



**HAL**  
open science

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

Lucas Sire, Rodolphe Rougerie, Laurent Larrieu, Annie Bézier, Béatrice Courtial, Christophe Bouget, Elisabeth Herniou, Carlos Lopez-Vaamonde

## ► To cite this version:

Lucas Sire, Rodolphe Rougerie, Laurent Larrieu, Annie Bézier, Béatrice Courtial, et al.. DNA Metabarcoding to quantify the response of insect diversity to mountain forest die-offs in the French Pyrenees. International Conference on Ecological Sciences (Sfécologie 2018), Oct 2018, Rennes, France. 825 p. hal-02736320

**HAL Id: hal-02736320**

**<https://hal.inrae.fr/hal-02736320>**

Submitted on 2 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

L. Sire<sup>1</sup>, R. Rougerie<sup>2</sup>, L. Larrieu<sup>3,4</sup>, A. Bézier<sup>1</sup>, B. Courtial<sup>5</sup>, C. Bouget<sup>6</sup>, E. Herniou<sup>1</sup>, C. Lopez-Vaamonde<sup>1,5</sup>

<sup>1</sup>IRBI, UMR 7261 – Université de Tours – Tours, France; <sup>2</sup>MNHN, UMR 7205 – Paris, France ; <sup>3</sup>INRA, UMR 1201 DYNAFOR – Castanet Tolosan, France; <sup>4</sup>CRPFOcc – Auzeville Tolosane, France; <sup>5</sup>INRA, UR0633 Zoologie Forestière – Orléans, France ; <sup>6</sup>IRSTEA, UR EFNO – Nogent-sur-Vernisson, France

**Lucas Sire**

2<sup>nd</sup> year of *Ph.D.* at IRBI – Tours

lucas.sire@univ-tours.fr

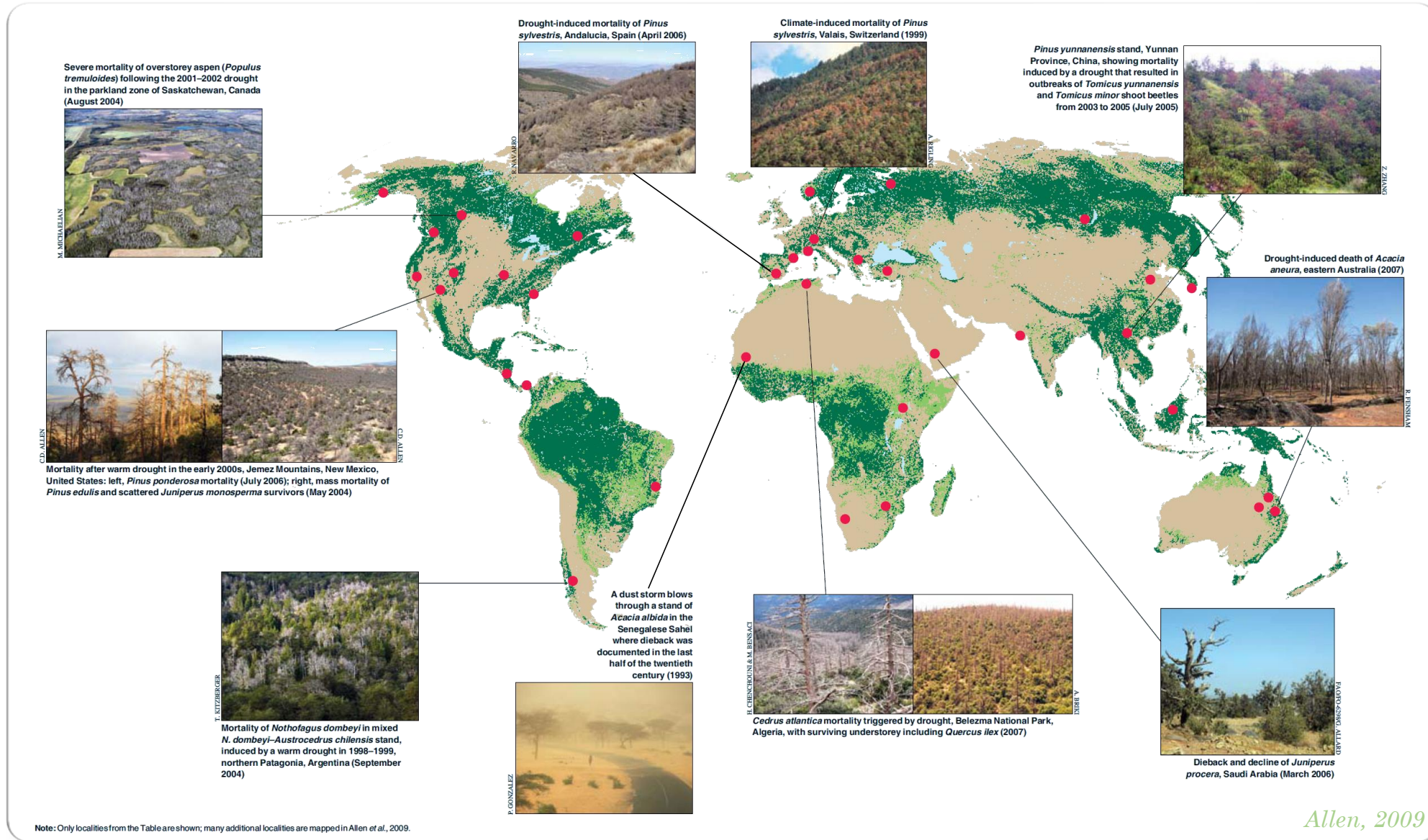


SFEcologie

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<sup>1\*</sup>, Martin Sorg<sup>2</sup>, Eelke Jongejans<sup>1</sup>, Henk Siepel<sup>1</sup>, Nick Hofland<sup>1</sup>, Heinz Schwan<sup>2</sup>, Werner Stenmans<sup>2</sup>, Andreas Müller<sup>2</sup>, Hubert Sumser<sup>2</sup>, Thomas Hören<sup>2</sup>, Dave Goulson<sup>3</sup>, Hans de Kroon<sup>1</sup>

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**

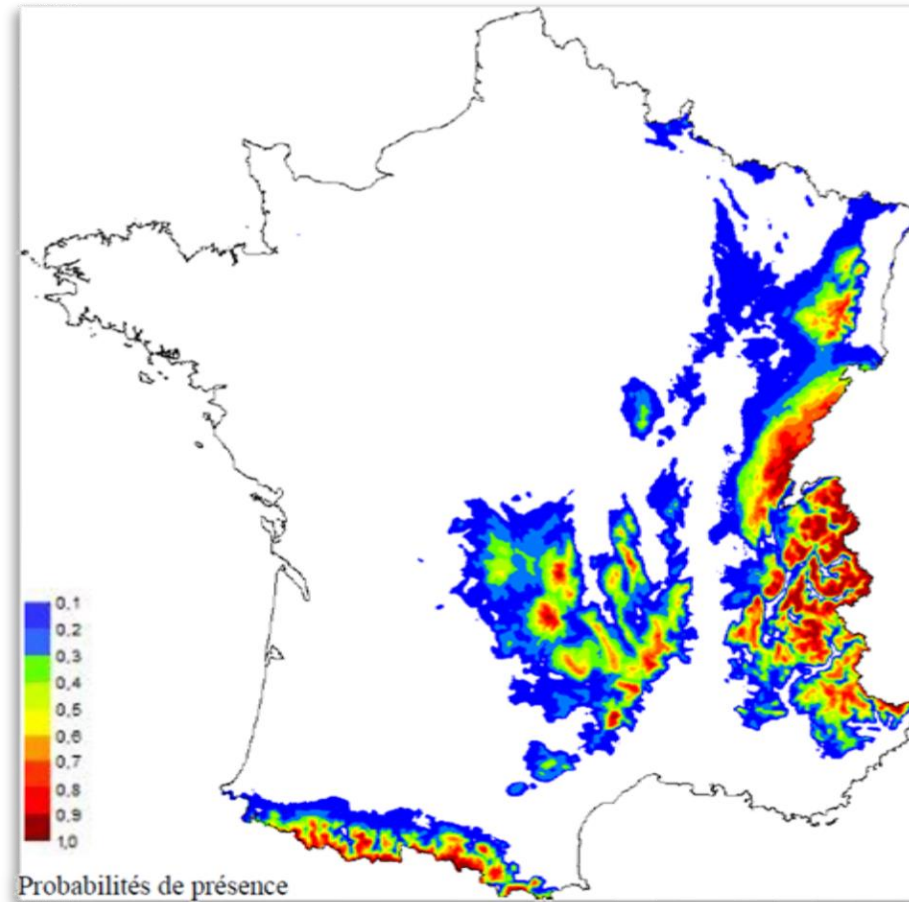
## This presentation will focus on:

