EURL for Insects and Mites

Between Bark and Barcode – Integrating Morphology and Molecular Tools in Forest Pest Diagnostics

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Why Integrated Diagnostics?

Need for high-quality DNA and contamination-free procedures

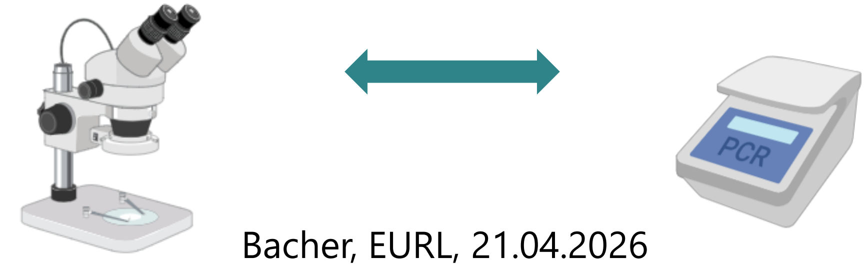
Importance of accurate species identification

Increasing diagnostic demands in regulated pests

Conflicting requirements:

- Morphology requires intact specimens
- Molecular tools require high-quality, contamination-free DNA

→ Further the complementarity of morphological and molecular methods



Sample Flow

Sampling

Morphological Analyses

Contamination !

Storage

Molecular Analyses

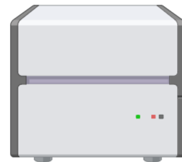
Sample damage !

Storage

Molecular Methods

Background information

Specific methods:



- Primers/probes that target **unique** DNA sequences
- Identification of specimens of **one particular taxon** (yes/no answer)
- Highly sensitive

Non-specific methods:

- Use primers that target **universally shared**, conserved genetic sequences with sufficient variability in-between binding sites (e.g. Barcoding)
- Identification of specimens with unknown/uncertain ID

Overview

Contents of the presentation

- Ethanol contamination (spike-and-recovery)
- Long-term storage tests
- Recommendations
- Ongoing → boiling test + NGS opportunities

Funded by the European Union

Ethanol Contamination

Why it matters

In routine diagnosis, the goal is usually **identification of whole insect specimens**, whereas detection of trace amounts is not the objective

Highly sensitive methods → risk of false positives, when dealing with contaminations

Shared storage media = risk for trace DNA transfer

How much of an impact can storage alcohol really have?

Ethanol Contamination

Spiking *Cydia pomonella* larvae with storage ethanol of *Thaumatotibia leucotreta*

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3x 1µl
3x 10µl
3x 100µl

Step	Treatment	Time
1	+contaminant	15min
2	+70% EtOH	RT 4 days
3	+dH2O	5min
4	+lysis buffer	56°C over night

→ Non-destructive DNA extraction → Analysis

1µl of liquid shown next to *Agrilus biguttatus* for scale

Bacher, EURL, 21.04.2026

Ethanol Contamination

Analysis & Results

Analysis:

- *T. leucotreta*-specific real-time PCR
- DNA Barcoding

→ **In all larval samples *Thaumatotibia leucotreta* DNA could be detected**

- ➢ even if only 1µl of storage ethanol
- ➢ even if washed before DNA extraction

1µl of liquid shown next to *Agrilus biguttatus* for scale

DNA barcoding still made the identification possible **BUT** higher amounts of contamination led to low sequence quality

Ethanol Contamination

Take-Away

Findings: accumulation + transfer potential is **real and measurable**

The spike and recovery experiment clearly shows the risk of storage ethanol being a source of contamination

Especially when working with non-destructive DNA-extraction

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Long-Term Storage Experiment

Non-destructive DNA extraction after 2 years of storage of larvae

3 biological replicates

Cydia pomonella larvae were used

99% EtOH	70% EtOH	Propylene glycol	Room temperature
99% EtOH	70% EtOH	Dry	-20°C

Multiple time points over 2 years

Analysis

Funded by the European Union

Long-Term Storage Experiment

Results Molecular Methods

DNA amount → storage temperature had the strongest impact, 99% ethanol performed best (at RT and -20°C)

DNA purity → temperature plays a role (especially short term), 99% ethanol performed best (at RT and -20°C), freezing dry → worst impact

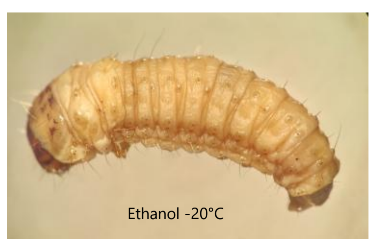
Real-time PCR → strongly correlates with DNA amount

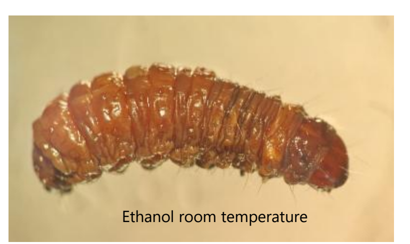
DNA barcoding → no significant differences

