Life cycle, ultrastructure and host-parasite relationships of *Angomonas deanei* (Kinetoplastea: Trypanosomatidae) in the blowfly *Lucilia sericata* (Diptera: Calliphoridae)

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Summary

This research describes the results obtained after the experimental per-os infection with monoxenous symbiont-containing trypanosomatid *Angomonas deanei* (isolate MN) of larvae and imagoes of the fly *Lucilia sericata*. It was discovered that larvae infected by this parasite do not develop a persistent infection. The imago's rectum was found to be the preferential site of colonization by flagellates where they form massive clusters until the death of an adult insect. Laboratory experiments on *A. deanei* transmission have demonstrated that the parasite may be transmitted horizontally between adult flies (via contaminated substrate and/or coprophagy). In a host's rectum, the parasites are detected on the cuticular lining, mainly at places near rectal glands and directly on the surface of the rectal glands. The attachment of the parasite is carried out through the apical or lateral surface of the flagellum with the formation of hemidesmosome-like junctions. Ultrastructural features of the parasite are consistent with the previous descriptions. However, the presence of a cytostome located in the distal part of the flagellate pocket was noted for the first time. The cytostome has the shape of a fossa, which is associated with cytoplasmic vesicles.

Key words: Trypanosomatidae, symbiont-harboring trypanosomatids, *Angomonas deanei*, parasite of Diptera, *Lucilia sericata*, life cycle, host-parasite relationships, experimental infection, ultrastructure

Introduction

The flagellates of the family Trypanosomatidae Doflein, 1901 are well-known uniflagellate parasitic protists of invertebrates and vertebrates, less often plants and even ciliates. Nowadays it is customary to divide the family into two groups of non-taxonomic rank – monoxenous (“one-host”) and dixenous (“two-hosts”) trypanosomatids, depending on whether one or two hosts are included in their life

cycle. Trypanosomatids have gained distinction mainly because of their numerous dixenous species, among which there are causative agents of serious diseases affecting humans (e.g. sleeping sickness, Chagas diseases, kala-azar, espundia, oriental sore, etc.), as well as wild and domestic animals (e.g. nagana, surra, or dourine) (Hoare, 1972).

The study of monoxenous trypanosomatids has remained on the sidelines of the most serious studies for a long time, which is explained by their

low practical significance. Only recently, the monoxenous species has started drawing a lot of researchers' attention due to the progress of molecular biology techniques. There is no doubt that monoxenous trypanosomatids are an interesting group to research not only from theoretical perspectives but also for practical reasons. It is now clear that monoxenous trypanosomatids parasitizing in insects are the ancestral forms of the entire family (Flegontov et al., 2013; Maslov et al., 2013; Lukeš et al., 2014, 2018; Frolov et al., 2015). An important evidence of this fact is the recent discovery and description of a new genus *Paratrypanosoma*. The monoxenous flagellates *P. confusum* were found in the digestive tract of mosquitoes. They are positioned at the base of the phylogenetic tree forming a branch between free-living kinetoplastids and a parasitic family Trypanosomatidae (Flegontov et al., 2013).

Furthermore, there is abundant evidence that monoxenic trypanosomatids are capable of causing opportunistic infections in humans (Dedet et al., 1995; Pacheco et al., 1998; Dedet and Pratlong, 2000; Garin et al., 2001; Chicharro and Alvar, 2003; Ghosh et al., 2012; Kraeva et al., 2015). They also negatively affect their immediate insect hosts, causing death or fertility decline of the latter (Bailey and Brooks, 1972a, 1972b; Schaub and Schnitker, 1988; Schaub and Jensen, 1990; Hamilton et al., 2015).

Angomonas deanei (Carvalho, 1973) belongs to the subfamily Strigomonadinae, which unites three genera of symbiont-harboring trypanosomatids (SHTs): *Angomonas*, *Strigomonas* and *Kentomonas*. To a large extent, the interest in studying SHTs is due to the presence of proteobacterial symbionts, which are vertically transmitted. They live in the cytoplasm of trypanosomatids, replicate synchronously with their hosts and change the shape of cell structures (Freytmüller and Camargo, 1981; Motta et al., 2010; Morales et al., 2016). The system "*Angomonas deanei* – symbiont *Candidatus Kinetoplastibacterium crithidii*" is a model for numerous studies of biochemical interactions between eukaryotic cells and bacterial symbionts, as well as metabolic features of symbiotic systems and the origin of endosymbiosis in trypanosomatids.

