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Report

Ph.D. thesis prepared by Magdaléna Kulich Fialová

***Trypanosomes* transmitted by mosquitoes: occurrence in hosts, transmission,
and specificity**

I have read Ph.D. THESIS prepared by Magdaléna Kulich Fialová with great interest. Ph.D. THESIS consist of an introduction;
a review of research and scientific literature that reviews known avian *Trypanosoma* species transmitted by mosquito vectors as well as *Trypanosoma theileri* group, possible vectors of this group and the impact of mosquitoes as vectors of one genetic lineage of this mammalian *Trypanosoma*;
aims of the thesis;
list of publications which consist of two publications in journal *Microorganism* and one publication being under preparation;
summary and
references.

The first publication was addressed to the investigation of avian parasite *Trypanosoma thomashancrofti* and mosquitoes as its vectors. The second publication provided data on the relationships between *Trypanosoma theileri* group and mosquitoes as possible vectors. The third publication (under preparation) described new *Trypanosoma* species and the possibility for mosquitoes to transmit these parasites.

Summarizing the dissertation - the data presented are new, interesting, based not only on field research, but also on experiments, which, I know, are not easy to perform in the laboratory, and it was a pleasure to read this work. Nevertheless, I have a few questions based on the first two publications that are already published. The third publication could still be improved, so I'll make a few comments below. I hope my comments will help the author in her future research.

First paper

It is written:

“Based on the high similarity of SSU rRNA sequences from Australian and Czech trypanosomes, *T. thomasbancrofti* was described as cosmopolitan, with the mosquito *Culex pipiens* being the suspected vector.”

It would be always good to provide the exact similarities or differences or genetic distances between sequences, how many percent or how many SNIPs are the differences between sequences or between genetic lineages.

How did You decide that 7 – 8 mosquito guts (not one or two) should be used for infection of birds? Was it a random number or did your practical research show this number to be the best for bird infection.

It is written:

“Out of the 771 dissected *Culex* mosquitoes, 49 individuals were infected with kinetoplastids: *T. culicavium* had the highest prevalence, with 35 positive individuals, 12 mosquitoes were infected with monoxenous kinetoplastids, and a single individual with *T. thomasbancrofti*, 1 *Cx. pipiens* had a mixed infection, and one harbored the mammalian species *T. theileri*. “ It is 50, not 49 infected individuals? It would be also good to know that species of monoxenous kinetoplastids have been detected.

It is written:

“1367 wild-caught mosquitoes were dissected from 5 different species. “

and later “All species of mosquitoes infected by kinetoplastids were tested, but avian trypanosomes were identified exclusively in *Culex* mosquitoes.”

It seems You did not find trypanosomatids in mosquitoes except *Culex* (it should be 596 mosquitoes belonging to 4 different species), did`n You?

Second paper

It is written:

“A total of 4051 mosquito females belonging to 18 species were caught in 2017–2019; from these, 3282 were tested by PCR in 560 pools, and 769 specimens were examined by dissection of the gut.”

How was it decided which mosquitoes and how many mosquitoes should be screened by PCR and which by dissection? Did You use cultivation after dissection? Was it (dissection) a sensitive enough method for detection of parasites? Can You provide some recommendation about the best methods to detect *Trypanosoma* in the wild for scientists based on Your experience?

It is very interesting that sometimes in the case of a negative PCR, parasites can still be detected using cultivation. I agree that PCR is a much faster and simpler method for detecting parasites, although it seems to be not sensitive enough, as *Trypanosoma* densities in the host can sometimes be very low. What can you say about this?

Tab 3 – have you observed statistically reliable morphological differences between parasites of the same species in different vectors?

Third paper

It is written:

“A part of positive guts was used for the cultivation of kinetoplastids, and the rest was stored in ethanol for barcoding of the parasite”

Was it as part of the same gut, used for different investigations? Or was it a part of all investigated guts?

It is written:

“After blood feeding, the blood fed to mosquitoes was always controlled under the microscope for the presence of live trypanosomes.”

Could You explain, please, I did not understand the sentence.

