



To: MC Chair, Andrei Mihalca and STSM coordinator, Dusan Petric

Murcia, 11/12/2015

STSM Scientific Report

Title of STSM: Sampling methods and genetic and morphometric speciation of mosquitoes.

Reference code: COST-STSM-ECOST-STSM-TD1303-261015-068695.

Period: 09/11/2015 to 20/11/2015.

Host institution: Hacettepe University, Department of Biology, Faculty of Science, Ecology Division, ESR Laboratories, Beytepe-Ankara-TURKEY.

Host professor: Bulent Alten: kaynas@hacettepe.edu.tr

Purpose of the STSM

As described by the host institution the aims of this STSM were five-fold:

- (a) Understand the principles of taxonomy, specimen acquisition, identification and preservation for both morphology and integrated systematic studies including rearing techniques of wild mosquito populations under laboratory conditions.
- (b) Become familiar with morphological identification keys.
- (c) Be able to make geometric morphometric analyses and manipulate geometric morphometric data with related state-of-the-art softwares.
- (d) Be able to manipulate DNA sequence data, database searches and sequence retrieval, analysis and alignment, extraction of basic sequence statistics and generate and interpret phylogenetic trees.
- (e) Be able to compare both geometric morphometric and barcoding data for understanding situation of the populations in given conditions.

Description of the work carried out during the STSM

During my STSM I had the opportunity to participate in the following activities:

1. Literature search and familiarity.
 - a. Eldridge BF (2005). Mosquitoes, the Culicidae. In: Marquardt WC. Biology of disease vectors. 2nd ed. USA: Elsevier Academic Press; pp 95-111.
2. Lectures.
 - a. Biology and Ecology of Target Mosquitoes: *Aedes*, *Culex*, *Anopheles*.
 - b. Classification, morphology and identification of mosquitoes.
 - c. Use of VectorNet website and vecmap phone application for field sampling.



3. Laboratory work.

- a. Morphological identification of mosquitoes and sandflies.
- b. Pinning mosquito samples.
- c. Colony rearing of mosquito and sandfly species.
- d. Molecular identification of mosquitoes.

Description of the main results obtained

1) Mosquito morphological identification, rearing and collection preparation.

I identified specimens of mosquitoes collected from a sampling field in Spain (Region of Murcia). A total of 11 mosquito species belonging to 4 genus and 2 subfamilies were identified using the taxonomic keys by Schaffner (2001) and Becker (2010) as shown in the table below:

Subfamily	Genus	Specie
<i>Anophelinae</i>	<i>Anopheles</i>	<i>An. maculipennis s.l.</i> <i>An. algeriensis</i> <i>An. marteri</i>
<i>Culicinae</i>	<i>Culex</i>	<i>Cx. pipiens s.l.</i> <i>Cx. theileri</i> <i>Cx. deserticola</i>
	<i>Culiseta</i>	<i>Cs. annulata</i> <i>Cs. longiareolata</i> <i>Cs. subochrea</i>
	<i>Ochlerotatus</i>	<i>Oc. refiki</i> <i>Oc. caspius</i>

The key morphological structures used for their identification included external structures like setae and scales distribution of the thorax, head morphology, color of the abdomen, color and length of the legs, wings morphology or genitalia male (Schaffner, 2001; Becker, 2010).

I couldn't identify several damaged specimens by morphological features. These samples were identified by barcoding methods. Furthermore, I learned how to pin mosquitoes for preparing collections.

I also had the opportunity to participate in rearing techniques of *Culex quinquefasciatus* and *Aedes aegypti* mosquito colonies, and rearing techniques of *Phelebotomus papatasi* sandfly colonies (Kasap, 2005; Chelby, 2007). This included:

- Blood meal supply for adult females on a rabbit (*A. aegypti*), a quail (*Cx. quinquefasciatus*), and an anesthetized mouse (*P. papatasi*).
- Food supply for male and female mosquito and sandfly adults (sugar cotton and grapes), mosquito instars (fish food) and sandfly instars (rabbit faeces).



- Egg and larval stage transfer to separate pots, and pupae stage transfer to adult cages.

2) Sandfly morphological identification

I had the opportunity to learn sandfly species of Turkey. I dissected male and female *P. papatasi* specimens. And I learned morphology of the following species: *P. tobbi*, *P. major s.l.*, *P. perfiliewi transcausicus* of *Larrousius* subgenus, and *P. simici* of *Adlerius* subgenus (Toprak, 2005).

3) Molecular identification of mosquitoes using barcoding methods

I used barcoding methods to identify 22 damaged specimens. First, I performed DNA extraction using the DNeasy® Blood&Tissue kit (QIAGEN). Then I did PCR amplification of cytochrome c oxidase subunit 1 and electrophoresis of the PCR product to visualize the amplified product. Subsequently the DNA was eluted from the gel and used for sequencing (Gunay et al, 2014).

I also learned to edit the DNA sequence using BioEdit and Sequencher sequence alignment editors, and to compare sequences using boldsystems.org website. Besides, I could generate and interpret phylogenetic trees using MEGA6 softwares.

References

- Gunay F, Alten B, Simsek F, Aldemir A, Linton YM (2014). Barcoding Turkish *Culex* mosquitoes to facilitate arbovirus vector incrimination studies reveals hidden diversity and new potential. *Acta Tropica*;
- Chelbi I, Zhioua E (2007). Biology of *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. *J Med Entomol*, 44(4):597-600.
- Kasap OE, Alten B (2005). Laboratory estimation of degree-day developmental requirements of *Phlebotomus papatasi* (Diptera:Psychodidae). *Journal of Vector Ecology*, 30(2):328-333.
- Toprak S, Ozer N (2005). Sand fly species of Sanliurfa province in Turkey. *Med Vet Entomol*, 19:107-110.
- Schaffner E, Angel G, Geoffroy B, Hervy JP, Rhaiem A, Brunhes J (2001). The Mosquitoes of Europe. Institute de Recherche pour le Développement (IRD), Montpellier, France. CD-Rom.
- Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, Kaiser A (2010). Mosquitoes and their control. 2nd ed. Springer Verlag Berlin Heidelberg.

Future collaboration with the host institution (if applicable)

The STSM has been a great opportunity to acquire knowledge on mosquito speciation and rearing techniques. It has been an opportunity to expand the ongoing collaboration between Hacettepe and Murcia University in the ecology and epidemiology of vector-borne disease. We intend to formalize this collaboration in the near future.



Foreseen publications/articles resulting from the STSM (if applicable)

The mosquito work carried out during my STSM will be part of a scientific publication describing *Culicidae* mosquito species in southeast Spain with shared authorship between the Hacettepe and Murcia groups.

Confirmation by the host institution of the successful execution of the STSM

I fully confirm that Clara Muñoz's STSM with the title of "Sampling methods and genetic and morphometric speciation of mosquitoes" of the COST project "EurNegVec" has been organized by Hacettepe University, Faculty of Science, Biology Department, Ecology Division, ESRL-VERG laboratories between November 9th-20th, and completed with success. My team members, Dr. Filiz Gunay, Research Assistant, and Yasemen Sarikaya, Gizem Oguz and Begüm Karaoglu have very much contributed the STSM with both theoretical and practical lectures.

This has been a further opportunity since the start of EURNEGVEG for successful collaboration between our institutions which will no doubt lead to shared scientific publications in the areas of mosquito and sandfly research.

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