Another feature of *A. deanei* that focuses the researchers' interest is unusually wide host specificity. The species was first described from the predatory bug *Zelus leucogrammus* (Hemiptera, Reduviidae) in the South America (Brazil) (Carvalho, 1973; Tei-

xeira et al., 2011); however, later it was repeatedly found in flies of the families Calliphoridae and Sarcophagidae around the world (Týč et al., 2013; Borghesan et al., 2018). Recently *A. deanei* has been found in the north of the Leningrad Region in the intestines of a blowfly *Lucilia* sp. (Ganyukova et al., 2017). This is the northernmost *A. deanei* record in the Palearctic to date.

Despite the fact that *A. deanei* had been the object of numerous studies in recent decades, the life cycle of this flagellate has not been studied yet. In this article, we analyze various stages of the *A. deanei* life cycle during an experimental infection of the widely distributed fly *Lucilia sericata* (Williams et al., 2016).

The choice of *L. sericata* as a model for research is due to a number of reasons. Firstly, *A. deanei* was detected in flies of this genus (Týč et al., 2013; Borghesan et al., 2018). Secondly, similarly to the MN isolate in the Leningrad region, it was also isolated from a fly *Lucilia* sp. (Ganyukova et al., 2017). However, in Russia flies from the genus *Lucilia* survive the cold period from November to April in the state of larvae or, less often, as pupae (Vinogradova et al., 1991). One of the aims of our experiments was to test the hypothesis that *A. deanei* develops a persistent infection in *L. sericata* larvae and may possibly persist during pupation. This assumption is largely supported by successful experimental infections of flies' larvae published previously. Such objects are *Jaenimonas drosophilae* from *Drosophila falleni* (Hamilton et al., 2015) and *Herpetomonas muscarum*, a parasite of eye gnat *Hipellates pusio* (Bailey and Brooks, 1972a, 1972b). Notably, both species, *J. drosophilae* and *H. muscarum*, are capable of forming massive aggregations in the endoperitrophic space of the midgut of larvae and adults, as well as persist during insect metamorphosis.

Material and methods

CULTIVATION OF TRYPANOSOMATIDS

Axenic culture of trypanosomatids *A. deanei* isolate MN (Ganyukova et al., 2017) was maintained in the bank of cultures of the Zoological Institute RAS (Malysheva et al., 2016) using the M199 medium without antibiotic solution at the temperature of 22 °C, being passaged every 14 days.

CULTIVATION OF INSECTS

We used the *L. sericata* fly as an experimental host. Laboratory culture of insects is maintained in the Laboratory of insect biopharmacology and immunology of Saint-Petersburg State University in the conditions described earlier (Vinogradova and Reznik, 2013). The insects were kindly provided by A.P. Nesin.

MAINTENANCE AND INFECTION OF LARVAE

To challenge larvae with trypanosomatids we collected pieces of beef liver with eggs from *L. sericata* oviposited overnight. Then we removed the pieces with eggs and placed them into 1 l glass jars covered with gauze for aeration at the temperature of 22 °C and 16L/8D photoperiod. Wet sawdust was used as a substrate. The next day we got one-day instar larvae.

These larvae were transferred to a Petri dishes 6 cm in diameter on a moist nutrient substrate, which consisted of *A. deanei* laboratory culture (3×10^8 cells/ml) and baby food “Baby puree FrutoNyanya from beef” (JSR “PROGRESS”) mixed in a 1:3 ratio. The infection was carried out according to two patterns. (1) Short-term infection: 1-day larvae were kept on the infected substrate during 10 minutes and then they were moved onto a normal nutrient substrate (the moment of moving was referred to as time zero). (2) Long-term infection: 1-day larvae were kept on the infected substrate, which was daily renewed, until the insect pupation.

MAINTENANCE AND INFECTION OF IMAGOS

The imagoes of *L. sericata* were kept in plastic containers L10×H15×W7 cm with a perforated lid at 22 °C and 16L/8D photoperiod. Troughs with drinking water and a lump of sugar provided to feed insects were kept in the containers during the whole time of the experiments. Adult flies received no protein nutrition.

Just before the infection, we kept insects without food and water for 24 hours. Then the flies were placed in separate Petri dishes 35 mm in diameter, where 15 µl of *A. deanei* laboratory culture (3×10^8 cells/ml) were added. After the fly finished drinking, the volume of the remaining drop was measured using a dispenser. The average volume of liquid drunk by one fly was about 5 µl. The moment the fly finished drinking was referred to as time zero.

