

Identification
of exotic *Agrilus*
in Europe:



The role of the European Union Reference Laboratory

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Reminder: what is EURL?



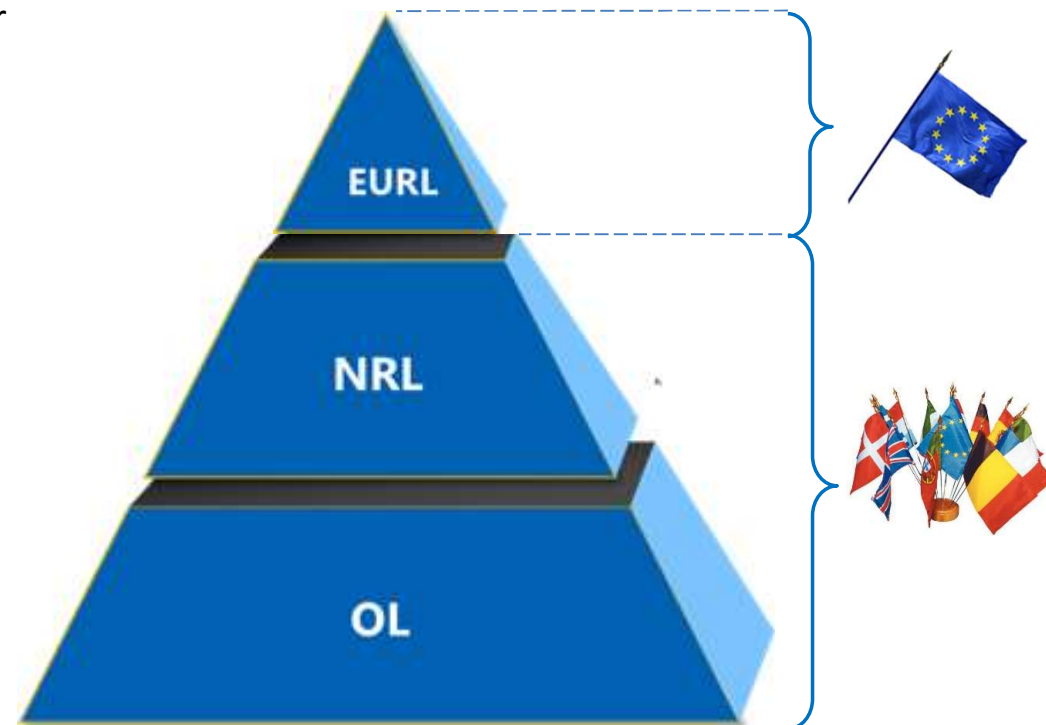
EURL and NRLs (+OLs)

EURL: European Union Reference Laboratory

- (EU) 2017/625: European Commission designates one EURL for each area of plant health (entomology, nematology...)
- Main role: technical assistance and proficiency testing of National Reference Laboratories (NRLs)
- Supervised by EU Commission

NRL: National Reference Laboratory

- (EU) 2017/625: each MS must designate one (or more) NRL(s)
- Main role: enforce the regulation on plant health
- Supervised by National Plant Protection Organization (NPPO)
- A NRL may in turn delegate part of its duty to one (or more) Official Laboratories (OLs)



The NRL network

EU Commission provided five EURL mandates for plant health

- Nematods, bacteria, fungi & oomycetes, viruses viroids & phytoplasmas... and insects & mites
- Insects & mites: mandate provided in 2019 to a consortium: ANSES (France) and AGES (Austria)
- ANSES and AGES are also NRLs for their respective country

The NRL network

- 28 laboratories involved:
- Sweden delegates to Denmark
- Ireland mandate extend to Northern Ireland
- Belgium & Slovenia designated two NRLs





EURL technical assistance to NRLs

1. Providing reliable identification methods

General information towards NRLS

- EURL newsletter (two issues/year)
- Website with restricted part for NRL
- **Annual workshop: 2024 devoted to *A. planipennis***



