OIST CORAL eDNA

(OIST Coral Reefs Conservation-Research Alliance using New eDNA Technology)


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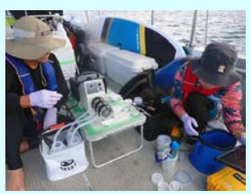
The Grand Survey of Coral Reefs in the Pacific Ocean

How can we monitor the health of coral habitats with environmental DNA (eDNA)?

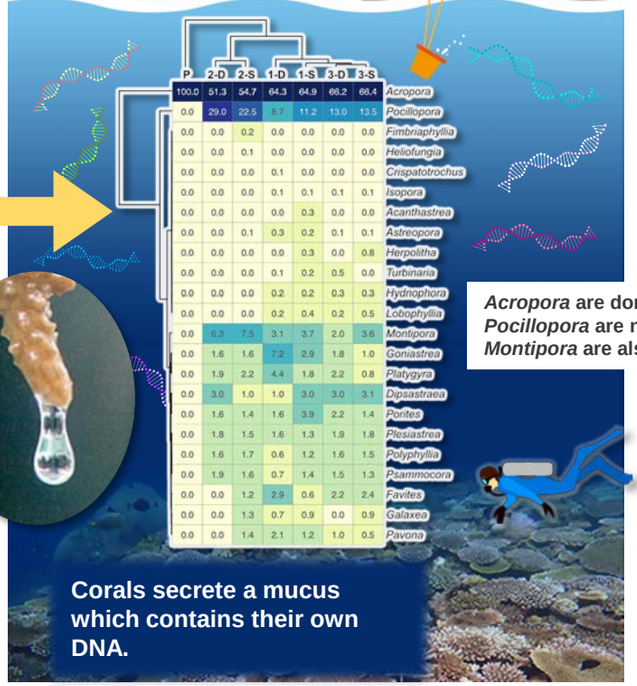
The DNA in seawater samples is amplified by a primer that specifically detects corals.

The amplified DNA is sequenced.

The sequenced DNA allows us to identify coral species and estimate the number of corals on the reef.



OIST researchers collect 2 liters of surface seawater for eDNA analyses.

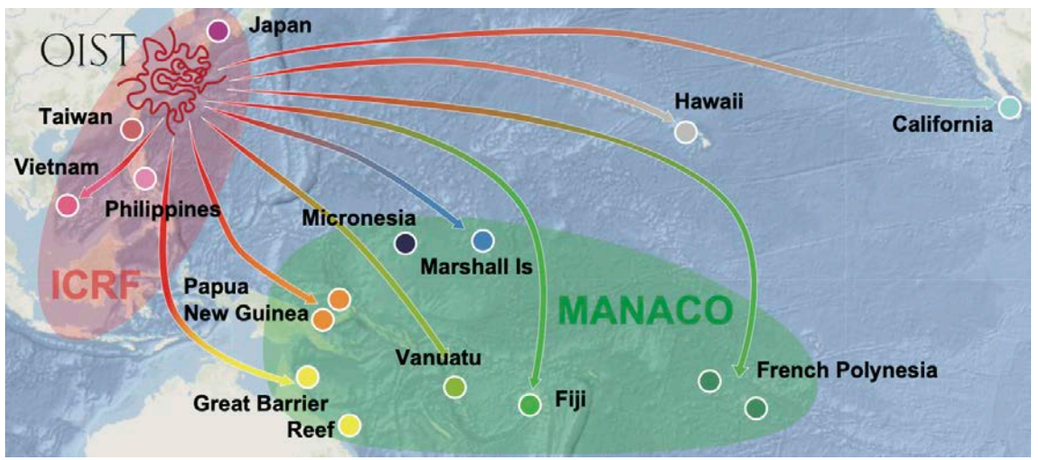


Corals secrete a mucus which contains their own DNA.

Acropora are dominant and
Pocillopora are next.
Montipora are also present in this reef.

