



**Telomere length dynamics in the coral *Stylophora pistillata* and in the natural populations of *Pocillopora* sp., *Porites* sp., and *Millepora* sp. coral genera across 32 island systems of the Pacific Ocean sampled during the TARA-Pacific expedition**

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# THÈSE DE DOCTORAT

Étude des variations de la taille des télomères chez le corail  
*Stylophora pistillata* et dans les populations sauvages des genres  
*Pocillopora sp.*, *Porites sp.*, et *Millepora sp.*  
récoltés sur 32 systèmes insulaires de l'Océan Pacifique au cours  
de l'expédition TARA-Pacifique

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Étude des variations de la taille des télomères chez le corail *Stylophora pistillata* et dans les populations sauvages des genres *Pocillopora sp.*, *Porites sp.*, et *Millepora sp.* récoltés sur 32 systèmes insulaires de l'Océan Pacifique au cours de l'expédition TARA-Pacifique

## Résumé

La linéarité des chromosomes eucaryotes implique la présence d'une chromatine à la structure particulière, appelée télomère, pour contrôler la stabilité du génome et conserver sa fonction. Des changements dans la structure des télomères peuvent déterminer la longévité, la résistance au stress et la susceptibilité de développer certaines pathologies. Notre compréhension sur la contribution des facteurs environnementaux sur la longueur des télomères n'en est qu'à ses balbutiements, tout comme notre connaissance de la diversité des mécanismes de régulation de la taille des télomères dans l'arbre de la vie. Les coraux constructeurs de récifs sont à cet égard des modèles de choix, leurs conditions de vie fixées les empêchant de fuir des conditions défavorables et leur endosymbiose obligatoire avec une micro-algue photosynthétique les exposant à un stress oxydant quotidien. Leurs télomères ne sont pourtant que peu étudiés. Dans ce travail de thèse, j'ai combiné une large étude de terrain sur 3 genres de coraux (deux scléractiniaires, *Pocillopora sp.* et *Porites sp.*, et un hydrozoaire *Millepora sp.*) et une étude en conditions contrôlées sur l'espèce modèle *Stylophora pistillata* dans le but de comprendre le lien entre la variation de la taille des télomères et l'environnement. J'ai observé que la taille des télomères des coraux ne diminuait pas avec l'âge, ni avec la taille des colonies, mais qu'une rupture de la symbiose induite à l'obscurité pouvait induire un raccourcissement des télomères. J'ai aussi montré que des espèces avec des stratégies évolutives différentes avaient des régulations de leurs tailles de télomères différentes, le genre *Pocillopora sp.* étant contraint à la fois par la génétique et l'environnement tandis que le genre *Porites sp.* démontrant un incroyable maintien de la taille des télomères en dépit des variations génétiques ou des catégories d'environnements. Cependant, malgré leurs différentes stratégies, la taille des télomères des deux genres étaient négativement corrélées aux variations environnementales, mettant à jour ce paramètre comme crucial et potentiellement perturbateur des mécanismes de maintien de la taille des télomères.

## Mots clés

Télomères, Coraux, Stress, Environnement, Changement climatique

Telomere length dynamics in the coral *Stylophora pistillata* and in the natural populations of *Pocillopora sp.*, *Porites sp.*, et *Millepora sp.* coral genera across 32 island systems of the Pacific Ocean sampled during the TARA-Pacific expedition

## Abstract

The linearity of eukaryotic chromosomes requires the presence of a particular terminal chromatin structure, named telomere, to control the stability and function of genomes. Changes in telomere structure during life can determine longevity, stress resistance and disease susceptibility. Our knowledge on the contribution of environmental factors on telomere length (TL) variability remains at its infancy, as well as the diversity of telomere maintenance mechanisms and ageing strategies existing in the tree of life. In this regard reef building corals are an interesting but yet poorly investigated model to tackle the question of telomere response to environment. Indeed those long lived animals cannot escape external environmental stressors due to their fixed life mode nor internal ones due to the symbiosis, within their gastrodermal cells, with a photosynthetic microalgae. Here I combined an extensive field case study on 3 coral genera (two scleractinians, *Pocillopora sp.* and *Porites sp.* and the hydrozoan *Millepora sp.*) as well as controlled conditions test on the coral model *Stylophora pistillata* to unravel the possible links between telomere DNA length variation and environmental stress. I found that an absence of TL shortening with age and size in all the investigated corals. I observed a possible impact of dark induced bleaching on TL and different TL dynamics in wild populations. *Pocillopora sp.* mean TL is constrained both by genetic and environment while *Porites sp.* has a remarkable ability to maintain its TL regardless of size, genetic and some environment disturbance. Yet both of genera TLs were negatively correlated with seasonal deviations, identifying this environmental parameter as a factor overcoming TL maintenance in genera with different TL dynamics and life-history strategies.

Keywords:

Telomere, Coral, Stress, Environment, Global Change

*A ma mère et ma grand-mère pour m'avoir transmis leur force et leur détermination et à ma sœur  
Marguerite et mes frères Toni et Félix pour me donner envie de regarder vers demain et comprendre  
un peu mieux le monde.*



# Avant propos

Every expedition weathered its storms and pitfalls but finally came back with more richness one could ever expect.

My expedition through the thesis manuscript ends here as your journey through it begins.

Because it is not easy to travel in the sea of doubts seeking for answers but it is worth seeing your world broken to be built back again richer, better and truer.

Have a safe trip !

Chaque expédition essuie ses tempêtes et ses écueils pour finalement revenir plus riche de savoir et d'expériences.

Mon voyage à travers l'écriture du manuscrit s'arrête ici mais le votre à travers sa lecture commence.

Parce qu'il n'est pas aisé de partir à la recherche de réponses en mer du doute mais qu'il n'est pas aussi de plus beau spectacle que de voir sans cesse son monde se déconstruire pour se rebâtir plus riche, plus complet et plus proche du vrai.

Bon voyage !

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# Chapter I-Introduction

## 1.Telomeres

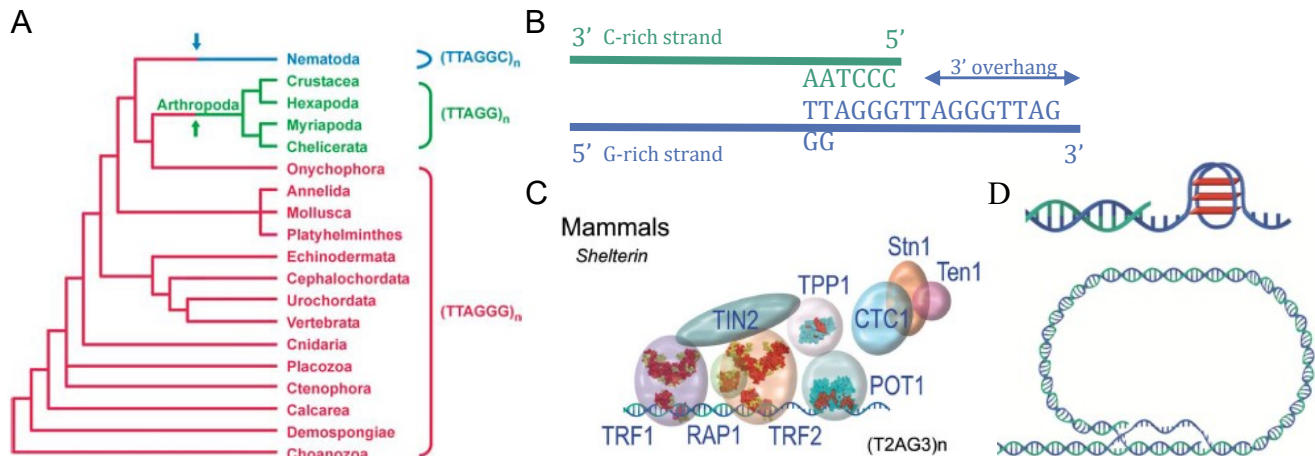
### 1.1. Discovery of telomeres

The name telomere was first given by Dr. Muller in the late 1930s from his work on fruit fly *Drosophila melanogaster*'s chromosome, compaction form of the DNA, when he realized that chromosome ends were less likely to experience fusion than X-ray induced double strand breaks (Muller, 1938). At the same time working on maize development Dr. McClinton discovered that during meiosis, the cellular division of diploid cells into haploid reproductive cells, called gametes, a broken chromatid ends could fuse with its sister chromatid and create a bridge that would prevent chromatid separation during anaphase and lead to chromosomal aberrations. They conceptualize that chromosomes must be capped at their ends, by what they expected to be genes at that time, to prevent chromosome fusion. It's only in 1978 with the work of Dr. Blackburn and Gall on the ciliate *Tetrahymena thermophila* that the first telomeric sequence was described, not as a gene but as a tandemly repeated non-coding sequence, (TTGGGG)<sub>n</sub> (E. H. Blackburn and Gall 1978). The human telomere sequence was described by Moyzis et al. in 1988 (Moyzis et al. 1988). Interestingly this sequence has been shown to be highly conserved among Metazoans (Fig1:A) with exceptions for nematodes and arthropods (Traut et al. 2007; Sinclair, Richmond, and Ostrander 2007). Human telomeric sequence (TTAGGG)<sub>n</sub> is oriented 5'-->3' and is therefore called the G-rich strand while its complementary strand (AATCCC)<sub>n</sub> is the C-rich strand (Fig1:B). Telomeric sequence strands in humans are asymmetric with the G-rich strand being longer and forming a 3' overhang, chromosome ends are therefore not blunt.

Muller and McClinton conceptualize the idea of "capped" chromosome ends within the concept of telomere and indeed a protective protein complex, the shelterin, composed (Fig1:C) of 6 proteins in human (TRF1, TRF2, TIN2, TPP1, POT1 and RAP1) (de Lange 2005; Giraud-Panis et al. 2013). This complex is bound to telomere DNA sequence to ensure its protection from repair machinery that would recognize it as a DNA damage. Telomeric-repeat binding factor 1 and 2 (TRF1 and TRF2) directly bind the duplex telomeric DNA repeats, Protection of telomere 1 (Pot1) to single stranded telomeric DNA, while TRF1-interacting nuclear factor 2 (TIN2) and TPP1 form a protein bridge between the double and single stranded parts of telomeric DNA. RAP1 is associated with TRF2.

The repeated characteristic of telomeric sequence enables the G-rich 3' overhang to fold back and hybridize with the complementary C-rich sequence of the upstream double strand, forming a protective structure called T-loop (Griffith et al. 1999) (Fig1:D). Moreover the high percentage of guanine in the telomere repeat sequence from the G-rich strand is conducive for G-quadruplex formation that is

another protective structure for telomeres (Griffith et al. 1999; Parkinson, Lee, and Neidle 2002) (Fig1:D).



**Figure 1 : Telomere sequence and capping.** (A) Telomere sequence in Metazoa from (Traut et al., 2007, Fig.14), (B) Human telomere sequence and 3' overhang. (C) Mammals shelterin protein complex association at telomeres and (D) G-quadruplex telomeric structure, telomere loop (T-loop) both from (Giraud-Panis et al., 2013)

## 1.2. Regulation of Telomere Length

Telomeric sequences being the end of chromosomes they are more likely to be recognized as breaks and should therefore be protected to avoid chromosome end-to-end fusion. We have seen that the shelterin complex is doing so by capping telomeric sequences, moreover some secondary structures such as T-loops or G-quadruplex can prevent recognition by the DNA repair machinery proteins.

Telomere structure prevents chromosome fusion to ensure proper chromosome segregation at the anaphase of mitosis, cellular division, but it also protects coding regions from erosion by the “end replication problem”.

### 1.2.a. End replication problem

To build multicellular organisms from a unique zygotic cell, cell proliferation, or doubling, is necessary. It takes place through cell division, a mechanism called mitosis, where a mother cell will divide in two equal daughter cells. To ensure the inheritance of the same genetic information encoded in DNA the mother cell is copying DNA strands through DNA replication. This process is in part carried out by a family of DNA polymerase enzymes that read one of the DNA strands from 3' to 5' and use it as the matrix to generate a new complementary strand. This requires the opening of the DNA double strand helix through a replication fork that is moving along with the replication of the leading strand. The opposite strand, lagging strand, is generated in the opposite direction of the replication fork movement. It generates small fragments, Okazaki fragments, of RNA primers (10 nt) and DNA that will be ligated together after removal of RNA primers. The leading strand is continuous and



(Wright et al. 1996; Lee et al. 1998). Telomerase gene is conserved among Metazoan but the enzyme activity regulation is highly species dependent (Gomes et al. 2011), and isn't only telomere dependent. Its non-canonical functions, telomere independent, have received less attention but are of importance as its transcription factor activity can modulate key signaling pathways (Ségal-Bendirdjian and Geli 2019).

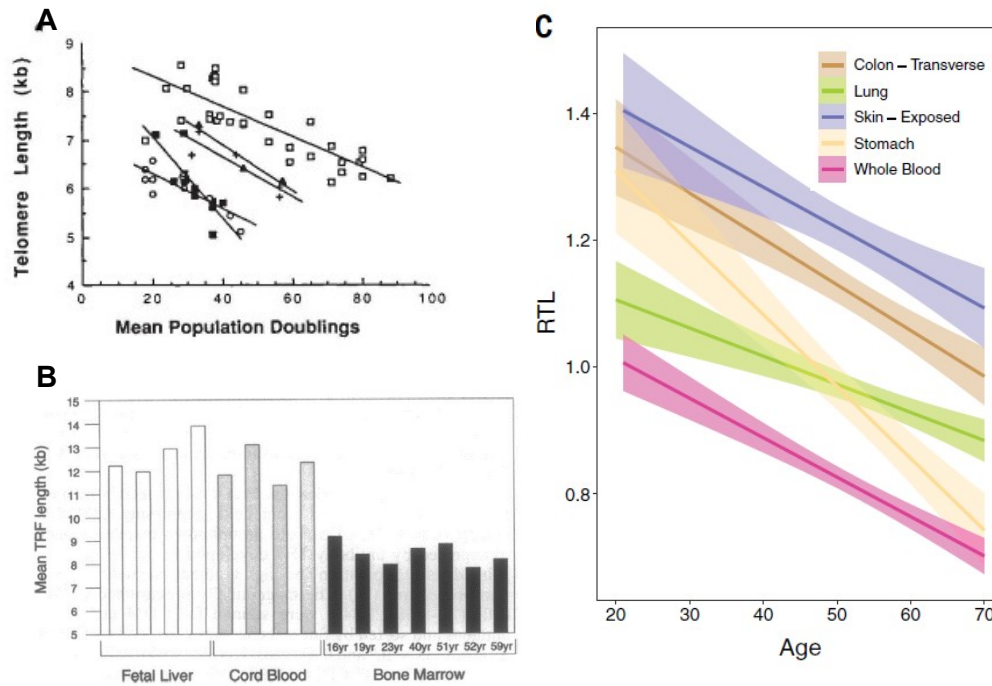
Some recombination based mechanisms known as alternative lengthening telomere (ALT) mechanisms have been shed in light and can lengthen telomeres in the absence of telomerase, but their occurrences in human cells is low (Gilson and Géli 2007; Cesare and Reddel 2010; Zhang et al. 2019).

### 1.3 Telomere and longevity

#### 1.3.a Telomere and cell proliferation

Telomere length has been reported to decrease with time in human cells (Fig3:A) (Harley, Futcher, and Greider 1990; Levy et al. 1992) and has been proposed to be part of a mitotic clock (Vaziri et al. 1994). Indeed excessive telomere shortening will result in unprotected DNA's ends and will trigger DNA damage response leading to growth arrest, replicative senescence, and eventually programmed cell death, apoptosis (Harley, Futcher, and Greider 1990; Zou, Sfeir, et al. 2004). In the meantime this process is preventing cancer by limiting cell proliferation (Artandi and DePinho 2000). Telomere attrition has been proposed to explain the Hayflick's limit (Shay and Wright 2000). In 1961 Hayflick and Moorhead (Shay and Wright 2000; Hayflick and Moorhead 1961) observed a decrease of *in vitro* human fibroblasts doubling with time before they entered a non replicative stage that will further be described as senescence. Hayflick's limit is the finite number of divisions that non immortalized *in vitro* cells can experience before entering in replicative senescence. This phenomenon has been described in several tissues and even if telomere length dynamic is tissue specific in humans the decreasing trend with age appears to be conserved (Fig3: B,C)(Demanelis et al. 2020).

Telomere homeostasis is therefore a key mechanism which leads to premature ageing or cancer when disrupted (Ye et al. 2014a; Aviv and Shay 2018) and has been listed as a hallmark of ageing (López-Otín et al. 2013).



