Metabarcoding for the detection and identification of invasive Cerambycides

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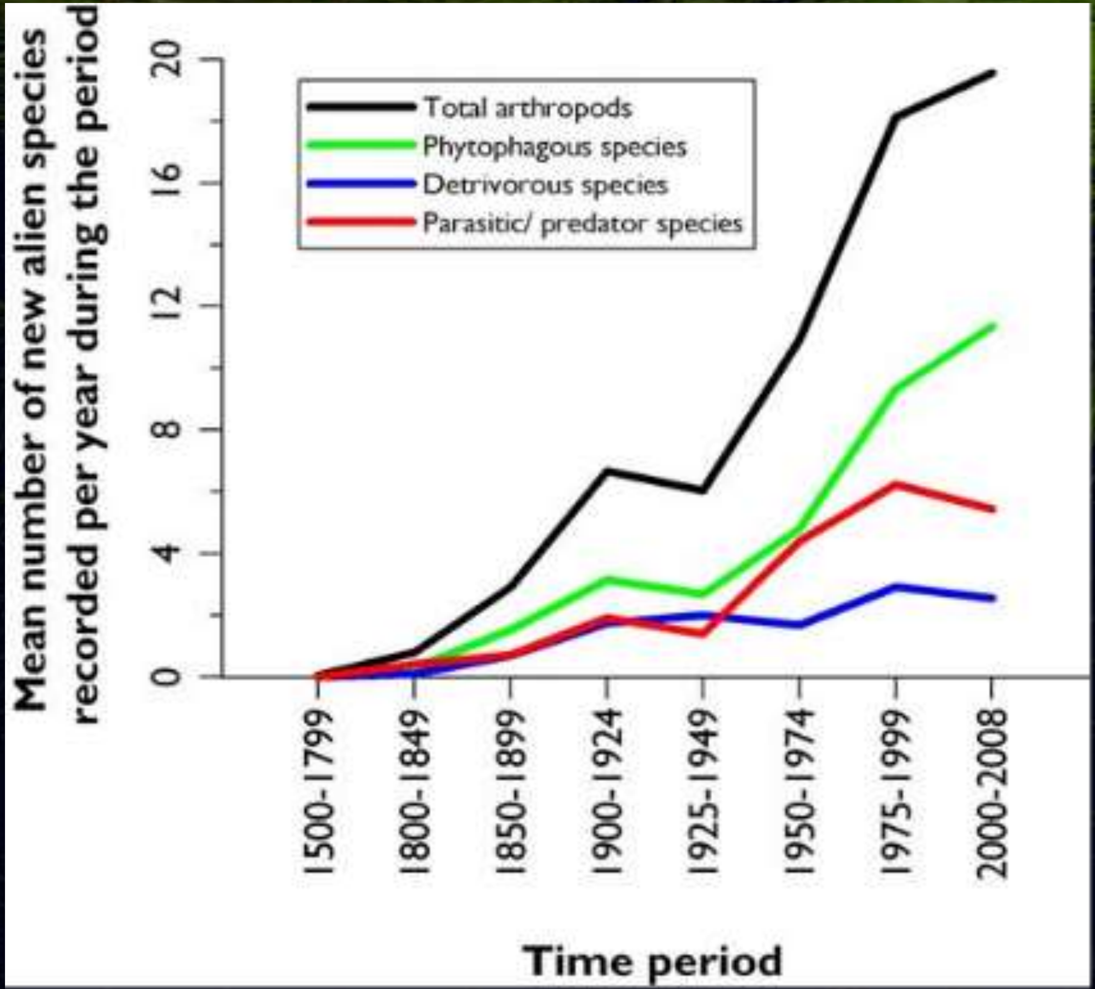
