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: Host feeding preferences and arboviral screening of mosquitoes in the Republic of Moldova.
STSM start and end date: 07/10/2019 to 06/12/2019
Host institution: Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany.
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PURPOSE OF THE STSM:

(max.200 words)

The mosquito host-feeding preferences are the important information to identify potential vector species under natural conditions. No study was performed, related to the host-feeding patterns of mosquitoes in the Republic of Moldova by the molecular identification of blood meal source in the mosquito gut. Only some behavioral observations of the mosquito species or species complexes were mentioned in the papers.
The arboviral screening of mosquitoes in Moldova was conducted between 1970s and 1990s. At that period Batai,Tahyna and West Nile viruses were isolated from mosquitoes. The updated data on mosquito-borne arbovirus circulation are absent in Moldova.
The purpose of the STSM proposal was to strength the collaboration between the Institute of Zoology and the Bernhard Nocht Institute for Tropical Medicine (BNITM); to connect to the infrastructure of the WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research in order to elucidate the blood-feeding patterns of the field collected mosquitoes, to identify sibling species, belonging to the Anopheles maculipennis and Culex pipiens complexes, and to find out if the local circulation of mosquito-borne viruses takes place in the Republic of Moldova.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

(max.500 words)
About 900 blood fed mosquito females were collected by CDC traps in 2016, 2017 and 2019, and more than 5000 unfed mosquitoes were collected in 2019 from about 50 localities belonged to 18 regions in the Republic of Moldova. Mosquitoes were identified to species/species complex level and transported frozen at the BNITM. Engorged mosquito females collected in 2016 and 2017 were fixed and transported in 96% ethanol. According to morphological identification the collected mosquitoes belonged to 21 mosquito taxa as follows *Anopheles maculipennis* s.l., *An. claviger, An. hyrcanus, Aedes annulipes, Ae. caspius, Ae. cantans, Ae. cataphylla, Ae. cinereus,*

Ae. dorsalis, Ae. flavescens, Ae. geniculatus, Ae. pulchritarsis, Ae. sticticus, Ae. vexans, Culex pipiens/torrentium, Cx. modestus/martinii, Coquillettidia richiardii, Cq. buxtoni, Culiseta annulata, Cs. longeariolata and *Uranotaenia unguiculata*.

The digestion status of mosquito blood meals was scored visually according to the Sella score to evaluate the impact of blood meal digestion status on blood meal identification by DNA sequencing. Blood fed mosquitoes were separated to the individual 2 ml tubes and DNA was isolated from the whole mosquito body. Frozen unfed mosquitoes were pooled by species, date and collection site for molecular screening on arboviruses. Up to 5-10 zirconia beads (BioSpec Products, Bartlesville, USA) and 700 µl of cell culture medium (high-glucose Dulbecco's modified Eagle's medium; Sigma-Aldrich, St. Louis, MO, USA) were added to the 2 ml tubes containing mosquitoes. The homogenization was performed with a Tissuelyser LT Qiagen, Hilden, Germany) for 3 min at 30 oscillations/s. The RNA/DNA extraction was performed with KingFisher™ Flex Magnetic Particle Processor using 5X MAGMAX^{tm} Pathogen RNA/DNA kit (Applied biosystems by Thermo Fisher Scientific).

PCR amplification of vertebrate host mitochondrial cytochrome b gene was conducted with the primers designed by Kitano et al., 2007: L2513: 5'- GCCTGT TTA CCA AAA ACA TCA C-3' and H2714: 5'- CTC CAT AGG GTC TTC TCG TCT T-3' (~244 bp). Another pair of primers was used to increase the chance to gain PCR amplicons:

L14841: 5' - CCATCCAACATCTCAGCATGATGAAA – 3' and H15149: 5'- CCCTCAGAATGATATTTGTCCTCA-3' (~358 bp) if no PCR amplicon was produced by primers of Kitano et al. The PCR reactions were performed according to the methodology described by Börstler et al., 2016.

The internal transcribed spacer 2 (ITS2) sequences were used to separate the sibling species of the Anopheles maculipennis complex in Moldova. PCR amplification of ITS2 was conducted with the following primers: forward: (5'-CTGCAGGACACATGAACACC-3', reverse: (5'-CAAGTTGAAACCTGGGGTTG -3') (Lühken et al., 2016).

A multiplex real-time PCR assay, targeting the gene locus for acetylcholinesterase 2 (ace2) to discriminate between *Culex torrentium* and *Cx. pipiens pipiens* and the CQ11 microsatellite locus for discrimination between *Cx. p. pipiens* biotypes pipiens and molestus, was performed (Rudolf et al., 2013).

Part of the material was screened for arboviruses of the family Flaviviridae using primers targeting the NS5 gene: mFU1: 5'-TACAACATGATGGGAAAGCGAGAGAAAAA-3' and CFD2 5'- GTGTCCCAGCCGGCGGTGTCATCAGC -3'.

Sanger sequencing was applied for all positive amplicons (LGC Genomics, Berlin, Germany), sequences pre-processed with Chromas 2.6.6. and compared to sequences from the GenBank database (National Center for Biotechnology Information, http:// blast.ncbi.nlm.nih.gov/Blast.cgi).

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Overall, 581 DNA sequences of vertebrate host mitochondrial cytochrome b gene have been obtained from the mosquitoes blood meal content. Currently, DNA sequences have been pre-processed with Chromas 2.6.6. and part of the material has been compared to sequences from the GenBank database. Preliminary data show that the main vertebrate hosts of mosquitoes from the Republic of Moldova are the cattle, poultry, human, canine, birds, etc. More detailed analysis of wild-caught mosquitoes' host-feeding patterns will provide important information on vector capacity of different mosquito species and associated Public Health risks for mosquito-borne diseases in Moldova.

In total, 298 DNA sequences of ITS2 gene have been obtained for the specimens, belonging to Anopheles maculipennis complex. Preliminary analysis revealed the presence of four sibling species: *Anopheles maculipennis* s.s., *An. messeae*, *An. atroparvus* and *An. daciae*. The presence of *An. sacharovi* in Moldova was not confirmed. More detailed analysis will show the geographical distribution and host-feeding preferences of sibling species in Moldova.

About 150 blood fed females, belonging to Culex pipiens complex have been identified using multiplex real-time PCR assay. The results showed a high prevalence of *Culex p. pipiens* biotype *pipiens* in the sample. Less abundant were *Culex torrentium* and *Culex p. pipiens* biotype

molestus. The blood meal analysis will show specific host–feeding preferences of Culex pipiens complex members in the conditions of the Republic of Moldova.
During the STSM the applicant was trained to screen the mosquitoes for arboviruses and this survey has been initiated for mosquitoes from Moldova. The arbovirology laboratory staff will continue the molecular screening of mosquitoes from Moldova for arboviruses of the families Flaviviridae, Bunyaviridae and Togaviridae.
The results obtained during the AIM-COST STSM will be publicated in peer-reviewed journals.

FUTURE COLLABORATIONS (if applicable)

To enhance research effectiveness, standardise and optimise the surveilance of artropod vectors and vector-borne diseases in the Republic of Moldova the joint research programm between the Institute of Zoology and BNITM has been planed.