Coral photosymbiosis: Linking phylogenetic identity to single cell metabolic activity in mixed symbiont populations

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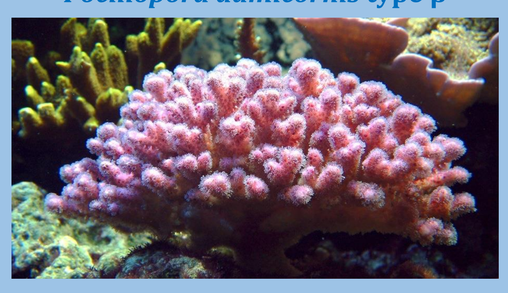
CONTEXT

Tropical reef-building corals (Scleractinia) live in nutrient-poor shallow oceanic waters. The ecological success of these heterotrophic animals lies in their symbiosis with photosynthetic dinoflagellates (*Symbiodinium*). Nine major clades (A-I) of *Symbiodinium* have been identified by molecular genetic analyses. Several dinoflagellate clades can simultaneously exist within a single coral colony. Physiological features of the different *Symbiodinium* clades influence the growth rates and the irradiance and thermal tolerance of the holobiont. **The objective of this project is to link the genetic identity of *Symbiodinium* to their metabolic performance in the intact symbiosis, at the cellular level.**

TROPICAL CORALS USED FOR THE STUDY

***Pocillopora damicornis* type β**

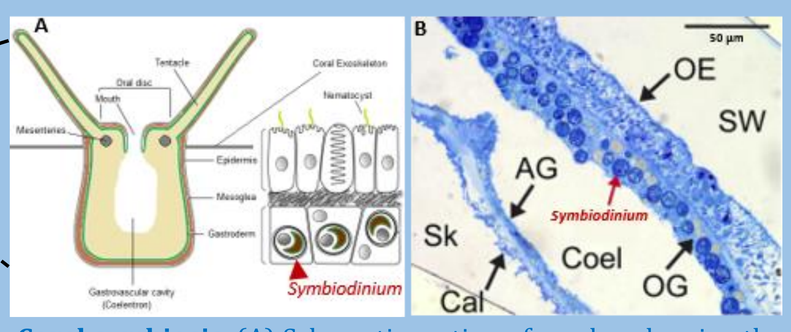


- Aquarium Porte Dorée, Paris, France
- hosts *Symbiodinium* clade C

Stylophora pistillata

- Red Sea, Eilat, Israel
- hosts varying proportions of *Symbiodinium* clade A and C as a function of depth

THE *SYMBIODINIUM*-CORAL HOLOBIONT

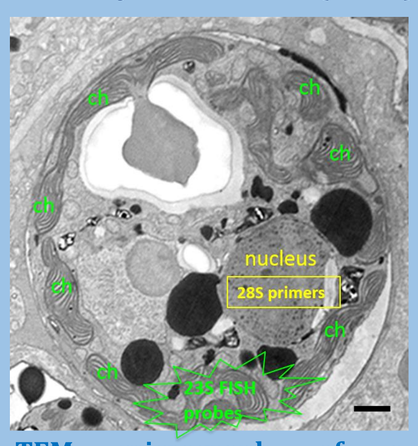


Coral symbiosis. (A) Schematic section of a polyp showing the location of *Symbiodinium* within coral gastrodermal cells and , (B) A tissue section from *Pocillopora damicornis* stained with methylen blue (Kopp et al. 2013).

The symbionts live inside the coral gastrodermal cells which line the gastric cavity of the polyp. Dinoflagellates contribute to the nutrition of their host; transferring up to 90% of photosynthates that are produced during the fixation of dissolved inorganic carbon (DIC) and nitrogen (either as nitrate or ammonium).

METHOD

1) **PCR genotyping** of *Symbiodinium* clades in corals.
2) Design clade-specific probes for Fluorescence *in situ* Hybridization (FISH).



TEM micrograph of a *Symbiodinium* section. ch: chloroplast. scale-bar = 500 nm.

