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

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

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

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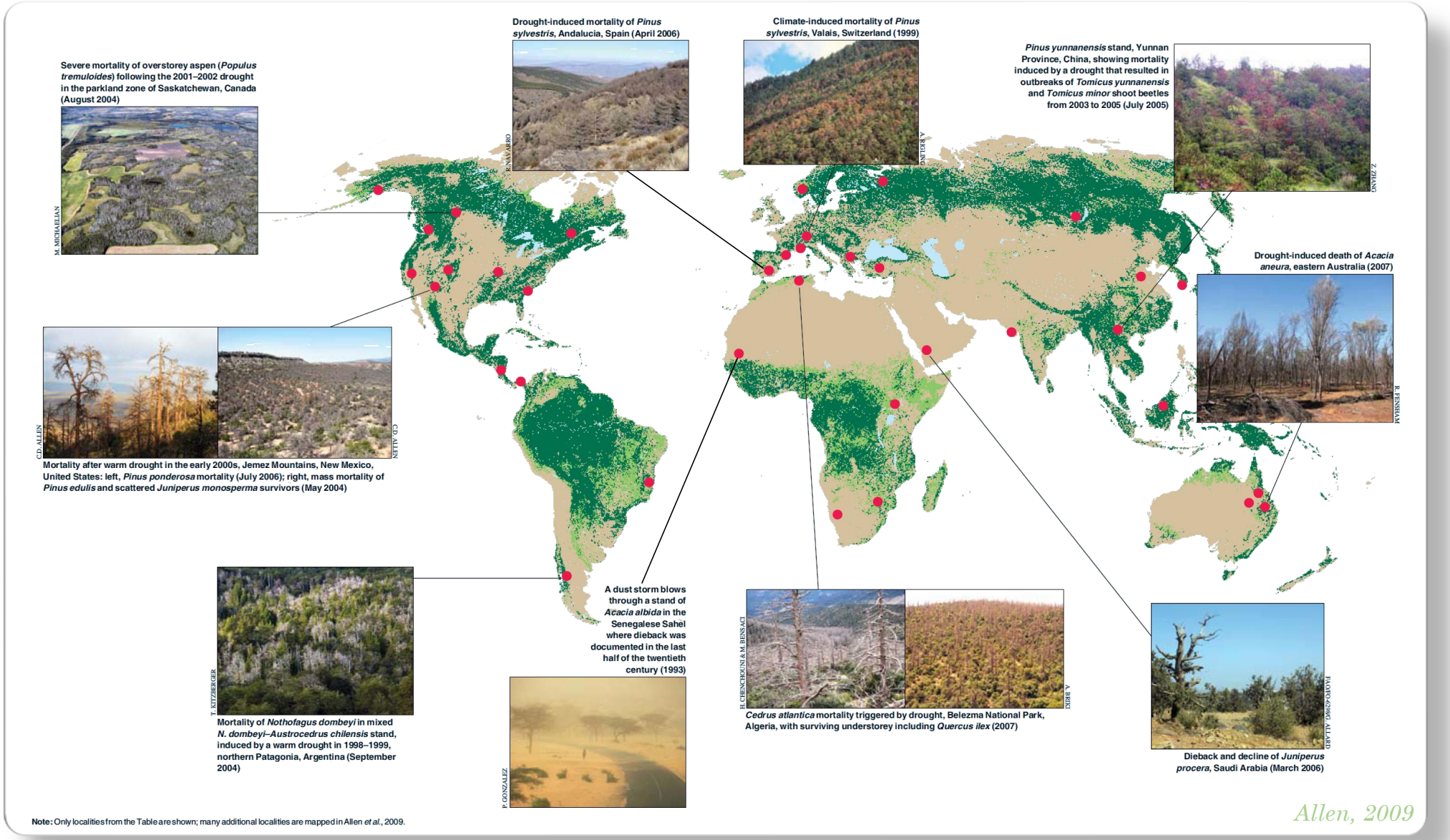
SFEcologie

Rennes – 23 Octobre 2018

Forest perturbations due to climate change



Allen, 2009

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 Sorg[2], Eelke Jongejans[1], Henk Siepel[1], Nick Hofland[1], Heinz Schwan[2], Werner Stenmans[2], Andreas Müller[2], Hubert Sumser[2], Thomas Hörren[2], Dave Goulson[3], Hans de Kroon[1]

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

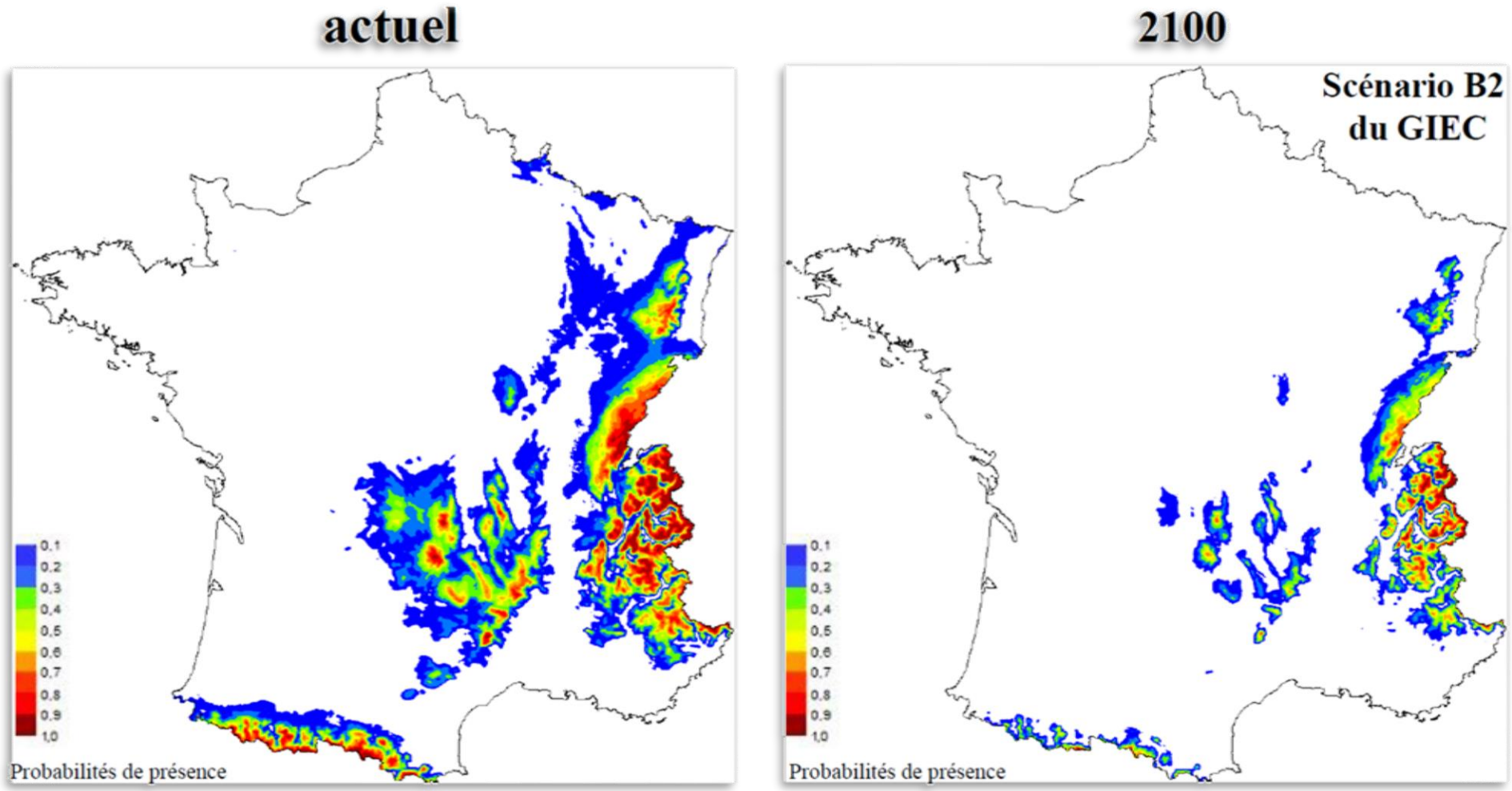
Thesis aims

- **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:

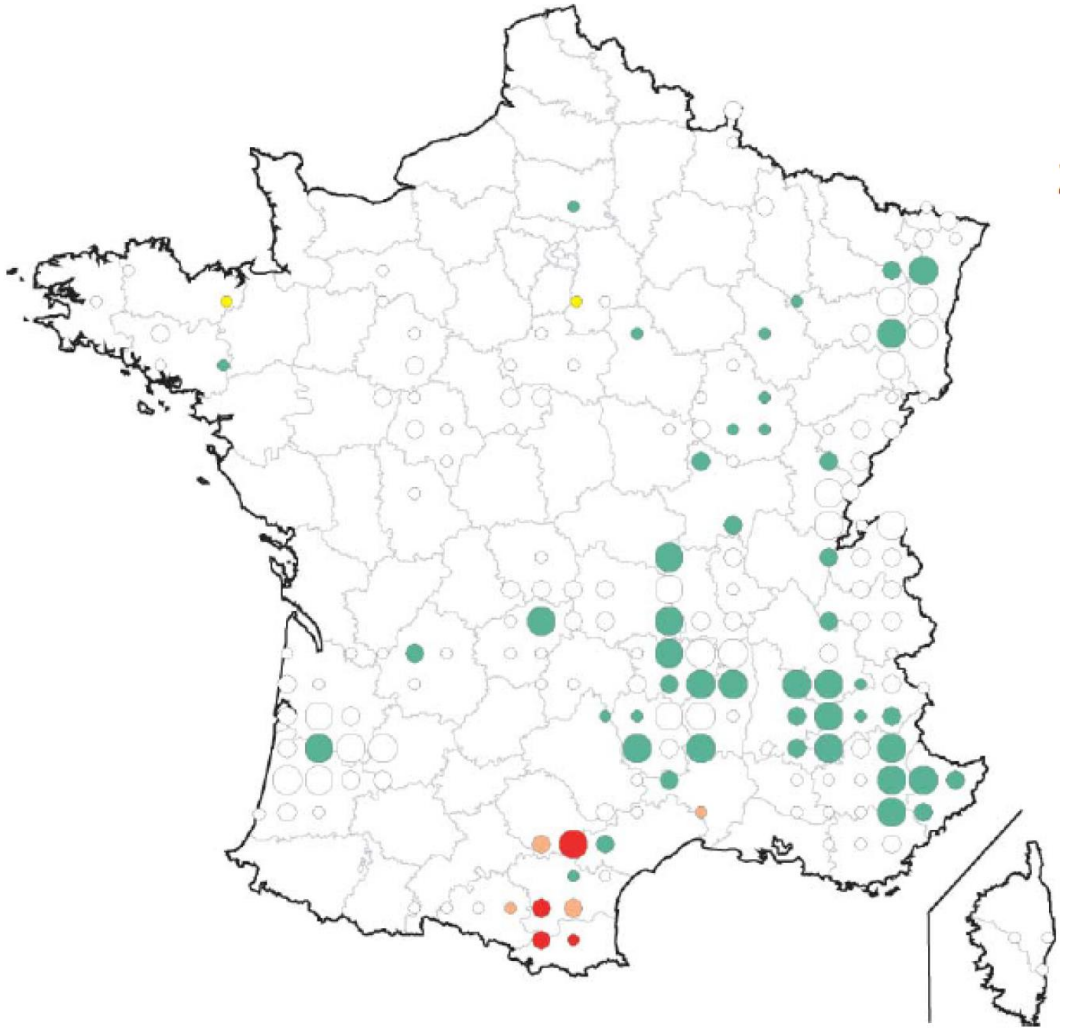
- What is the species richness in arthropods ?
- Temporal turnover
- Test DAMe bioinformatic pipeline

