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DNA Metabarcoding to quantify the response of insect diversity to mountain forest die-offs in the French Pyrenees

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Lucas Sire

2nd year of *Ph.D.* at IRBI – Tours

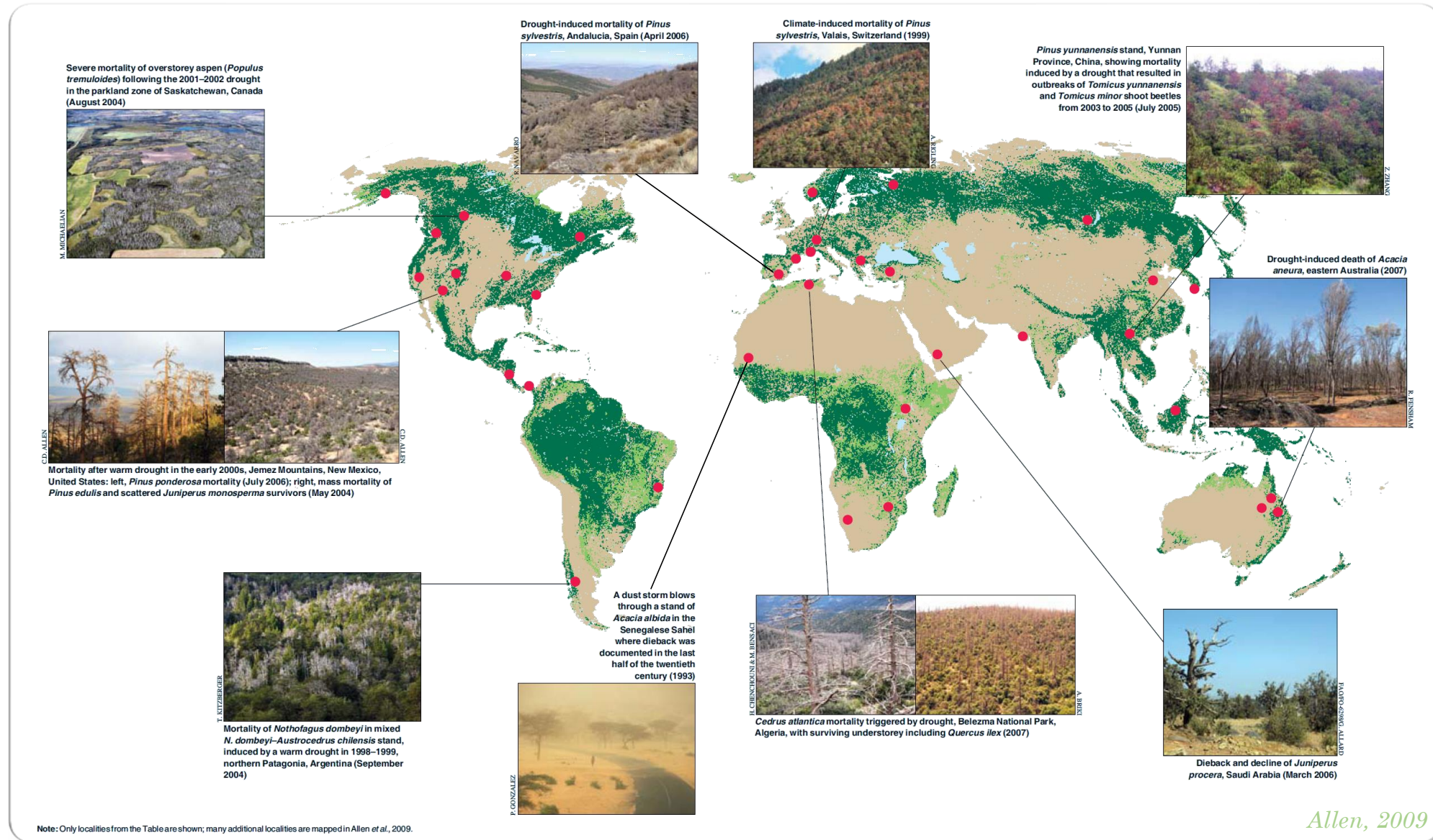
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SFEcologie

Rennes – 23 Octobre 2018

Forest perturbations due to climate change



Dead wood management

- How to **manage forest diebacks** is a significant concern for forest stakeholders.
- What should be done with the large volumes of dying trees and fresh deadwood (salvage logging, sanitation salvage) ?



DOES SALVAGE LOGGING MAKE
THINGS BETTER OR WORSE?

January 14, 2015 | Conservation This Week | 2 Comment

Activists Protest Logging In Poland's Ancient Forest

© 29 days ago



Global insects decline ?

WHERE HAVE ALL THE INSECTS GONE?

Surveys in German nature reserves point to a dramatic decline in insect biomass. Key members of ecosystems may be slipping away

More than 75 percent decline over 27 years in total flying insect biomass in protected areas

Caspar A. Hallmann^{1*}, Martin Sorg², Eelke Jongejans¹, Henk Siepel¹, Nick Hofland¹, Heinz Schwan², Werner Stenmans², Andreas Müller², Hubert Sumser², Thomas Hören², Dave Goulson³, Hans de Kroon¹

Insectageddon: farming is more catastrophic than climate breakdown
George Monbiot

Warning of 'ecological Armageddon' after dramatic plunge in insect numbers

Three-quarters of flying insects in nature reserves across Germany have vanished in 25 years, with serious implications for all life on Earth, scientists say

Insects

A giant insect ecosystem is collapsing due to humans. It's a catastrophe



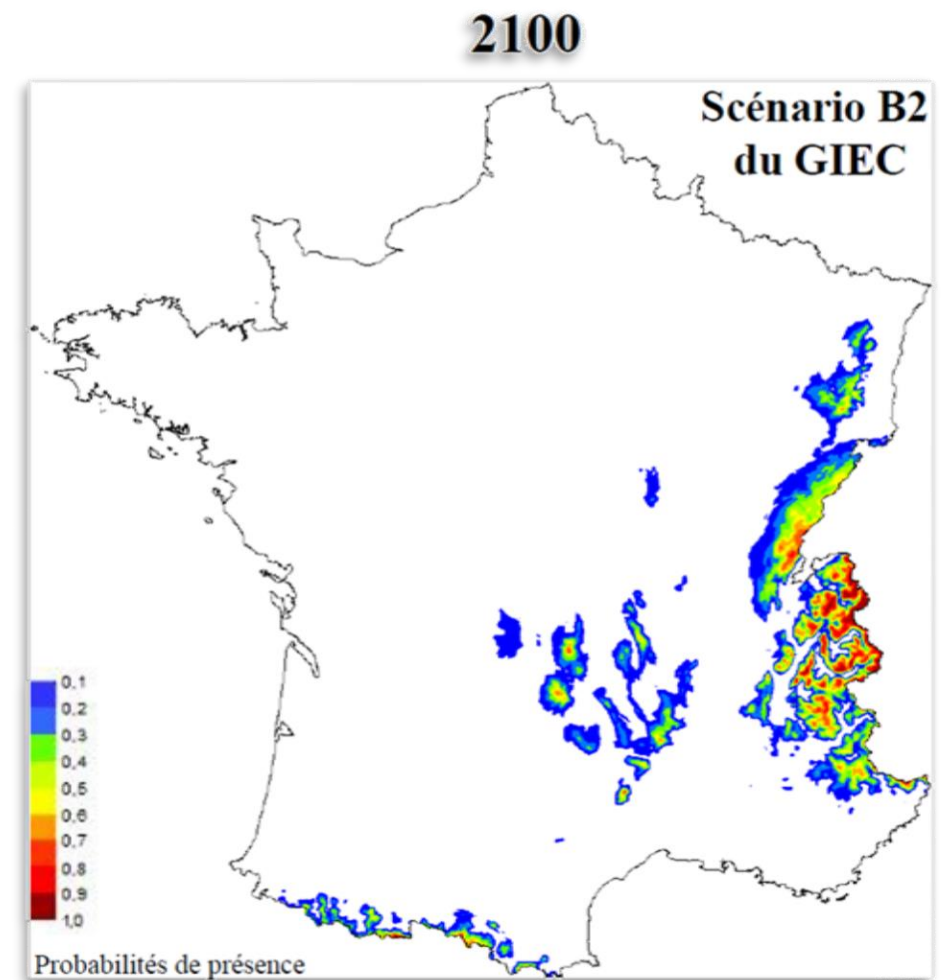
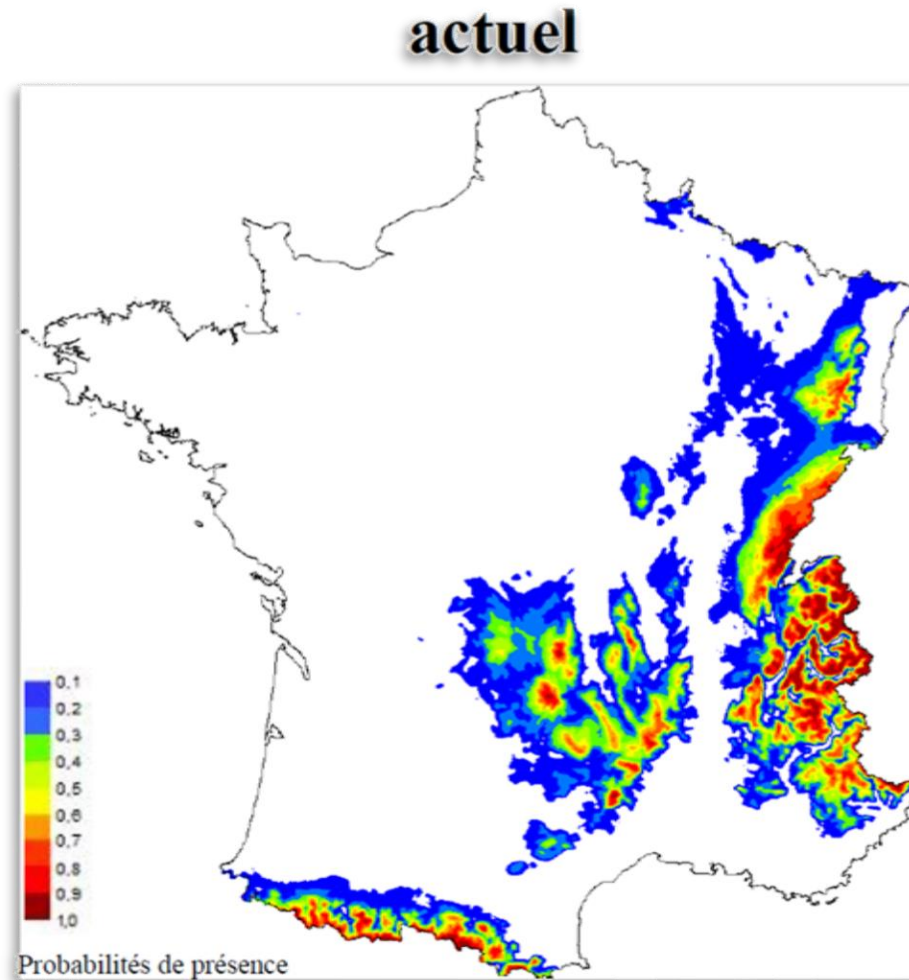
Thesis aims

