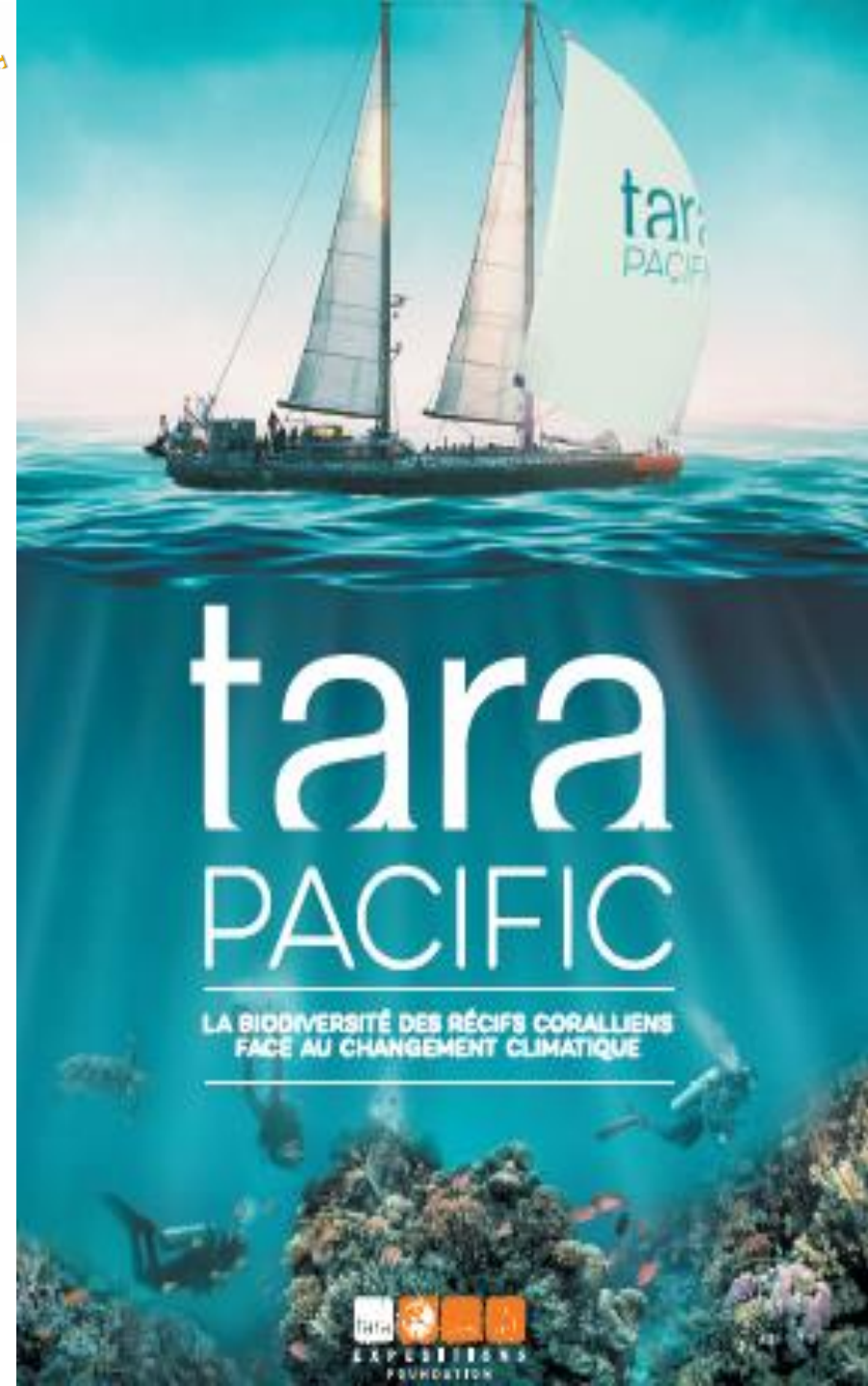


Workshop MinIon
13 december 2017

Coral holobiont analysis with MinION sequencer onboard *Tara*

Quentin Carradec Julie Poulain

Laboratoire d'analyses
génomiques des eucaryotes
CEA/Genoscope

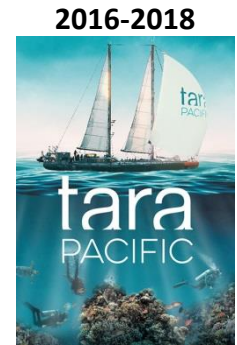
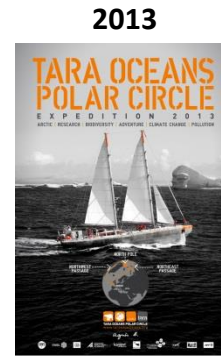


- **15 years of scientific expeditions**

- **Study and understand the impact of climate change on the world's oceans**

- **Multi-disciplinary**
 - Oceanography
 - Chemistry
 - Biology (genomics, imaging)

- **Communication**
 - Inform populations about consequences of global warming
 - Education programs for children (500 French teacher involved)



« Genetic complexity of coral holobiont across the Pacific »

- 100,000 km
- More than 100 researchers
- 23 research institutes (France, Monaco, USA, Germany, Switzerland, South Arabia, Japan)
- 15,000 samples of corals and other species