Situation of Silver fir in France



Badeau et al., 2005

Situation of Silver fir in France



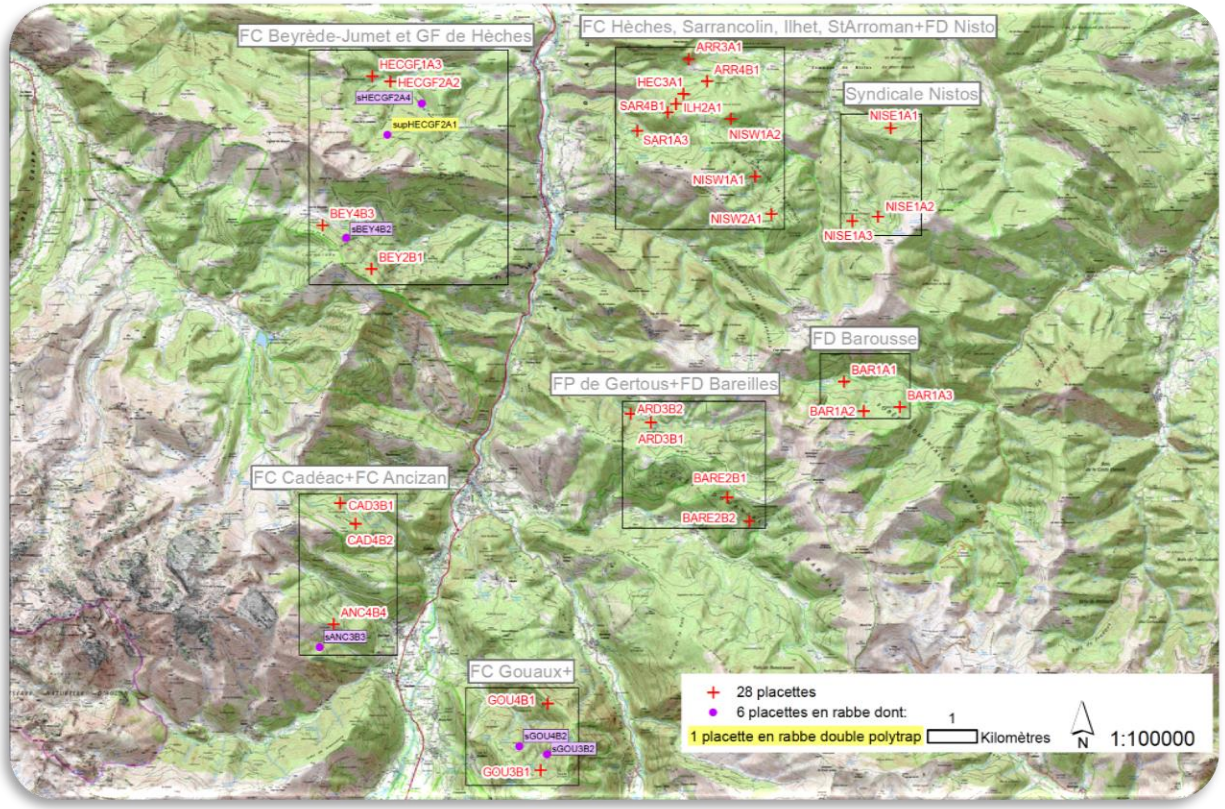
Proportion of dominant trees with more than 25% of dead branches

- More than **35%**
- From **20** to **35%**
- From **10** to **20%**
- Less than **10%**
- No tree in that case

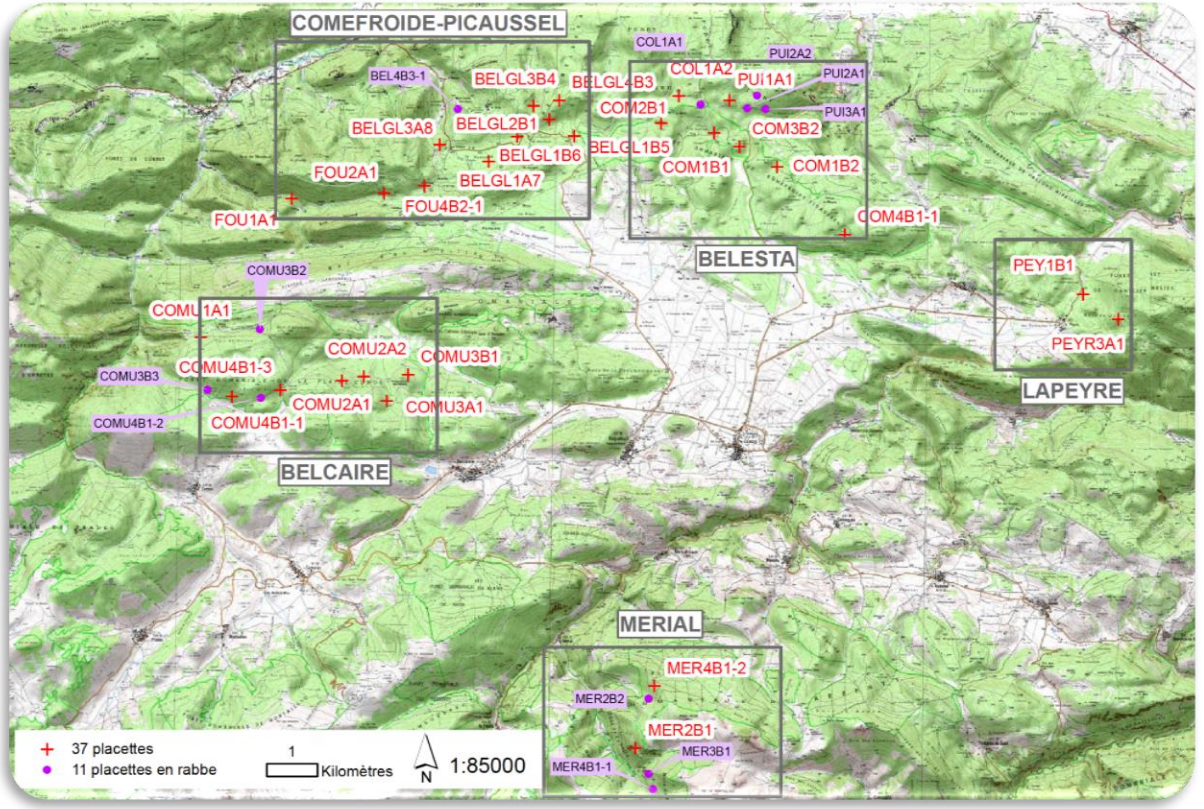
Number of living and dominant trees

- More than **100**
- From **50** to **100**
- From **25** to **50**

Experimental design



Vallée d'Aure

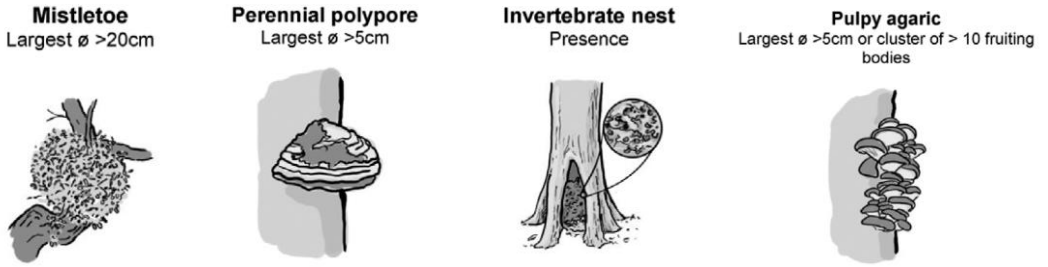


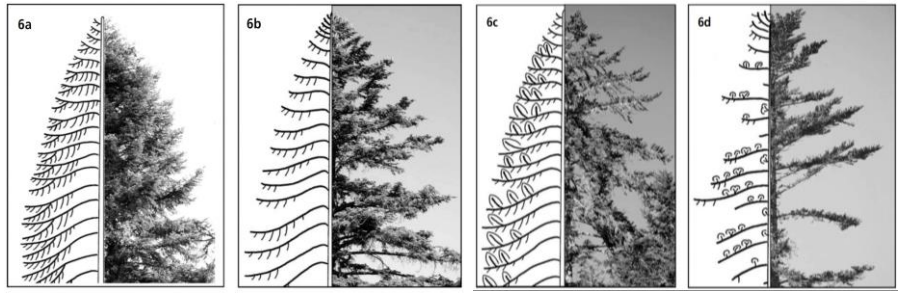
Pays de Sault

N = 57 plots

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

Ecological parameters





Micro-dendrohabitat assessment

Larrieu et al., 2018

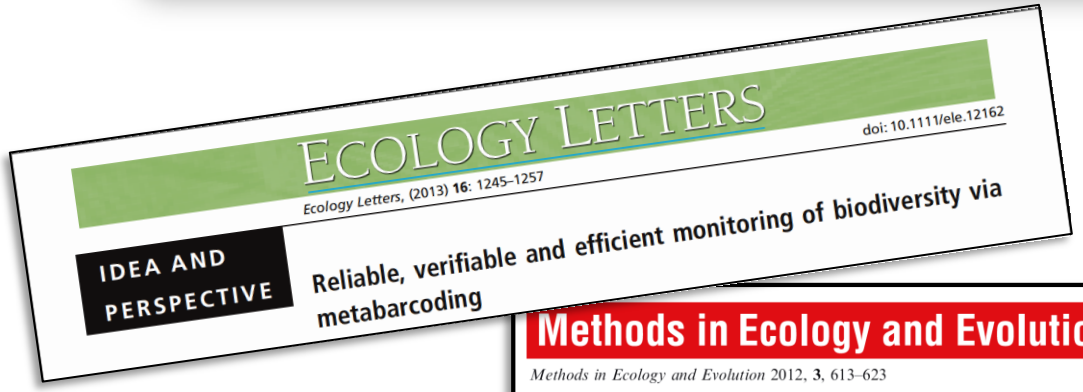
ARCHI method (Assess health state of dominant trees)