PROLONGED IMMOBILIZATION OF FLIES

As part of the experiment to determine the duration of infection of the flies, 3-days imagoes (after emerging from puparia) were placed in plastic tubes (Kostygov et al., 2020) after single infection. The front-opening of the tube was covered with gauze with a mesh of ~ 1 mm. The size of the mesh allowed the insect to pull the proboscis and receive 15% of the sugar syrup from the external drinking trough.

The trough with sugar syrup was daily renewed. The back of the tube containing the fly was plugged with a piece of cotton wool, which did not allow the fly to move along the longitudinal axis of the tube and absorb insect feces. The cotton wool was changed daily. The movement of insects in the tubes was limited, which excluded the possibility of autoinvasion of flies through their own feces.

TRANSMISSION OF INFECTION BETWEEN IMAGOS

We tested the adult-adult transmission by co-housing of 5 uninfected flies and 1 infected fly (7 vials in total contained 35 flies of uninfected group). All these insects were dissected and screened for trypanosomatids by light microscopy after 3 days of cohabitation in the container. To differentiate flies from each other we clipped a wing of the infected fly.

DISSECTION OF INSECTS

The insects were euthanized in chloroform vapors and dissected in a drop of physiological saline. The intestines were examined under a Leica DM2500 microscope (Leica Microsystems, Wetzlar, Germany). Isolated intestine fragments including their contents were studied for infection. Isolated parts of the digestive tract were studied separately. In case of detection of trypanosomatids, parts of the infected intestine were used for subsequent studies (dry smears, TEM and SEM fixation).

LIGHT MICROSCOPY

Dried smears prepared from insect intestinal fragments were fixed for 10 minutes with 96% ethanol. After drying, the slides were stained according to Romanowsky-Giemsa (pH 6.8) for 30 minutes and washed with water. The studies were performed with a Leica DM 2500 microscope. Microphotographs were obtained with 14 MPs USB camera UCMOS 14000KPA (TOUPCAM). Flagellate's cell sizes

were measured with UTHSCSA ImageTool v. 2.0. Statistical data (the mean and standard error of the mean) were processed with LibreOffice Calc.

TRANSMISSION AND SCANNING ELECTRON MICROSCOPY

The rectums of infected flies were prepared for TEM as described earlier (Frolov et al., 2016a). Ultrathin sections were examined using Morgagni 268-D microscope (FEI Company/Thermo Fisher Scientific, Hillsboro, OR, USA) with accelerating voltage of 80.00 kV.

The rectums of infected flies were prepared for examination by the scanning electron microscopy according to the method mentioned earlier (Ganyukova et al., 2019). Then they were examined under the microscope in Tescan Mira3 LMU with accelerating voltage of 25.00 kV.

Results

COURSE OF INFECTION IN THE DIGESTIVE TRACT OF *L. SERICATA* LARVAE

The structure of the digestive tract of *L. sericata* larvae is shown in Fig. 1, A. The foregut includes the following departments: a pharynx, an oesophagus, a large blind-closed food reservoir (a crop), which is connected to the oesophagus through a thin long tube (not shown in the Fig. 1, A), and an expanded proventriculus. The midgut is represented by a long tube, which has poor external differentiation into sections. A characteristic feature of the midgut is the presence of a peritrophic membrane that separates the food bolus from the intestinal wall. The border between the midgut and hindgut is marked by a slight narrowing – the pyloric valve. The hindgut of larva is poorly differentiated on the outside. It is customary to divide it into an anterior hindgut, where the Malpighian tubules join the digestive tract, and a posterior hindgut, which ends with the anus.

Experiment 1^{La}. Development of *A. deanei* with short-term single infection.

The purpose of the first experiment was to study the dynamics of infection spread along the larval digestive tract immediately after the per os infection (Table 1). 1-day larvae of *L. sericata* were kept on the substrate infected with *A. deanei* for 10 minutes

Table 1. Short-term single infection of 1-day larvae of *Lucilia sericata*.

Time after infection	Dissected	Infected
5-30 min	20	20 (100%)
30 min – 2 h 30 min	25	25 (100%)
4–24 h	23	23 (100%)
1-2 days	18	7 (38.9%)
3 days	19	0 (0%)

(see “Materials and Methods”), after that they were transplanted onto a normal nutrient substrate (beef liver) and dissected at regular intervals. Twenty larvae were dissected within 5-30 min after the infection. Free-floating flagellates were present in the lumen of the foregut and the anterior parts of the midgut in all dissected maggots.

