

A DNA-based pipeline for species-level identification of Belgian mosquitoes



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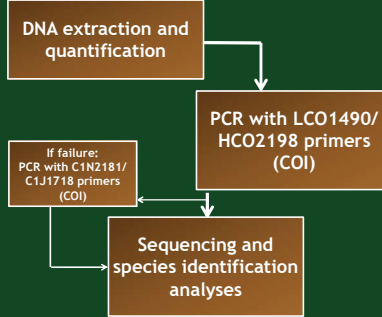
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Early detection of exotic mosquito species (EMS) is of vital importance to prevent local transmissions of mosquito-borne diseases. From 2017 to 2020, the Belgian federal government and regional authorities funded the MEMO project (Monitoring of Exotic Mosquitoes in Belgium), which aimed at detecting and evaluating the occurrence of EMS in Belgium. During three years, 23 locations where EMS are expected to enter were monitored (collection of adults, larvae and eggs).



52,478 mosquito specimens, representing 32 species (or species complexes) and five genera were collected. To implement management strategies, especially regarding potential disease vectors, accurate and reliable species-level identification of all mosquito life stages is essential. Therefore, a pipeline for DNA based identification was developed in the context of this framework. Morphological species identifications of a subset of samples, including all EMS, were validated using DNA-based identifications.

N= number of specimens validated, displayed in pie chart below

IF species identified as:

- 1 - *Aedes communis* N=2
- 2 - *Aedes detritus* N=1
- *Aedes flavescens*
- 3 - *Aedes geniculatus* N=192
- 4 - *Aedes punctator* N=9
- 5 - *Aedes rusticus* N=6
- 6 - *Aedes sticticus* N=9
- 7 - *Aedes vexans* N=38
- 8 - *Anopheles claviger* N=13
- 9 - *Anopheles plumbeus* N=500
- 10 - *Culex hortensis* N=10
- 11 - *Culex modestus* N=1
- 12 - *Culex territans* N=3
- 13 - *Culiseta annulata* N=51
- 14 - *Culiseta fumpennisi* N=2
- 15 - *Culiseta morsitans* N=19
- *Culiseta ochroptera*
- 16 - *Coquillettia richiardii* N=16

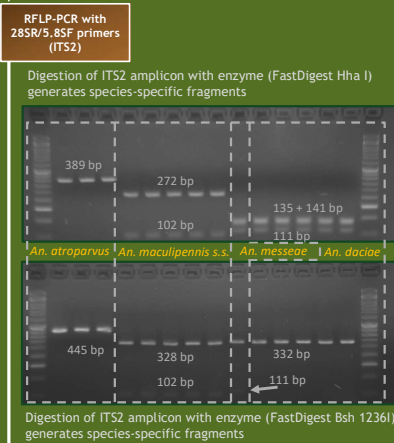
EMS

- *Aedes aegypti*
- 17 - *Aedes albopictus* N=96
- *Aedes atropalpus*
- *Aedes triseriatus*
- 18 - *Aedes japonicus* N=301
- 19 - *Aedes koreicus* N=144
- 20 - *Anopheles pharoensis* N=1
- 21 - *Culiseta longiareolata* N=7

Reliable ID: STOP

IF species identified as:

Anopheles maculipennis species complex



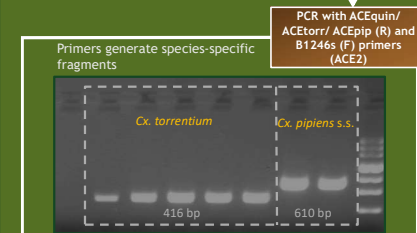
IF species identified as:

- 22 - *Anopheles atroparvus* N=6
- 23 - *Anopheles maculipennis* s.s. N=52
- 24 - *Anopheles daciae* N=29
- 25 - *Anopheles messeae* N=15

Reliable ID: STOP

IF species identified as:

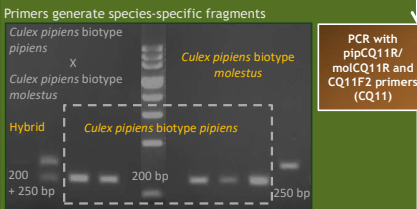
Culex pipiens s.l.* N = 37 OR *Culex torrentium*



IF species identified as:

- *Culex quinquefasciatus*
- 26 - *Culex torrentium* N=403
- Culex pipiens* s.s. * N=37

Reliable ID: STOP



IF biotypes identified as:

- 27 - *Cx. p.* biotype molestus N=228
- 28 - *Cx. p.* biotype pipiens N=953
- 29 - Hybrid N=30

Reliable ID: STOP

IF species identified as:

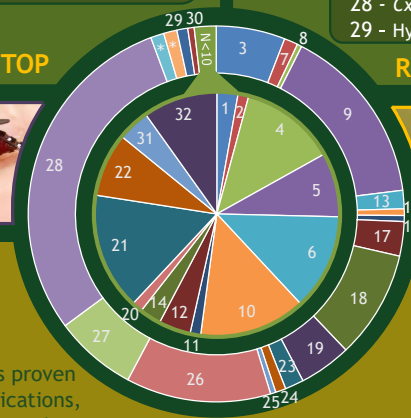
- 30 - *Aedes annulipes* N=18
- *Aedes cantans*
- 31 - *Aedes caspius* N=3
- *Aedes dorsalis*
- 32 - *Aedes cinereus* N=7
- *Aedes geminus*
- *Culiseta subochrea*

Further tests needed

In case of *Aedes caspius*/*Ae. dorsalis* and *Ae. cantans*/*Ae. annulipes* species complexes, further study to develop species specific identification techniques is required.

A third species complex, *Ae. cinereus*/*Ae. geminus* cannot yet be resolved due to the lack of available sequences for *Ae. geminus* in online reference databases.

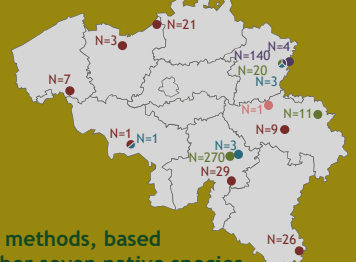
In case of *Culiseta subochrea*, not enough sequence data is available ($N_{seq} = 1$) in online reference databases.



About 3,300 specimens were barcoded using multiple markers to obtain an identification at species level (COI, ITS2, ACE2, CQ11).

The designed DNA-based identification pipeline has proven its usefulness to validate the morphological identifications, especially in case of damaged specimens or immature stages.

24 native Belgian mosquito species and the eight investigated EMS can be reliably identified using DNA-based methods, based upon data analyses of online repositories and subsequent testing. Further test are required to identify the other seven native species to species level. Early interceptions of EMS and a rapid DNA-based identification are thus feasible and will help the authorities in their decision process. Ultimately, all generated sequences will be made available on online sequence databases (GenBank).



INTRODUCTION

MATERIAL/METHODS AND RESULTS

CONCLUSION