**Figure 3: Telomere length decrease with age in human cells and tissues** (A) Telomere length (kb) decrease upon in vitro cellular doubling in human fibroblast from (Harley et al., 1990, Fig2). (B) Telomere length (kb) in different tissues and in different donor age group from (Vaziri et al., 1994, Fig1). (C) RTL (Relative Telomere Length) depending on donor age in different human tissues (colors) from (Damenalis et al., 2020, Fig3). (D)

### 1.3.b Telomere and ageing theories

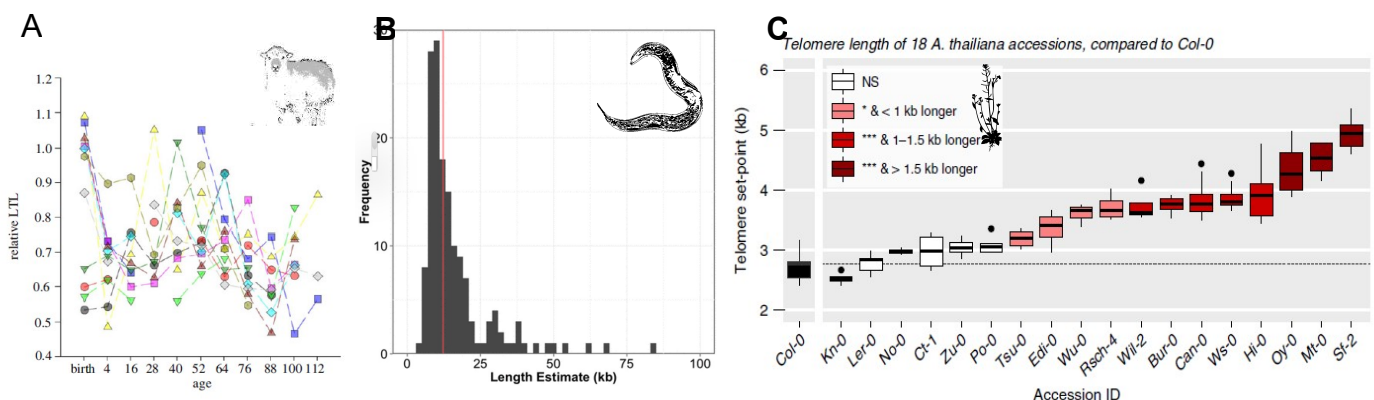
In the early 1890 Weismann developed a theory of a programmed ageing that would limit lifespan to increase the survival of the species over the individual. Following the same idea Kirkwood elaborated the soma/germline theory of ageing in which an organism's goal is to ensure the reproductive success of the species and therefore protect the germline while somatic cells are disposable and perishable (Kirkwood 1977; Kirkwood and Holliday 1979).

Other theories are also based on an accumulation of damage but that arise or from an exhaustion of metabolism machinery, which can be rescue or delayed by low caloric diet (Harman 1992) or from an accumulation of errors, whether in the DNA (Medawar, 1952) whether at the transcriptional or translational level leading to proteins dysfunction (Orgel 1973), or even at every molecular level where each error adding to the other will create a deleteriome that will lead to ageing (Gladyshev 2016). Ageing has also been theorized to be the consequences of antagonistic pleiotropic genes, genes that would have a beneficial effect early in life but would be detrimental later on (Williams 1957).

Besides current debate in the field of ageing to figure out the causes, evolutionary origins and mechanisms at stake, telomeres are ancient and evolutionary conserved structures that guard the genome from being cut and balance cell proliferation. Therefore, telomere length dynamics in wild population studies in a wide variety of taxon are key elements to unravel the evolution of ageing and the role of telomeres in preventing it or triggering it.

### 1.3.c Telomere shortening rate, lifespan and natural variation

Telomere length shortening rate seems negatively correlated with lifespan in vertebrates (Bize et al. 2009; Whitemore et al. 2019), with species experiencing fast telomere shortening having a shorter lifespan than the ones with a slow telomere sequence attrition, regardless of their absolute telomere length. For example within rodents a lot of species have long telomere, like the house mouse and the norway rat with a mean TL ( ~50kb) but a short lifespan (4 years old and 5 years old) while humans have mean TL of (~9kb) are living much longer (~73 years old) (Gomes et al. 2011; Gorbunova, Bozzella, and Seluanov 2008).



**Figure 4 : Telomere length variation in individuals and between wild population.** (A) Leukocyte relative telomere length from 11 Ovis aries sheep individuals measured along their life, each color and symbol combination represents an individual, from (Dugdale and Richardson, 2018, Fig3, with data from Fairlie et al., 2016). (B) Histogram of telomere length (kb) frequency in wild populations of *C. elegans* from (Cook et al., 2016). (C) Boxplot of telomere length (kb) variation in *Arabidopsis thaliana* ecotype (4 to 17 individuals per accession ID) statistically compared to the Col-0 population (Tukey's HSD) (\*)0.005<P<0.05, (\*\*\*) P<0.005) from (Abdulkina et al., 2019)

But if telomere shortening rate is variable between species it is also between individuals of the same species (Fig4:A)(Dugdale and Richardson 2018). This individual telomere length variability in organisms sharing the same chronological age, time since they were born, has been conceptualized as biological age, the distance from reaching an ageing triggering checkpoint. It has raised a concern on telomere length regulation, among them the question of the genetic determination of telomere length and its shortening rate, compared to the effects of environment and lifestyle (Monaghan and Haussmann 2006).

Telomere length variation in wild populations of nematode *Caenorhabditis elegans*, (Fig4:B) is largely due to a genetic factor, a nucleotide mutation in POT2 gene, the homologous of human POT-1 protein (Cook et al. 2016) and in the plant *Arabidopsis thaliana* different genetic populations can exhibit a telomere length variation (Fig4:C) (Abdulkina et al. 2019).

But telomere length dynamics is also sensitive to stressors and the individual's environment. In humans it has been shown that poor early life conditions resulted in individuals with shorter TL later in adulthood than the control group (Kananen et al. 2010; Shalev et al. 2013). In (Fig4:A) individuals from a female sheep *Ovis aries* cohort were experiencing greater TL loss, early in life (Fairlie et al. 2016; Dugdale and Richardson 2018) and it was shown by (Nettle et al. 2017) that early life nutritional restrictions were deleterious in the european starling *Sturnus vulgaris*, shortening telomeres and increasing DNA damage. Furthermore it has been demonstrated in the long lived bats, *Myotis myotis*, through a longitudinal study that individuals TL variations were more correlated to environment than heritability (Foley et al. 2020a).

Environment and efforts to compensate for the lack of positive environmental conditions, or parental care for early life steps, increase the rate of telomere shortening in early life for many species but also in adults. In (R. C. Young et al. 2017) they investigated the diving behavior of a sea bird, the thick-billed murre *Uria lomvia*, and found that individuals that were spending more time diving in winter were experiencing greater telomere loss. Hence increased effort is linked to shorter telomeres and lifespan. Signals transduction underlying those environmental effects are far to be fully understood but one mechanism that is linked to telomere attrition through DNA damage is the production of reactive oxygen species (ROS). Indeed they found an increase of oxidative damage in ((Robin et al. 2020; R. C. Young et al. 2017) linked to telomere attrition and recent work have shown that even in human somatic non dividing cells ROS could lead to telomere shortening (Robin et al. 2020) reinforcing the analysis on multiple stressors impact performed in (Chatelain, Drobniak, and Szulkin 2020) that unraveled the correlation between telomere length attrition and ROS production.

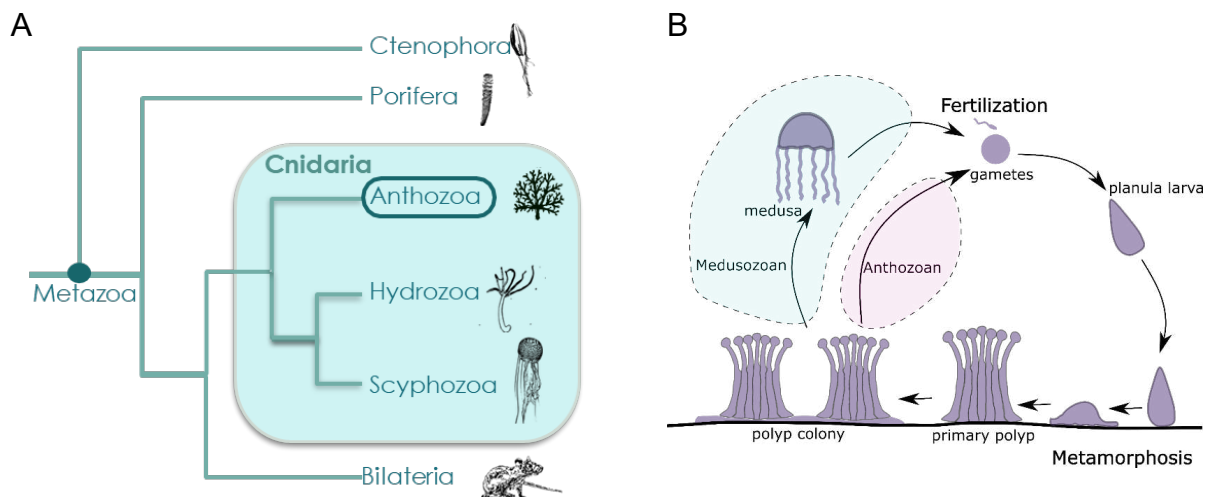
Telomere length decreases with time and this attrition rate is linked to species lifespan. Genetic determinants of absolute telomere length inheritance or telomere protection introduce variability among individuals telomere length attrition over time. So do environmental factors that through metabolic stress can influence telomere attrition rate. However, our knowledge on telomere length variation and maintenance is majoritarly based on vertebrates studies in which most species experienced ageing while it remains unclear for invertebrates such as cnidarians from which some species are claimed to be immortal.

## 2. Cnidarians

### 2.1 Diversity, synapomorphy and phylogeny

Cnidarians arose (~800-750) million years ago (MA) (dos Reis et al. 2015), after Ctenophores (Li et al., 2020) and Sponges (Simion et al. 2017), and host 13 300 known aquatic species. Cnidarians are the sister group of bilaterians (Fig5:A), they shared a common character, synapomorphy, in the presence of stinging cells, cnidocyte, that contains a specific organite, nematocyst or cnidocyst, with an invaginated harpoon that is release upon mechanical or chemical signal and use for predation or defense (McAuley, Thorington, and Hessinger 2020). Nevertheless this aquatic group diversity of morphology, lifestyle and reproduction is tremendous. Cnidaria are divided in Anthozoa, fixed, sessile, organisms and Medusozoa, animals with an ancestral jellyfish stage in their life cycle (Zapata et al. 2015; Kayal et al. 2018).

In Medusozoa the asexual life stage is ensure by a colony of polyps, called zooids, with separated functions, usually polyps colony have a sessile life mode but some, like Siphonophores, are free living planctonic colonies (Munro et al. 2018). All the zooids are linked together by a stolon and form a colony. They are named according to their functions, the most frequent ones are gastrozooids, feeding polyps, and gonozooids, reproductive polyps producing the sexual life stage, the jellyfish (Houliston, Momose, and Manuel 2010). The medusa is carrying the gonads and is releasing the gametes in the water column, oocyte and spermatozooids, to achieve sexual reproduction, depending on the species medusae can be hermaphrodites or gonochorique (Leclère et al. 2012; Gold et al. 2019; Khalturin et al. 2019). After fertilization a swimming larvae, called planula, will remain couple of hours or days in the water column before undergoing metamorphose on a substrate, rocks, sea bed, algae, etc, to give rise to the primary polyp that will grow clonal secondary polyps to generate a new colony (Fig5:B). The medusa formation, morphology as well as zooids colony organisations are specific traits to medusozoa branches.



**Figure 5 : Simplified cnidarian phylogeny and life cycle.** (A) Phylogenetic tree showing Cnidaria position in the Metazoa phylogeny. Cnidaria is the Bilateria sister group and is divided in Anthozoa (anemone and corals) and Medusozoa from which only Hydrozoa and Scyphozoa classes are represented. (B) Simplified lifecycle of Cnidaria. Fertilization of an oocyte by a spermatozooids, gametes, will give rise to a swimming ciliated planula larva that will settle on the substrate to undergo metamorphosis. Primary polyp will grow other polyps to form a polyp colony that will spawn gametes in the water column in Anthozoan (pink), or that will produce medusa in Medusozoan (blue). In Medusozoan the medusa stage is the reproductive stage that will spawn the gametes in the water column.

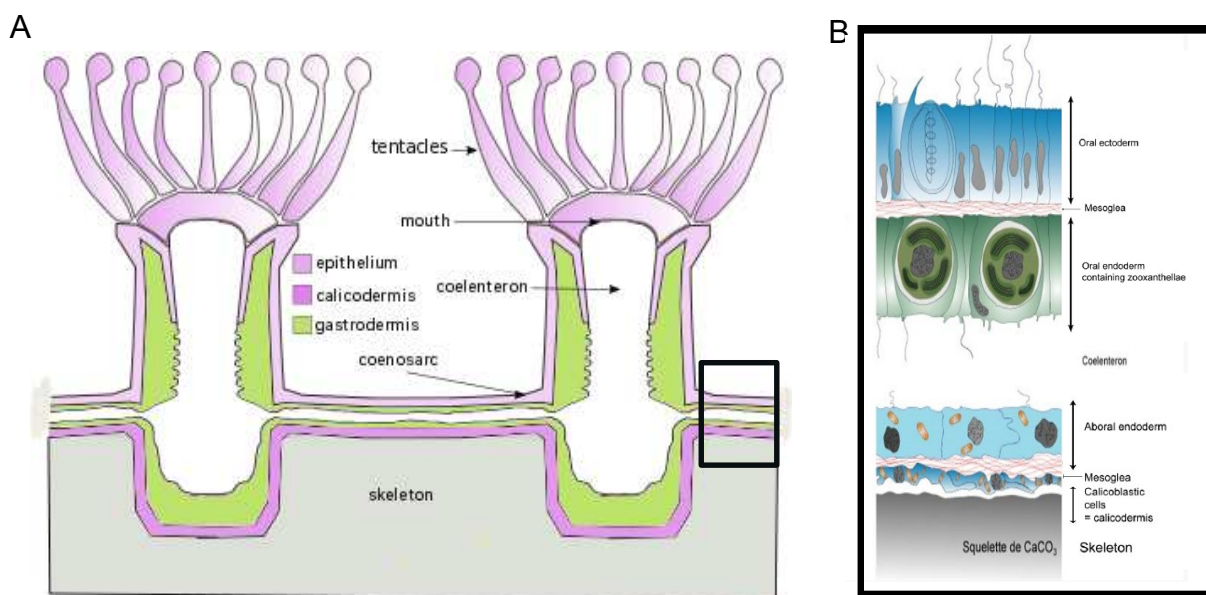
Anthozoans on the other hand, do not have medusa stage and their dispersion is ensured by the gametes release in the water column and the planula larval stage (Thompson et al., 2018). They are composed of polyps that are able to perform feeding, defense and reproduction function. They are divided based on their symmetry, between octocorals (hosting the red corals) and hexacorals. Within hexacorallia we can find sea anemonea mostly composed of solitary polyps that perform sexual reproduction or asexual one by fission, laceration, etc...(Geller, Fitzgerald, and King 2005). Reef building corals belong to scleractinians, an order of hexacorallia, they are composed of colonial polyps linked together by a stolon. Reproduction modes are quite variable in corals and will be developed later.

## 2.2 Polyp structure and symbiosis

### 2.2.a General polyp structure

A polyp is composed of two embryonic layers, an epidermis and a gastrodermis (Fig6:A). A mouth surrounded by tentacles covered in cnidocyte, allows it to catch some prey and feed or defend itself. The gastrodermal cavity, coelenteron, is linked between polyps by a stolon also called coenosarc. The gastrodermis tissue layer is forming structures called mesenteries that are following the body symmetry (a multiple of six in Hexacorallia). Coral polyps can feed using their stinging cells, cnidocytes, located on their tentacles to catch some prey and bring them to the mouth to be digested by the mesenteries. Coral polyps are able to produce a calcareous skeleton through a modified and specialized basal epidermis, called calicodermis (S. Tambutté et al. 2011).

The coenosarc is producing a flat and smooth skeleton while polyps are building a cavity, the calyx, with septa separating mesenteries. It has a species-specific morphology (J. (charlie) E. N. Veron and John (charlie) E 2011). Polyps bodies being soft and fragile, those calyxes allow them to retract inside as a defense mechanism against predators or during colony fragmentation carried through waves or hitting objects. It also offers a protection against dryness for shallow corals that are exposed to air in intertidal zones. However, this skeleton secretion requires an energy cost that coral polyps do not easily cover with their predation, mostly in waters with low concentration of prey. Polyps being sessile, they cannot chase their prey and the establishment of a symbiosis have allowed them to overcome this issue.