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

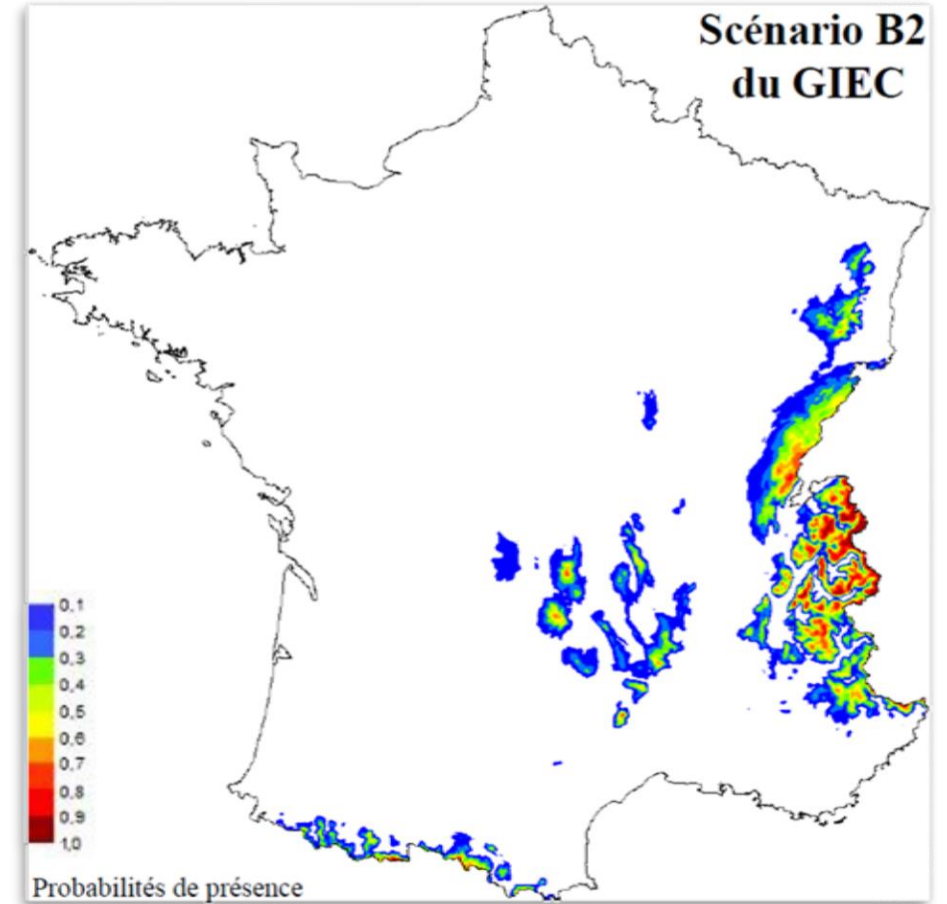
# Situation of Silver fir in France



actuel

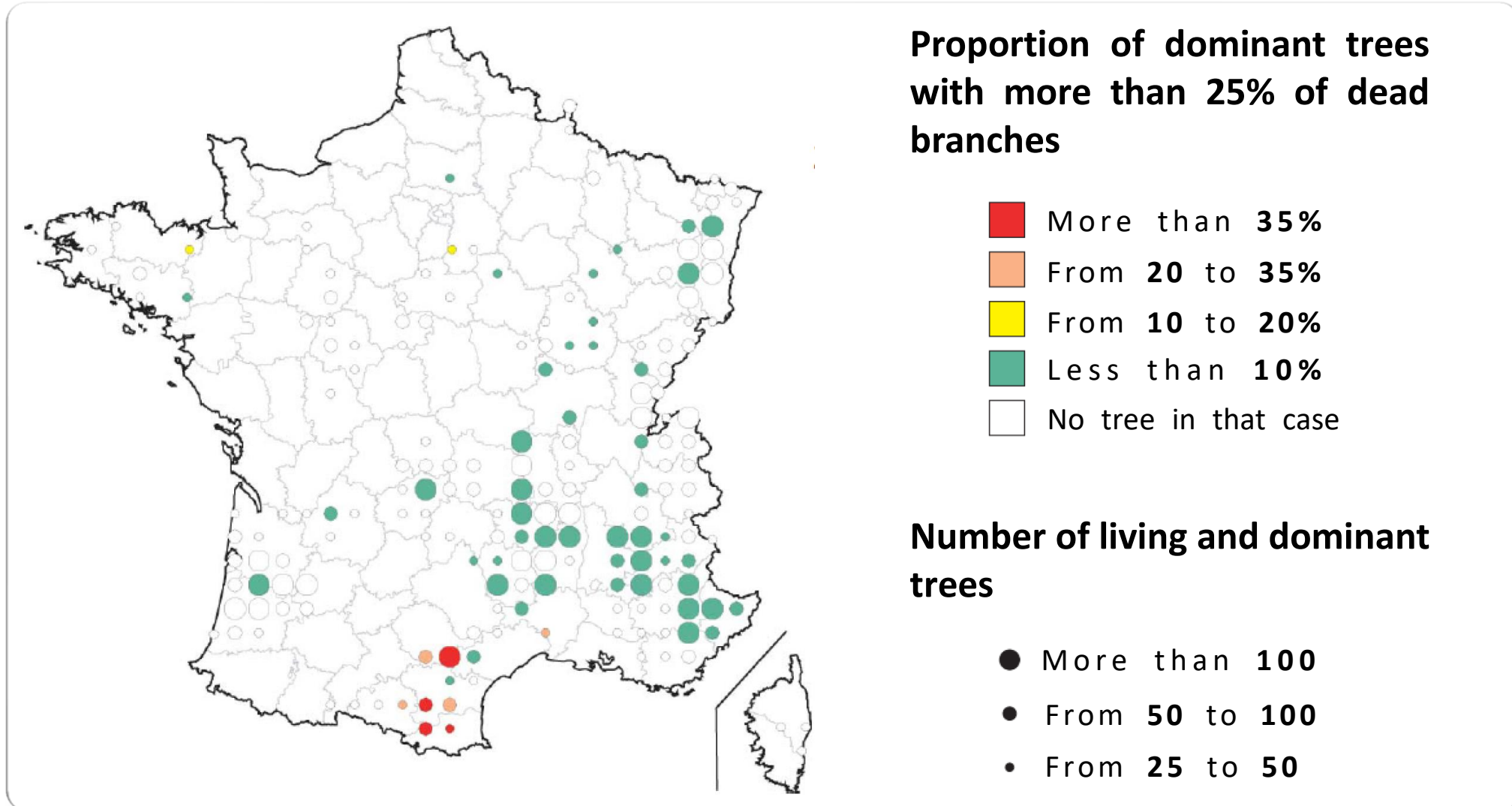


2100



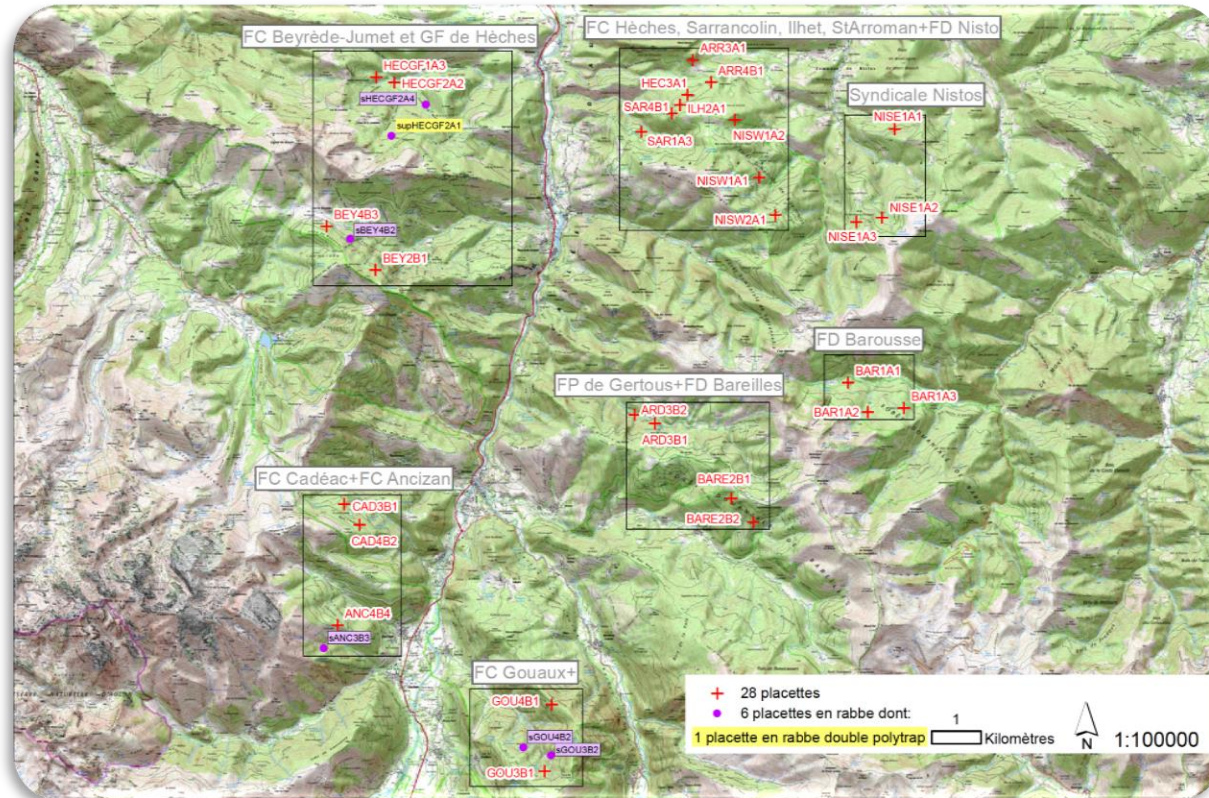
*Badeau et al., 2005*

# Situation of Silver fir in France

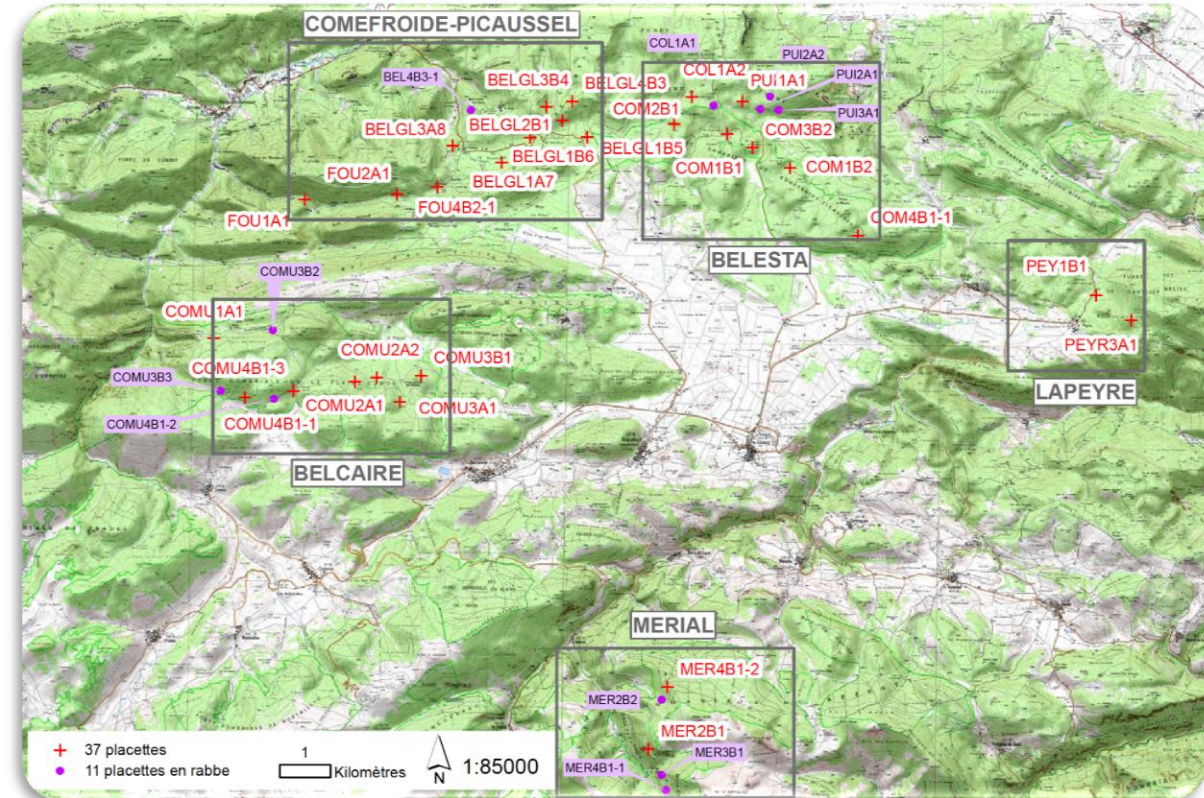




# Experimental design



Vallée d'Aure



Pays de Sault

N = 57 plots

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





# Ecological parameters

**Mistletoe**  
Largest  $\phi$  >20cm



**Perennial polypore**  
Largest  $\phi$  >5cm



**Invertebrate nest**  
Presence

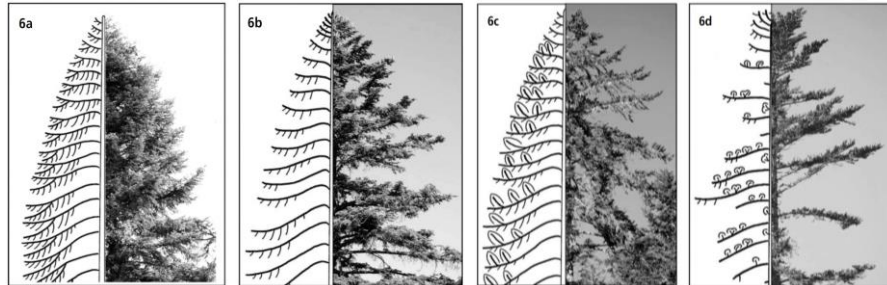


**Pulpy agaric**  
Largest  $\phi$  >5cm or cluster of > 10 fruiting bodies



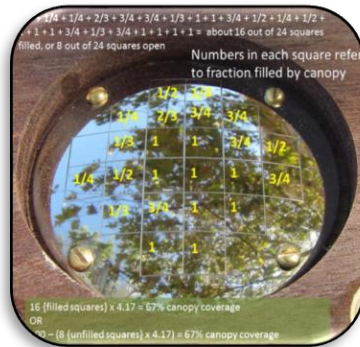
## Micro-dendrohabitat assessment

*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



## Methods in Ecology and Evolution

Methods in Ecology and Evolution 2012, 3, 613–623

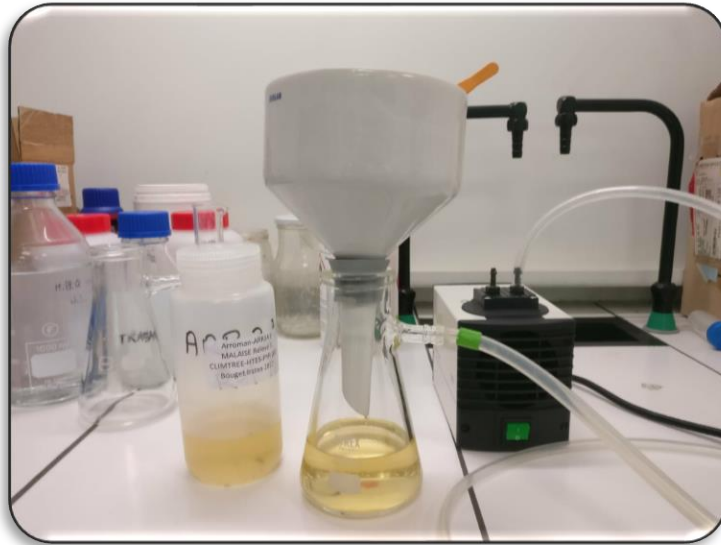
doi: 10.1111/j.2041-210X.2012.00198.x

### Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring

Douglas W. Yu<sup>1,2\*†</sup>, Yinqiu Ji<sup>1†</sup>, Brent C. Emerson<sup>2‡</sup>, Xiaoyang Wang<sup>1</sup>, Chengxi Ye<sup>1</sup>, Chunyan Yang<sup>1</sup> and Zhaoli Ding<sup>3</sup>



