NRCPD-OUAVM Joint Research Report

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1. Principal investigator

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Position: Researcher

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2. Project title:

Molecular identification of fly vector of haemoparasites in Mongolia

3. Collaborating research group members at NRCPD

Name: Shinya Fukumoto

Position: Associate Professor

4. Research period (in mm/dd/yyyy, and total number of years)

04/01/2019 – 03/31/2021 (one year)

5. Purposes and objectives

Insect vectors are responsible for the transmission of important parasitic diseases, causing millions of deaths every year and endangering approximately 3 billion people and animals around the world. The types of *Tabanidae* and *Aedes* or *Culex* spp are vector of cameline trypanosomiasis and filariasis, respectively. These diseases are economically important infectious diseases affecting the camel industry, especially in the camel rearing areas of the world including Mongolia. Therefore, aim of this study was to detect the major *Trypanosoma evansi* and *Dipetalonema evansi* parasites in insect vectors from different areas of Mongolia.

6. Outline of research process

In this study, a total of 135 specimens of mosquitoes and flies captured in two provinces, namely Umnugovi and Dundgovi in Mongolia. Mosquitoes and flies were selected to have a complete body structure, especially still has a head, thorax, legs and abdomen then insect vectors were identified with the aid of taxonomic keys based on morphological observation using microscope. Further, total genomic DNA was isolated from single whole mosquito and fly sample using Nucleo spin tissue kit following manufacturer's instructions. All gDNAs of insect vectors were screened by PCR assay using the *T. evansi-ITS1* and *D. evansi−COI* genes.

In addition, a subset of mosquito specimens was selected for identification and confirmation using PCR targeting the *COI* gene. PCR products were sequenced using Sanger technology with ABI BigDyeTM Terminator v3.1 chemistry and phylogenetic analysis was performed.

7. Outline of research achievements

During this research, we morphologically identified the most epidemiologically important *Glossina* fly and *Aedes*, *Culex* and *Anopheles* genera of mosquito. Molecular analysis of 10 *COI* sequences was exclusively comparable with the morphological identifications of *Aedes caspius*, *Culex pipiens* and *Anopheles messeae* species. As illustrated in Fig. 1, *COI*-based phylogenetic analysis showed distinct clustering of individual species within each genus with strong bootstrap support. Our *Culex pipiens* – *COI* gene sequences clustered with those of similar species from other regions, Singapore, Brazil, Uganda and Iran reported in the NCBI database. In contrast, Mongolian *Aedes caspius*–*COI* gene sequences determined in this study was found in the different clade.

In this study, all gDNAs of flies and mosquitoes were negative for *Trypanosome* and/or *Dipetalonema evansi*, except only one mosquito DNA sample that was positive for *Trypanosome* spp. (Fig. 2). In the future, a comprehensive epidemiological survey of fly vectors of haemoparasites in different regions of Mongolia is required.

8. Publication of research achievements

The paper is under writing for publication.

Figure 1. Phylogenetic tree based on *COI* sequences of *Aedes*, *Culex* and *Anopheles* species mosquitoes. Numbers shown at branch nodes indicate booster values.

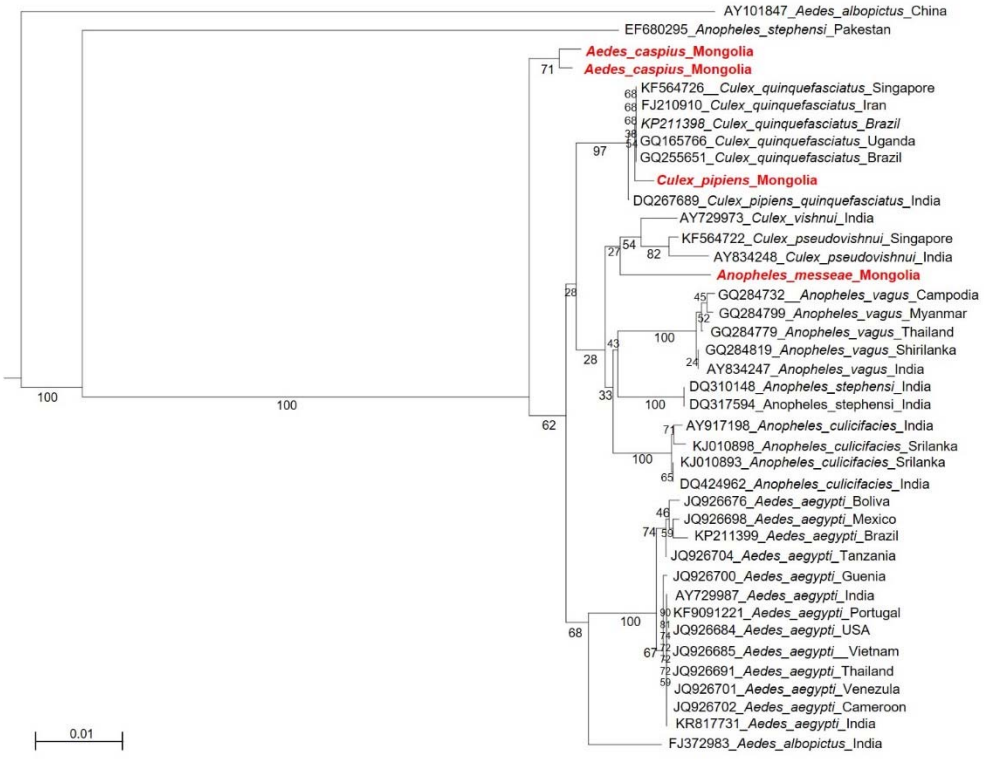

Figure 2. PCR result. M, Marker; Lanes 1-6, gDNA of fly; Lanes 7-9, gDNA of mosquitoes.