**Figure 6 : Schematic coral polyp structure and tissue organization.** (A) Scheme of a longitudinal view of 2 polyps from a coral colony. Tentacles are surrounding the mouth and can be used to catch preys or defend against predators thanks to specialized stinging cells (cnidocyte). The mouth is used to bring the food to the gastric cavity, coelenteron where the mesenteries (in green) will digest it, or to spawn the gametes during reproduction events. Polyps are oriented through an oral/aboral axis. The tissue layers are displayed in colors based on their developmental origins, gastrodermis is in green, epithelium is in pink and the aboral epithelium that specialized in skeleton secretion, calicodermis, is in dark pink. Polyps are linked by a cavity called coenosarc. (B) Scheme of polyp tissue organization, epithelium cells and cnidocyte from the oral ectoderm, epithelium, are in blue separated from the oral endoderm, gastroderm, by a mesoglea. Oral endoderm are represented with photosynthetic endo-symbionts, called here zooxanthella. The aboral endoderm is separated by a mesoglea from the aboral endoderm, also called calicodermis, that is secreting the calcareous skeleton from (Tambutté et al., 2011)

### 2.2.b Symbiosis

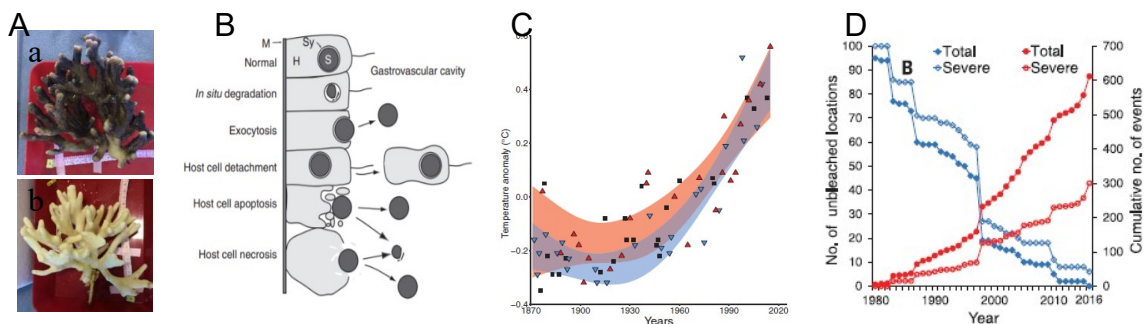
Coral polyps have evolved a mandatory intracellular symbiosis with a photosynthetic unicellular algae that belongs to Symbiodiniaceae. Symbiodiniaceae are part of the alveolata group that includes toxoplasma parasites and tetrahymena (Keeling and Palmer 2001), first organism in which the telomere sequence was described (E. H. Blackburn and Gall 1978). They belong to dinoflagellate and can have a free living stage and a coccoid form when they lose their 2 flagella to establish the symbiosis. The symbiosis takes place inside gastrodermal cells in which one or several algae can be hosted. Seven genera of Symbiodiniaceae, previously called zooxanthellae, have been described corresponding to previous clade clustering (*Symbiodinium* sp., former clade A, *Breviolum* sp., former clade B, *Cladocopium* sp., former clade C, *Durisdinium* sp., former clade D, *Effrenium* sp., former clade E, *Fugacium* sp., former clade F, *Gerakladium* sp., former clade G ), however the taxonomic position of few Symbiodiniaceae remains to be clarified and those species are still grouped under clades (T. C. LaJeunesse et al. 2018). Symbionts from several genera can be found in one coral host depending on its specificity. Some corals are called specialists as they only establish symbiosis with a specific species of Symbiodiniaceae while others, generalists, can host a patchwork of symbionts species or genera, in various proportions. Symbionts can be inherited vertically if they are already inside the oocyte, or horizontally at the larval stage or later on, after the metamorphosis. In corals, symbionts transmission varies between species (Simon K. Davy and Turner 2003). The symbiosis offers a shelter, inorganic nutrients from metabolic waste of the host and access to light for the algae. On the other hand coral polyps can retrieve the photosynthesis product which enables them to have access to a constant and sufficient energy supply, and therefore enhance their skeleton secretion (Gattuso, Allemand, and Frankignoulle 1999). Tropical waters are usually poor in available nutrients, oligotrophs, and coral symbiosis is therefore even more important in those areas to provide them the necessary energy to sustain and grow.

### 2.2.c Bleaching

When the symbiosis is broken, the loss of the symbionts and their photosynthetic pigments reveals the white color of the calcareous skeleton visible over the transparent polyps. That is why this phenomenon is called bleaching (Fig7:A).

Bleaching can be triggered by increasing sea water temperature, increasing light intensity or persistent darkness (C. A. Downs et al. 2013; Sully et al. 2019). Other factors such as hyposalinity (Craig A. Downs et al. 2009), exposure to pollutants like sun cream (Danovaro et al. 2008) or pathogens infection (Kushmaro et al. 1996; E. Rosenberg et al. 2009; Y. Rosenberg et al. 2017). Symbiosis breakdown can occur via symbionts phagocytosis, symbionts exocytosis, host cell apoptosis, host cell necrosis, or host cell detachment (Fig7:B) (Weis 2008; S. K. Davy, Allemand, and Weis 2012).

In the wild if the animal stays bleached for more than a couple of weeks and is unable to restore its symbiosis by horizontally acquiring new symbionts, it dies. In experimental conditions if food is provided to the animal it can survive for longer in a bleaching state but will eventually experience body mass loss, stop of calcification, growth arrest, polyp mortality, etc...(Lyndby et al. 2020; Gardner et al. 2017). Global climate change is resulting in global ocean sea surface temperature warming and an increase in strength and frequency of climatic events thus causing massive bleaching events and threatening coral reefs all over the world (Fig7:C,D) (Hughes et al. 2018).



**Figure 7: Bleaching of coral colonies, mechanisms and future threats for coral reefs.** (A) *Stylophora pistillata* from the Centre Scientifique de Monaco (CSM) (a) healthy (b) bleached. (B) Cellular mechanisms of coral bleaching over symbiosis breakdown. (M) mesoglea, (S) symbionts, (H) host, symbiosis breakdown can happen by in situ degradation from the host cell, exocytosis of the symbionts or over host cells degradation either by cell detachment, apoptosis or necrosis, from (Weiss 2008). (C) Sea surface temperature anomalies over the last 45 years, relative to a 1961-1990 baseline. El Niño events are represented as red triangles, la Niña with blue ones and neutral periods with black squared. from (Hughes et al., 2018, Fig1) (D) Number of non bleached location (left axis, blue) and cumulative bleaching events (right axis, red) per year for severe bleaching events (empty circles) to total bleaching events (full circles) from (Hughes et al., 2018, Fig2)

## 2.3 Coloniality, clonality and growth

The problem in a colonial organism is that the border between the concept of clonality, or asexual reproduction, and growth is thin and can overlap. However, telomere length regulation being linked to cellular proliferation and our study being at the colony level it is of importance to understand what those mechanisms imply and the evidence that tips the scale in favour of either of them.

### 2.3.a General concept

Coral reproduction is prone to generate confusion about sexual and asexual reproduction. Sexual reproduction in eukaryotes implies a meiosis event and fertilization of gametes from two individuals. In the case of gonochoric individuals, with separated sex, this fertilization from two different genetic background will lead to a new and unique combination of genes that will be or deleterious for the new individual in its environment where it will, most likely, not survive, or beneficial leading the individual to reproduce and spread its genes among the population. This natural selection process conceptualized by Darwin is a major driver of species adaptation and evolution. In hermaphrodites individuals, producing male and female

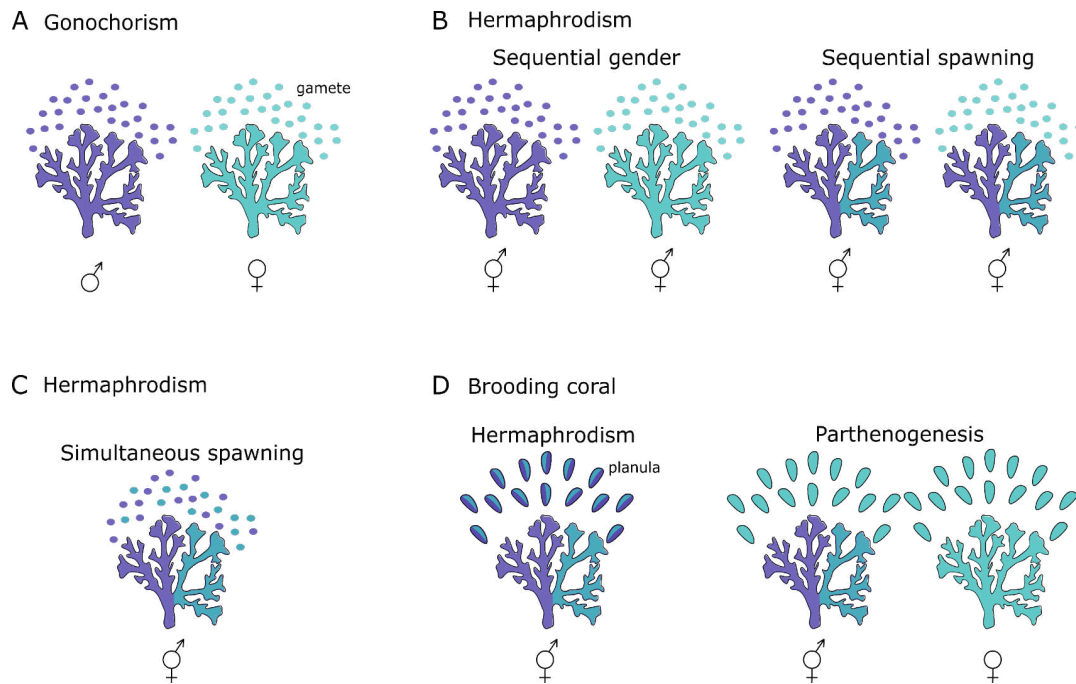
gametes, if self fertilization is prohibited, the genetic mixing will be the same as in gonochoric species. In hermaphrodite individuals with self-fertilization the genetic mixing will be less important but on the other hand it will increase the fertilization success, mostly in species with external fertilization, increasing the number of offspring.

At the cellular level sexual reproduction is performed by cells that have undergone meiosis, in most metazoan this function is performed by a cellular lineage that is specified early in life, the germline (Kumano 2015). This early segregation of the germline from the non reproductive cells, somatic cells, is taking place to prevent the transmission to the offspring of the mutation accumulation and damage that occurs in the individual before reaching sexual maturity.

### 2.3.b Coral reproduction

In corals some species are gonochoric, with separated sex, and release their gametes in the water column for an external fertilization (Fig8:A) (Kerr, Baird, and Hughes 2011). Others are hermaphrodites. Within the hermaphrodites some can be simultaneously hermaphrodite, with one polyp developing male and female gametes (Fig8:C), and some can be sequential, in space when each polyp produce only one type of gametes (Fig8:B), or in time when the colony alternates between female or male during its life (Siebert and Juliano 2017). Moreover fertilization isn't always external with the release of gametes in the water columns as some corals are "brooding" coral, which means they release an already formed larvae rather than gametes (Fig8:D)(Harrison 2011). This phenomenon can be the result of self-fertilization in hermaphrodites or of parthenogenesis, division of the oocyte without a spermatozoid fertilization.

Parthenogenesis is cited as one of the asexual reproduction mechanism of cnidarians in (Lively and Johnson 1994; Bocharova and Kozevich 2011; Siebert and Juliano 2017) based on the assumption that larvae with identical genotype than their mother colonies are produced by asexual reproduction. In corals one of the evidence underlying this claim is based on data from (Ayre and Resing 1986) where they interpreted the proportion of planula with the same genotype as their parents colony (*Tubastrea diaphana* and *T. coccinea* corals) as a proof for parthenogenesis. Nonetheless the genotyped determination based on electrophoresis remains elusive and therefore inconclusive. In (Yeoh and Dai 2010) they performed genotyping using microsatellites regions and found identical genotypes between mother colonies and the brooded larvae of *Pocillopora damicornis*. The hypothesis of sequential hermaphroditism in time, with the disappearance of male gonad at the brooding, can still explain genetically identical larvae and the lack of other cellular and molecular evidence for parthenogenesis leave this question unsolved.



**Figure 8 : Schemes of the possible reproduction mode in corals.** (A) Gonochorism designate colonies of separated genders that spawn their gametes (spermatozoid or oocyte) simultaneously (broadcast spawners). (B) Sequential hermaphrodism designate or colonies that will change their gender during their lifetime (sequential gender) or colonies that will spawn male gametes not as the same time as female ones to avoid self-fertilization. (C) Simultaneous hermaphrodism designate a colony that will spawn both gametes, male and female, at the same time which can lead to self-fertilization. (D) Brooding corals designate corals that spawn the planula larvae, mechanism underlying it are still unclear but it can be explain or by internal fertilization of an hermaphrodite colony or parthenogenesis (division of the oocyte) in female colony or hermaphrodite colony.

### 2.3.c Germline and soma in corals

Regarding clonal reproduction it is necessary to get insights from stem cell determination as well as somatic lineage and germinal lineage specification.

The bilaterian germ line and somatic are segregated early in life to prevent the inheritance of mutations that accumulate in somatic cells over time. In cnidarian the limit between stem cell, germ line and somatic cell is still unclear in some groups. Most of the research had been focusing on hydrozoan model systems (*Hydra sp.*, *Clytia sp.*, *Hydractinia sp.*, etc...) (Siebert and Juliano 2017).

Germline differentiation in hydrozoan is linked to stem cells that were first characterized by (Weismann, 1883). In *Hydra* three distinct stem cell population are described, two epithelial stem cells, one unipotent population in the epidermis another in the gastrodermis, and the multipotent interstitial stem cells (ISC or i-cells) (Bode 1996; Watanabe et al. 2009); (Schenkelaars et al. 2018). A subpopulation of interstitial stem cells can differentiate into a germline stem cells, or a female one or a male one, this germline lineage cannot dedifferentiate back into multipotent ISCs but in case of germline depletion the ISCs can give rise to a new germline (Nishimiya-Fujisawa and Kobayashi 2012; Siebert et al. 2019).

In the hydrozoan model *Clytia hemisphaerica* mRNA of characteristic germline genes *vasa*, *nanos* and *piwi* were detected in the jellyfish gonads as well as in the maternal mRNAs pool in the oocyte and in late planula larvae (Leclère et al. 2012) which suggest that in this species multipotent stem cells (i-cells) can be segregated at the embryonic level. However, evidence of survival of such cells after metamorphosis or localization in the polyp colony are still lacking. Whether those genes are specific to a segregated germline rather than multipotent stem cell is to put in light of the results of (DuBuc et al. 2020). Working with the colonial hydrozoan *Hydractinia symbiolongicarpus* they highlighted the role of Tfp2 (transcription factor AP2) gene in inducing germ cell fate in i-cells and gonads formation. Tfp2 mutants were lacking both and only Tfp2 expression in Piwi positive cells, Piwi being a marker of i-cells, was able to rescue the phenotype. All together those results suggest that the germ line in hydrozoans is continuously segregated in sexually mature adults from induced multipotent stem cells.

In corals it is possible to describe gametes in histology sections, (Rinkevich and Loya 1979) however if those gametes arose from a segregated germ cells niche or from multipotent stem cells remains to be clarified. Evidence of such segregation was investigated in comparing adult colonies' genotypes to their gametes, revealing at the same time two interesting mechanisms that are common in corals, mosaicism and chimerism.

Mosaicism is the differential accumulation of somatic mutations with life, leading to differentially acquired genotypes in cells from the same tissue. Mosaicism is quite common in highly proliferative tissue, such as human intestinal cells (Guo et al. 2015; Siudeja et al. 2015) and cancer (Jacobs et al. 2012). In corals it was proposed to be a source of genetic variability and acclimation, adaptation in the time frame of the individuals, or even adaptation considering fragmentation as an asexual reproduction (van Oppen et al. 2015).

Chimerism is the fusion of two genetically distinct individuals or tissues. It is controlled by the immune system of the two individuals, it is highly restricted in vertebrates conducting to artificial immune depression of grafted human patients but it is more common in invertebrates (Frank et al. 1997; DuBuc et al. 2020).

Both mosaicism and chimerism were reported in (Maier et al. 2012) in 27 of the 155 colonies of investigated coral *Seriatopora hystrix*. In (Maximilian Schweinsberg et al. 2015) they demonstrated intracolony genetic variability, mostly due to mosaicism, in 3 species of *Acropora* genus and in *Pocillopora sp.* and *Porites australiensis*. In (M. Schweinsberg et al. 2014), they already reported mosaicism in *Acropora hyacinthus* colonies and this time they were able to see an inheritability of those somatic intercolony mutations to the gametes (sperm or eggs bundle). They interpreted it as evidence for a non segregated germline that would differentiate from any somatic cells. Yet in (Barfield, Aglyamova, and Matz 2016), they compared somatic mutations from 2 opposite parts of colonies from the massive coral *Orbicella faveolata*, and mutation in the sperm from those colony sections. They found a

higher rate of somatic mutations in the coral colony compared to the spawn sperm and they interpreted this evidence as a clue for the presence of a segregated germ line.

In anthozoans no evidence of homology between the hydrozoans' i-cells and some cell population were found (Gold and Jacobs 2013). Some high proliferative tissue and structure had been highlighted in the gastrodermis of *Pocillopora damicornis* coral (A. Lecointe et al. 2013) as well as in *Stylophora pistillata* coral (Agathe Lecointe et al. 2016) where the pharynx structure was clearly showing a fast proliferation rate in primary polyp and in adults but those cells remain to be characterized. It is likely that anthozoans possess multipotent stem cells that would allow their regenerative abilities (Amiel et al. 2015), in (Sebé-Pedrós et al. 2018) they isolated from the sea anemone *Nematostella vectensis* progenitor cells expressing stem cell marker genes (*Myc*, *Nanos1*, *Vasa*, *Piwi*) but whether they segregate early a germline or continuously give rise to germ cells is still not elucidated.