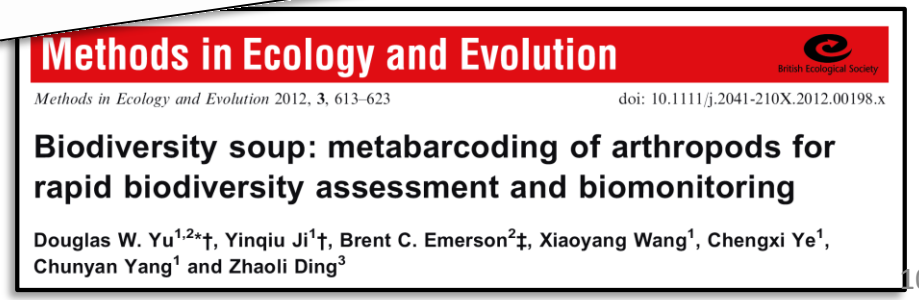
Drénou et al., 2013

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

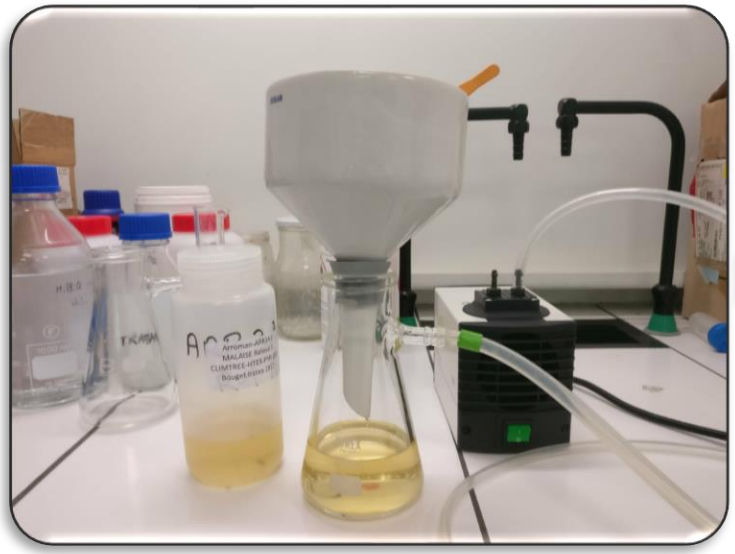
Biomonitoring using mass-trapping samples

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


ECOLOGY LETTERS
Ecology Letters, (2013) 16: 1245–1257
doi: 10.1111/ele.12162
IDEA AND PERSPECTIVE
Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding

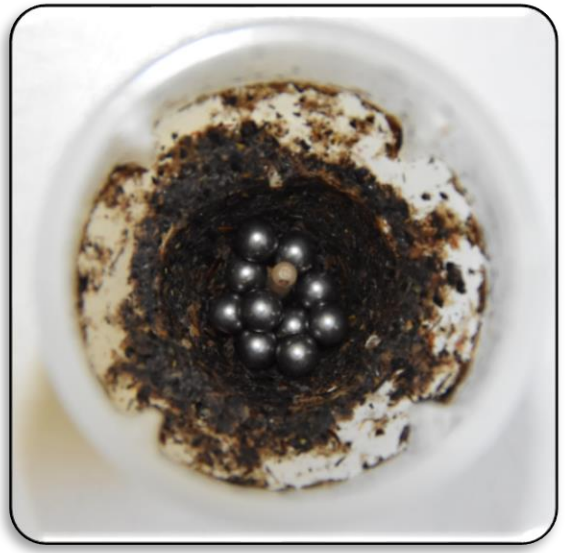

Methods in Ecology and Evolution
British Ecological Society
Methods in Ecology and Evolution 2012, 3, 613–623
doi: 10.1111/j.2041-210X.2012.00198.x
Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring
Douglas W. Yu[1,2*†], Yinqiu Ji[1†], Brent C. Emerson[2‡], Xiaoyang Wang[1], Chengxi Ye[1], Chunyan Yang[1] and Zhaoli Ding[3]

Sample preparation



Trap filtration

Drying and homogeneization



Grinding

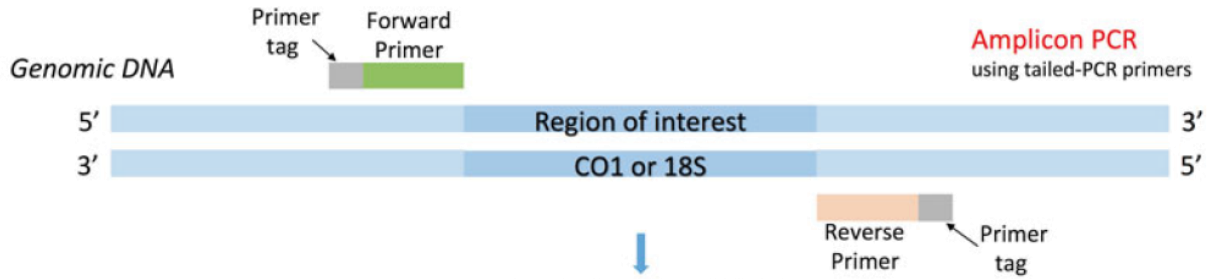
DNA extraction

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



Leray et al., 2016 (Chap 14)

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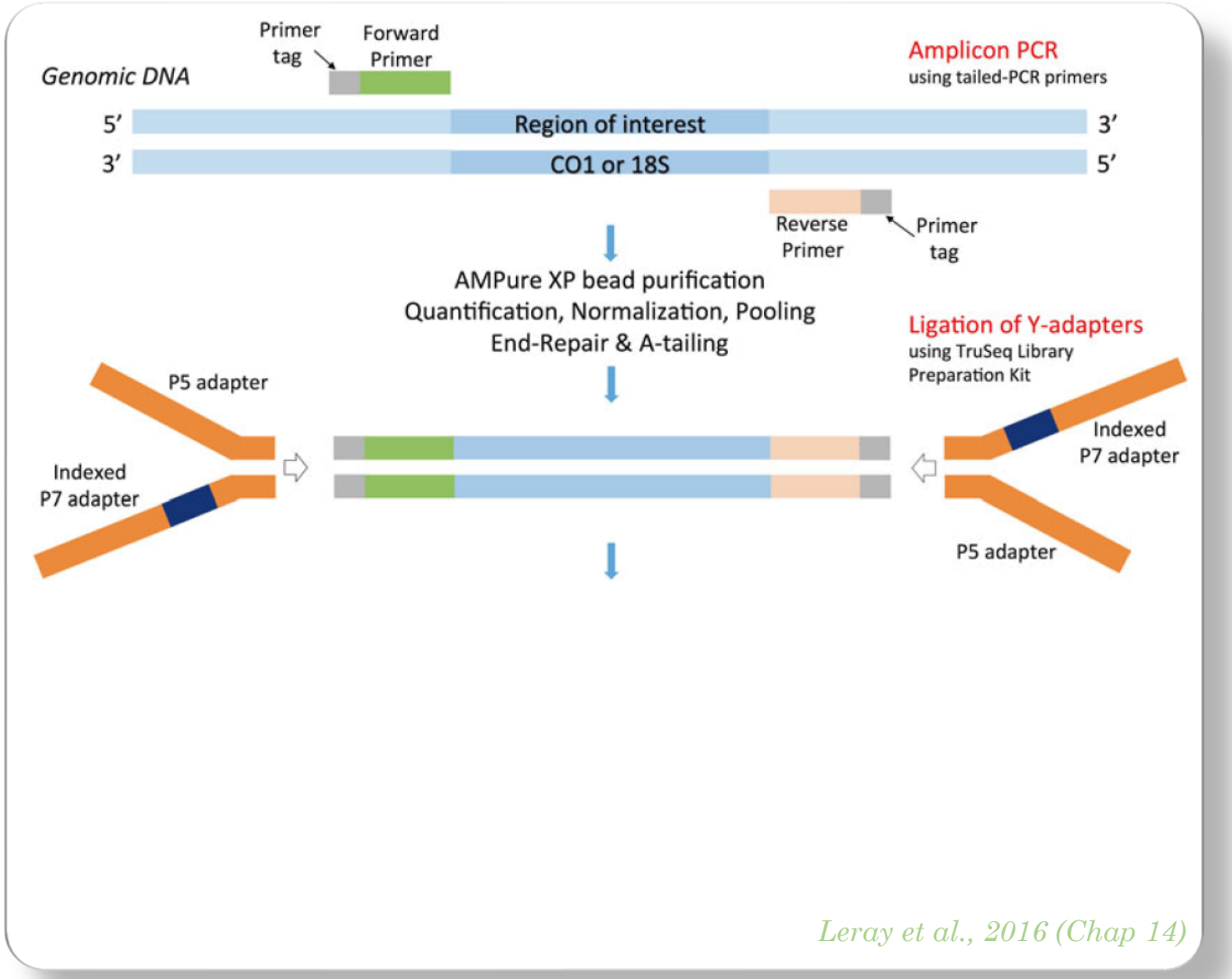