Coral sampling

Water sampling



→
**on board
freezing**

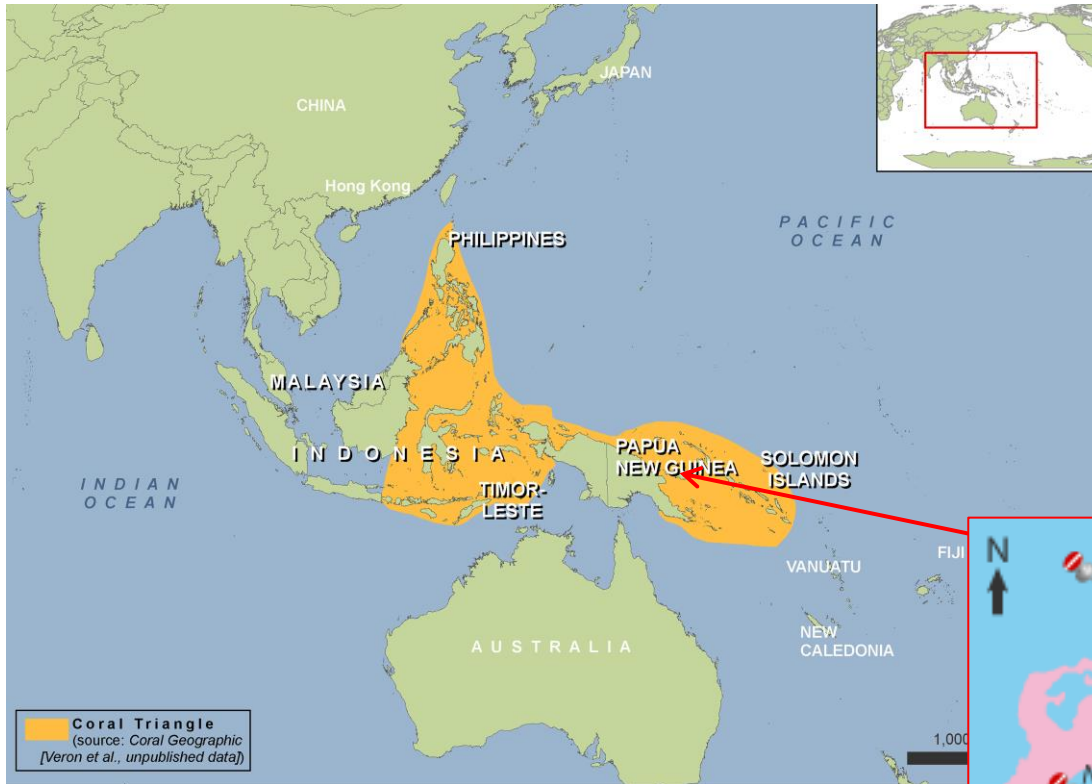


→
shipping



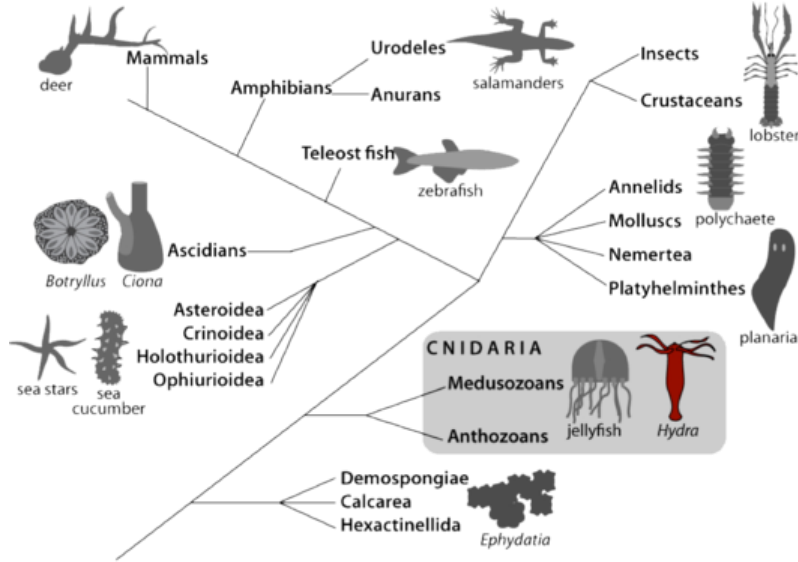


- Species identification via marker gene sequencing
- Direct RNA sequencing to evaluate coral health and response to stress.

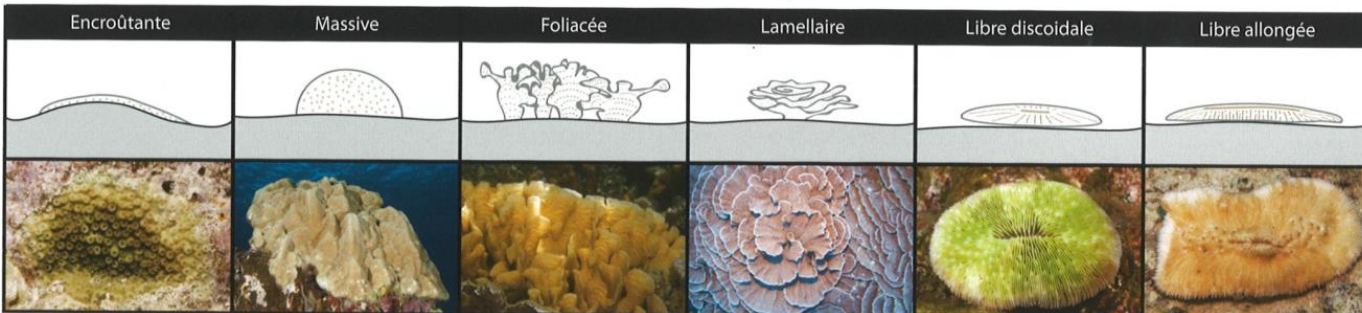
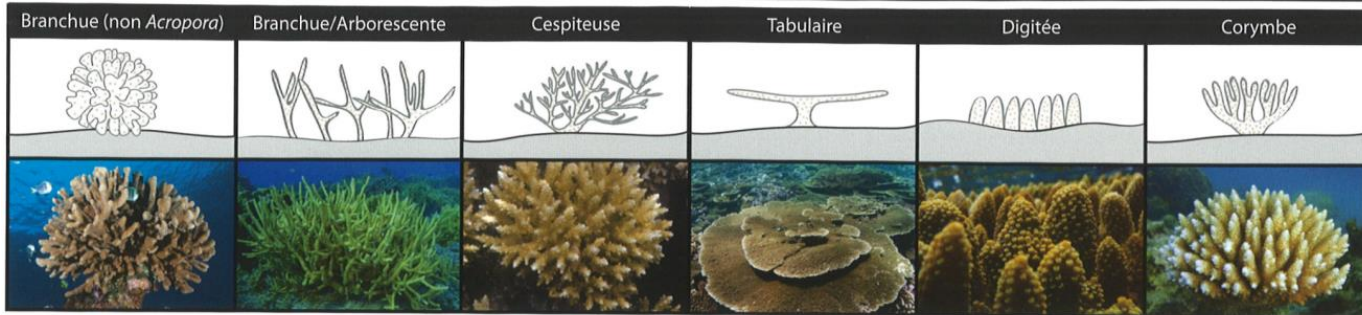


50% of total coral diversity



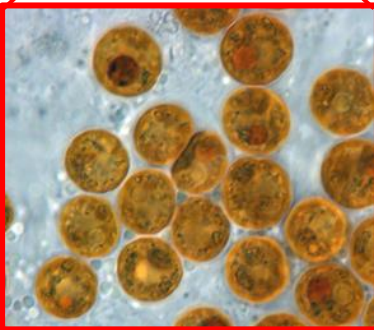
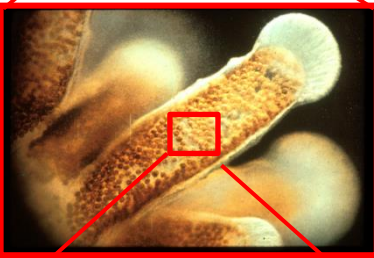
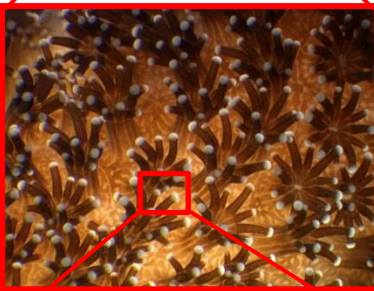
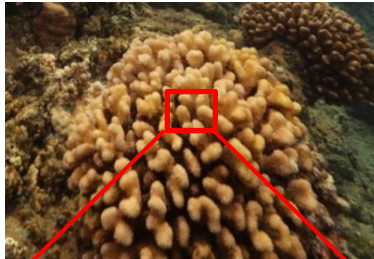