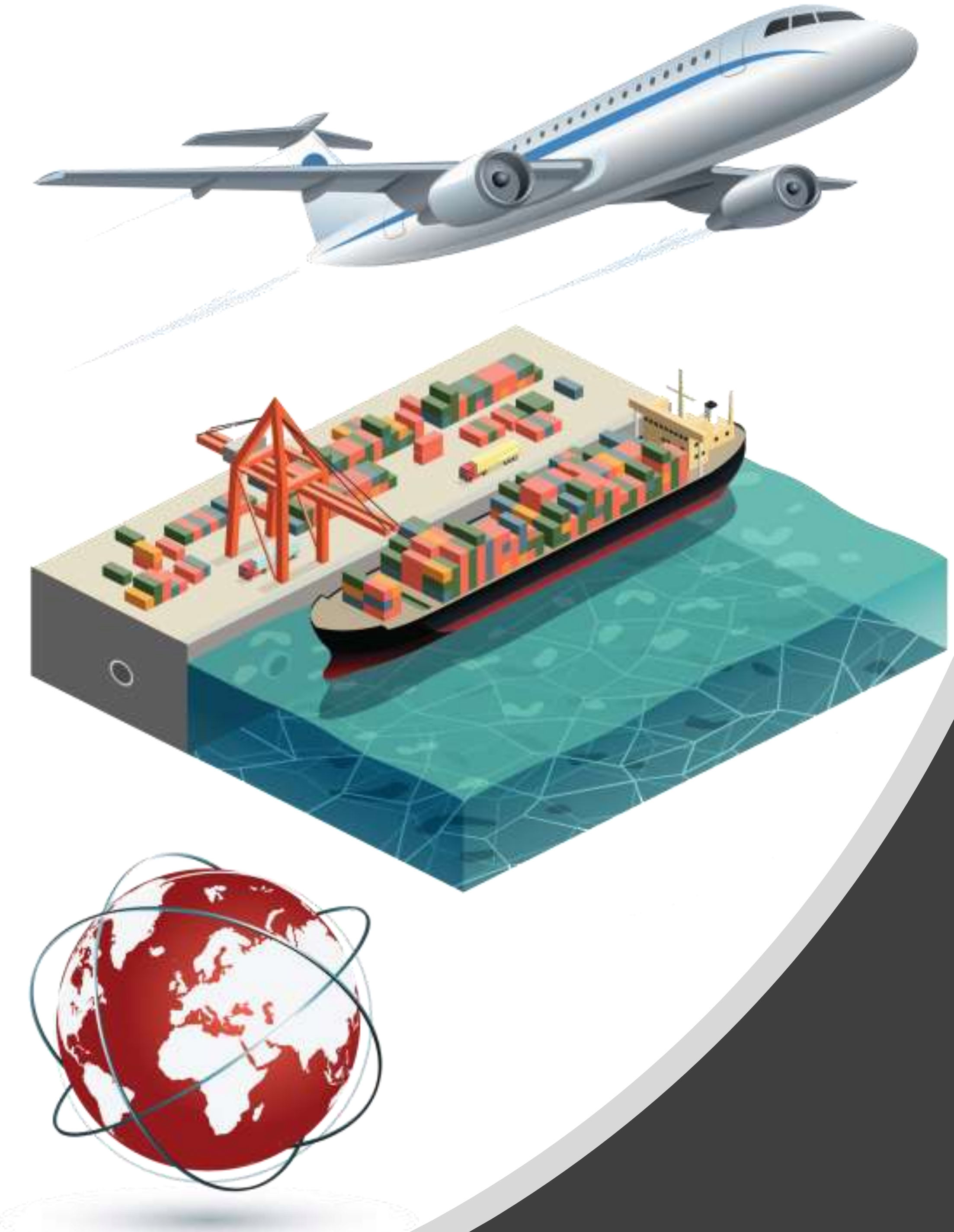
INRAE URZF UNITÉ DE RECHERCHE DE ZOOLOGIE FORESTIÈRE HOMED IRBI Institut de Recherche sur la Biologie de l'Insecte