- **Genetic identification by PCR:** clade-specific primers were developed from the nuclear 28S rDNA
- **Genetic identification by FISH:** clade-specific DNA probes were developed from the chloroplast 23S rDNA

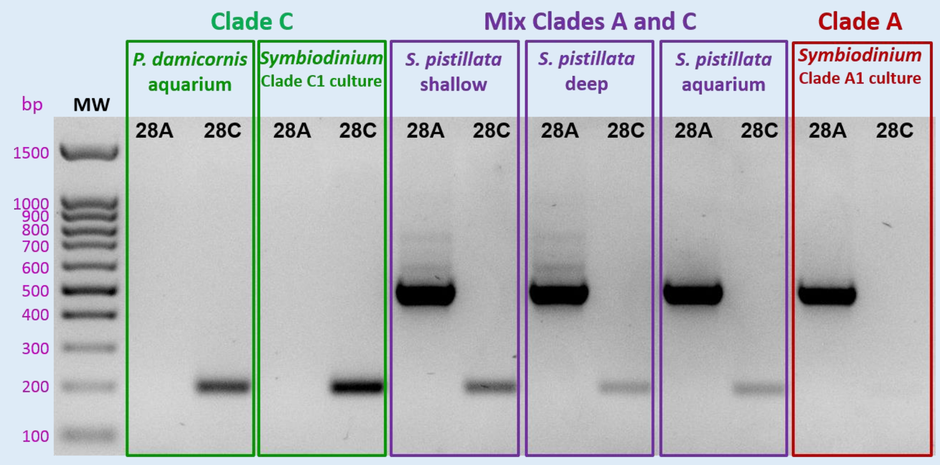
3) **Pulse-labeling** of coral colonies with stable isotopes (^{13}C-bicarbonate/^{15}N-nitrate).
4) **Ultrastructural analysis** of holobiont tissue sections (Electron Microscopy).
5) **FISH visualization** of *Symbiodinium* clades in holobiont tissue sections.
6) **NanoSIMS quantification of isotopic enrichment** at the single cell level (individual symbiont metabolic activity) on the same coral sections.

1. GENOTYPING OF *SYMBIODINIUM* CLADES (PCR)

- 28S rDNA (LSU) markers were developed to discriminate *Symbiodinium* Clade A from Clade C using direct PCR run on crude holobiont tissue extracts.
- This method was validated on pure and mixed cultures of *Symbiodinium* strains.

➢ ***P. damicornis* (from aquarium) hosts *Symbiodinium* Clade C exclusively.**

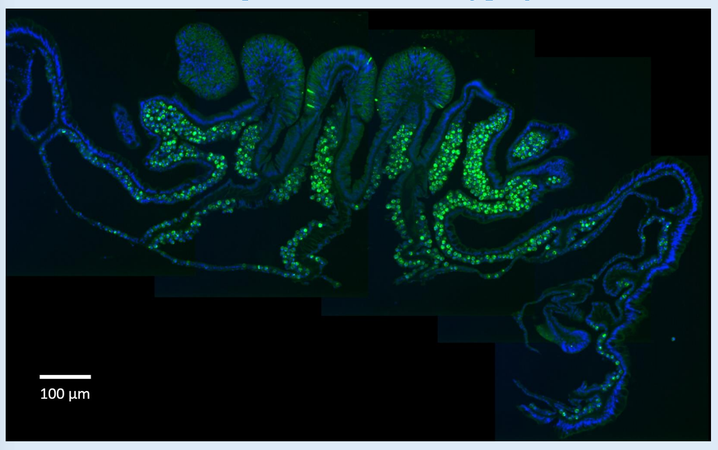
➢ ***S. pistillata* (from aquarium or Eilat) hosts both *Symbiodinium* Clades A and C.**



Genetic identification of *Symbiodinium* clade by PCR with 28S rDNA markers. MW: molecular weight of the markers in base pairs (bp), 28A: Clade A-specific primers, 28C: Clade C-specific primers.