Phylum	Class	Subclass	Order
Cnidaria	Hydrozoa		<ul style="list-style-type: none"> Hydroidea (hydroids) Milleporina (fire corals) Stylasterina
		Anthozoa	<ul style="list-style-type: none"> Octocorallia
	Anthozoa	Hexacorallia	<ul style="list-style-type: none"> Actiniaria (simple sea anemones) Zoanthidia (colonial anemones) Corallimorpharia (corallimorpharians) Scleratinia (true stony corals) Rugosa (Paleozoic coral - now extinct) Tabulata (Paleozoic corals - now extinct)
		Ceriantipatharia	<ul style="list-style-type: none"> Antipatharia (black corals) Ceriantharia (tube anemones)



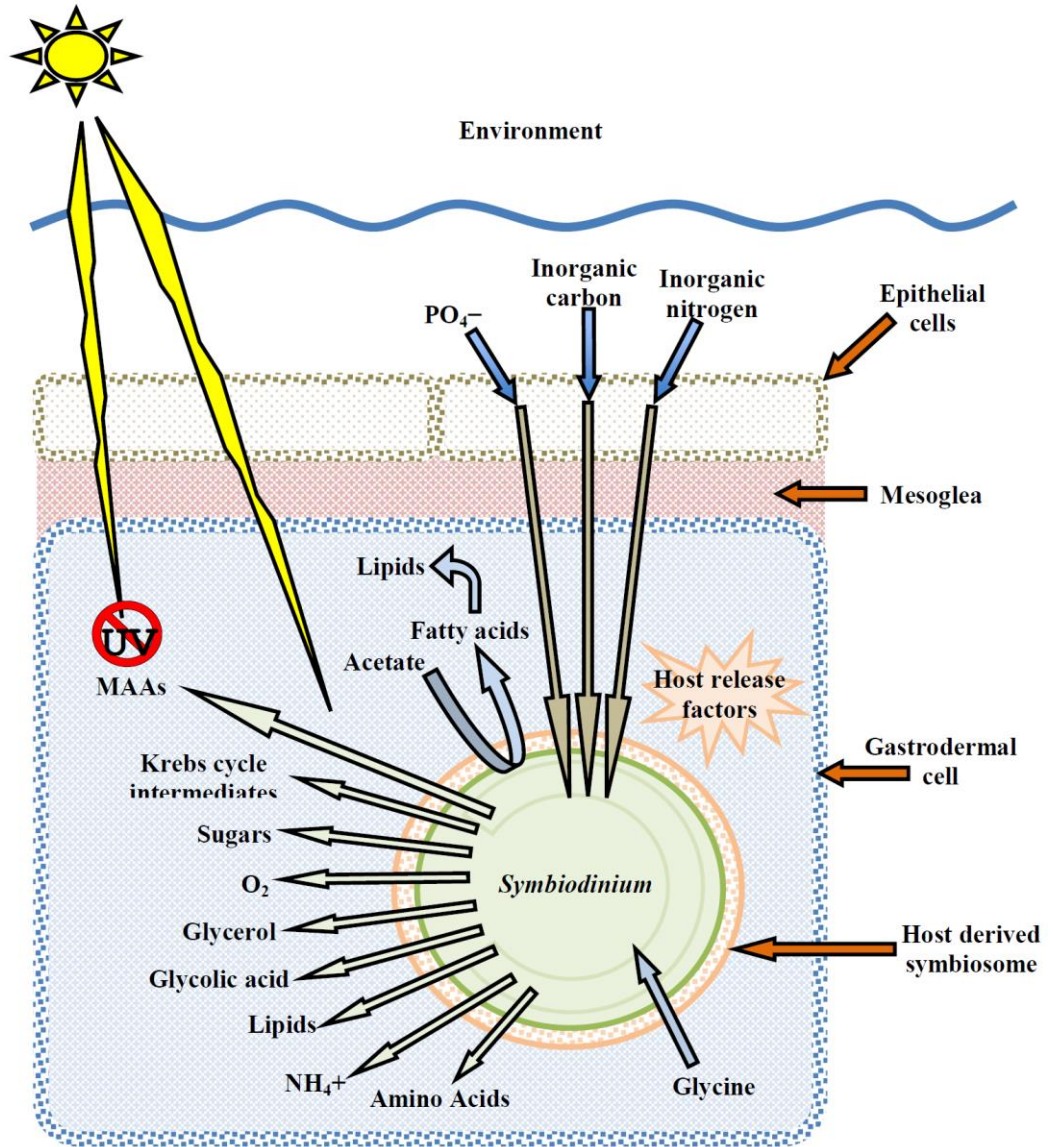
798 species identified

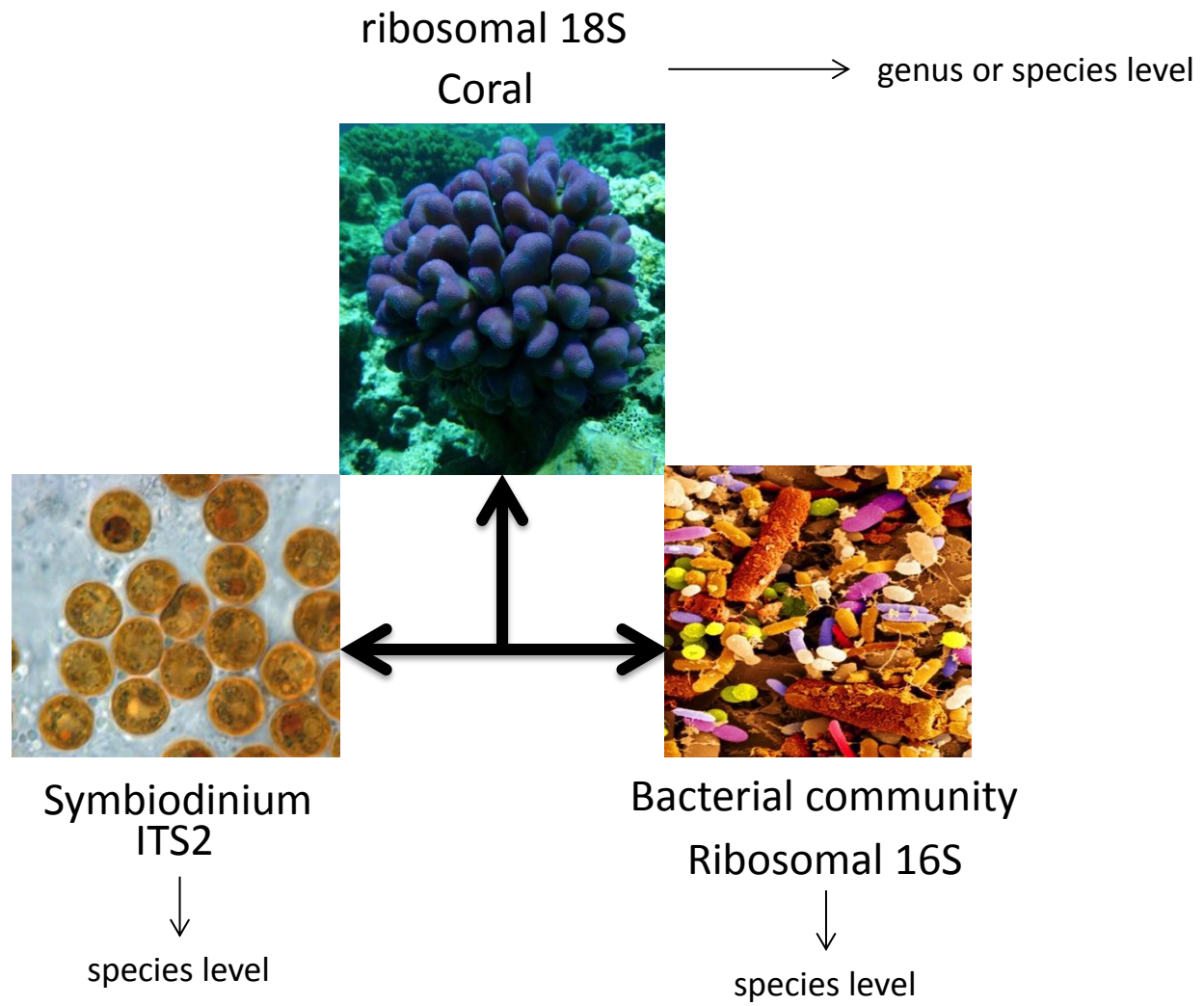
Pocillopora



Symbiodinium

>150 species







Emilie Boissin



Corals sampling

Grinding (1minute)



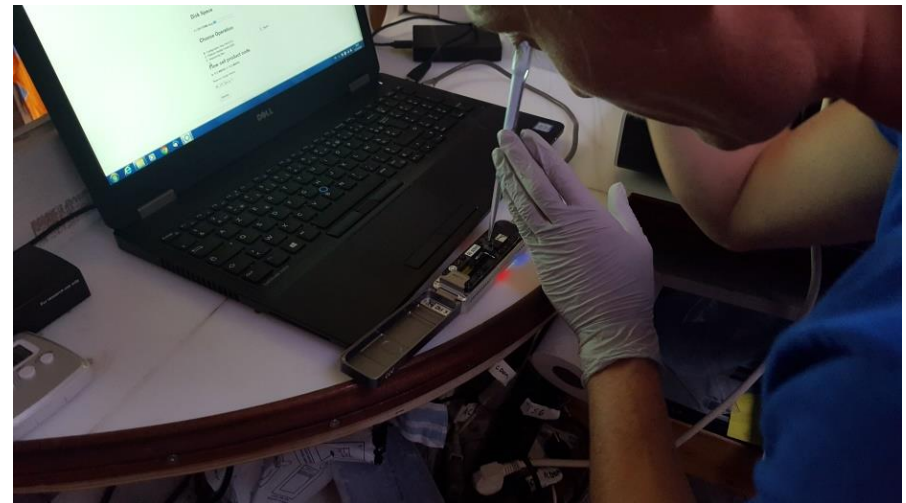
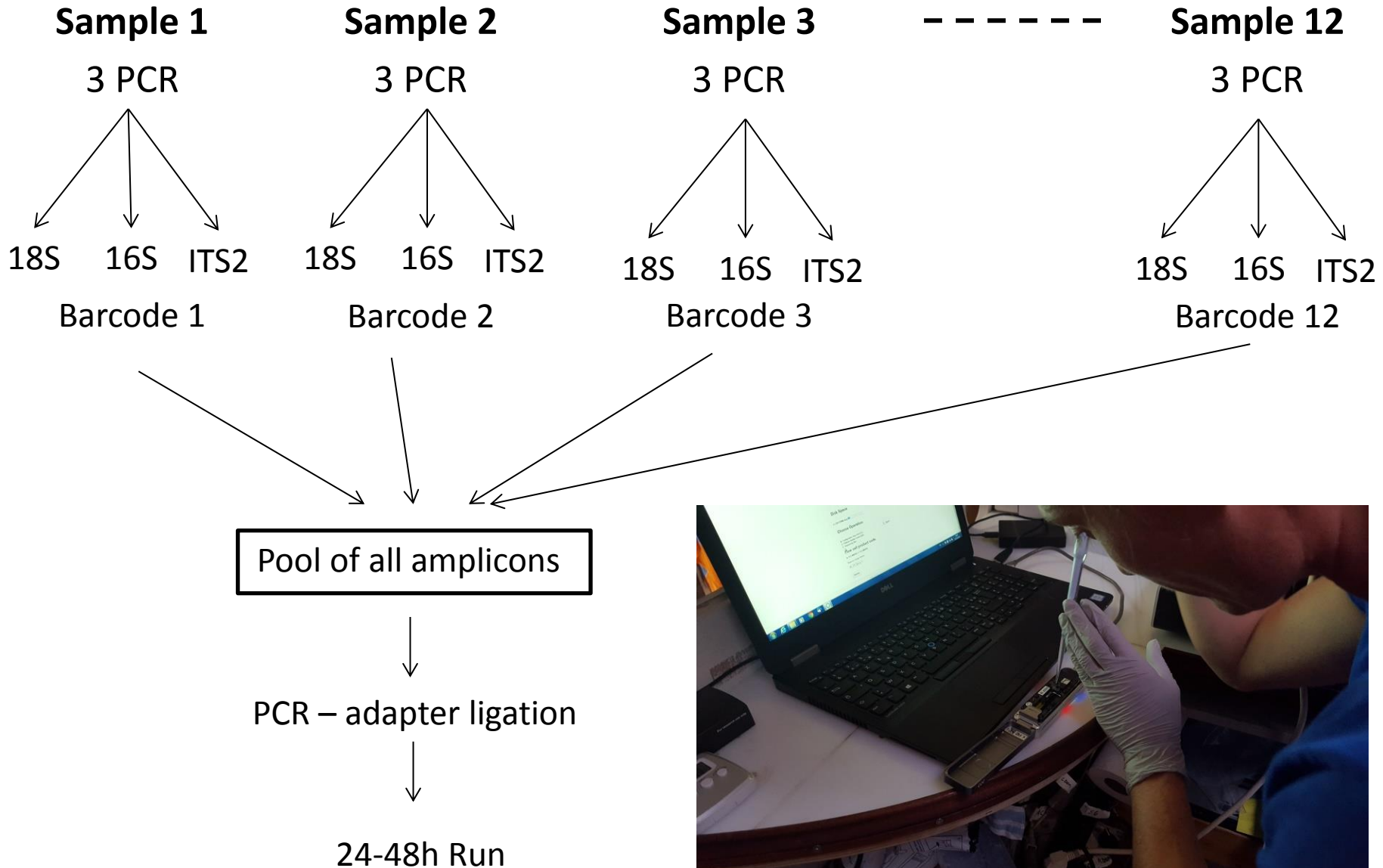
TerraLyzer