Roques, 2010 Biorisks

Increasing biological invasions over the years, without saturation

Can cause environmental, health and economic damage

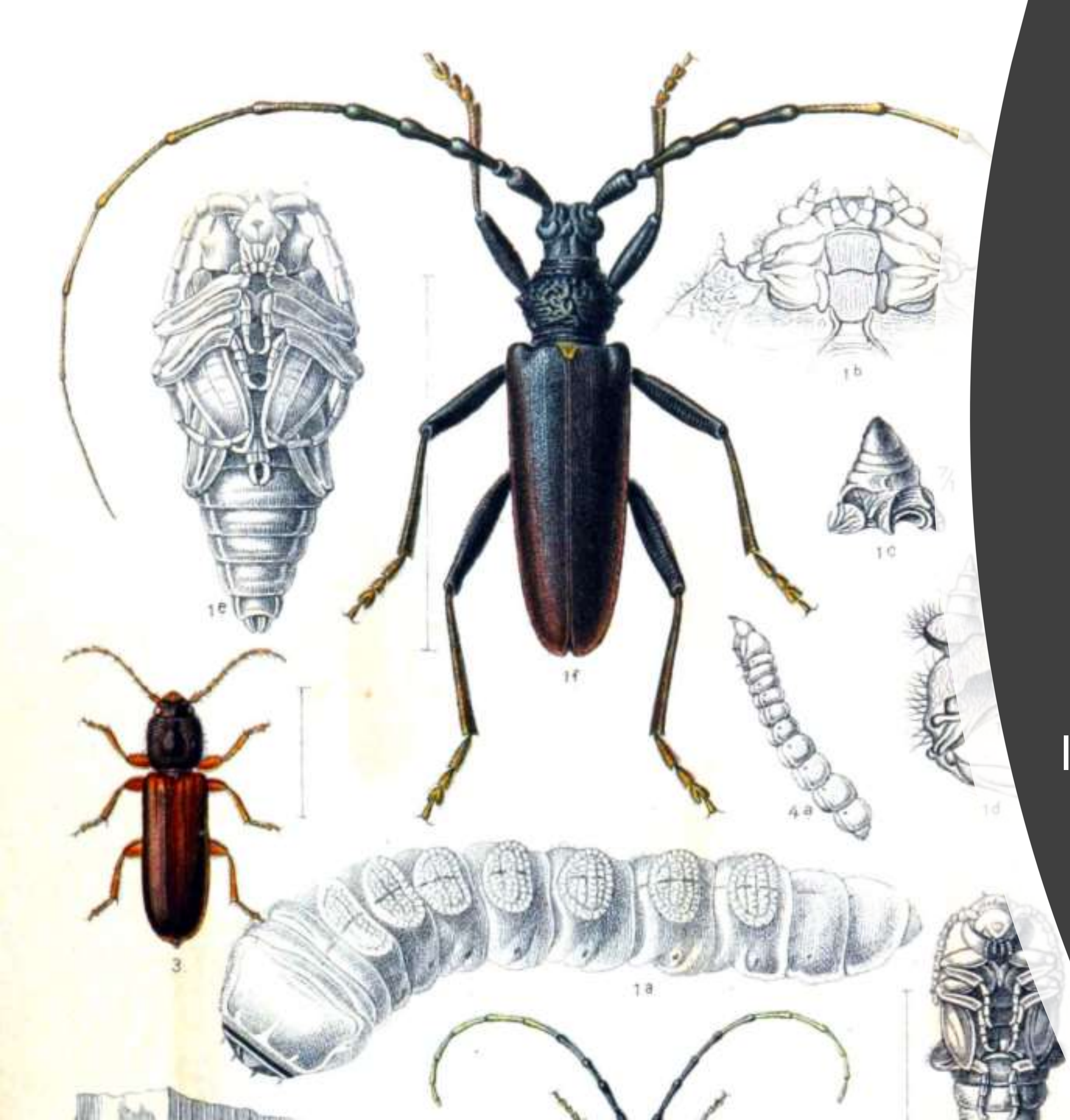


Interception of insects on arrival using pheromone traps

Morphological identification :

- Time consuming
- Requires a high level of taxonomic expertise
- Delay between sample collection and identification

Complementary approaches to detection and identification ?



Cerambycids

+35 000 species described

Important for ecosystems

Long life cycle (month / years)

