

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17108 Aedes Invasive Mosquitoes

STSM title: Microsatellite-based population analysis of Aedes albopictus (Diptera: Culicidae) from Romania

STSM start and end date: 03/05/2021 to 21/05/2021

Grantee name: Cintia Horváth

PURPOSE OF THE STSM:

The main purpose of this STSM was to gain experience in microsatellite-based population genetic analysis of invasive mosquito species at the Institute of Hygiene and Tropical Medicine, in Lisbon.

Another important objective was to learn about the basics of population genetics.

Firstly, it was my aim to be able to perform microsatellite based molecular analysis and to interpret data as this method is not yet implemented at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, therefore it represents a valuable knowledge as in the future I can use it for my PhD thesis, since there are no previous studies in population genetics on invasive mosquito species in Romania.

Secondly, another purpose of this STSM, as stated above, was to become familiar with performing molecular analysis based on microsatellite markers, and to learn the use of several population genetics- and statistical softwares.

Both aims were fulfilled, and will significantly contribute to further involvement of the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca in WGI activities under the umbrella of AIM COST Action.

Finally, this STSM provided the opportunity for me to acquire a set of practical skills for the laboratory work and also improve my dexterity, and not at last the theoretical part helped me to acquire knowledge in the basics of population genetics. I consider gaining experience in this field as a beginner will help my future development as a researcher. Not only this STSM allowed me to do better my practical work but also offered me the possibility to really understand the theoretical aspects behind each step of the process I was interested in.

In conclusion, I firmly believe that this Short Term Scientific Mission at the Institute of Hygiene and Tropical Medicine, under the guidance of Professor João Pinto will help me to perform microsatellite analysis in the future and to interpret the data collected using appropriate population genetics softwares, as well as interpreting statistical analysis, increasing the quality of my scientific work.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The training at the Institute of Hygiene and Tropical Medicine, was divided into two parts: a theoretical and a practical one, which were included on an everyday basis; additionally after the completion of the scheduled tasks for the day, time was allowed for background research or documentation on the tackled topics. Below I am including a detailed work plan for the three weeks of my stay.

In the first week (03.05-07.05.2021), the focus was mainly on PCR based microsatellite analysis. I started the practical session by performing PCR-based genotyping of microsatellites: laboratorial procedures, fragment analysis and genotypic database preparation. The genetic polymorphism was assessed at six microsatellite markers: Aealbmic6, Aealbmic7, Aealbmic8, Aealbmic11, Aealbmic12 and



Aealbmic13. PCR was prepared in 96 well plates, DNA was amplified in 19 μ l reaction mixes (mix per well: 20 μ l). The end of each forward primer was labeled with a fluorescent dye (6-FAM, HEX or NED) (Sigma, USA). Cycling conditions were set in case of each primer, as they have specific annealing temperature. For fragment analysis another two 96 well plates were prepared with 9 μ l of Formamide and 1 μ l of each PCR product, to obtain a total volume of 12 μ l. The plates were sealed and centrifuged, each plate was provided with a specific identification number, then shipped to Yale University for fragment analysis.

In the second week (10.05.2021- 14.05.2021) I started analyzing the data. Microsatellite alleles were scored with GeneMarker software (SoftGenetics, LLC) and data was extracted into an excel file. Results were analyzed in GENEPOP (version 4.7.5, Rousset 2008), F-Stat (version 2.9.4, Goudet 2003) and GeneClass2 (Piry S et al. 2004). Hardy-Weinberg equilibrium (HWE) tests, allelic frequencies for each locus and linkage disequilibrium (LD) tests were computed using GENEPOP, as well the observed (Ho) and expected (He) heterozygosity. F-Stat was used to calculate Fst for each population pair and across populations. Allele frequency graphs were created in excel after importing data from GENEPOP. GeneClass2 was used to calculate the number of f0 migrants.

The third week (17.05.2021- 21.05.2021) was dedicated to theoretical tutorials on population genetics, interpreting genotypic data, finalizing my database with all the results from the performed tests and writing a scientific report summarising the main finding on Aedes albopictus microsatellites analysis from Romania.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During my stay at the Institute of Hygiene and Tropical Medicine, I believe that I have accomplished the main goal of my Short Term Scientific Mission, which was to gain experience in microsatellite-based population genetic analysis of invasive mosquito species.

I have learned about basic population genetics, I practiced laboratory work- PCR for genotyping microsatellites and scoring microsatellite alleles with specific softwares. I also had a chance to gain practice with statistical programmes and learned to work with new population genetics softwares. During my Short Term Scientific Mission I gained experience and knowledge in fragment analysis and genotypic database preparation as well as interpreting results in different tests performed with several softwares (GeneMarker, GENEPOP, F-Stat, GeneClass2, CONVERT), which I consider the most important part of this experience, because I had the opportunity to learn everything about how to start analyzing microsatellites from genotyping them to interpret data extracted from different population genetics software. This knowledge is useful for understanding the potential of colonization dynamics and establishment, geographic patterns of invasion and range expansion, and potential for evolutionary responses to novel environments as well as the relationships among invaders and native mosquito species.

This experience will help in increasing capacity of our working group at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, which will further contribute to our involvement in activities of WGI of AIM COST Action.

FUTURE COLLABORATIONS (if applicable)

Our future plans focus on analyzing the remaining samples and also new field collected data should be included in this study. We intend to publish these results. I received some advice for my PhD project in Romania from Professor Pinto which was very useful in designing the study's further data collection. Hopefully we will establish a collaboration for further research on this topic between our University and the researchers team involved in this STSM from the Institute of Hygiene and Tropical Medicine.

The STSM grantee

Cintia Horváth

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Date: 25/05/2021

Cluj-Napoca,
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Signature:

