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A study of the life cycle of *Trypanosoma neveulemaire*, a blood parasite of the marsh frog *Pelophylax ridibundus*, in the organism of the leech *Hemiclepsis marginata*

M.A.Guseinov

Institute of Zoology, Azerbaijan National Academy of Sciences (Baku, Azerbaijan)
gus_mair@mail.ru

The development of *Trypanosoma neveulemairei*, a blood parasite of the marsh frog *Pelophylax ridibundus*, in the leech *Hemiclepsis marginata* was studied. The description of different stages of consecutive development of the trypanosomes was given. It completes in the multiple division (schizogony) creating rosettes with an enormous number of metacyclic trypanosomes.

Key words: *leech, rosettes, metacyclic trypanosomes, Devechi firth of the Caspian Sea.*

Изучение жизненного цикла *Trypanosoma neveulemaire* – паразита крови озерной лягушки *Pelophylax ridibundus* в организме пиявки *Hemiclepsis marginata*

М.А.Гусейнов

Изучено развитие *Trypanosoma neveulemairei* – паразита крови озерной лягушки (*Pelophylax ridibundus)* в организме пиявки *Hemiclepsis marginata*. В статье приводится описание различных стадий последовательного развития трипаносом, заканчивающегося множественным делением (шизогония), с образованием розеток и отходящих в огромном количестве (сотни, тысячи) от них метациклических трипаносом.

Ключевые слова: *пиявка, розетки, метациклические трипаносомы, Девечинский лиман Каспийского моря.*

Introduction

Currently the blood parasite biology of amphibians is known much worse than their fauna. We can state with certainty that the life cycles of the trypanosomes parasitizing amphibians are very poorly studied.

It is known that principal vectors of the amphibian blood parasites are leeches but some authors consider also blood-sucking mosquitoes as vectors (Baily, 1962; Desser et al., 1973; Miyata, 1976).

The papers devoted to the life cycles of batrachian trypanosomes in the leech organisms are very scarce. In the Miyata's paper (1976) (Fig. 1) the supposed life cycle of *Trypanosoma rotatorium* living in the blood of *Rana rugosa* and transmitted to the tadpoles by the leach *Hirudo nipponia* is outlined. The author presented drawings of the trypanosome metacyclic forms but he depicted none of the development stages of the flagellate in the leech organism.

The absence of reliable and detailed information on the development of the amphibian blood parasites in their vector organisms motivated us to undertake the study on the development of the blood parasite *Trypanosoma neveulemairei* Brumpt, 1928 of the marsh frog in the leech *Hemiclepsis marginata*.

Materials and methods

For the purpose of the experiment we reared in the laboratory the leeches of the species *Hemiclepsis marginata.* The leeches in the amount of 6 samples were collected from the Devechi firth of the Caspian Sea (Azerbaijan). The leeches were fed on the blood of aquarium fishes free of blood parasites (the fishes were examined beforehand for the presence of the blood parasites).

After 1–1.5 month every leech laid cocoons (30–40) on the aquarium's walls. The young leeches having hatched from the cocoons fed on the blood of the fishes mentioned above. After 3–5 times of feeding on the blood the young leeches were big enough to be used in the experiment. To have the leeches infected 12 individuals of the leeches were placed into the vessel where a marsh frog was. To make sure that the frog was infected with the blood parasites blood was taken from the frog's femoral artery just before the experiment and examined under microscope for the presence of the blood parasites (11 individuals of *Trypanosoma neveulemairei* were detected) (Fig. 2). The young leeches that were placed into the vessel immediately attached to the frog's body.

To detect and describe various development stages of the trypanosomes we dissected one or sometimes two leeches on the 2nd, 4th, 5th, 6th, 7th, 8th and 9th day after placing into the vessel with the infected frog.

A small portion of the stomach contents of the dissected leeches was observed directly under microscope and the rest of it was used for making smears. The latter were stained with Giemsa's azur-eosin. During the whole period of the study photos and videos were done.