Organisms easily morphologically identifiable

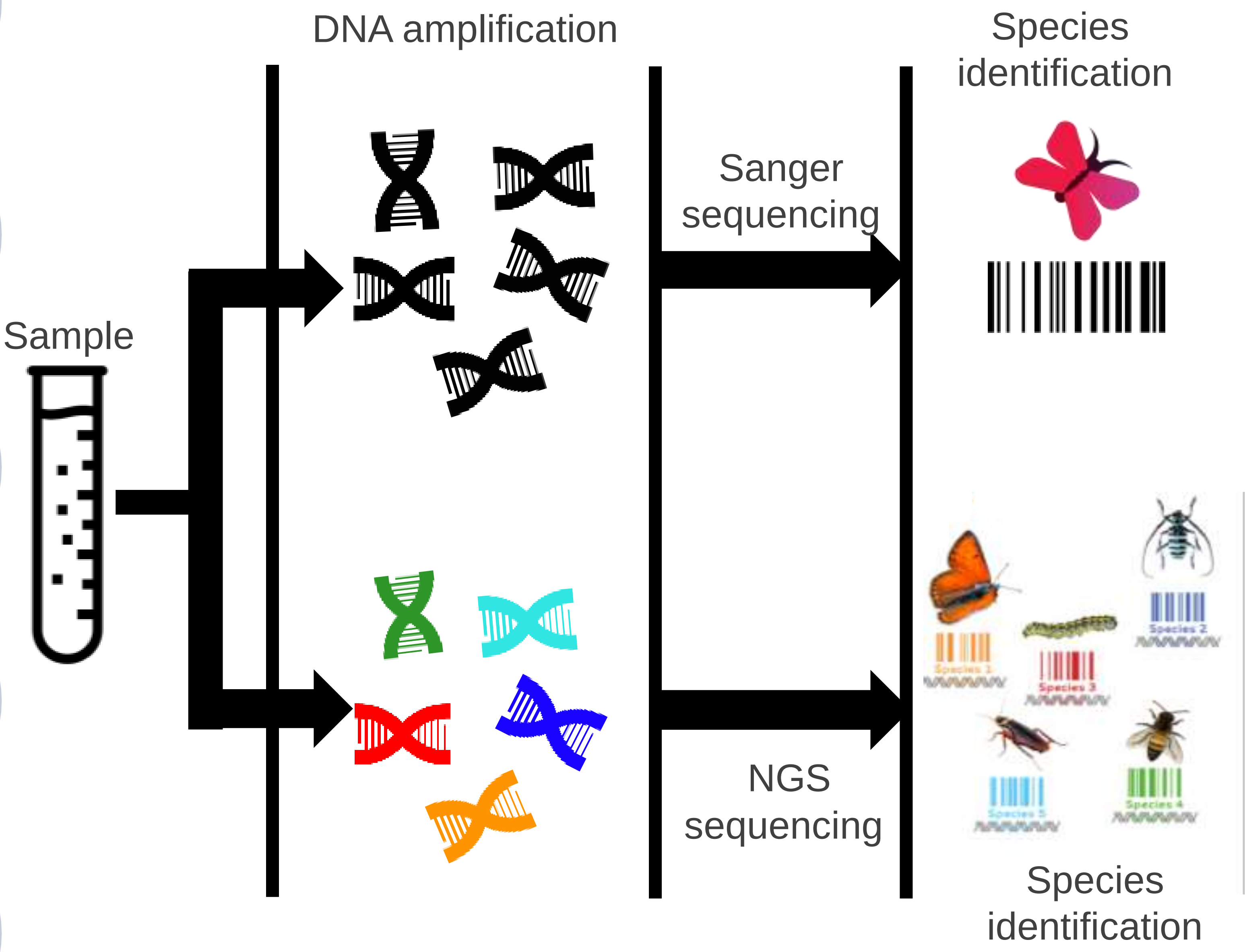
Ideal for developing the method and then applying it to more "complex" organisms

