EURL for Insects and Mites

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Interplay of Morphological and Molecular Methods in Entomology

Pohn Chiara[1], Bacher Elena L. T.[1], Gottsberger Richard A.[1], Sun Hao[1], Wernicke Matthias[1], Kolkmann Kerstin[1], Reisenzein Helga[1], Reynaud Philippe[2]

1 AGES 2 ANSES

Background

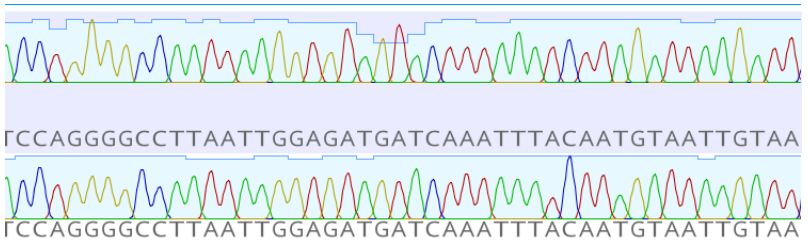
Who we are and what we do

EU Reference Laboratory for Insects and Mites

https://eurl-insects-mites.anses.fr/

Reference specimens:

- Morphologically analysed
- In pristine condition
- Available in collection
- High quality DNA and sequences
  - Non-destructive DNA extraction



How to achieve a workflow that suits both disciplines?

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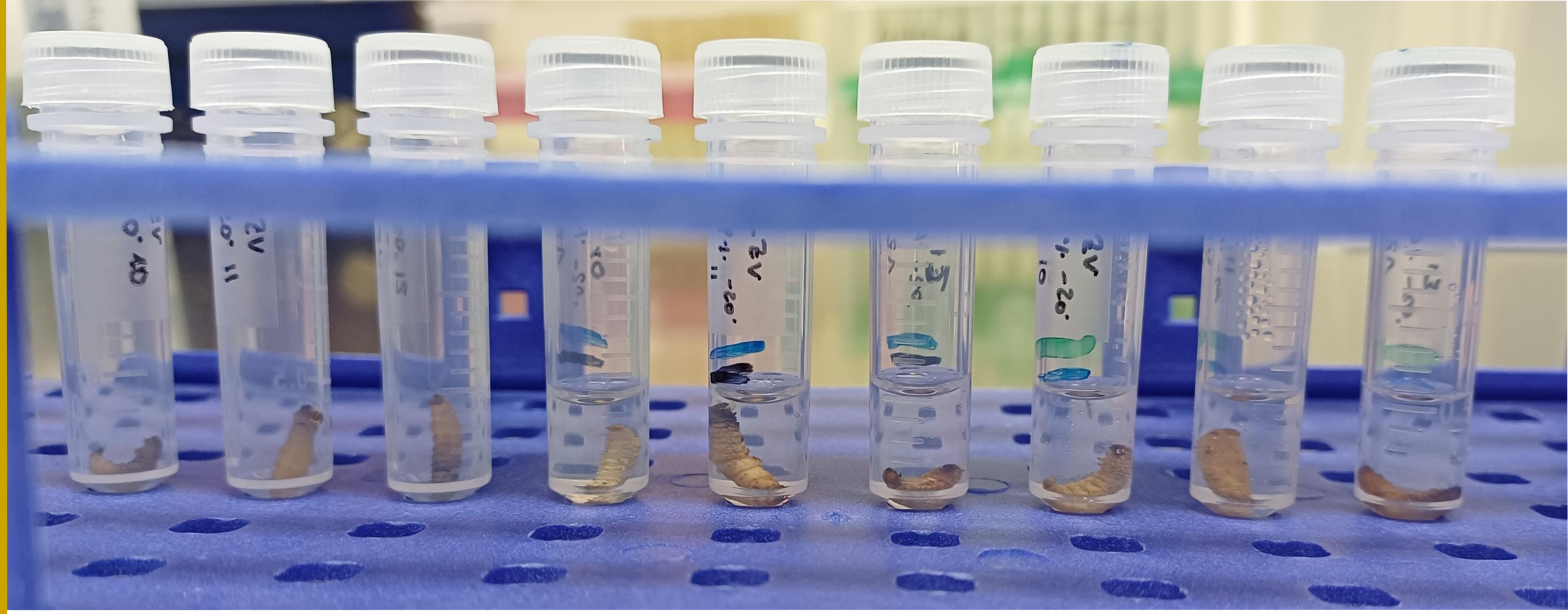


Long-term storage experiment

Non-destructive DNA extraction after 2 years of storage under different conditions

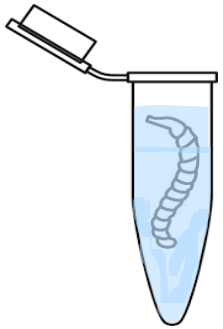
Long-term storage experiment

Workflow

3 larvae (biological replicates) per condition

Cydia pomonella

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



Non-destructive DNA extraction

Multiple time points over the course of 2 years

Evaluation

- DNA amount/purity
- Suitability for generic real-time PCR
- Suitability for DNA barcoding
- Morphology

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Long-term storage experiment

Results – DNA amount and purity

DNA amount

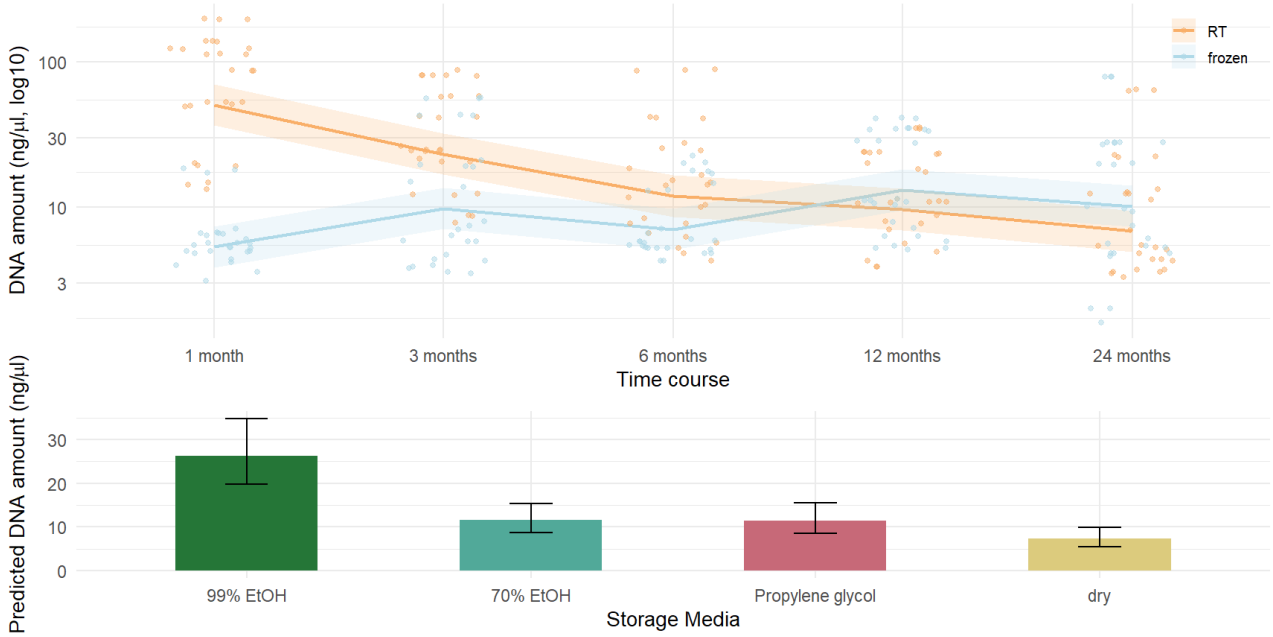
Storage temperature had a significant effect

Especially pronounced at early time points

Convergence and even inversion over time

99% Ethanol performed best at both temperatures

Freezing samples dry performed worst



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Long-term storage experiment

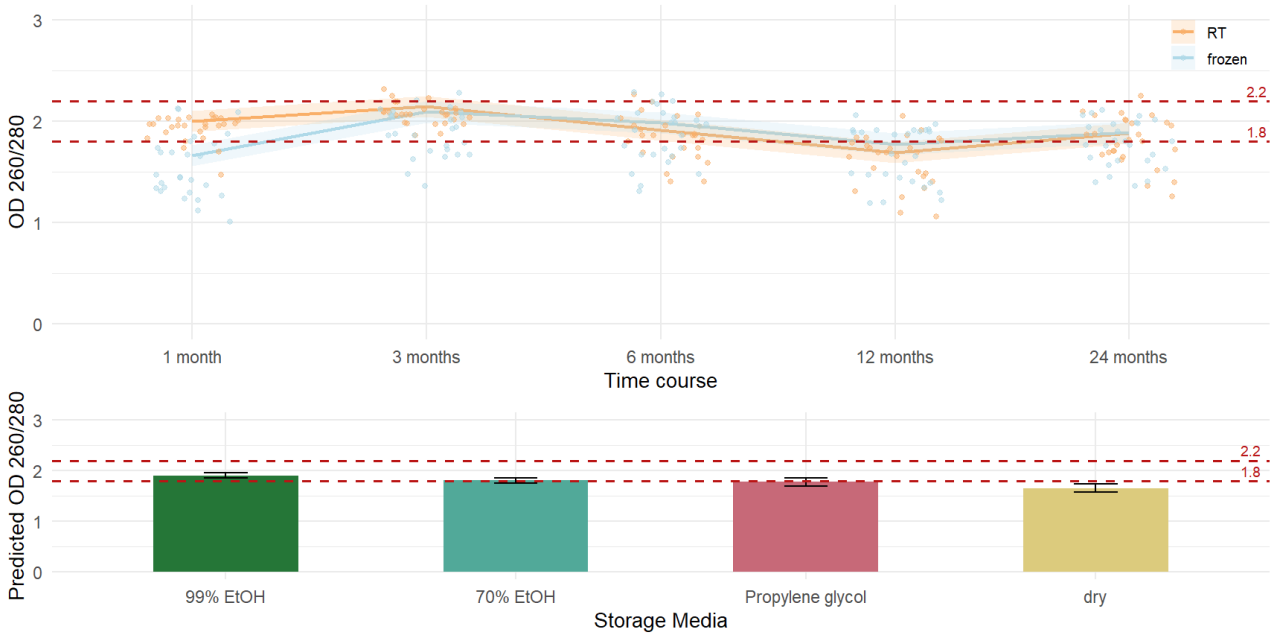
Results – DNA amount and purity

DNA purity

Significant difference in the beginning and convergence over time

99% Ethanol performed best at both temperatures

Freezing samples dry performed worst, with most samples below OD 1.8 (“not pure”)



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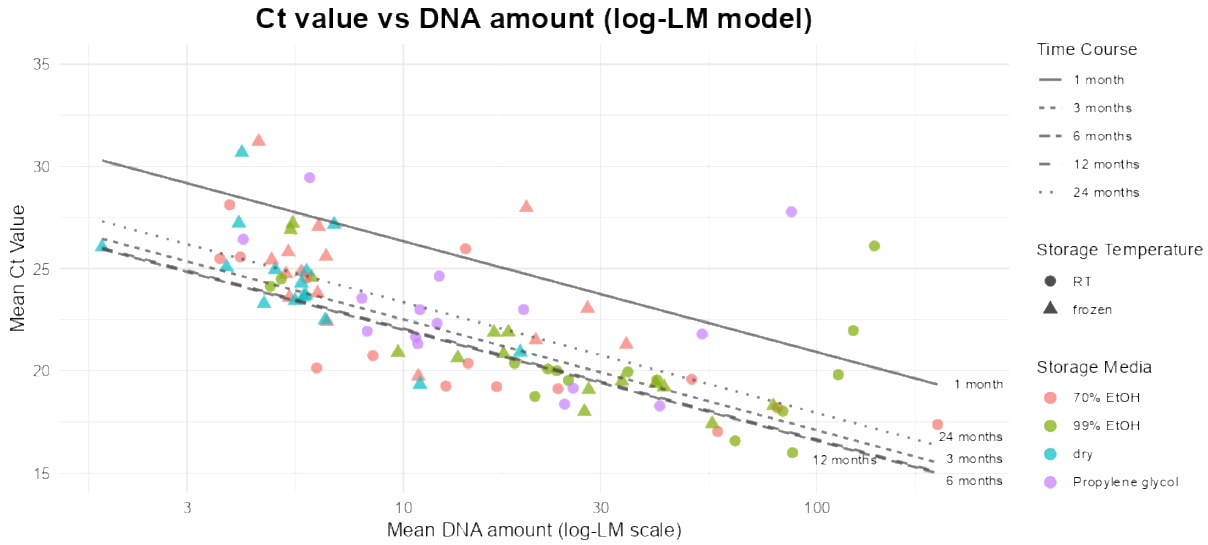
Long-term storage experiment

Results – real-time PCR and DNA barcoding

Real-time PCR

Storage medium, time and especially temperature had significant impact on Ct value

Strongly correlating with DNA amount, as would be expected



DNA barcoding

No significant differences detected

All conditions proved suitable for subsequent real-time PCR and DNA barcoding

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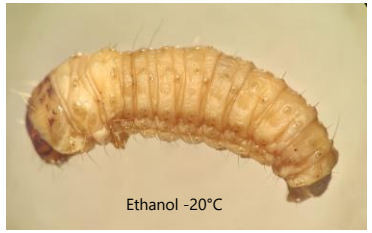
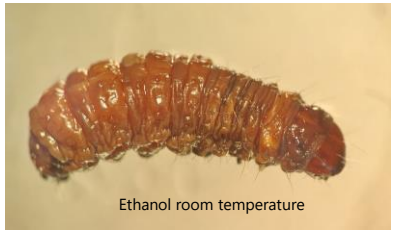
Long-term storage experiment

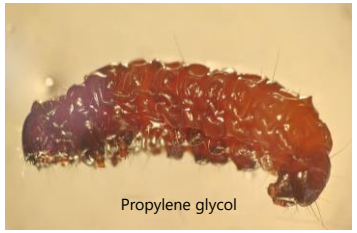
Results – Morphology after 2 years of storage

Main observed issues:

Discoloration, drying out and loss of flexibility



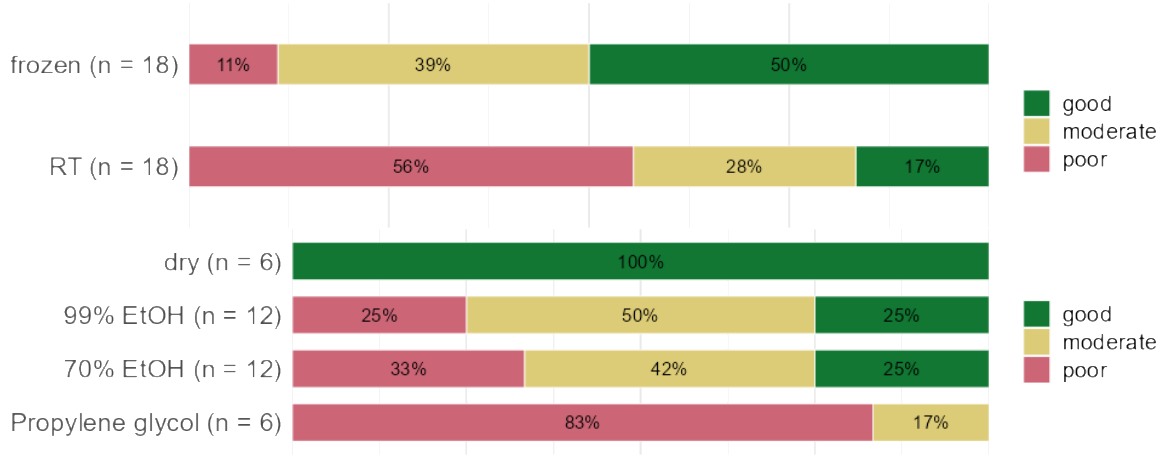




Room temperature performed worse than storage at -20°C, especially for color

Freezing dry performed best

Propylene glycol led to the strongest desiccation and discoloring overall



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Long-term storage experiment