- Quantify the ecological impact of climate-induced forest die-offs and salvage logging on arthropod communities
- Develop biomonitoring pipelines for forest biodiversity

This presentation will focus on:

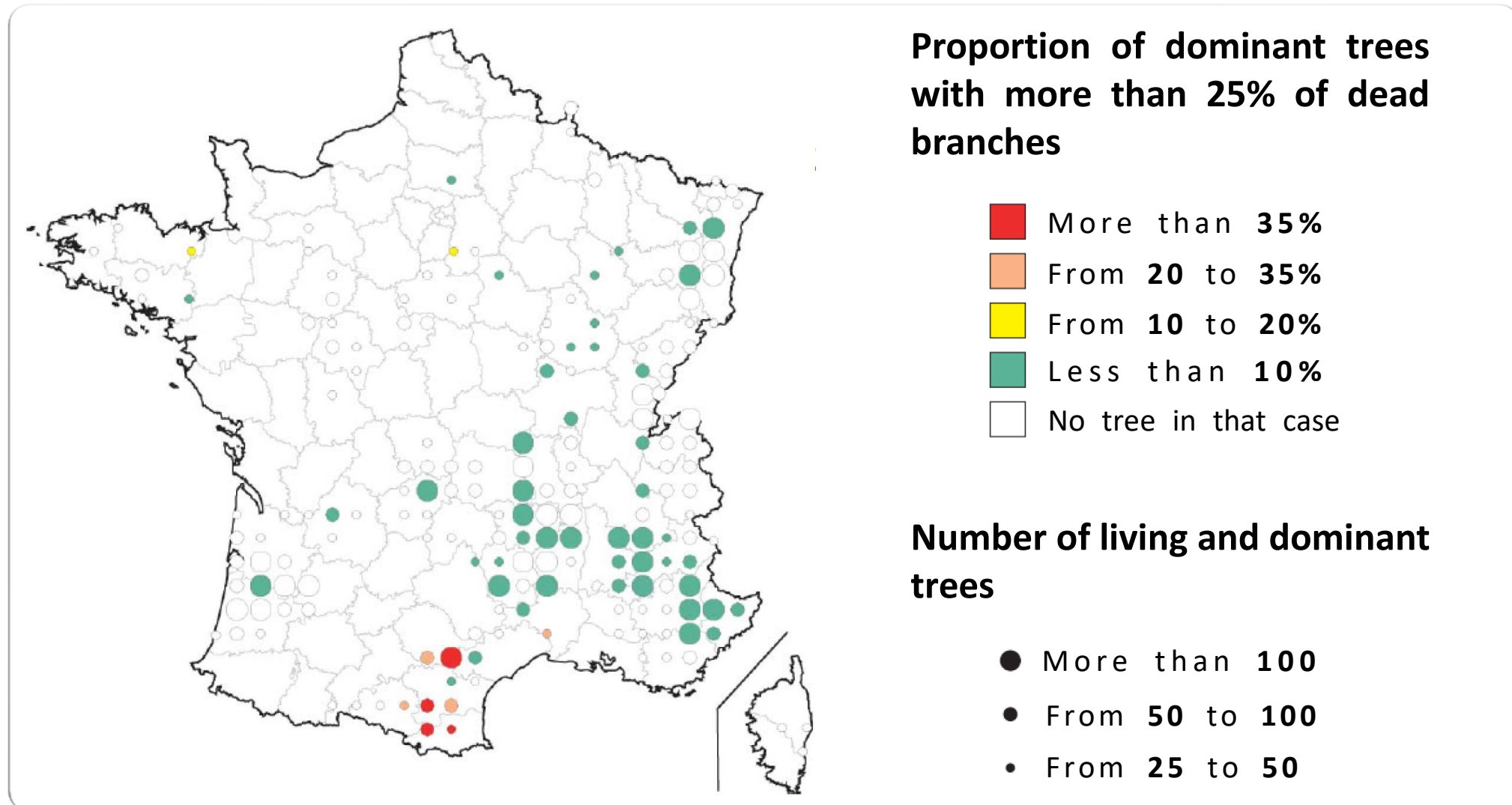
- What is the species richness in arthropods ?
- Temporal turnover
- Test DAME bioinformatic pipeline

Situation of Silver fir in France

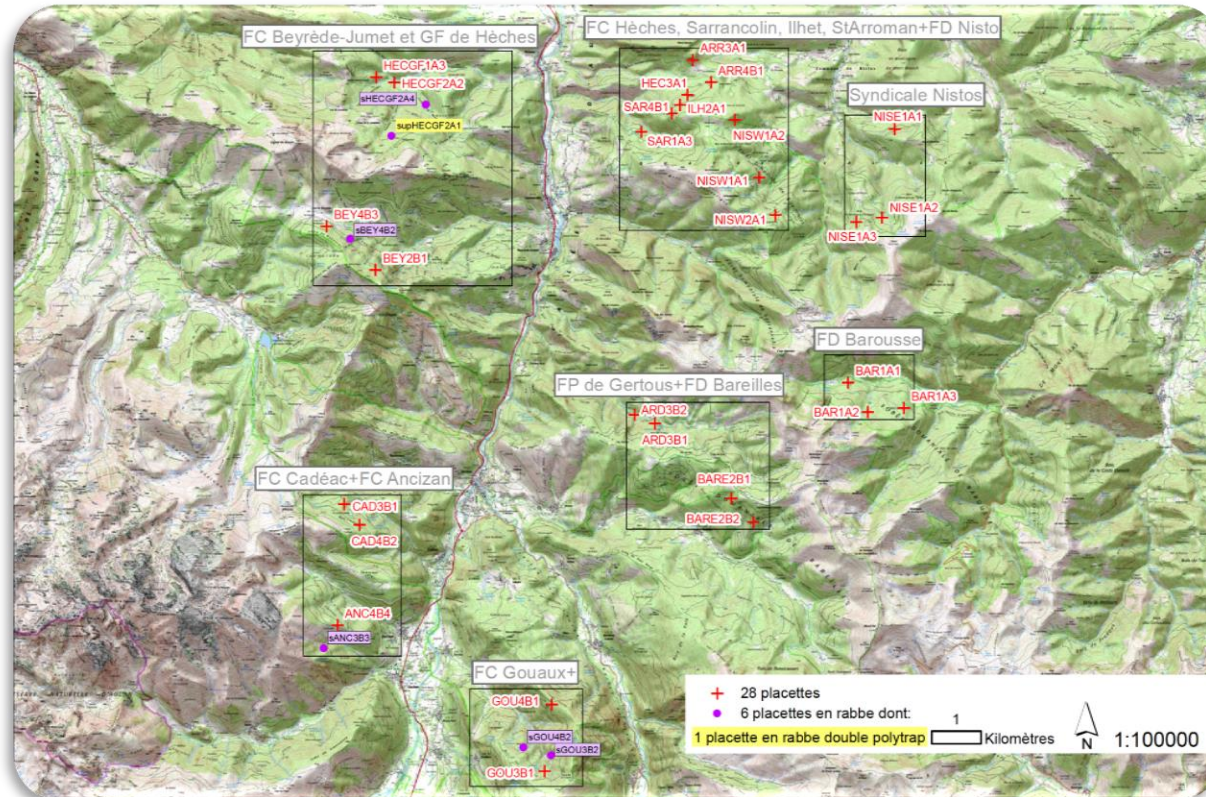


Badeau et al., 2005

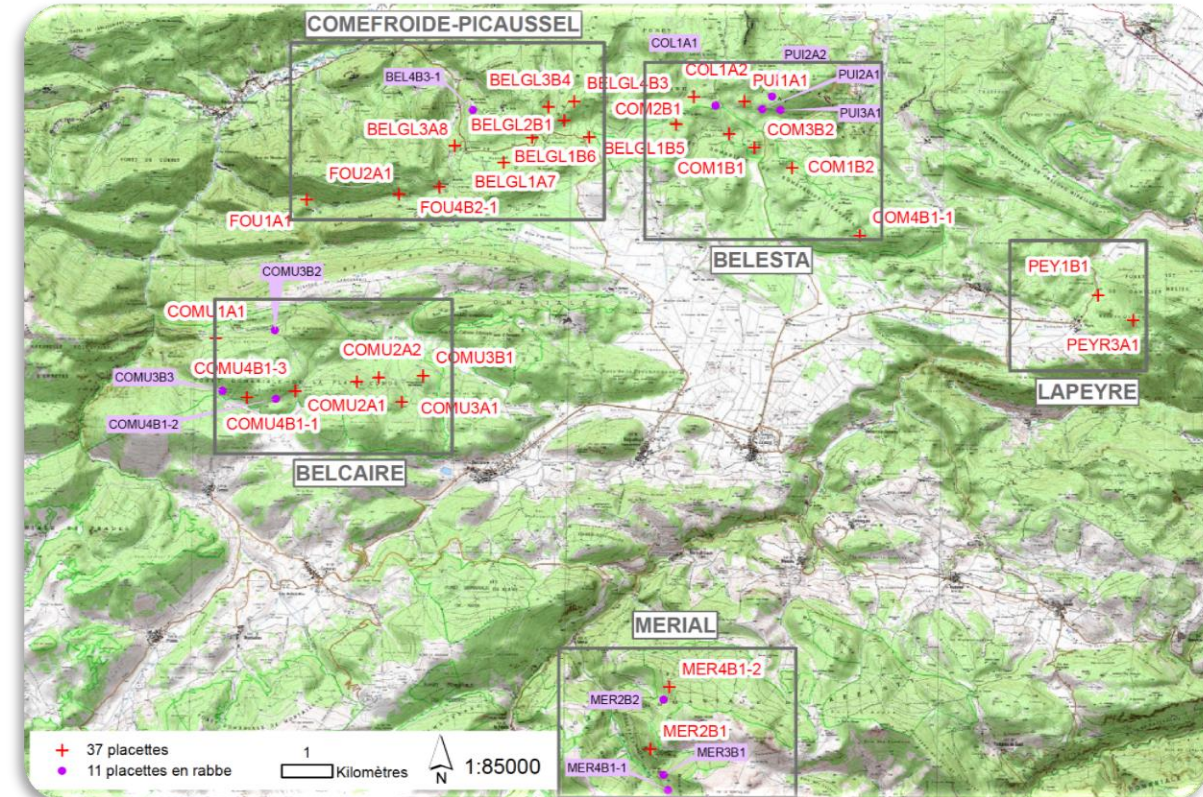
Situation of Silver fir in France



Experimental design



Vallée d'Aure



Pays de Sault

N = 57 plots

Healthy plots | Low / high level diebacks | No harvesting / Salvage logging



Ecological parameters

Mistletoe
Largest $\varnothing$ >20cm



Perennial polypore
Largest $\varnothing$ >5cm



Invertebrate nest
Presence



Pulpy agaric
Largest $\varnothing$ >5cm or cluster of > 10 fruiting bodies



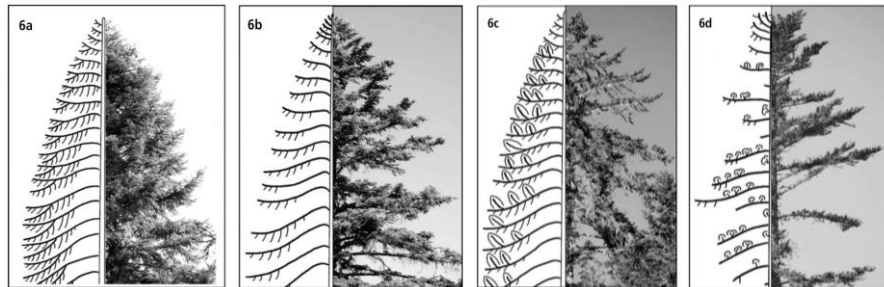
Micro-dendrohabitat assessment

Larrieu et al., 2018

ARCHI method (Assess health state of dominant trees)

