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: Microsatellites based analysis of genetic variation and population structure of *Aedes albopictus* in Spain.
STSM start and end date: 15/09/2019 to 30/09/2019
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PURPOSE OF THE STSM:

The Asian tiger mosquito *Aedes albopictus* is a major threat to public health due to its ability for dengue, chikungunya and Zika virus transmission. In Europe, it is extended to 26 countries, including the Iberian Peninsula, since 2004, in Spanish territory and with several cases of Dengue since 2018.

In 2018, *Ae. albopictus* was recorded for the first time in the Extremadura region of Spain (Bravo-Barriga et al. 2019). Preliminary genetic analyses suggest that mosquitoes collected derive from multiple source populations. However, due to the lack of information about the Iberian Peninsula there were difficulties in establishing the geographical origin and colonization routes of these introductions.

The aims of this work plan are therefore:

1. To evaluate the genetic variation and population structure of *Ae. albopictus* in Spain.
2. To determine the geographical origin and colonization routes of the recent introductions of the Tiger mosquito in Extremadura, Western Spain.
3. To contribute for a microsatellites-based genetic variation database of *Ae. albopictus* for the Iberian Peninsula.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During the two-weeks short-term scientific mission, the following laboratory activities were carried out at the IHMT in Lisbon:

1. Meetings were held to analyse geographical coverage of the already existing samples, identify further candidate locations for additional sampling in Spain and to select representative subsamples of individuals for the microsatellites study.
2. The DNA of individual mosquitoes was extracted from the samples by different protocols.
3. The PCR amplification of 16 microsatellite loci (Manni et al. 2015) of the samples and the preparation of plates for automated fragment analysis was initiated.
4. Training on microsatellites genetic data analysis was carried out with already existing datasets from the Iberian Peninsula. Analyses included:
   - Analysis of electropherograms and allele scoring using GENEMARKER, and preparation of genotypic databases for subsequent analysis.
   - Basic genetic variation estimates and summary statistics were performed with MICROCHECKER, FSTAT and GENEPOP
   - Analysis of population structure using Bayesian Clustering Methods (STRUCTURE) and Discriminant Analysis of Principal Components (ADEGENET).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The work carried out during these two weeks of STSM allowed for the following advances in this genetic study of *Aedes albopictus* populations for the Iberian Peninsula.

1. Initial preparatory meetings allowed for selection of 268 mosquitoes from 59 collection sites in Spain. The DNA of these individuals was extracted using the following protocols
   a. A commercial kit (DNeasy Blood & Tissue Kit, QIAGEN). N= 59
   b. A CTAB 2% protocol. N= 209

2. PCR amplification with fluorescent-labelled primers of 16 microsatellite loci was carried out on 40 individual mosquito samples, using protocols adapted from Manni et al. (2015). Amplified products were organized into 96-well microplates to be shipped to the DNA analysis Facility at Yale University for fragment analysis in an automated sequencer.

3. Finally, I have carried out an initial apprenticeship to microsatellite genetic data analysis. Using appropriate population genetics softwares, I have analysed the genotypes of 150 individuals from seven Iberian Peninsula populations to estimate genetic variation and population structure. Figures 1 and 2 illustrate the main results opbtained from these analyses.
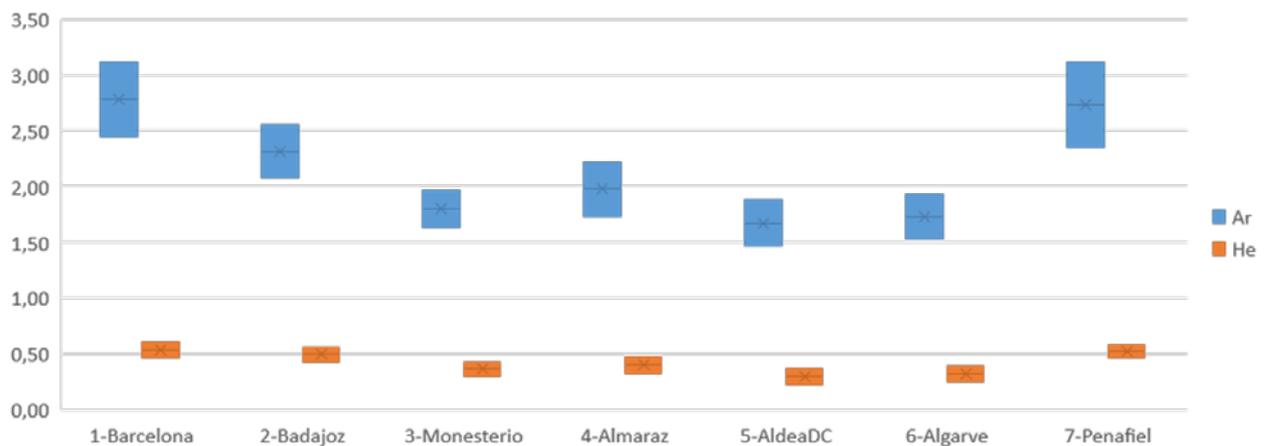

Figure 1. Mean over-loci estimates of allele richness (Ar) and expected heterozygosity (He) for *Aedes albopictus* from the Iberian Peninsula.
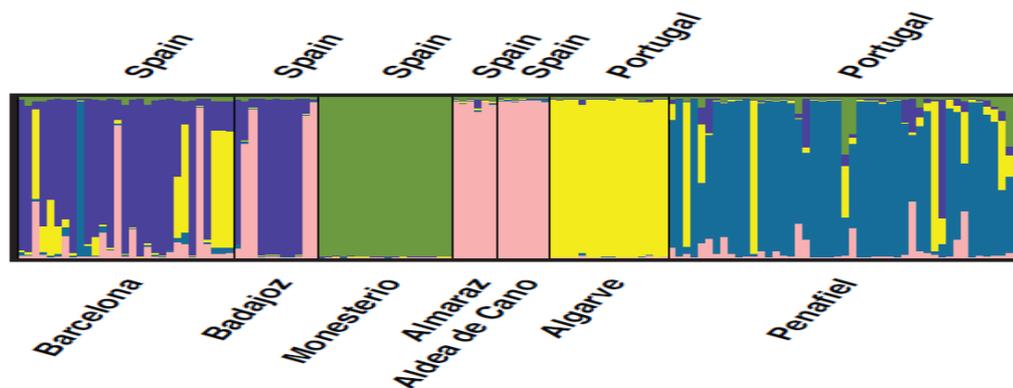

Figure *2*: Results of Bayesian Clustering Analysis implemented by STRUCTURE for *Aedes albopictus* from the Iberian Peninsula. The figure shows assignement of genotyped individuals into five distinct genetic clusters.

FUTURE COLLABORATIONS (if applicable)

Continuity will be given to the analysis of a large collection of *Aedes albopictus* samples from the Iberian Pensinsula, representing both long-established populations and recent introductions. To overcome a few gaps in the sampling coverage, collaborations with the Health Department of Madrid and Bilbao have been established in order to obtain *Ae. albopictus* from these regions.

In addition to the ongoing microsatelites analysis analysis of population structure, our groups plan to further collaborate in a study aimed at determining the genetic diversity and phylogeny of *Wolbachia* strains in these Iberian populations. The results of this study will add knowledge to the growing literature on *Wolbachia* and could be used as a reference to explore its potential as a biological control agent.

Finally, we will continue to collaborate in the genetic data analysis and preparation of publications based of the data generated in these analyses.