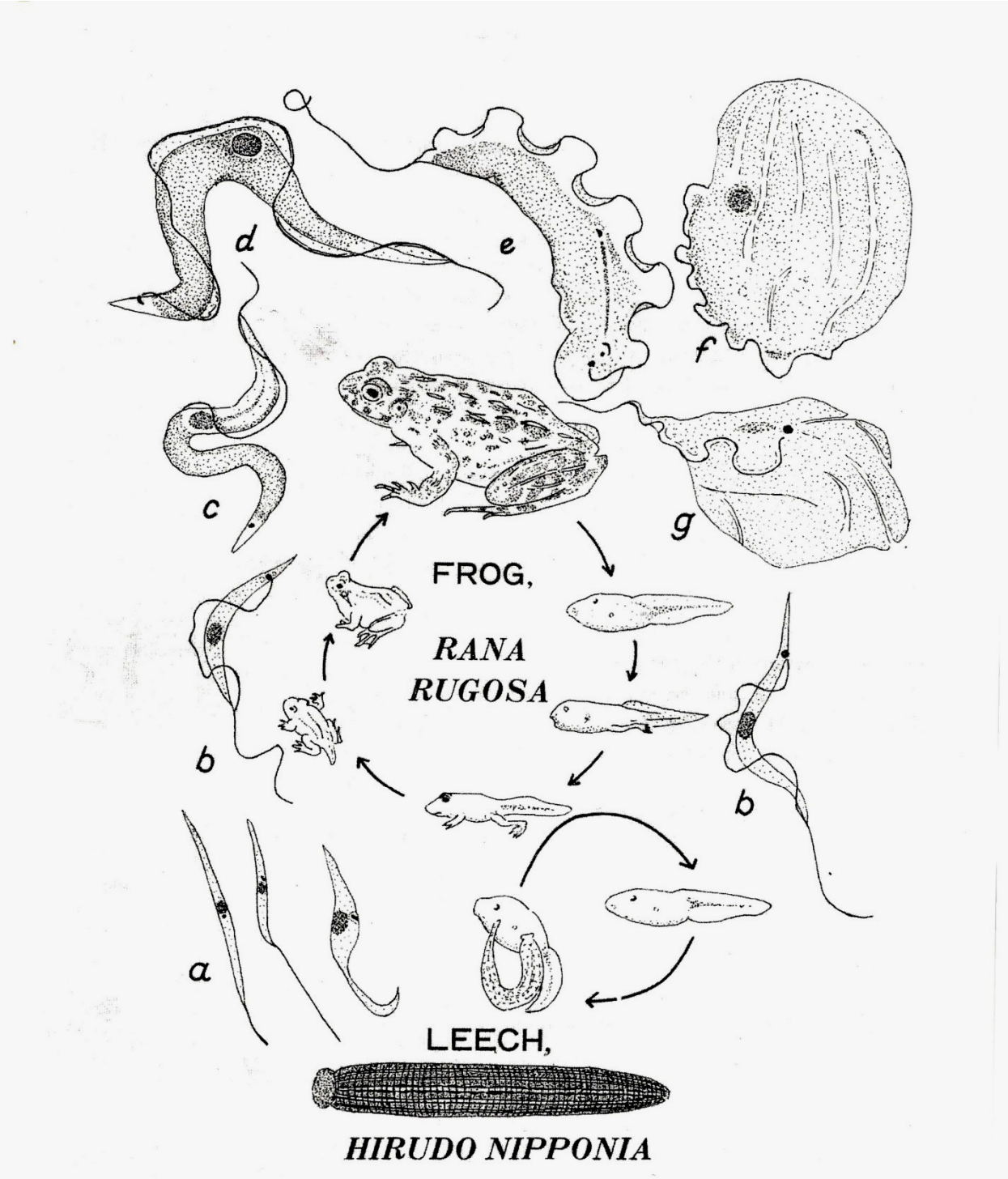

Fig. 1. Possible life cycle of *Trypanosoma rotarium* (according to Miyata, 1976)

Results and discussion

On the 2nd day of the experiment we found in the stained smears made from the leech stomach contents rounded trypanosomes which had nuclei and kinetoplast but undulating membranes and flagella were absent (Fig. 3). Sometimes in smears several rounded trypanosomes were present in a group as if they formed a small colony (Fig. 4).

On the 4th day division of the kinetoplast took place followed by the division of the nuclei and the rounded trypanosomes enlarged.

On the 5th day of the experiment we observed cyst-like body which was quite unlike the blood stages of the trypanosomes (Fig. 5).

Fig. 2. *Trypanosoma neveulemairei* from the frog's blood

Fig. 3. Rounded trypanosome in the leech's organism on the 2nd day of the experiment