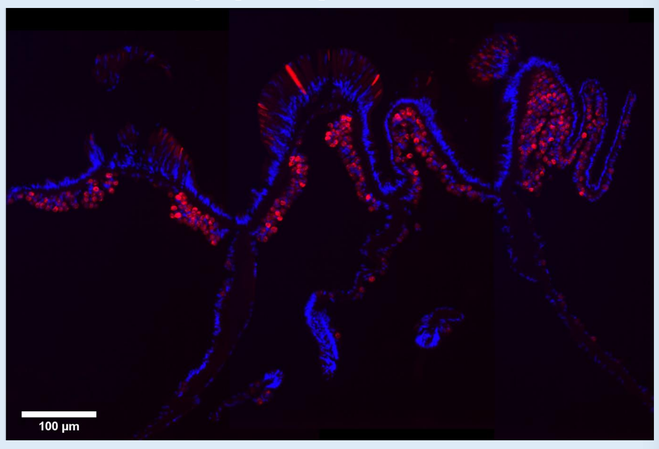
2. *IN SITU* VISUALIZATION OF *SYMBIODINIUM* GENOTYPES (FISH)

***Pocillopora damicornis* type β**



Symbiodinium Clade C labeling (green) in *P. damicornis* tissue section. Nuclei are stained with DAPI (blue).

Stylophora pistillata



Symbiodnium Clade A labeling (red) in *S. pistillata* tissue section. Nuclei are stained with DAPI (blue).

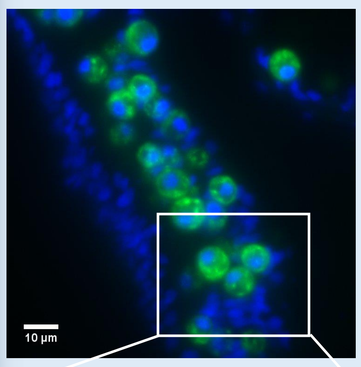
3. *ULTRASTRUCTURAL AND NanoSIMS METABOLIC ANALYSES AT THE SINGLE CELL LEVEL*

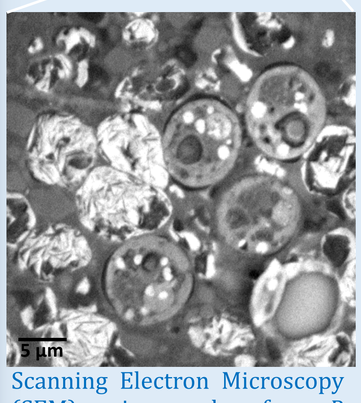
- FISH visualization of isotopically-labeled tissue (pulse experiments with ^{13}C-bicarbonate and ^{15}N-nitrate)

➢ Attribution of a particular photosynthetic assimilation capacity to a specific clade of *Symbiodinium*.

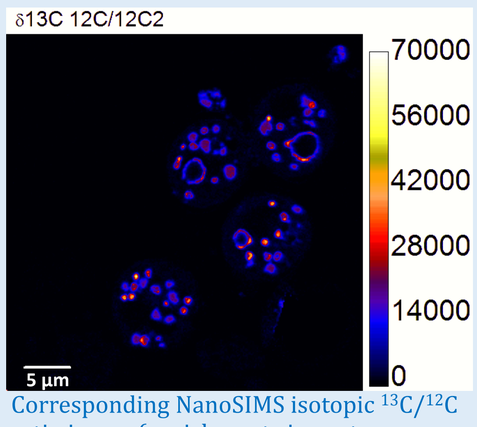
- Precise correlation between SEM and NanoSIMS isotopic enrichment maps

➢ Sub-cellular quantification of the turnover and translocation of metabolites within the holobiont.





Scanning Electron Microscopy (SEM) micrograph of a *P. damicornis* section



Corresponding NanoSIMS isotopic ^{13}C/^{12}C ratio image (enrichments in parts per thousand over the natural ratio)

CONCLUSIONS

Due to the complex and stress-sensitive nature of the coral photosymbiosis, the ability to link phylogenetic identity to metabolic activity of specific *Symbiodinium* clades is crucial to evaluate the adaptive response (plasticity) of the coral holobiont to current climate change. The correlation between *in situ* hybridization genotyping and NanoSIMS mapping of the metabolic activity of individual *Symbiodinium* cells paves the way towards developing studies of clade-specific metabolic interactions, in the intact symbiosis. This analytical breakthrough opens an entirely new area for understanding the dynamics of interactions between animals and the microbial world.

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FNSNF Fonds National Suisse Schweizerischer Nationalfonds Fondo Nazionale Svizzero Swiss National Science Foundation

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