

Development of Trypanosomatids in the Phytophagous Insect *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae). A Light and Electron Microscopic Study

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Experimental infections of the phytophagous Hemiptera Dysdercus peruvianus with different trypanosomatids were studied for up to 55 days by light microscopy while the course of infection with Leptomonas seymouri and the Leptomonas isolate 49/553G.O. was analyzed by electron microscopy.

Rates of infection of D. peruvianus varied according to the infecting flagellate. The lower part of the midgut was found to be the preferential site of colonization where most flagellates were found isolated or arranged in clumps or rosettes. Specialized junctional structures with host cells were never observed. Flagellates could also be seen inside midgut cells within a parasitophorous vacuole. Infection of haemocoel and salivary glands was also observed.

Key words: *Dysdercus peruvianus* - *Leptomonas* - *Phytomonas* - trypanosomatids

Monoxenous trypanosomatids may colonize a wide range of phytophagous insects (Wallace 1966). Among Hemiptera of the family Pyrrhocoridae, *Leptomonas* spp. were described in *Dysdercus nigrofasciatus* and *Pyrrhocoris apterus* (Wallace 1966), in *D. suturellus* (Wallace 1977), in *Odontopus nigricornis* (Vaidya & Ranade 1984) and in *Dysdercus* spp. (Sbravate et al. 1989).

Several aspects of the host-parasite association have been studied in insects naturally infected with trypanosomatids (Molyneux 1977, McGhee & Cosgrove 1980, Schaub 1992), but only in a few cases, investigations involved phytophagous bugs (Hanson & McGhee 1963, Laugé & Nishioka 1977, Tiezen et al. 1986, 1989, Freymuller et al. 1990).

Ultrastructural studies of *Leptomonas*-host association comprise only those of *Leptomonas oncopelti* in *Oncopeltus fasciatus* (Laugé & Nishioka 1977), *Leptomonas lygaei* in *Lygeus pandurus* (Tiezen et al. 1989), and *Leptomonas* spp. in Siphonaptera (Molyneux & Ashford 1975, Molyneux et al. 1981, Beard et al. 1989).

The present study reports on the receptivity of *D. peruvianus* to different trypanosomatids, and light and electron microscopy observations of the gut and salivary glands infected with *L. seymouri* and a new *Leptomonas* isolate.

MATERIALS AND METHODS

Organisms - Cultures of trypanosomatids were obtained from various sources: *Phytomonas serpens* from the phytophagous *Phytia picta* (Jankevicius et al. 1989); *Phytomonas* sp. from the host plant *Citrus bergamia* (Conchon et al. 1989), *L. seymouri* from *D. suturellus* (ATCC 30220); isolate 24/268G.O. from *Dysdercus* sp. (Sbravate et al. 1989); 49/553-G.O. from *D. ruficollis* (Moraes 1991). Flagellates were cultured in biphasic medium consisting of 2% blood agar base and an overlay of LIT or FYTS medium at 28° as before (Camargo 1964).

Insect colony - *D. ruficollis*, *D. maurus*, *D. peruvianus*, and *D. honestus* were collected from the environs of Campinas (State of São Paulo, Brazil). Insects were cultured in glass vials, on cotton seeds, at room temperature.

Infection of phytophagous insects - Clean, thirsty adult insects were artificially fed on small pieces of cotton which were saturated with log-phase culture flagellates (1×10^7 organisms/ml). After 48 hr exposure to cultures, the dissection of insects on sterile saline was initiated. Groups of four insects were dissected at 2, 7, 15, 21, 30, 45, and 55 days post infection (p.i.). Development of infection was monitored by phase-contrast microscopy on squashes of gut, haemolymph, and salivary glands. Density of parasites was estimated on four insect groups based on the number of flagellates founded in ten microscopy fields, using a X40 objective lens (+: up to ten flagellates; ++: 10-100

flagellates; +++: higher than 100 flagellates). Follow-up of isolate 24/268G.O. was not included due to the low level of infection as well as to its short duration (until the 30^o day p.i.). Histology and electron microscopy studies were done with *L. seymouri* and isolate 49/553-G.O.