#### 2.3.d Asexual reproduction and colony extension

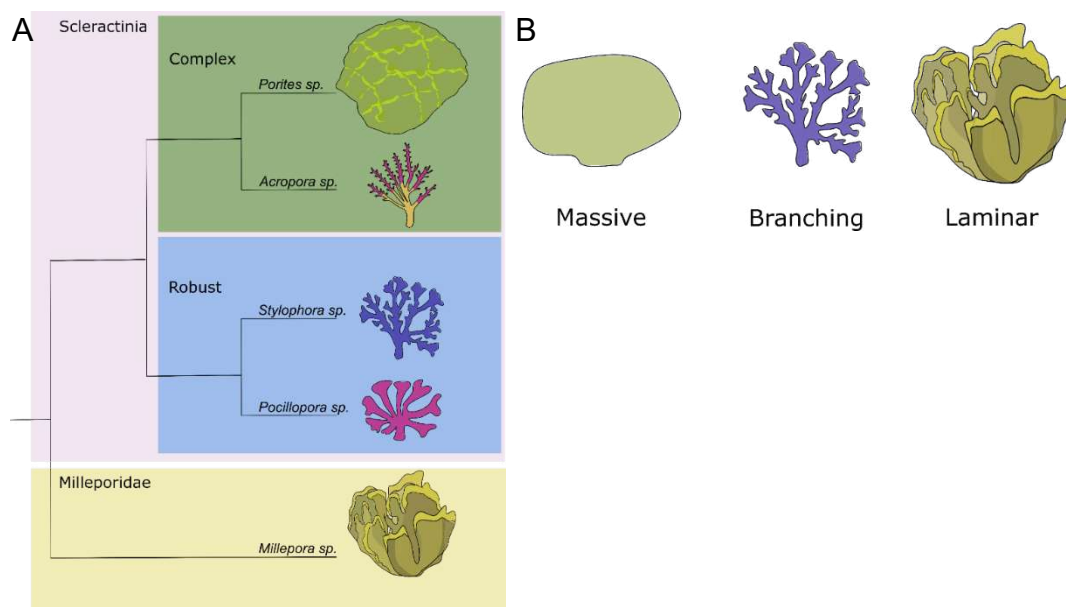
Coral growth, or colony extension, happens by polyp budding or fission which is, according to Buss' (Buss, 1983) definition, an asexual reproduction mode. But it is also the growing mechanism of coral colonies. Even if each polyp can ensure all vital function for the colony and therefore be independent, contrary to hydrozoans colonies where polyps are specialized, if isolated a lonely polyp will grow back a colony.

Polyp isolation can occur by a mechanical fragmentation of a branch, that will be called a ramet, created or by waves, boat anchors, divers palms, etc... Some studies have tried to isolate coral polyps in microchips to document the calcification process (Shapiro et al. 2016) or some metabolic process (Pang et al. 2020) but they were eventually growing other polyps or dying after 15 days. Those studies were taking advantage of a phenomenon called polyp bail out. It can occur when a colony is under a stress threat and expulses a polyp from the colony (Sammarco 1982; Fordyce, Camp, and Ainsworth 2017), the expelled polyp can grow another colony if it survives. This mechanism and behaviour is still under investigated in coral and could be an active asexual reproduction mode. At the cellular and molecular level no evidence has demonstrated that either coral polyp budding or fission involved a reset that would give rise to a younger polyp than the one from which it originated. Therefore, in colonial coral I will consider the colony as the individual because even if the polyp is the functional unit it is unable to fulfil its lifecycle on its own and will indubitably regenerate the colony structure by growing new polyps. This conceptual point of view implies that I will be considering chronological age at the colony level, starting from metamorphosis, and that new polyp formation whether it is part of the colony growth or asexual reproduction, and whether it derives from a germ cell, a multipotent stem cell or a somatic, do not necessarily implies a biological ageing reset until proven.

## 2.4 Coral Reef

Scleractinian species emerged ~ 240 million years ago (MA) (J. E. N. Veron 1995) and are the key stone of the coral reef ecosystem. The constant nutrient support provided by their symbiosis with photosynthetic algae had empowered them to settle in low nutrient environments such as tropical waters. The coral reef structure is an ecosystem that offers shelter for a huge number of species, it is used as much as a nursery as a reproduction hotspot or a habitat. It also protects shore from waves and is of importance for human survival and economic sustainability (P.-Y. Chen et al. 2015; Fisher et al. 2015) acting as a food supplier and accounting for most of the touristic resources of some remote areas.

Among the reef building coral, 5 will be investigated in this thesis (*Stylophora pistillata*, *Pocillopora meandrina*, *Porites lobata*, *Acropora digitifera* and *Millepora platyphylla*) and therefore described below.



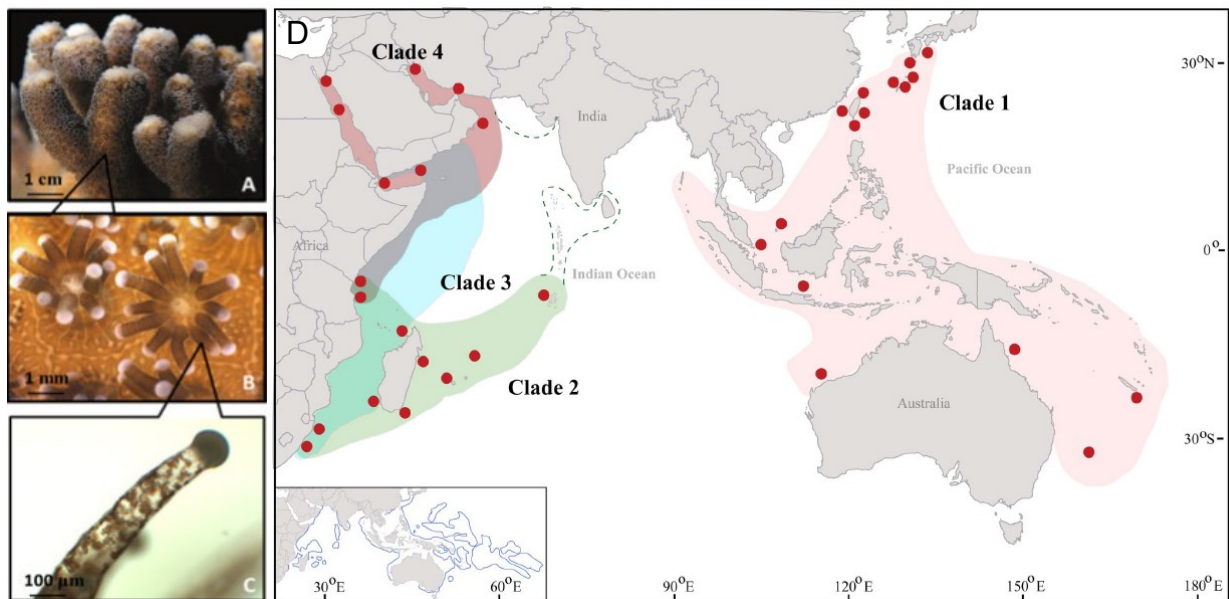
**Figure 9 : Phylogeny of the 5 reef building coral genera investigated.** (A) Within Scleractinia the Complex lineage is grouping *Porites sp.* and *Acropora sp.* while *Pocillopora sp.* and *Stylophora sp.* belong to the Robust lineage. *Millepora sp.* belong to a group of hydrozoan. (B) Scheme of 3 morphology found in corals. Massive form that usually exhibit horizontal growth and are robust to fragmentation. Branching and lamina form that have a vertical growth pattern and are more sensitive to fragmentation.

Within scleractinian two lineages have diverged and had been highlighted thanks to molecular and genetic evidence yet their names were chosen based on a skeleton morphological trait, robust and complex (S. L. Romano and Palumbi 1997). Differences between robust and complex corals is noticeable at the genomic level with differentially expanded and duplicated groups of genes, more often and in wider proportions in complex corals, with genes related to innate immunity (Bhattacharya et al. 2016; Voolstra et al. 2017) even if an ancestral histidine pathway is retained in robust corals but absent in complex corals and sea anemone (Ying et al. 2018) (Fig9:A).

## 2.4.a.1 Robust corals

### a. *Stylophora pistillata*

*Stylophora pistillata* (Esper, 1797) is a branching (Fig10:A,B,C) scleractinian model species. In the wild this species is spread (Fig10:D) from the Red Sea to the North Pacific Ocean, passing through Indian Ocean and West Pacific Ocean with 4 genetic populations (Keshavmurthy et al. 2013). *Stylophora pistillata* is a sequential hermaphrodite in time and is a brooding coral that spawns directly its planula larvae (Rinkevich and Loya 1979; Shefy, Shashar, and Rinkevich 2018).



**Figure 10 : *Stylophora pistillata* a coral model species.** (A) Picture of a *Stylophora pistillata* colony, from (Tambutté et al., 2007). (B) Close up of a *Stylophora pistillata* polyp, from (Tambutté et al., 2007). (C) Close up of a *Stylophora pistillata* polyp tentacle, visible brown circle are intracellular symbionts, from (Tambutté et al., 2007). (D) Map of the geographic distribution of 4 genetic clade, population, of *Stylophora pistillata* in the wild from (Keshavmurthy et al., 2013)

*Stylophora pistillata* is supposed to be more sensitive due to its branching form with some reports of bleached colonies after a heat-event in wild populations of Sesoko island, Japan (Loya et al. 2001) and of higher sensitivity to heat-stress, not surviving above 30°C, compared to other species (Dias et al. 2019).

However it seems that the Centre Scientifique de Monaco strain, originally coming from the Red Sea (clade 4, Fig10:D) shows a higher tolerance to acidification and temperature with an optimal photosynthesis and calcification rate in 29°C (A. A. Venn et al. 2019; Bernardet et al. 2019).

### b. *Pocillopora meandrina*

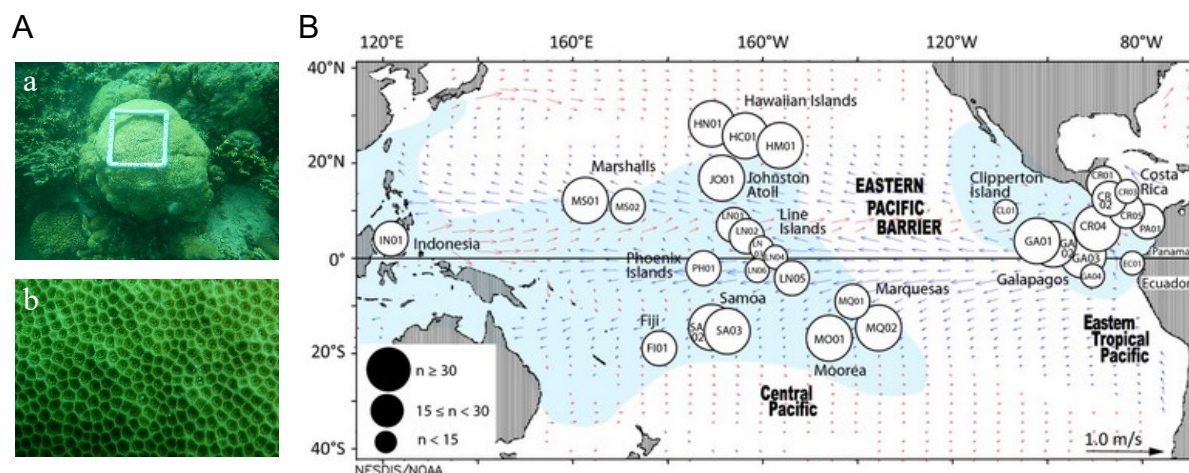
*Pocillopora* genus (Lamarck, 1816) is widely spread across from the Red Sea to Eastern Tropical Pacific Ocean. Species delimitation was first based on morphological features but phenotypic plasticity of this branching coral genus leads

to confusion in species assessment (Pinzón et al. 2013; Nakajima et al. 2017; Johnston et al. 2017). *Pocillopora meandrina* is a hermaphroditic and broadcast coral (Kerr, Baird, and Hughes 2011), its symbiodinium acquisition is vertical as the oocytes are “seeded” with maternal symbionts (Marlow and Martindale 2007). The most studied species in this genus is *Pocillopora damicornis*, which has been shown to be quite sensitive to thermal stress, acidification and bleaching (Courtial et al. 2018; Dias et al. 2019) as well as other species such as *Pocillopora verrucosa* (Pogoreutz et al. 2017, 2018) which makes this genus quite sensitive to environmental change and disturbance.

#### 2.4.a.2 Complex corals

##### a. *Porites lobata*

*Porites lobata* is a massive encrusting coral species (Fig11:A) with gonochoric individuals that spawns their gametes in the water column, broadcast coral (Kerr, Baird, and Hughes 2011). It is widespread across the Pacific Ocean with some genetic population at the island level, however the Eastern Pacific *Porites lobata* population seems to be highly isolated from the rest of the Pacific Ocean (Baums et al. 2012; M. Schweinsberg, Tollrian, and Lampert 2016) (Fig11:B).



**Figure 11 : *Porites lobata* geographic .** (A) Picture of *Porites lobata* from Palau Republic sampling of the TARA-Pacific expedition, (a) colony, (b) close-up (credits Fondation TARA Ocean). (B) Map of the geographic distribution of genetic population of *Porites lobata* in the Pacific Ocean (circle size stand for the number of individuals and names for the genetic population) from (Baums et al., 2012).

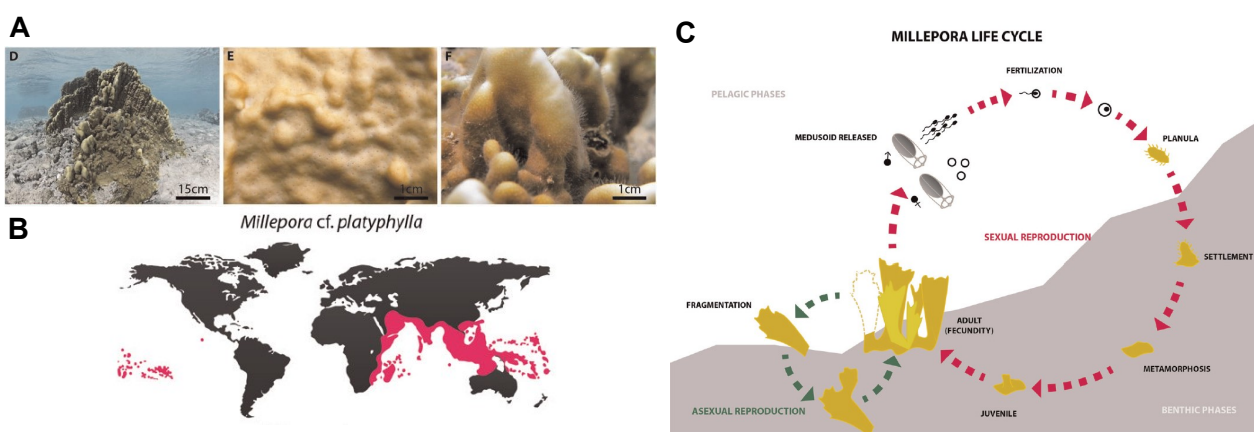
*Porites lobata* is known for its toughness and resilience to bleaching allowed by its active predation on zooplankton to recover (Levas et al. 2013), it is also able to survive partial mortality thanks to regeneration of the outer colony from tissue buried deep inside the skeleton (Roff et al. 2014). This ability might be a key for its high tolerance to sedimentation (Stafford-Smith 1993). *Porites lobata* is preferentially associated with one specific symbiont, *Cladocopium*, previously *Symbiodinium* clade C15, it is a specialist coral (T. LaJeunesse et al. 2004; Barshis et al. 2010).

### a. *Acropora digitifera*

*Acropora* genus is a branching hermaphrodite and broadcast spawner (Kerr, Baird, and Hughes 2011) the genus is quite sensitive to bleaching (Loya et al. 2001) and belongs to a 'competitive' group of corals, like *Pocillopora* sp. or *Stylophora* sp., that are fast growing branching corals that can easily dominate a reef but are at the same time quite sensitive and less resilient than massive ones like *Porites* sp. (Darling et al. 2012).