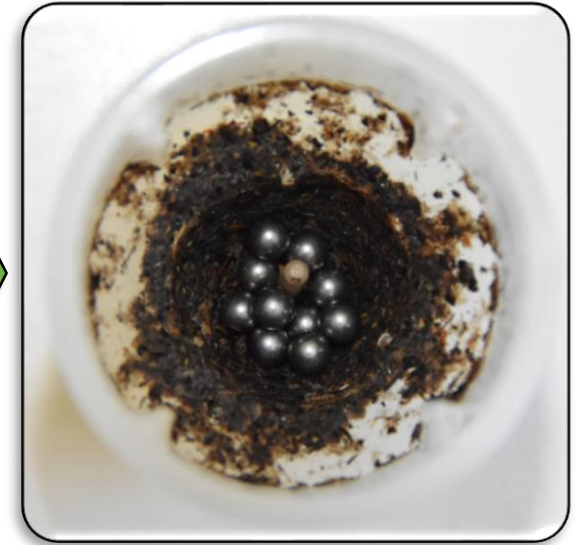
# Sample preparation



Trap filtration



Drying and  
homogenization



Grinding

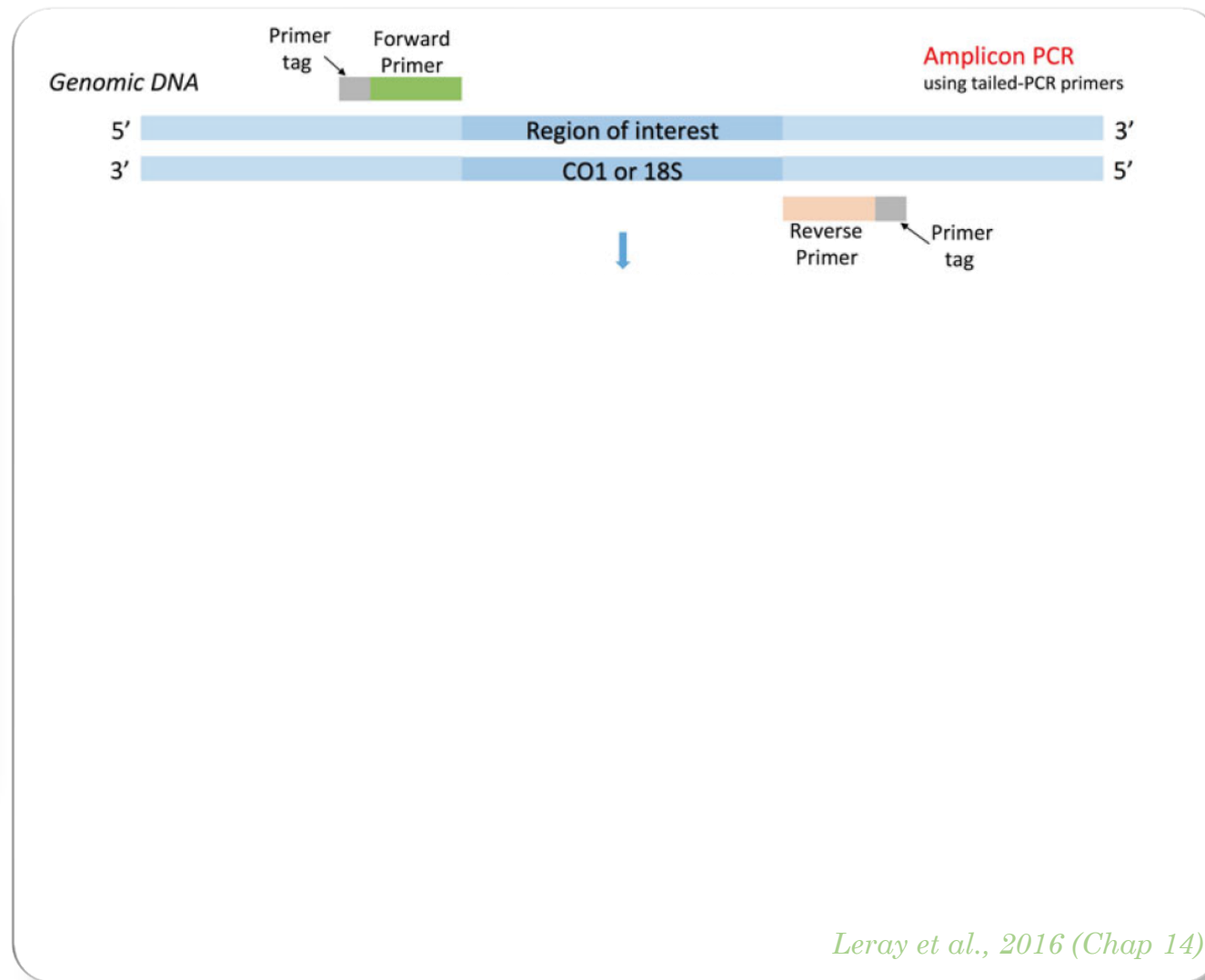
↓  
DNA extraction

# 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*



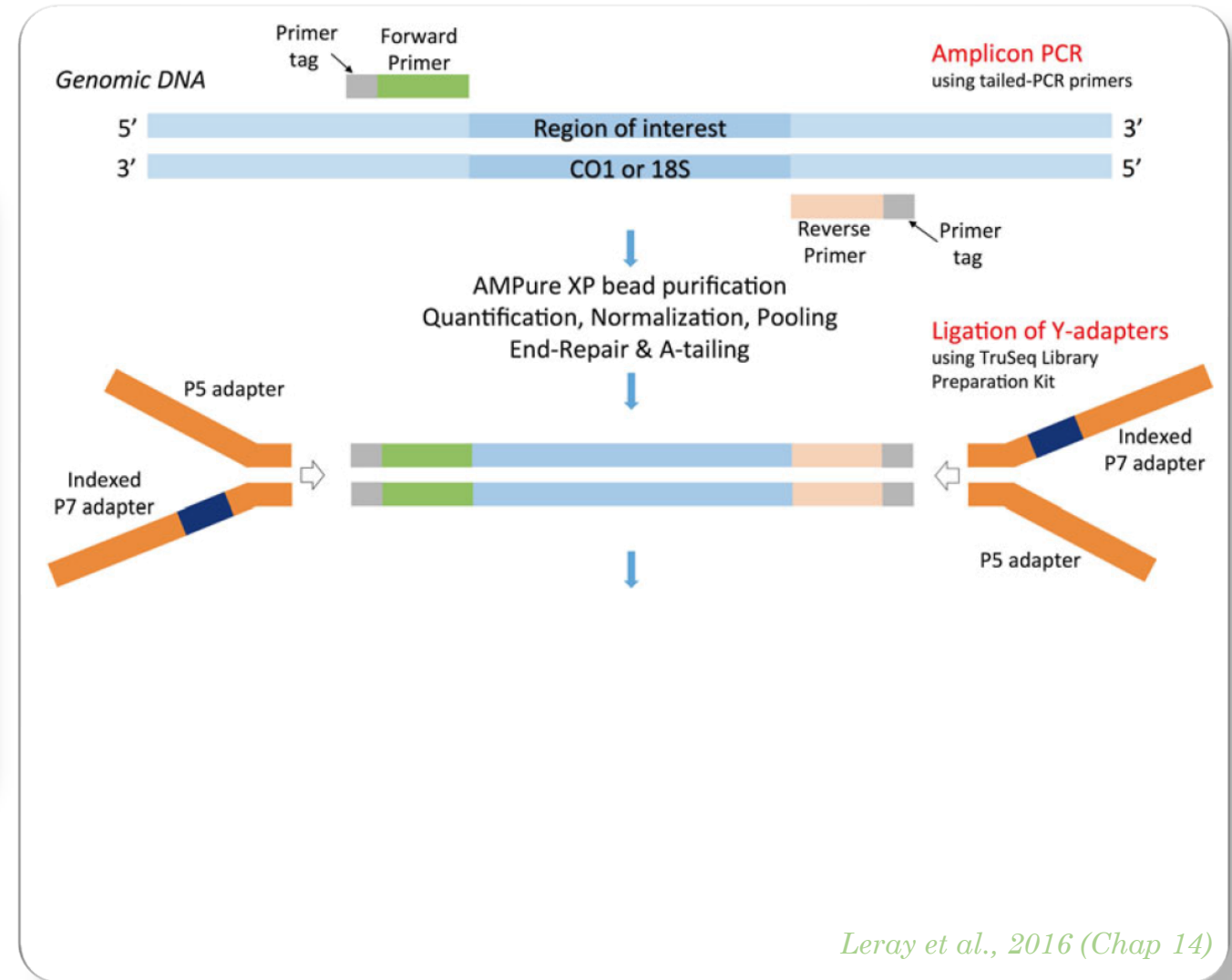
*Leray et al., 2016 (Chap 14)*

# Twin-tagging dual indexing PCRs

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

*Leray et al., 2013*

*Geller et al., 2013*



