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VERIFICATION OF MOLECULAR IDENTIFICATION METHODS – THE CASE OF *ANOPLOPHORA GLABRIPENNIS*

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ANOPLOPHORA GLABRIPENNIS

Coleoptera, Cerambycidae
- native to **eastern Asia**,
- **EU: Quarantine pest** (Annex II B; Priority pest for EU, invasive)

RELIABLE IDENTIFICATION

A key tool for preventing the import and spread of new organisms harmful to plants

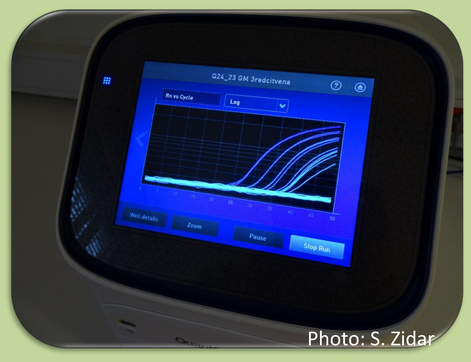
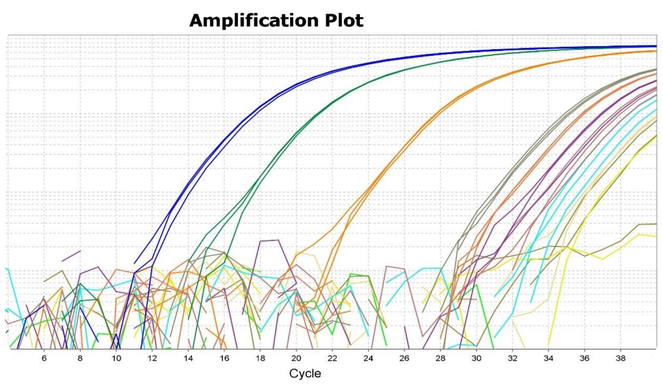


Photo: S. Zidar

A. GLABRIPENNIS ID AT THE SLOVENIAN FORESTRY INSTITUTE



Before implementation of molecular methods - **morphological identification**

✓molecular identification (**specific qPCR** according to EPPO standard PM7/149 (1) - Taddei et al., 2021)

+ internal isolation control - 18S universal qPCR (Ioos et al., 2009

CHALLENGE

<u>trouble accessing reference material</u>

- *A. glabripennis* DNA extract from EURL
- *A. glabripennis* larvae from other sources

↓

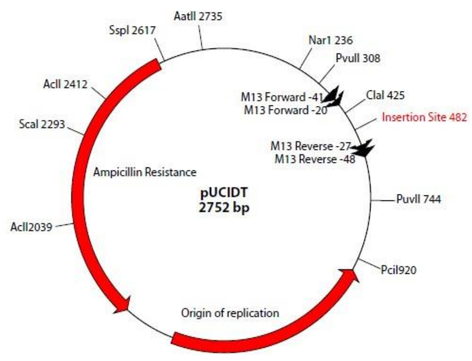
stage not suitable for morphological ID

SOLUTION

confirmation of ID via barcoding
+
confirmation by EURL
(barcoding and specific qPCR)

CHALLENGE

<u>Trouble accessing enough reference material for continuous use as positive amplification control</u>



SOLUTION

target sequence inserted into a commercial plasmid

plasmid used as both positive amplification control and limit of detection (LOD) control

CHALLENGE

<u>Adult specimens not available for DNA extraction</u>

total DNA extraction
↓
tested on specimens of ***Monochamus galloprovincialis***

qPCR
↓
tested with *M. galloprovincialis* adult DNA extract **spiked** with *A. glabripennis* larva DNA extract

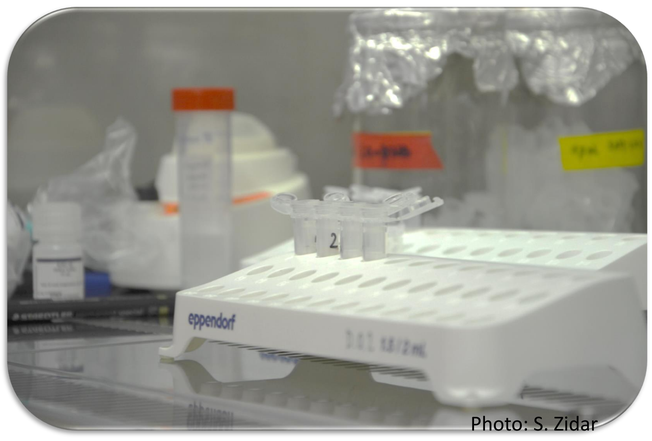


Photo: S. Zidar

RESULTS

✓ Analitical sensitivity (Limit of detection):
- Larva - Around **10 mg**
- Plasmid HM062991.1: **220 copies per reaction**

✓ Analitical specificity:
- Exclusivity **100%**
- Inclusivity **100%**

✓ Repeatability **100%**

✓ Reproducibility**100%**

CRITERIA – EPPO PM7/76 (5)

Specificity supported by *in silico* analysis

FOR THE FUTURE

It is crucial:
- **To further improve availability of entomological reference material**
- **To make sure the methods of identification used, especially for quarantine and priority pests, are robust and reliable.**

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