### 2.4.a.3 *Millepora platyphylla*

Unlike the previously described corals, *Millepora platyphylla* do not belong to Scleractinia, it is an hydrozoan belonging to the Milleporidae family (Linnaeus 1767, Boschma, 1948). Consequently *Millepora*'s colonies, that can be found from 0 to 40 m depth, are composed of specialized polyps (1.2.a) and its life cycle contains a short planktonic reproductive jellyfish stage (Lewis 2006) (Fig12:C). The jellyfish are gonochoric and contain oocytes with symbionts henceforth the symbiotic inheritance in *Millepora* sp. is primarily vertical (Dubé et al. 2020). Moreover Milleporidae have evolved the ability to secrete a calcareous skeleton that is similar to scleractinian ones (Tissier and Le Tissier 1991) which enable them to be part of reef building coral. They are called "fire coral" because of their particularly irritating nematocyst venom for humans. Some reports of *Millepora* sp. growth rate exists and mean age colonies were estimated to be around 5 or 6 years old, with the maximum one being of 10 years old (Lewis 1991). *Millepora platyphylla* is majoritary localized in the center Indian Pacific Ocean (Dubé et al. 2020) but can also be found in the Eastern part of the Pacific Ocean (Boissin et al. 2019) (Fig12:B).



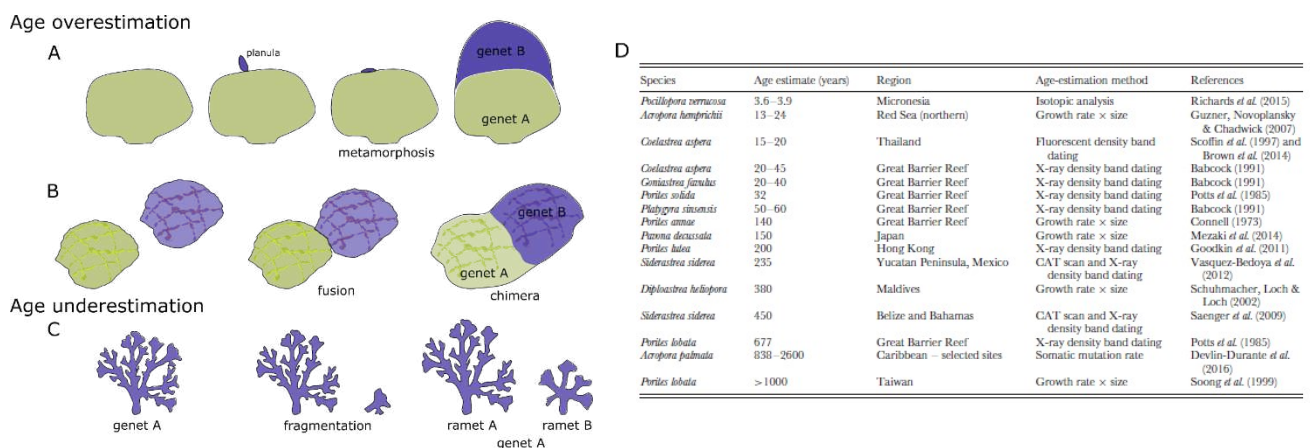
**Figure 12 : Extracted figures on *Millepora platyphylla* from (Dubué et al., 2019)** (A) Morphology of *Millepora platyphylla* colonies (Figure 2 in Dubué et al., 2019, "(D) photograph being a courtesy of Gilles Siu"), (B) World map of *Millepora platyphylla* geographic repartition (Figure 4 in Dubué et al., 2019), (C) Drawing of the schematic life cycle of *Millepora platyphylla* (Figure 9 in Dubué et al., 2019)

## 2.5 Estimation of age in wild corals

For several reasons that were discussed earlier it is even more complicated in corals, than in some other animals, to precisely estimate their chronological age in the wild. One method is to use the isotopic ions trapped in the skeleton and the bands left by skeleton accumulation that forms, like in trees, growing bands. This method enables scientists to date the skeleton but also to have information on the past climate and the past growth rate of the colony (Buddemeier & Kinzie, 1976 , (Bessat and Buigues 2001; Lough and Cantin 2014; Cantin and Lough 2014). Age records from sclerochronology were reviewed in (Bythell, Brown, and Kirkwood 2018) (Fig 13:B). Nonetheless this method is quite invasive because a skeleton core must be drilled from the colony hence being more suitable to massive coral species rather than branching ones.

Somatic mutation within a colony, in other words the degree of mosaicism of the colony, was investigated in (Devlin-Durante et al. 2016) but the use of it to estimate colony age was inconclusive and could be highly species dependent. Moreover in (Fig13:B) *Acropora palmata* ages are ranging from 800 years old (yo) to 2'600 yo based on the mutation rate, but it has previously been discussed that corals are highly permissive to mosaicism and that it is consider a possible adaptation way to cope with sessile condition and low reproduction rate (van Oppen et al. 2015), thus mutation rate is not suitable for coral age estimation.

The size of the colony can also be used to estimate the age knowing the growing rate (inferred from sclerochronology) of the investigated species. Because calcification and symbiosis in coral is sensitive to the environment using a growth rate measured in the same conditions or location as the studied colonies ensure a better precision in the age estimation. Several biases underpin this method and will be discussed (Fig13:A:B:C).



**Figure 13 : Scheme of possible bias in age estimation based on colony size.** (A) When a planula larvae metamorphosis on an adult the fusion leads to a chimera that will be estimated to be older than it is based on its size. This is likely to happen in different coral morphology. (B) When two colonies fused to form a chimera the age of the colony will be overestimated. Chimerisms is highly species dependent. (C) Fragmentation will divide one genetic colony in two ramets with the same genetic background and coming from the planula metamorphosis. However in the field they can be mislead as two different colonies and ramet B age based on its size will be underestimated.. This is more likely to happen in branching morphology. (D) Table of estimated age of corals based on literature review by (Bythell et al., 2017)

If the coral colony studied is a chimera, either from the fusion of two lateral colonies (Fig13:B) or the fusion with a larva metamorphosing onto an adult (Fig13:A), its age will be overestimated. If the studied colony is the result of the development of a broken branch, a ramet, or a polyp bailed out, its age will be underestimated because it would not account for the size of the first colony, the genet (Fig13:C). Another argument used in (Devlin-Durante et al. 2016) would be to consider that the budding of a polyp represents a chronological birth. First, as we discussed in (1.2.c) no evidence of an ageing reset in coral polyp budding had been shed to light until now. Second, if we consider a new polyp being younger chronologically it is not inappropriate to use growth rate to estimate age, it will just estimate the age of the oldest polyp, the primary polyp. Or in other words, the time since settlement, metamorphosis, which is at least in our case what we are interested in. Hence colony size measurement remains the most straightforward available method to estimate the chronological age parameter. It is needless to add that in controlled conditions monitoring a colony from its metamorphosis is the best way to estimate its age.

### 3. Telomere in Cnidarian

Telomere in cnidarian had mostly been investigated in hydrozoan jellyfish or in corals, first in comparative studies to find the conserved telomeric sequence of metazoan (Traut et al. 2007; Sinclair, Richmond, and Ostrander 2007), demonstrating that cnidarian animals have the conserved telomeric repeat sequence (TTAGGG)<sub>n</sub> while it was demonstrated later on in (Zielke and Bodnar 2010) that their symbionts have a different telomeric repeat, the *Arabidopsis thaliana* one (TTTAGGG)<sub>n</sub>. Even though telomeric repeat sequences in other dinoflagellates (*Prorocentrum micans* and *Amphidinium carterae*) had already been identified as (TTTAGGG)<sub>n</sub> (Alverca et al. 2007).

In Sinclair et al. 2007 they described telomere length for the complex *Acropora surculosa* (~4kb) and *Acropora digitifera* (~8.9kb) in (Hiroki Tsuta et al. 2014), and the robusts *Favia pallida*, *Leptoria Phrygia* and *Goniastrea retiformis* (~9kb) (Sinclair, Richmond, and Ostrander 2007). They found longer mean telomere length in *Galaxea fascicularis* (15.kb) in (H. Tsuta and Hidaka 2013), or in robust *Madracis aurentenra* and complex *Agaricia fragilis* (~19.5kb) from (Zielke and Bodnar 2010).

In (Ojimi et al., 2012) they investigated the correlation between the weight and the mean TL of the solitary coral *Ctenactis echinata* without finding any correlation. Few studies investigated telomere length in different life stage and in the complex coral *Galaxea fascicularis* (H. Tsuta and Hidaka 2013) they found no significant differences between sperm (17.9 kb), planula larvae (7.5±2.7 kb) or adult (15.6±3.9 kb). Nevertheless, a non-significant decrease in the planula larvae was observed and restored in the adult, suggesting important DNA repair or cell clearance during the key step of metamorphosis (Seipp et al. 2006, 2010). Later on in (Hiroki Tsuta et al. 2014) they found the opposite results with this time a significant difference between life stages telomere length in the complex *Acropora digitifera*. Its telomere length decreases between sperm (16.1± 2.7 kb), planula larvae (11.7± 3.4 kb) and adult colonies (8.9± 1.8 kb). Interestingly longer telomere were measured in the center of adult colonies compared to the colony border which can be linked to mosaicism.

In (Ojimi and Hidaka 2010) they investigated telomere length in different stages of the scyphozoan jellyfish *Cassiopea andromeda* comparing polyps and reproductive polyps (propagule) to several anatomical part of the jellyfish stage (arm, beel and gonads). Telomeres were longer in the jellyfish and mostly in the jellyfish bell while polyps were having shorter telomeres, nonetheless telomere length was not different between the jellyfish gonads and the propagule. However telomerase activity wasn't significantly different between the jellyfish bell and the polyps (Ojimi, Isomura, and Hidaka 2009). Surprisingly few studies have investigated telomere length in the most used hydrozoan solitary model, *Hydra sp.*, with telomere length only being reported in (Traut et al. 2007) measuring approximately (~20kb), no

evidence of telomerase activity had been reported yet but a differential expression of TERT in some populations of stem cell could explain long telomere maintenance in *Hydra sp.* (Wenger, Buzgariu, and Galliot 2016; Schenkelaars et al. 2018).

It was stressed out in Bythell et al. 2017 that coral ageing must be a next priority in the field to unravel the presence of an ageing mechanism in cnidarian and understand how this parameter can be an asset to acclimatize to global climate change.

## 4. Presentation of the PhD work

My PhD work was mainly motivated by the will to understand the determinants of telomere DNA length regulation in reef corals.

I performed extensive studies on telomere DNA length (TL) variation, using the telomere restriction fragment assay, in 5 coral genera ( *Millepora sp.*, *Porites sp.*, *Acropora sp.*, *Pocillopora sp.*, *Stylophora sp.*) in different parts of colonies of different age, size and across 32 island systems of the Pacific Ocean as part of the Tara-Pacific consortium.

To get insight on the conservation of genomic telomeric features I will investigate telomere sequence localization in the genome of (*S. pistillata*, *P. meandrina* and *P. lobata*) to describe and compare their interstitial telomeric sequence.

Next I will investigate the relationship between telomere length, age and size in *S. pistillata* at the colony level and the branch level to further on document the effect of separate abiotic stress treatments (acidification, temperature increase and dark-induced bleaching) in controlled and natural conditions in *S. pistillata* and *Acropora sp.*.

Finally, I will research the genetic and environmental determinants of TL variation of wild population from 3 coral genera (*Millepora sp.*, *Porites sp.*, *Pocillopora sp.*) at the Pacific Ocean scale.

# Chapter II-Telomeres and interstitial telomere sequence in coral genomes

## 1. Introduction

Telomeres are composed of short repeated DNA sequences located at the end of linear eukaryotic chromosomes. The telomeric repeat motif (TTAGGG) $_n$  is highly conserved among Metazoan with the exceptions of Nematodes and Arthropods (Traut et al. 2007). In many organisms, the telomeric DNA sequences are bound by a protective protein complex, the shelterin, composed of 6 proteins in human (TRF1, TRF2, TIN2, TPP1, POT1 and RAP1) (Traut et al. 2007; de Lange 2005; Giraud-Panis et al. 2013). Telomeric-repeat binding factor 1 and 2 (TRF1 and TRF2) directly bind the duplex telomeric DNA repeats, Protection of telomere 1 (Pot1) to single stranded telomeric DNA, while TRF1-interacting nuclear factor 2 (TIN2) and TPP1 form a protein bridge between the double and single stranded parts of telomeric DNA. RAP1 is associated with TRF2. Since the replication of DNA is incomplete, telomeric DNA shortens at each round of cell's division leading to DNA damage activation pathways and senescence when they reach a critically short length (Harley, Fletcher, and Greider 1990; Gilson and Géli 2007; Aviv and Shay 2018). Telomeres act as breakwaters, protecting the genome from replication attrition. But telomeric repeat sequences are also found in extra-telomeric locations, defined as interstitial telomeric sequences (ITS) (Meyne, Ratliff, and Moyzis 1989; Bolzán and Bianchi 2006).

ITSs are mostly located in intergenic regions or introns, rather than in exons and their density is usually higher in subtelomeres regions (Bolzán 2017; Pryde, Gorham, and Louis 1997). The chromosomal location of ITSs is poorly conserved between species (Ruiz-Herrera et al. 2002; Nergadze et al. 2007). Some can arise from insertions at fragile sites or during double strand breaks repair (Ruiz-Herrera et al. 2008) while others from ancient chromosome fusions (Ijdo et al. 1991; Hartmann and Scherthan 2004). The repeated telomeric sequences of ITSs are often degenerated, even more in long ones, most likely to avoid the instability produced by pure tandem repeats (Aksenova et al. 2015; Ye et al. 2010). Nevertheless, the presence of telomeric DNA repeats within the genome allows shelterin proteins to bind. This was shown by chromatin immunoprecipitation studies for RAP1 (Martinez et al. 2010) and TRF1 and TRF2 (Simonet et al. 2011; Yang et al. 2011).

The 3' overhang of telomeric DNA is able to fold back and hybridizes with its complementary sequence in the more internal telomeric double stranded DNA, a process assisted by TRF2 and forming a protective structure called T-loop (Griffith et al. 1999; Benarroch-Popivker et al. 2016). The 3' overhang could also hybridize with internal ITSs, leading to large subtelomeric loops. Thus, one function of the subtelomeric ITS could be to enhance telomere protection, an hypothesis suggested

by interactions between TRF2 and subtelomeric ITSs (Wood et al. 2014; Robin et al. 2020). Noteworthy, the repression of subtelomeric gene expression exerted by telomere proximity (Telomere Position Effects or TPE) can be extended toward more centromere proximal genes thanks to ITSs, being handovers for the telomere initiated chromatin spread (Fourel et al. 1999; Steinert, Shay, and Wright 2004; Robin et al. 2020).

A genome-wide function of ITSs can be gene expression regulation. Indeed, there is a set of evidence that the binding of shelterin subunits to ITS contributes to the regulation of genes located nearby or directly associated to an ITS (Martinez et al. 2010; Yang et al. 2011; Biroccio et al. 2013; Cherfils-Vicini et al. 2019; Robin et al. 2020). It was hypothesized that the action of shelterin at ITSs can be modulated by the telomere state, coupling telomere function to the regulation of a network of ITS-associated genes (Ye et al. 2014a). This might result from modulations in the formation of subtelomeric chromatin loops, from dispersion of shelterin throughout the nucleoplasm upon telomere shortening or from telomere length-dependent regulation of shelterin expression (Ye et al. 2014a).

If the presence of ITS is widespread among eukaryotes, very little is known about the presence and function of ITSs outside yeast, human and mouse. Here, we investigate the presence and distribution of ITSs in four reef coral genomes.