Drénou et al., 2013

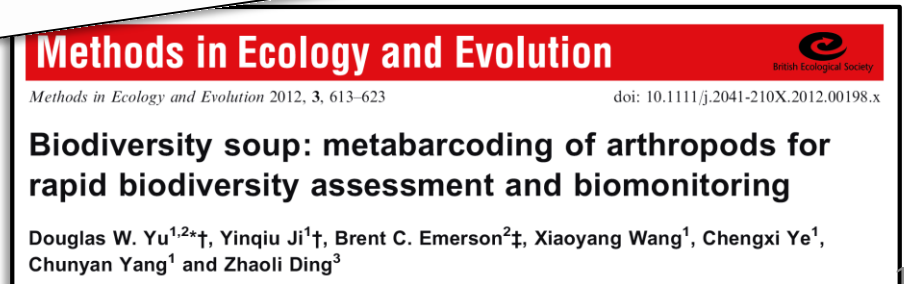
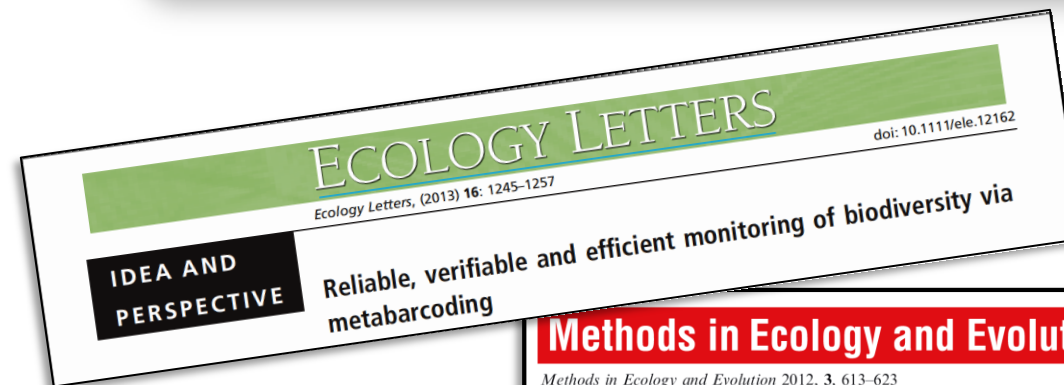
Stand structure, canopy openness, dead wood volume, large tree density...



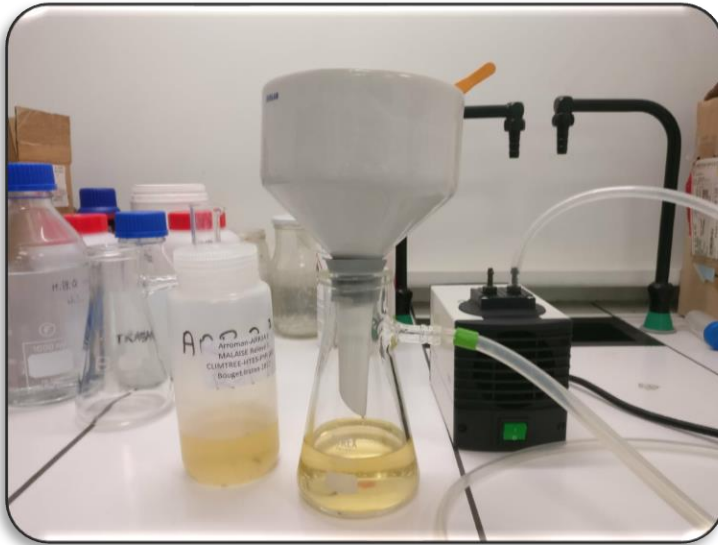
Biomonitoring using mass-trapping samples



- Sampling **each month** over **4 months** (from May to September)
 - **224 Malaise** samples
 - 448 Polytrap samples



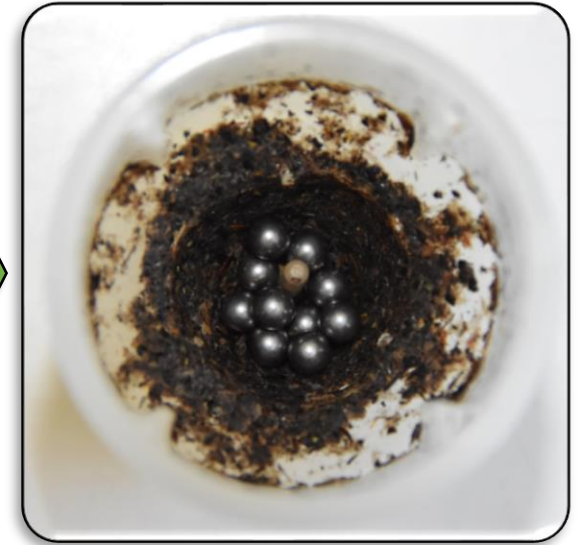
Sample preparation



Trap filtration



Drying and
homogeneization



Grinding



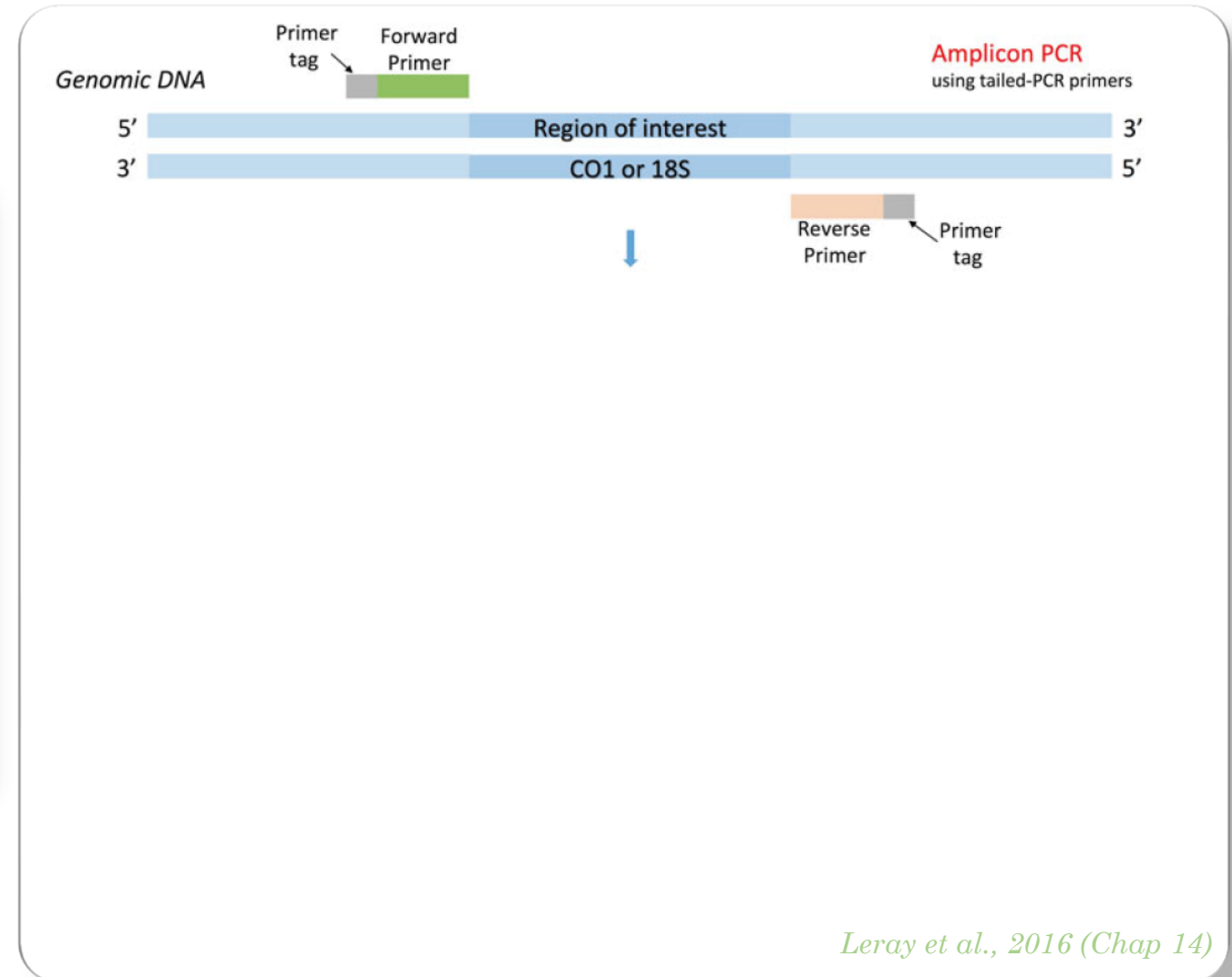
DNA extraction

Twin-tagging dual indexing PCR

- DNA Quality control post extraction
- 313bp COI barcode fragment
- Highly degenerated primers