Summary

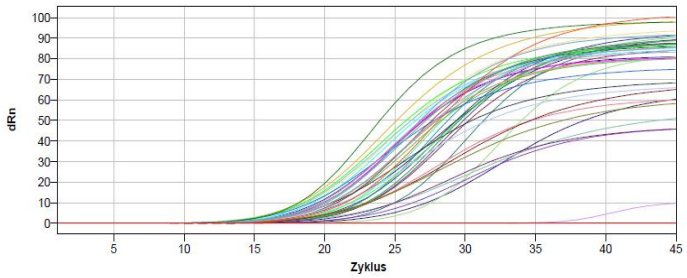
Molecular:

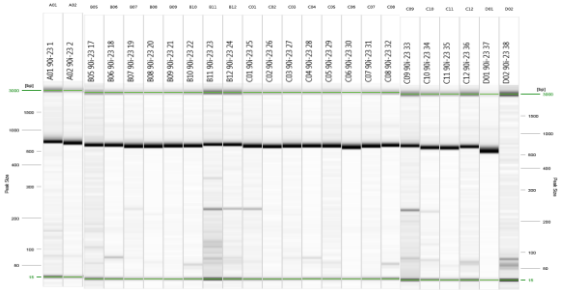
99% Ethanol best for both DNA amount and purity

Temperature matters most in the beginning

Freezing dry and propylene glycol performed worst

All conditions proved suitable for both real-time PCR and DNA barcoding





Morphological:

Frozen > room temperature

Freezing dry performed best

Propylene glycol performed worst

Identification often impossible

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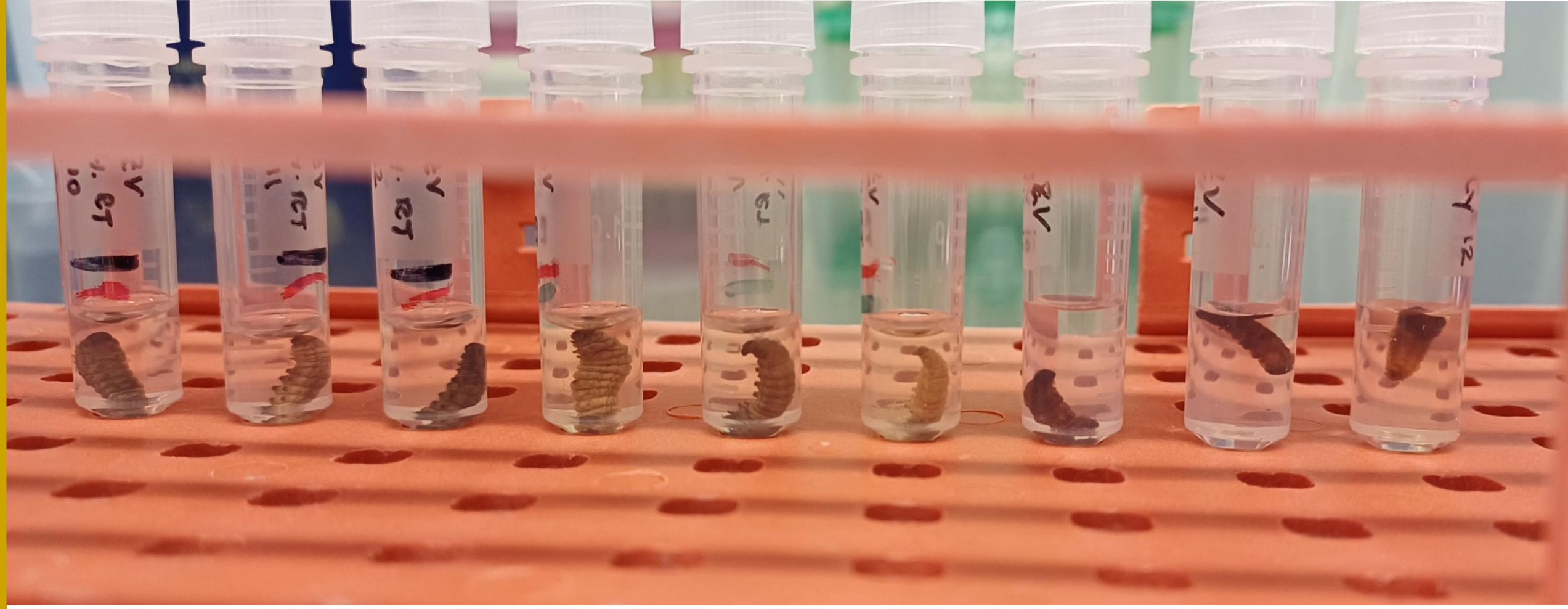