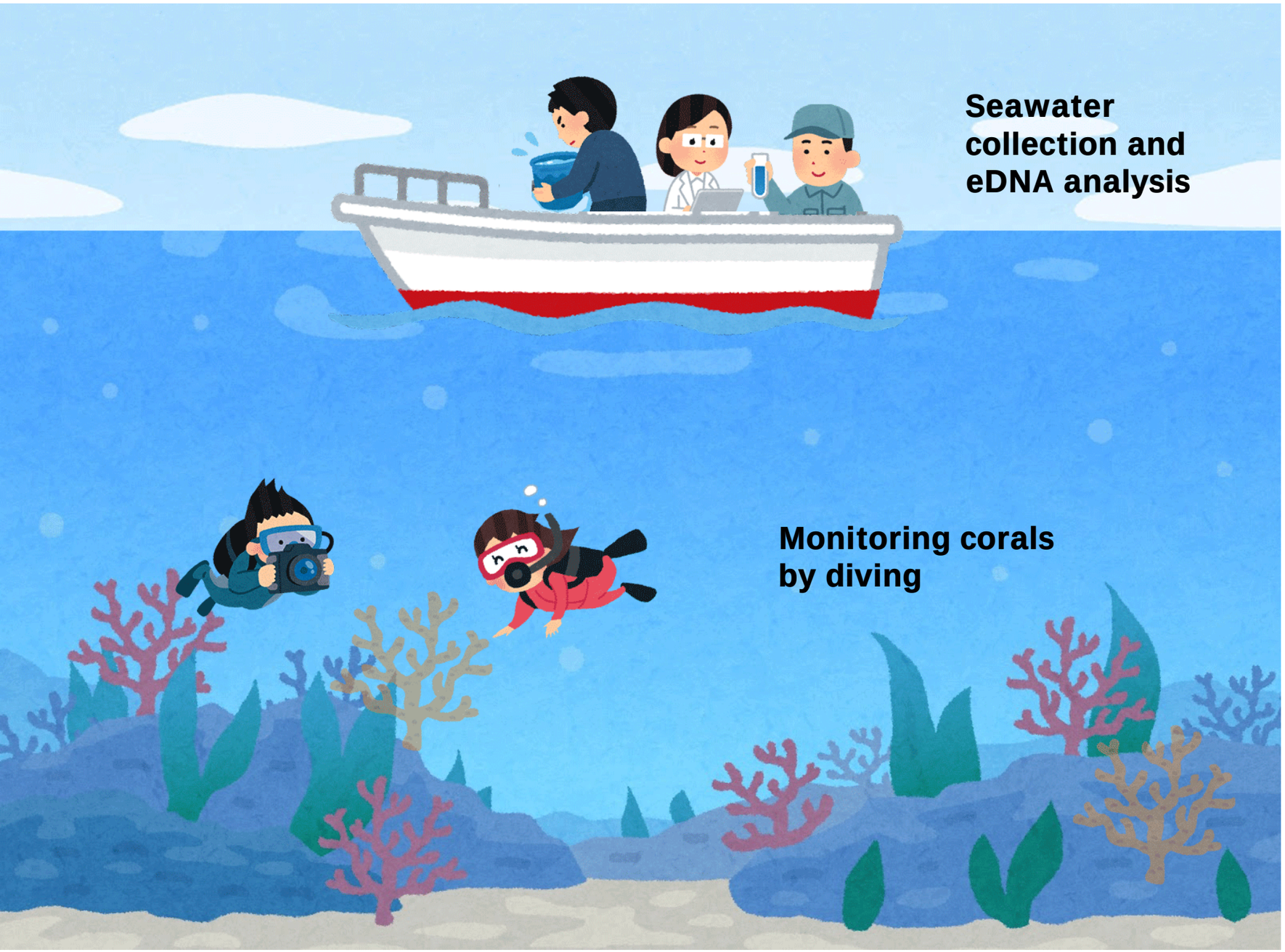
We compared eDNA analyses with the observation of coral specialist divers: how many and what species of corals are there on a given coral reef?

eDNA analyses of a small amount of seawater can capture more species information than what divers can observe.



Priority Areas for the Grand Survey Project: Covered by MANACO Consortium and International Coral Reef Forum (ICRF)

The efficiency of this eDNA method has been proven by a survey of 38 spots of the Okinawa Island.



Reef-building corals (Scleractinian corals)