Light and electron microscopy - For histological studies, gut fragments and salivary glands were fixed in 0.1 M phosphate-buffered 2.5% (v/v) glutaraldehyde, dehydrated in an alcohol series, embedded in Histo-resin (LKB), and sections stained with 1% Giemsa for 50 min. For transmission electron microscopy (TEM), samples were fixed for 3hr in 2.5% glutaraldehyde and 1.5% paraformaldehyde (v/v) in 0.1 M cacodylate buffer, pH 7.3, at 4°C, post-fixed in 1% OsO₄ for 2 hr, stained in uranyl acetate overnight, dehydrated in an ethanol series and propylene oxide and embedded in Spurr resin. Ultrathin sections were stained in uranyl acetate and lead citrate and examined in a Jeol 100 CX electron microscope. For scanning electron microscopy (SEM) hindgut specimens were fixed, postfixed and dehydrated as above described, critical-point dried in a Balzer apparatus, shadowed with gold and examined in a Jeol JSM 5300 scanning electron microscope.

RESULTS

The rates of infection of *D. peruvianus* varied according to the flagellate's species. Higher infec-

tivity rates were obtained with *L. seymouri* (80%) followed by the *Leptomonas* isolate 49/553G.O. (74%) and isolate 24/268G.O. (31%) (Table I). Infection with *P. serpens* and *Phytomonas* sp. isolated from *C. bergamia* were negative throughout the experiments.

Besides gut colonization, infection of haemocoel and salivary glands was observed for *L. seymouri* (6%) and isolate 49/553G.O. (10.8%) at any time p.i. (Table I). Infection of both, haemocoel and salivary glands, were always simultaneous, although higher number of flagellates were always found in salivary glands (Table II).

TABLE I

Experimental infection of *Dysdercus peruvianus* to different trypanosomatids

Flagellates	Insects exposed to infection		
	Total Number infected	Gut infection	Haemolymph /salivary glands infection ^a
<i>Leptomonas seymouri</i>	83	67	2
49/553 G.O.	46	34	3
24/268 G.O.	95	30	0
<i>Phytomonas serpens</i>	14	0	0
<i>Phytomonas</i> sp.	14	0	0