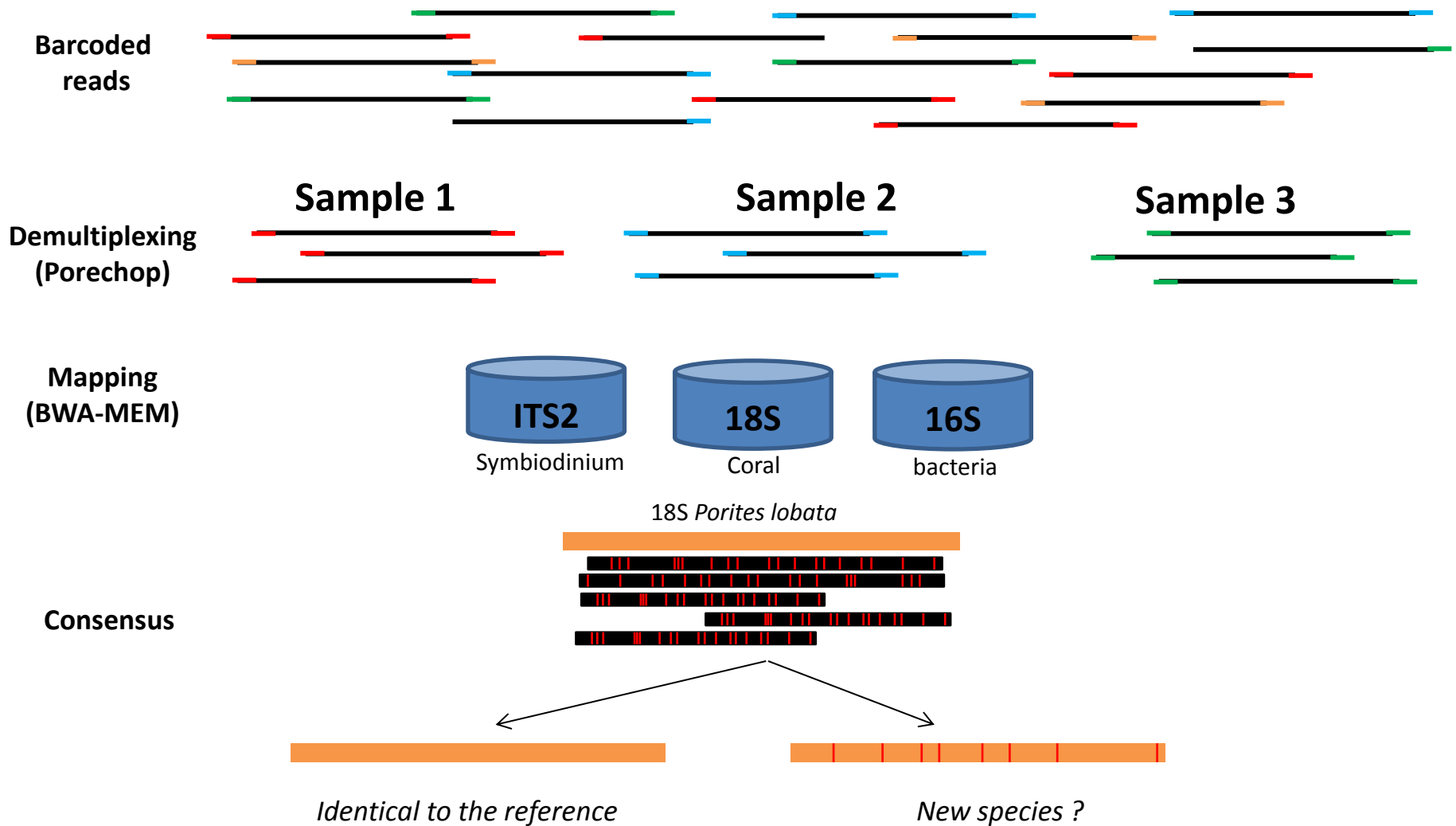
DNA extraction (2h30 for 12 samples)