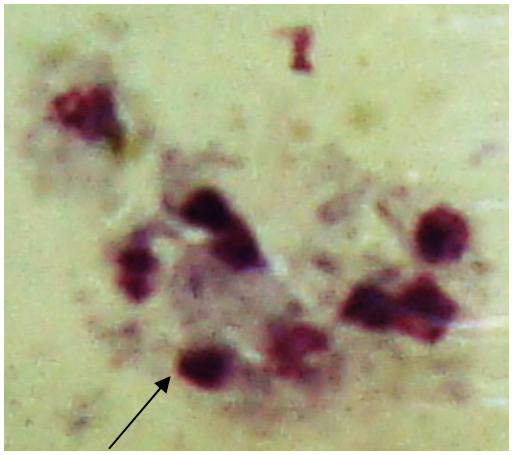

Fig. 4. Several rounded trypanosomes formed a colony

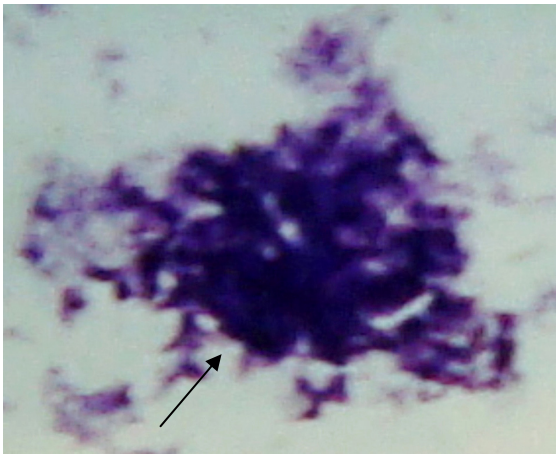

Fig. 5. A cyst-like body formed after division of the kinetoplast and nuclei

On the 6th day rosettes started to form from the cyst-like body observed before (Fig. 6).

On the 7th day we found in the smears both small rosettes and big ones. Enormous amounts (from hundreds to thousands) of metacyclic trypanosomes with kinetoplasts, nuclei and flagella but without the undulating membranes extended from the latter (Fig. 7, 8, 9).

On the 8th and 9th day we observed in every field of vision of the microscope 40–50 metacyclic forms of trypanosomes separated from rosettes, every smear was about 200 fields of vision (Fig. 11, 12). Later on a portion of the flagellates presumably migrates into the leech proboscis and while sucking blood by the leech from a frog trypanosomes get into the blood stream of the vertebrate host. Then they adopt typical appearance of the parasite with kinetoplast, nuclei and strongly pronounced undulating membrane.

In the Fig. 13 the scheme of the *Trypanosoma neveulemairei*'s life cycle in the leech *Hemiclepsis marginata* is presented.