^a: haemolymph/salivary gland infection detected together with gut infection

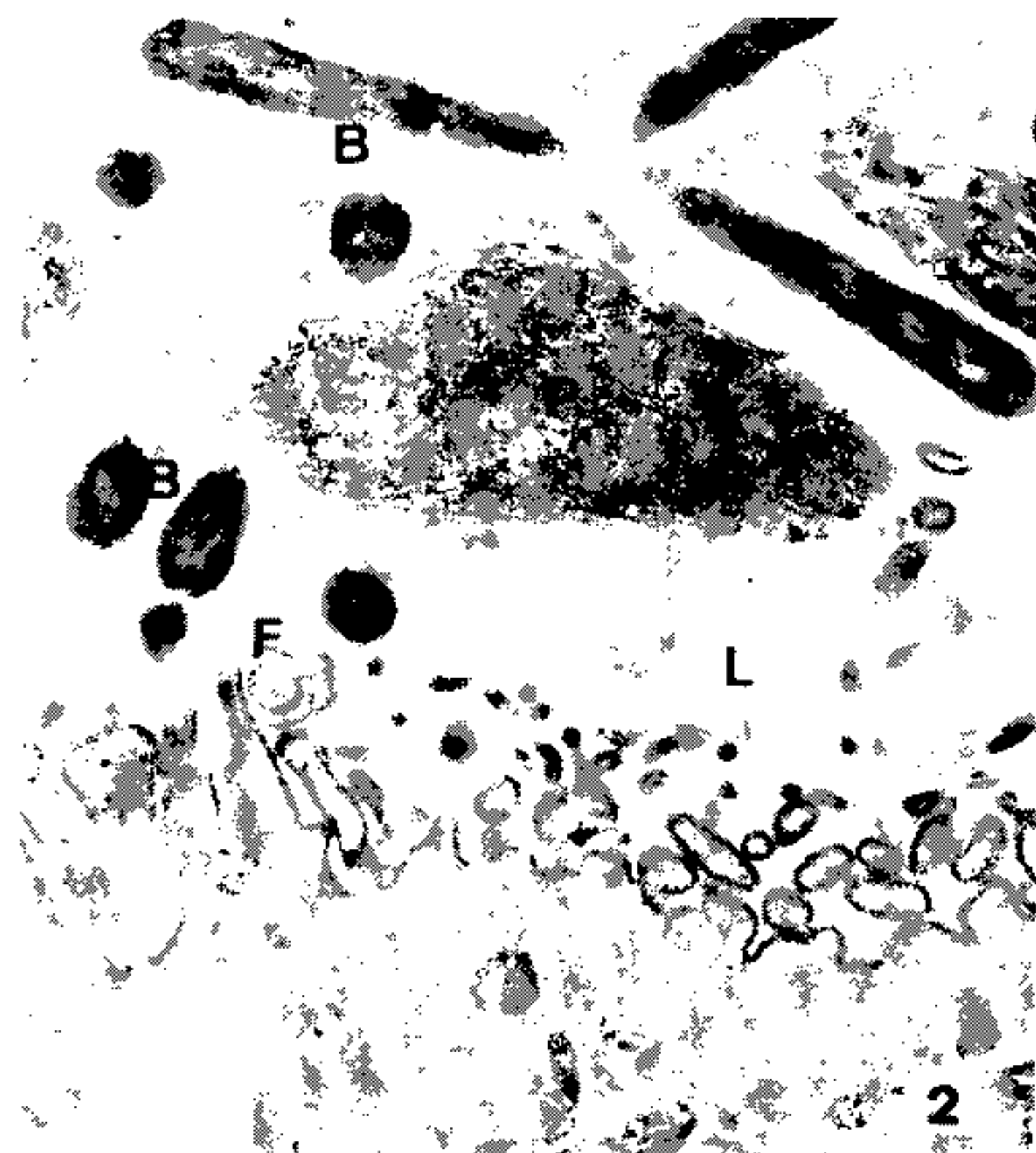


Fig. 1: TEM. *Leptomonas seymouri*. 8th day p.i. Free parasite (P) in midgut lumen near cell microvillus (MV); ecml: extracellular membrane layers; B: bacterium. X19,300. Fig. 2: TEM. *Leptomonas seymouri*. 21st day p.i. Parasite (P) and bacteria (B) in midgut lumen (L). Flagellum (F) in close contact with cell microvillus but without any attachment structure. X10,200.

TABLE II

Development of *Leptomonas seymouri* and isolate 49/553G.O. after experimental infection of *Dysdercus peruvianus*

Days p.i. ^a	Midgut		Hindgut		Haemolymph ^b		Salivary glands ^b	
	a	b	a	b	a	b	a	b
02	+	+	0	0	0	0	0	0
07	++	++	+	+	+	0	+++	0
15	++	+++	+	++	0	+	0	+++
21	+++	+++	++	++	0	0	0	0
30	+++	++	+	+	+	+	+++	+++
45	+	++	+	+	0	+	0	+++
55	+	++	+	+	0	0	0	0

Average level of infection: 0 no flagellates detected; +: up to 10 flagellates; ++: 10-100 flagellates; +++: higher than 100 flagellates

a - *Leptomonas seymouri*

b - isolate 49/553G.O.

a: days post-infection

b: infection found in only one insect of the corresponding period

Gut density of flagellates reached the maximum between 15 and 21 days p.i. Thereafter, their number began to decrease although insects remained infected until the end of experiments (Table II). In the midgut, most flagellates occurred as free parasites in the lumen, near lining cells (Figs 1, 2), frequently in close association with extracellular membrane layers (Figs 1, 2) and usually displaying large flagellar pockets. Sometimes, trypanosomatids could be seen arranged in clumps in close proximity to the midgut epithelial border. However, specialized junctional structures with host cells were never seen (Fig. 2). Colonization was always higher in the lower part of the midgut which corresponds to its widened storage part.

In the rectum, flagellates intermixed with numerous bacteria were located only at the rectal wall level (Fig. 3). Most flagellates were found isolated (Figs 4, 6) or forming rosettes (Fig. 5), free in the lumen (Figs 4, 6) or appensed to the rectal cells (Fig. 3). As in midgut, attachment with host cells through junctional complexes was never seen.

Penetration of the midgut epithelium by *L. seymouri* and isolate 49/553G.O. occurred through an intracellular route, although pathological alterations of the epithelium were never observed (Fig. 7). Outside gut wall, large number of flagellates were seen in close proximity and lined-up along the basement membrane, but with no signs of physical association (Fig. 8). In the haemolymph flagellates were never seen inside haemocytes.