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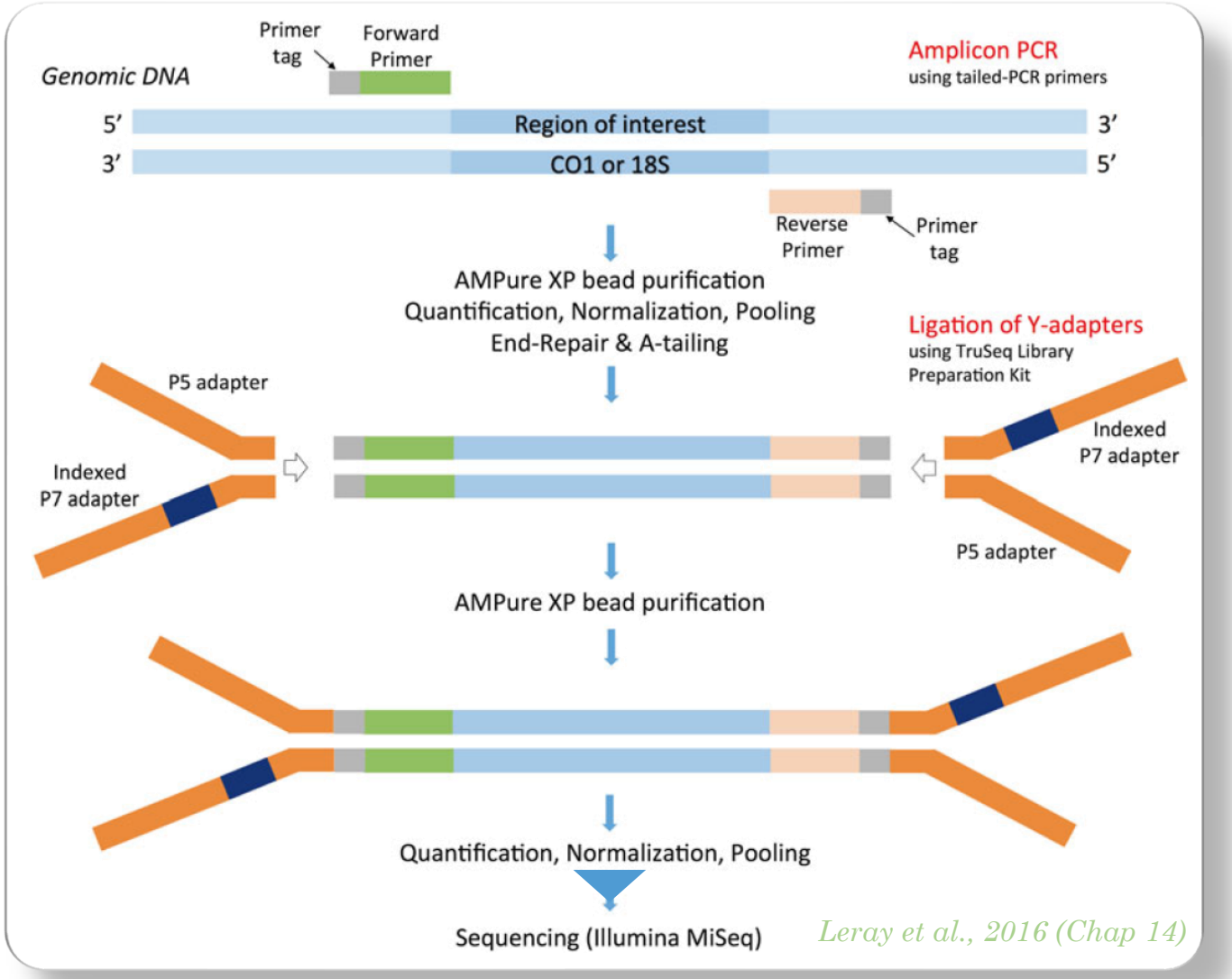
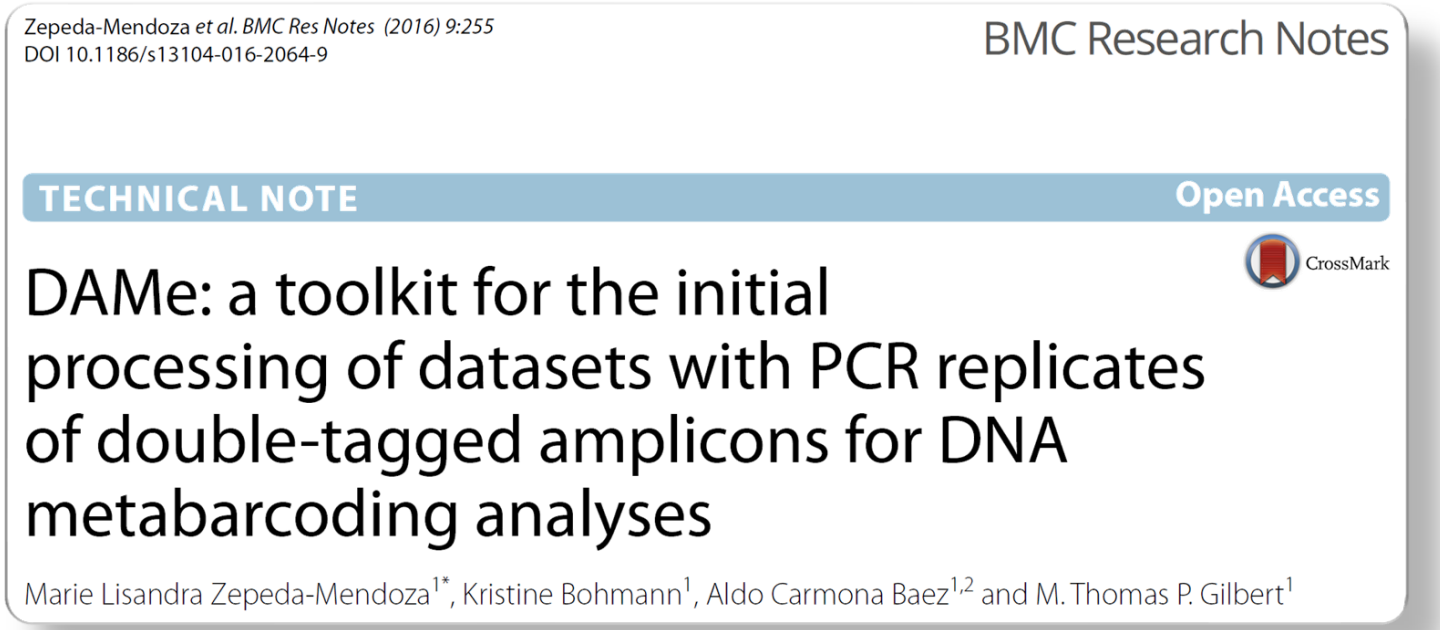


Leray et al., 2016 (Chap 14)