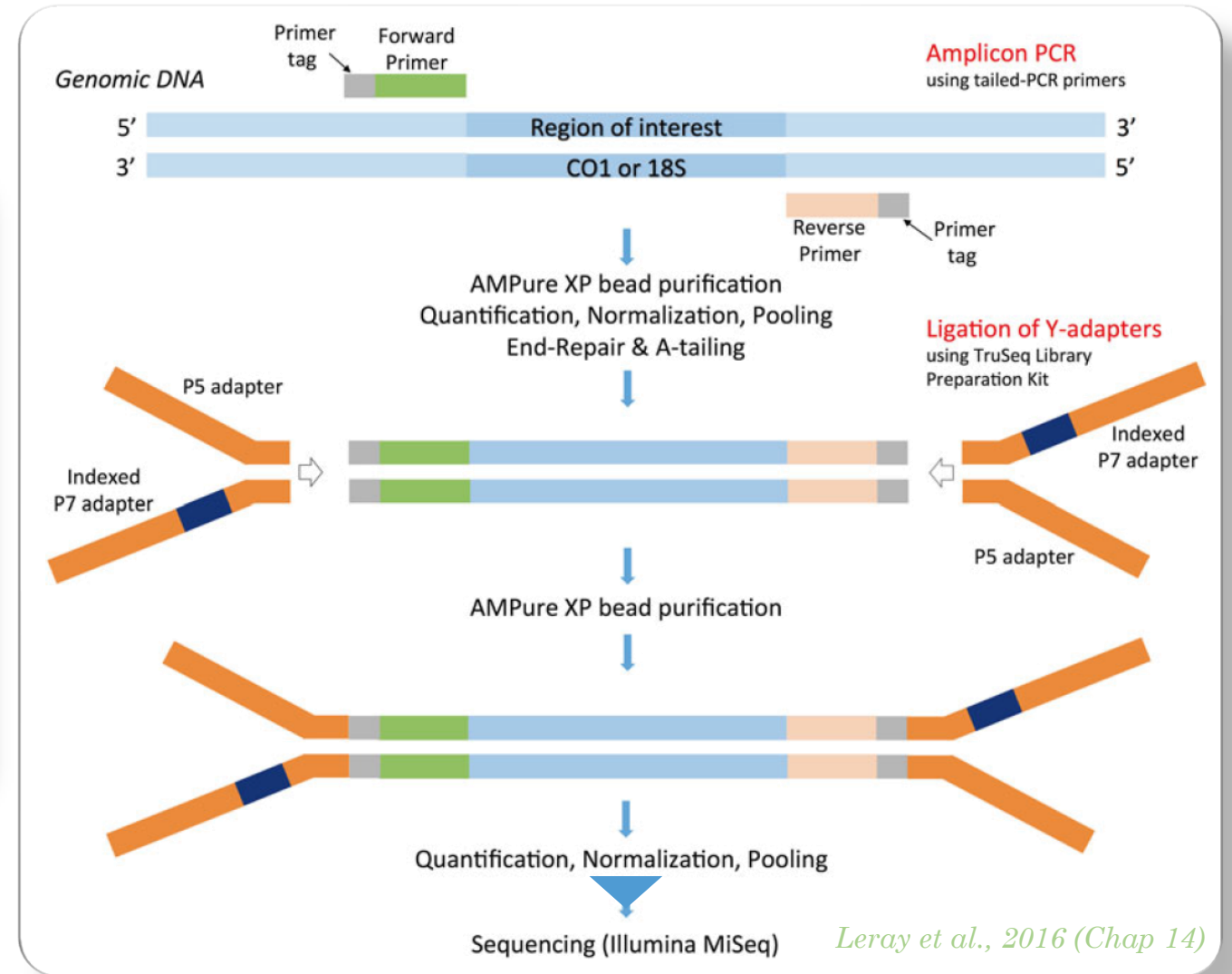


# Twin-tagging dual indexing PCRs

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

*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

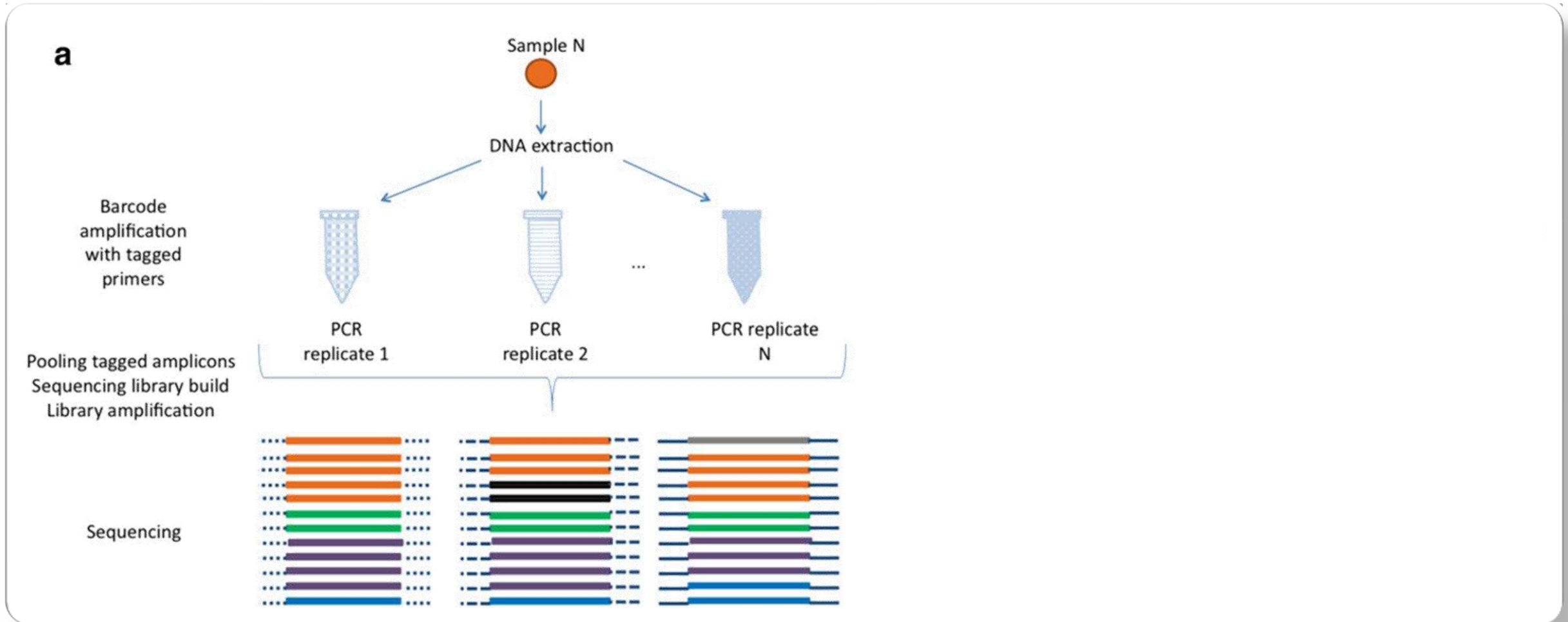
Open Access



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

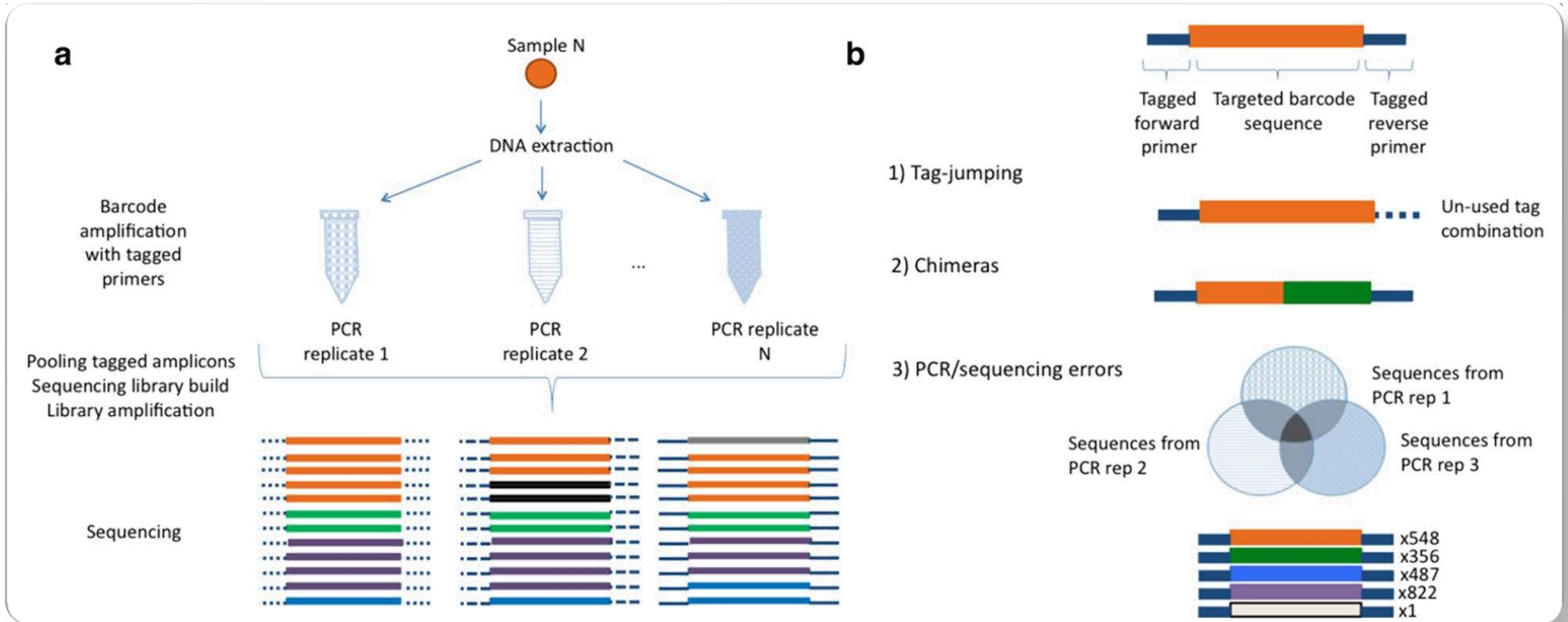
Marie Lisandra Zepeda-Mendoza<sup>1\*</sup>, Kristine Bohmann<sup>1</sup>, Aldo Carmona Baez<sup>1,2</sup> and M. Thomas P. Gilbert<sup>1</sup>