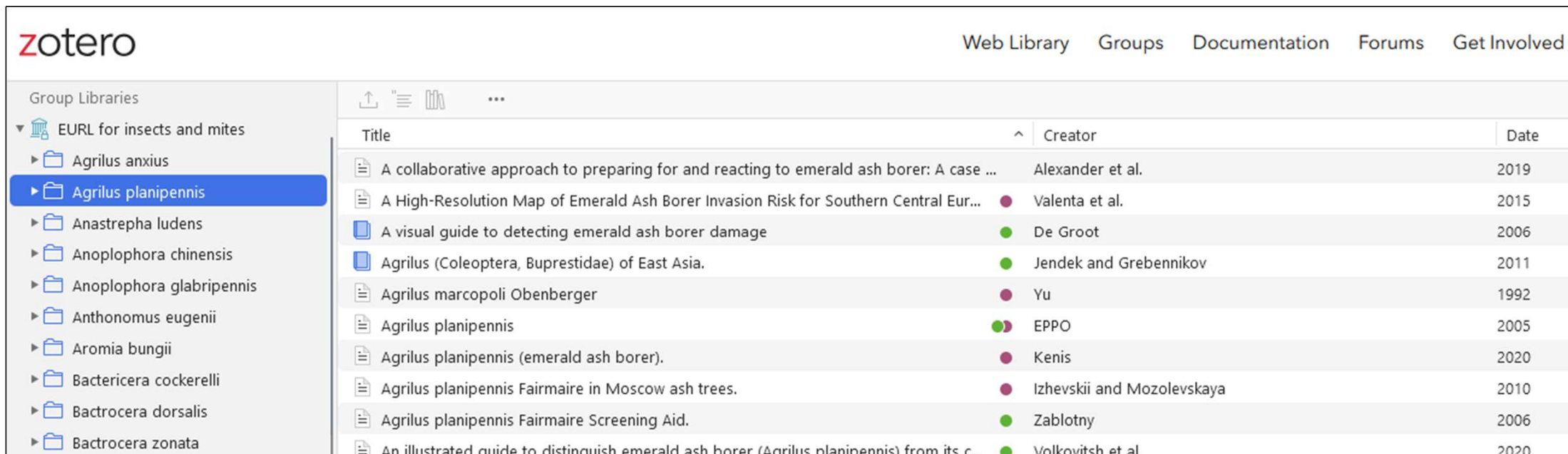
2024: the NRL network welcomed in AGES facilities

The image shows a screenshot of the EURL Workshop 2024 website. At the top, there are logos for the European Union Reference Laboratory for Insects and Mites (EURL), ANSES, and AGES. The website has a navigation menu with links for HOME, PRESENTATION, EURL ACTIVITIES, DOCUMENTS, NRLS WORKPLACE, and CONTACT US. The main heading is 'EURL WORKSHOP 2024' with the subtext 'Vienna is calling back!'. Below this, it states 'From 25 June 2024 to 25 June 2024' and 'EVENT CONCLUDED'. A paragraph of text describes the workshop, mentioning a keynote speech by Aurelien Sallé on the genus Agrilus (Coleoptera, Buprestidae) and the main topic being Agrilus planipennis. It also mentions the Eppo network and a validation study presented by the EURL. At the bottom of the screenshot is a group photo of approximately 30 people posing in front of a modern building with a glass facade and the AGES logo.

1. Providing reliable identification methods

Extensive Systemic Literature Search

- Periodic interrogation of OVID® Scopus® and CAB Direct® databases to retrieve publications on diagnostics, distribution and outbreaks report
- Coupled with EFSA Horizon scanning and EPPO Reporting Service outputs
- Bibliography available for NRLs on EURL website (Zotero database)
- ***Agrilus* spp: currently 88 references available**



The screenshot shows the Zotero web interface. On the left, a sidebar lists 'Group Libraries' with 'EURL for insects and mites' expanded to show 'Agrilus planipennis' selected. The main area displays a table of references with columns for Title, Creator, and Date.

