

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

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

Action number: CA17108

STSM title: Morphological identification of invasive mosquito species and principles in surveillance planning

STSM start and end date: 26/07/2021 to 15/08/2021

Grantee name: Gregoris Notarides

PURPOSE OF THE STSM:

(max.200 words)

As an early career researcher who is investigating mosquito biodiversity and abundance in Cyprus, I managed to get familiar with mosquitoes' biology and their surveillance. However, due to the demand for implementing a vector control strategic plan, it was important to extend my knowledge of surveillance planning and acquire skills for the identification of Invasive Mosquito Species.

Hence, this Short Term Scientific Mission was the right course for me at the right time in my career to improve and evolve my knowledge in the surveillance of mosquitoes. Therefore, the visit to the Laboratory for Medical and Veterinary Entomology of the University of Novi Sad had the purpose to improve my skills and advanced research quality in the surveillance of mosquitoes but also in their morphological identification, sampling methods, and their colony rearing. Additionally, the training in the Veterinary Institute of Novi Sad aimed for me to become versed in the molecular detection of arboviruses in mosquitoes.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

The STSM started with fieldwork, which included the sampling of the two higher risk areas in the province of Vojvodina, for the West Nile surveillance program. In the area higher risk number 1, a total of 30 NS2 traps and 70 ovitraps were set up at a 420km total travel distance for each day of sampling. On the other hand, in the area higher risk number 2, 20 NS2 traps and 50 ovitraps were set up at a 350km total travel distance for each day of sampling instead. Before each sampling day preparation was required, as all the batteries of the traps needed to be charged at least 12hrs and the nets to be cleaned. The sampling duration for NS2 traps is 24hrs, so the traps were collected the next day of each sampling. All the collected samples were placed immediately to dry ice (to avoid virus degradation). Ovitrap will be collected in the next sampling period of the WNV program (after one month), as they need an extended period to collect larvae.

*After the fieldwork, laboratory work followed. The specimens which were collected from the sampling were identified morphologically using specialized keys and stereoscopes, while during the whole process dry ice was being used to keep the samples in a cold environment. All the specimens were counted by species for each sampling site, and at the same time, the *Culex pipiens* complex individuals were sent for further RNA*

analysis, as they are the main vectors of WNV. In parallel with the morphological identification of the adults, we worked also with larvae that were collected from previous samplings and I was introduced to the technique of their breeding, and the separation of males from females as well. In addition, the process of the mosquito colony rearing was thoroughly presented to me, as we were doing the daily tasks to guard the fitness of the mosquito colony. Mosquito larvae were being fed with special nourishment high in proteins and low in fats. The surface of the bucket's water with the eggs was cleaned every time from the fat layer which prevents mosquito breathing. Additionally, the mosquitoes at the stage of pupa were being collected and transferred to cages where they develop into adults. In the interior of the cages, there are small tanks of water where the mosquitoes eventually oviposit their eggs. At this stage, the eggs were being collected and transferred into the initial buckets with water, to start a new breeding cycle.

For the third part of my STSM, I visited Veterinary Institute in Novi Sad to watch the whole process of WNV detection. At first, the specimens that were chosen for RNA analysis in the lab were put into tubes were prepared with materials for the tissue destruction of the mosquitoes. Then, the tubes with the specimens were centrifuged vertically and horizontally and only the supernatant was selected for the further process. Later, the RNA extraction and purification methods were made following the instructions of commercial kits. Apart from the manual methods, an instrument that offers automated extraction and purification system was used, due to the increased numbers of samples. Finally, samples for the RT-PCR were prepared carefully to avoid contamination. After the completion of the RT-PCR reactions, the results were evaluated to check whether the virus was present in the tested samples or not.



DESCRIPTION OF THE MAIN RESULTS OBTAINED

First and foremost, I became familiar with the morphological identification of invasive mosquito species (*Ae. albopictus*, *Cq. richiardii*, *An. hyrcanus*, *Ae. japonicus*, etc). Secondly, I extended my knowledge about their surveillance and the sampling methods being used (what are the ideal sites for sampling, type of traps and specific usage of attractants, special handling of samples, etc). Thirdly, I learned the process of molecular analysis for the detection of diseases from the collected specimens. Lastly, I was introduced to techniques like mosquito colony rearing, the breeding of collected larvae from ovitraps, and the separation of males from females. In conclusion, my visit to the Laboratory for Medical and Veterinary Entomology of the University of Novi Sad, and at the Veterinary Institute, made me more efficient and better qualified with

mosquito surveillance and gave me valuable experience with the Invasive Mosquito Species. The knowledge gained and skills acquired through this STSM will be applied for implementing a vector control strategic plan in Cyprus.

FUTURE COLLABORATIONS (if applicable)

We established collaboration between our research group and the researchers' group involved in this STSM from the Laboratory for Medical and Veterinary Entomology of the University of Novi Sad. Our cooperation is about an upcoming research project which concerns the identification and distribution of mosquito vectors of medical importance on the island of Cyprus. Among others, we will continue to exchange ideas about mosquito surveillance and always seek better results, as a part of our productive collaboration.

For the STSM grantee

Gregoris Notarides
Cyprus University of Technology
Date: 21/08/2021

Signature