Leray et al., 2013

Geller et al., 2013



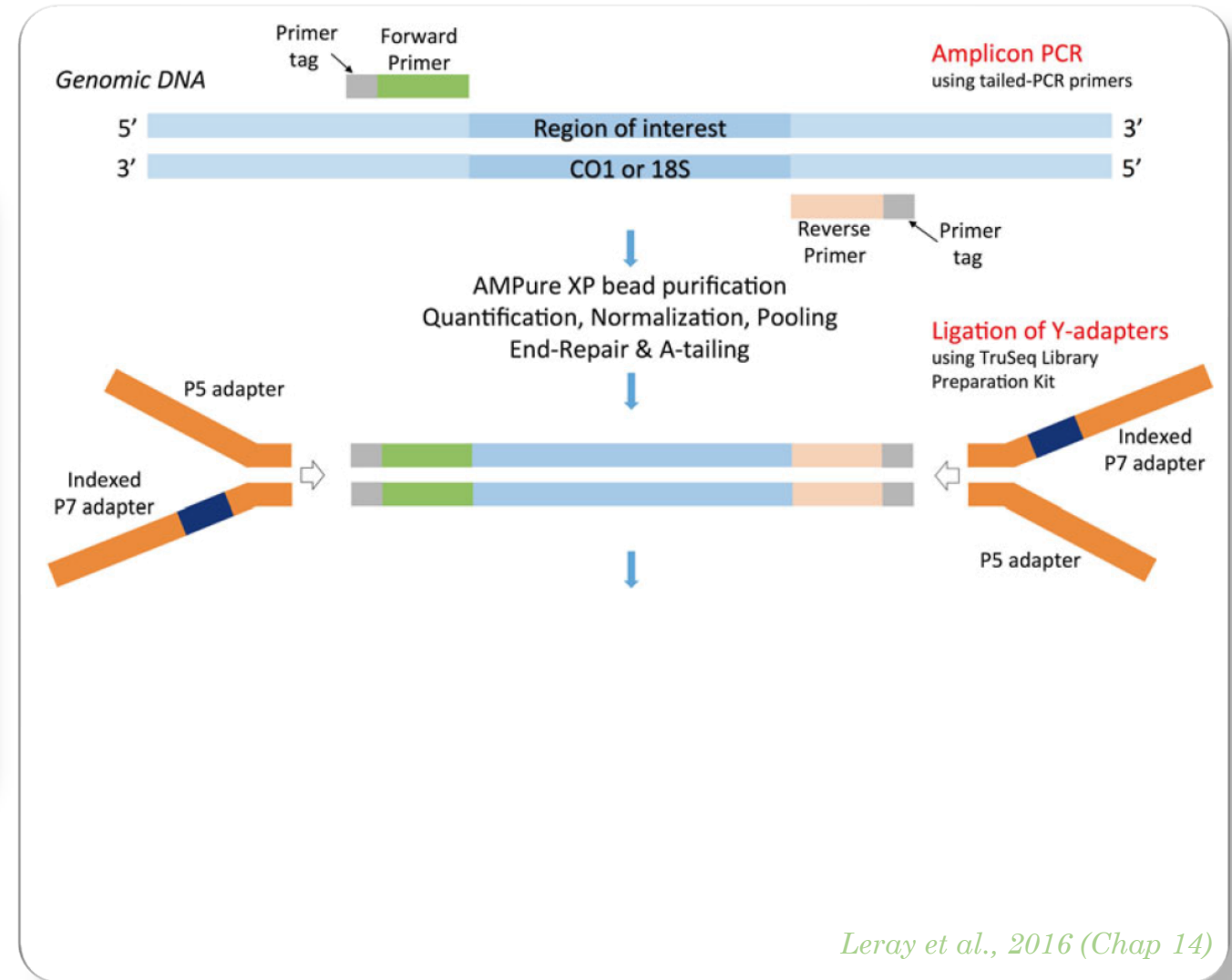
Leray et al., 2016 (Chap 14)

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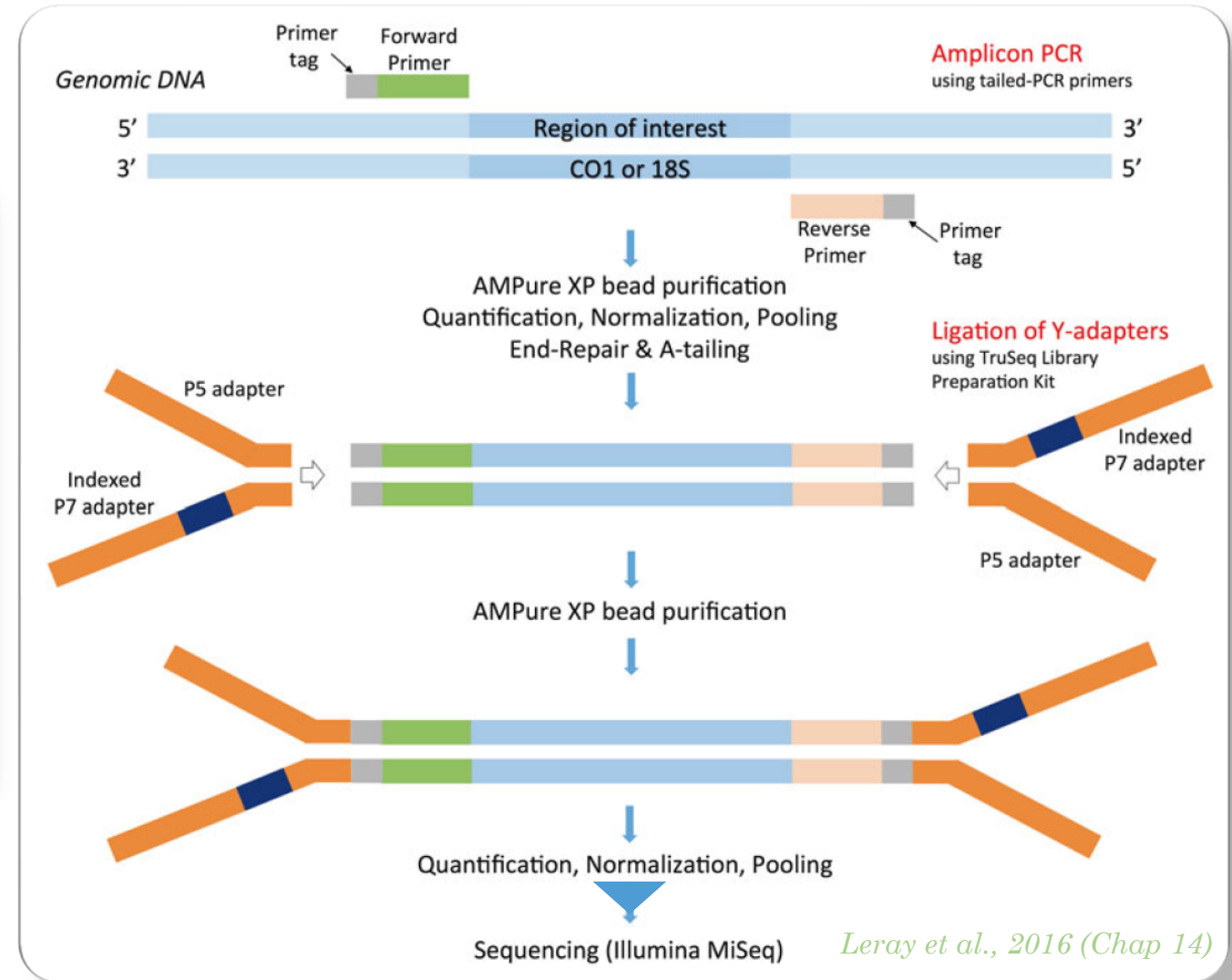
Leray et al., 2016 (Chap 14)