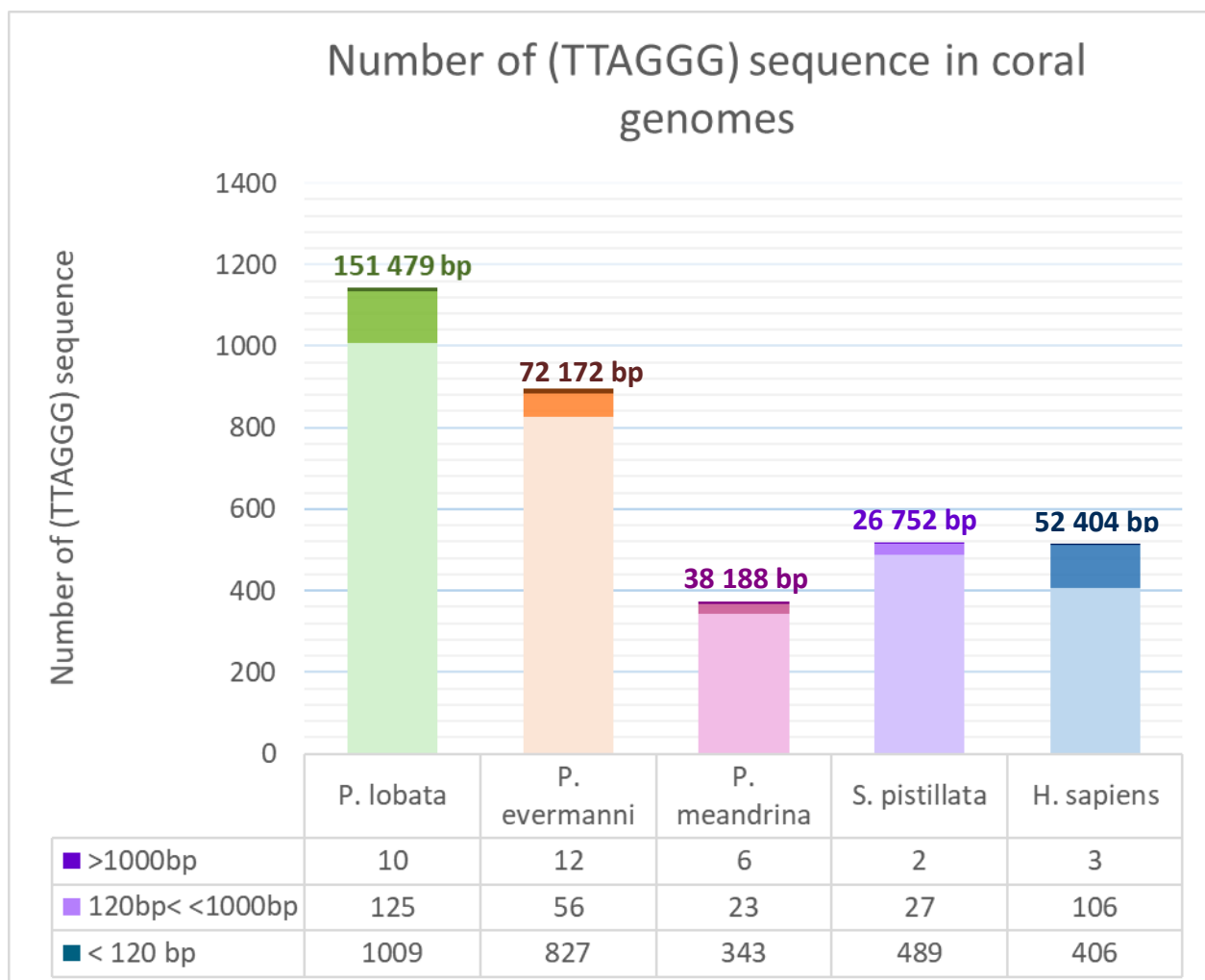
## 2. Results

Here we investigated the distribution of ITSs in the coral model *Stylophora pistillata* genome (Voolstra et al. 2017), in two targeted species from TARA-PACIFIC expedition *Pocillopora meandrina* and *Porites lobata*, and in another coral *Porites evermanni*. We looked for sequences covering at least 75% of the (TTAGGG)<sub>4</sub>, allowing two mismatches and grouping under one ITS identification, sequences that were distant from less than 400 nt. In order to distinguish the (TTAGGG)<sub>n</sub> sequences located at chromosome termini (telomeres) from ITSs, we considered the (TTAGGG)<sub>n</sub> repeats located at one end of a contig (less than 10 nucleotides (nt) from the extremity) as being part of a telomere and the others as ITSs.

### ITS characterization in coral genome

The two Pocilloporidae corals, *S. pistillata* and *P. meandrina*, have a similar number of ITSs in their genome (Fig14), with a majority of short sequences (respectively 489 and 343), few middle length sequences (respectively 27 and 23) and a few long ones (respectively 2 and 6). Noteworthy, sequencing of *P. meandrina* genome using long read technology did not increase the number of short or middle length ITSs but increased the number of long ITSs by 3 fold. This can be interpreted as the short/middle ITSs can easily be assembled with short reads while it is more uncertain or even impossible for longer ITSs. Indeed, when we looked for ITSs'

closest genes it appears that 42% of ITSs are located within the genes, both in *P. meandrina* and *S. pistillata*, while respectively 45% and 57% are located in intergenic regions (Fig16D). In *S. pistillata* the 2 long ITSs are located into the 14th intron of the coadhesin-like gene (XM\_022926987.1) and the first intron of NMDA 1-like glutamate receptor ionotropic gene (XM\_022925250.1). Only two genes were found close to long ITSs in *P. meandrina*, a Dopamine D (1) receptor like (Pmea\_00029636) and a LINGO1 gene (Leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1) (Pmea\_00026759), both being involved in neurons function in humans. Additionally to the 6 long ITSs, 3 long (TTAGGG)<sub>n</sub> sequences and 2 middle ones are located at contig extremities and thus considered as telomeres. In these 5 predicted telomeres, 3 of them are located at less than 10kb from long ITSs (ITS\_11, ITS\_62, ITS\_151) that are likely part of a subtelomeric region in contig\_51, contig\_62 and contig\_195, respectively.

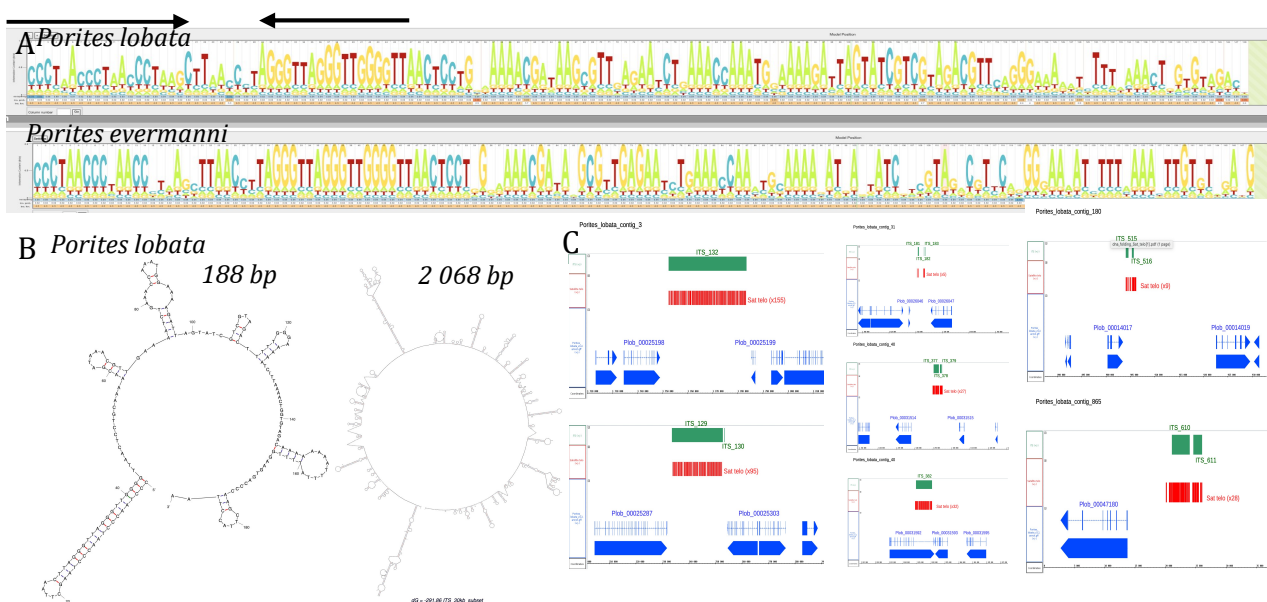


**Figure 14 : Histogram of corals *P. lobata*, *P. evermanni*, *S. pistillata*, *P. meandrina* and human number of interstitial telomeric sequence in the genome, ranging by 3 class size.**

The second target species of the TARA-Pacific expedition, *P. lobata*, has more ITS (1144) than the other investigated species and even a little more than its fellow genera *P. evermanni* (895) (Fig14). In proportion to genome size, the content in ITSs is twice as high in *P. lobata* than in *P. meandrina* (0.02467063 % versus 0.01355775%). *P. lobata* genome has proportionally more middle length ITS (10%) than the 3 other corals (6%) but no more than *H. sapiens* (20%). Based on their position at contig extremities (contig\_42, contig\_357, contig\_514), 3 long (TTAGGG)<sub>n</sub> sequences were considered as telomeres (ITS\_264, ITS\_306, ITS\_871) and 2 were grouped with other ITS that might compose the subtelomeric region of the contig.

### Porites genus specific palindromic ITS

Noteworthy, *P. lobata* genome contains 2 ultra long ITSs (19kb and 31kb), while the longest in *P. evermanni* is 6kb. Long reads sequences bridging those ultra long ITSs confirmed their interstitial localization within the chromosome. They are composed of a tandem array of a 188 nt repeated unit, containing 6 TTAGGG sequences in dyad symmetry thus capable to fold into a stem loop structure (palindrome) (Fig15). The rest of the repeated unit exhibits a marked AT-richness. This sequence was named “palindromic ITS unit” on its specific architecture, the consensus sequence between *Porites lobata* and *evermanni* is shown in (Fig15). This “palindromic ITS unit” is specific to *Porites* genus. Interestingly, 6 out of the 7 sequences of *P. lobata* composed of an array of palindromic ITS units were intergenic, occupying a large part of the region separating two genes (Fig15). To our knowledge, there is no other known example of such a tandemly repeated sequence containing a palindromic ITS. Interestingly, the palindromic nature of these ITSs should allow the coral shelterin TRF to bind when the sequence is single-stranded, for example during replication, or if it is transcribed as RNA.



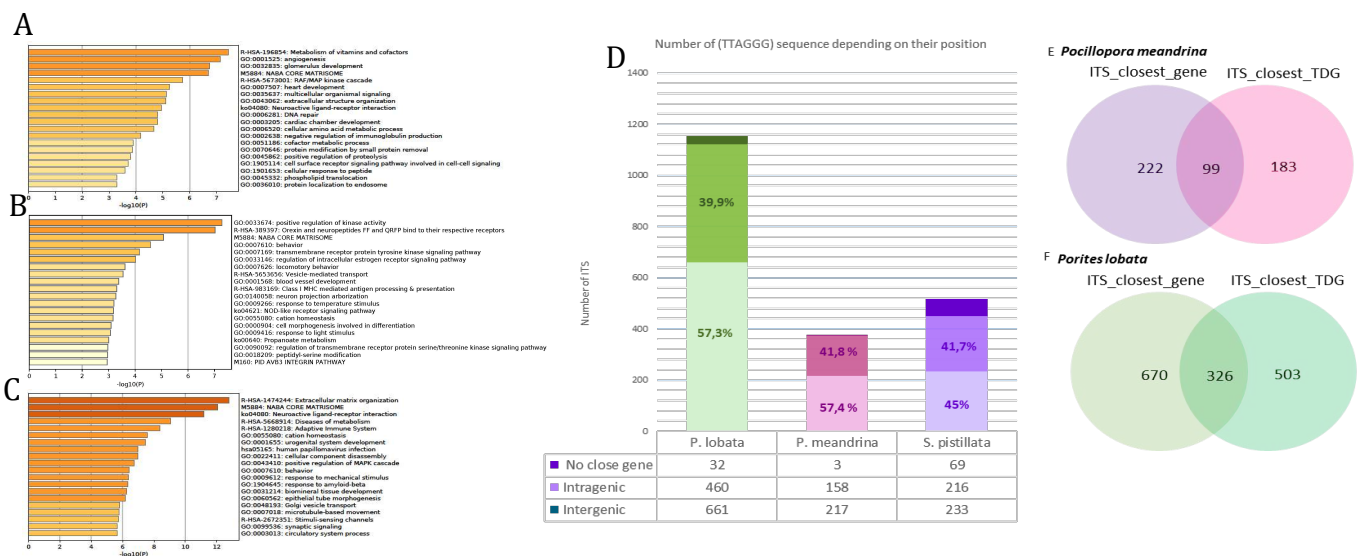
**Figure 15 : Long palindromic ITSs in *Porites* genus.** (A) Sequence of long palindromic ITS sequence of *Porites lobata* and *Porites evermanni*. (B) Folding and 1D structure of one repeat (188bp) of the palindromic ITSs and structure of 11 repeats (2068 bp) in *Porites lobata*. (C) Localization of palindromic sequence ITS (red) blast on the genome of *P. lobata*.

## ITSs associated to tandemly repeated genes

Among the ITS associated genes, 26.3% for *P. meandrina* and 28.4% for *P. lobata* were actually tandemly repeated genes (TDG) (Fig16: E: F). Comparing those tandemly repeated genes groups between the two species allowed the identification of conserved TDG groups, referred as orthogroups. Among them, 4 are associated with ITSs in the 2 species (OG0002611, OG0000232, OG0000178, OG0012279).

For the TDG orthogroup (OG0012279), it is associated to 2 ITSs in *P. meandrina*, the predicted telomere (ITS\_150) located less than 10kb to (ITS\_151), but the TDG function was uncharacterized (Pmea\_00009991). The same structure can be found for *P. lobata* with 2 ITS (ITS\_211 and ITS\_216) separated by 10 kb distance for which the closest genes (Plob\_00021963, Plob\_00021936) are belonging to the same uncharacterized orthogroup than in *P. meandrina* (OG0012279). The 3 other orthogroups contained genes with predicted functions (DNA polymerase delta subunit for *P. meandrina* and beta gamma crystallin isoform for *P. lobata*, histamine H2 receptor-like and lymphocyte antigen 6 complex).

When extending the search beyond the closest genes to reach the closest TDG it appears that among the 95 found, 42 TDG clusters for *P. lobata* and 30 for *P. meandrina* belong to 26 common orthogroups (Fig16: E, F).



**Figure 16: Gene Ontology of Interstitial Telomeric Sequence (ITS) closest genes in (A) *S. pistillata* genome, (B) *P. meandrina* genome, (C) *P. lobata* genome, (D) Histogram of the localization of ITS to their closest genes in *P. lobata* (green), *P. meandrina* (pink) and *S. pistillata* (purple), with an intragenic or intergenic position as well as the absence of a nearby gene. Venn diagram of ITS closest genes that belong to Tandemly Duplicated Genes (TDG) in (E) *P. meandrina* (F) and *P. lobata*.**

### Gene Ontology of ITSs associated genes

The gene ontology analyses of the ITS-associated genes were performed for *S. pistillata*, *P. meandrina* and *P. lobata* and the 20 most enriched terms are displayed in (Fig16: A, B, C). This analysis reveals conserved functional features between the 3 species related to extracellular components, possibly skeleton synthesis or symbiosis interaction, and exogenous signal sensing. These include the functional annotations of gene sets encoding core extracellular matrix components (in addition to M5884, which is conserved among the three coral species analyses, there is in *P. meandrina* M160, in *P. lobata* HSA 1474244/GO0031214 and in *S. pistillata* GO:43062) as well as signal transduction factors (particularly gene sets related to the MAPK pathway : *P. meandrina*/GO:0033674/GO:0007169/GO:0090092, *P. lobata*/GO:0043410 and *S. pistillata*/R-HSA-5673001 but also in *P. meandrina* GO:0033146/ko04621 and in *P. lobata* GO:0009612/R-HSA-2672351) (Fig16: A, B, C).

Moreover, in *P. meandrina* some GO terms linked to environment sensing such as response to light (GO:0009416) or temperature (GO:0009266), are enriched in ITS -associated genes. In further agreement with enriched gene sets involved in sensing exogenous stimuli, several GO terms in the 3 corals are related to the immune system (*P. meandrina*/R-HSA-983169, *P. lobata* hsa05165/R-HSA-1280218, *S. pistillata* GO:0002638/GO:1901653) and to the nervous system (*S. pistillata*/ko04080/GO:1905114, *P. meandrina*/R-HSA-389397/GO:0140058 and *P. lobata*/ko04080/GO:0099536).

Overall, the ITS-associated genes of diverse corals have in common to form a network of genes involved in extracellular structure and sensing.

### Conserved Pif1 helicase family sequence

To find conserved genes specific to corals compared to their symbionts we compared the genomes of 3 symbionts species to the corals genome. We identified a long (7kb) and highly conserved (99%) sequence between the 3 corals investigated *S. pistillata*, *P. lobata* and *P. meandrina*. This sequence corresponds to 2 predicted mRNA coding for a PIF1 helicase family protein in *S. pistillata* (XM\_022946233.1 and XM\_022936671.1). The corresponding sequences in *P. lobata* and *P. meandrina* were not part of the closest genes of some ITSs however in *P. lobata* one of the conserved sequence located in contig\_1 was flanked by 2 ITSs (ITS\_1010 and ITS\_1011) and was overlapping with a RNAseq transcript (RNAT00117003001).

### 3. Discussion

We were able to identify interstitial telomeric sequence (ITS) in the genome of four scleractinian species, 2 complex corals, *Porites lobata* and *Porites evermannii* and two robust corals *Pocillopora meandrina* and the previously sequenced *Stylophora pistillata* (Voolstra et al. 2017). Even if their absolute number is variable between species, the proportion of short ITSs (Fig14) and the localization to the closest gene (Fig16), whether the ITS is in an intergenic region or within the gene, was highly conserved in scleractinians. However as previously demonstrated in other animals (Ruiz-Herrera et al. 2002; Nergadze et al. 2007) the precise localization of the ITSs was not always conserved between species.

Indeed, the 2 long ITSs found in *S. pistillata* that are located in the introns of a gene coding for a predicted NMDA 1-like glutamate receptor (XP\_027051052.1) and a coadhesin-like protein (XP\_027050960.1) were not present in the homologous genes in *P. meandrina* genome, respectively (Pmea\_00000911) and (TDG\_787). The presence of ITSs in those genes was specific to *S. pistillata*.