According to methods You used two *Trypanosoma* strains (CUL5 and PAS416), but in experimental infection chapter (lines 117-136) only CUL5 was mentioned. In chapter about Prediuresis (lines 139-145) only PAS416 was mentioned.

In “Results” it was written that both strains have been used for the mosquito infection (lines 215-217), but this information should better be provided in “Methods”, not in “Results”.

Lines 161-162.

“Amplification of trypanosome SSU rRNA gene from bird blood and dissected guts of 162 mosquitoes was done by specific nested PCR”. What does it mean “specific nested PCR”, no references were provided.

Line198.

What species of monoxenous kinetoplastids were detected in *Aedes* and *Mansonia*.

Lines 236-238 (Prediuresis)

It would be good to provide the name of the strain used in this investigation even if it is known to be PAS416 from previous chapters.

General text

I think in some sentences more references should be provided as for example the sentence:

“Mosquitoes (Diptera: Culicidae) are in focus as vectors of pathogens causing infections: viruses (dengue fever, yellow fever, West Nile virus), protists (malaria), and helminths (lymphatic filariasis)” was without references, but this statement, I think, should have references.

“In addition to dixenous trypanosomes, mosquitoes also harbour monoxenous kinetoplastids (Wallace, 1943; Flegontov et al., 2013, Kostygov et al., 2021).”

It would be good to mention that not only mosquitoes can harbor monoxenous kinetoplastids, but also other insects used in this research.

I was written:

“In nature, *T. culicavium* has been isolated from two subspecies of *Culex* mosquitoes – *Culex (Culex) pipiens* and *Culex (Barradius) modestus*.”

The question is if *Culex (Culex) pipiens* and *Culex (Barradius) modestus* are two subspecies or two species.

T. theileri has also been found in *P. perfiliewi* in Italy. Page 17.

This insect species was mentioned here for the first time, so the full genus name should be provided.

I have found that some references, cited in the text, were not mentioned in the “References”. These are:

Baker, 1963; Desser et al., 1973; Hoare, 1967; Kučera 1983;
Novy et al., 1907; Rodrigues et al., 2011; Scheuerlein and Rickles, 2004;
Sharma et al., 2021; Svobodová and Votýpka, 2004;
Votýpka and Svobodová, 2014; Votýpka and Svobodová, 2024; Votýpka
et al., 2021;

Two references, mentioned in the reference list were not cited in the text:

Ferreira, R. C., De Souza, A. A., Freitas, R. A., Campaner, M., Takata, C. S., Barrett, T. V., Shaw, J.J. & Teixeira, M. M. (2008). A phylogenetic lineage of closely related trypanosomes (Trypanosomatidae, Kinetoplastida) of Anurans and Sand Flies (Psychodidae, Diptera) sharing the same ecotypes in Brazilian Amazonia 1. *Journal of Eukaryotic Microbiology*, 55(5), 427-435.
and

Nzelu, C. O., Kato, H., Pupilampu, N., Desewu, K., Odoom, S., Wilson, M. D., Sakurai, T., Katakura, K. & Boakye, D. A. (2014). First detection of *Leishmania tropica* DNA and *Trypanosoma* species in *Sergentomyia* sand flies (Diptera: Psychodidae) from an outbreak area of cutaneous leishmaniasis in Ghana. *PLoS Neglected Tropical Diseases*, 8(2), e2630.

Two references were cited the same way, although there are different literature sources published in the same year:

Garcia et al., 2011;

Garcia, H. A., Kamyngkird, K., Rodrigues..... and

Garcia, H. A., Rodrigues, A. C., Martinkovic, F., Minervino....

and

Rodriguez et al., 2010;

Rodrigues, A. C, Garcia, H. A., Ortiz, , P. A., Cortez

Rodrigues, A. C., Garcia, H. A., Batista, J. S. ,Minervino

As I have already mentioned, the dissertation is interesting, presenting new data, publications are of good quality published in high-level scientific journals. Magdaléna Kulich Fialová is a good, perspective specialist of mosquitoes and *Trypanosoma* parasites and her thesis meets the requirements for a doctoral dissertation.

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2024 03 15