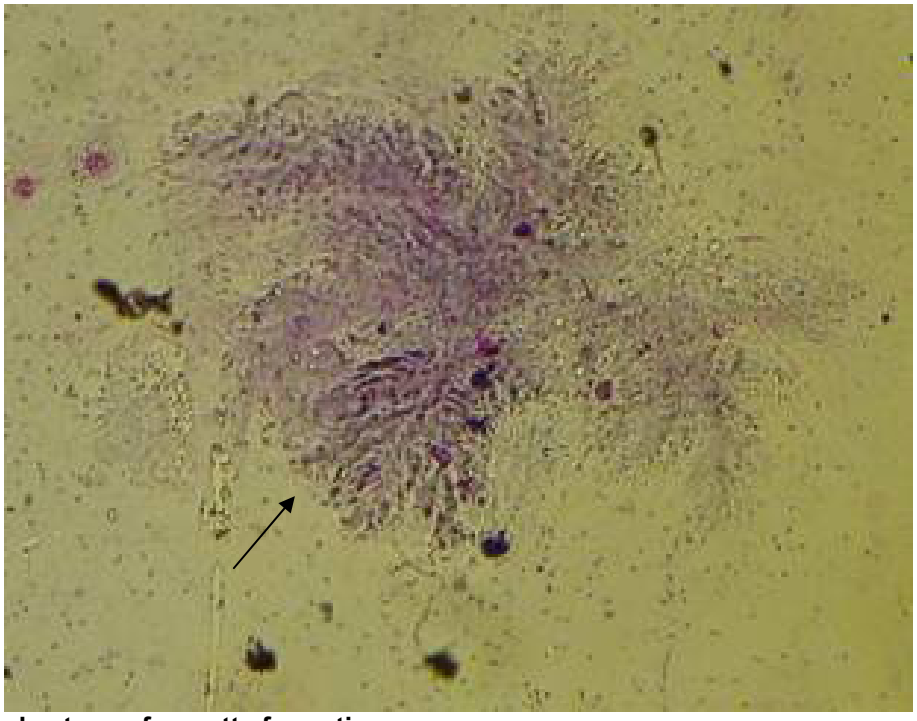

Fig. 6. Early stage of rosette formation

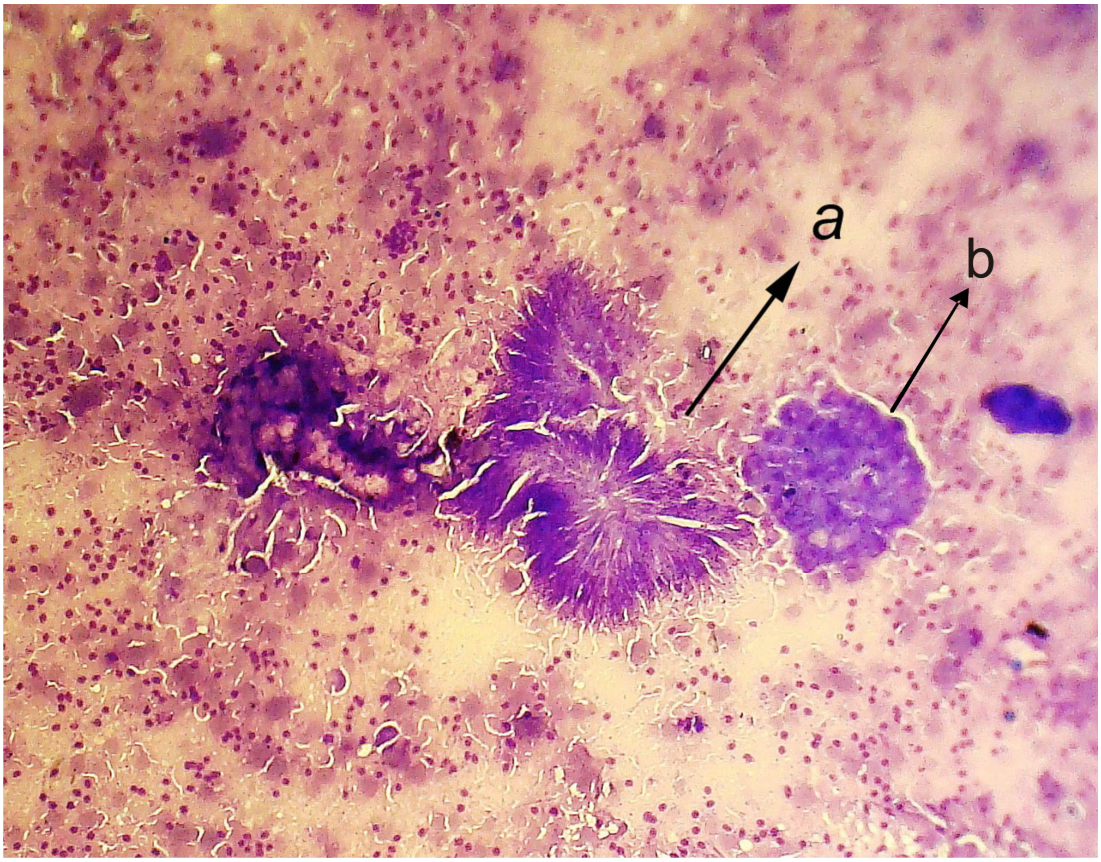

Fig. 7. A small rosette with metacyclic trypanosomes (a) and a cyst-like body (b)

