate is examined the facility with which a tsetse fly can infect an animal will no longer be a matter of wonder.

Finally, from the table it will be seen that in no case were the salivary glands invaded.

The Methods used in the Examination of the Flies.

The flies were dissected as described in a previous paper.* An additional method of examining the contents of the hypopharynx was to isolate infective flies by putting each fly into a separate tube, numbering it, and feeding the fly on a susceptible animal with a corresponding number on its cage. The numbers on the cages of animals which became infected indicated the tubes containing infective flies. These, when thus identified, were starved for 24 hours, in order to make them hungry. A tube containing one of the infective flies was then taken, and its mouth being covered with mosquito netting was applied to a large cover-glass placed on a man's finger. The hungry fly at once attempted to feed through the glass, and in poking about with its proboscis smeared the surface of the cover-glass with saliva. This was immediately fixed, stained with Giemsa, and examined.

The Trypanosomes found in the Proboscis.

Reference to the table above will show that in Experiment 754 two infected flies were found, one on the 37th day after feeding on an infected monkey, and one on the 50th day, and that the labial cavities of both flies were infected.

The fly that died on the 50th day was the one which no doubt actually infected the healthy monkey, since the animal showed trypanosomes seven days after the death of this fly and no other infected fly was found. As these two flies died before they were isolated, the method of inducing them to salivate on a cover-glass was not used. When, however, the two proboscides were examined in a drop of normal saline solution under a cover-glass,

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 513.

trypanosomes attached to the labrum were seen growing in colonies in the labial cavity. They were moving freely and some detached individuals were swimming actively up and down the lumen of the tube.

It is to be regretted that the contents of the hypopharynx were not specially noted. These were two of the earliest experiments, and at that time the contents of the labial cavity and the hypopharynx were not differentiated.

In Experiment 1847 seven infected flies were found. It will observed (Table VI) that the first was dissected on the 16th day after the last infected feed and that the proboscis was not infected. Another fly dissected on the 32nd day had also no infection of the proboscis. A third fly dissected on the 31st day had the labial cavity of the proboscis infected but not the hypopharynx. The remaining four were found to contain swarms of trypanosomes in both the labial cavity and the hypopharynx. On examination it was observed that there were two distinct varieties. One found in the hypopharynx closely resembled small blood forms of *T. simiæ* (Plate 8, figs. 18 to 21). They swarmed in the narrow tube, which had the appearance of being blocked up by their enormous numbers. These small blood forms of the parasite were readily distinguishable from those growing in the labial cavity. Those growing in the labial cavity resemble *Leptomonas*, and are peculiar in having their non-flagellated extremity prolonged to a snout-like extension (Plate 8, figs. 12 to 16). They are assembled in clusters and attached by their flagella to the inner surface of the labrum, their prolonged free extremity moving vigorously in the lumen of the tube (Plate 6, fig 1).

The contents of the hypopharynx of a living infective fly isolated from Batch 2 was examined by inducing the fly to salivate on a cover-glass as described above. On examination of the stained preparations typical blood forms of *T. simiæ* were seen embedded in the saliva which the fly had ejected on the cover-glass in its efforts to reach the skin (Plate 8, figs. 22 to 25). Another infective fly was taken alive from its glass tube and its proboscis gently squeezed until a minute drop of fluid was observed at its tip, which was then lightly rubbed over a cover-glass. Here again typical blood forms of *T. simiæ* were found embedded in the salivary secretion (Plate 8, figs. 26 to 29).

In Experiment 1847 it is seen that a positive result is associated with the finding of infected flies in which *T. simiæ* resembling those found in the blood of infected animals are found in large numbers blocking up the tube of the hypopharynx.

The experiment of tempting the infective fly to feed through a cover-glass demonstrates the fact that when the fly salivates, as it undoubtedly does in puncturing the skin, these blood forms of the parasite are washed out of the

hypopharynx with the saliva and are injected with it under the skin of the fly's victim.

Further examination of flies by inducing them to salivate on cover-glasses revealed the fact that sometimes the long, narrow intestinal forms of trypanosomes are ejected in large numbers on to the cover-glass (Plate 8, figs. 1 to 11). There is no doubt, therefore, that an infected fly has the power of regurgitating the contents of its proventriculus and intestines forward into the labial cavity and probably into the blood stream of the bitten animal. It is conceivable that in this way the proboscis first becomes infected by the intestinal forms of trypanosomes, which attach themselves to the inner surface of the labrum and enter the lumen of the hypopharynx, which they invade, however, only as far as the entrance of the two salivary ducts. Here in the chitinous hypopharynx they establish themselves and, bathed in the salivary secretion, finally complete their development into the infective blood form of the parasite.

It is a curious fact that neither the salivary glands nor even the salivary ducts beyond the hypopharynx have ever been found infected with *T. simiæ*.