Title	Creator	Date
A collaborative approach to preparing for and reacting to emerald ash borer: A case ...	Alexander et al.	2019
A High-Resolution Map of Emerald Ash Borer Invasion Risk for Southern Central Eur...	Valenta et al.	2015
A visual guide to detecting emerald ash borer damage	De Groot	2006
Agrilus (Coleoptera, Buprestidae) of East Asia.	Jendek and Grebennikov	2011
Agrilus marcopoli Obenberger	Yu	1992
Agrilus planipennis	EPPO	2005
Agrilus planipennis (emerald ash borer).	Kenis	2020
Agrilus planipennis Fairmaire in Moscow ash trees.	Izhevskii and Mozolevskaya	2010
Agrilus planipennis Fairmaire Screening Aid.	Zablotny	2006
An illustrated guide to distinguish emerald ash borer (<i>Agrilus planipennis</i>) from its c...	Volkovitsh et al.	2020

1. Providing reliable identification methods

Validation Study (VS) on existing methods

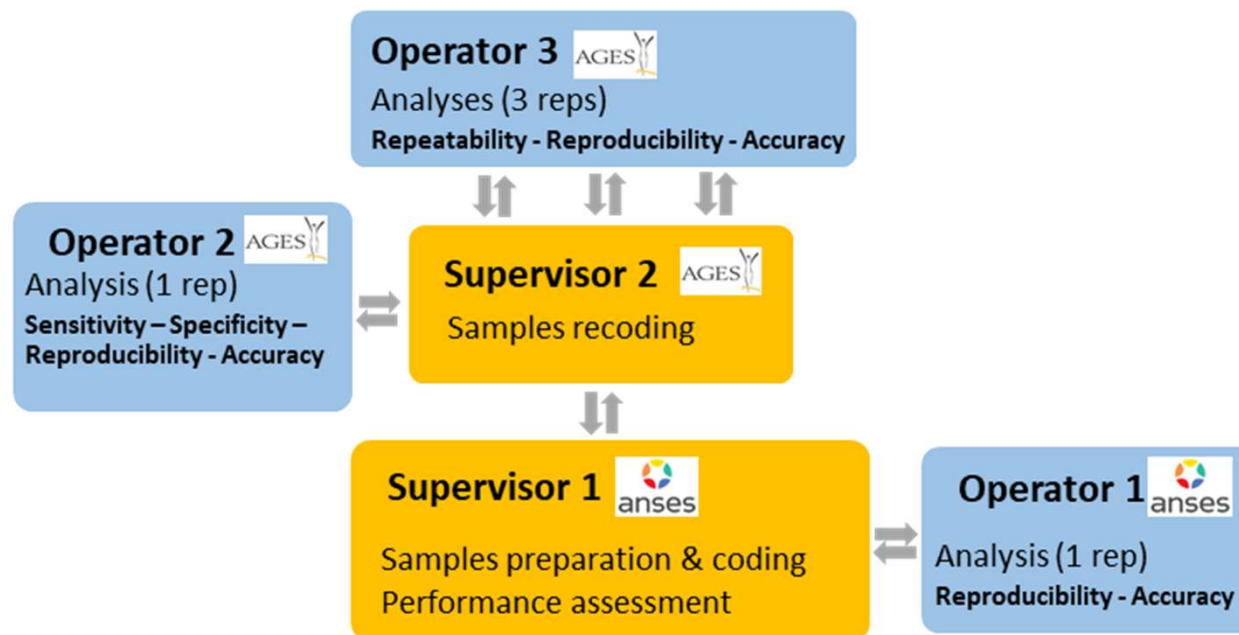
- Performance criteria (i.g. analytical and diagnostic sensitivity/specificity) are assessed for tests included in international diagnostic protocols (IPPC, EPPO) or published tests
- Objective: to test method (not lab proficiency)
- Coded samples sent by Supervisors to at least three Operators
- Operators identify them using method under validation
- Based on the obtained performance characteristics, suitable tests are recommended to EU-NRLs
- Interdisciplinary exchange by performing joint validation of morphological and molecular identification protocols



1. Providing reliable identification methods

EPPO PM 7/154(1) (*Agrilus planipennis*)