Twin-tagging dual indexing PCR

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Leray et al., 2013

Geller et al., 2013



Bio-informatic pipeline

Zepeda-Mendoza et al. *BMC Res Notes* (2016) 9:255
DOI 10.1186/s13104-016-2064-9

BMC Research Notes

TECHNICAL NOTE

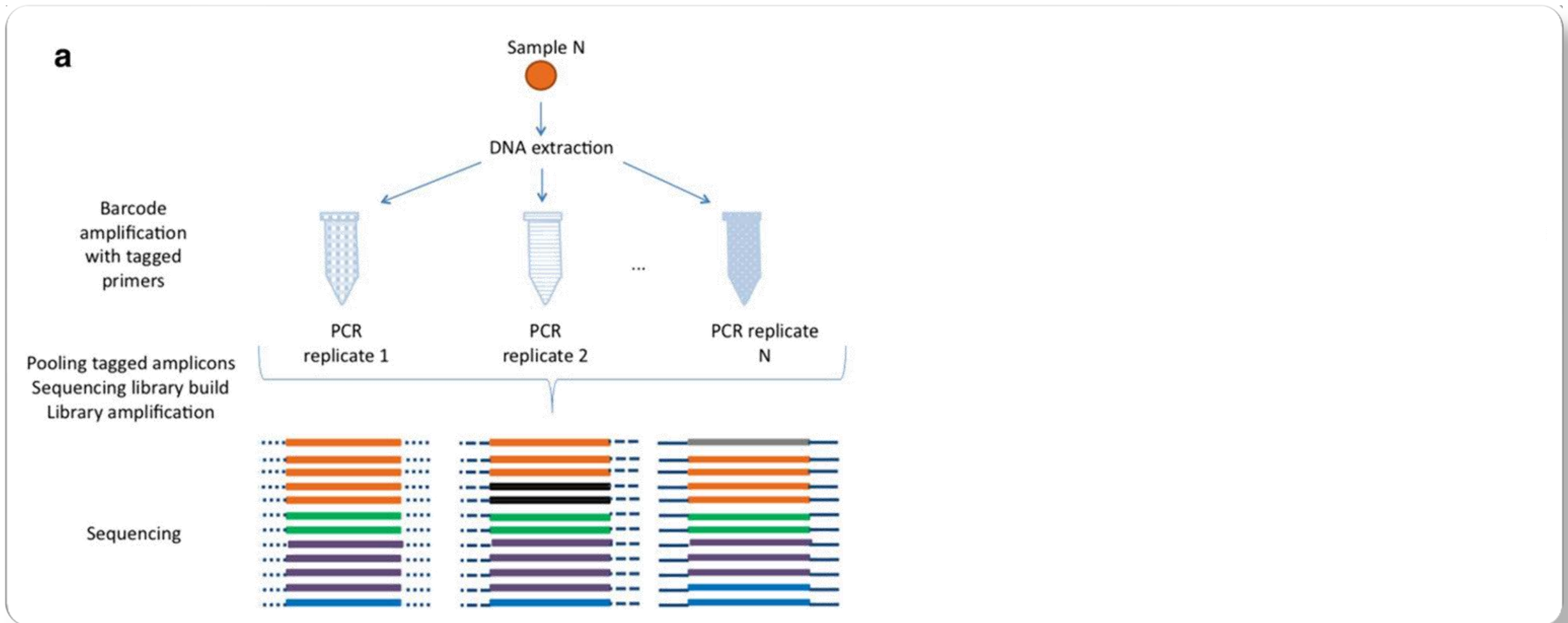
Open Access



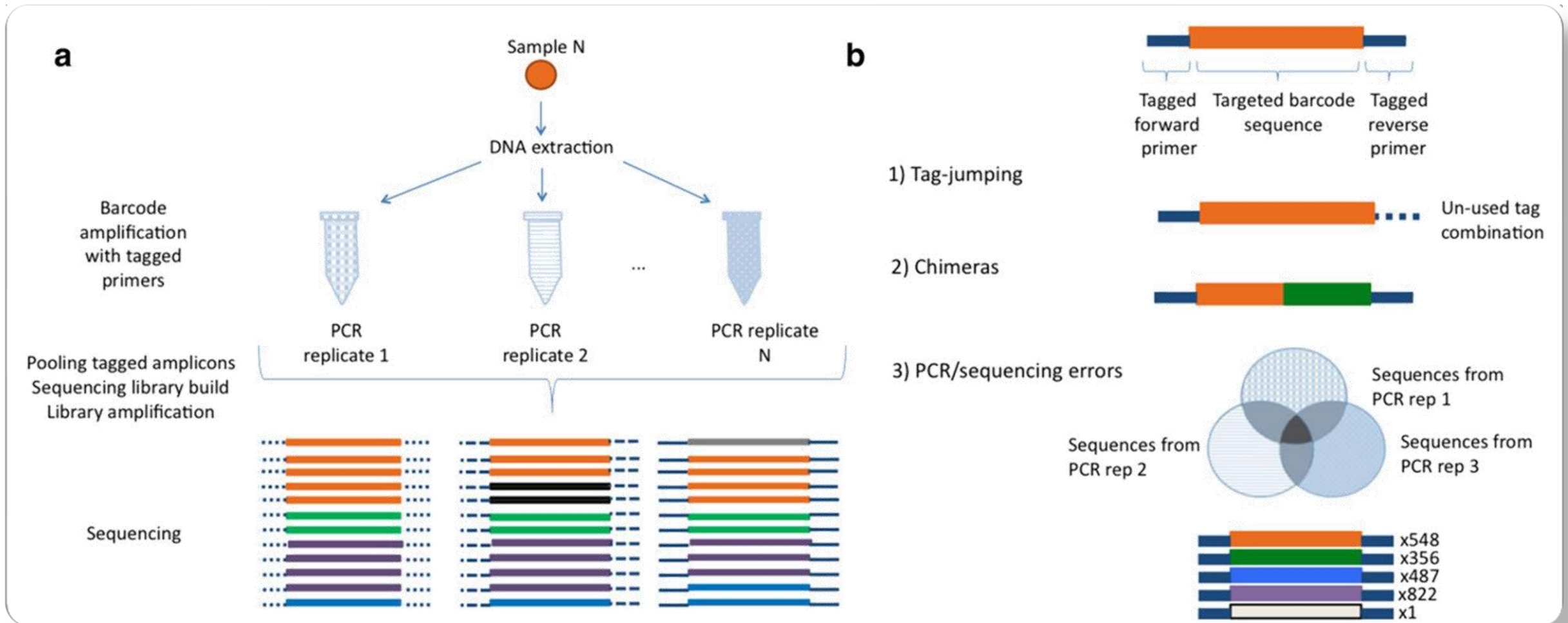
DAMe: a toolkit for the initial processing of datasets with PCR replicates of double-tagged amplicons for DNA metabarcoding analyses

Marie Lisandra Zepeda-Mendoza^{1*}, Kristine Bohmann¹, Aldo Carmona Baez^{1,2} and M. Thomas P. Gilbert¹