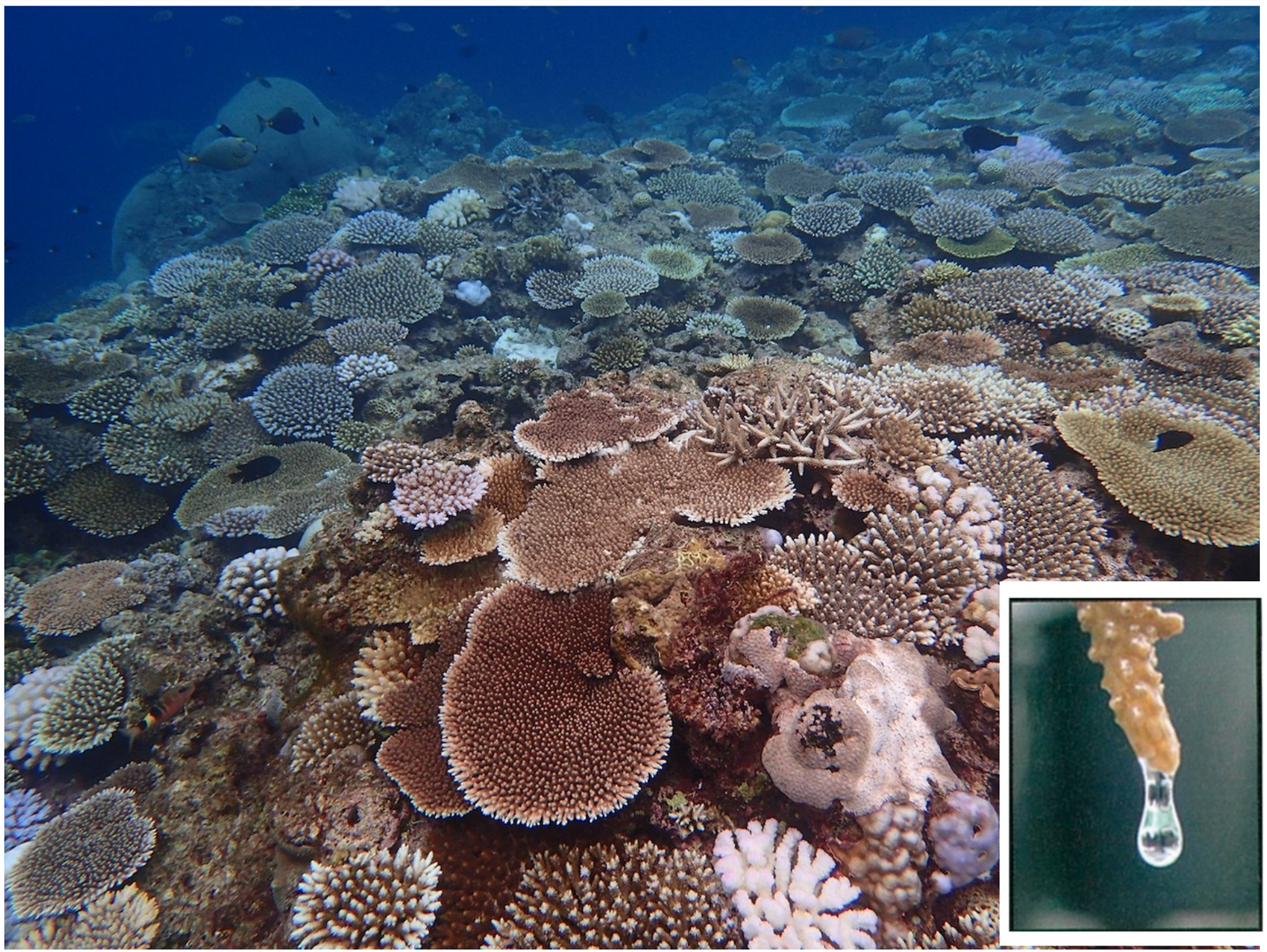
~1,300 species
239 genera
25 families

(based on ITIS, www.itis.gov)

If eDNA method becomes feasible, we may chase corals without (tough) dividing into the sea and more broadly.

An urgent challenge !

Chasing Coral Reefs : Challenge using environmental DNA (eDNA) method

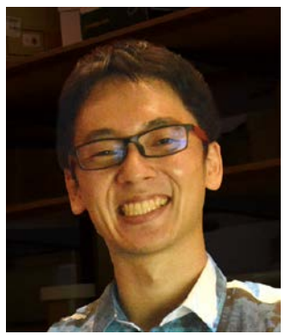


From H. Yamashiro

eDNA barcoding method

1. Corals secret mucus, which likely contains DNA specific to given corals.
2. Mucus floats near surface of sea.
3. Collect surface seawater that possibly contains mucus.
4. Extract DNA and amplify mitochondrial DNA with coral-specific primers.
5. Sequence DNA to identify corals in the samples.

Challengers: Marine Genomics Unit of OIST and AORI of the University of Tokyo



Koki Nishitsuji

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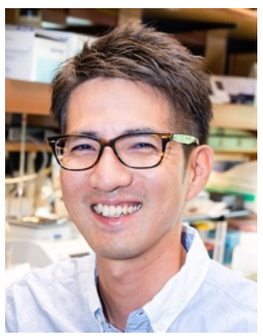
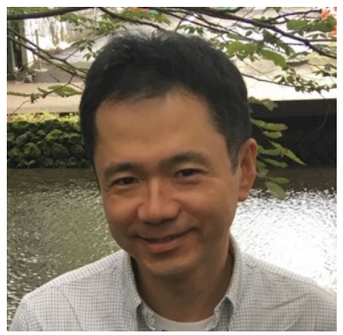