# Bio-informatic pipeline



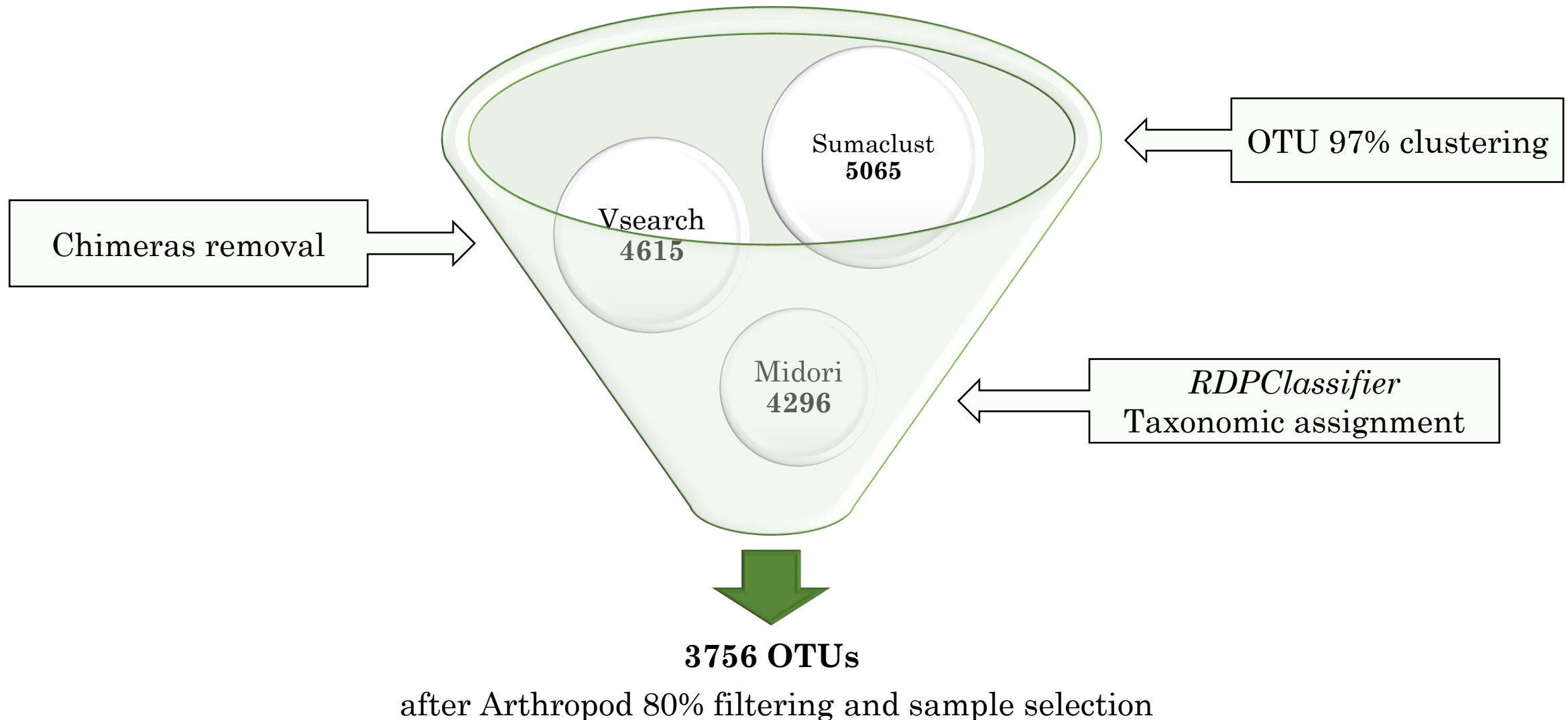


# Bio-informatic pipeline



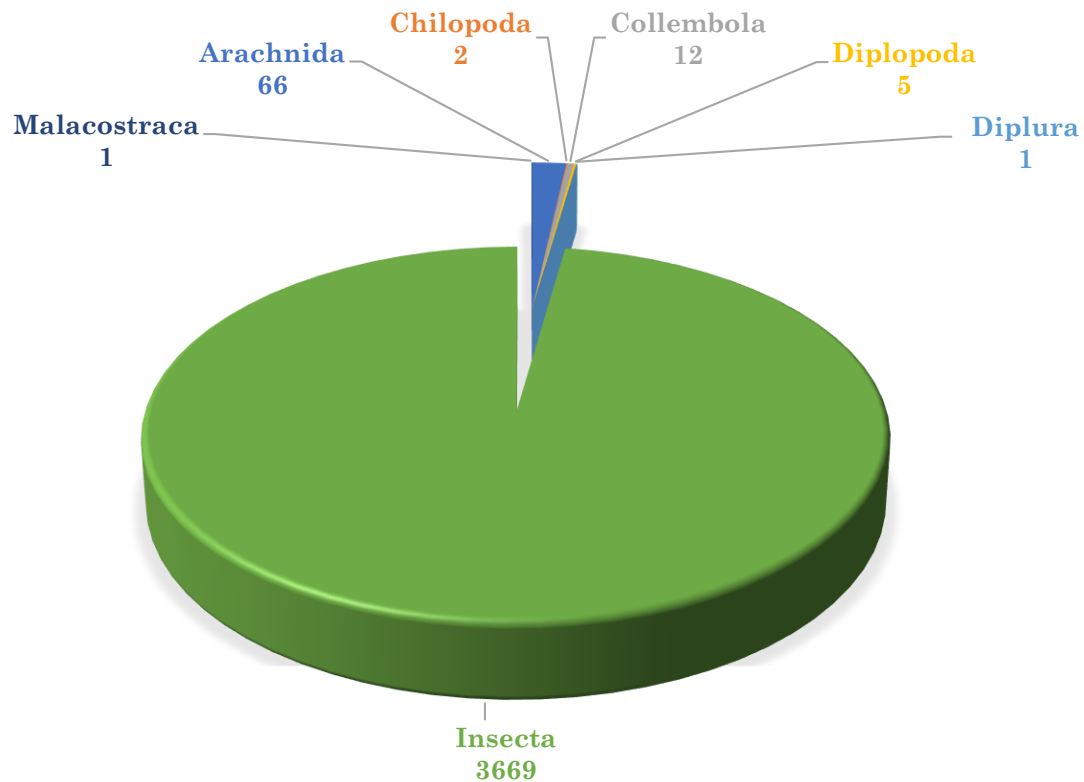
# Bio-informatic pipeline

Minimum 4 reads in each of the 3 PCR replicates

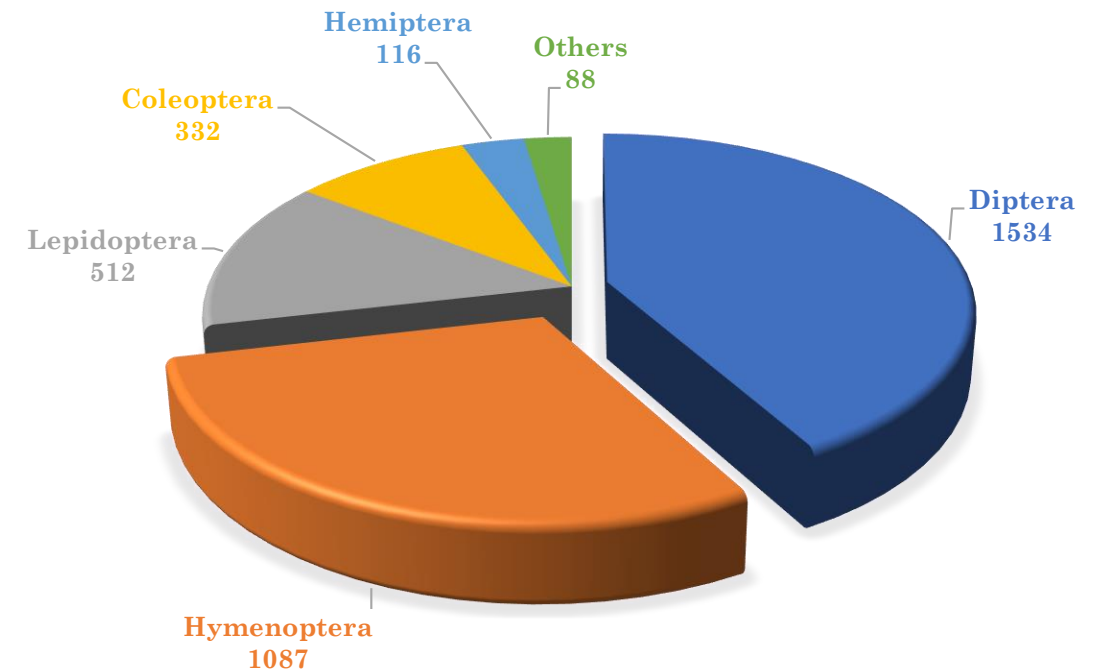


# Species richness for Malaise traps

3756 OTUs found



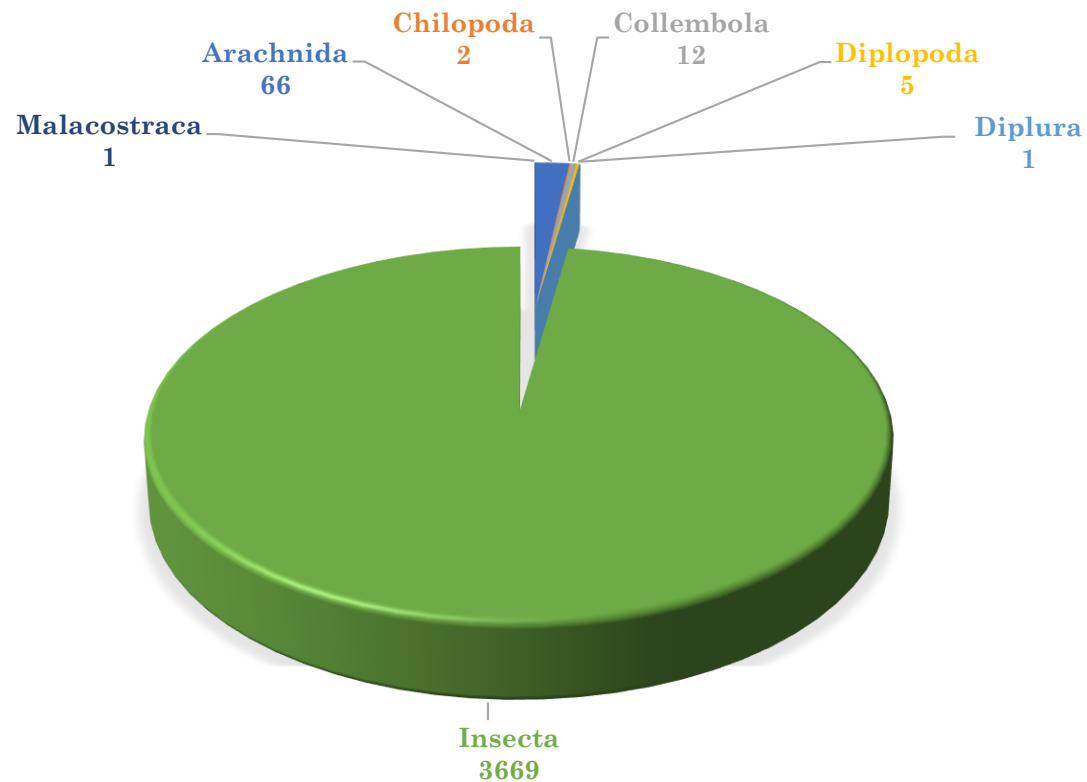
18 Insecta orders represented



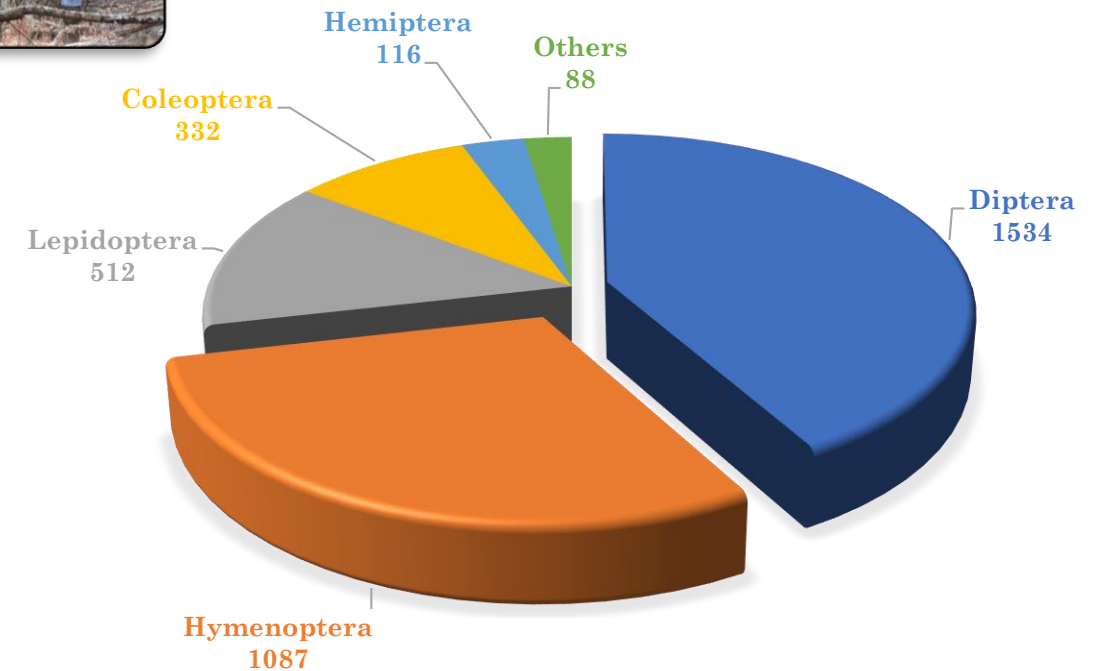


# Species richness for Malaise traps

3756 OTUs found