- Double VS (morphological / molecular) in 2024
- Molecular: barcoding and LAMP both met 100% performance criteria -> recommended to NRLs
- Morphological: also 100% for criteria, but numerous comments -> update recommended to EPPO



Outline of the VS procedure for EPPO PM7/154(1) (morphology)

2. Developing new methods

Contribution to EPPO PM 7/154(2) *Agrilus planipennis*

- Update of EPPO PM 7/154(1) drafting coordinated by Maarten de Groot (Slovenian Forestry Institute)
- Suggestions following VS comments:
 - Point out reddish tergites as valuable diagnostic character
 - clarify and better illustrate several characters used in diagnostic table



Left to right: *Agrilus planipennis*, *A. biguttatus*, *A. viridis*

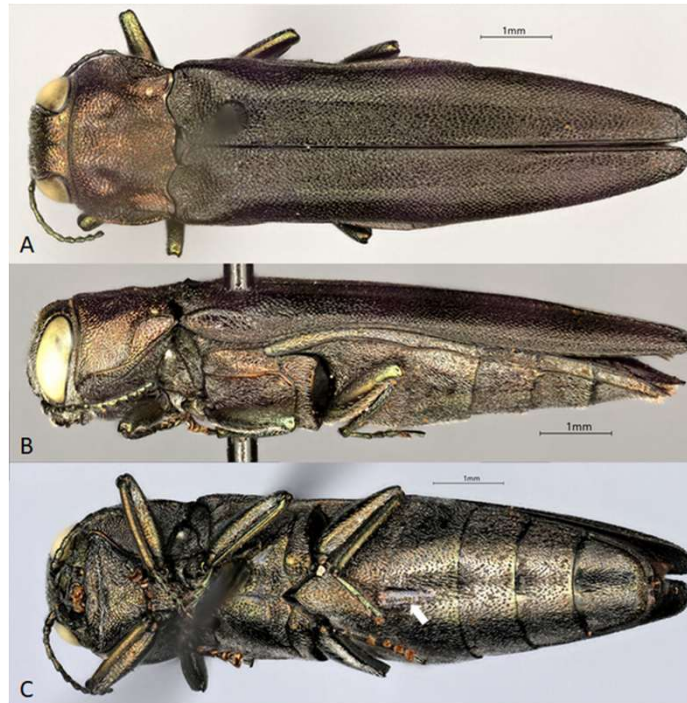
2. Developing new methods

Contribution to EPPO PM 7/XXX *Agrilus anxius*

- Incoming EPPO method, drafting also coordinated by Maarten de Groot
- EURL part of the drafting team
- Publication late 2026 or 2027?