Infections of salivary glands were always intense displaying lumen and gland wall cells completely filled with flagellates (Fig. 9). Usually, only one or two out of four gland lobes were parasitized. Trypanosomatids could be seen between lay-

ers of the basement/haemocoelomic membrane system and gland cells. Flagellates were also seen inside gland cells within a parasitophorous vacuole (Fig. 11) as well as in gland lumen (Fig. 10).

DISCUSSION

Experimental infection of *D. peruvianus* was highly successful with *L. seymouri*, but failed with *Phytomonas* spp. This is in agreement with Sbravate et al. (1989) who could not disclose *Phytomonas* spp. in *Dysdercus* after a large survey for infection in bugs.

In our study with *D. peruvianus*, flagellates in the midgut were frequently seen surrounded by amorphous material and extracellular membrane layers similar to that described in other phytophagous Hemiptera (Tiezen et al. 1986, 1989) and reported to originate from the delamination of the outer unit membrane of microvilli (Tiezen et al. 1989).

Ultrastructural studies of the host-parasite relationship between *Leptomonas* and phytophagous insects (Laugé & Nishioka 1977, Tiezen et al. 1989), reported the attachment between flagellum and specialized regions of the hindgut such as the rectal gland cells, frequently through hemidesmosomes. This was also true for the *Leptomonas*/flea association (Molyneux et al. 1981, Beard et al. 1989). Differently, in our study, even when in close proximity to cell wall, flagellates remained free in the gut lumen as described by Wallace (1977) for *L. seymouri* in *D. suturellus*. Even when clumped along the gut wall no signs of structural association with host cells were detected.

Analogous to the infection of tsetse flies by *Trypanosoma brucei* (Ellis & Maudlin 1985) and

of *Rhodnius prolixus* by *T. rangeli* (Hecker et al. 1990), parasites were seen inside a parasitophorous vacuole in midgut cells. Free flagellates in midgut cell cytoplasm were absent.

After reaching haemocoele, most flagellates remain lined up, side by side with the basement membrane. A similar type of association was observed in *R. prolixus* infected with *T. rangeli* in the region of

the basal lamina of midgut epithelial cell (Hecker et al. 1990). During haemocoelomic invasion, *Leptomonas* were never detected within haemocytes as reported for *T. rangeli* (Añez 1983). No pathological effects, such as reduction of extracellular membrane layers, microvilli and vacuolated cells were seen, as described for *Triatoma infestans* infected with *Blastocrithidia triatomae* (Jensen et al.