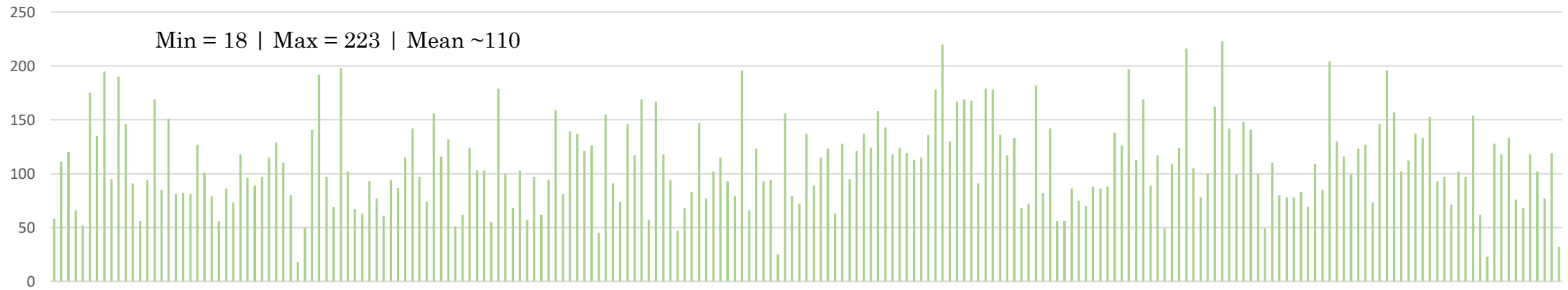
18 Insecta orders represented



# Trap composition

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

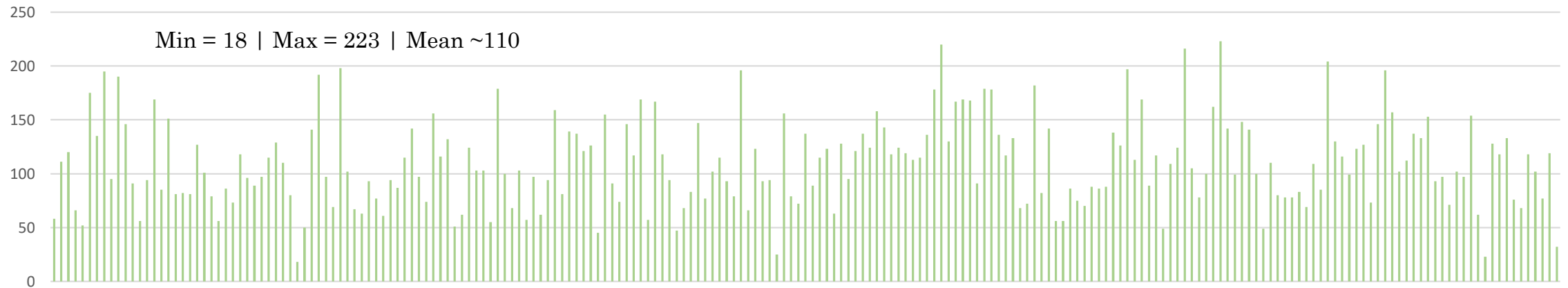
Number of OTUs per bulk sample



# Trap composition

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

Number of OTUs per bulk sample

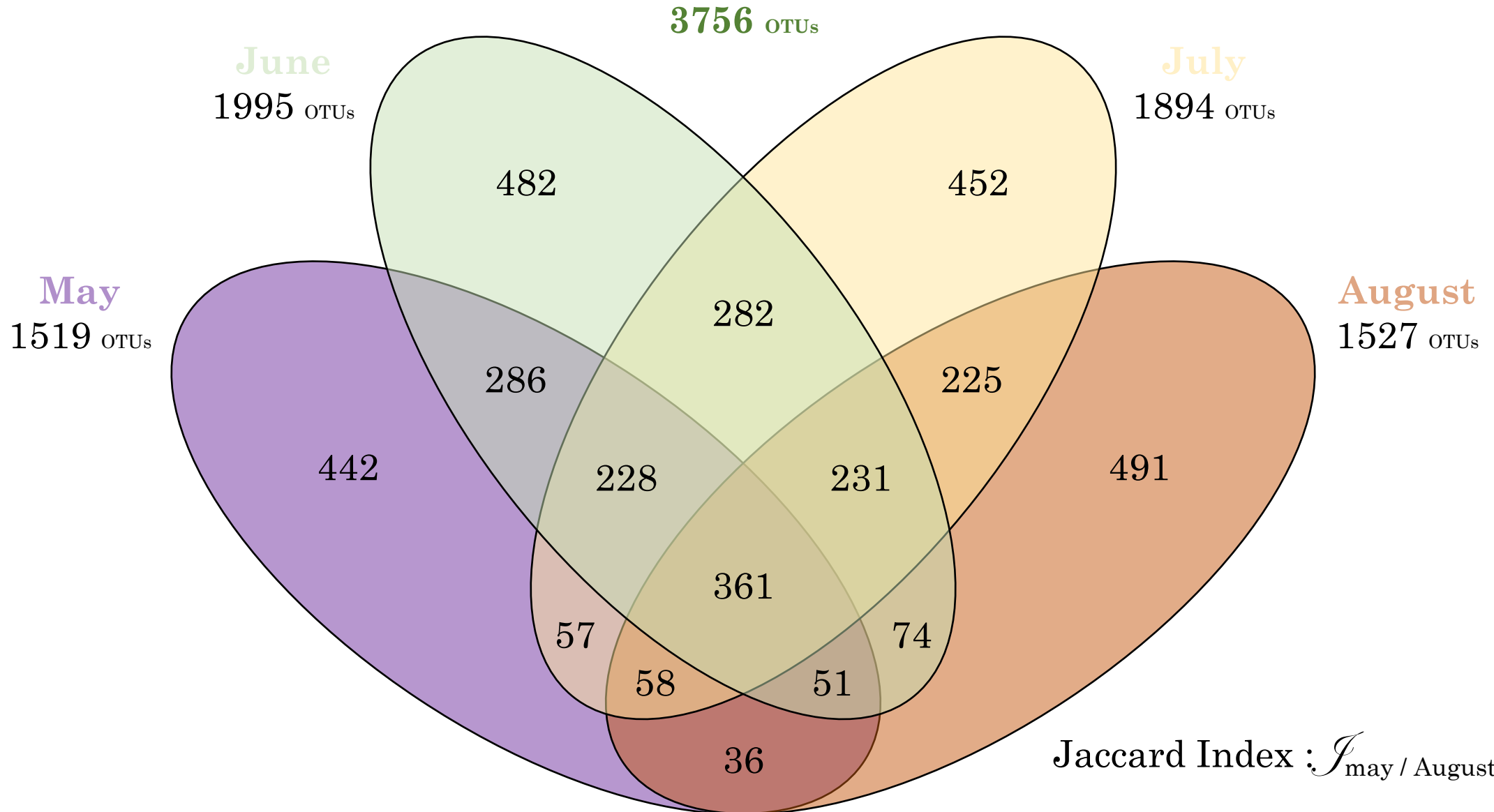


Number of bulk samples per OTU