Chuya Shinzato



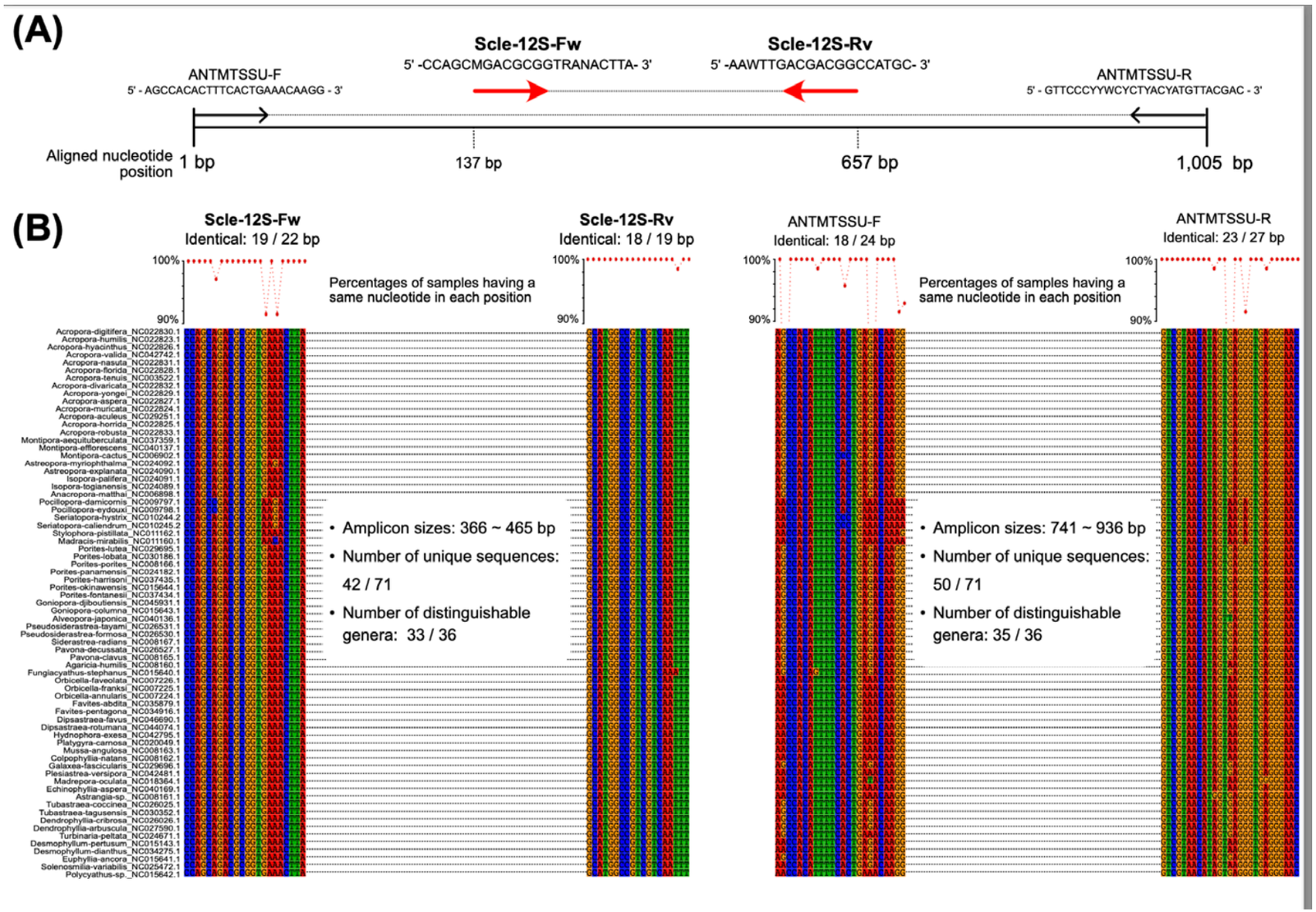
Jun Inoue

Nori Satoh

✓ Previously, we carried out an aquarium-level experiment using conventional primers and found that eDNA method is applicable to *Acropora* corals (Shinzato et al., 2018).

1. We wish to develop **primers that cover most genera of reef-building corals** (not only *Acropora*).
2. We wish to **test feasibility of this method at natural seashore**.

Development of a new universal primer pairs I : Scle-12S-Fw and Scle-12S-Rv



Mitochondrial genomes (NCBI: May 1, 2021)