Nonetheless, long ITSs were found in 2 genes with a related function in *P. meandrina*, a predicted D1 Dopamine receptor and collagen-alpha-chain-like coding gene (Pmea\_00029636/XP\_027051052.1 and Pmea\_00012474/XP\_027050960.1). Supporting the notion that ITS-associated genes have conserved functional features between coral species (Fig16), we found an enrichment of GO-Terms related to extracellular matrix and exogenous signal sensing. Corals have evolved specialized extracellular matrices in order to build their calcareous skeleton (Bhattacharya et al. 2016; Voolstra et al. 2017). On the other hand they also have developed specialized extracellular receptors such as Toll like receptors, to enable them to initiate their symbiosis and recognize the suitable symbiont (S. K. Davy, Allemand, and Weis 2012). Finally, extracellular proteoglycan is involved in the stabilization to their receptors of extracellular signaling master regulators, such as Wnt or Hedgehog. Whether the ITS-associated genes are specifically involved in one of these functions remains to be elucidated as well as the control of their expression by the associated ITS.

Nevertheless, it is impressive that the function of the ITS-genes in coral is reminiscent of the human situation where several ITS-associated genes are involved in proteoglycan biosynthesis and glycome structure (HS3ST4, GPC6, VCAN and SULF2). Interestingly, in human cells, these genes are under the control of the shelterin subunit TRF2 during oncogenesis, modifying cancer cell microenvironment and immune-surveillance (Biroccio et al. 2013; Cherfils-Vicini et al. 2019; Zizza et al. 2019). Therefore, coupling the telomere state to cellular microenvironment via a specific network of ITS-associated genes could be conserved through evolution as a mechanism regulating organismal fitness and adaptation to environmental changes

(Ye et al. 2014). In the case of coral, it would be of interest to study if this mechanism is involved in microbial population regulation and stress/disease resistance as suggested by the high number of tandemly duplicated gene (TDG) (Bhattacharya et al. 2016; Voolstra et al. 2017).

A high proportion of ITS-association genes corresponds to TDGs, both in *P. lobata* and *P. meandrina*. Some of these TDG exhibit redundant functions and could be clustered in orthologous groups. Some orthogroups were conserved between the 2 species. Interestingly for the lymphocyte antigen 6 complex orthogroup, *P. meandrina* has only one ITS-associated gene (ITS\_27, Pmea\_00034871) while *P. lobata* has 2 ITSs (ITS\_335, ITS\_336) associated with 2 genes (Plob\_00040959, Plob\_00040961) belonging to the same TDG group (TDG\_2546) and another ITS (ITS\_156) in another contig associated to 2 genes (Plob\_00050194, Plob\_00050193) of a different TDG group (TDG\_1139) but belonging to the same orthogroup (OG0000178). Therefore, we could make the hypothesis that this TDG has been duplicated in *P. lobata* or lost in *P. meandrina*, along with associated ITSs. *P. lobata* is a well known specialist coral, it preferentially establishes symbiosis with one species of Symbiodiniaceae (*Cladocopium sp.*, (T. C. LaJeunesse et al. 2018; Barshis et al. 2010)) while *P. meandrina* is more permitting. Therefore, we can wonder if this orthogroup linked to innate immunity and antigen recognition could be involved in *P. lobata* symbiosis specificity.

Looking for a conserved sequence in corals that was not present in symbionts we found an interesting 7kb conserved and duplicated sequence in scleractinians genomes that is predicted to code for a Pif1 helicase family protein in *S. pistillata*. This finding is intriguingly recalling the results from (Bhattacharya et al. 2016; Voolstra et al. 2017) where they identified a specific DNA mismatch repair (MutS) conserved in basal metazoans but lost in bilaterian. DNA mismatch repair protein (MutS) and Pif1 helicase share a particular structure called a P\_loop\_ NTPase involved in the hydrolysis of the beta-gamma phosphate bond of a bound nucleoside triphosphate (NTP). Interestingly, Pif1 is required for genome maintenance in a large variety of organisms. A conserved function of the Pif1 helicase family proteins is to unwind G-quadruplexes (Paeschke et al. 2013), structures that are formed at G-rich regions at telomeres, ITS and other chromosomal sites. Pif1 has also been shown in yeast to prevent telomere lengthening by preventing telomerase access (Eugster et al. 2006; Stinus, Paeschke, and Chang 2018). Recently, a study on *Drosophila melanogaster* has shown that the Pif1 helicase counteracts replication stress by promoting double strand gap repair (Kocak et al. 2019). Altogether those results suggest that corals have conserved a Pif1 helicase to reinforce their mechanisms of genome maintenance and telomere length regulation. Interestingly, this conserved Pif1 sequence could be used as an animal-specific reference gene.

*P. lobata* was showing some peculiarities, having proportionally more ITSs in its genome than *P. meandrina* or another species from its genera *Porites evermanni*. We found a 188nt length sequence that contains a palindromic telomeric repeat forming ultra long and conserved ITSs at the *Porites* genus level. These repeated DNA sequences are to our knowledge new and unique to *Porites* sp.. Whether they are involved in specific traits of this genus, like their stress resistance (Darling et al. 2012), by regulating the expression of their flanking genes is an interesting hypothesis to investigate.

ITSs function was hypothesized to enable a telomere length dependent gene expression regulation by their association to shelterin proteins whose availability is modulated by telomere DNA length (Ye et al. 2014). Here we found 18 genes whose transcription is correlated to *P. meandrina* mean telomere length (see chapter V); 16 of them were more expressed when telomere length was short. However, those genes were not significantly overrepresented or underrepresented in the correlated gene dataset, therefore the fact that those genes contain ITSs within their sequence (15 genes) or upstream or downstream (3 genes) might be due to chance. Nevertheless, 2 negatively correlated genes' expression to telomere length (Pmea\_00005670 and Pmea\_00005732) with intronic ITSs belong to the same contig\_14 which could suggest a local coordinated regulation.

In *P. meandrina* none of the closest genes to the 6 identified telomeres had an expression correlated to mean host TL, nevertheless characterisation of telomere sequence and more complete subtelomere region structure needs to be investigated to see if the Telomere Position Effect (TPE) is conserved in corals. The direct transcriptional effect of shelterin proteins upon telomere shortening still needs to be unraveled in corals but the highly conserved features between distantly related corals species and humans is opening an exciting field of investigation to undermine the molecular regulation of coral resistance to stress and longevity.

Even if corals seems to have evolved specific genomic features to adapt to their sessile and symbiotic life mode (Bhattacharya et al. 2016; Voolstra et al. 2017) they have conserved structural telomeric ones with a conserved (TTAGGG)<sub>n</sub> motif and ITSs. How they regulate their telomere length with time and upon environmental stressors remains to be elucidated.

# Chapter III-Telomere length variation with age and size in corals

## 1. Introduction

Telomere attrition had been demonstrated to be a good predictor of lifespan in vertebrates (Bize et al. 2009) and to be a hallmark of ageing (López-Otín et al. 2013). In corals two opposite results demonstrated a shortening from larval stage to adult (Hiroki Tsuta et al. 2014) while no shortening between solitary coral of different size, used as an age proxy, was reported in (Ojimi et al., 2012). Concerning ageing in corals a study on *S. pistillata* described a shorter cell turnover (2 days) than the one in *Hydra sp.* (Agathe Lecointe et al. 2016) and (Rinkevich and Loya 1986) notified *in situ* ageing phenotype in *S. pistillata*. But to this date no clear evidence of ageing in coral colonies was characterized. The high proliferative ability of *S. pistillata* could be dependent on a telomerase telomere lengthening but in (Gorbunova, Bozzella, and Seluanov 2008) they investigated the paradox found in rodents where some species have very long telomeres, usually due to telomerase activity, compared to their short lifespan. Whereas no correlation was observed between telomerase activity and lifespan (15 species with lifespan ranging from 4 years to 28 years), they show a negative correlation between telomerase activity and body mass.

Taken together and knowing that *S. pistillata* can be considered as a fast growing coral we investigated TL in corals of different age as well as a RNA-seq to search for a molecular ageing signature and shed light on the apparent lack of ageing phenotype.

## 2. Telomere length in *Stylophora pistillata* colonies of different ages

Here we investigated telomere length in *Stylophora pistillata* colonies of known age to unravel the telomere length regulation in a coral species. We sampled 3 branches, or nubbins (n), of 3 colonies of approximately 1-year-old (A1), 2 colonies of approximately 2 years old (A2), 1 colony of 4 years old (A4) and 2 different mother colonies that were introduced in the Centre Scientifique de Monaco (CSM) aquarium 30 years old ago (S1 and S2) (Fig17). We considered the group of 1yo, 2yo and 4yo as the young group compared to the old group of colonies from a different genetic background (S1 and S2). Telomere length was measured twice using TRF assay and average and we performed a RNA-seq of selected branches in each group of age to characterize a potential ageing transcriptome in *S. pistillata*.



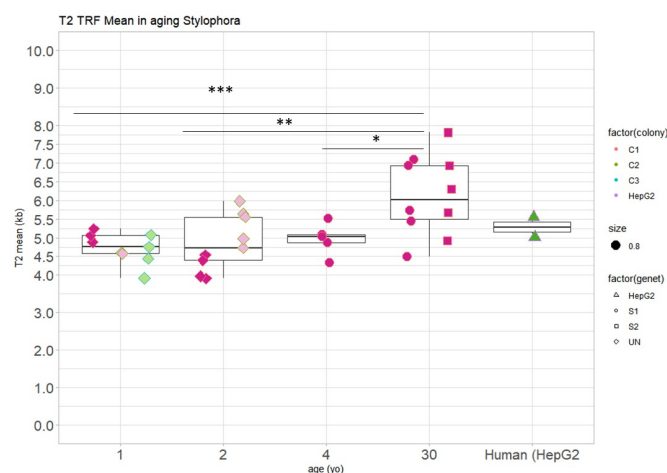
**Figure 17: *Stylophora pistillata* of different age.** Colonies (C1, C2, C3) of 1-year-old (A1) are displayed on top with orange borders, 2 years old (A2) are shown together with green borders, the only colony of 4 years old (A4) is displayed with purple border and the adult one (S1) with a pink border on the right.

## 2.1. Results

### Telomere length analysis

We found that contrary to what could have been expected (Fig18), the mean T2L doesn't decrease in *S. pistillata* with age. On the opposite, the mean T2L of the older colonies (S1 and S2) was significantly higher than the younger ones with a positive slope of ( $y=4.5713334 \times T2L+0.0009114$ ).

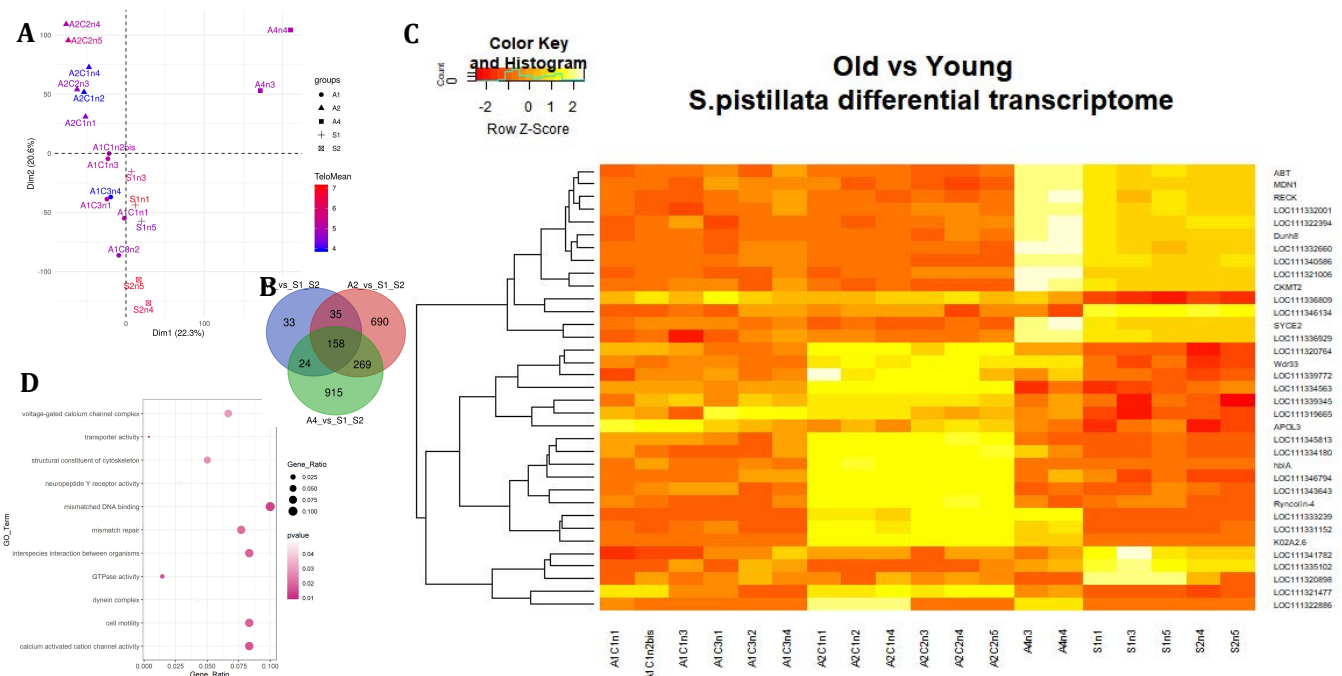
We were also able to observe an increase of telomere length variability within one colony (dots with same shapes) with age which can be related to previously reported increase in mosaicism in coral colonies (Maier et al. 2012).



**Figure 18 : Boxplot of Telomere length with age in coral *Stylophora pistillata*.** Shapes are representing genet of colonies, colonies are differentiated with colors, each points represent a branch of a colony. Pairwise Wilcoxon test results are displayed (\*' p<0,05, '\*\*' p<0,01, '\*\*\*' p<0,001)

## Differential transcriptome in older corals

In order to have a deeper understanding of the mechanisms underlying this telomere length maintenance with age we extracted the RNA from 3 nubbins (n) and 2 colonies per age group. We performed a mRNA paired-end sequencing (Illumina) and normalized each gene expression to the total gene expression in each sample. The differentially expressed genes (DEG) were extracted comparing the young group (A1, A2, A4) to the old group (S1, S2). The colony of 4 years old was expressing a pool of different genes compared to the (Fig19:A) and the colonies were not differentiated by their mean T2L. We identified 158 DEG shared between the young colonies compared to the old ones, among them 35 were significantly differentially expressed (Fig19:B:C).



**Figure 19 : Transcriptome analysis with age in coral *Stylophora pistillata*.** (A) Principal component analysis (PCA) of *S. pistillata* coral samples based on their transcriptome, (A1=age1, A2=age2, A4=age4, S1=adult colony from S1 genetic, S2=adult colony from S2 genetic, C=colony, n=nubbins (branch), 2 colonies of 1-year-old (A1), 2 colonies of 2 years old (A2), one colony of 4 years old (A4) and two colonies of more than 30 years old (S1 and S2) were sequenced with 3 branches per colony, except for the (A4) that only had 2 branches. Age groups are colored according to their telomere length. (B) Venn diagram of the shared differentially expressed genes (DEG) between the colonies of different ages and the controls (S1 and S2), 158 genes were in common but only 35 genes were significantly DEG. (C) Heat map of the 35 significantly DEG between young colonies (A1, A2, A4) and old colonies (S1 and S2). (D) Significantly Enriched Gene Ontology (GO) pathways (p<0,05) (biological process, cellular component and molecular function). Ion transport and transporter, cell motility, interactions and DNA repair are enriched in younger compared to older colonies.

When we looked at the normalized count for this ageing core transcriptome we found that within each age group the sign of the fold change is well conserved as well as between branches, showing low individual variability (Fig19:C). Each group of age has its own expression pattern, however a moving increase or decrease in gene counts could be observed with age. Moreover, it appears that the DEG pattern of the colony of 4 yo (A4) was quite similar to the two older ones (S1 and S2) surprisingly setting the age threshold between 2 yo and 4 yo for a coral colony.

For example, the Synaptonemal complex central element protein 2 (SYCE2) was under expressed in young colonies (A1 and A2), highly expressed in A4 and still expressed but to a lesser degree in S1 and S2. The protein encoded by this gene is involved in chromosome segregation during meiosis, it has been reported to be conserved in basal metazoans and cnidarians (*Hydra vulgaris* and *Nematostella vectensis*) and specifically expressed in *Hydra vulgaris* testis both at the mRNA and protein level (Fraune et al. 2013). This gene could be a good candidate to investigate the question of the germline segregation in corals and isolate meiotic cells.

Among the DEG some were classed as non coding RNA like (LOC111336809, LOC111319665) that are progressively underexpressed with age or (LOC111320898, LOC111341782) that are overexpressed in adult colonies. Those long coding RNA could be involved in translation regulation or part of some ribonucleic complex, none of them were containing the (TTAGGG) motif.

Several genes were overexpressed in adults compared to young ones with some of them with predicted functions such as (ABT, RECK, CKMT2). CKMT2 (Mitochondrial creatine kinase) is involved in phosphorylation of creatinine and might be important for mitochondrial biogenesis, it could be the sign of a switch in metabolism in adult corals, like the mitochondrial ADP/ATP carrier (ABT). APOL3 (apolipoprotein L gene family) is progressively underexpressed with age and is known to be involved in cytoplasmic lipid trafficking and RECK is an extracellular protein that have been reported to have a protease inhibitor function.