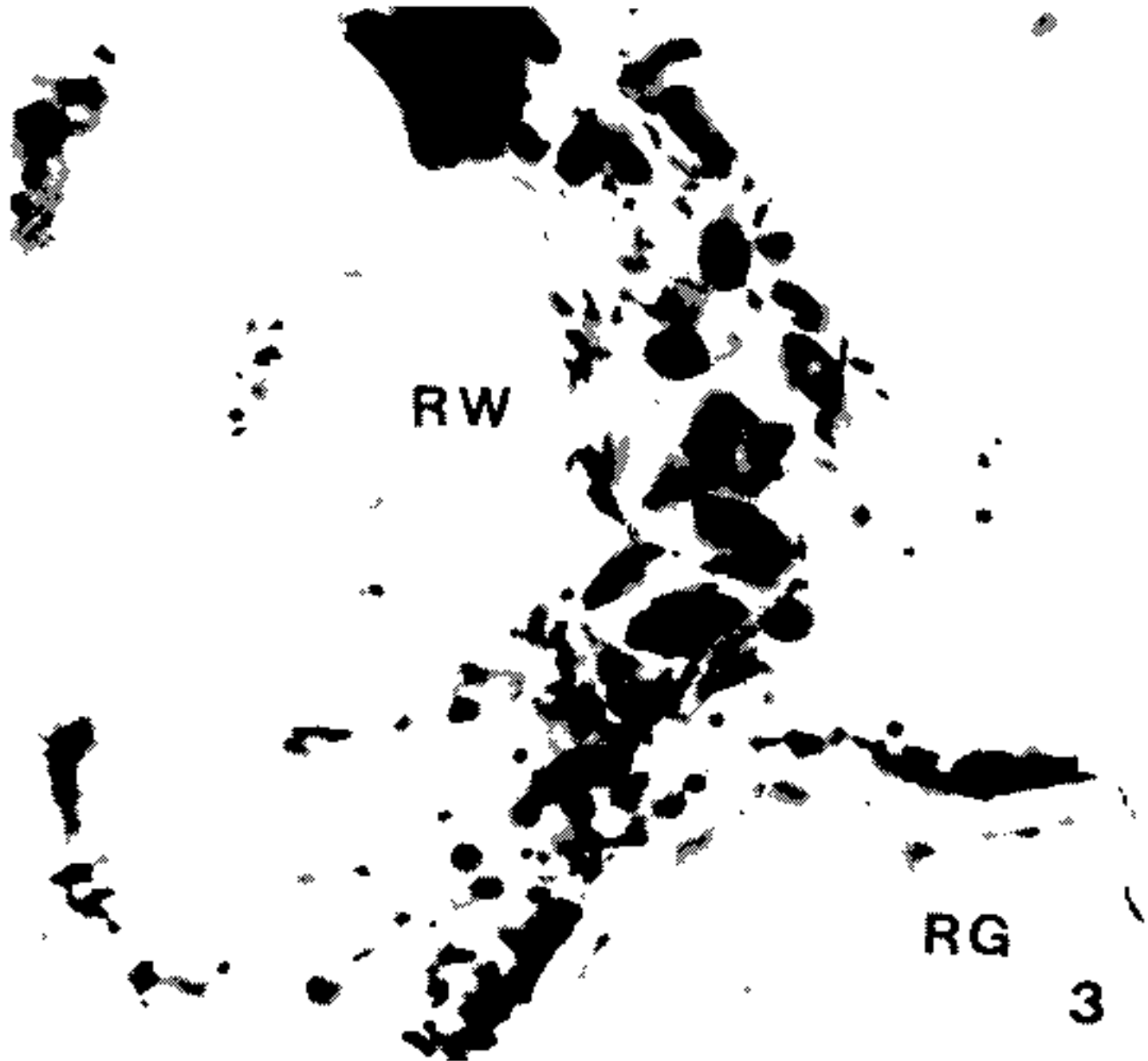


Fig. 3: light microscopy of rectum. *Leptomonas seymouri*. 21st day p.i. Flagellates along rectal wall (RW) intermixed with numerous bacteria which also cover rectal glands (RG). X1,500. Fig. 4: TEM. Isolate 49/553G.O. 21st day p.i. Parasite with enlarged flagellar pocket (fp) in the lumen of the rectum. Numerous bacteria (B); C: cuticle. X17,200. Fig. 5: SEM. Isolate 49/553G.O. 21st day p.i. Flagellates forming rosettes on the rectal lumen. RW: rectal wall. X10,000. Fig. 6: SEM. Isolate 49/553G.O. 21st day p.i. Few parasites (P) in the rectal lumen. RW: rectal wall. X10,000.

1990, Schaub et al. 1992). The development of flagellates in salivary glands looks similar to that found in *R. prolixus* infected with *T. rangeli* (Ellis et al. 1980, Hecker et al. 1990) and in *P. picta* infected with *P. serpens* (Freymuller et al. 1990).

Developmental patterns of trypanosomatids in their host insects vary according to the host as well as to the infecting trypanosomatid. Accordingly, different developmental features were displayed by

Drosophila melanogaster infected with different species of *Herpetomonas* (Rowton & McGhee 1983) and by houseflies infected with three distinct *Herpetomonas* isolates (Hupperich et al. 1992). Our findings reinforce this fact by showing that infection of phytophagous insects is restricted to a few species of trypanosomatids and that the course of infection is peculiar to each host/parasite infection.