Within the next two hours, 25 larvae were dissected. All of them turned out to be infected. Parasites were present in all parts of the intestine from the pharynx to the rectum.

In the period from 4 to 24 h after feeding on the infected substrate, some flagellate cells were observed in the intestines of 23 dissected larvae. However, they were found only in the lumen of midgut and hindgut and did not form any clusters. Within the next two days (24–72 h after the infection), 18 larvae were dissected. The infection was noted only in 7 larvae. Only single floating cells were present in the midgut and hindgut of the infected larvae.

The dissection of 19 larvae held during 3 days after the infection did not reveal even a single cell of the parasites.

Experiment 2^{La}. Development of *A. deanei* with long-term infection.

The purpose of the next experiment was to identify the ability of parasite development in the digestive tract of larvae during long-term invasion. We maintained *L. sericata* larvae on the infected substrate from the first day after hatching until pupation (Table 2).

Some of the larvae were dissected to check infestation during the larval period of development. We found cells of the parasite in 21 individuals out of 23 dissected 5-days larvae. We failed to find mass clusters of parasites in any part of the digestive tract; however, individual cells of the parasite were observed in all parts of the alimentary tract.

Fig. 1. A – Digestive tract of *L. sericata* larva; B – digestive tract of *L. sericata* imago; C-D – rectum of infected *L. sericata* imago. Arrowhead indicates clusters of attached trypanosomatids; stick arrowhead indicates clusters of free-swimming trypanosomatids.

On the 6-7th day after hatching the larvae stopped feeding and proceeded to the post-feeding stage. They moved away from the nutrient substrate on which they had been previously fed and started to look for a suitable place for pupation. During this period, we dissected 18 larvae that were fed on the infected substrate and we found single parasite cells in 2 insects' midguts. On the 8th day, we dissected 20 individuals in a pre-pupal phase and found no infection in any of the individuals.

Experiment 3^{1a}. The impact of infection on the viability and development of the larvae.

Among other experiments, we evaluated the effect of infection on the larval viability and development. We had two groups of insects (control and infected), which included 35 individuals each (Table 3). One-day larvae were maintained on substrate with *A. deanei* for infection in the same way described above. Individuals of the control group were maintained on non-contaminated beef

liver. Twenty-seven larvae from the control group were dissected at the ages of 1-day and 3-days after hatching before the start of the experiment; those were not included in the number of insects recorded during the experiment. The dissection did not reveal any infections in the individuals from this group.

On the 6-7 days, the insects from both groups switched to the post-feeding stage and moved away from the nutrient substrate. They climbed forming clusters into the wet sawdust or under the gauze covering the glass jar. By the beginning of the 8th day after hatching all larvae had been inactive and their cuticles had started to get darker. By the end of the 8th day, the larvae had formed dense puparia. We counted 30 puparia in the group of the infected larvae and 31 puparia in the control group.

After 12-14 days from the moment of pupation adults emerged. There were 29 and 28 individuals in the infected and the control groups, respectively. The dissection of the emerged imagoes of both groups did not reveal any infection in any of the adult insects.

Table 2. Long-term infection of 1-day larvae of *Lucilia sericata*.

Age	Dissected	Infected
5-days larvae	23	21 (100%)
6-7-days larvae (post-feeding stage)	18	2 (11.1%)
8-days larvae (pre-pupal stage)	20	0 (0%)

COURSE OF INFECTION IN THE DIGESTIVE TRACT OF ADULTS OF *L. SERICATA*

The digestive tract of the *L. sericata* imago has a typical organization for brachyceran flies (Fig. 1, B, C). The foregut is represented by a pharynx, an oesophagus (not shown in the Fig. 1, B) and a short dilated proventriculus. In addition, the foregut includes a large blind-closed food reservoir (a crop), which is connected to the distal part of the oesophagus through a thin long tube. The long midgut has poor morphological differentiation into sections. Its characteristic feature is the presence of a peritrophic membrane that encloses the food bolus inside. A pyloric valve marks the border between the midgut and hindgut. The hindgut is differentiated into three sections. A pylorus located between the pyloric valve and the pyloric sphincter is the confluence of the Malpighian tubules. It is followed by an elongated ileum that ends with a rectal valve. A large dilated rectum, which is also called a rectal ampulla, has 4 prominent rectal glands. The rectum ends with an anus.

Experiment 1^{Im}. Development of *A. deanei* in the imago.