# 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





# Detection of pests and invasive species

ICONOGRAPHIA COLEOPTERORUM POLONIAE  
Copyright © by Lech Borowiec



0.5mm

*Hylesinus toranio*



1.0mm

*Xylosandrus germanus*

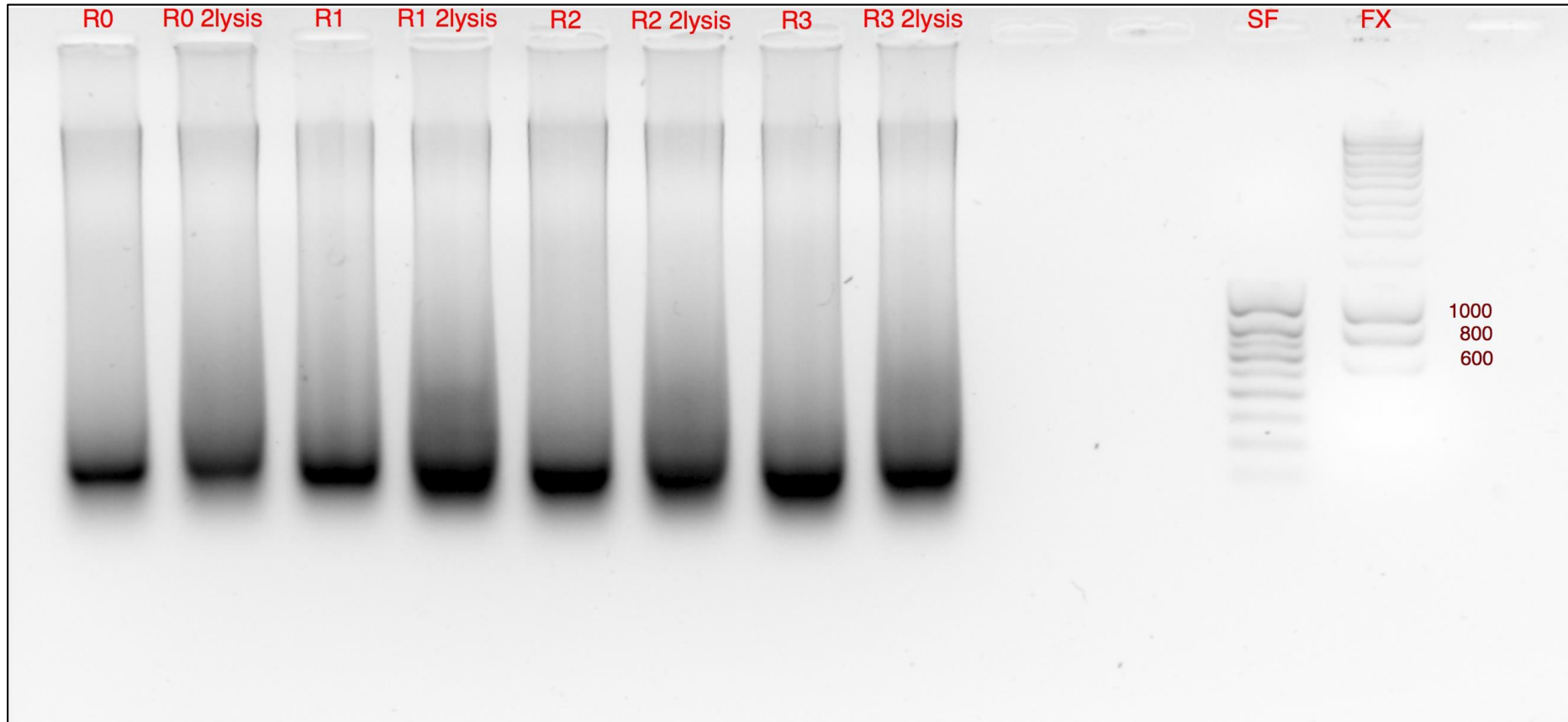


1.0cm

*Vespa vetulina*

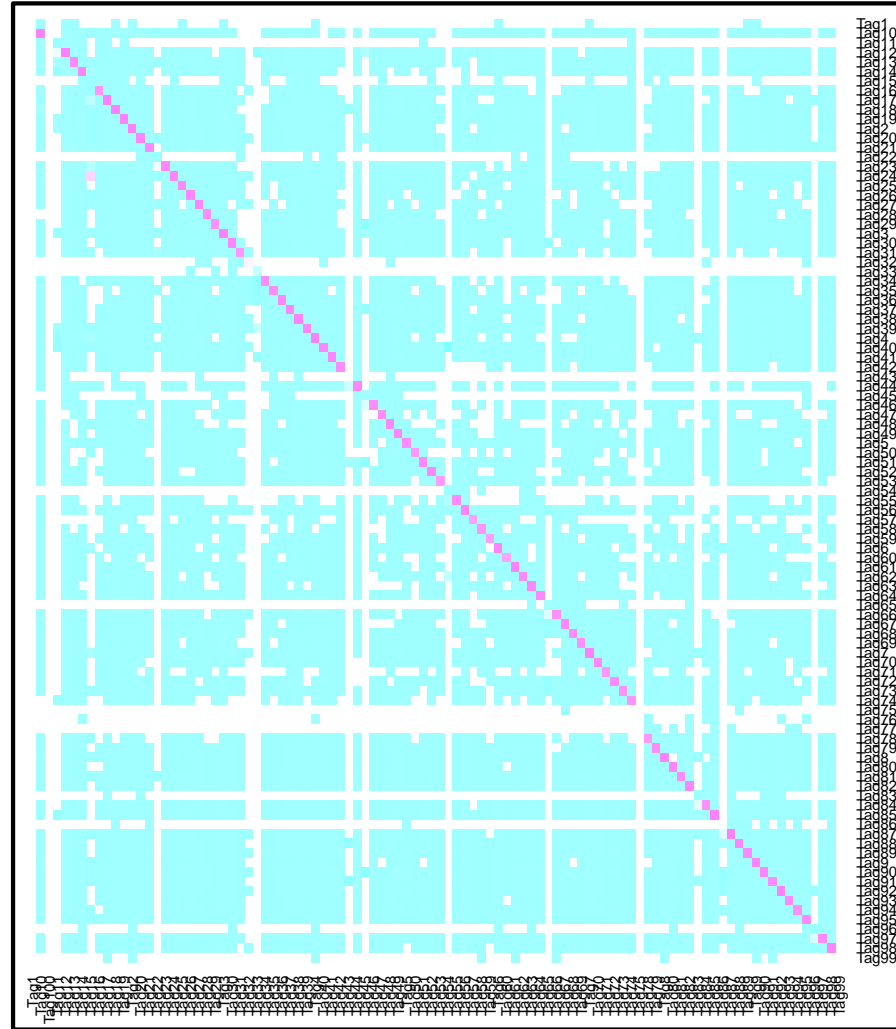
# Degradation of samples

## 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

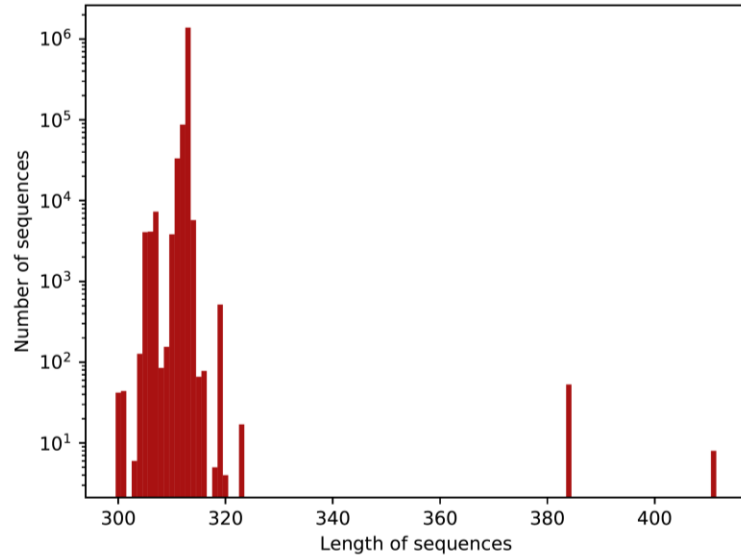
# Heatmap of primer-tag combinations



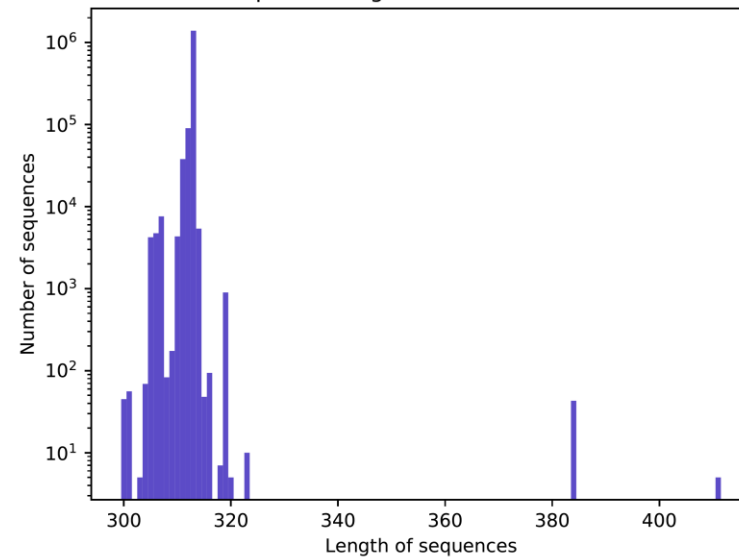