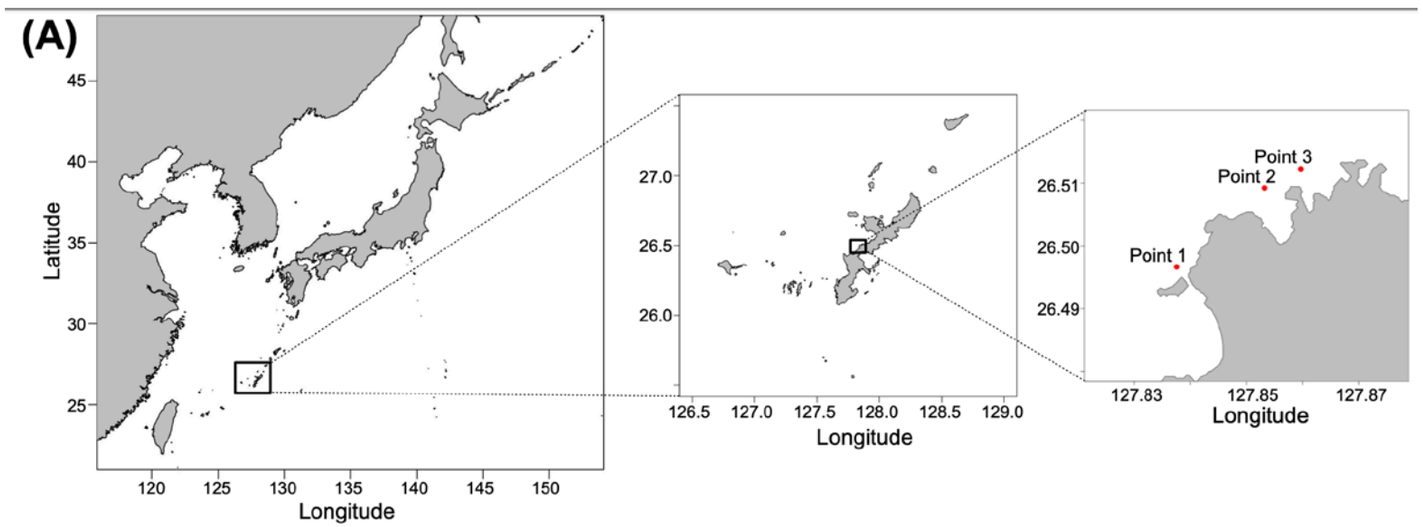
36 genera
15 families
71 species

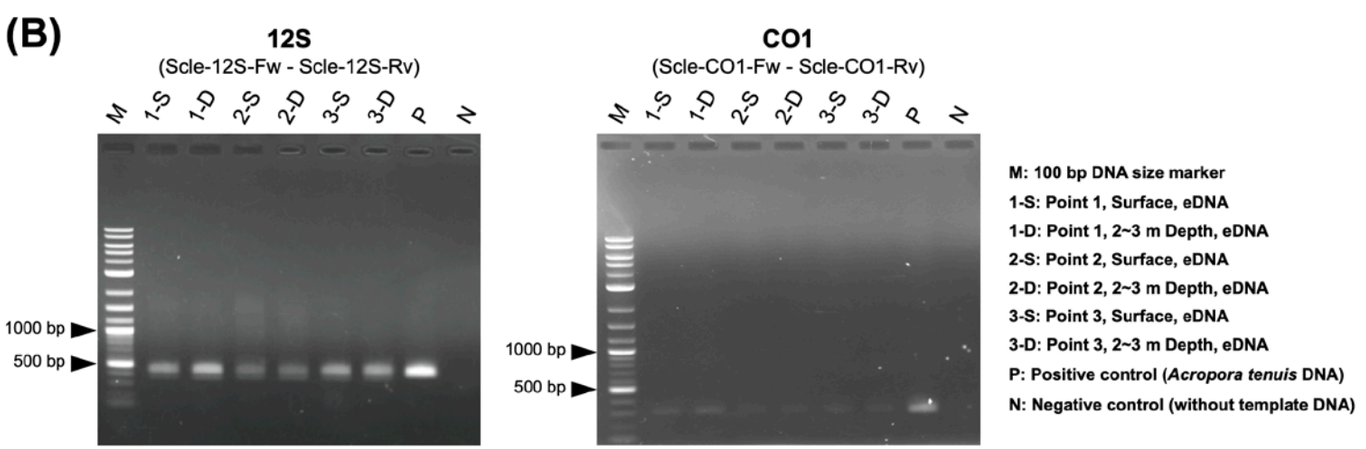
Compare sequences to develop a best primer-set

12S rDNA gene

(Shinzato et al., Front. Marine Sci. 8: 758207 2021)