Bio-informatic pipeline

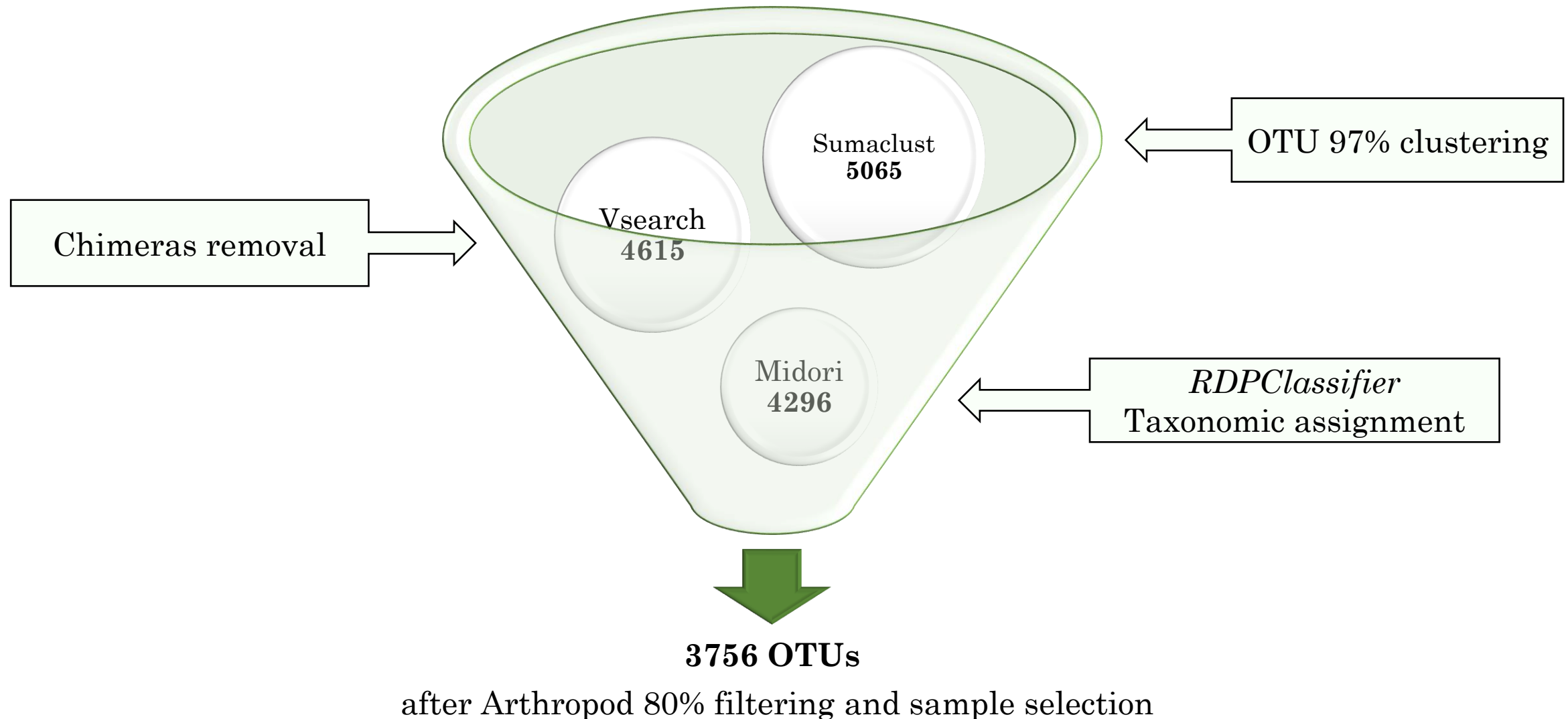


Bio-informatic pipeline



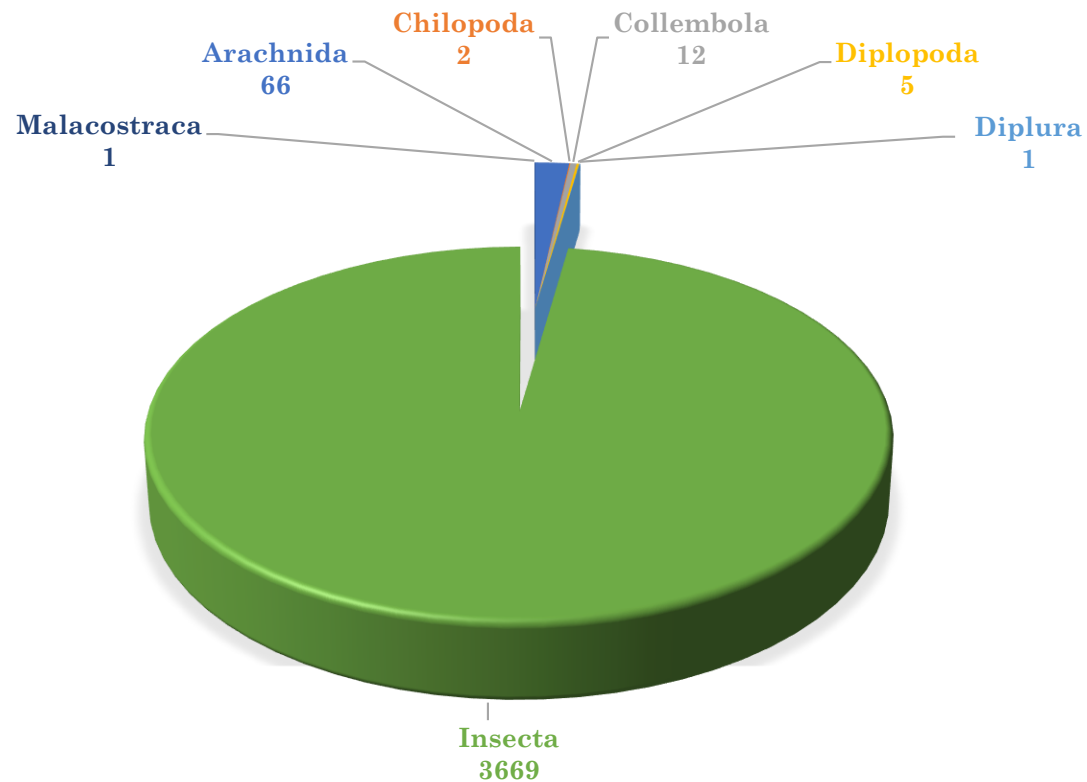
Bio-informatic pipeline

Minimum 4 reads in each of the 3 PCR replicates

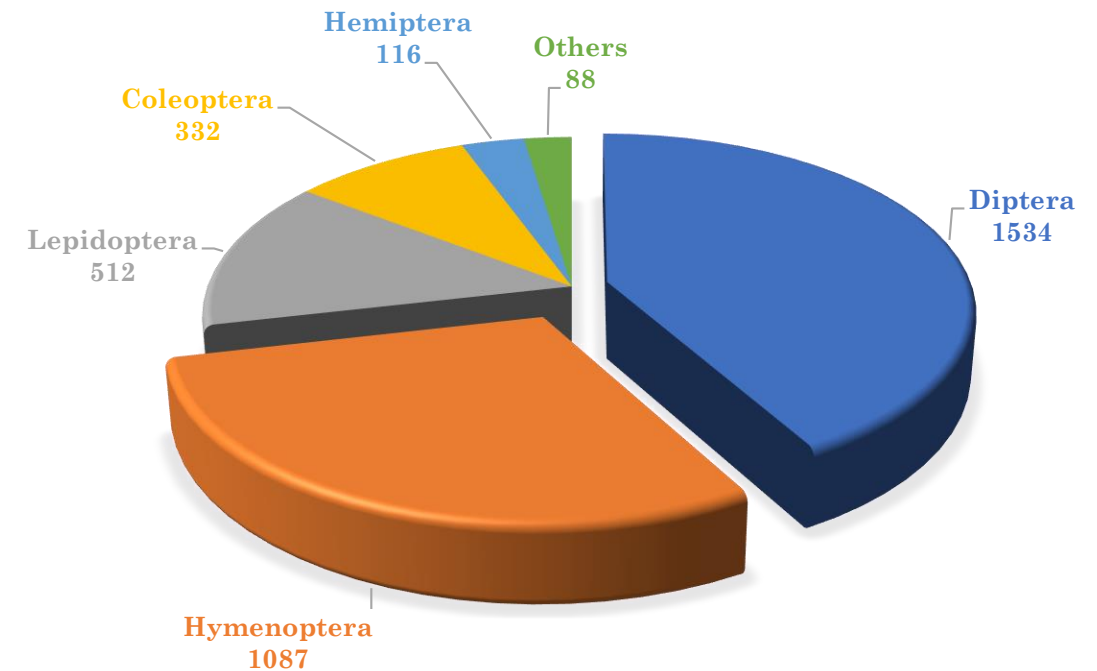


Species richness for Malaise traps

3756 OTUs found

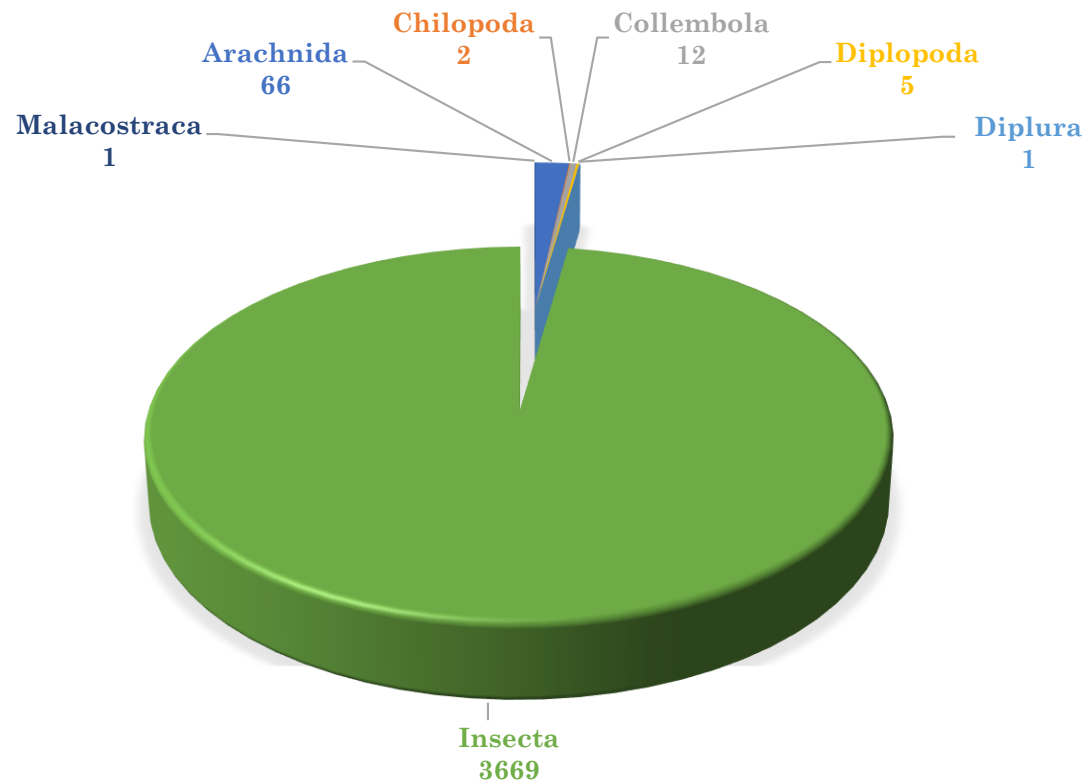


18 Insecta orders represented

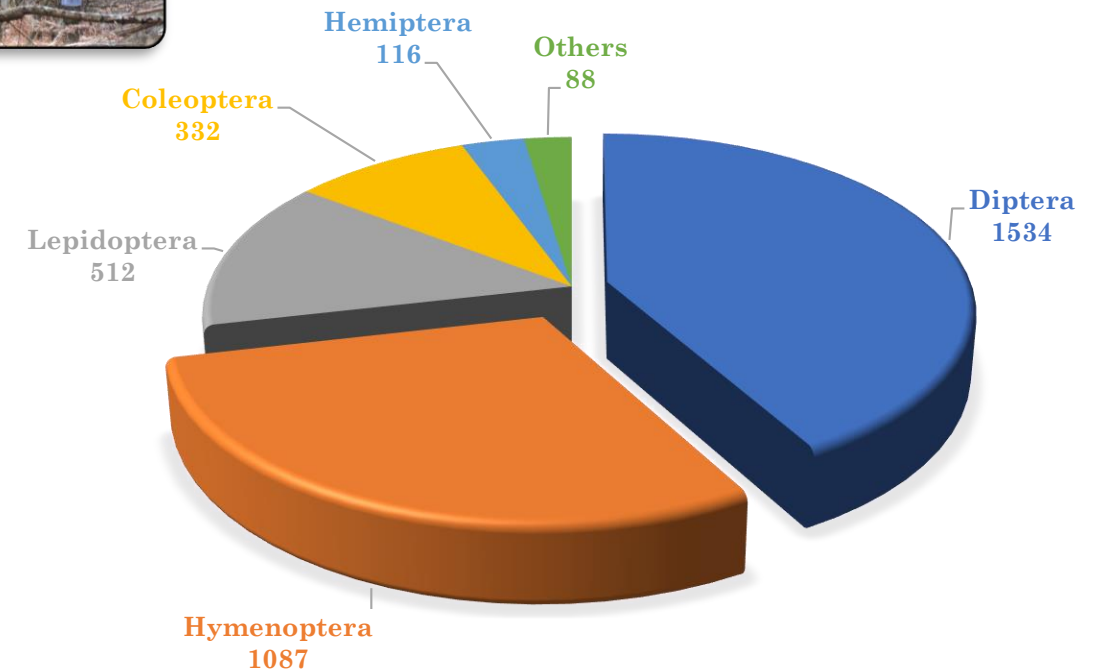


Species richness for Malaise traps

3756 OTUs found



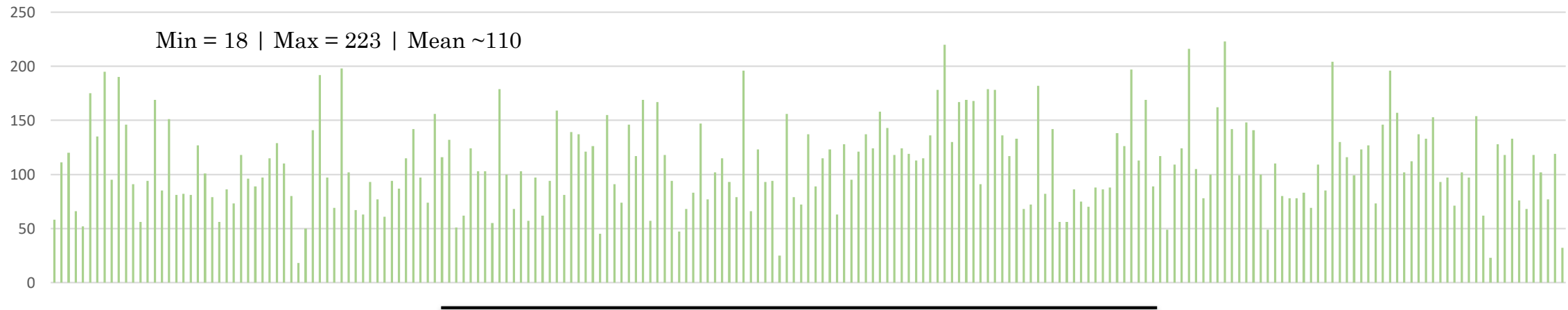
18 Insecta orders represented



Trap composition

$N_{\text{bulk samples}} = 211$

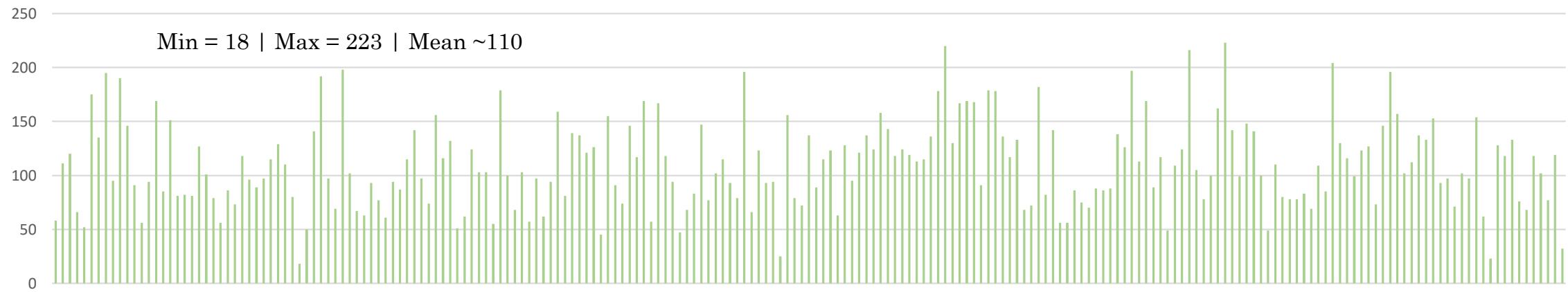
Number of OTUs per bulk sample



Trap composition

$N_{\text{bulk samples}} = 211$

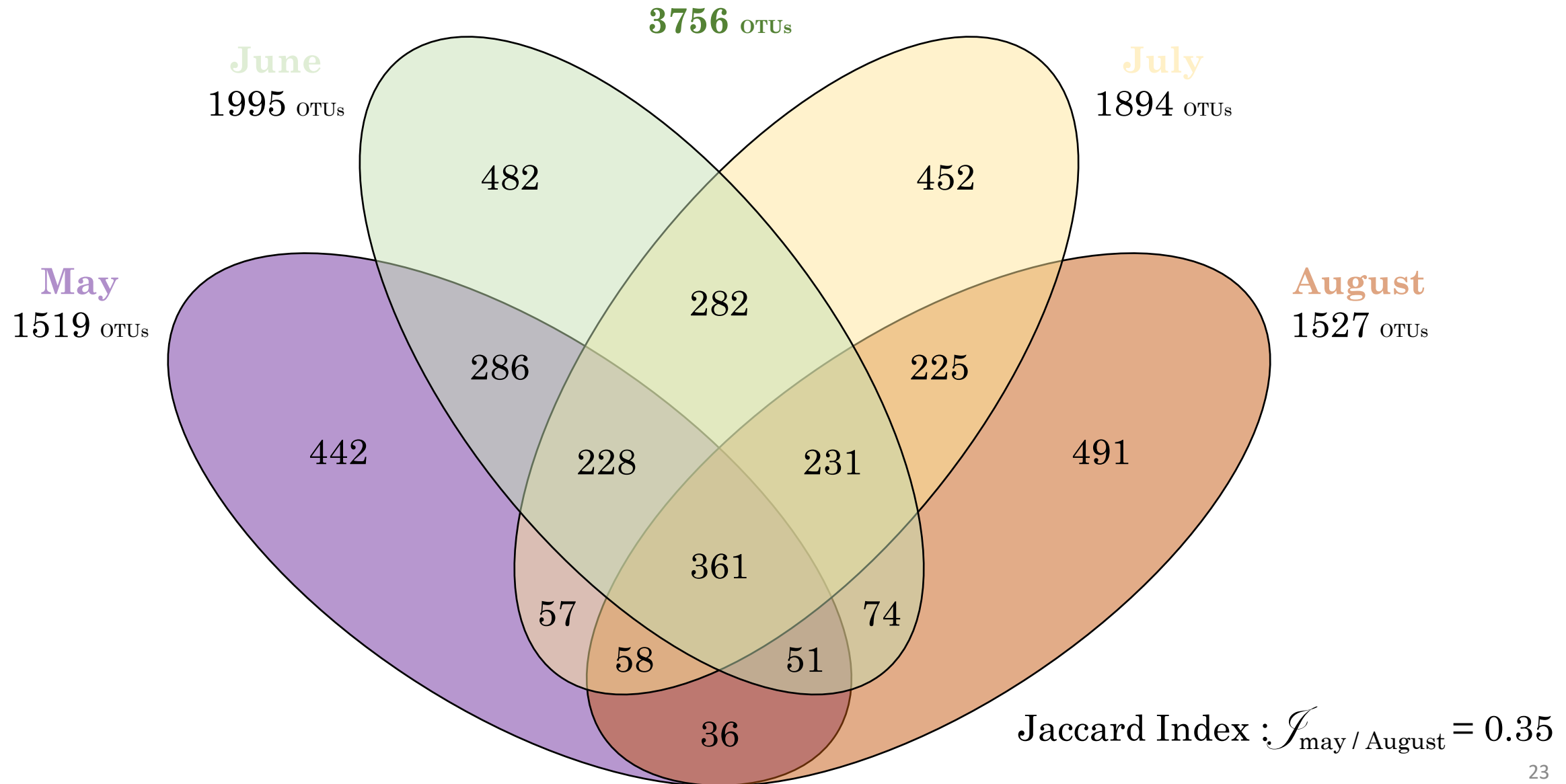
Number of OTUs per bulk sample



Number of bulk samples per OTU



High temporal turnover from May to August



Future work

- Expand reference libraries (saproxylic beetles)