Agrilus anxius, larva



Agrilus anxius, adult

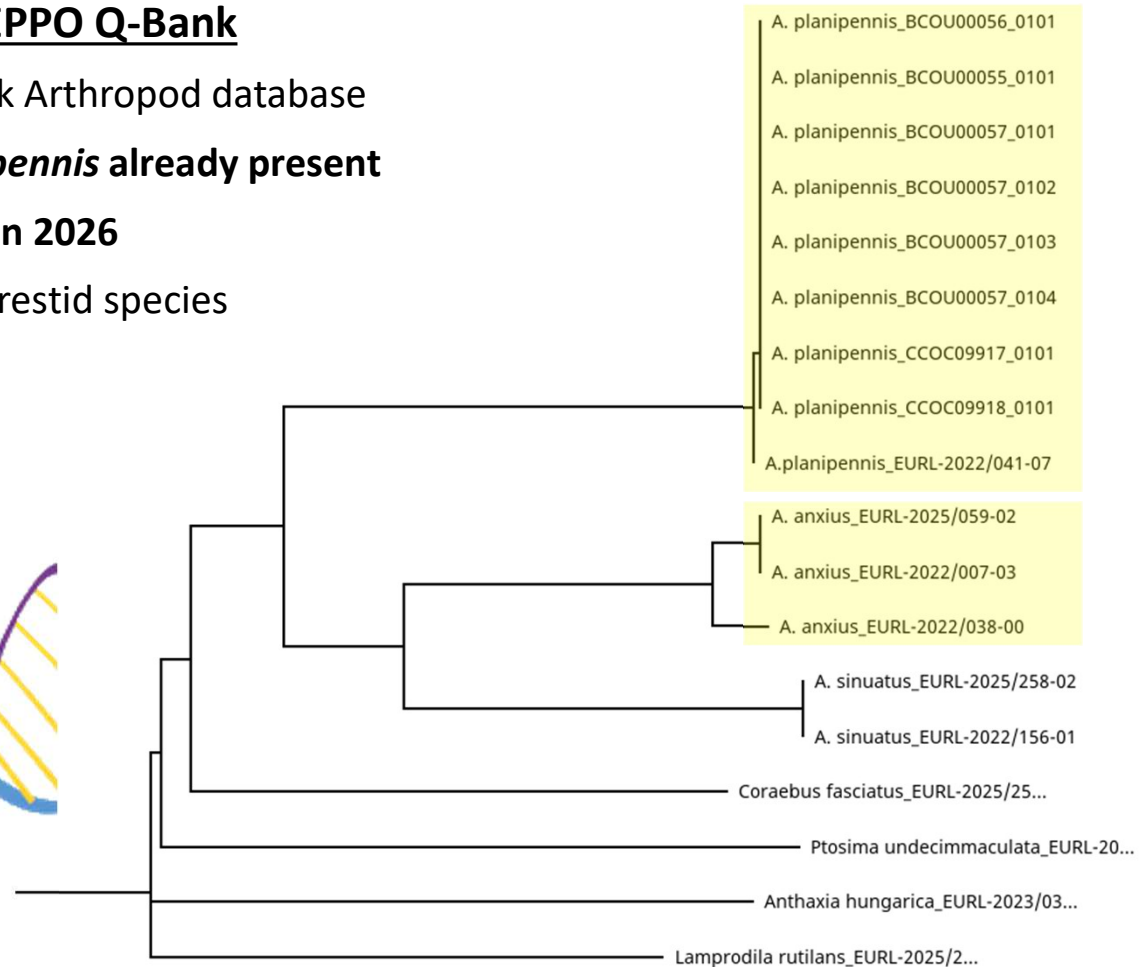


Agrilus anxius, aedeagus

2. Developing new methods

Provision of reference COI sequences to EPPO Q-Bank

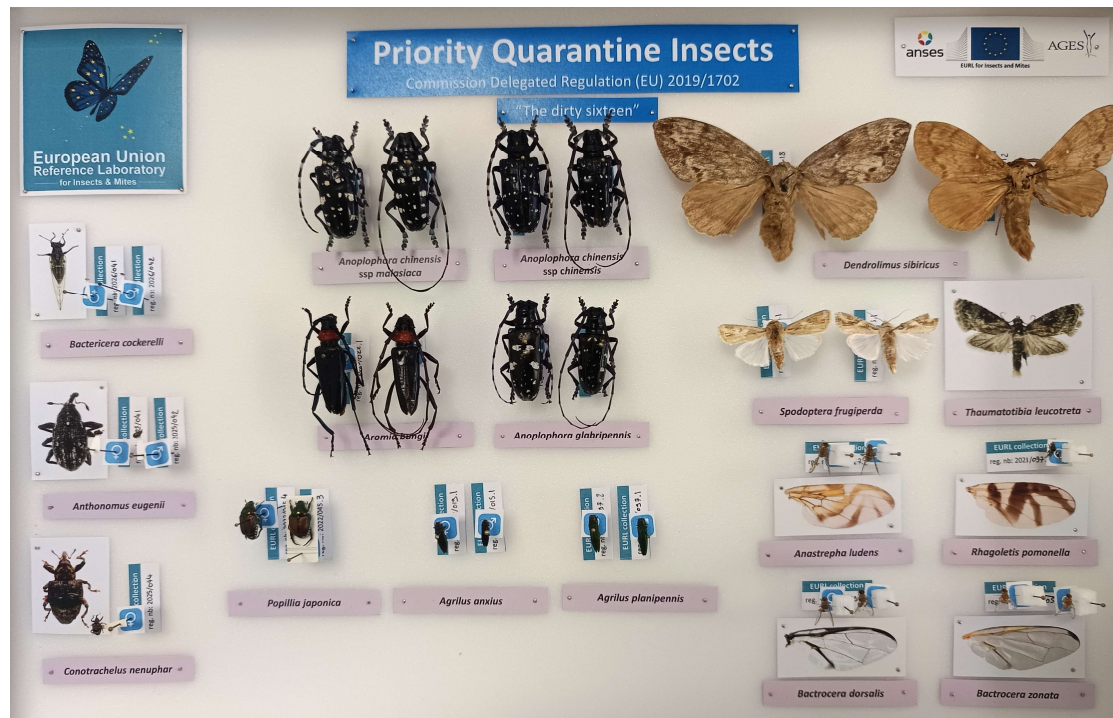
- EURL contributes to curation of EPPO-Q-bank Arthropod database
- **8 COI public COI sequences of *Agrilus planipennis* already present**
- **3 *Agrilus anxius* sequences to be uploaded in 2026**
- Along with COI of morphologically close buprestid species