This experiment aimed at studying the dynamics of the infection spread in the intestines of adult flies. For this purpose, 3-day imagoes after emerging from puparia were kept without water for 24 hours, after that they drank a drop of *A. deanei* culture with a volume of about 5 µl (see “Materials and methods”). Then the flies were dissected at regular intervals (Table 4).

The dissection of 20 adults showed that in all infected individuals the parasites’ cells spread following the liquid along the foregut including the crop and the proventriculus and the anterior part of midgut after 5 min from the moment of infection. However, 1 h later the flagellates occupied the entire digestive tract reaching the hindgut sections

Table 3. The impact of infection on the viability and development of the larvae with long-term infection of *Angomonas deanei*.

<i>Lucilia sericata</i>	Infected (N=35)	Control (N=35)
Pupation	30	31
Emerged imago (12-14 days)	29	28
Infected imago	0	0

(the ileum and the rectum). We noted this pattern in 18 individuals which were dissected 1 h after the infection.

In 4 h after the experiment had begun, the trypanosomatids finally left the foregut (including the crop) and the anterior part of the midgut. In 24 h after the infection and during the next 3 days the flagellates were observed only in the rectums in 42 dissected flies (Fig. 1, C, D). Apparently, the cells of *A. deanei* were fixed in the rectum and did not leave this section actively proliferating there.

Experiment 2^{Im}. Duration of *A. deanei* persistence in the fly’s rectum

We placed the flies in plastic tubes with the diameter of 5 mm immediately after the infection (see “Materials and methods”). The design of the “houses” made it possible to isolate the infected flies from other insects and excluded the possibility of movement of the fly itself. Thus, we completely prevented the possibility of insect autoinvasion through the substrate contaminated with their own excrement. We kept 30 infected adults and 30 control flies in the immobilization conditions. During the experiment, we changed the troughs with sugar syrup every day and checked the condition of the insects. The dead flies were removed and dissected. In total, we carried out three replications of the experiment.

The lifespan of the flies both in the infected and in the control groups is described by a declining linear graph (Fig. 2). The maximum lifespan of the infected insects under immobilization conditions was 21 days, the same as in the control group. The maximum total lifespan including 3 days after hatching (see “Materials and methods”) was 24 days. It is worth noting that the maximum lifespan of the mobile flies kept in containers under the same temperature and light conditions was 47 days.

The dissection of the dead insects demonstrated the presence of intense infection of their rectum throughout the entire period of the experiment.

Table 4. Infection dynamics of *Angomonas deanei* isolate MN in the digestive tract of adult *Lucilia sericata*.

Section of the intestine	5 min N=25 Infected=25	1 h N=18 Infected=18	4–24 h N=21 Infected=20	After 24 h N=42 Infected=42
Foregut	+	+	–	–
Midgut	+	+	+	–
Hindgut	–	+	+	+

Notes: “+” cells were detected in the section of the digestive tract; “–” cells were not detected in the section of the digestive tract.

In other parts of the intestine, no flagellates were observed.

Experiment 3rd. horizontal transmission of *A. deanei* in *L. sericata*.

We have experimentally shown the possibility of a horizontal transmission of parasites between the adults of *L. sericata*. One infected fly was marked with the wing clipping and placed in a container with five uninfected imagoes. We dissected all insects after three days of cohousing. As a result of 7 experiments, the infection was detected in 13 individuals out of 35 experimental flies (37.1%).

MORPHOLOGY OF *A. DEANEI* IN THE RECTUM

Approximately 24 h after the infection of the insect the parasite cells were observed only in the rectum of the adult flies (Fig. 1, C, D; Fig. 4, A, E, F). The trypanosomatids were irregularly distributed. The most massive aggregations of the attached cells were located on the surface of the rectal glands. Some of the free flagellates were floating in the lumen of the posterior part of the rectum.

In the host's rectum, *A. deanei* cells attached to the surface of the cuticular lining through their flagella (Fig. 4, A–F, H–L). The structure of the rectum cuticle at the attachment sites (Fig. 4, H–L) did not differ from the areas free of parasites (Fig. 4, G). The attachment of *A. deanei* to the surface of the rectum was carried out by the tip of the flagellum or their lateral surface and was accompanied by the formation of hemidesmosomes-like structures (Fig. 4, I–L).

