

DZS – AKTION

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Final report

Project code: **92p14**

Project title: **A survey of *Pyrhocoris apterus* parasites in Austria with a focus on monoxenous trypanosomatids**

Project program: AKTION Czech Republic – Austria

Project duration: 1.7. – 31.12.2022

Type of supported activity: Research trip to obtain scientific material, research activities for students and 2 workshops/seminars for students

Project partners:

Prof. MSc. Vjačeslav Jurčenko, Ph.D., University of Ostrava, Czech Republic

Hans-Peter Fuehrer, Dr.rer.nat. Priv-Doz., University of Veterinary Medicine, Vienna, Austria

Research trip in Austria to collect scientific material

The adult firebugs *Pyrhocoris apterus* were collected in 12 locations within four Austrian cities at the end July 2022. All insects were kept in individual Eppendorf tubes until dissection to prevent their cross-contamination via coprophagy or cannibalism. For dissection, insects were euthanized by submerging them into 96% ethanol for 10 seconds. This was also needed for the sterilization of the outer surface of the insect body to decrease the chances of culture contamination. Each individual was dissected using a pair of disposable wooden toothpicks. The contents of the gut were carefully examined under a light microscope. In the case of trypanosomatid presence, three following samples were typically prepared 1) a culture seeded to Schneider *Drosophila* medium supplemented with 10% fetal bovine serum and 100 units/ml of penicillin and 100 µg/ml of streptomycin; 2) portion of the infected gut for the subsequent DNA isolation preserved in a solution of 2% SDS, 0.1M EDTA, and with 10mM Tris pH 7.5); 3) methanol-fixed smear for the subsequent staining with Giemsa stain, if a control of cell morphology will be required. If the infection intensity was low (only a few cells detected by microscopy), cultures were not seeded.

In total 84/126 (67%) bugs were found to be positive for trypanosomatids (Table 1). The estimated prevalence within particular locations varied in the widest possible range i.e. from 0 to 100%. We detected only a single location where none of 12 dissected firebugs was infected, probably because it was a small isolated colony (under a single tree in the center of Vienna very far from other suitable biotopes), which could be founded by a single uninfected female. Out of other cases, the lowest trypanosomatid prevalence was detected in the Augarten in Graz, where only five male firebugs could be found under a single tree in the whole park. The prevalence in other locations could be also affected by the presence of unidentified bacterial infections, which may interfere with trypanosomatids. Interestingly, to the best of our knowledge, we were the first to document the

presence of nematodes in firebugs: the bodies of two normally looking individuals from Salzburg in addition to trypanosomatids contained ~10 cm long mermithid worms, which partially destroyed internal organs of the insects.

Collected	City	Location	Coordinates	Prevalence
7/24/2022	Vienna	(VI1) single tree near Rossauer barracks	48°13'2"N, 16°22'3"E	0/12 (0%)
		(VI2) small garden near Augarten bridge	48°13'7"N, 16°22'18"E	6/8 (75%)
		(VI3) Augarten, entrance from Untere Augartenstrasse, outer alley	48°13'19"N, 16°22'37"E	7/7 (100%)
		(VI4) Kaiserwiese	48°12'59"N, 16°23'38"E	2/2 (100%)
7/27/2022	Gratz	(GR1) Augarten, tree at the very entrance	47°3'50"N, 15°26'6"E	1/5 (20%)
		(GR1) Stadtpark, memorial stone	47°4'32"N, 15°26'39"E	12/24 (50%)
7/29/2022	Salzburg	(SA2) Moenhsberg, lawn surrounded with trees	47°47'41"N, 13°2'29"E	5/6 (83%)
		(SA3) Moenhsberg, tree on the alley	47°47'47"N, 13°2'23"E	5/9 (55%)
		(SA1) Moenhsberg, single tree on the slope near Siegmundstor West	47°47'55"N, 13°2'19"E	14/16 (88%)
7/30/2022	Innsbruck	(IN1) Gramarstrasse 3-7	47°17'10"N, 11°23'49"E	24/29 (83%)
		(IN2) Alpenzoo, dirt wall near wolf	47°16'54"N, 11°23'53"E	4/4 (100%)
		(IN3) Alpenzoo, dirt wall near the cross between moose and ibex	47°16'55"N, 11°23'50"E	5/5 (100%)
			Total	84/126 (67%)