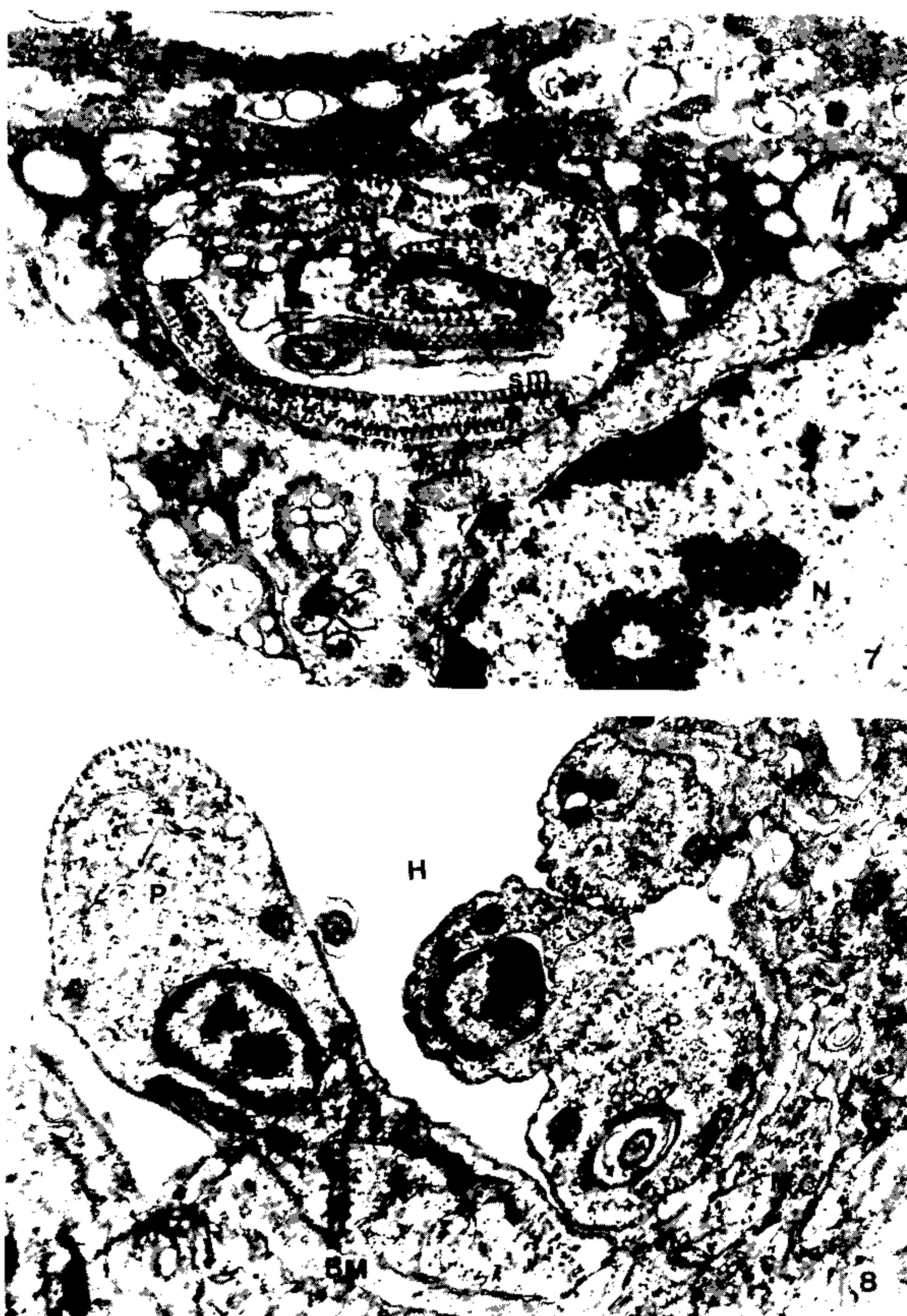


Fig. 7: TEM. Isolate 49/553G.O. 21st day p.i. Folded flagellate inside gut wall. F: Flagellum; sm: subpellicular microtubules; N: nucleus of the host cell. X25,000. Fig. 8: TEM. Isolate 49/553G.O. 21st day p.i. Parasites (P) are seen in the haemocoelomic cavity (H) lined up along the basement membrane (BM) of the gut; MC: midgut cell. X22,200.