The cells of *A. deanei* in the rectum of the host were small (Table 5). Based on the position of the kinetoplast in the cell we have identified three morphotypes that were found in the intestine of the *L. sericata*: promastigotes, paramastigotes and opistomastigotes (Fig. 3). The largest group was

Table 5. Cell morphometry of *Angomonas deanei* isolate MN in the rectum of *Lucilia sericata*.

Characteristic	Promastigotes	Paramastigotes	Opistomastigotes
Cell length (μm)	5.12±1.27 (3.42–7.51)	3.93±0.64 (2.72–5.85)	3.50±0.43 (2.54–4.74)
Cell width (μm)	2.78±0.39 (1.89–3.72)	2.81±0.33 (2.21–3.76)	2.60±0.41 (1.79–3.54)
Free flagellum length (μm)	4.01±1.49 (1.25–7.36)	3.45±1.37 (1.50–6.86)	1.85±1.03 (0.68–5.19)
Nucleus length (μm)	1.27±0.20 (0.87–1.64)	1.18±0.19 (0.77–1.72)	1.16±0.18 (0.79–1.51)
Nucleus width (μm)	1.17±0.27 (0.68–1.79)	1.16±0.19 (0.81–1.67)	1.05±0.20 (0.68–1.51)
Anterior end to nucleus distance (μm)	2.55±0.44 (1.71–3.88)	1.77±0.31 (1.21–2.39)	1.72±0.45 (0.68–3.10)
Anterior end to kinetoplast distance (μm)	1.40±0.39 (0.71–2.37)	1.72±0.50 (0.73–3.05)	2.61±0.42 (1.86–3.46)

paramastigotes (80.8%), where nucleus is located in the middle of the cell and the position of the kinetoplast varies. It can be located both near the nucleus and at the level of its posterior edge (Fig. 3, B). The proportion of elongated promastigotes with an anterior kinetoplast position was 10.8% (Fig. 3, A). The least frequent type was opistomastigotes, in which the kinetoplast is located behind the nucleus. Opistomastigotes were registered with a frequency of 8.4% (Fig. 3, C). Flagellate pockets of the cells of all morphotypes were wide and clearly visible.

The ultrastructural organization of *A. deanei* cells in the host intestine did not differ from the previously published descriptions based on cells from laboratory cultures.

Flagellates have a number features common for subfamily Strigomonadinae: large kinetoplast with a relatively loose network of DNA fibrils (Fig. 5, A, B), cytoplasmic bacterial symbionts in symbionthophore vacuoles (Fig. 4, H, I; Fig. 5, A–C, F, H), irregularly located microtubules of the tubulemma, which interspersed with mitochondrion branches (Fig. 5, F). The paraflagellar rod is absent except for the widened base of the flagellum where this structure can be seen in a reduced form (Fig. 5, D, E). Noteworthy is the presence of a contractile vacuole, which connects to the distal part of the flagellar pocket through a long tube (Fig. 5, G).

We also detected the cystostomal complex elements in the flagellar pocket of *A. deanei* (Fig. 5, H–I). In the anterior part of the flagellar pocket, there was a small fossa (cystostome), which was surrounded

Fig. 2. The lifespan of infected (left) and uninfected (right) *L. sericata* imagoes during immobilization experiment. The X-axis indicates the day of the experiment, the Y-axis indicates the number of alive flies.

by a dense felt-like material that formed the pre-oral ring. A system of small vesicles was connected to the bottom of the cystostome. This organelle complex was also reinforced with microtubules.

Discussion

In this research, we have studied the life cycle of *A. deanei* in *L. sericata* flies. The flagellates transit through all sections of the insect's digestive tract and accumulate in the rectum. The prolonged infection of flies is subsequently associated with the development of parasites in the rectum of adult insects. The cells of *A. deanei* quite quickly reach the posterior intestine of the imago. Within an hour after the infection, we found the flagellates in the hindgut of the insect, and after 24 h the parasite completely left the foregut and midgut sections. Meanwhile, "rectal" trypanosomatids are known to have an ability to linger in the anterior parts of the digestive tract for a longer period. For example, *Herpetomonas samuelpessoai* finally leaves the crop and the midgut of *Musca domestica* only 8 days after the onset of the infection (Hupperich et al., 1992).

