DNA-barcoding: an efficient tool for rapid identification of native and exotic mosquito species intercepted in Belgium

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INTRODUCTION

Due to international trade, tourism, and climate/ecological changes, mosquito species are transported, dispersed, introduced and may eventually become established in new territories. The introduction of potential disease vector species constitutes a threat to human and animal health. Since July 2017, a nationwide three year monitoring project funded by the Belgian federal authorities and the federated entities, is ongoing in Belgium (MEMO: Monitoring of Exotic Mosquitoes), and is coordinated by the Institute of Tropical Medicine (ITM). DNA-based technologies are used to validate the morphological identifications of intercepted exotic mosquito species (EMS), as well as of a 5% subset of the yearly sampling (quality control). Additionally, a DNA sequence reference database is being compiled.

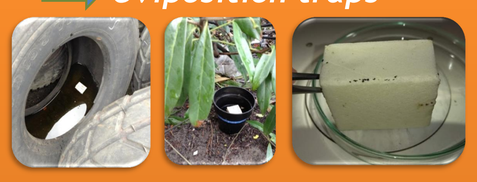
Aim MEMO project: Detecting and evaluating the occurrence of exotic mosquito species in Belgium + risk analyses.

MATERIAL, METHODS AND RESULTS

Sampling: 23 PoEs (Point of Entry)