Bio-informatic pipeline



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

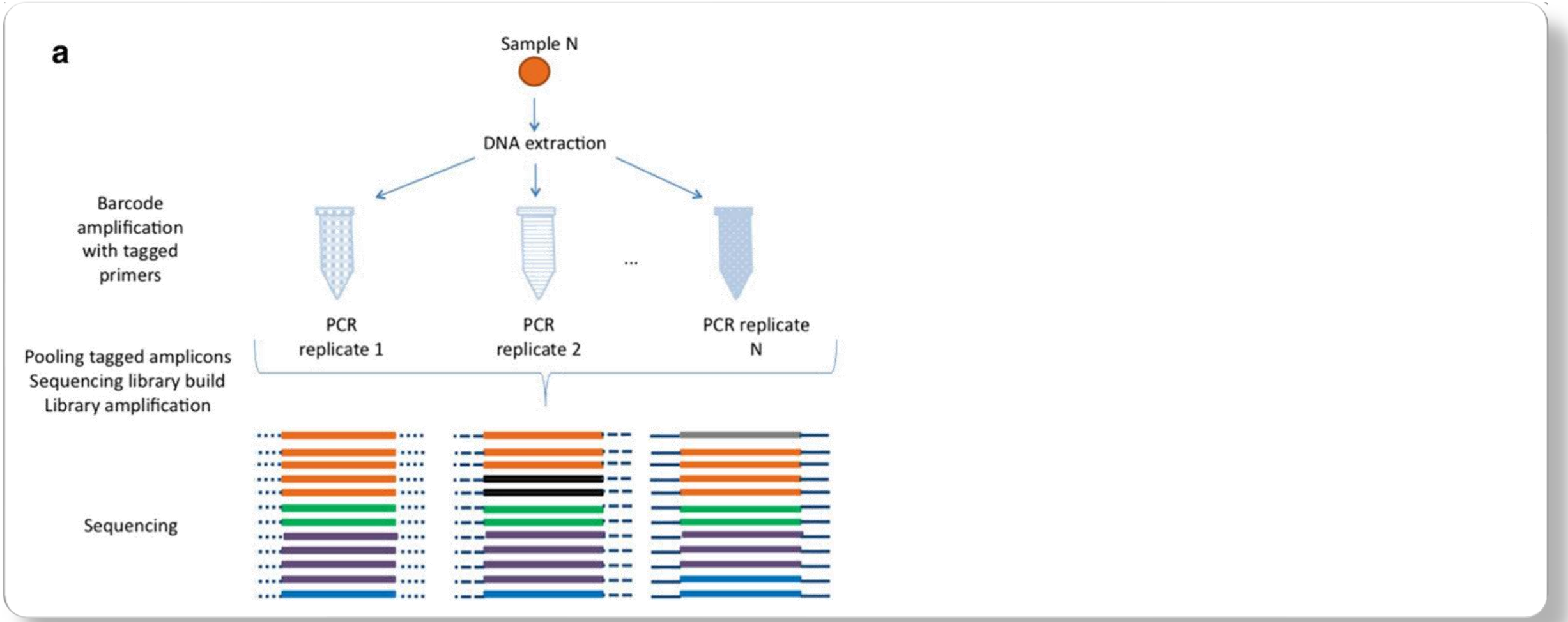
BMC Research Notes

TECHNICAL NOTE Open Access

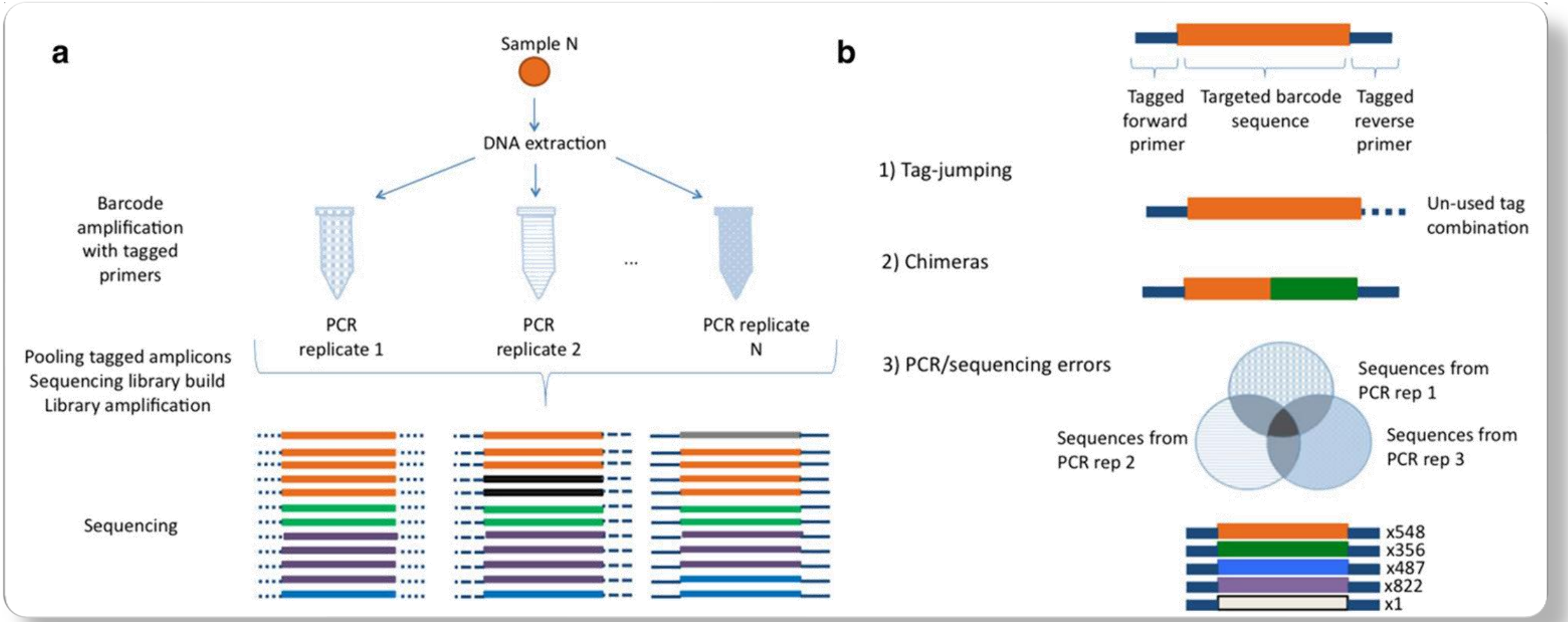
DAMe: a toolkit for the initial processing of datasets with PCR replicates of double-tagged amplicons for DNA metabarcoding analyses

Marie Lisandra Zepeda-Mendoza[1*], Kristine Bohmann[1], Aldo Carmona Baez[1,2] and M. Thomas P. Gilbert[1]

Bio-informatic pipeline

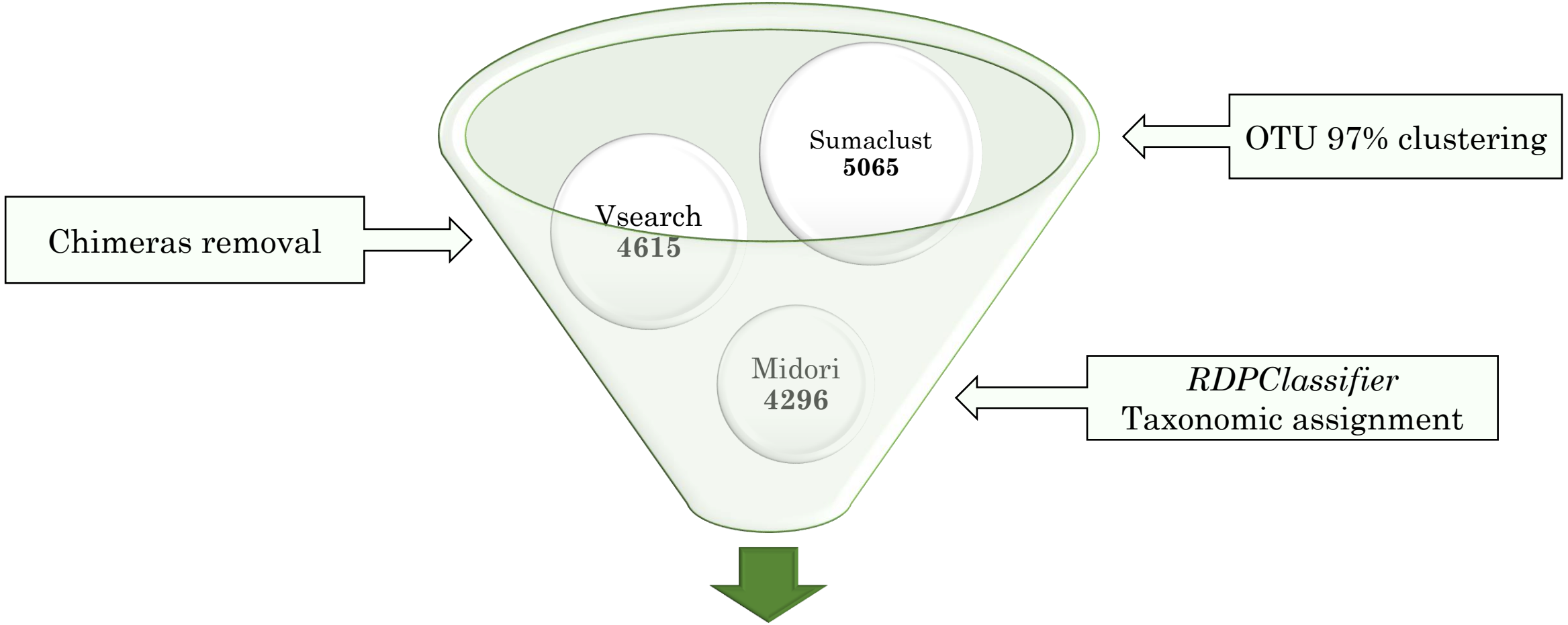


Bio-informatic pipeline



Bio-informatic pipeline

Minimum 4 reads in each of the 3 PCR replicates

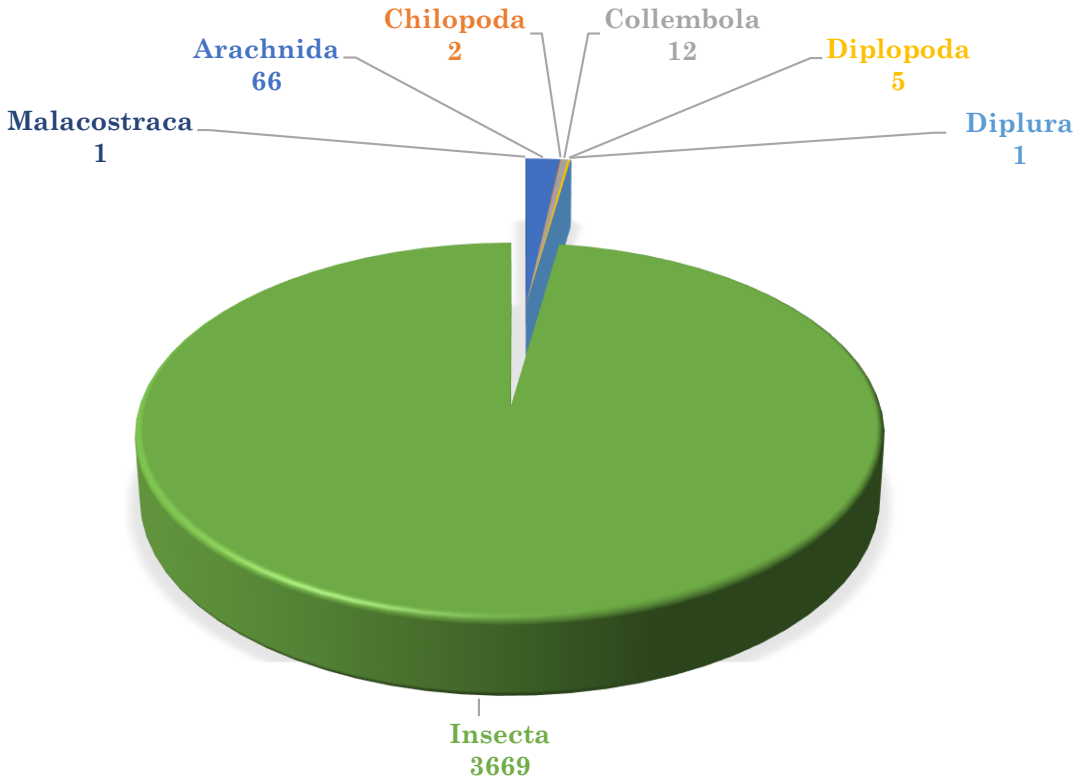


3756 OTUs

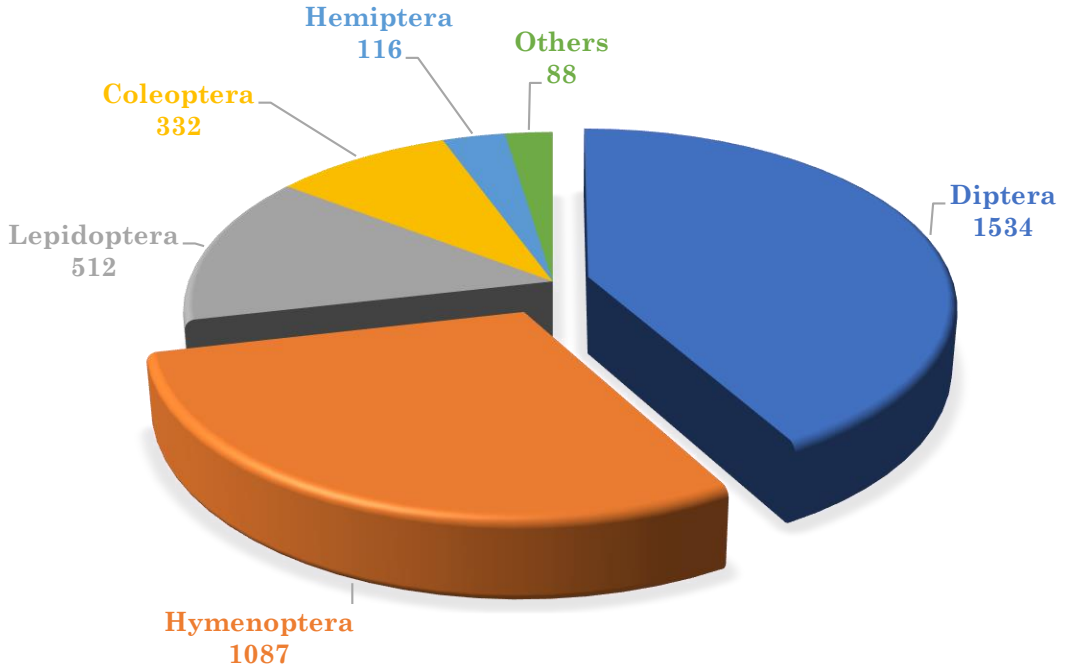
after Arthropod 80% filtering and sample selection