The localization in the insects' rectum is frequent in both monoxenous and dixenous trypanosomatids (Wallace, 1966; Molyneux, 1977; Schaub, 1992). It is a very common phenomenon among parasites of brachyceran flies. It has been demonstrated that many species prefer to localize directly on the rectal glands of the insect (Molyneux, 1977; Schaub, 1992; Frolov, 2016b). The exception is *H. samuelpessoai*, which actively attaches to the cuticular lining specifically at the bases of the rectal glands, but not on them (Hupperich et al., 1992). According to our results, *A. deanei* forms "pile capets" over the entire

surface of the rectum cuticle, but huge multilayered clusters are observed directly on the rectal glands. The rectal glands are multifunctional, but not a well-researched organ. They are known to play an important role in water and salt reabsorption (Gupta and Berridge, 1966), absorption of residual amino acids (Wall and Oschman, 1975) and in the release of sex pheromones (Khoo and Tan, 2005) of insects.

The attachment of *A. deanei* to the host's epicuticle is carried out with the flagellum. We noted the formation of hemidesmosome-like junctions on the flagellum. This type of attachment in the host for retention is widespread among trypanosomatids with rectal localization (Molyneux, 1977; Schaub, 1992; Frolov, 2016b; Lukeš et al., 2018). It is also believed that the flagella attachment to the epicuticle may be a result of a hydrophobic interaction (Schmidt et al., 1998).

The morphology and ultrastructure of *A. deanei* in the rectum of *L. sericata* does not differ from the previous descriptions performed on cells from laboratory cultures (Freymuller and Camargo, 1981; Motta et al., 1997; de Sousa and Motta, 1999; Gadelha et al., 2005; Motta et al., 2010; Teixeira et al., 2011; Ganyukova et al., 2017). However, an important finding is the cytostomal fossa associated with the vesicle system. These organelles can be characterized as a reduced cytostome–cytopharynx complex (Frolov and Karpov, 1995). There is a possibility that this structure in *A. deanei* is actively involved in the intracellular transport and, perhaps, in phagotrophic nutrition as well (Chasen et al., 2020). Previously the cytostome–cytopharynx complex was not detected during a detailed ultrastructural analysis of flagellates of the subfamily Strigomonadinae (Bombaça et al., 2017; Loyola-Machado et al., 2017) and its existence has

Fig. 3. Cells of *A. deanei* isolate MN in the rectum of *L. sericata* on Giemsa-stained smears. A – Promastigote cells; B – paramastigote cells; C – opistomastigote cells. *Abbreviations:* fl – flagellum; k – kinetoplast; nu – nucleus.

been questioned (Harmer et al., 2018). It is possible that further studies of this structure can shed light on the functionality of this complex. It is not out of the question that this structure plays an important role in the development of bacterial symbiosis in the Strigomonadinae.

It is known that some monoxenous trypanosomatids are capable of negatively affecting their insect hosts, causing their death or fertility decline (Bailey and Brooks, 1972a, 1972b; Schaub and Schnitker, 1988; Schaub and Jensen, 1990; Hamilton et al., 2015). Despite the high abundance of *A. deanei* in the intestine of the host, we did not observe any decrease in the viability of the infected adults in comparison with the control group. We also did not notice any destruction in the structure of the rectum cuticle at the sites of the flagella attachment. The lifespan of the insects in the condition of the immobilization experiment was no longer than 21 days, while under standard conditions the lifespan of *L. sericata* adults can be up to a month and a half (Ring, 1973). Since a similar shortened lifespan was also noted among uninfected insects of the control group, we tend to associate this fact with the conditions created in the framework of the experiment meaning the isolation and the immobilization of the insects.

The life cycle of *A. deanei* appears to be simple and is associated with cell proliferation on the surface of the rectum cuticle. We did not spot any special expansion stages in *A. deanei*, which are inherent in a number of other monoxenous trypanosomatids (Schaub and Pretsch, 1981; Malysheva and Frolov, 1995; Takata et al., 1996; Frolov et al., 2017). The horizontal transmission of the infection most likely occurs when flies are fed on a substrate contaminated

with feces of an infected insect containing non-specialized *A. deanei* cells. Imagoes of calliphorid and sarcophagid flies prefer to be fed on liquid and semi-liquid substrates (Artamonov, 2011). The humid conditions of such substrates can contribute to a more or less prolonged preservation of the viability of flagellates, which was confirmed during our experiments.

Interestingly enough, a number of symbiotic bacteria from the intestines of blowflies synthesize metabolic compounds after being released with feces onto nutrient substrates. These compounds attract oviparous females of different species (Chaudhury et al., 2010; Tomberlin et al., 2017). This behavioral trait of adults may facilitate the transmission of *A. deanei* among insects, which forms a necrophages/coprophages complex on wet decaying substrates.