# Length control of the reads

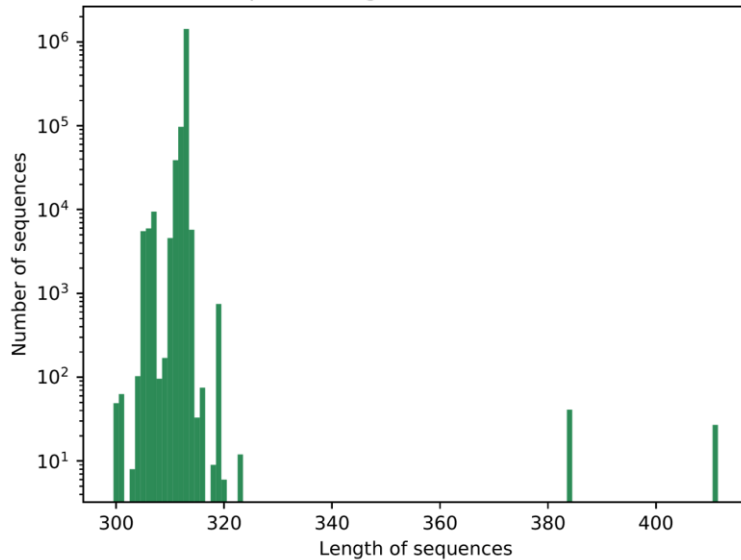
Sequence length distribution: Pool 1



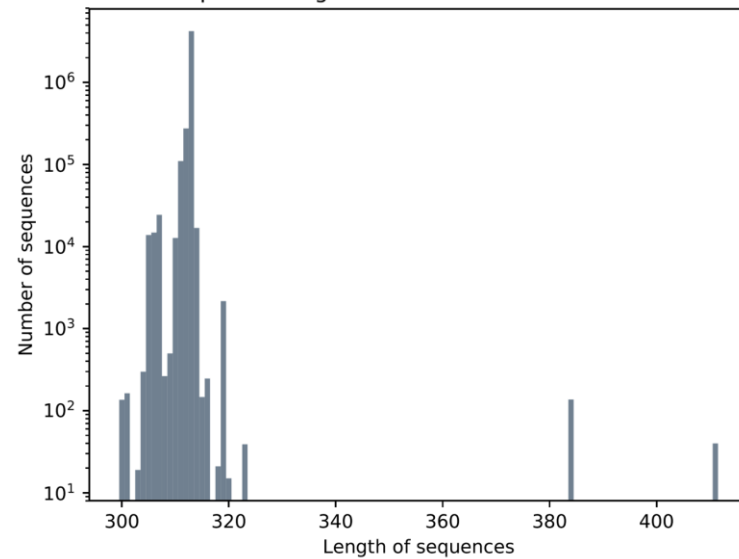
Sequence length distribution: Pool 2



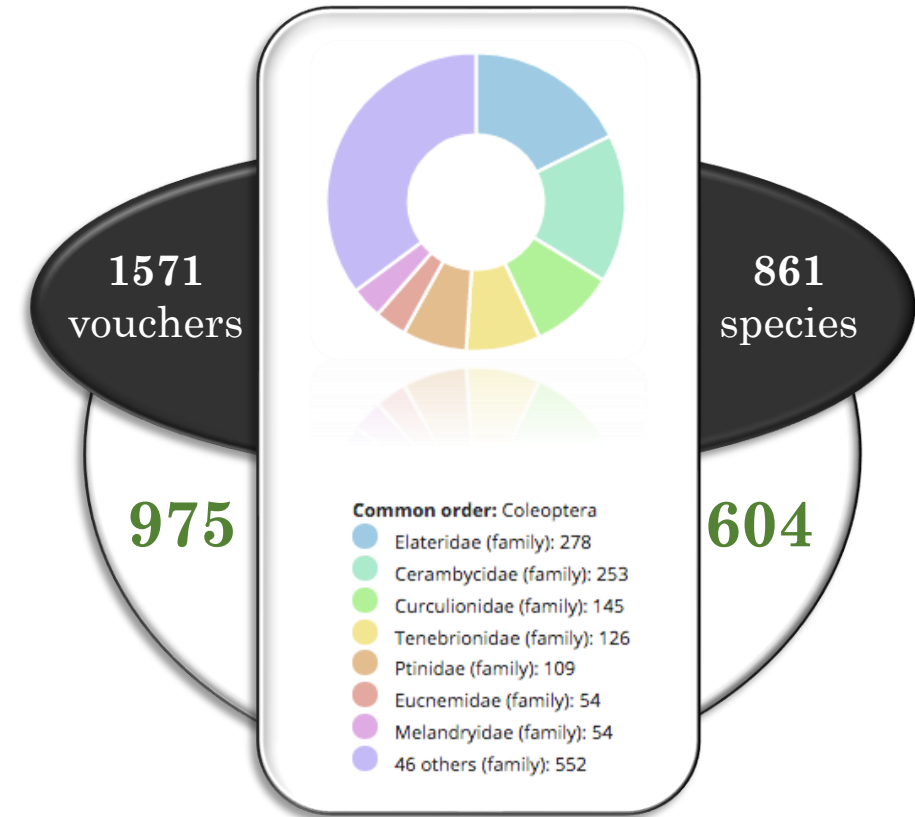
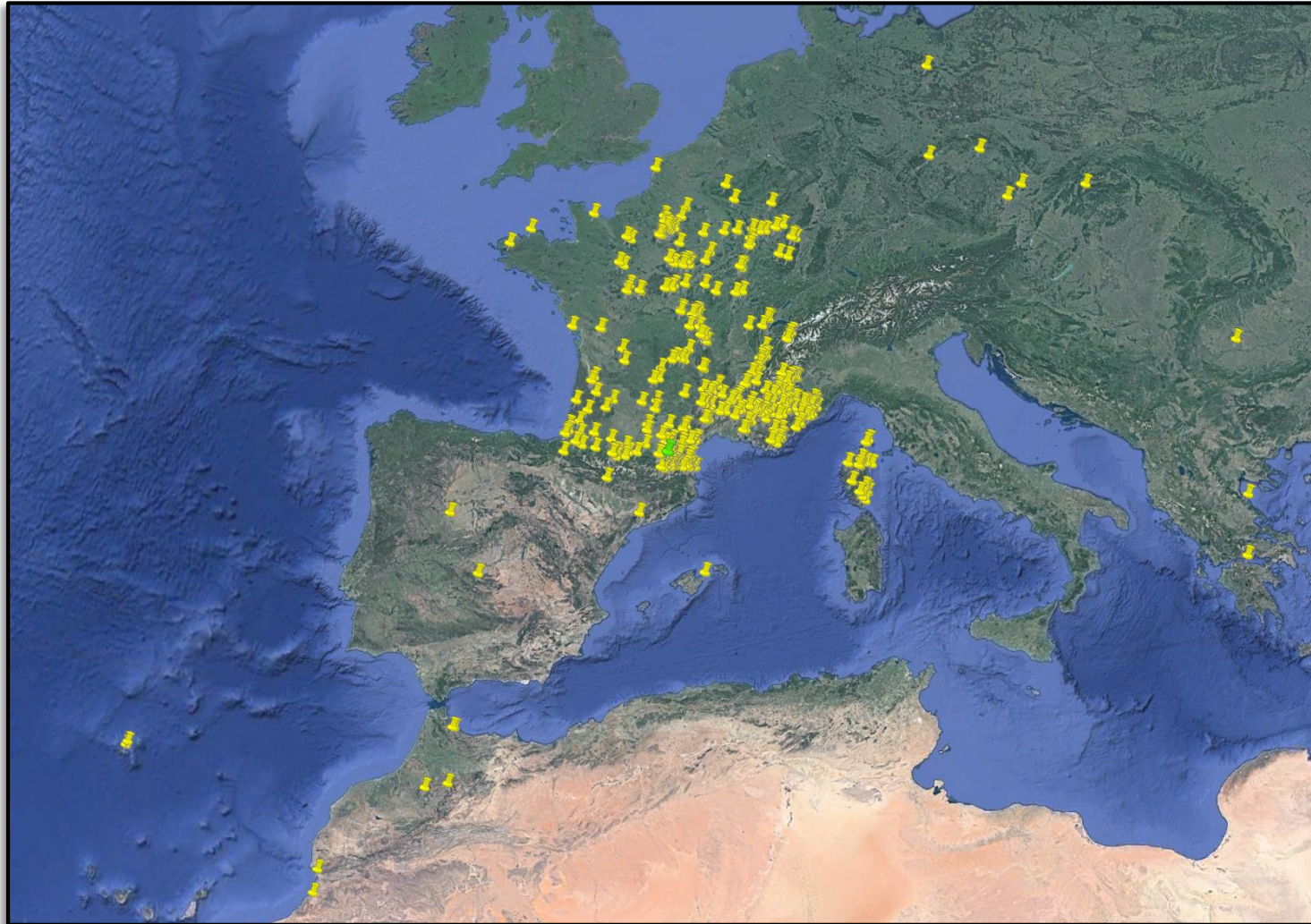
Sequence length distribution: Pool 3



Sequence length distribution: Pools combined



# Reference library for saproxylic beetles



*Rougerie et al., 2015*

*Sire et al., in prep.*