Contamination experiment

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

Contamination experiment

Workflow

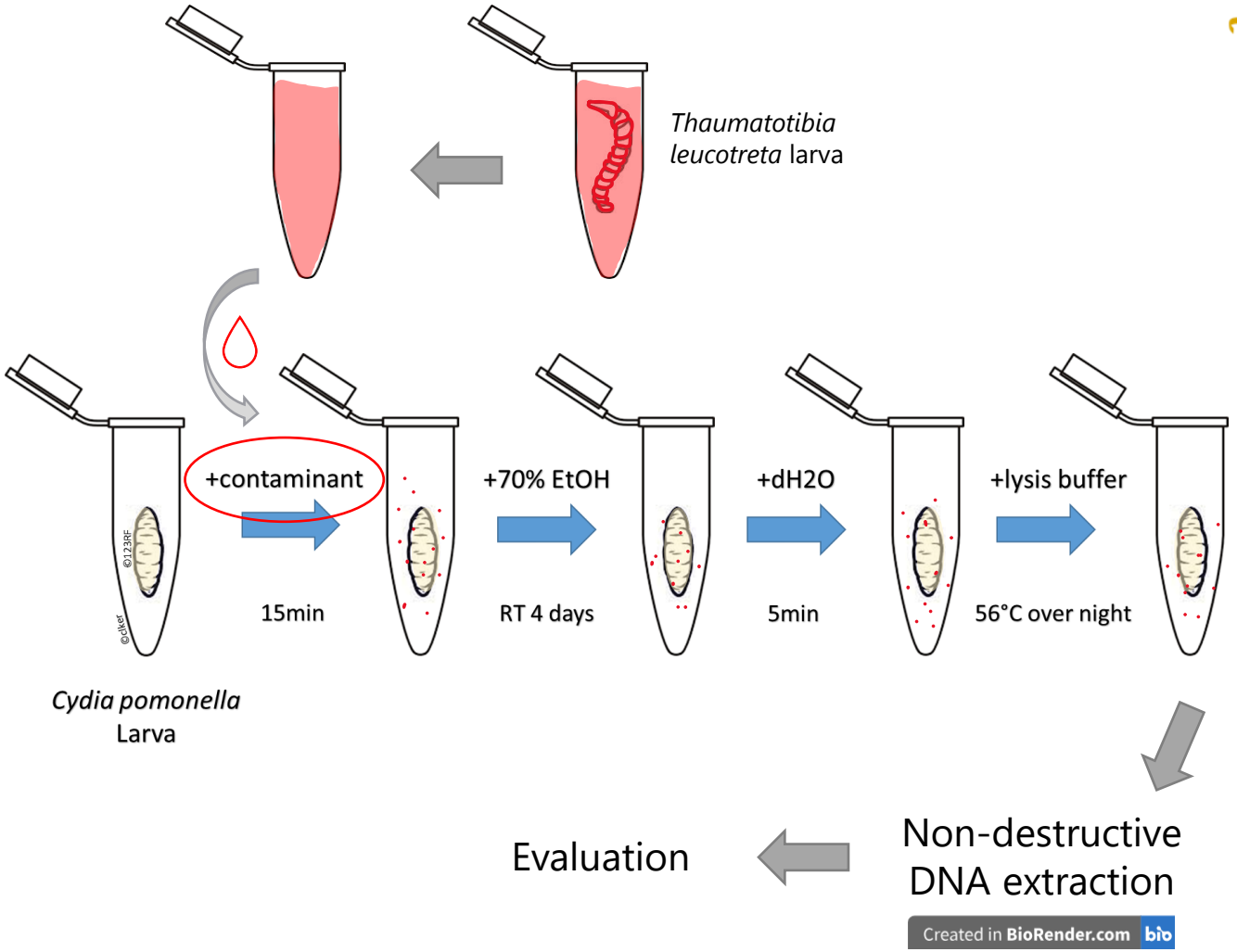
Spiking *Cydia pomonella* larvae with different amounts of storage ethanol previously containing *Thaumatotibia leucotreta*

Non-destructive DNA extraction

Evaluation:

T. leucotreta-specific real-time PCR

DNA barcoding



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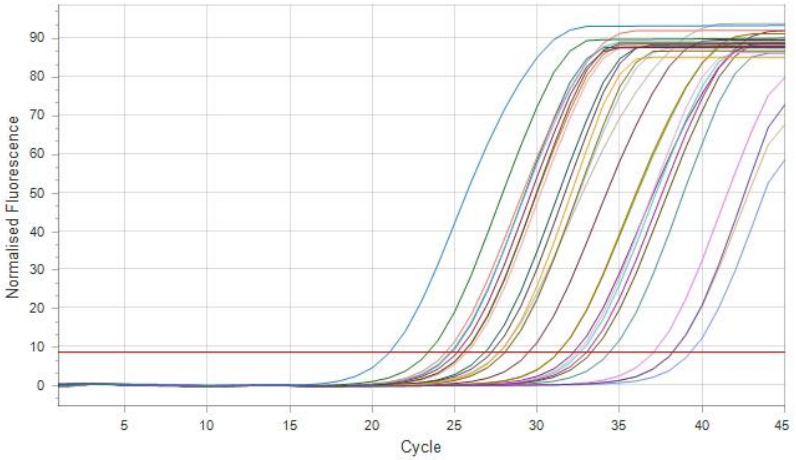
Contamination experiment

Results

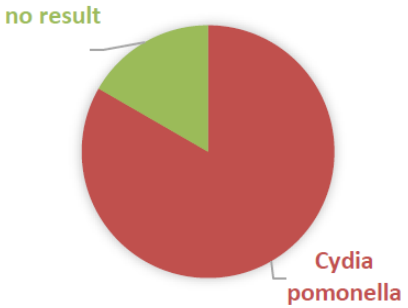
All spiked *Cydia pomonella* larvae tested positive for *Thaumatotibia leucotreta* (real-time PCR)

Correct identification possible via barcoding

But: contamination at high amounts led to low sequence quality (composite samples)



LARVAE



Non-destructive DNA extraction in combination with highly sensitive pest-specific real-time PCR is very susceptible to **contaminations**

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Interplay of morphological and molecular methods

Summary

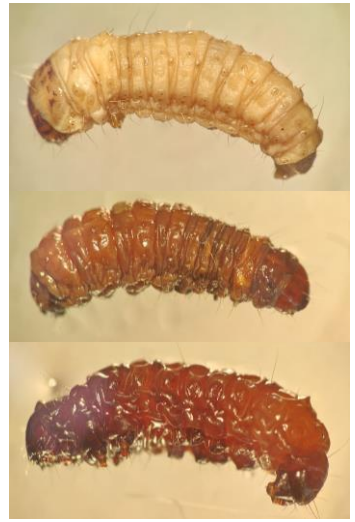
Ideal storage conditions

(for larvae)

Molecular: 99% Ethanol at room temperature (short term) or -20°C (long term)

Morphological: Freezing without any storage medium (dry)

Highly concentrated ethanol at -20°C

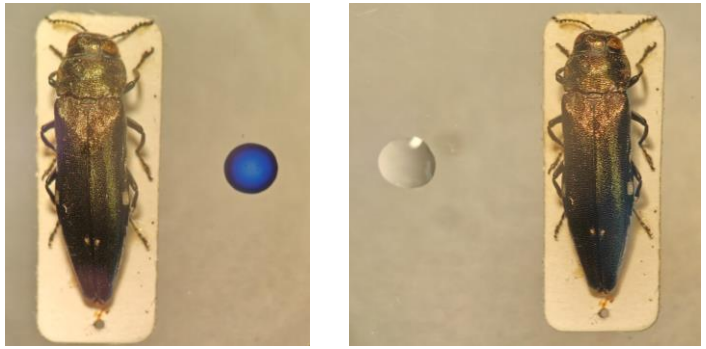


Contamination risks

Sterile ≠ DNA-free!

Storage ethanol
Even in small amounts!