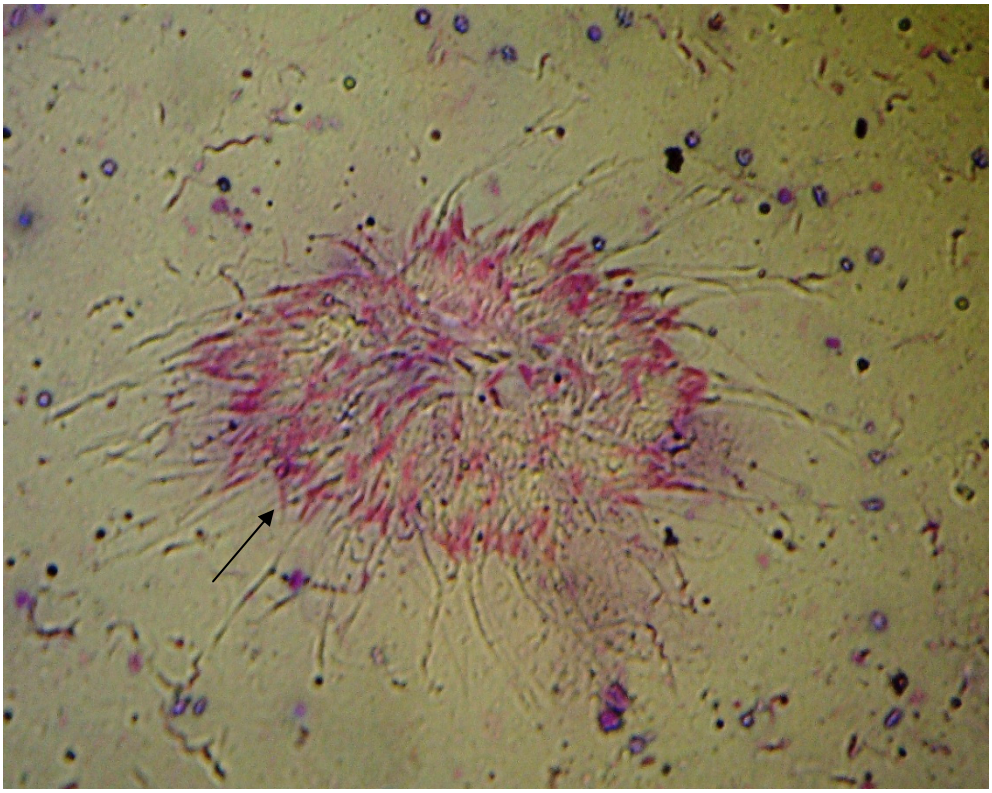

Fig. 8. A small rosette with trypanosomes

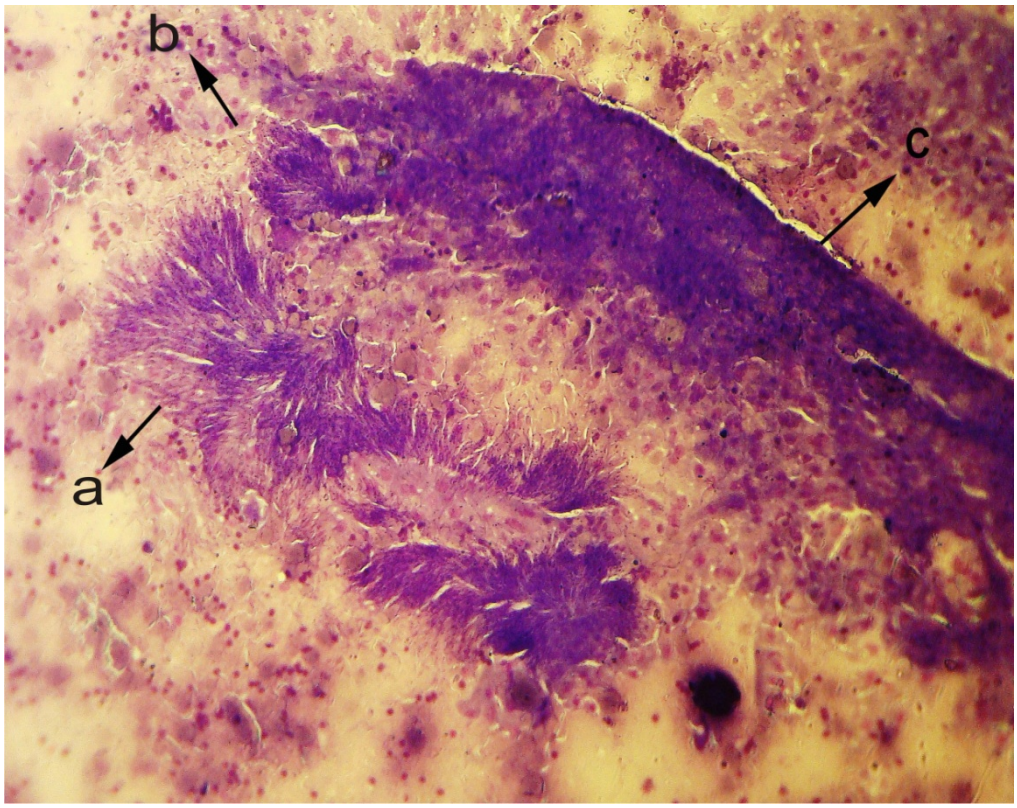

Fig. 9. A large rosette (a), an early stage of rosette formation (b) and cyst-like body (c)