Total genomic DNA was extracted from all samples using GeneJET Genomic DNA Purification Kit following the manufacturer's protocol. The DNAs were used for PCR amplification of partial 18S rRNA gene (from all samples) and SL RNA gene repeats (from the selected ones), which were sequenced for species identification. As expected from the previous studies of trypanosomatids in firebugs (Votýpka et al., 2012; Frolov et al., 2017;2018) the detected flagellates belonged to two different taxa: *Leptomonas pyrrocoris* and *Blastocrithidia papi*. The first species was detected in all specimens, while the second one was present only as an admixture in different proportions, therefore the prevalence of this species could not be reliably estimated. The analysis of SL RNA gene sequences is still ongoing.

Based on the results of assessment of the geographical distribution of parasite haplotypes, we expect to shed light on the population dynamics of *Leptomonas pyrrocoris*. Out of 84 infected firebugs, we obtained 71 trypanosomatid cultures. Most of these cultures proved to be contaminated with fungi (yeast or mold). Those 12 that were not contaminated, have been already tested for the presence of double-stranded RNA and were found to be positive. The analysis of NGS data is still ongoing.

Note: we originally planned to collect about 75 specimens of fire bugs, but managed to collect more (126 in total) justifying higher expenses for sequencing analysis, as reflected in the budget.

References

- Frolov, A.O., Malysheva, M.N., Ganyukova, A.I., Yurchenko, V., and Kostygov, A.Y. (2017). Life cycle of *Blastocrithidia papi* sp. n. (Kinetoplastea, Trypanosomatidae) in *Pyrrocoris apterus* (Hemiptera, Pyrrhocoridae). *Eur J Protistol* 57, 85–98.
- Frolov, A.O., Malysheva, M.N., Ganyukova, A.I., Yurchenko, V., and Kostygov, A.Y. (2018). Obligate development of *Blastocrithidia papi* (Trypanosomatidae) in the Malpighian tubules of *Pyrrocoris apterus* (Hemiptera) and coordination of host-parasite life cycles. *PLoS One* 13, e0204467.
- Votýpka, J., Klepetková, H., Yurchenko, V.Y., Horák, A., Lukeš, J., and Maslov, D.A. (2012). Cosmopolitan distribution of a trypanosomatid *Leptomonas pyrrocoris*. *Protist* 163, 616–631.

Lecture by prof. Jurčenko at the University of Veterinary Medicine, Vienna, Austria. 25.07.2022

Title: **Evolution of monoxenous Trypanosomatidae: origin and molecular peculiarities (viruses, unique genetic code, virulence factors, bacterial endosymbionts, and beyond)**

Lecture by Priv.-Doz. Hans-Peter Führer at the University of Ostrava, Ostrava, Czechia. 02.09.2022

Title: **Vectors and VBDs. Global Change – Parasites and Vectors**

List of all team members: as planned in the proposal

- 1) prof. MSc. Vjačeslav Jurčenko, Ph.D., University of Ostrava, Czech Republic
- 2) Priv.-Doz. Hans-Peter Führer, Dr.rer.nat., University of Veterinary Medicine, Vienna, Austria
- 3) Alexei Kostygov, Ph.D., University of Ostrava, Czech Republic
- 4) Julia Reichl, M.Sc., University of Veterinary Medicine, Vienna, Austria

Evaluation of cooperation: the grant 92p14 (title: A survey of *Pyrrhocoris apterus* parasites in Austria with a focus on monoxenous trypanosomatids) provided by the AKTION Czech Republic – Austria has allowed to establish a productive collaboration between two excellent teams led by prof. prof. MSc. Vjačeslav Jurčenko, Ph.D., University of Ostrava, Czech Republic and Priv.-Doz. Hans-Peter Führer, Dr.rer.nat., University of Veterinary Medicine, Vienna, Austria. In the frame of this project, 126 specimens of firebugs were collected in Austria and jointly analyzed by both teams in Austria and Czech Republic (once the analysis is complete, a scientific publication will be prepared). But (and probably more importantly!) this led a foundation for further collaborative projects between these two teams on several scientific topics.

In Ostrava on January 2022

doc. RNDr. Jan Hradecký, Ph.D., dean
(electronically signed)