Handling
Instruments, surfaces, human DNA



Agrilus biguttatus

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Interplay of morphological and molecular methods

Recommendations

Ideal storage conditions

(for larvae)

Highly concentrated ethanol at -20°C

Preventing contamination

No re-using of storage ethanol
Changing gloves and cleaning tools
Decontaminating surfaces in contact with specimen or storage ethanol

Bleach
UV-radiation
Commercial solutions
To be tested: heat

Clean work, safe storage and non-destructive DNA extraction allow for both complete reference specimens and high-quality sequences

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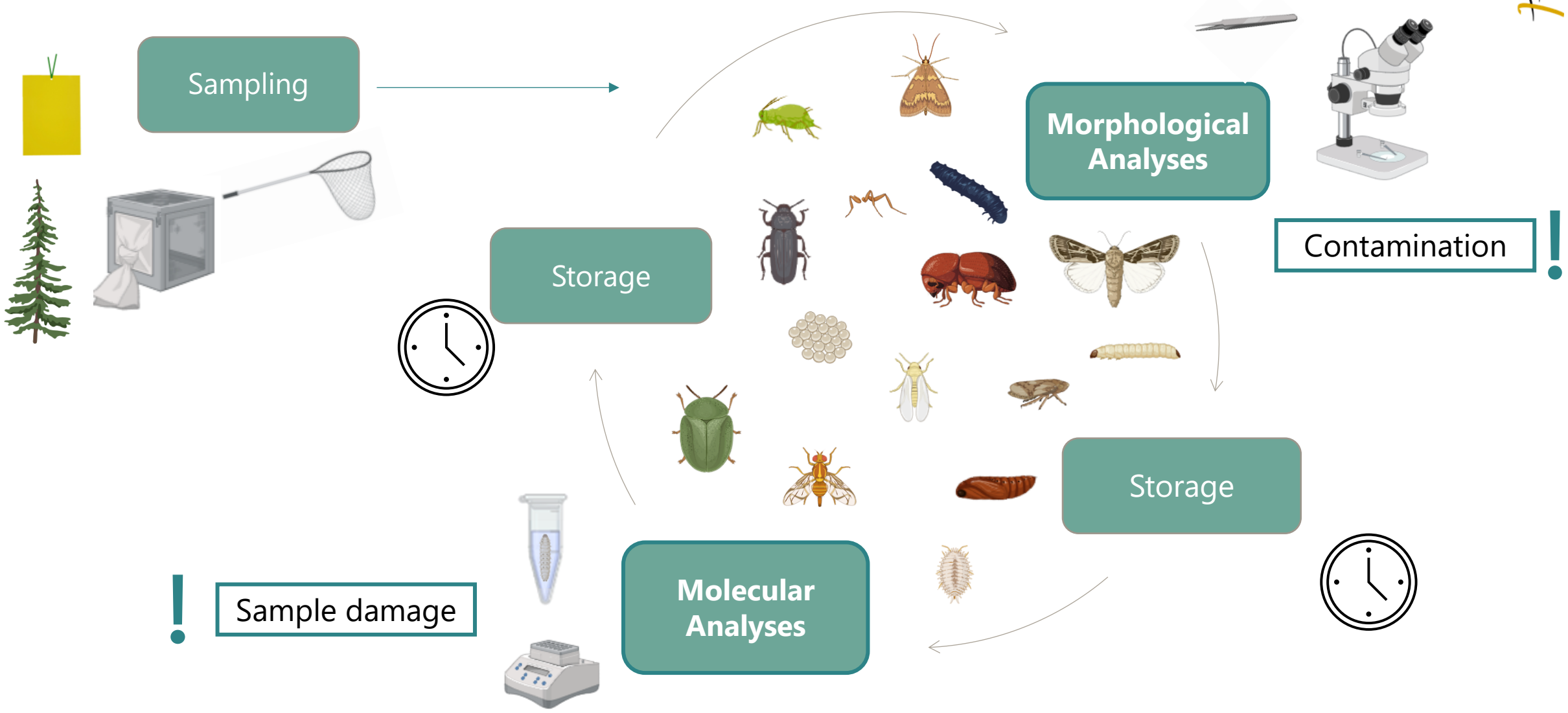
Interplay of morphological and molecular methods

Graphical summary



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Pohn Chiara – LWT/NPP/PURE/MOPS

Spargelfeldstraße 191

A-1220 Wien

T +43 (0) 50 555-33355

chiara.pohn@ages.at