



To: MC Chair, Andrei Mihalca and STSM coordinator, DusanPetric

Murcia, 10/11/14

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

Reference code: COST-ONLINE_STSM-TD1303-6736

Period: October 20th-November 3rd, 2014.

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 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. Phlebotomine Sand Flies, the Psychodidae, biology, ecology, distribution.
- 1.2. Medical Impact of Mosquitoes.
- 1.3. Sand Flies and their medical importance and aspects: Leishmaniasis and Phleboviruses.
- 1.4. Taxonomy, Systematics and Classification of sandflies and mosquitoes.
- 1.5. Collection and preservation of sand fly and mosquitoes for integrated study.
- 1.6. Laboratory breeding of mosquito and sand fly populations.
- 1.7. What is geometric morphometrics? Theoretical presentations on principals of morphometrics; sample studies on sand fly and mosquito species.



- 1.8. Introduction to morphometrics software and statistics.
- 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 morphometric 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:

Hacettepe University, Ankara

Ecology department: Bulent Alten, Osge Erisoz, Filiz Gunay and Gisem Oguz

Molecular Biology department: Cagatay Karaaslan

Technopolis INC, Hacettepe University: Murat Aytekin.

Medical School: Koray Ergunay.

Ege University, Izmir

Medical entomology: 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 *Larrosius*, *Phlebotomus* and *Paraphlebotomus* groups including following species: *Phlebotomus perniciosus*, *P. longicuspis*, *P. tobii*, *P. major*, *P. papatasi*, *P. sergenti*, *P. alexandri*, *Sergentomyia dentata* and *S. minuta*. I also had the opportunity to recognize adults, eggs and instars 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 Ecology department. This included providing food for instars kept separately from adults, 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. It involved dissecting the wings from the rest of the body, staining sandflies wings with a methylene blue solution, removing scales from mosquito wings and mounting the wings on a glass coverslip 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, Morpheus 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 analysed corresponded to landmarks 13-15 situated in the center of the wing.

Molecular speciation of sandflies using barcoding methods and phylogenetic analysis of sandflies species

I performed molecular speciation on three *P. papatasi* 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 almost 700bp of the target sequence and the BLAST search retrieved other *P. papatasi* sequences with up to 98% sequence homogeneity with my *P. papatasi* specimens.



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)

The STSM has provided me the gateway to collaborating with one of the best sandfly ecology groups, and its leader, Prof. Alten, has expressed his intention to visit Murcia University and help us design and carry out a province-wide ecological study of sandfly distribution and abundance in the coming spring. Furthermore, he kindly offered to take on students from Murcia and I can envisage future collaboration in geometric morphometric analysis of sandflies. From our side we offer our expertise in Leishmania infection and epidemiological data analysis and look forward to host members of his group willing to gain experience in these areas.

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 20th and November 3th and completed with success. Three participants have been involved the STSM, Prof.Dr. Eduardo Berriatua Fernández de Larrea from Murcia University, Spain, Slavica Vaselek from Novi Sad University, 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 Aytakin and Assoc. Prof. Dr. Koray Ergunay, and their team members have very much contributed the STSM with both 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.

Bülent

Prof.Dr. Bülent Alten
The STSM Coordinator

Other comments (if any)

From Murcia's perspective the STSM was very fruitful and our laboratories and expertise remain open to further STSM within COST action TD 1303.

E. Berriatua

Eduardo Berriatua Fernández de Larrea
Professor of Animal Health.



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