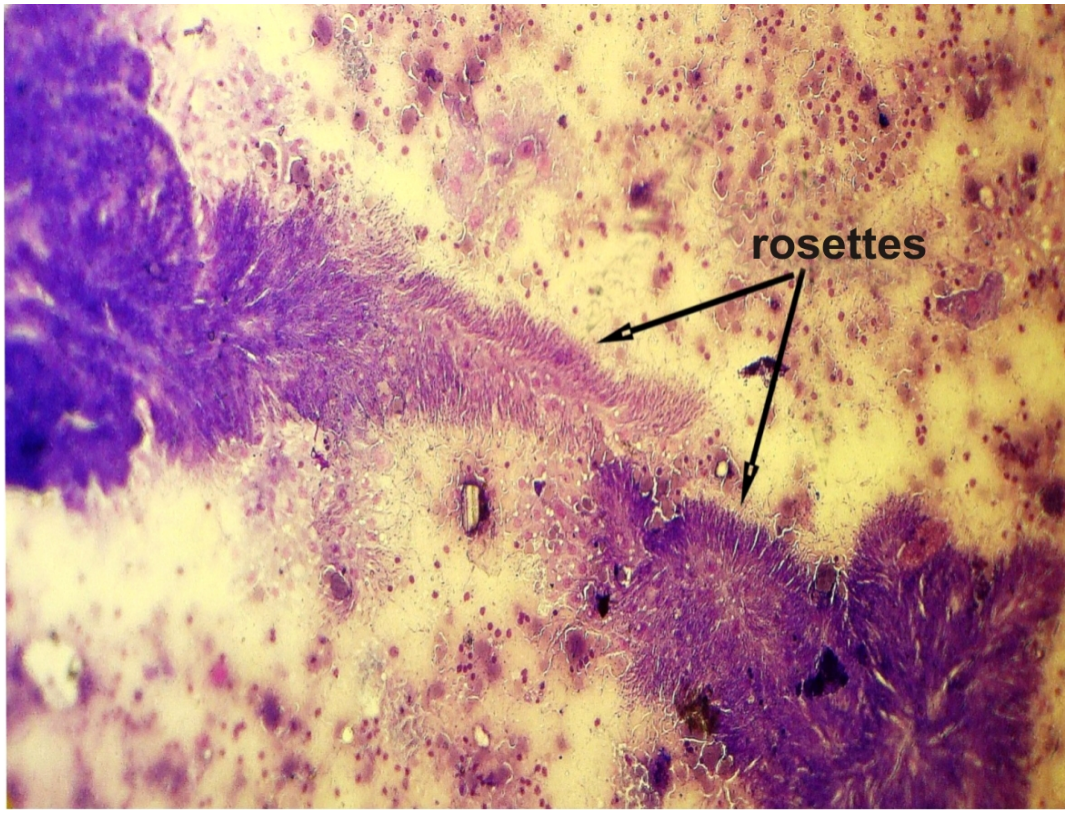

Fig. 10. A large rosette with an enormous number of metacyclic trypanosomes attached to it

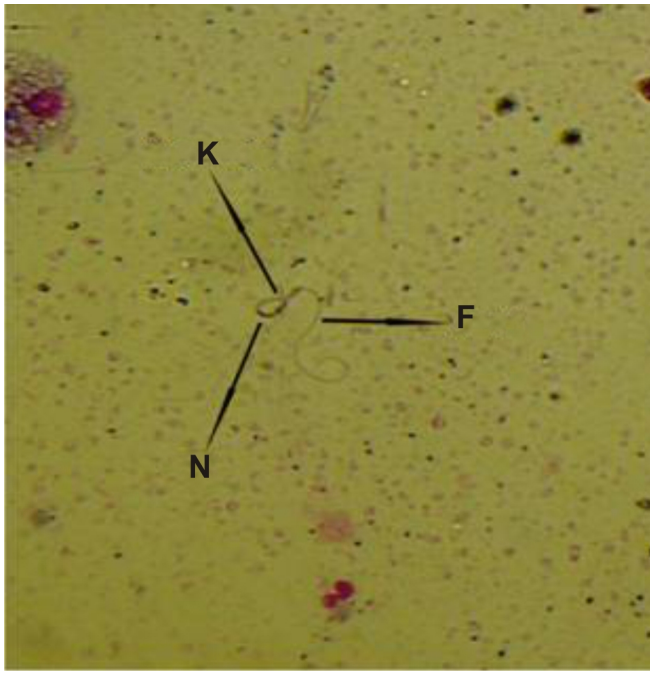

Fig. 11. A metacyclic trypanosome with nucleus (n), kinetoplast (k) and flagellum (f)