It was proved by the Commission in Uganda that the blood forms of *T. gambiense* developing in the salivary tract were the virulent forms of the parasite, and it now seems also proved that the developmental forms of *T. simiæ* found in the hypopharynx represent the last and infective stage of development of this species of trypanosome in the "fly."

It may be noted here that in the negative Experiment 1477, in which an infected fly was found (Table V), the labial cavity was infected with the long forms of the parasite attached to the labrum, but most careful search failed to reveal infection of the hypopharynx with blood forms. In this fly the parasite had not attained the final and essential stage of its development—the reversion to the blood type—and so the fly was harmless.

The Trypanosomes found in the Alimentary Canal.

The intestines of infected flies were generally packed full of trypanosomes from the proventriculus to the mid-gut. Sometimes the infection extended to the hind-gut, but never beyond.

Little need be said in regard to the developmental forms found in the intestines. One curious fact, however, emerges and that is, that it is impossible to differentiate one species of trypanosome from another by the study of these intestinal forms. Whether it is *T. brucei* or *T. gambiense*, *T. pecorum* or *T. simiæ*, they present the same appearance. Perhaps on further work some differences may become apparent, but at present no difference has been found to exist. The most numerous forms are long,

slender, ribbon-like, very active trypanosomes, which in a former paper* were called the normal reproductive type. By comparing Plate 7 with the insets of that paper, the resemblance between the intestinal developmental forms of *T. simiæ* and *T. gambiense* will be apparent.

Conclusions.

1. That *T. simiæ* can be transmitted from infected to healthy animals by the tsetse fly *G. morsitans.*

2. That *T. simiæ* multiplies in the intestines and in the labial cavity of the proboscis of the "fly." Here only developmental forms are found, never infective forms.

3. That the *T. simiæ* growing in the intestines of the "fly" has no specific characters by which it can be distinguished from other species of pathogenic trypanosomes found in tsetse flies.

4. That the final stage of the development takes place in the hypopharynx, wherein the infective form of the parasite, similar in shape to the trypanosome found in the blood of infected animals, is produced.

5. That the flies do not become infective until about 20 days after their first infected feed.

DESCRIPTION OF PLATES.

Plate 6.

Fig. 1.—Appearance of the labial cavity of the proboscis of *Glossina morsitans* with *Trypanosoma simiæ* growing in clusters attached by their flagellar extremities to the inner surface of the labrum. Living and unstained, × 500.

Fig. 2.—Appearance of the hypopharynx in the same fly, showing innumerable small and active *T. simiæ* almost blocking up the lumen of the duct. Living and unstained, × 500.

Plate 7.

Intestinal developmental forms of *T. simiæ.* These do not differ in appearance from the developmental forms of other species of pathogenic trypanosomes found in the intestinal tract of tsetse flies.

Plate 8.

Developmental forms of *T. simiæ* from the labial cavity and hypopharynx of *G. morsitans.*

Figs. 1–11.—Trypanosomes ejected by a living *G. morsitans* on attempting to feed through a cover-glass. These are supposed to be intestinal forms pressed up into the proboscis and on to the glass by the muscular contraction of the fly.

Figs. 12–16.—*T. simiæ* growing in the labial cavity of the proboscis of *G. morsitans.*

Fig. 17.—Aberrant form from hypopharynx.

Figs. 18–29.—Blood forms of *T. simiæ* found in the hypopharynx. These form the final stage in the developmental cycle of this species of trypanosome and are the only infective forms. Stained Giemsa, × 2000.

* 'Roy. Soc. Proc.,' 1911, B, vol. 83, p. 513.