Molecular Barcoding

Identification of a unique individual through a short DNA fragment

Metabarcoding

Identification of all species present in a sample using a short DNA fragment



Barcoding

Sample

DNA amplification

Sanger sequencing

Species identification

NGS sequencing

Species identification

Metabarcoding

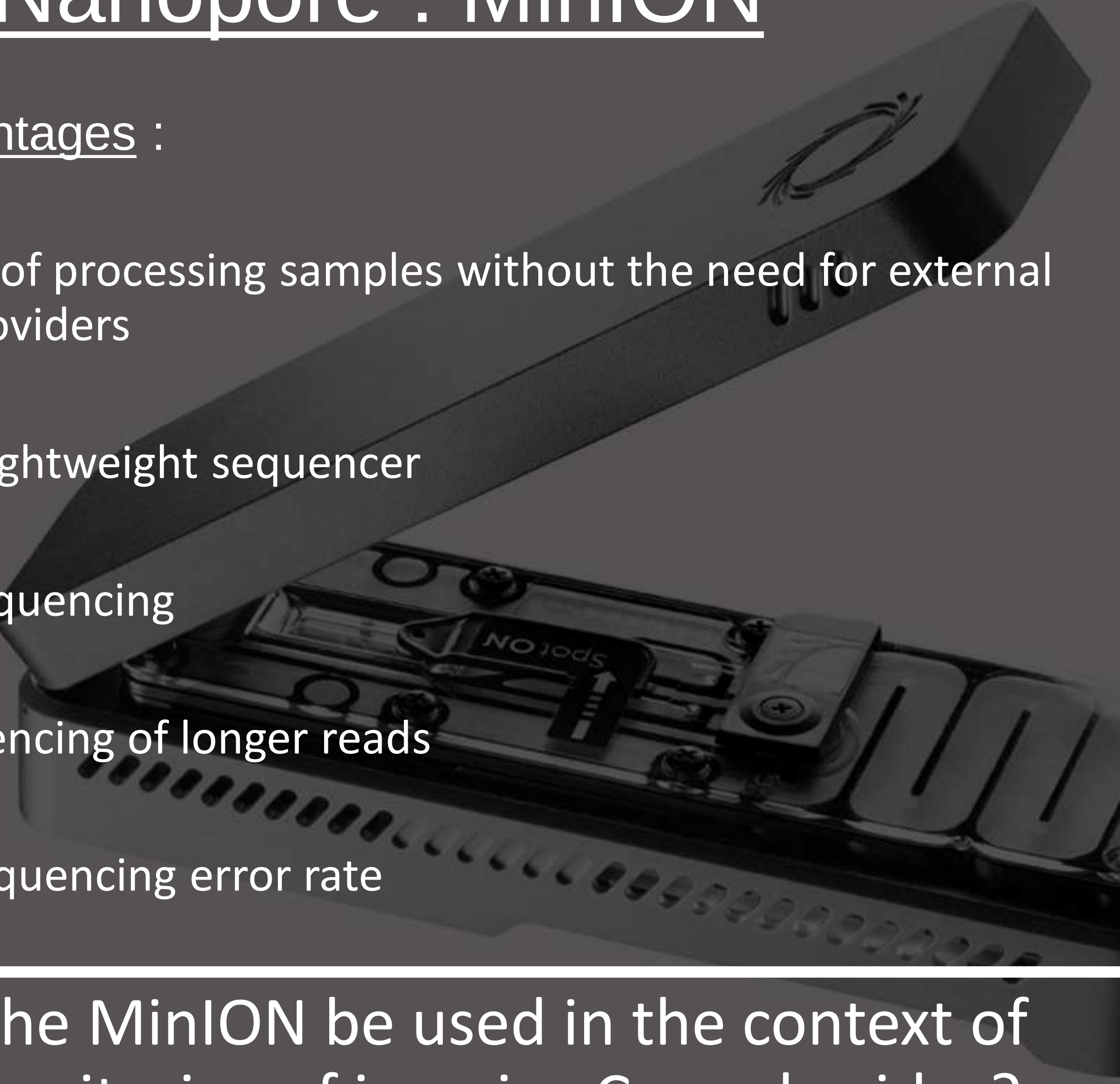
Metabarcoding with Illumina Miseq

- Expensive sequencing
- Often requires the involvement of external providers
- Limited read size
- Need for sophisticated laboratories for library construction

Limited accessibility

Illumina Miseq Sequencer

Oxford Nanopore : MinION



Several advantages :

- Possibility of processing samples without the need for external service providers
- Small and lightweight sequencer
- Cheaper sequencing
- Allows sequencing of longer reads

BUT: Higher sequencing error rate

Can the MinION be used in the context of biomonitoring of invasive Cerambycides?

Can the MinION differentiate between genetically related species?

Monochamus sartor and *sutor*

Xylotrechus smei and stebbingi

Construction of fictitious traps composed of closely related species

Construction of two sample bulks of closely related species

(2 legs per individual)

Bulk 1
Arhopalus rusticus
Arhopalus ferus
Arhopalus syriacus
Xylotrechus chinensis
Xylotrechus stebbingi
Xylotrechus arvicola
Xylotrechus undulatus

Bulk 2
Monochamus galloprovincialis
Monochamus sutor
Monochamus scutellatus
Monochamus carolinensis
Phymatodes amoenus
Phymatodes testaceus
Phymatodes varius
Phymatodes aereus
Phymatodes dimidiatus

MinION sequencing

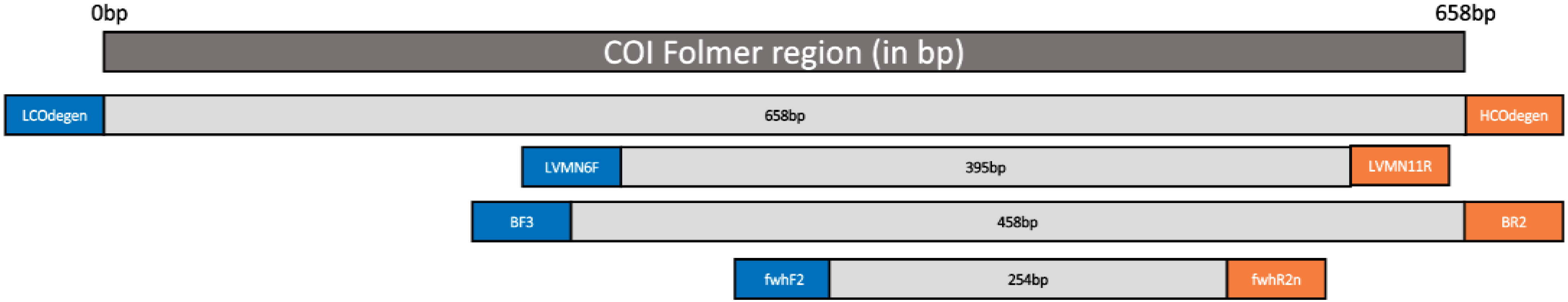
Primers used
HCOdegen / LCOdegen 658 pb
LVMN6F / LVMN11R 395 pb
BF3 / BR2 458 pb
FwhF2 / fwR2n 254 pb