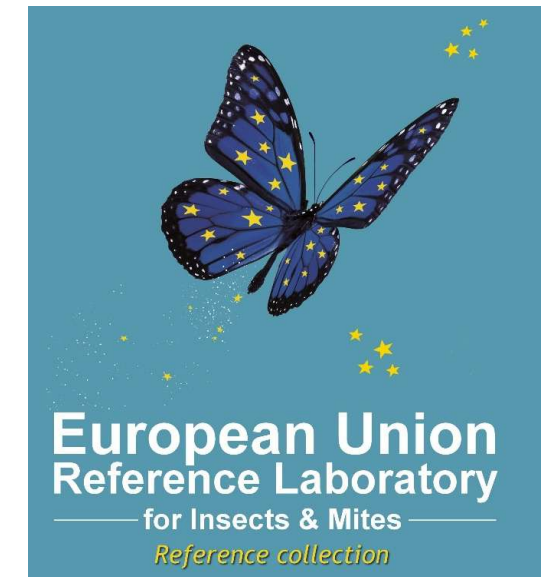
3. Providing reference material

EURL reference collection

- Specify™ database for a shared AGES/ANSES management
- ***Agrius*: 587 specimens, 42 COI sequences, 28 species**
- 48 specimens lent to 11 NRLs



Specify 7



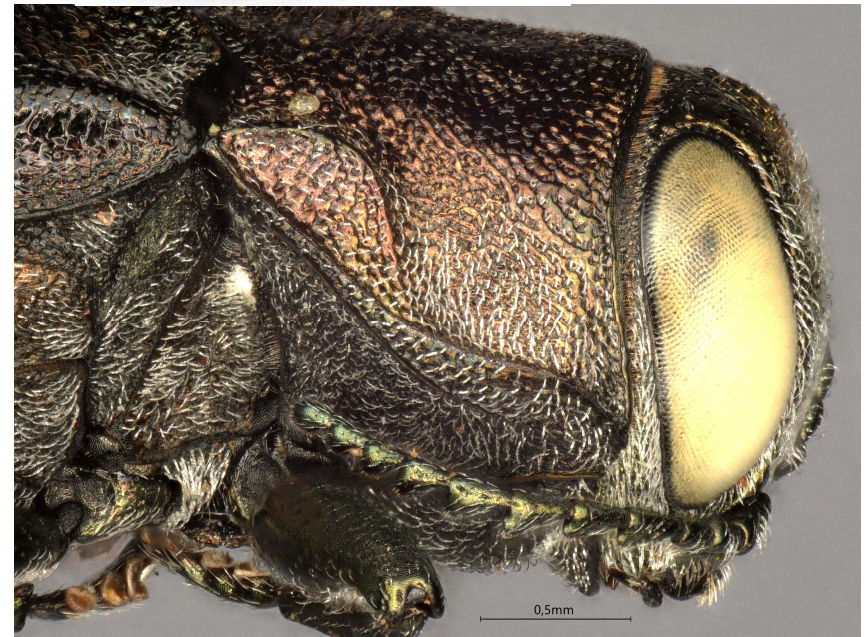
3. Providing reference material

High resolution pictures database

- HD pictures taken with Keyence™ system on reference specimens
- Available on EURL website