**96 samples
in 12 days**

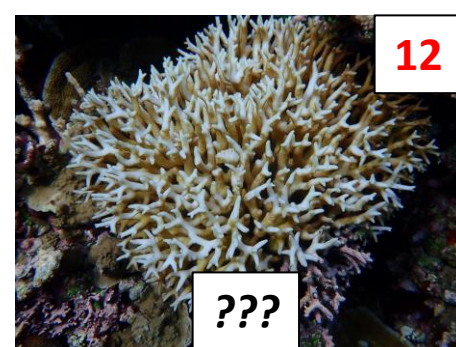
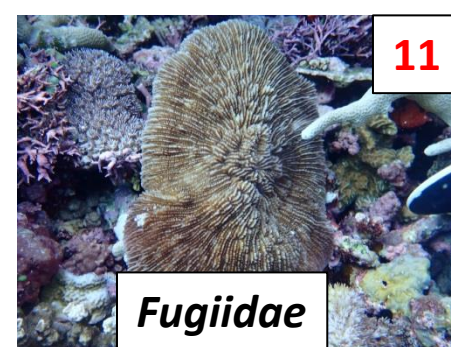
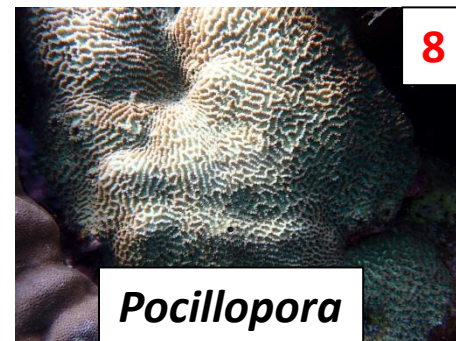
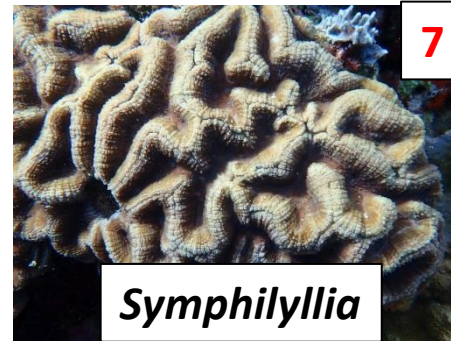
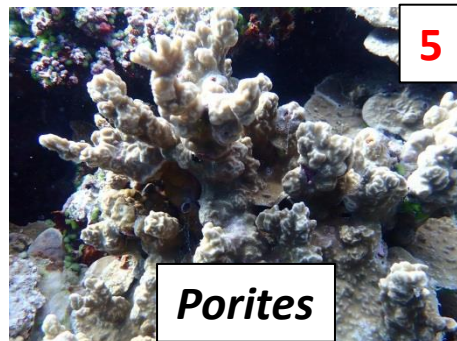
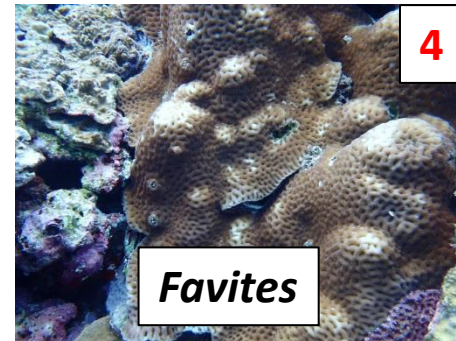
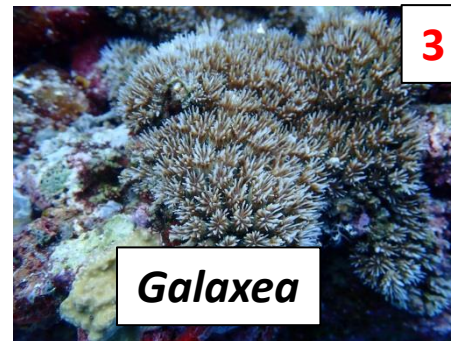
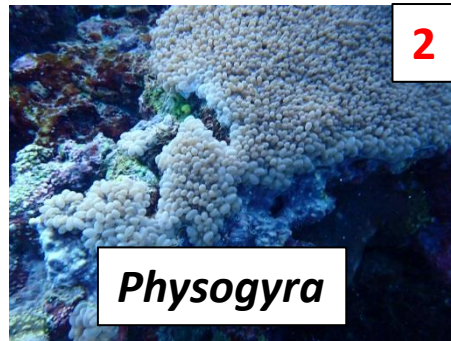
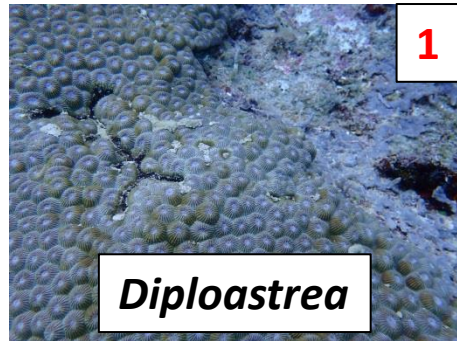