Bioinformatic steps

MSI

Egeter et al., 2022

- **Preprocess**
- **Polish**
- **Cluster**
- **Classification**



Results for bulk 1

Bulk 1 - Detection ?	🟩	🟩	🟥	🟩	🟩	🟥	🟩
Species	**Arhopalus rusticus**	**Arhopalus ferus**	**Arhopalus syriacus**	**Xylotrechus stebbingi**	**Xylotrechus chinensis**	**Xylotrechus arvicola**	**Xylotrechus undulatus**

Results for bulk 2

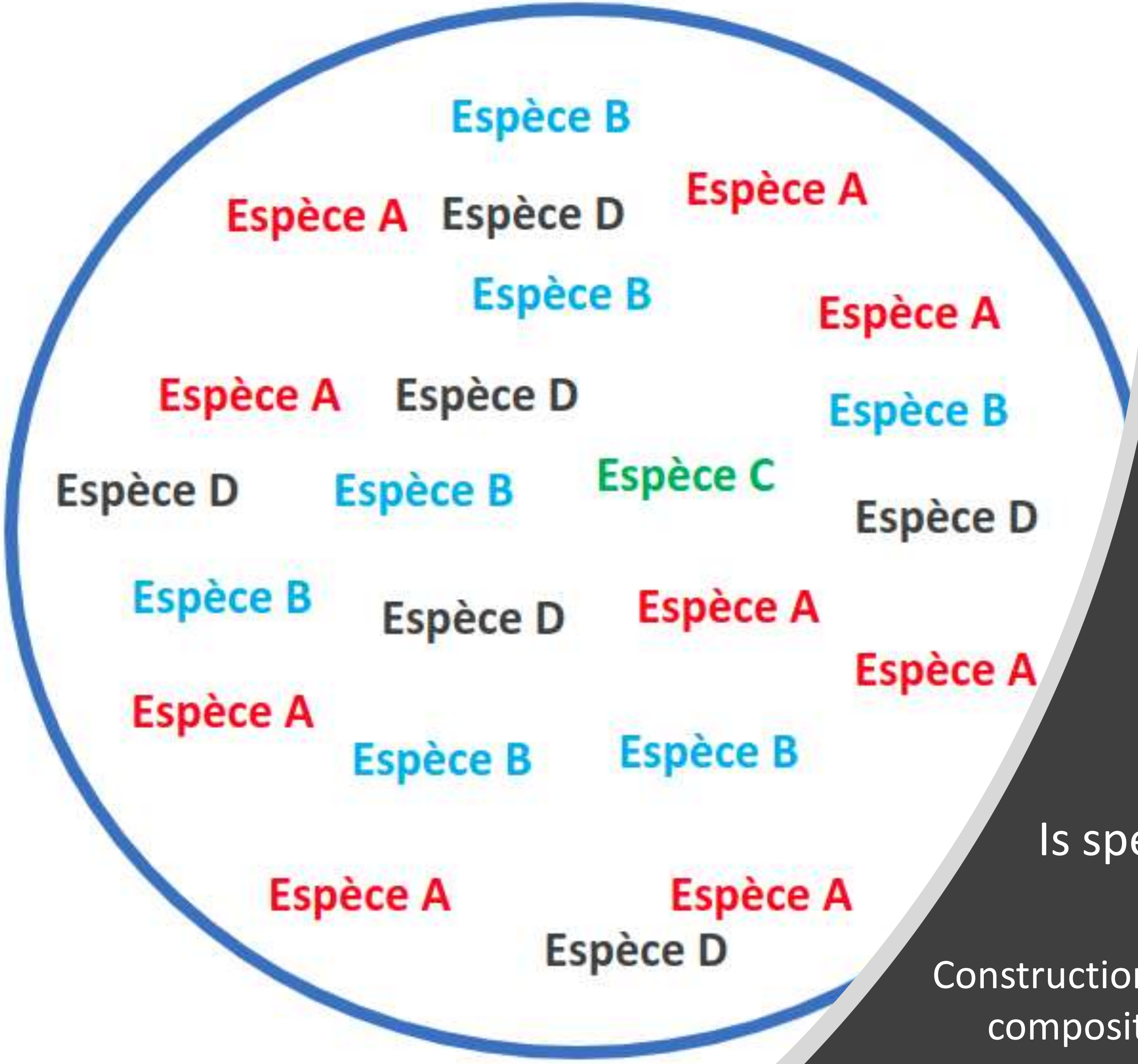
Bulk 2 - Detection ?	🟩	🟩	🟥	🟥	🟩	🟥	🟥	🟥	🟥
Species	**Monochamus galloprovincialis**	**M. sutor**	**M. scutellatus**	**M. carolinensis**	**Phymatodes amoenus**	**P. testaceus**	**P. varius**	**P. aereus**	**P. dimidiatus**

🟩 Species level detection

🟥 No detection

➡ Several species of the same genus have been detected, identified and differentiated

➡ Bulk 2: Few species detected. Poor quality DNA ? Problems with laboratory handling ?