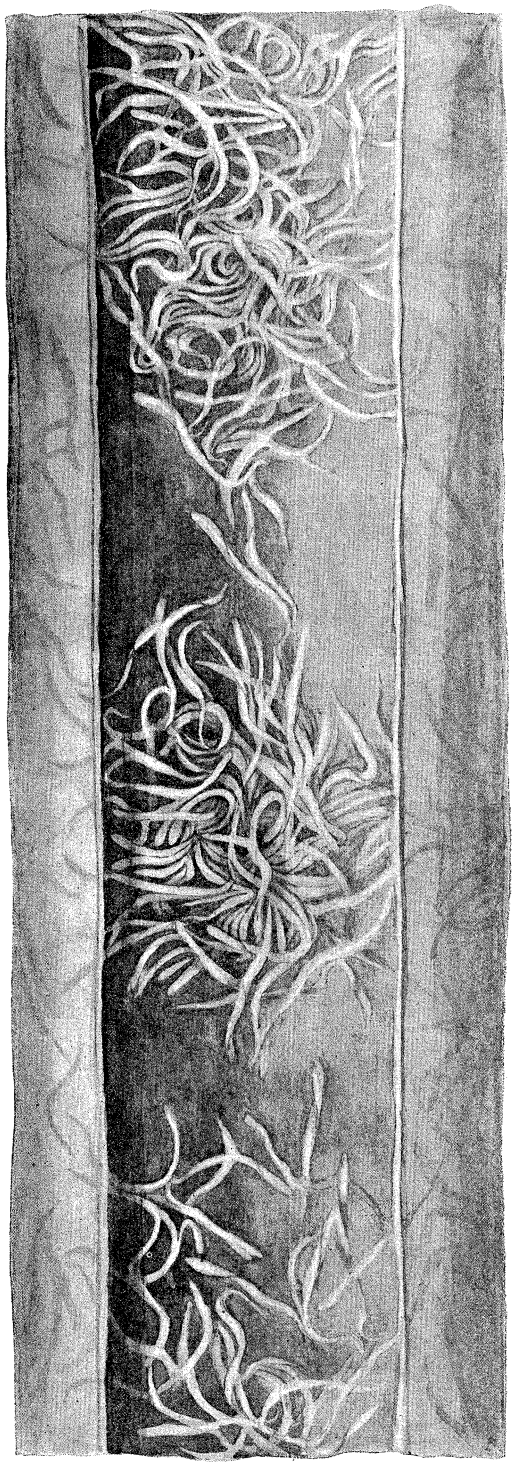
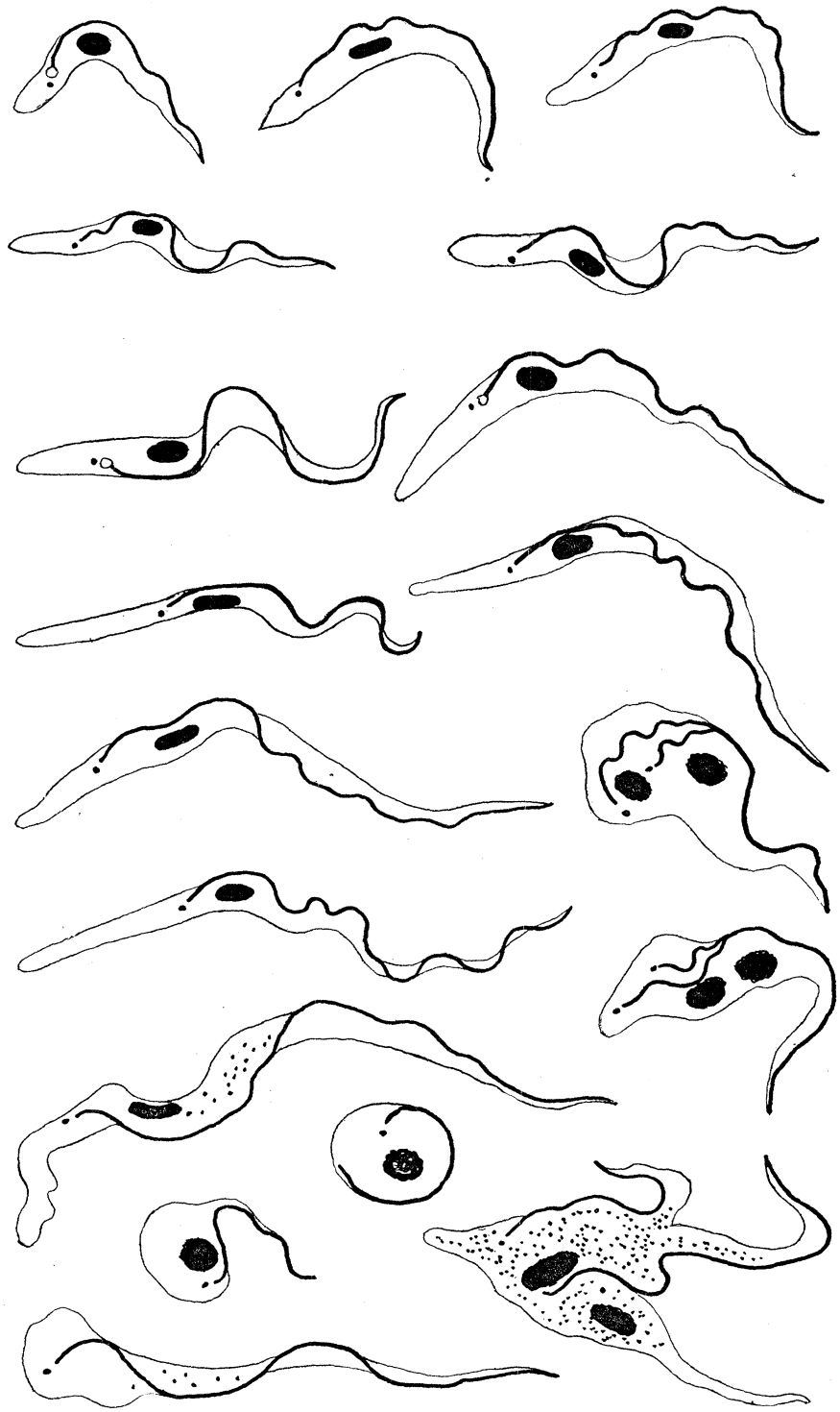


FIG. 1. FIG. 2.

