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

Novi Sad, 12/11/14

## **STSM scientific report**

**Title of the STSM:** Genetic and phenotypic comparison of sand fly and mosquito populations using geometric morphometrics (size and shape analyses) and barcoding methods with relevant softwares

**COST STSM Reference Number:** COST-STSM-TD1303-20730

**Reference code:** ECOST-STSM-TD1303-201014-048137

**Period:** October 20th-November 2nd, 2014

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

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

**WG:** WG1

## **Purpose of the STSM**

As described by the host institution the aims of this course 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 sand fly and mosquito populations under laboratory conditions, (b) become familiar with 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 stay I attended the following lectures and had an active participation in practical laboratory and computer sessions described below.

### **1. Lectures:**

- 1.1. Taxonomy, Systematics and Classification of sandflies and mosquitoes.
- 1.2. Collection and preservation of sand flies and mosquitoes for integrated study.

- 1.3. Laboratory breeding of mosquito and sand fly populations.
- 1.4. What is geometric morphometrics? Theoretical presentations on principals of morphometrics; sample studies on sand fly and mosquito species.
- 1.5. Introduction to morphometrics software and statistics.
- 1.6. Biology, ecology and distribution of sand flies.
- 1.7. Sand flies and their medical importance and aspects: Leishmaniasis and Phleboviruses.
- 1.8. Medical impact of mosquitoes.
- 1.9. Mosquito Barcoding Initiative and BoLD database.
- 1.10. Introduction to Bioinformatics for DNA sequence analysis.
- 1.11. Database sequence interrogation.
- 1.12. Correct sequence alignment: the basis of good phylogenies.
- 1.13. Phylogenetic reconstruction.

## **2. Laboratory Practical Exercises**

Sandfly and mosquitoes identification and rearing:

- 2.1. Preparation, taxonomic identification of specimens with classical methods.
- 2.2. Colony rearing and breeding of sand fly and mosquito species.

Sandfly and mosquito morphometrics:

- 2.3. Preparation of wings for morphometry: dissection, descaling and staining.

Sandfly DNA analysis:

- 2.4. Specimen preparation for DNA extraction.
- 2.5. DNA extraction.
- 2.6. PCR analysis.
- 2.7. DNA electrophoresis.
- 2.8. DNA clean up for sequencing.
- 2.9. Sequencing.

## **3. Computer Practical Exercises**

- 3.1. Photograph techniques for morphometric analysis (wing slides).
- 3.2. Installation of landmarks on wings.
- 3.3. Image processing.
- 3.4. Statistical analysis and assessment of morfometric results: principal components analysis (PCA) and canonical variates analysis (CVA).
- 3.5. Manipulation of DNA raw sequence data.
- 3.6. Sequence database interrogation and submission.
- 3.7. Sequence alignment and phylogenetic tree building.

**The following scientists participated as teachers in the course:**

*Haccetepe University, Ankara*

Department of Biology (Ecology Division): Prof. Dr. Bulent Alten, Dr. Ozge Erisoz Kasap, Research Assistant M.Sc. Filiz Gunay and Research Assistant B.Sc. Gizem Oguz

Department of Biology (Applied Biology): Associate Prof. Dr. Murat Aytekin

Molecular Biology department: Associate Prof. Dr. Cagatay Karaaslan

Medical School: Associate Prof. Dr. Koray Ergunay

*Ege University, Izmir*

Department of Parasitology: Prof. Dr. Yusuf Ozbel

**Results obtained from laboratory and computer practicals**

**Sandfly and mosquitoes identification and rearing:**

I dissected and morphologically identified female and male specimens of sandflies from the genus *Phlebotomus* (subgenus *Larrousius*, *Phlebotomus* and *Paraphlebotomus*) and *Sergentomyia* including following species: *Phlebotomus perniciosus*, *P. longicuspis*, *P. tobbi*, *P. major*, *P. papatasi*, *P. sergenti*, *P. simici*, *P. alexandri*, *Sergentomyia dentata* and *S. minuta*.

I also had the opportunity to recognize eggs, instars and adults of the genus *Culex*, *Aedes* and *Anophles* mosquitoes.

I participated in the routine to maintain the *P. papatasi* sandfly colony and the *Culex pipiens molestus*, *C. quinquefasciatus* and *Aedes aegypti* mosquito colonies held at the ESRL laboratories. This included providing food for instars (both mosquito and sandfly), transferring the mosquito pupae stage and the newly emerged sandfly to adult cages and providing a blood meal for adult female sandflies on an anesthetized mouse.