Agrilus anxius, larva, detail of terminal process (dorsal)



Agrilus anxius, adult, detail of pronotum (profile)

4. Testing the proficiency of NRL

Proficiency Tests (PTs) ISO 17043

- (EU) 2017/625: EURL must assess NRLs' proficiency to identify quarantine organisms (QO)
- PTs organized according to ISO 17043 standard
- Coded samples sent by EURL to NRLs
- NRLs identify them (positive/negative) using any method
- EPPO/IPPC method recommended if available (and preferably validated)
- Participation of EU NRLs mandatory, third countries may participate
- In case of non-conformity, NRL must proceed to cause-analysis and propose corrective actions
- EURL may propose trainings as corrective actions

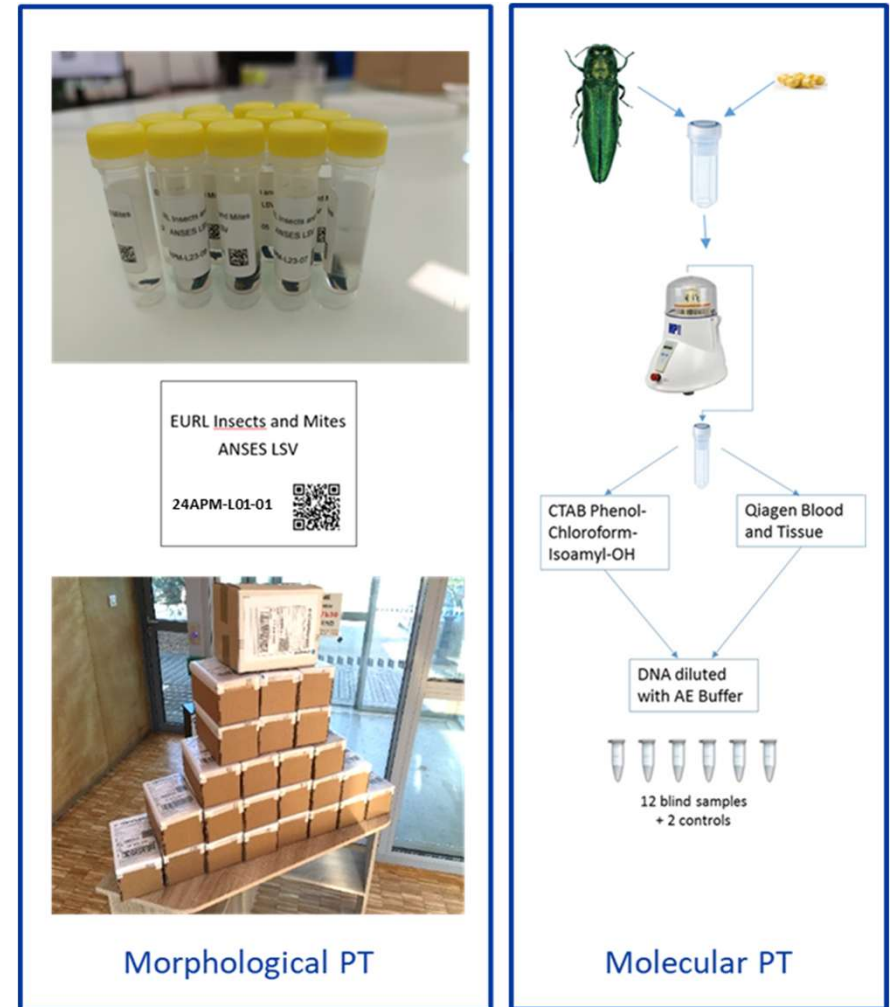


Outline of the PT 24APM (morphological ID of *A. planipennis*)

4. Testing the proficiency of NRL

Two PTs organized in 2024 for *Agrilus planipennis*

- Morphological identification of adult (ANSES)
- Molecular identification (AGES)
- Recommended method: EPPO PM 7/154(1)
- Additional instructions provided from preceding VS comments
- Results classified...