Can an invasive species present in a small proportion in a trap be detected with the MinION?

Exemple :

Species A : 9 individuals (39%)
Species B : 7 individuals (30%)
Species C (invasive) : 1 individual (5%)
Species D : 6 individuals (26%)

Is species C detectable and identifiable ?

Construction of fictitious traps with a known composition and proportion of insects

Construction of two bulks samples from DNA extractions

Bulk 3
Pyrrhidium sanguineum (51%)
Xylotrechus stebbingi (21%)
Monochamus galloprovincialis (13%)
Xylotrechus chinensis (7%)
Chlorophorus glabromaculatus (5%)
Phymatodes testaceus (3%)

Bulk 4
Monochamus sutor (46%)
Arhopalus ferus (21%)
Xylotrechus stebbingi (16%)
Aegomorphus francottei (9%)
Xylotrechus chinensis (5%)
Monochamus galloprovincialis (4%)

MinION sequencing

Primers used
HCOdegen / LCOdegen 658 pb
LVMN6F / LVMN11R 395 pb
BF3 / BR2 458 pb
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Bioinformatic steps

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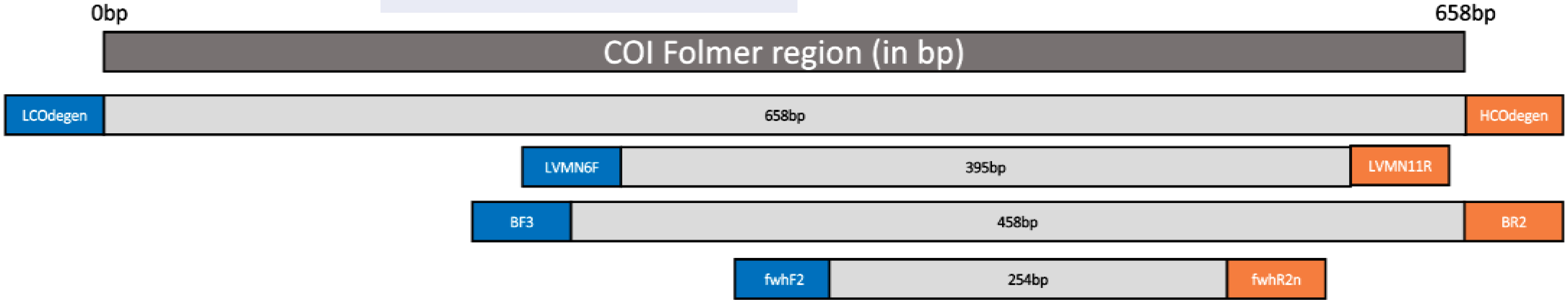
Egeter et al., 2022

- Preprocess
- Polish
- Cluster
- Classification



Results – Bulk 3

	Pyrrhidium sanguineum	*Xylotrechus stebbingi*	*Monochamus galloprovincialis*	*Xylotrechus chinensis*	*Chlorophorus glabromaculatus*	*Phymatodes testaceus*
Detected ?	🟩	🟩	🟩	🟩	🟩	🟩
Proportion of DNA	**51%**	**21%**	**13%**	**7%**	**5%**	**3%**

Results - Bulk 4

	Monochamus sutor	*Arhopalus ferus*	*Xylotrechus stebbingi*	*Aegomorphus francottei*	*Xylotrechus chinensis*	*Monochamus galloprovincialis*
Detected ?	🟩	🟩	🟩	Aegomorphus sp.	🟩	🟩
Proportion of DNA	**46%**	**23%**	**15%**	**8%**	**5%**	**4%**

➡ All species were detected and identified to species level (except A.francottei which was identified to genus)

➡ Detection of *Phymatodes testaceus* and *Monochamus galloprovincialis* despite low representativity (3% and 4%)

Construction of two bulks samples mimicking the composition of real traps with samples of different provenance and dating