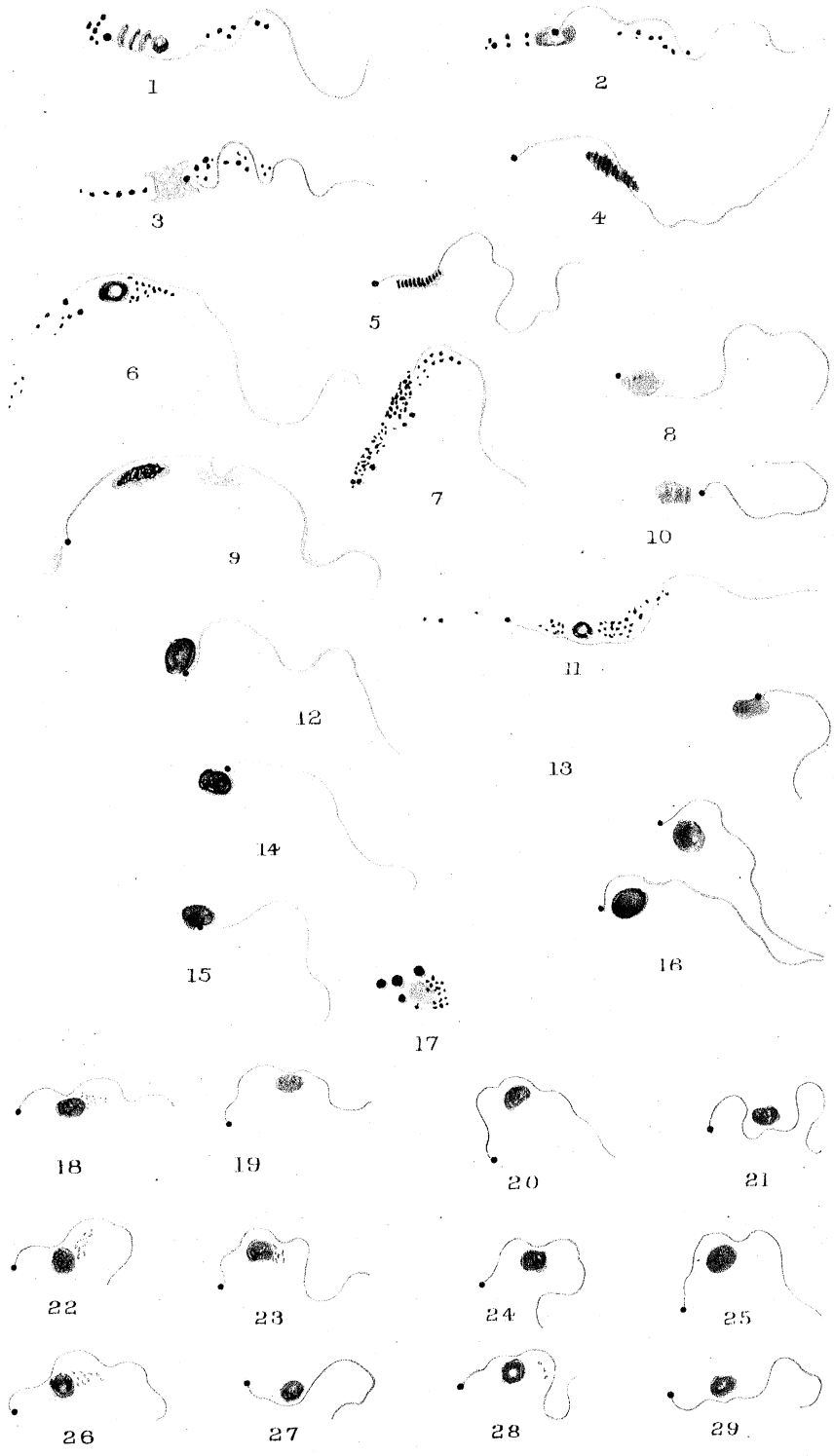
T. simiæ in labrum and hypopharynx.

M. E. Bruce, del. ×750.



T. Simiæ in gut of fly.

Major Hamerton, del x 2000.



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T. simiæ from proboscis & hypopharynx.

FIG. 1.

FIG. 2.

T. simiæ in labrum and hypopharynx.

T. Simiæ in gut of fly.

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T. simiæ from proboscis & hypopharynx.