Larval sampling

Oviposition traps

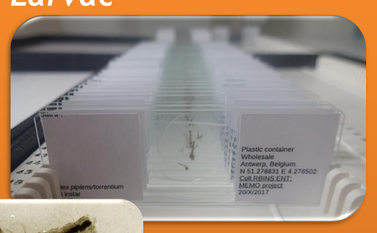


Adult traps

Voucher collection

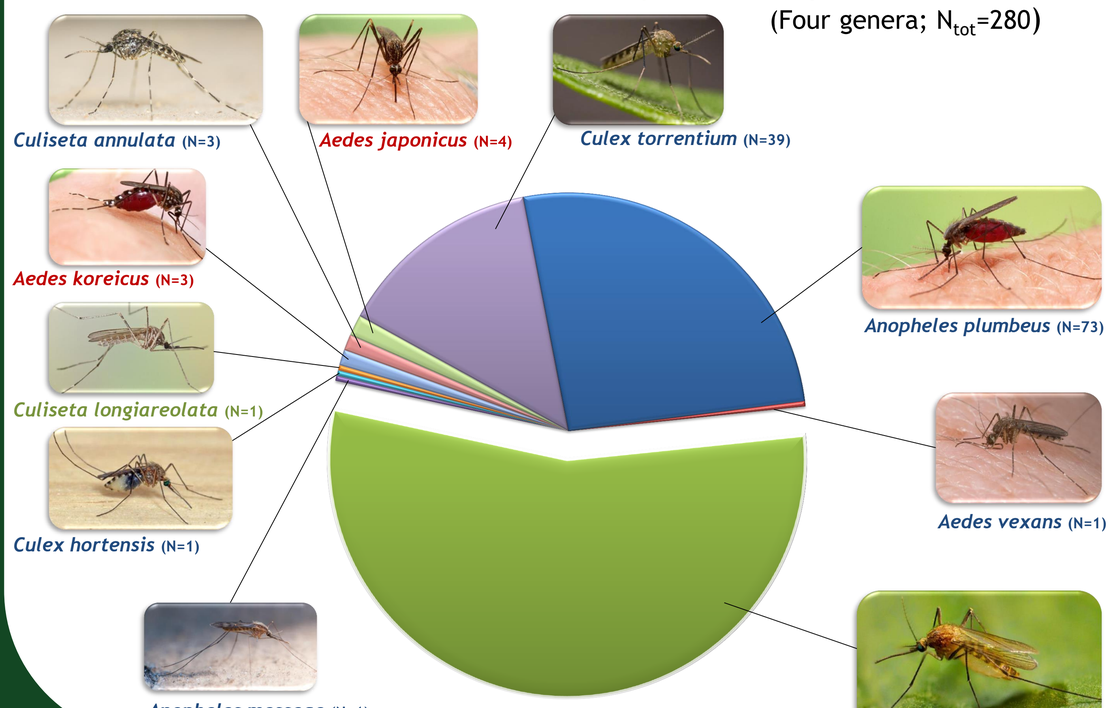
Adults

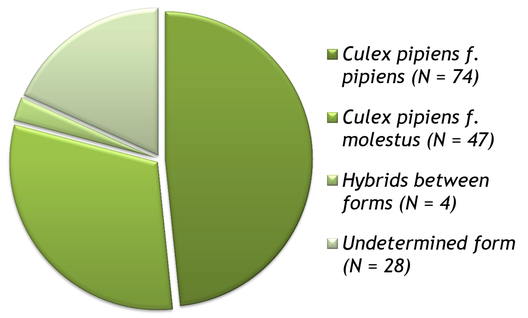
Larvae



2017 annual species validation

5% of the collected individuals validated:
Seven **native**, two **exotic** and one **newly** recorded species validated
(Four genera; N_{tot}=280)

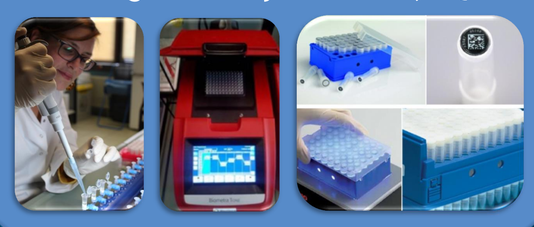




Species identification
Morphology-based

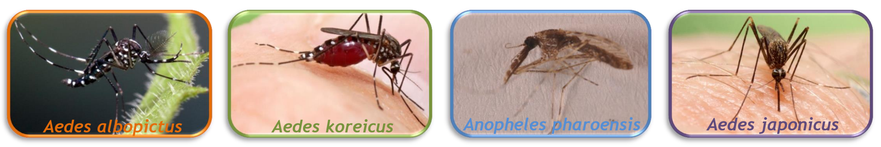
Sp identification validation
DNA-based

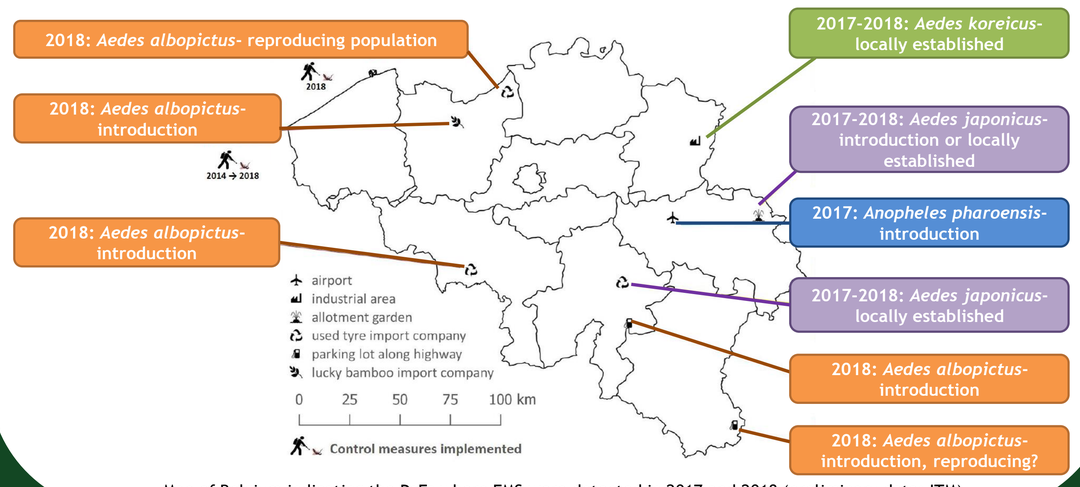
PCR and sequencing of COI, ND4, ITS2
Size fragment analyses of ACE2, CQ11



Sp validation of intercepted exotic mosquitoes

Four EMS collected once or multiple times at one or multiple PoEs





Map of Belgium indicating the PoEs where EMS were detected in 2017 and 2018 (preliminary data; ITM)

CONCLUSION

➔**Annual validation:** The DNA-based identifications up to species complex level were in agreement with the morphology, validating the morphology-based species identifications. Yet, DNA-based methods allowed to discriminate between species of the same complex.

➔**EMS validation:** EMS collected at the nine distinct PoEs were validated using DNA-based techniques. EMS eggs were most often morphologically miss-identified with *Aedes geniculatus* eggs (native). EMS seem to enter effectively via different introduction pathways: through lucky bamboo, tyre transport and ground traffic, but possibly also by natural dispersal. Early interceptions and rapid DNA-based verifications should help the authorities in their efforts to contain the spread and eradicate EMS populations.