

Final report of grant KH_130379

The actuality and importance of the research topic is well indicated by the fact that already in the initial year of the application we were invited to several conferences (as an invited speaker) where we were able to communicate the application and social utility of our research, the interconnectedness of mosquito research and mosquito control actions. During the presentations we discussed the significance and importance of Integrated Mosquito Control, as well as the data of the automatic mosquito counter used during the project, thus introducing the possibility of a new measurement technology and its reliability in Hungary to the professional community. Its importance in the implementation was emphasized by measuring the development of mosquito populations in different areas with different managements (chemical or biological).

Unfortunately, the restrictions related to the Covid pandemic significantly limited both field and laboratory processes, yet the goals set out in the project plan were achieved. Contrary to what was planned, the arrival and collection of samples was partially hampered, but the objectives were still achieved.

Mosquito monitoring work was continued and expanded throughout the period, both in terms of the areas studied and the test methods used, depending on the pandemic circumstances. Within the framework of the Aedes Invasive Mosquito COST-Action network, we joined a Europe-wide surveillance program, in 2020 we carried out mosquito surveys in about 6 Hungarian settlements (Baranya, Tolna, Pest counties), this year (2021) Baranya, Tolna, Pest, Veszprém, Győr-Moson-Sopron counties). The data obtained in this way not only provide new information on the situation of invasive species in Hungary, but also make a significant contribution to the knowledge of the European landscape. The analysis of the European survey data from more than 20 countries has started, and the manuscript is expected to be published in 2021. This excellent networking achievement is a direct result of the current grant.

In addition, we built an excellent professional relationship within the country, together with the ELKH Ecological Research Center we launched the Mosquito Monitor citizen science program, both in the professional part (translation of Mosquito Alert mobile application into Hungarian, validation of data from Hungary, coordination and implementation of further monitoring work throughout the country) and we have a significant role to play in comprehensibly informing the civilian population.

We performed multipurpose studies with the *Aedes koreicus* mosquito, which is an important vector species in our country. Importance of this species means we are able to conduct focused and extensive scientific observations on the establishment of an alien mosquito species in novel territories, since we detected the emergence of this mosquito in the early phase. During the early phase of this research we assessed its ecological requirements and optimal environmental factors for breeding and survival. We intended to build on these observations and provide a universal model for the temperate climate region to better understand its dispersal possibilities to other geographic regions and therefore provide the baseline knowledge for counter measurements as well. We used our field measurement and sampling data and finally examined the topic in strong collaboration with Italian colleagues from the Research and Innovation Centre Fondazione Edmund Mach, Department of Biodiversity and Molecular Ecology (San Michele all'Adige, Trento, Italy) and published a collaborative paper in 2020 (Int J Environ Res Public Health).

Within the framework of the grant we started the population genetic analysis of *Aedes koreicus*, both with domestic (Hungarian) specimens and with mosquito samples from other countries, therefore sequence data were available for analysis from all European countries (different populations) where the species has already appeared or settled. It should be emphasized that a similar study on the species was not yet available in Europe before, only entomologic data was available. Based on the expanded analyzes of the mitochondrial gene COI, 32 haplogroups occurring in Europe was identified, as well as the possible distribution routes of the species between the examined countries. Our results shed light on the importance and significance of coordinated cross-border monitoring.

An extraordinary result of this grant is that of the invasive species of *Aedes* that appeared in continental Europe, so far only the complete genome of *Aedes albopictus* has been published. Within the framework of the application, we determined the total nuclear and mitochondrial genome sequence of *Aedes koreicus* using Oxford Nanopore long read sequencing technology and Illumina sequencing technology. Sequence data have been uploaded to the NCBI public database (nuclear genome: SUB9093984, mitochondrial: MZ460582), and their availability will become public in parallel with the submitted publication. We have integrated this result into previous work of COI-based haplotype analysis, the manuscript expected to be published in 2021. The *Aedes* Invasive Mosquito COST network has already indicated its intention to design a genetic analysis based on SNP (single nucleotide polymorphism) using the genome we have created, in which our research team will be actively

involved as well. This is a good indication of the importance of the result and its founding effect on broadening future research portfolios.

Another main topic of the grant was the complex study of diseases transmitted by native mosquitoes. As part of this, we performed a comprehensive risk assessment of the Hungarian West Nile Fever virus, with special regard to the role of *Culex pipiens* mosquitoes as the main vector of this virus. As a new approach, we also examined the role of mosquitoes in the wintering of the virus at a site with several confirmed veterinary cases during the 2018 West Nile virus epidemic. This study, in which we measured the population of wintering mosquitoes in the area of infections, was also combined with the study of the presence of other microbes, especially the symbiont *Wolbachia* bacteria.

Although West Nile virus was not described in the wintering mosquito population, but species-specific *Wolbachia* bacteria were successfully detected in the *Culex pipiens* species. The scientific significance of these bacteria is growing in the control of mosquito-transmitted viruses and in the control of the mosquito populations, so their recognition and description can serve as a basis for several future research topics or innovative purposes. The results were published in *Viruses MDPI* journal, representing the first large-scale West Nile virus data from our country.

We have also performed *in vitro* isolation of viruses found in local mosquitoes using the C6/36 *Aedes albopictus* cell line. As a result we currently have one of the largest mosquito virus collection in Europe in our laboratory. These were isolated only from native mosquito species and also the viral genome sequences were already partially determined. This result is an extremely important achievement for understanding the viruses carried by the domestic flora and for accurately understanding their diversity, human or animal health significance. Unfortunately this workflow was extremely affected by the work on the COVID-19 epidemic, since we used the laboratory capacity of the Virological Research Group at the Szentágotthai Research Centre, who were subjected to national coronavirus research. Nevertheless the virological results achieved within the framework of the tender will enable extremely important basic research in the field of communicable diseases in Hungary in the future.



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