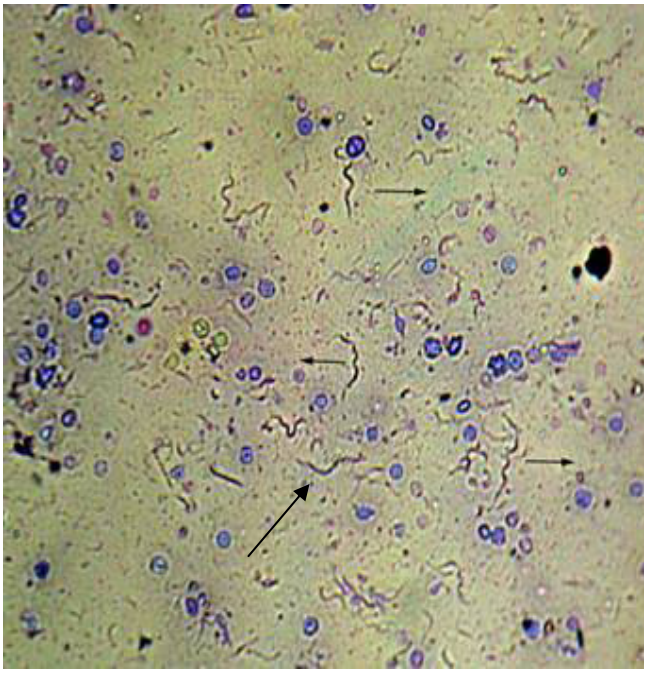

Fig. 12. Metacyclic trypanosomes separated from rosettes

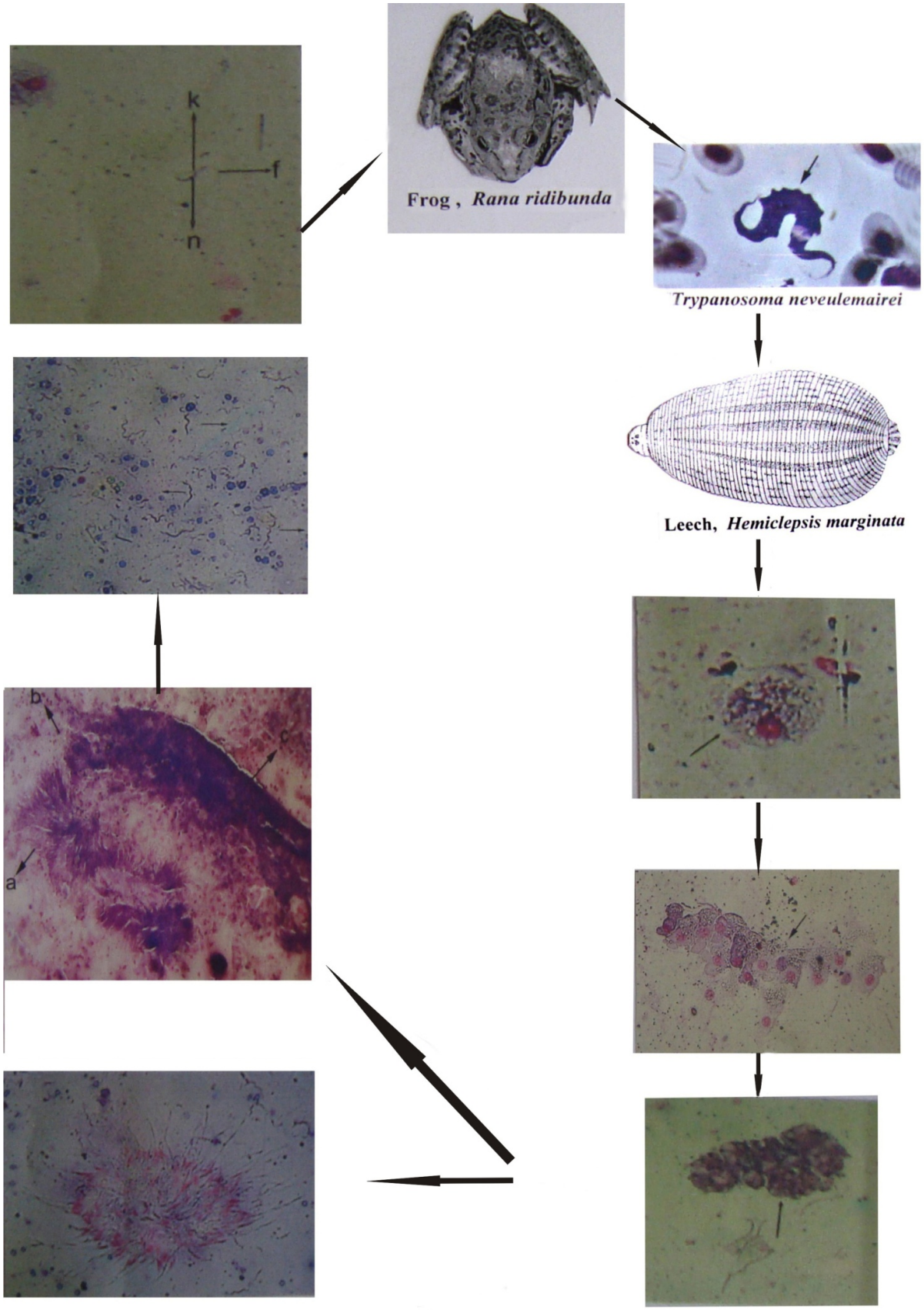

Fig. 13. The scheme of the *Trypanosoma neveulemairei's* life cycle in the leech *Hemiclepsis marginata*

