

Molecular validation of native and invasive mosquito species in Belgium

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Background- Since 2017, a nationwide three year exotic mosquito species (EMS) monitoring project is running in Belgium (MEMO), funded by the federal government and the federated entities. Special attention is given to *Aedes* species, since some can be efficient vectors of, for example, the dengue, Zika and chikungunya viruses. In Belgium, 23 points of entry (PoE's) are investigated, including used tyre and lucky bamboo import companies, airports and parking lots along highways, using adult trapping, as well as egg and larval sampling. The critical point in monitoring projects is the correct identification of the collected specimens, which might be especially difficult in the case of damaged specimens, immature stages or cryptic species. Therefore, in addition to morphological identifications, DNA-based techniques are applied to validate species identification. Collected EMS are all barcoded to verify their morphology-based species identifications. Also, 5% of the annual mosquito specimen collection is verified using DNA-barcoding technology (about 1,000 specimens each year), as quality control measure of the morphology-based species identification.

Results- In 2017, 12 native species/complexes were morphologically identified, of which 11 were molecularly validated (7 were verified during the annual quality control procedure). In 2018, 18 native species/complexes were morphologically identified and validated using DNA-based techniques (11 were verified during the annual quality control procedure). The use of specific molecular marker(s) also allowed to resolve some species complexes, as for example the identification of the members of the *Anopheles maculipennis* group. Additionally, all intercepted EMS could be distinguished from the 28 native culicids known to occur in Belgium (culicid checklist from Boukraa et al., 2015). Presently, four EMS were collected once or multiple times at one or multiple PoE's: *Aedes koreicus*; *Ae. japonicus*; *Ae. albopictus*; and *Anopheles pharoensis*. In 2018, *Ae. albopictus* was intercepted at five PoE's, three of which were locations where the species has never been detected before.

Significance- EMS were found to enter Belgium effectively and repetitively through different introduction pathways: via lucky bamboo and used tyre transport, vehicular traffic, but also potentially through natural dispersal. In this perspective, MEMO will contribute to a better understanding of the introduction process of the different EMS by providing information on their status (introduction, establishment or spread), which is essential to guide surveillance and control. Also, the DNA-based approach is essential to ensure the quality of the morphological identifications and confirm the presence of EMS in case of damaged specimens or immature stages.