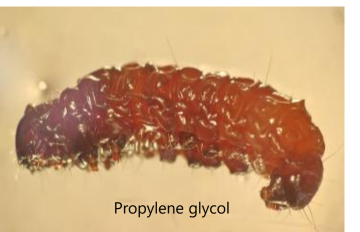
Long-Term Storage Experiment

Results Morphology

Cydia pomonella larvae

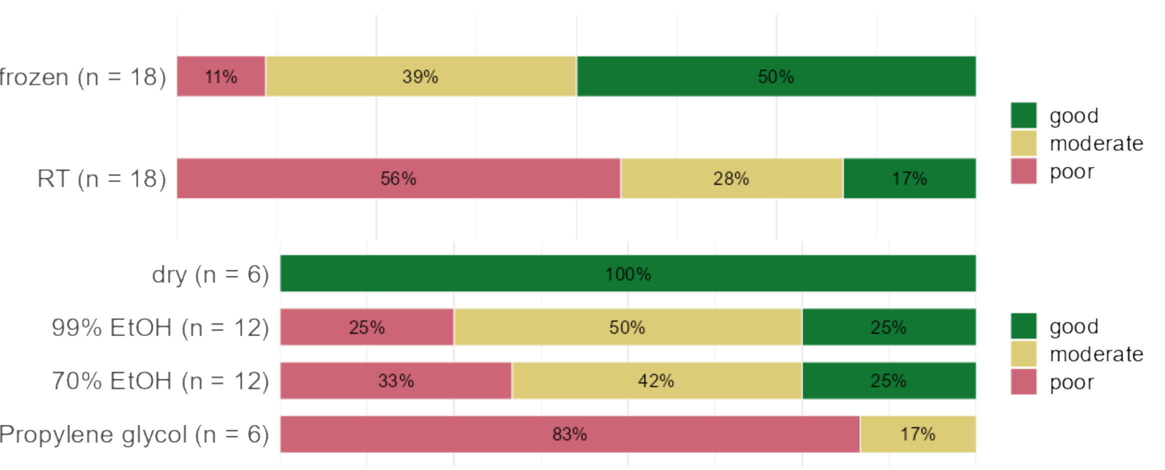






After 2 years of storage in the various media:

Issues → discoloration, drying out, loss of flexibility



Dry freezing leads to the best results

Storage at -20°C generally preserved color best

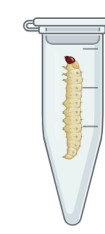
Proylene glycol yielded the least satisfying results (discoloration and desiccation)

Recommendations

Storage of larvae

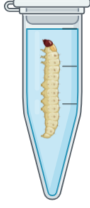
For **morphological** purposes:

Best preservation of larva with dry freezing



For **molecular** purposes:

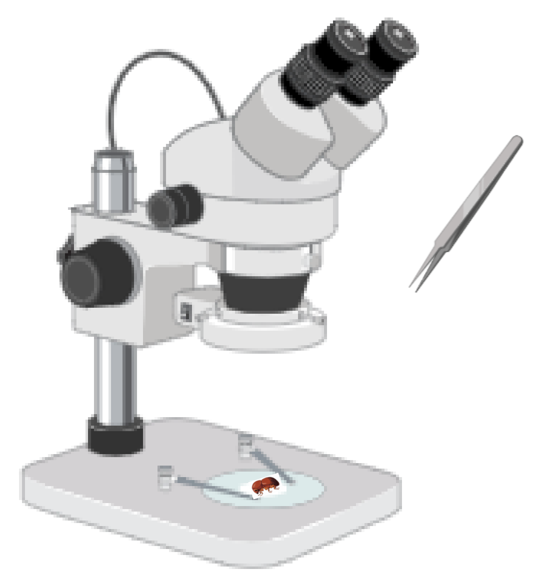
99% ethanol at RT (short term), 99% ethanol at -20°C (long-term)



Best overall: 99% ethanol at -20°C to allow analyses from both disciplines

Recommendations

Practical Recommendations



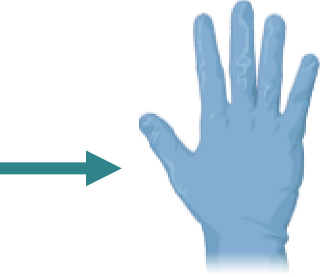
Sterile does not mean DNA-free!

Clean for molecular analysis means:

- At least 20min under UV-light after wiping with ethanol
- Cleaning with bleach or commercially available DNA-degrading solutions

True for everything that **comes into physical contact** with your sample, or has **contact through liquids**

If non-specific methods are used → they target universally shared, conserved genetic sequences → you too have these in your DNA ☻



Recommendations

Integrating Methods for Reliability

Handling in the **field** → naturally **not contamination free**!

As soon as possible → switching to a **workflow** that **ensures future useability for a variety of tests** both of morphological and molecular nature

This ensures:

- Optimal synergy of the two disciplines
- Most reliable results

Ongoing Studies & Outlook

Ongoing Experiments

- **Impact of larval boiling** on morphology and DNA
- **NGS metabarcoding** approaches: contamination detection

In Planning:

- Comparison of **dry-pinned** and **dry-frozen** specimens (flies and beetles)

Open for suggestions and ideas!!!

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