Julie Poulain

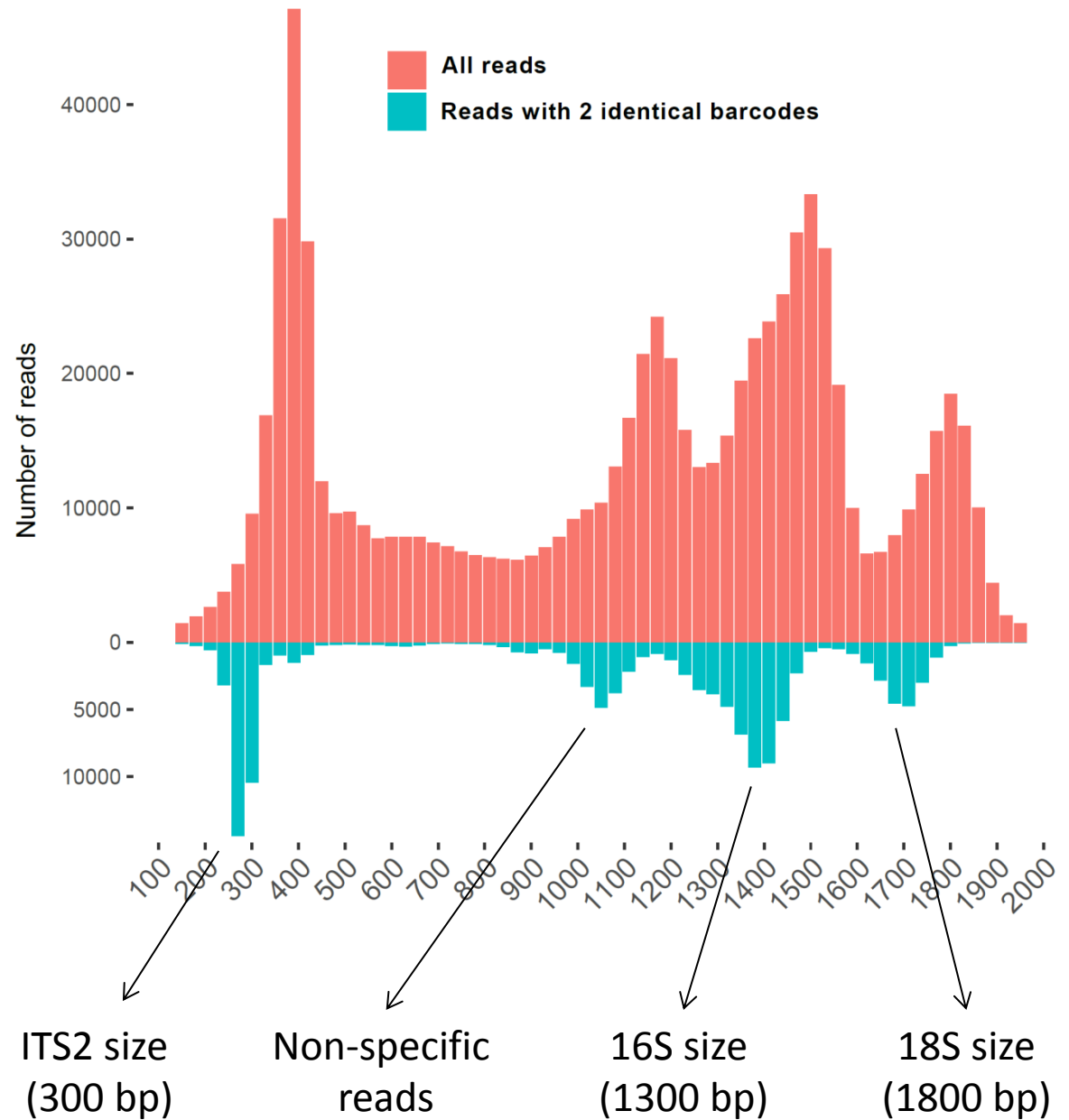


1 RUN = 12 samples and 3 Marker genes / sample



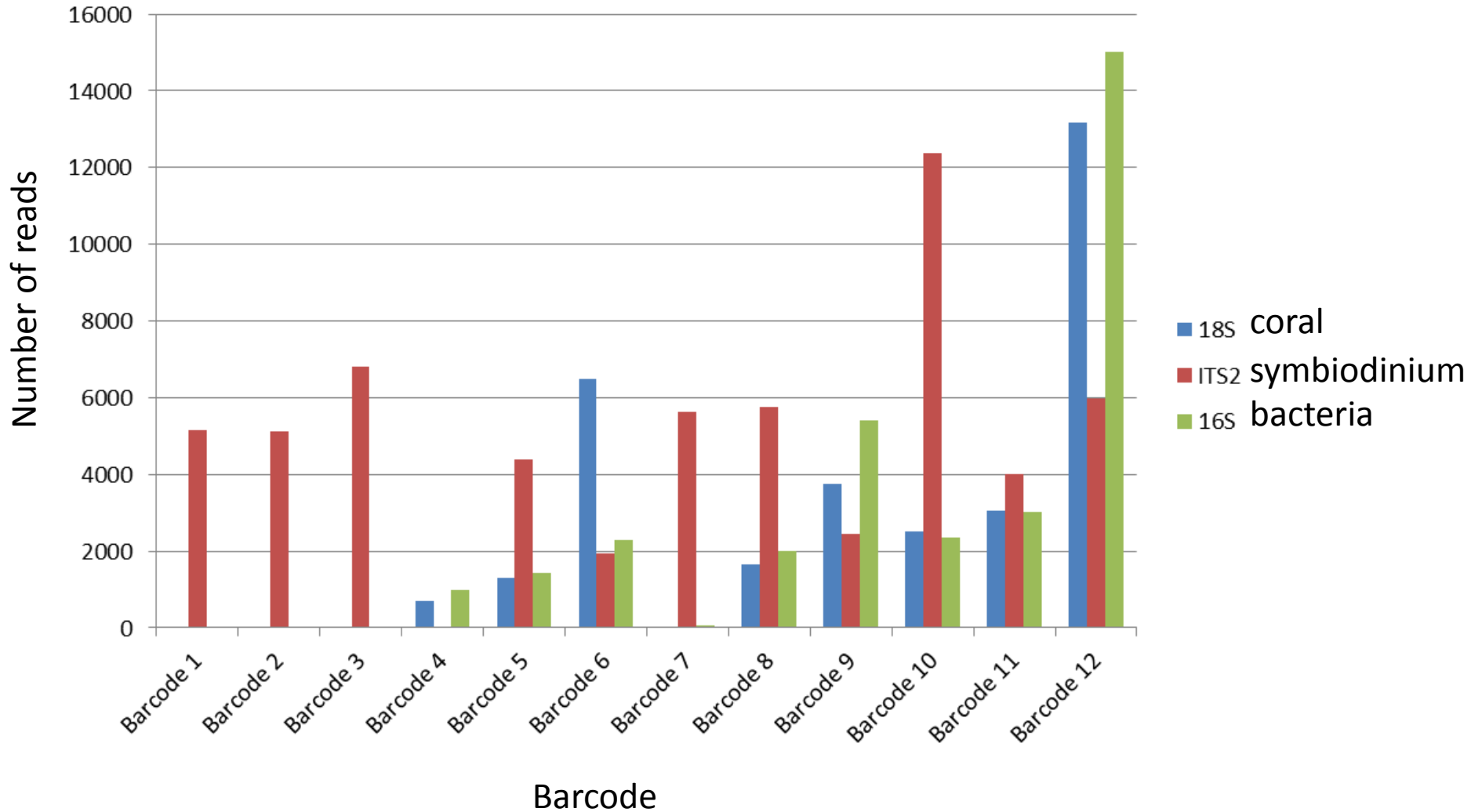


- 862 639 reads
- 127 974 (15%) with detected barcodes on each side

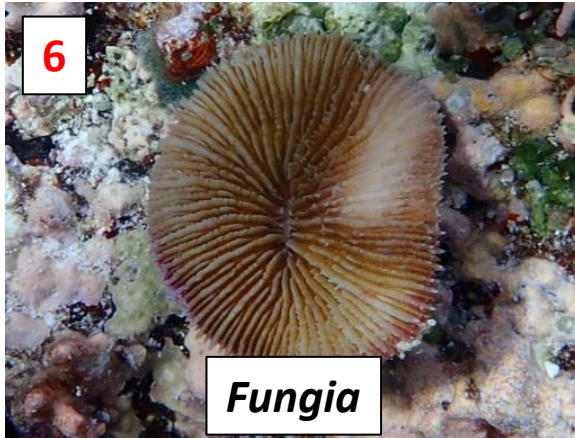


BWA :

- 80% of read length aligned
- >70% of identity



Morphology



18S sequencing

- *Fungia paumotensis*
- *Fungia horrida*

ITS2 sequencing

- Symbiodinium -Clade C161 (+3 SNPs)
- Clade C2r (+4 SNPs)

16S sequencing

Bacteria community : not yet analyzed....

Conclusion:

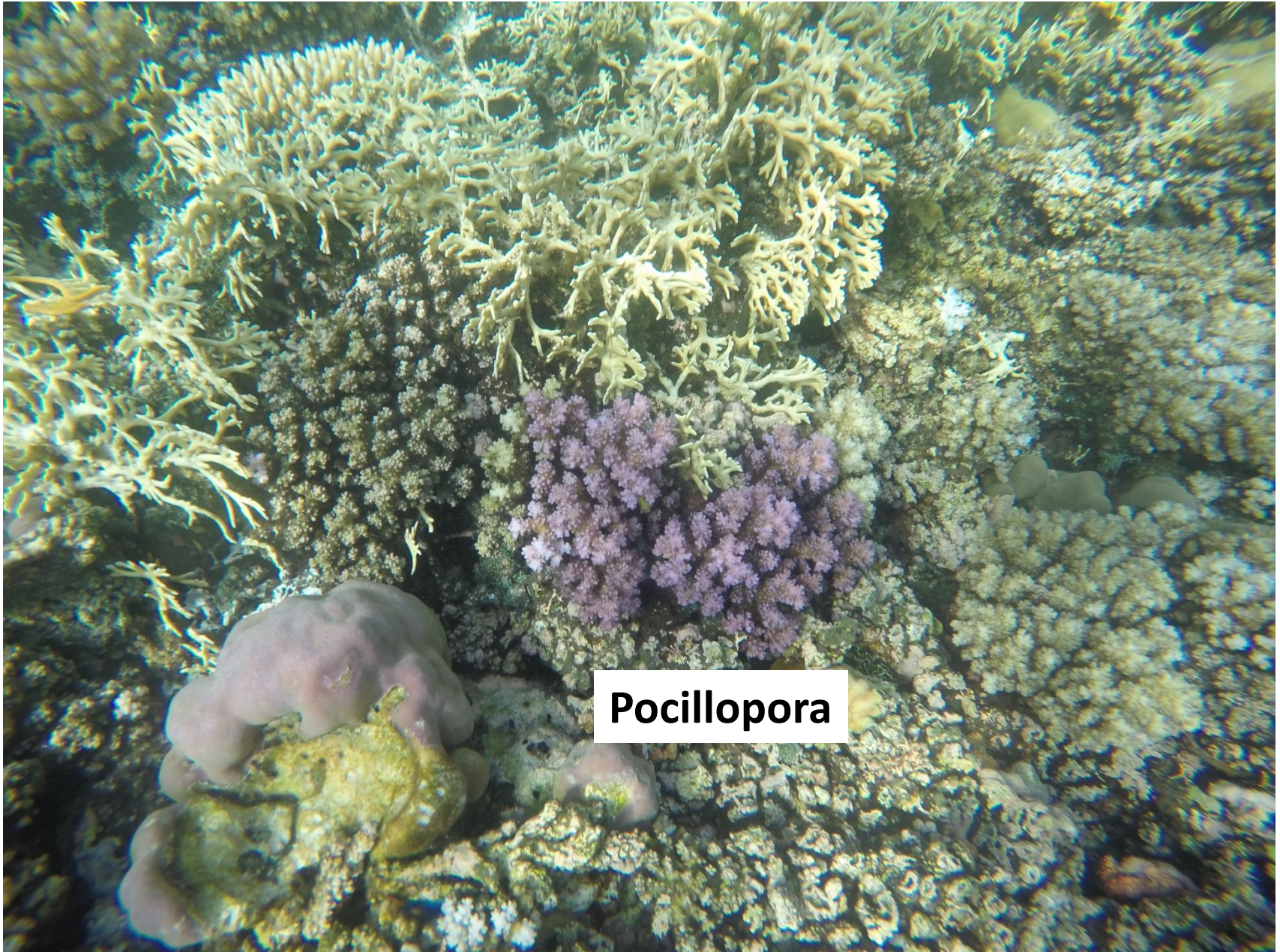
- 96 DNA extractions
- 5 MinION runs
- More than 30 corals identified + symbiodinium + bacterial community.