- Improve taxonomic assignment into the bioinformatic pipeline
- Reanalyze with new version **DAMe 2.0** incoming
- Compare with other bioinformatic methods (**mBRAVE**, **CROP...**)
- Analyze Polytrap (**ethanol eDNA** metabarcoding)

Thank you for your attention !



Doug Yu's team



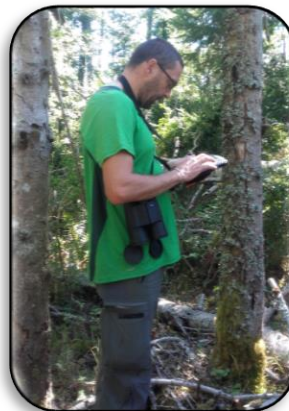
CLIMTREE consortium



Paul Schmidt



Susan Mbedi



AGENCE NATIONALE DE LA RECHERCHE
ANR

BELMONT
FORUM



BeGenDiv

Detection of pests and invasive species

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0.5mm

Hylesinus toranio



1.0mm

Xylosandrus germanus

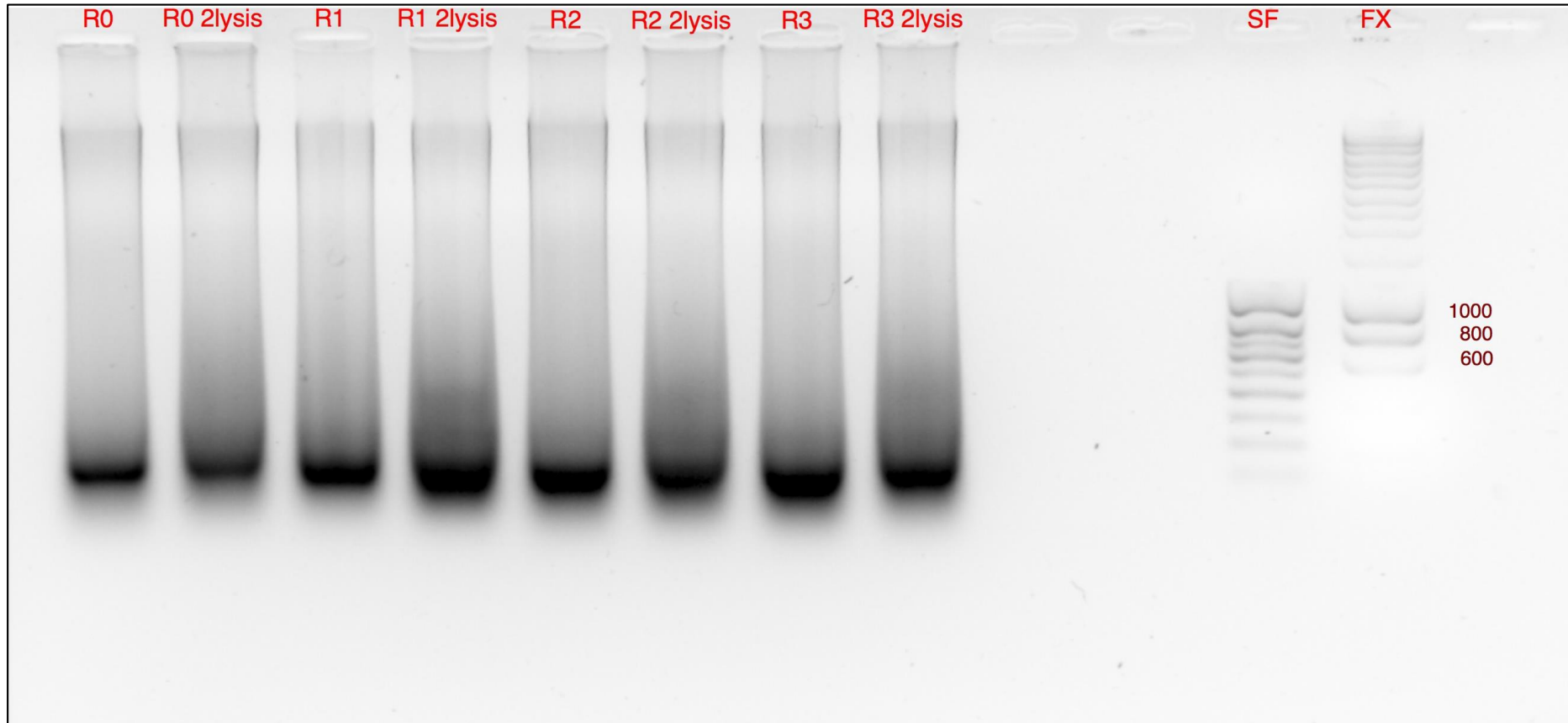


1.0cm

Vespa vetulina

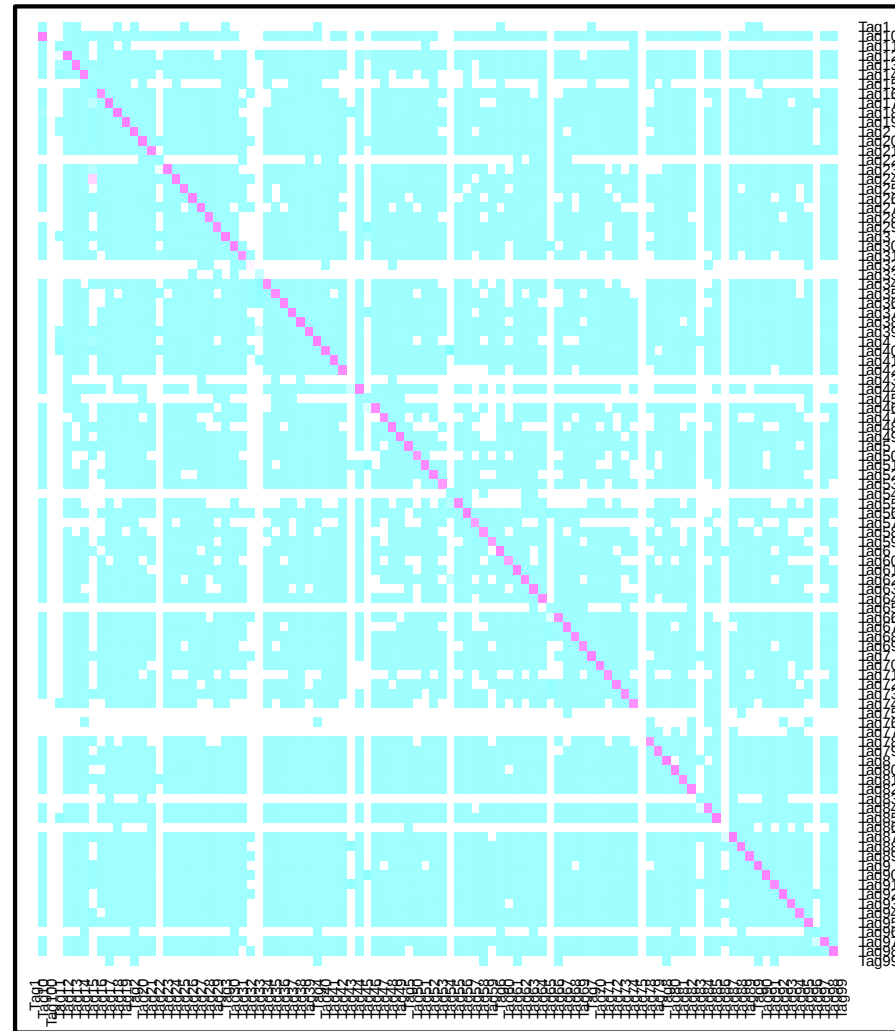
Degradation of samples

Pre-PCR migration and rincing test for MPG removal



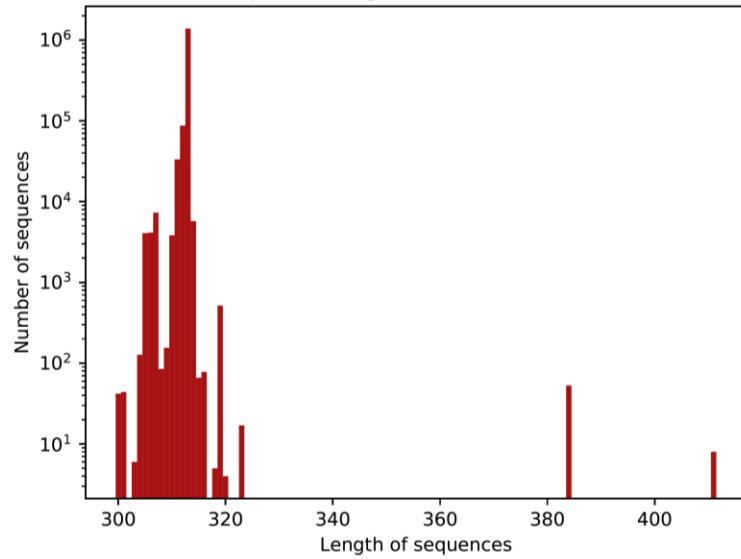
R0: no rincing | R1: rincing with 2x100mL water | R2: rincing with 2x100ml ethanol | R3: rincing with 100ml ethanol + 100ml water