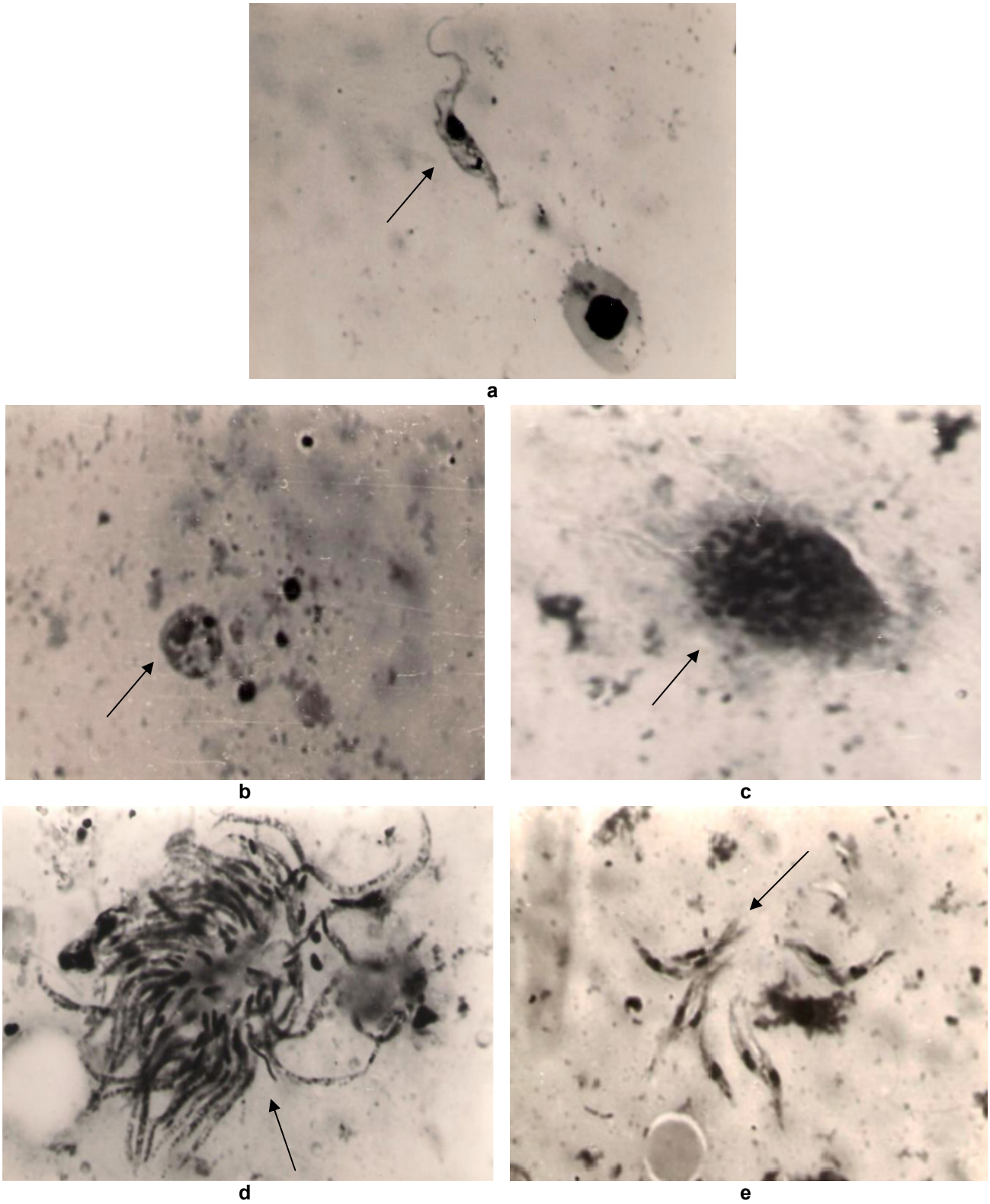

Fig. 14. Different stages of the life cycle of *Trypanosoma danilewskyi* *(a – Trypanosoma danilewskyi from carp blood; b – rounded trypanosome; c – cyst-like body; d – rosette; e – metacyclic trypanosomes detached from a rosette)*

During our experimental work, it was found that the process of reproduction of the marsh frog trypanosome *Trypanosoma neveulemairei* within the leech *Hemiclepsis marginata* was nearly identical to the

reproduction of the fish trypanosome *Trypanosoma danilewskyi* within the leech *Piscicola geometra* (Khaibulaev, Guseinov, 1983).

Thus, in both species, the flagellates became rounded on the second day of the experiment. After that, kinetoplast division started and was followed by nuclei division. On the fourth and fifths days, the parasites enlarged to form cyst-like bodies that were totally different from blood stages of trypanosomes and gave rise rosettes with a great number of outgoing metacyclic trypanosomes (Fig.14).

References

Khaibulaev K.Kh., Guseinov M.A. Izuchenie razvitiya tripanosom (Trypanosoma) i kriptobiy (Cryptobia) karpa i linya v piyavke *Piscicola geometra* (A study of the development of trypanosomes (Trypanosoma) and cryptobians (Cryptobia) of carp and tench in the leech *Piscicola geometra*) // Parazitologiya. – 1983. – Vol.19, No.1. – P. 75–77. [In Russian]

Baily J.K. *Aedes aegypty* as possible new intermediate host for frog trypanosomes // Exptl. Parasitol. – 1962. – Vol.12. – P. 155–163.

Desser S.S., McIver S.B., Ryckman A. *Culex territans* as a potential vector of *Trypanosoma rotatorium*. 1. Development of the flagellate in the mosquito // J. Parasitol. – 1973. – Vol.59. – P. 353–358.

Miyata A. Anuran haemoprotozoa found in the vicinity of Nagasaki City. 1. *Trypanosoma rotatorim* (Mayer, 1843) // Trop. Med. – 1976. – No.18. – P. 125–134.

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