The successful transmission of *A. deanei* through a contaminated substrate was confirmed in a wide range of hosts. This species has been repeatedly found in flies from the families Calliphoridae and Sarcophagidae all over the world (Týč et al., 2011; Ganyukova et al., 2017; Borghesan et al., 2018). However, it is not yet obvious whether *A. deanei* can successfully carry out its life cycle in the rectum of all these insects. It is possible that at least in some species of flies the parasites can only persist for some time. Nevertheless, the fact that *A. deanei* was first described from the intestines of the predatory reduvid bug *Zelus leucogrammus* (Carvalho, 1973; Teixeira et al., 2011) admit the transmission of infection by predation or necrophagy.

An indirect confirmation of the ability of *A. deanei* to realize its life cycle in a wide range of hosts might be the fact that flies of *L. sericata* in central

Fig. 4. *A. deanei* isolate MN in the rectum of *L. sericata*. A-D – SEM micrographs of *A. deanei* cells attached to the cuticle of the host rectum. The extended bases of the flagella are marked with asterisks; E-F – fragment of transverse semi-thin section of an infected rectum in the region of the rectal gland (methylene blue staining, BF); G – intact cuticle at the base of the rectal gland of *L. sericata* (TEM); H – accumulation of attached flagellates on the base of the rectal glands (TEM); I-L – different variants of trypanosomatids' attachment by the flagellum to the epicuticle of the rectum (TEM). **Abbreviations:** al – apical leaflets; ax – axoneme; CorC – cortical epithelium cells; cu – rectum cuticle; des – desmosome-like contacts; en – endocuticle; ep – epicuticle; fg – flagellum; ft – tip of flagellum; hde – hemidesmosome-like junctions; inf – infundibulum; lm – lateral plasma-membranes of the cortical epithelial cells; lu – rectum lumen; MedC – medullary cells; mit – mitochondria of host cells; RecC – rectal epithelium cells; sb – bacterial symbiont; Tr – trypanosomatids.

Fig. 5. Ultrastructure of *A. deanei* in the rectum (TEM). A–C – Longitudinal section through cells of *A. deanei*; D – cross section through the base of the flagellum; E – cross section through the distal part of the flagellum; F – cross section of the cell; G – contractile vacuole of *A. deanei*; H – cross section through the flagella pocket and cytotome; I – cytotome of *A. deanei* (enlarged section of the fig. H). *Abbreviations:* ax – axoneme; c – cytotome; cv – contractile vacuole; des – desmosome-like contacts; fex – extended part of flagella; fg – flagellum; fp – flagellar pocket; gr – granule; kp – kinetoplast; m – mitochondrion; nu – nucleus; pm – plasma membrane; POR – pre-oral ring; rpr – reduced paraxial rod; sb – bacterial symbiont; smt – subpellicular microtubules; ve – vesicles.

Russia overwinter exclusively at the larval or pupa stage (Vinogradova, 1991). As we have demonstrated above, *A. deanei* does not develop in the larvae and puparia of these flies, and the parasite's cells are completely removed from the host's intestine at the post-feeding stage. It is possible that adult flies from the same ecological niche overwintering at the imaginal stage are the reservoir for the persistence of the infection during the winter diapause of hosts. Such species are not known among the Sarcophagid flies of the middle part of Russia; however, many

Calliphorids may well claim this role (Vinogradova, 1991).

The larvae of many species of flies, especially calliphorids, tend to form massive clusters (Boulay et al., 2013), which should hypothetically contribute to the successful larva-larva transmission of infection. Meanwhile, the question why *A. deanei* does not develop in *L. sericata* larvae remains open. We have two main assumptions as putative explanations. The first one is associated with the probable effect of antimicrobial peptides and various immune res-

ponses of insects on parasites (Hu and Aksoy, 2006; Hamilton et al., 2015) and, in particular, the direct action of antibacterial agents of larvae (Mumcuoglu et al., 2001), which can suppress the development of infection. The second assumption, which also seems probable, is based on the fact that the structure of the hindgut of the fly larvae is different from adult flies. It has a poorly differentiated elongated tube that does not have a pronounced expansion like in adult flies and is devoid of rectal glands (Lowney, 1890–1892; Fox et al., 2010). The inability to attach in the gut of the larva due to its anatomical features coupled with physio-biochemical conditions that differ from those in the hindgut of the imago, may also be the reason why *A. deanei* is unable to develop in the digestive tract of *L. sericata* larvae.

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