Species richness for Malaise traps

3756 OTUs found



18 Insecta orders represented



Species richness for Malaise traps

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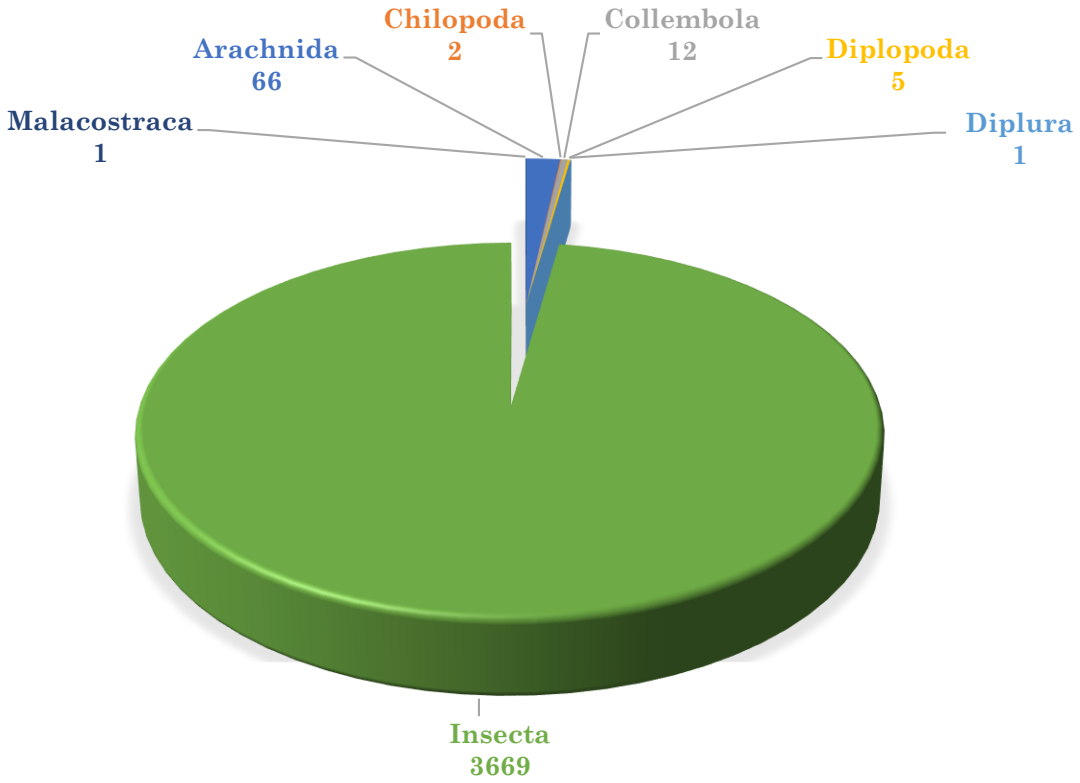

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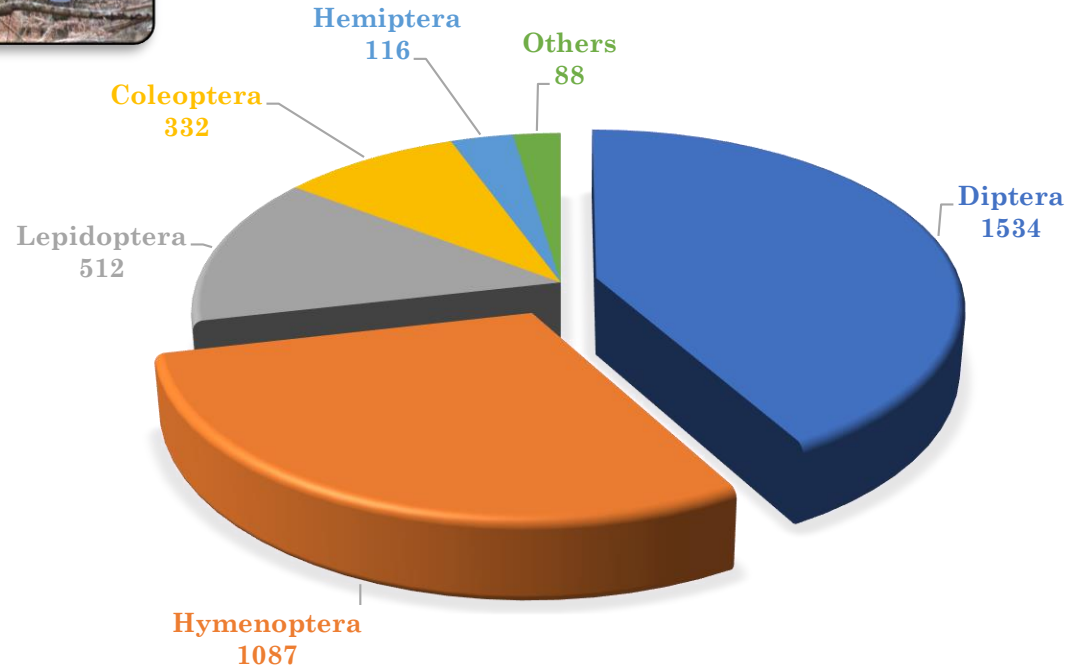
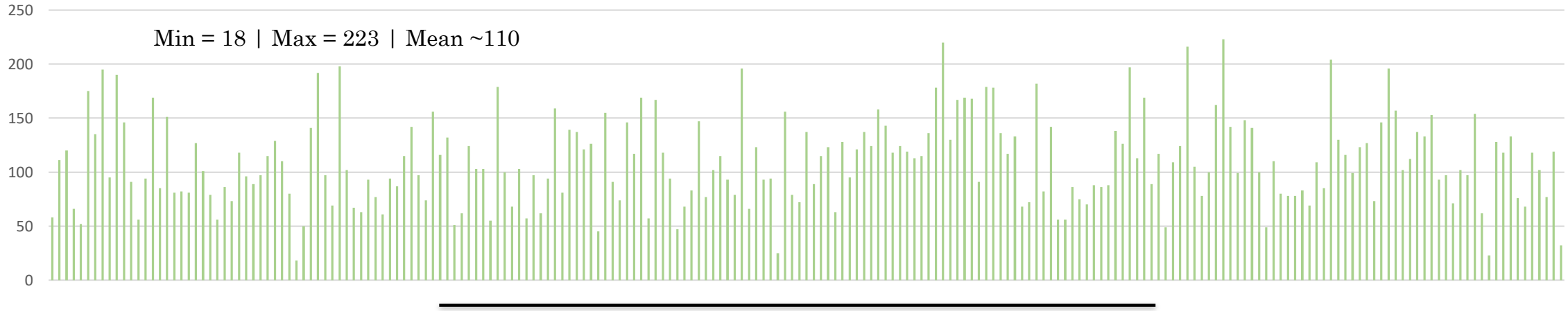


Trap composition

$N_{bulk\ samples} = 211$

Number of OTUs per bulk sample



Trap composition

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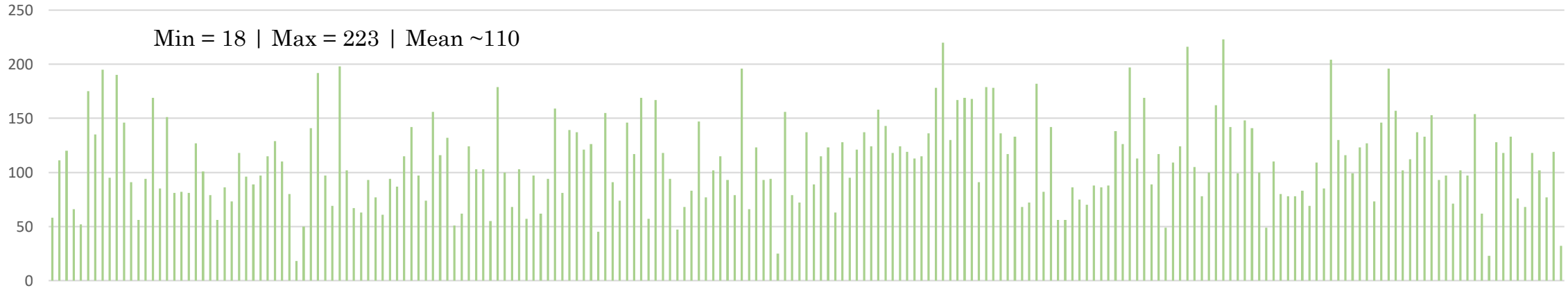
Number of OTUs per bulk sample



