# SHORT TERM SCIENTIFIC MISSION (STSM)
# SCIENTIFIC REPORT

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

**Action number:** **CA17108-44617**
**STSM title:** **Population genetics and insecticide resistance mechanisms in *Aedes aegypti* and *Aedes albopictus* from Turkey**
**STSM start and end date:** **03/11/2019 to 14/11/2019**
**Grantee name:** **M. Mustafa AKINER**

## PURPOSE OF THE STSM:

Specimens with known insecticide resistance status collected from two *Ae. aegypti* and five *Ae. albopictus* populations from Turkey were processed to: (i) detect knockdown resistance mutations associated with target-site resistance by allele specific PCR; (ii) estimate copy number variation (CNV) of genes involved in metabolic resistance using a qPCR approach; (iii) assess genetic variation, geographical origin and population structure using 17 microsatellites markers and related it to each population insecticide resistance profile.
As main outcomes we intent to characterize resistance phenotypes and underlying mechanisms in order to clarify possible causes of inefficacy of insecticide-based control measures implemented in Turkey and to assist heath authorities in the planning of new interventions. By assessing genetic variation, we aim to better understand the origin and structure of these two invasive species in this region. In addition, genetic information produced in this work will be incorporated into genetic databases helping to unveil the dispersal pathways and prevent new introductions of these species into Europe.

## DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

**Sample selection and study design**
According to the work plan, three different populations of *Aedes albopictus* and another three of *Aedes aegypti* were seleted:

*Aedes albopictus* populations were selected considering three different entry points into Turkey:

- Aliaga Western population from Izmir province
- Igneada Thrace population from Kırklareli province
- Hopa Eastern populations from Artvin province

As to *Aedes aegypti,* we seleted three populations that have just been found in Eastern Black Sea area:

- Arhavi Artvin province
- Pazar Rize province
- Hamidiye Rize province

**DNA extraction**

Two different protocols were performed for DNA extraction: CTAB protocol, routinely used at the host institute and a Qiagen DNA isolation kit. A total of 15 specimens from each *Aedes albopictus* were used for DNA extration (5 using CTAB protocols and 10 using Qiagen kit) to assess genetic variation, geographical origin and population structure. Ten specimens of each *Aedes aegypti* population were seleted for DNA extraction using CTAB protocol, to detect knockdown resistance mutations associated with target-site resistance by allele specific PCR and to estimate copy number variation (CNV) of one gene involved in metabolic resistance.

***Aedes albopictus* genetic variation, geographical origin and population structure.**

A panel of thirteen of polymorphic microsatellite loci were used to assess genetic variation, geographical origin and population structure of the three seleted population. DNA amplification was performed according to standart protocols and amplicons were sent to Yale DNA Analysis Facility for sequencing.

In a training exercise, microsatellite alleles were scored with Genemarker (Softgenetic LLC) and population genetic estmates (e.g. allele richness, effective population size, expected heterozygosity) were calculated using Genalex and NeEstimator softwares.

***Aedes aegypti* kdr mutations and analysis of CYP9j23-gene copy number variation (CNV).**

Detection of three different kdr mutations (F1534C, V1016I and V410L) in *Aedes aegypti* specimens was carried-out using standartized allele-specific PCRs.

PCR amplified products were size fractionated using gel electrophoresis, visualised under UV and photographed for analysis.

For CYP9j23 CNV analysis , DNA content of each sample was measured with Qbit. To be further prosseced by qPCR, DNA samples were diluted to obtain a final concentration of 0,1 ng/µl. Only one target gene (CYP9j23) and two reference genes (CYP4D39 e Cl prot channel) were analysed. Specimens from a susceptible colony (Rockfeller strain) were used as controls and processed using the same protocols. The estimation of CYP9j23´s CNV of Turkish specimens was performed by ΔΔCt analysis using CFX Maestro ™ v. 4.0.2325.0418 (Bio-Rad) software and Rockfeller specimens for comparison.

## DESCRIPTION OF THE MAIN RESULTS OBTAINED

As to *Aedes albopictus* studies we are still waiting for microsatellite sequencing results in order to proceed with population genetic analysis.

Regarding *Aedes aegypti kdr* mutiations, with the exception of three samples that were not successfully amplified, all specimens reveled to be homozygotic for the susceptible alleles. As to CNV analysis of CYP9j23, no significant differences were found between Turkish and the reference susceptible (Rockefeller) specimens. However, the technique showed some discrepancies that will be addressed in the near future as a collaborative effort between the two institutions. These molecular data confirm previously obtain results regarding insecticide resistance assays that have shown that all populations analyzed were susceptible to all compounds tested.

**FUTURE COLLABORATIONS**

In the course of this STSM we have carried-out two distinct studies: one regarding population genetic of *Aedes albopictus* and another related to insecticide resistance in *Aedes aegypti*. During this period, three different molecular procedures were performed. In a near future, we aim to establish these molecular techniques as routine procedures of our laboratory. Due to time limitation, not all the collected populations were analyzed. Thus, the collaborative work initiated with this STSM will be continued in order to finalize the studies proposed and publish the results.

Future collaboration will also address qPCR methodology for CNV detection of genes associated to metabolic resistance. Optimization to process single specimens (current technique is designed to use pools of five mosquitos) still needs to be addressed for successful CNV analysis, and comparison of results obtained by the labs of two institutions will be carried-out in order to validate protocols.