Seawater samplings and mtDNA amplification by new primer sets

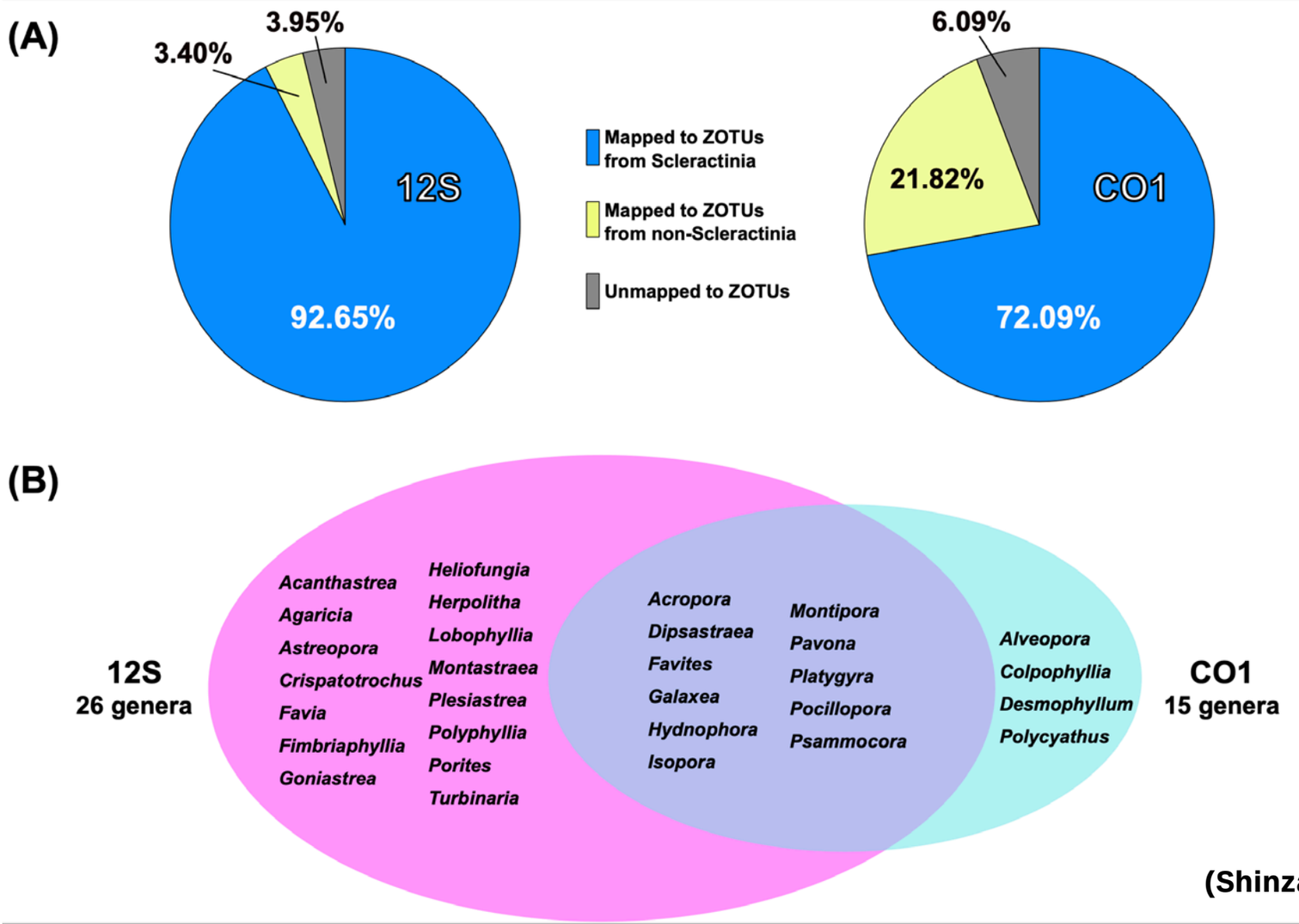




both resulted in positive signals

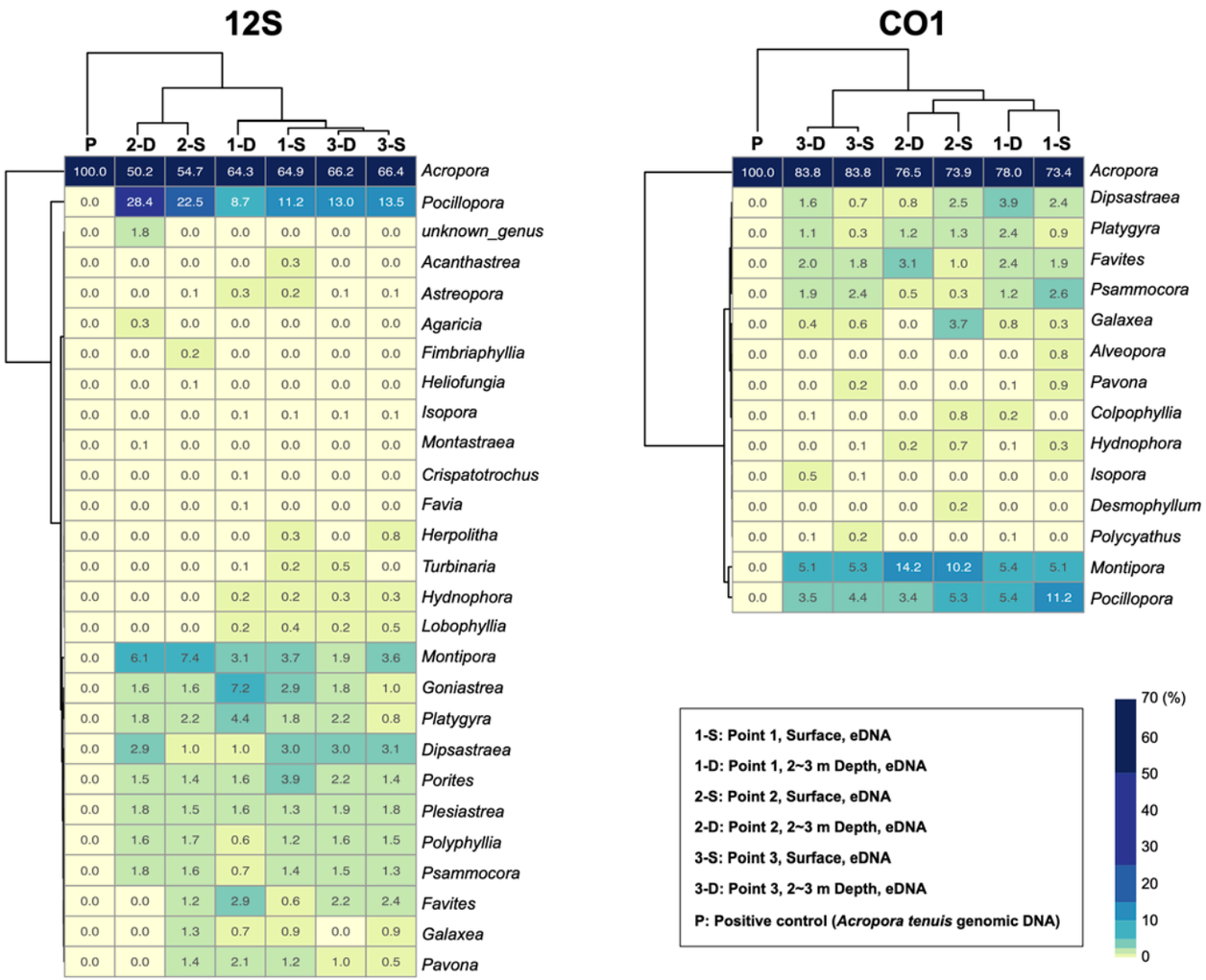
(Shinzato et al., 2011)

PCR amplicon sequences and detection of coral genera



(Shinzato et al., 2021)

Percentage of sequence reads mapped to coral genera in each eDNA sample



Dominant species
Acropora

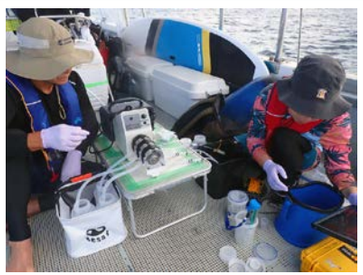
Subdominant species
Pocillopora

Others
Montipora, Galaxea

(Shinzato et al., 2021)