limitations:

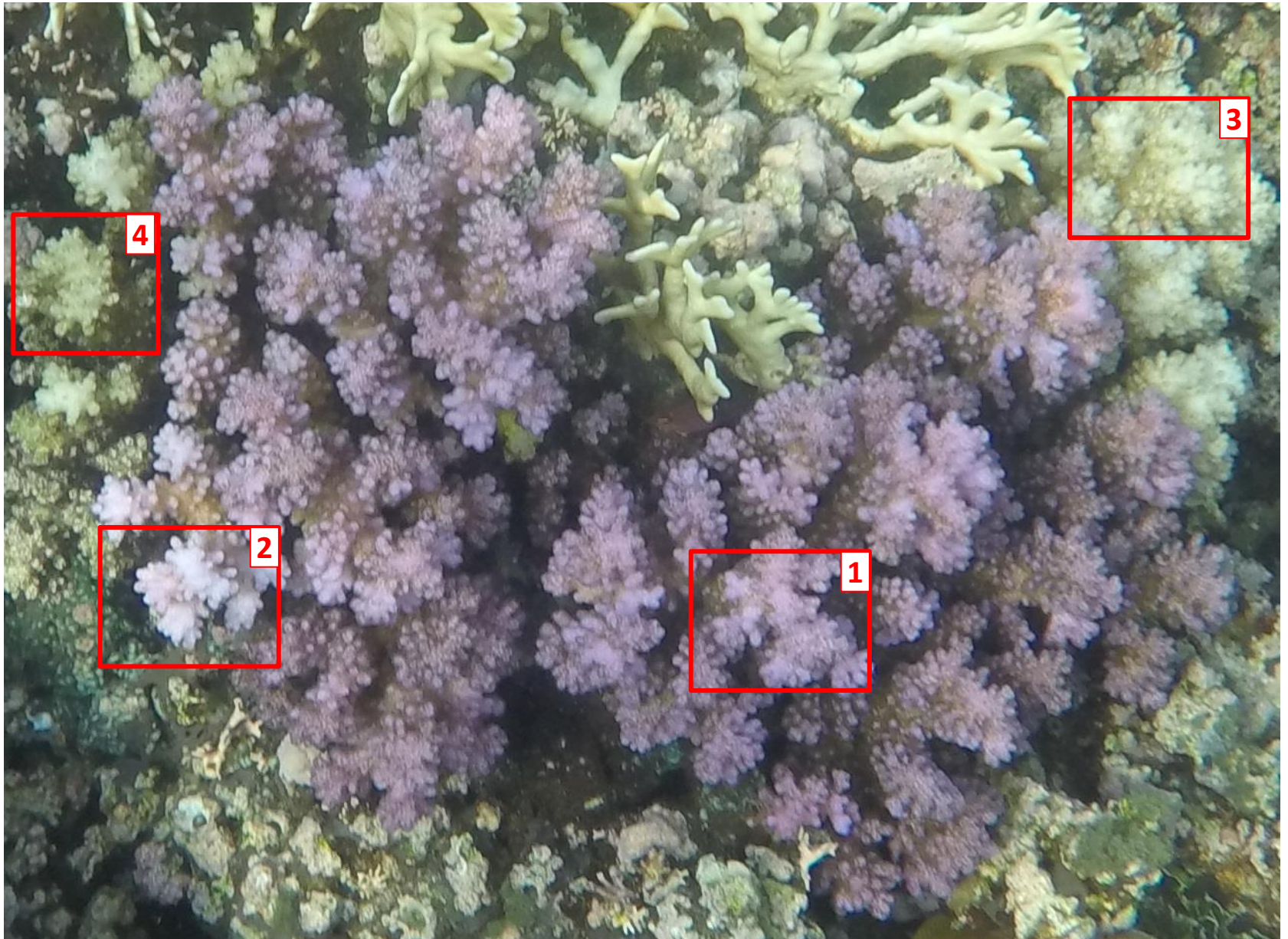
- Flow cells quality (over than 2 month at 4°C)
- Reactive conservation at low temperature.
- Parallelization of library preparation (VolTrax ?).

Prospects:

- Daily use of MinION for species identification.
- Less samples to conserved onboard
- Less samples to send.
- Immediate answer.



Pocillopora





RNA extraction

RNA extraction

RNA extraction

RNA extraction

PolyA RNA selection

PolyA RNA selection

PolyA RNA selection

PolyA RNA selection

Library preparation
for RNA sequencing

Library preparation
for RNA sequencing

run 1

run 2

27 712 reads

34 452 reads

mapping on *Pocillopora*
reference transcriptome

11 325 reads aligned

18 247 reads aligned

Genoscope, CEA Patrick Wincker

Corinne Cruaud (LBioMeg)

Valerie Barbe (LBioMeg)

Arnaud Lemainque (LBioMeg)

Stefan Engelen (R&D bioseq team)

Denis Debaussart (informatic team)

CRIOBE, Perpignan

Emilie Boissin

Thank you for your attention

Tara Pacific Consortium

Serge Planes (CNRS)

Denis Allemand (Centre scientifique de Monaco)

web site: <http://oceans.taraexpeditions.org/>