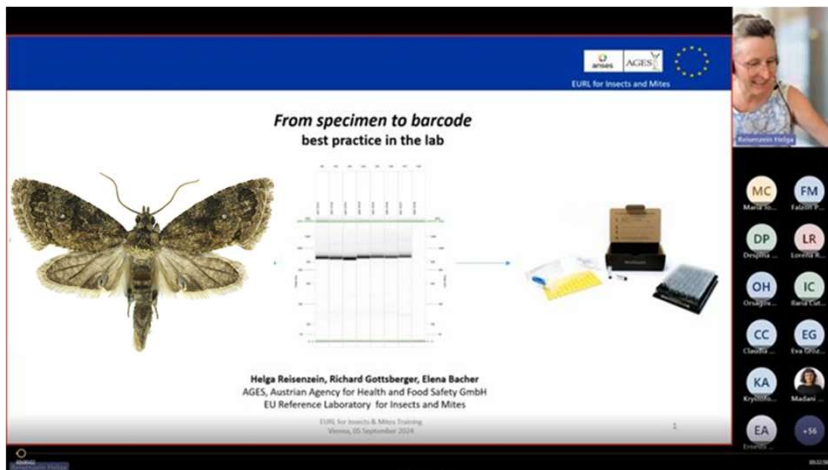
5. Training sessions

Three formats proposed by EURL

- Hands-on trainings
- Webinars
- Three levels (webinar / “homework” / online debriefing)
- In order to prepare PTs... or as follow-up actions
- **No general training conducted so far on *Agrius* spp.**



2024: hands-on training for the morphological ID of tephritid larvae



2022: 3L training for the molecular ID of *T. leucotreta*



2025: interactive webinar for the morphological ID of *Bactrocera* spp.

6. Confirmatory analyses

In case of first detection of QO in EU country

- EURL may assist NRLs to confirm outbreak diagnostic
- Both morphological and molecular confirmation provided
- Several examples so far (*S. frugiperda* in several Mediterranean countries, *Pseudips mexicanus* in Ireland, *Dacus ciliatus* in Cyprus...)
- **No request for *Agrilus* spp. so far...**
- ... but for how much longer?



The screenshot shows the EPPO Global Database interface. At the top, there is a search bar with the text "Search by name or EPPO Code..." and a "Go!" button. Below the search bar, there are navigation links: "Home", "Standards", "Photos", "Reporting Service", "Explore by", and "Download user guide". The main content area features a report titled "First report of *Dacus ciliatus* in Cyprus". The report text states: "The NPPO of Cyprus recently informed the EPPO Secretariat of the first finding of *Dacus ciliatus* (Diptera: Tephritidae – EPPO A2 List) on its territory. This is the first record for the European Union, but in the EPPO region, the species is already present in Israel and Türkiye. In June 2024, suspicious symptoms were observed by a private agronomist on cucumbers (*Cucumis sativus*). Samples were immediately collected by the NPPO and sent to the EURL laboratory (AGES for molecular analysis). The molecular analysis of larvae samples identified the insect as *Dacus ciliatus*. Surveys were conducted on the island and the pest was detected in several sites in Larnaca district and in one field in Ammochostos district (south coastal area of Cyprus). The crops which were found to be infested were cucumber (*Cucumis sativus*), zucchini (*Cucurbita pepo*), watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*). The pest was also detected in the weed *Echallium elaterium*. The total infested area is 6.11 ha. Surveys are continuing throughout the island and a poster has been prepared to increase awareness amongst producers. The pest status of *Dacus ciliatus* in Cyprus is officially declared as: **Present, under eradication.**" Below the text, there is a "Sources" section listing "NPPO of Cyprus (2024-07)".