eDNA barcoding method

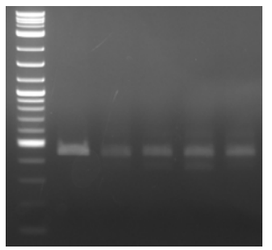
① collect seawater and filtration



② isolation of DNA

③PCR with primers we devised for mtDNA 12S and CO1

④ electorophoresis



⑤ library preparation

⑥ sequencing by Illumina MiSeq

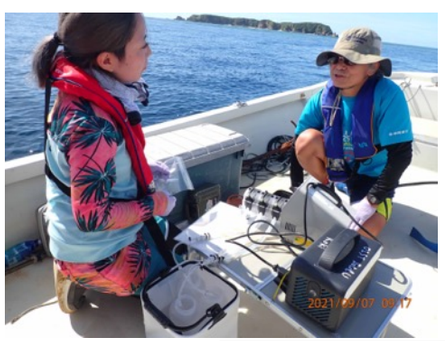
⑦ sequence analysis by computer

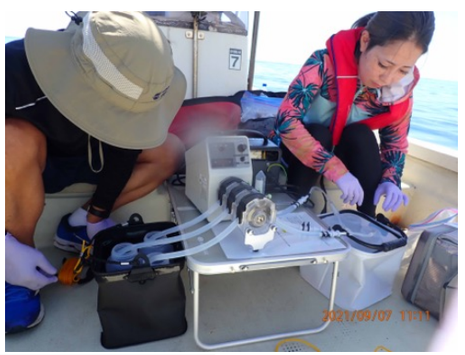
⑧ coral identification

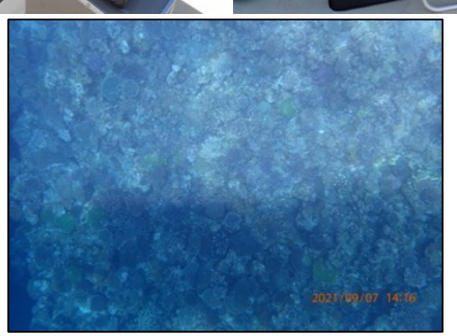
	P	2-D	2-S	1-D	1-S	3-D	3-S
Acropora	100.0	51.3	54.7	64.3	64.9	66.2	66.4
Pocillopora	0.0	29.0	22.5	8.7	11.2	13.0	13.5
Fimbriaphyllia	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Heliofungia	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Crispatotrochus	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Isopora	0.0	0.0	0.0	0.1	0.1	0.1	0.1
Acanthastrea	0.0	0.0	0.0	0.0	0.3	0.0	0.0
Astreopora	0.0	0.0	0.1	0.3	0.2	0.1	0.1
Herpolitha	0.0	0.0	0.0	0.0	0.3	0.0	0.8
Turbinaria	0.0	0.0	0.0	0.1	0.2	0.5	0.0
Hydnophora	0.0	0.0	0.0	0.2	0.2	0.3	0.3
Lobophyllia	0.0	0.0	0.0	0.2	0.4	0.2	0.5
Montipora	0.0	6.3	7.5	3.1	3.7	2.0	3.6
Goniastrea	0.0	1.6	1.6	7.2	2.9	1.8	1.0
Platygyra	0.0	1.9	2.2	4.4	1.8	2.2	0.8
Dipsastraea	0.0	3.0	1.0	1.0	3.0	3.0	3.1
Porites	0.0	1.6	1.4	1.6	3.9	2.2	1.4
Plesiastrea	0.0	1.8	1.5	1.6	1.3	1.9	1.8
Polyphyllia	0.0	1.6	1.7	0.6	1.2	1.6	1.5
Psammocora	0.0	1.9	1.6	0.7	1.4	1.5	1.3
Favites	0.0	0.0	1.2	2.9	0.6	2.2	2.4
Galaxea	0.0	0.0	1.3	0.7	0.9	0.0	0.9
Pavona	0.0	0.0	1.4	2.1	1.2	1.0	0.5

Shinzato et al. (2021) Front. Marine Sci. 8: 758207

Monitoring corals by two diver-specialists together with three eDNA sampling staffs of MGU







5-15 m in depth

eDNA analysis

Monitoring corals
by diving

Results were similar between
the two methods

Acropora 72 %

Porites 16 %

Montipora 10 %

Reef-building corals (Scleractinian corals)

~1,300 species
239 genera
25 families

(based on ITIS, www.itis.gov)

The eDNA method is feasible, and we may chase corals more broadly in near future.

OIST Coral eDNA: Ongoing Challenges

(1) Deepen research further to sub-phototrophic reefs

- Conventional diving limit: ~25 m in depth
- Deepen to 40 or 50 m depth?
- Combination of underwater robot with eDNA (in collaboration with NTT DoComo)

underwater robot;
50 m in depth

Collect 500 ml
SW over the reefs

(2) Prediction of bleaching by eDNA

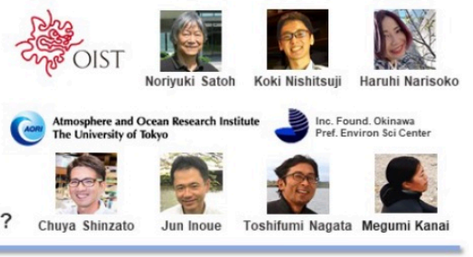
- Corals secrete mucus constantly.
- If under increasing stress condition such as seawater temperature raise, corals secrete more mucus than usual, can we detect this by eDNA?
- If eDNA method can detect this change, a few weeks prior to real bleaching, we may prepare something against bleaching.

OIST OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY
沖縄科学技術大学院大学

OIST eDNA Workshop
for young coral biologists in the Pacific region

OIST CORAL eDNA

(OIST Conservation-Research Alliance using New eDNA Technology)



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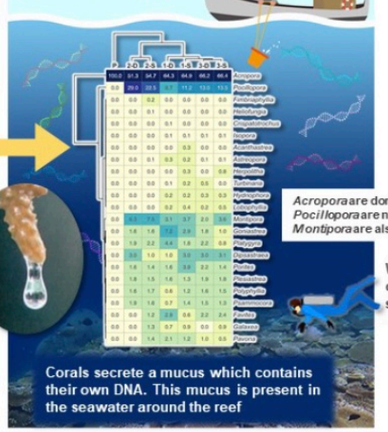

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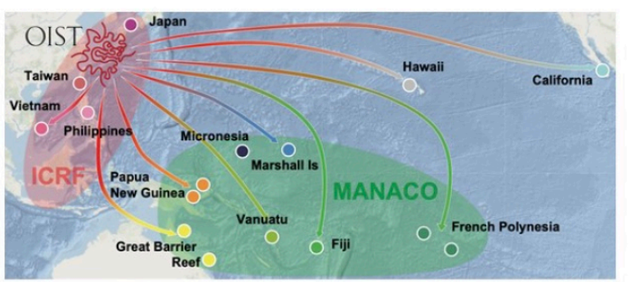

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