**Sandfly and mosquito geometric morphometrics:**

I prepared sandfly and mosquito wings for morphometric analysis, a technique that is particularly useful to differentiate populations of the same species. For sandflies it involved dissection of the wings from the rest of the body, staining wings with a methylene blue solution and mounting the wings using entellan resin. For mosquitoes it involved dissection of the wings, removing scales from the wings and mounting the wings using entellan resin.

Wings were photographed using a digital camera and images were used to carry out the geometric morphometric analyses to measure wing size and shape, using the TPS, IMP, Morphueus and Morphologika software packages.

The first step involved plotting 16 anatomical landmarks at the intersections of wing veins. The coordinates of the landmarks were digitized and images were superimposed to visualize directional and quantitative changes in shape using Generalized Procrustes Analysis. We then used multivariable PCA and CVA to compare individual and group-level variation, respectively, in wing shape. This allowed identifying the landmarks with the greatest contribution to changes

in wing shape which in the case of the sandfly wings analyzed corresponded to landmarks 13-15 situated in the center of the wing.

#### **Molecular speciation of sandflies using barcoding methods and phylogenetic analysis:**

I performed molecular speciation on three sandfly specimens. The steps followed in individual specimens included: (i) DNA purification, (ii) PCR amplification of cytochrome c oxidase subunit 1, (iii) electrophoresis of the PCR reaction to visualize the amplified product, (iv) purification of the amplified product, (v) dideoxynucleotide sequencing of both the forward and reverse DNA strands, (vi) sequence editing using BioEdit sequence alignment editor to obtain consensus sequence (vii) sequence similarity search using BLAST in NCBI (GenBank).

I was able to obtain between 600bp and 700bp (depend on the specimen) of the target sequence and the BLAST search retrieved other sequences with up to 98-99% sequence homogeneity with my specimens (*P. papatasi* and *P. tobbi*).

Finally, I had the opportunity to have a brief insight into the construction of phylogenetic trees of *Phlebotomus* spp. and *Lutzomyia* spp., downloaded from GenBank, using MEGA6 software. We used distance based Neighbor Joining (NJ) and character based Maximum Likelihood (ML) analysis to construct trees and bootstrapping sampling to judge the strength of support for clades in the phylogenetic trees.

#### **Future collaboration with the host institution (if applicable)**

This STSM has provided me the opportunity to strengthen collaboration between University in Novi Sad and Hacettepe University especially collaboration between Laboratory for Medical and Veterinary Entomology in Novi Sad and ESRL Laboratory in Ankara.

For summer of 2015 it is planned extensive field work in Balkan countries, one of the counties is Serbia and our laboratory is going to be host institution.

Furthermore, Prof. Alten agreed to be a supervisor on my PhD thesis.

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

The STSM was a great opportunity for me to learn a variety of sandfly and mosquito ecological research tools and to strengthen our friendship and interest for future collaborations and common scientific publications.

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

I fully confirm that the STSM with the title of “Genetic and phenotypic comparison of sand fly and mosquito populations using geometric morphometrics (size and shape analyses) and barcoding methods with relevant softwares” of the COST project “EurNegVec” has been organized by Hacettepe University, Faculty of Science, Biology Department, Ecology Division, ESRL-VERG laboratories between October 20<sup>th</sup> and November 2<sup>nd</sup> and completed with success.

Three participants have been involved in STSM, Prof. Dr. Eduardo Berriatua Fernández de Larrea from Murcia University, Spain, M.Sc. Slavica Vaselek from University of Novi Sad,

Serbia and Nikolina Sokolovska from Ministry of Health, Skopje, Macedonia. ESRL-VERG laboratories with accommodation and daily expenses have supported Mrs. Sokolovska.

My team members, Dr. Özge Erisöz Kasap, Research Assistants Filiz Gunay, Research Assistant Gizem Oguz and three academicians from Biology Department Prof. Dr. Hatice Mergen, Associate Prof. Dr. Murat Aytekin and Assoc. Prof. Dr. Koray Ergunay, and their team members have very much contributed this STSM both with theoretical and practical lectures.

I also confirm that the writing about the topics of the STSM has been finished during the course interval.

As ESRL-VERG laboratories, we have got quite much scientific feedbacks from the participants. It was also very good opportunities to make future plan, collaboration and student exchange programs with Murcia and Novi Sad University and Macedonia Ministry of Health.



Prof. Dr. Bülent Alten  
THE STSM Coordinator



M.Sc. Slavica Vaselek