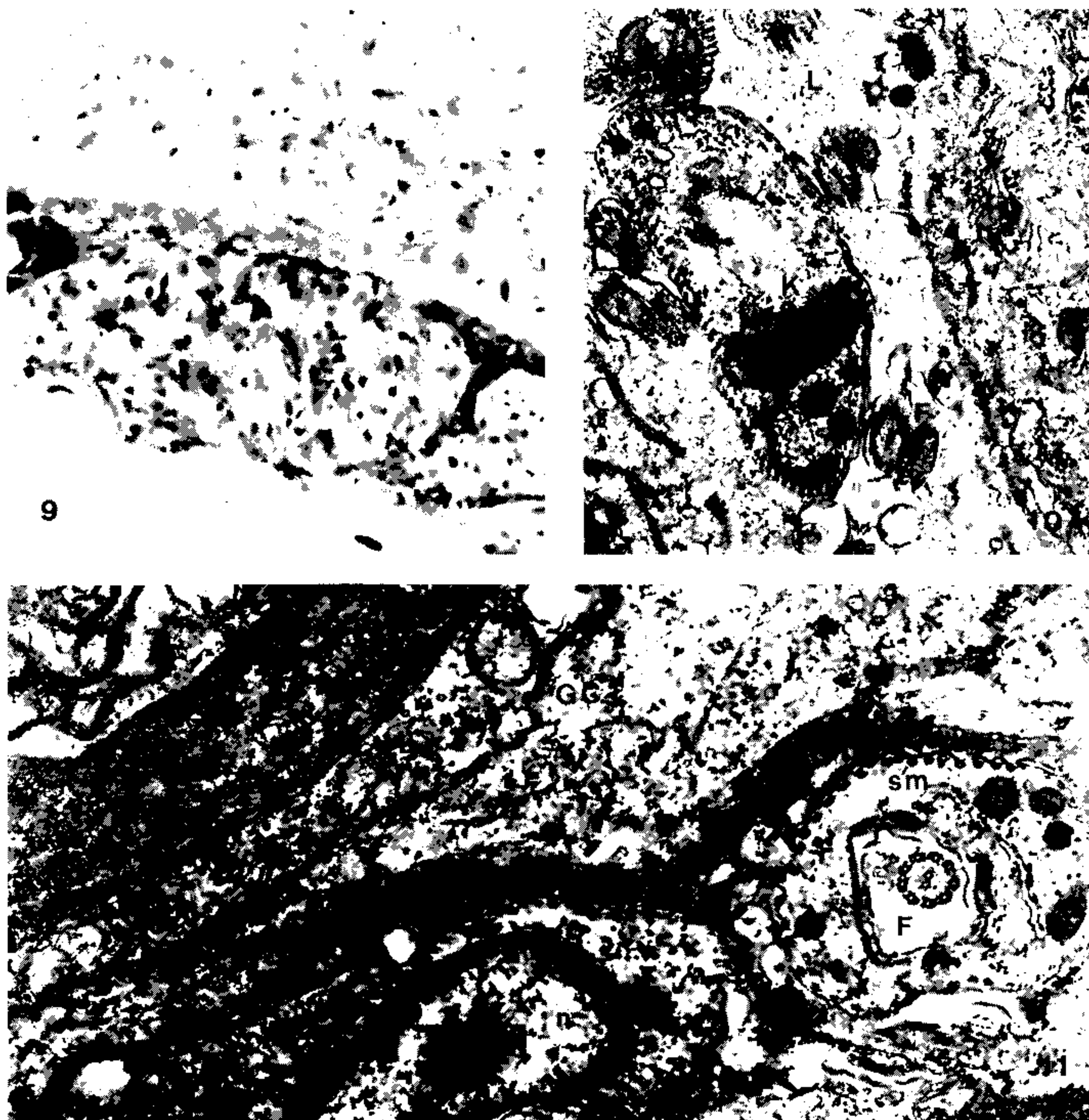


Fig. 9: light microscopy. *Leptomonas seymouri*. 30st day p.i. Heavy infection of salivary gland which is completely filled with parasites. X1,500. Fig. 10: TEM. Isolate 49/553G.O. 21st day p.i. Flagellates in the gland lumen (L) immersed in desintegrated material. F: flagellum; K: kinetoplast. X30,000. Fig. 11: TEM. Isolate 49/553G.O. 21st day p.i. Trypanosomatids in salivary gland located between layers of the basement/haemocoelomic membrane system (*) and gland cell (GC); F: flagellum; n: parasite nucleus; sm: subpellicular microtubules. X37,000

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