Heatmap of primer-tag combinations

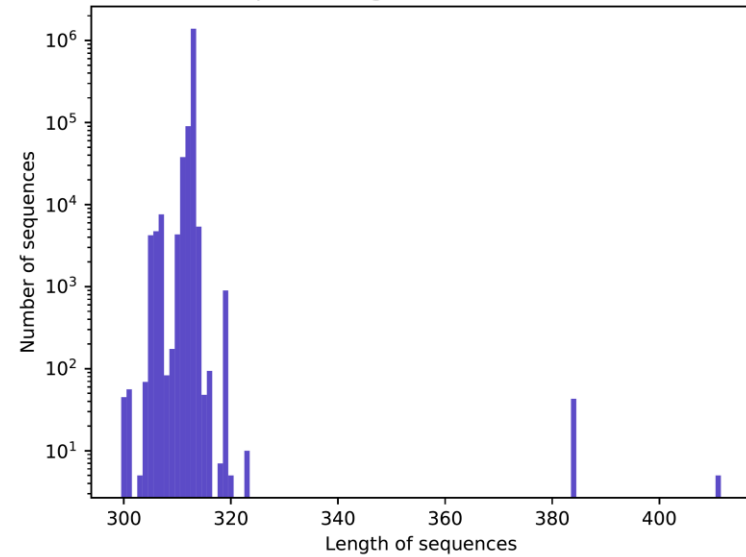


Length control of the reads

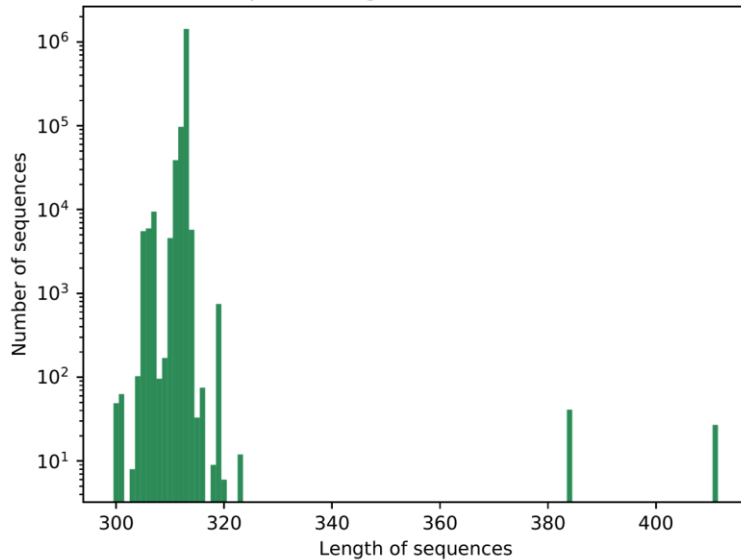
Sequence length distribution: Pool 1



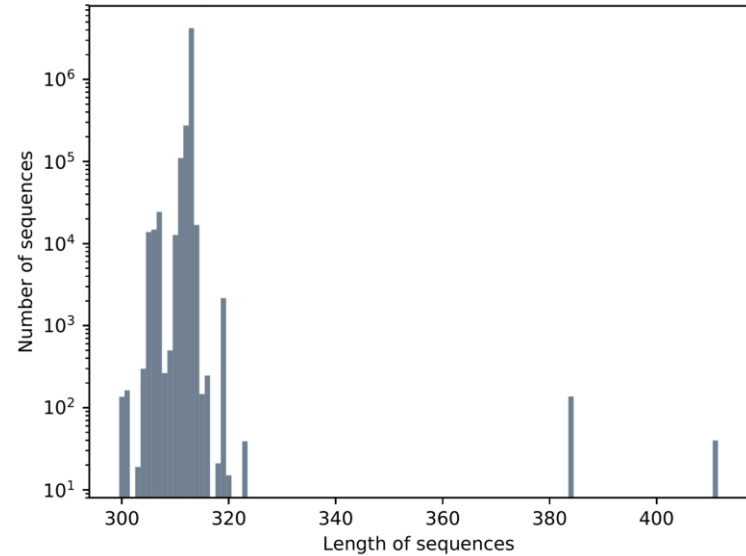
Sequence length distribution: Pool 2



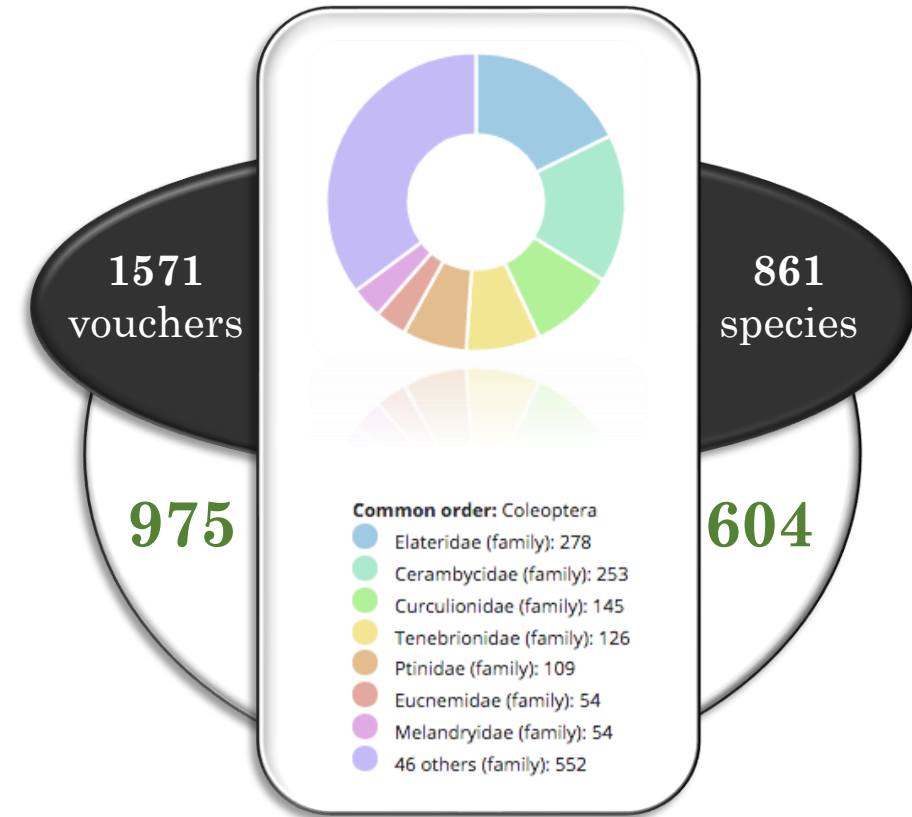
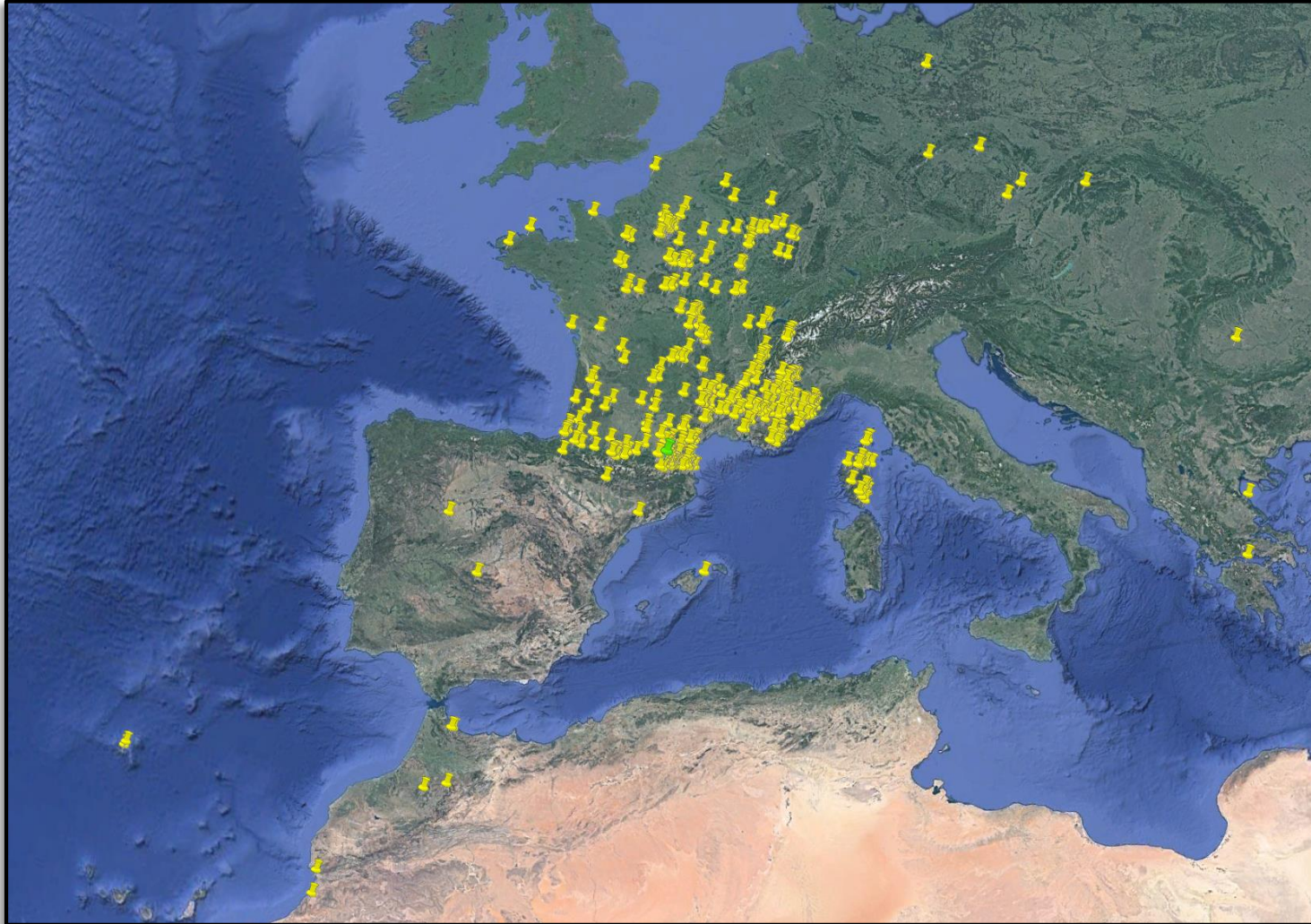
Sequence length distribution: Pool 3



Sequence length distribution: Pools combined



Reference library for saproxylic beetles



Rougerie et al., 2015

Sire et al., in prep.