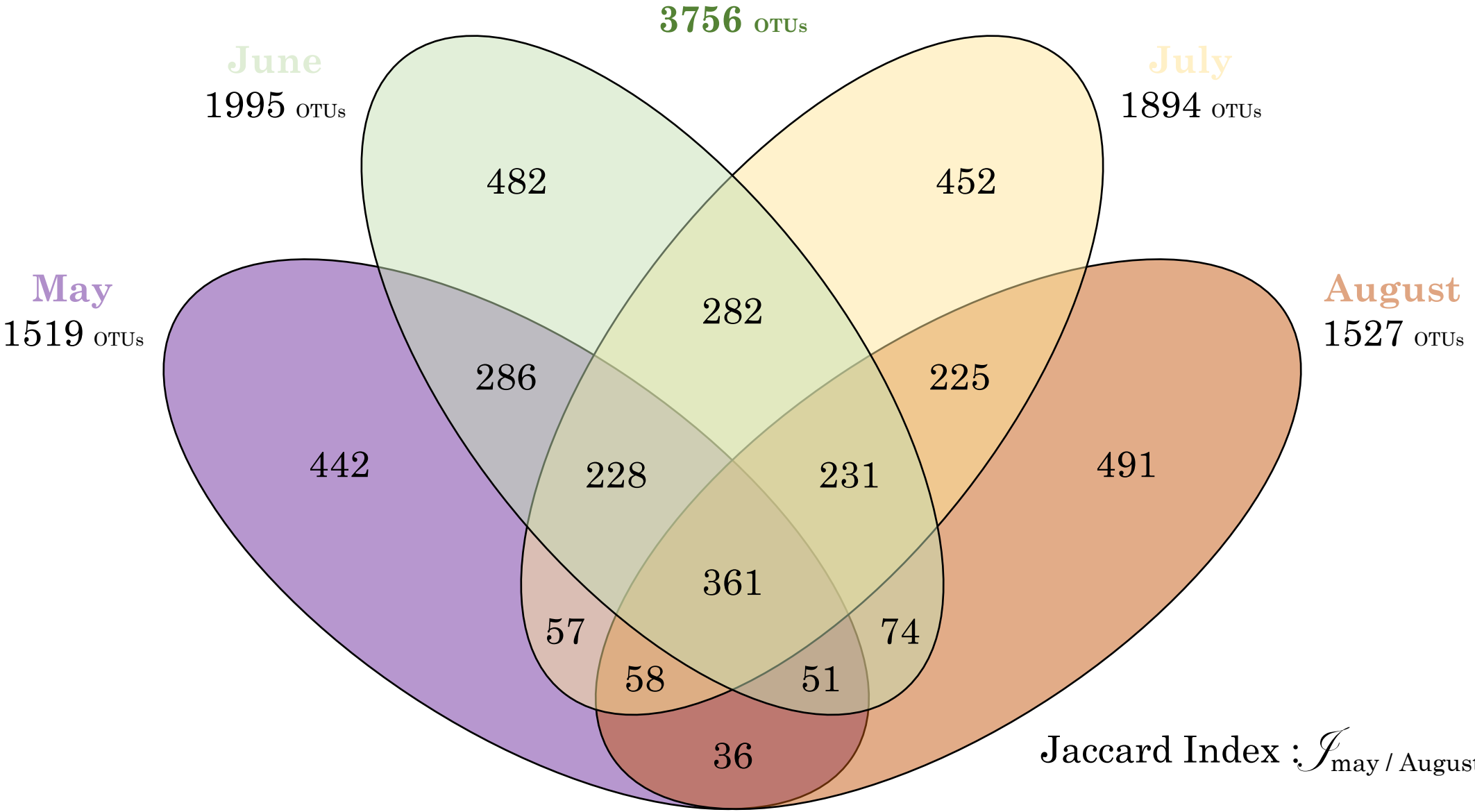
Number of bulk samples per OTU

Vespula vulgaris

Bombus lucorum

Bombus pascuorum

High temporal turnover from May to August



Jaccard Index : $\mathcal{J}_{\text{may / August}} = 0.35$

Future work

- Expand reference libraries (saproxylic beetles)
- Improve taxonomic assignment into the bioinformatic pipeline
- Reanalize 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

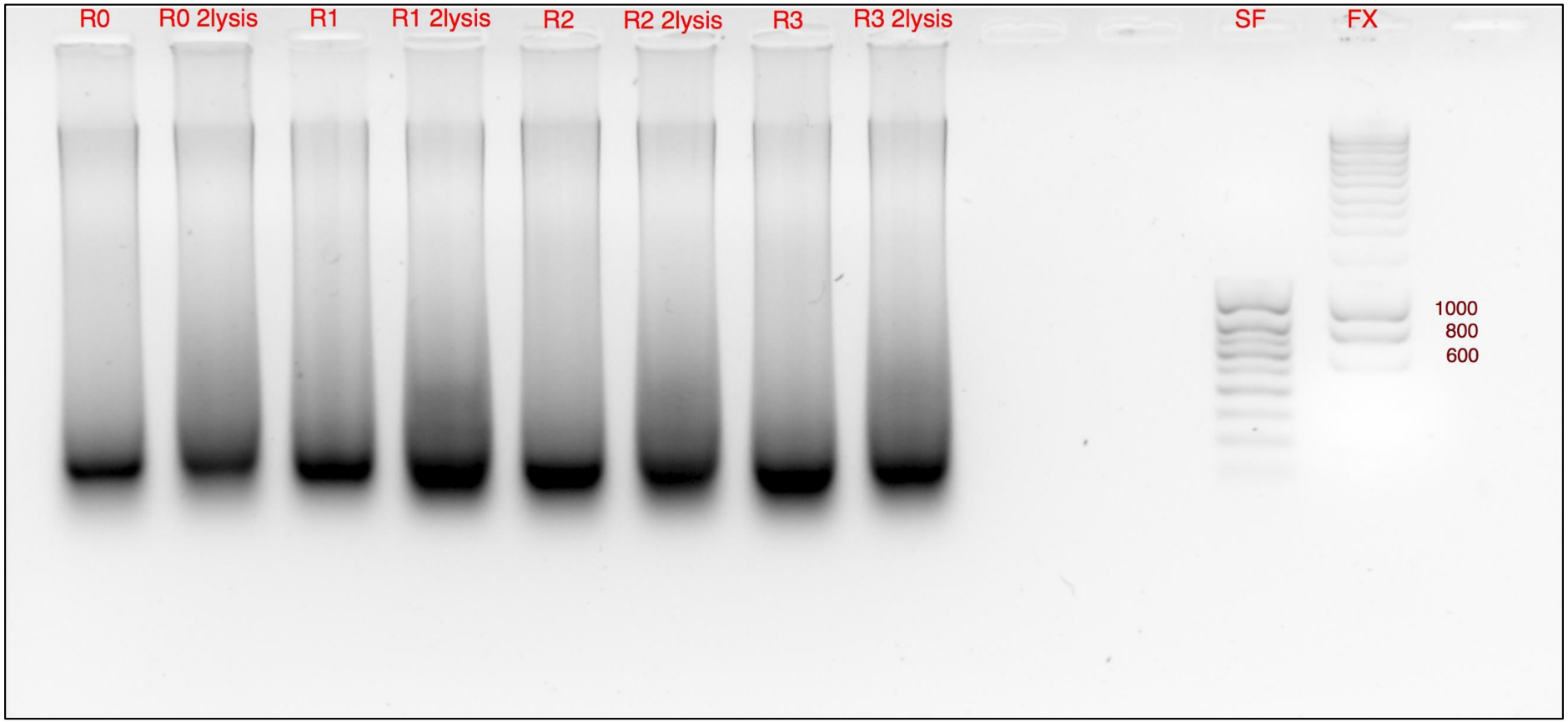
Hylesinus toranio

Xylosandrus germanus

Vespa vetulina

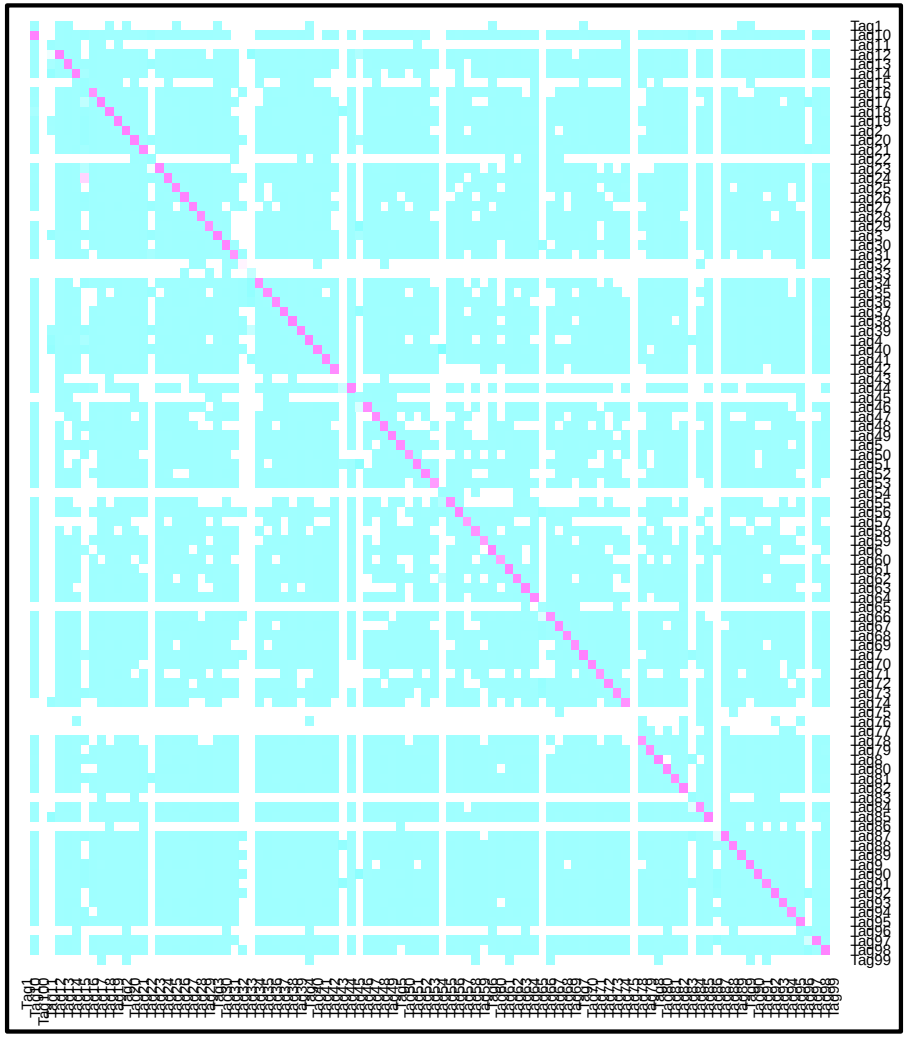
Degradation of samples

Pre-PCR migration and rincing test for MPG removal

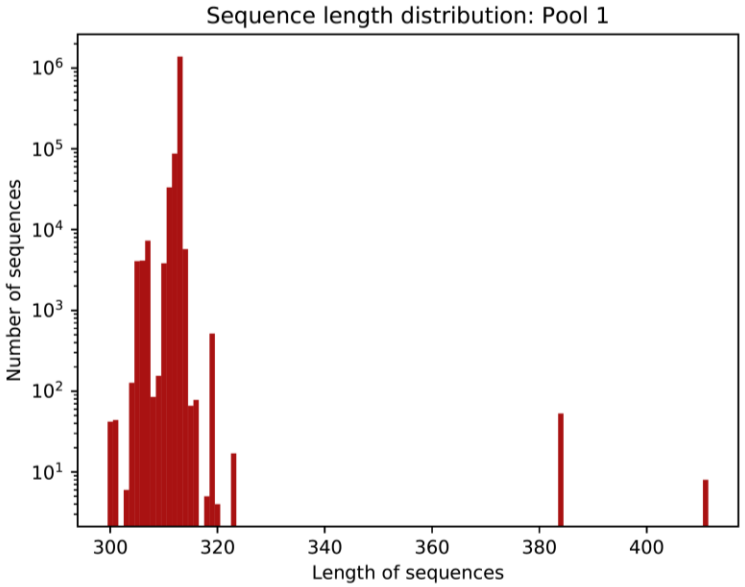


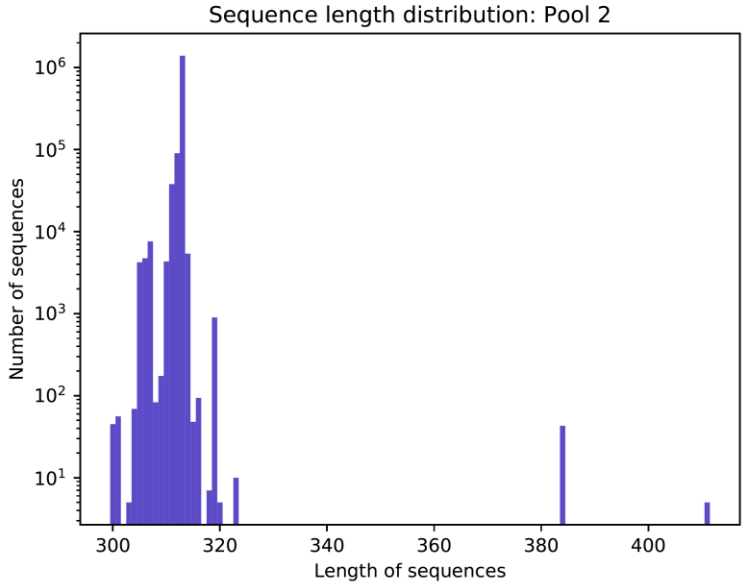
R0: no rincing | R1: rincing with 2x100mL water | R2: rincing with 2x100ml ethanol | R3: rincing with 100ml ethanol + 100ml water