Results – Bulks 5 and 6

Bulk 5	*Cordylomera spinnicornis*	*Cerambyx scopolii*	*Pachyta bicuneata*	*Batocera rubus*	Pyrrhidium sanguineum	Strictoleptura cordigera	Leiopus femoratus	Leiopus linnei
Detected ?	🟩	🟥	🟥	🟥	🟩	🟩	🟩	🟩
Date	2020	2020	1987	2012	2021	2021	2020	2020
Number of legs	1	1	1	1	6	6	5	1
Origin	France	France	Chine	Chine	France	France	France	France

Bulk 6	Arhopalus rusticus	Xylotrechus chinensis	Plagionotus detritus	Plagionotus arcuatus	Xylotrechus stebbingi	Arhopalus syriacus	Arhopalus ferus	Xylotrechus colonus	Chlorophorus ruficornis	Phymatodes testaceus	Prionius coriarius	Phymatodes amoenus
Detected ?	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟩	🟩	🟥
Date	2020	2019	2020	2020	2020	2020	2020	2019	2021	2021	2020	2019
Number of legs	5	1	1	1	5	6	1	1	1	12	6	1
Origin	France	Grèce	France	France	France	France	France	USA	France	France	France	USA

➡ Samples from China and the USA were not detected

➡ "Minority" samples were detected and closly related species differentiated

➡ Arhopalus syriacus was detected (unlike Bulk 1)

Results – Bulks 5 and 6

Bulk 5	***Cordylomera spinnicornis***	***Cerambyx scopolii***	***Pachyta bicuneata***	***Batocera rubus***	**Pyrrhidium sanguineum**	**Stictoleptura cordigera**	**Leiopus femoratus**	**Leiopus linnei**
Detected ?	🟩	🟥	🟥	🟥	🟩	🟩	🟩	🟩
Date	**2020**	**2020**	**1987**	**2012**	**2021**	**2021**	**2020**	**2020**
Number of legs	**1**	**1**	**1**	**1**	**6**	**6**	**5**	**1**
Origin	**France**	**France**	**Chine**	**Chine**	**France**	**France**	**France**	**France**

Bulk 6	**Arhopalus rusticus**	**Xylotrechus chinensis**	**Plagionotus detritus**	**Plagionotus arcuatus**	**Xylotrechus stebbingi**	**Arhopalus syriacus**	**Arhopalus ferus**	**Xylotrechus colonus**	**Chlorophorus ruficornis**	**Phymatodes testaceus**	**Prionius coriarius**	**Phymatodes amoenus**
Detected ?	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟩	🟩	🟥
Date	**2020**	**2019**	**2020**	**2020**	**2020**	**2020**	**2020**	**2019**	**2021**	**2021**	**2020**	**2019**
Number of legs	**5**	**1**	**1**	**1**	**5**	**6**	**1**	**1**	**1**	**12**	**6**	**1**
Origin	**France**	**Grèce**	**France**	**France**	**France**	**France**	**France**	**USA**	**France**	**France**	**France**	**USA**

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Results – Bulks 5 and 6

Bulk 6	Arhopalus rusticus	Xylotrechus chinensis	Plagionotus detritus	Plagionotus arcuatus	Xylotrechus stebbingi	Arhopalus syriacus	Arhopalus ferus	Xylotrechus colonus	Chlorophorus ruficornis	Phymatodes testaceus	Prionius coriarius	Phymatodes amoenus
Detected ?	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟥	🟥	🟩	🟩	🟥
Date	**2020**	**2019**	**2020**	**2020**	**2020**	**2020**	**2020**	**2019**	**2021**	**2021**	**2020**	**2019**
Number of legs	**5**	**1**	**1**	**1**	**5**	**6**	**1**	**1**	**1**	**12**	**6**	**1**
Origin	**France**	**Grèce**	**France**	**France**	**France**	**France**	**France**	**USA**	**France**	**France**	**France**	**USA**

Bulk 1 - Detection ?	🟩	🟩	🟥	🟩	🟩	🟥	🟩
Species	**Arhopalus rusticus**	**Arhopalus ferus**	**Arhopalus syriacus**	**Xylotrechus stebbingi**	**Xylotrechus chinensis**	**Xylotrechus arvicola**	**Xylotrechus undulatus**

Portugal (2019)

➡ Samples from China and the USA were not detected

➡ "Minority" samples were detected and closly related species differentiated

➡ Arhopalus syriacus was detected (unlike Bulk 1)

Conclusion - Use of the MinION

- Detection of species present in very low quantities in the traps
- Distinguishing between closely related species

- Need to add data to databases to avoid false negatives
- Good quality DNA samples are essential (preservation of individuals)

Comparison of results with Illumina Miseq sequencing

Thank you for
your attention