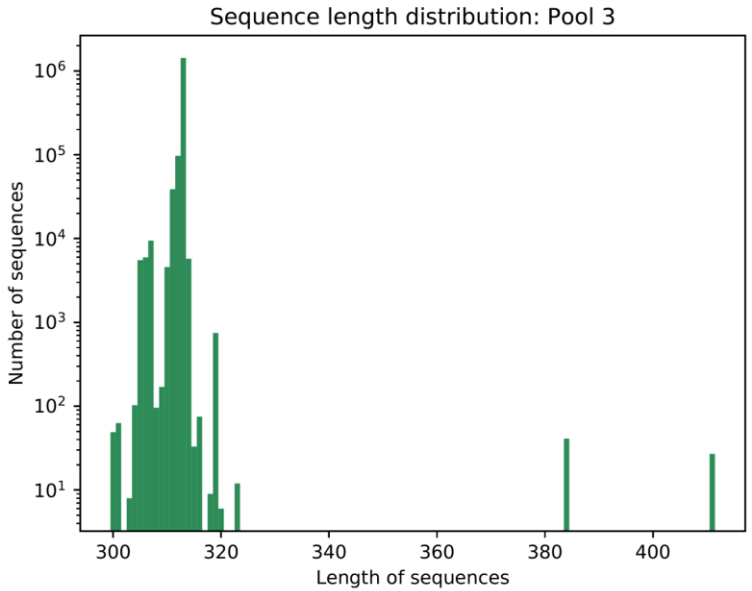
Heatmap of primer-tag combinations

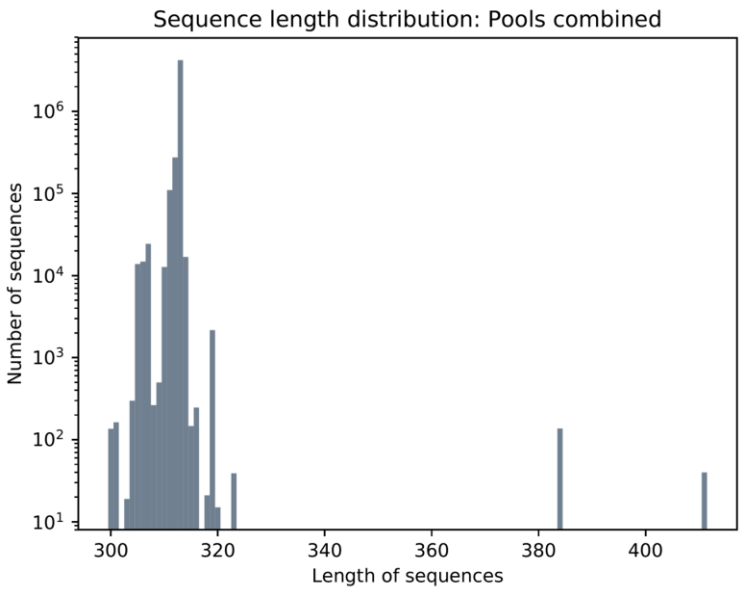


Length control of the reads

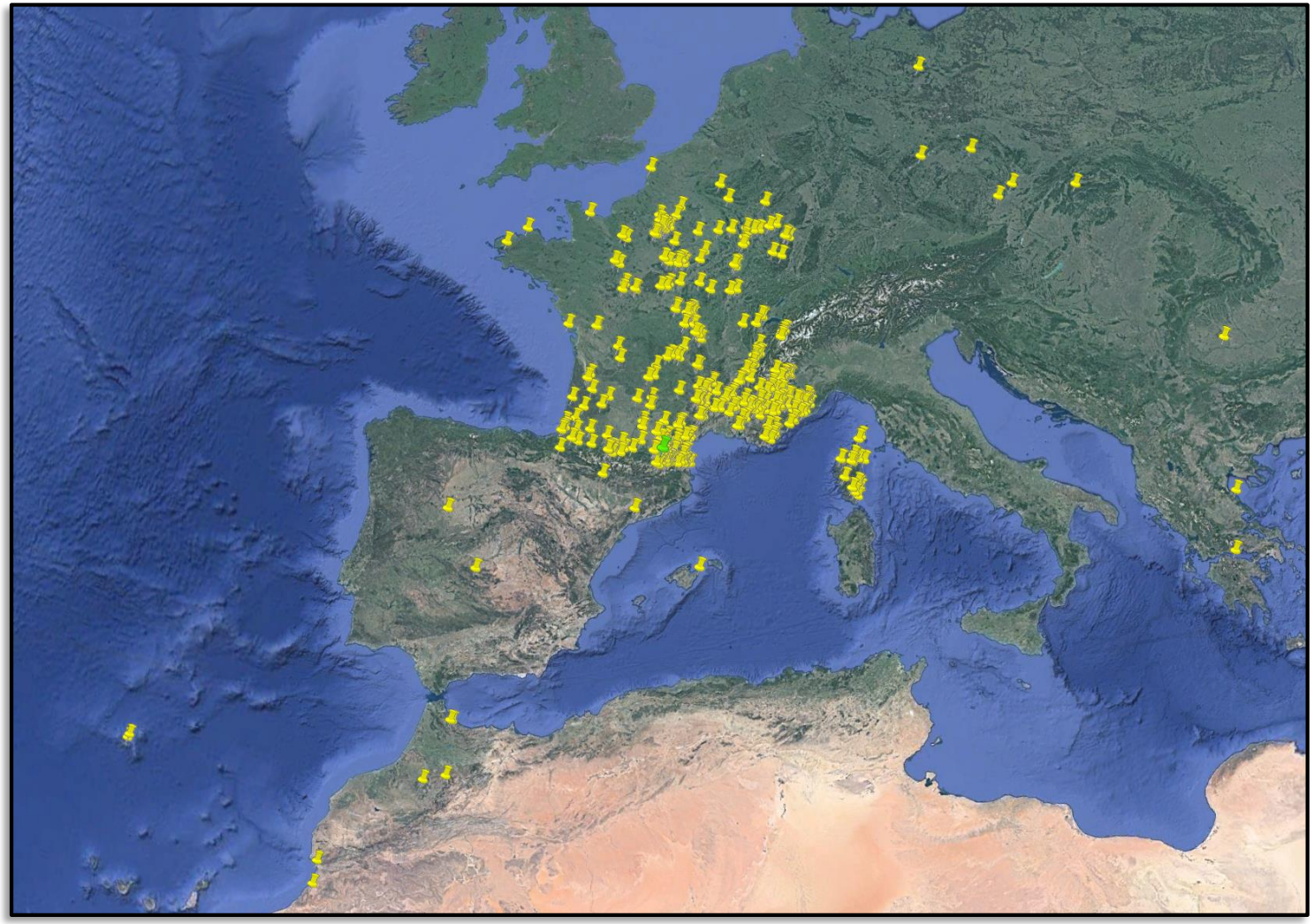


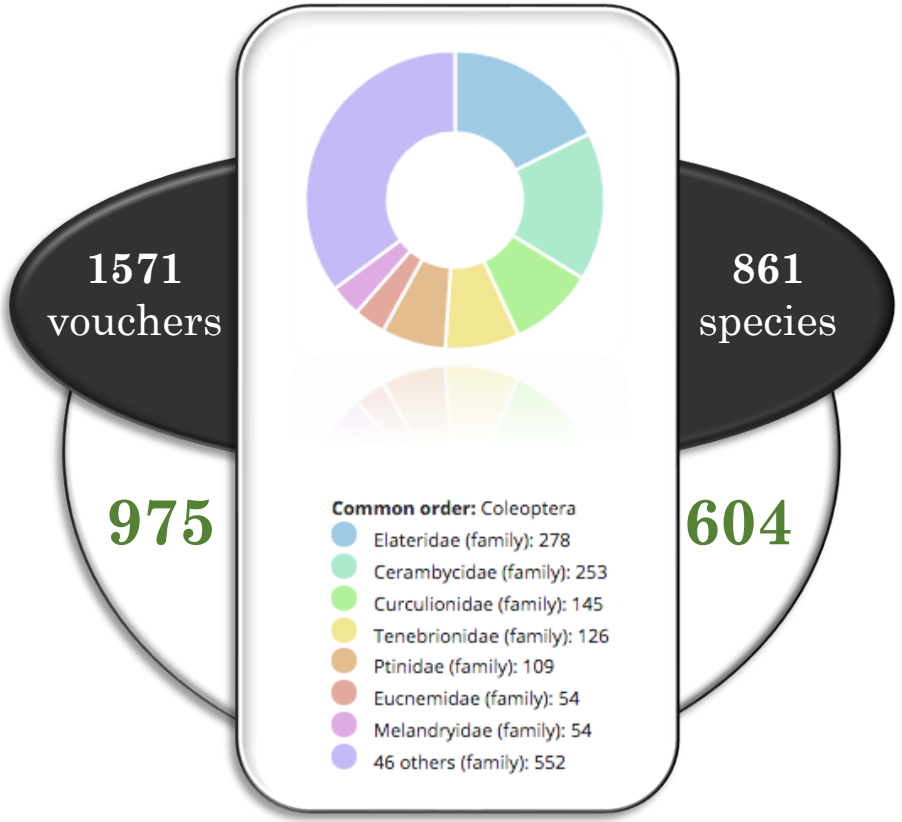






Reference library for saproxylic beetles





Rougerie et al., 2015

Sire et al., in prep.