Among the gene ontology (GO) pathways significantly enriched (Fig15:D) in adults, the mismatch repair pathway containing (LOC111332660) a predicted mutS protein homolog 4-like that was overexpressed in (A4) and (S1S2) was reminiscent of (Bhattacharya et al. 2016) where they described a basal metazoan conserved gene repair such as mutS proteins. This specific transcript was not part of their phylogenetic analyses but it could be a good candidate to explain the coral telomere length maintenance with age, as it was overexpressed in the 4 years old colony (A4) and adult ones (S1 and S2). What was automatically described as a centrosome associated zinc finger protein (MDN1/LOC111328456) was also progressively more expressed with age and could be involved in genome stability.

A predicted platelet-derived growth factor receptor alpha-like (PDGFRa/LOC111346134) was also progressively overexpressed with age and in humans it has been shown that TRF2 is able to act as a positive transcription factor of PDGFRb (El Maï et al. 2014). However, in this case PDGFRa is overexpressed when telomeres are longer, potentially sequestering coral TRF protein at telomeres, nevertheless investigating ITSs that would allow TRF binding in the (LOC111346134) environment could be interesting to undermine the conserved mechanism of this gene family transcription regulation by shelterin.

## 2.2. Discussion

Overall, we have shown that in the coral *S. pistillata* T2L was not decreasing with age and that 35 genes were differentially expressed with age setting the maturation age of colonies between 2 years old and four years old. We found that a mutS mismatch DNA repair coding gene was overexpressed in the colonies with longer T2L and could be involved in an age dependent TL maintenance. We also identified an age dependent DEG involved in the synaptonemal complex formation SYCE2 that was overexpressed in the 4 yo colony and in the older adults compared to younger colonies. Moreover when we compared only the 4 yo colony to the older adults we found significantly over expressed (LOC111321815/Piwi like protein) and SYCP1 synaptonemal complex protein 1 (LOC111322006 ) in the 4 years old colony. SYCP1 was already described as a highly conserved gene among metazoans (Fraune et al. 2013), altogether it suggests a sexual maturation of *S. pistillata* between 2 years and 4 years after settlement. We believe that such genes could be good candidates to investigate and label the germ line in corals.

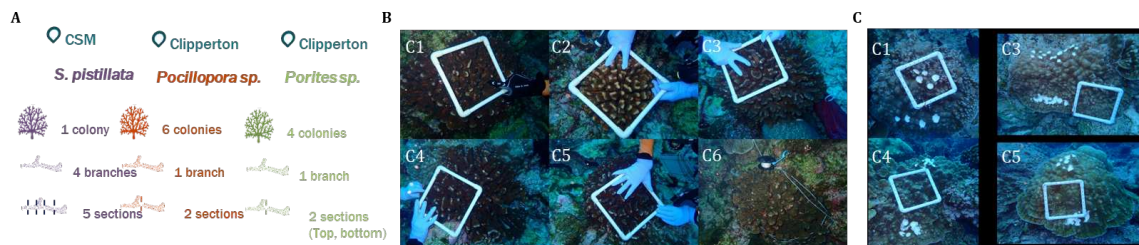
We did not find evidence of ageing in the studied colonies, however some reports (Rinkevich and Loya 1986) of *Stylophora pistillata* colonies, looking senescent, exist and we cannot exclude that ageing mechanism can take place later in life of corals colonies or upon stress condition.

To gain more insight on the TL dynamics within the colony structure and relatively to the growing hot spots, we investigated the TL along a branch.

### 3. Telomere length along a branch

#### 3.1. Introduction

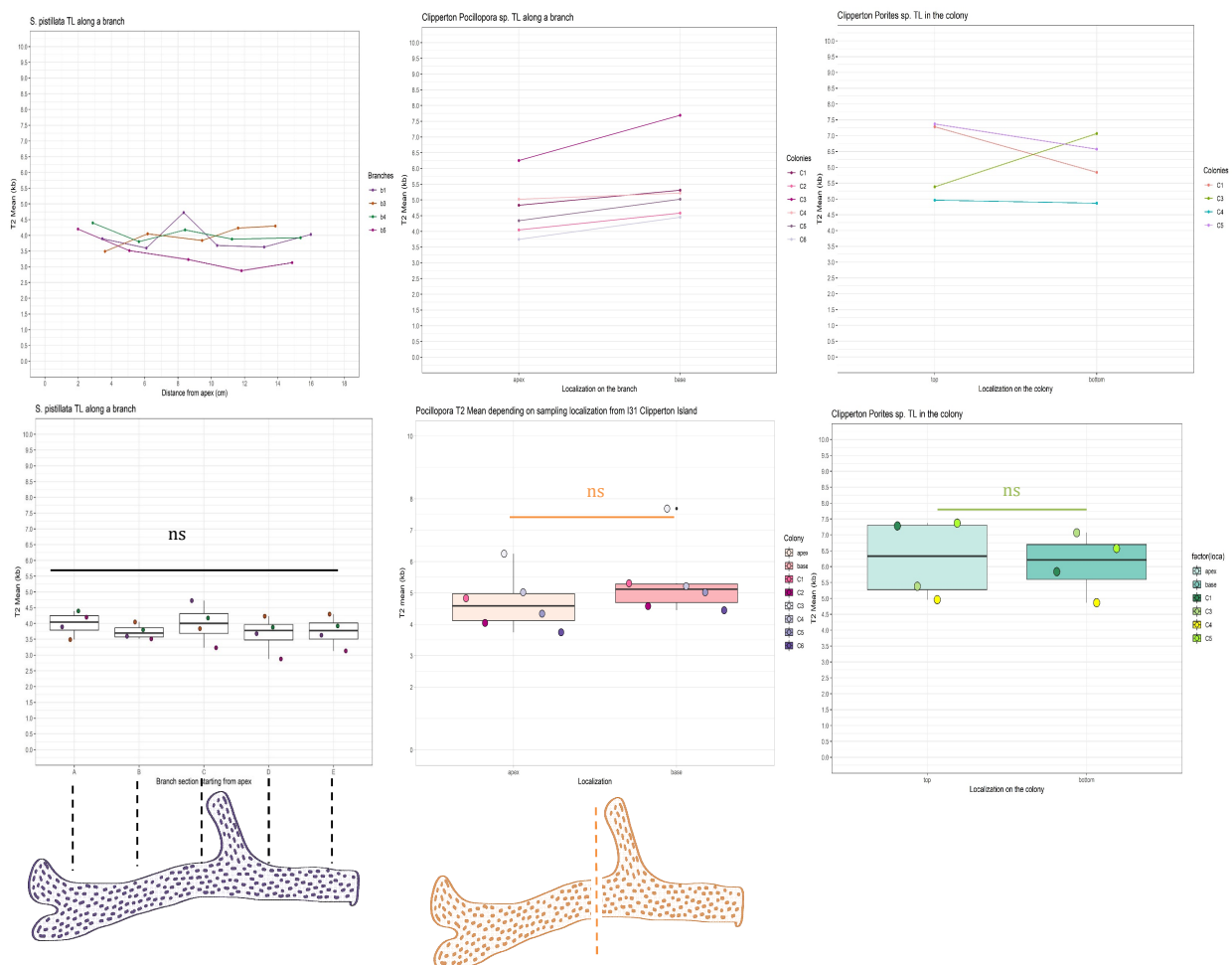
Linear extension in branching coral takes place at the tip of a branch which was raising the concern of an impact of growth rate and proliferation on measuring TL from this area. Moreover, the absence of clear evidence on cells involved in colonial growth, or asexual reproduction, as discussed in (Chapter I. 2.3) led us to ask the question of a differential ageing along a branch. To test whether formed polyps were having a different TL than the ones at the bottom of a branch we measured the TL in different sections of a branch.



**Figure 20: Experimental set up for branches section sampling in *S. pistillata* from Centre Scientifique de Monaco (CSM), *Pocillopora sp.* and *Porites sp.* from Clipperton.** (A) Experimental design for each of the species, with the number of colonies investigated and branches per colony and section per branches. (B) Pictures of *Pocillopora sp.* colonies labelled with their numbers (C1, etc..)(credits : Alexis Pey). (C) Pictures of *Porites sp.* colonies (credits : Alexis Pey).

### 3.2. Results

Four branches of a colony of *Stylophora pistillata* from the CSM were sampled and cut into 5 sections of (~2 cm) (Fig20:A). DNA was extracted from each section and TL was measured using TRF assay. Six colonies (Fig 20:B) of *Pocillopora* sp. from Clipperton island were sampled and cut into 2 sections, the tip or apex, and the base of the branch. Finally, four colonies (Fig 20:C) of *Porites* sp. from Clipperton island were sampled in the middle or top part of the colony, corresponding to the tip or apex, and at the border or bottom of the colony, corresponding to the base.



**Figure 21 : Mean T2L along a branch.** (A) Mean T2L in individual branches of *S. pistillata* (B) Boxplot of mean T2L of branches segments of *S. pistillata*. (C) Mean T2L in individual branches of *Pocillopora* sp. (D) Boxplot of mean T2L of branches segments of *Pocillopora* sp. (E) Mean T2L in individual branches of *Porites* sp. (F) Boxplot of mean T2L of branches segments of *Porites* sp. (ns= not significant)

### Telomere length does not increase along a branch

We were able to observe that neither *S. pistillata*, *P. lobata* nor *Pocillopora* sp. (Fig 21:B,C,E) have different TL regarding the sample position on a colony branch (Wilcoxon test,  $p>0.05$ ). In *S. pistillata* and *P. lobata* branches were having different behaviors with some having shorter TL at the tip of the branch compared to the base, some were flat and others have longer telomere at the tip rather than at the base.

In *P. meandrina* somehow this phenomenon wasn't observed and branches exhibit a similar pattern with shorter mean telomeres at the apex of a branch, which is a growing area, rather than at the bottom of it. They found similar results in *Acropora hyacinthus* (Hiroki Tsuta et al. 2014) with colonies having longer TL at the center rather than at the border of the colony.

### 3.3. Discussion

We looked at TL along branches of 3 coral species to see if TL was decreasing along the branch as it could have been expected if polyps were arising from stem cells with longer telomeres and if the newly formed polyp was therefore having longer telomeres. This linear ageing within a colony that would go along with the colony extension was discussed in (Bythell, Brown, and Kirkwood 2018) where they propose a model of cell proliferation pattern in coral growth based on (Lough and Cooper 2011) and (Lough and Cooper 2011; Darke and Barnes 1993). But no evidence of such mechanisms could be found at the telomere level in our results and on the contrary an increasing trend was observed in *Pocillopora* sp., yet non significant.

## 4. Discussion and conclusion

In this chapter we found that even though corals were sharing telomeric genomic similarities with human, having interstitial telomeric sequence in genes related to the same function of extracellular sensing and extracellular matrix, they were not showing a significantly decrease of TL with age or size. Moreover, the coral *S. pistillata* was exhibiting longer telomeres in older colonies. These differences were related to DEG involved in metabolism and DNA mismatch repair such as a mutS protein like.

But mean TL had been measured on fragments coming from the tip of colony branches and those areas are the growing tips of colonies' linear extension. This raised a concern on the bias it could involve in TL measurements as well as the probability of a branch TL gradient that would correspond to individual polyp age if they were to arise from stem cells with long telomeres. We didn't find differences in TL along branches for neither of the 3 species investigated (*S. pistillata*, *P. meandrina*, *P. lobata*).

In order to understand if external stressors and environment could impact coral TL maintenance that was shown to be robust against time, we investigated TL in corals put under several abiotic challenging conditions.

# Chapter IV-Impact of abiotic factor on coral telomere length

## 1. Acidification

### 1.1. Introduction

Atmospheric CO<sub>2</sub> dissolved in seawater into dissolved inorganic carbon (DIC), such as carbonates (CO<sub>3</sub><sup>2-</sup>), bicarbonates (HCO<sub>3</sub><sup>-</sup>) or carbonate acid (H<sub>2</sub>CO<sub>3</sub>). An increase of dissolved CO<sub>2</sub> results in a decrease of seawater pH, a phenomenon referred to as ocean acidification. Coral skeleton is an aragonite calcium carbonate structure (CaCO<sub>3</sub>) produced by their specialized epithelium, calicoderms, from the seawater dissolved DIC that they concentrate into a calcifying fluid from which they keep a lower pH than the surrounding water to optimize carbonates precipitation (McCulloch et al. 2012; Alexander A. Venn et al. 2013; S. Tambutté et al. 2011).

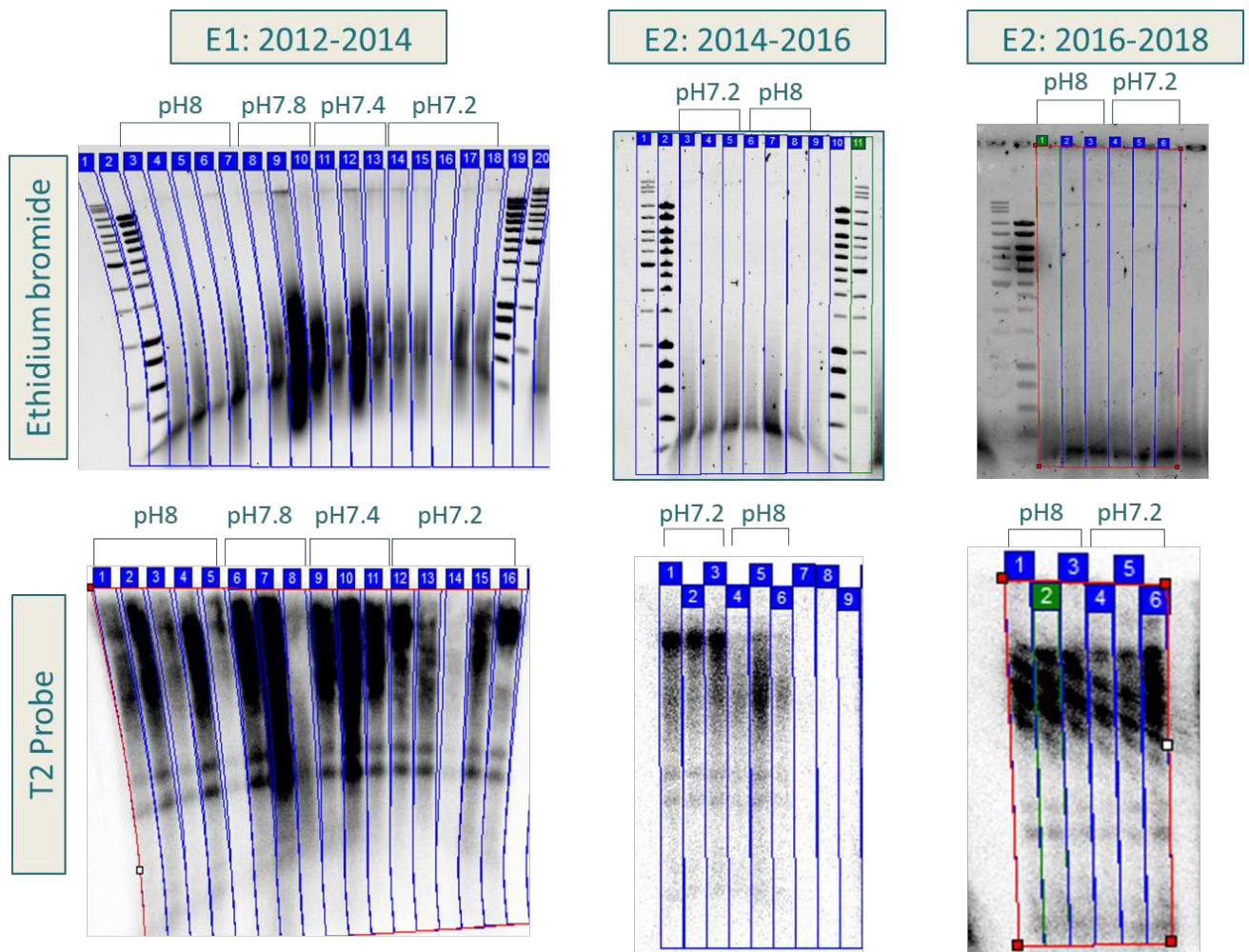
Decrease of seawater pH is resulting in a decrease of the aragonite saturation state. Aragonite saturation state ( $\Omega$ ) measures the level of solubility of the calcium carbonate structure, the higher it the more stable aragonite is in its solid form, when it falls below 1 aragonite structures starts to dissolve.

The increasing sea temperature and ocean anthropogenic CO<sub>2</sub> uptake is leading to a global ocean pH decrease, raising more and more concerns about the fate of coral reefs (Gattuso, Allemand, and Frankignoulle 1999). Several studies have reported a decrease in coral calcification and growth upon seawater acidification in an array of species, some being more sensitive than others. Two parameters are defining coral growth, the skeleton density and the extension. *Pocillopora* sp., *Porites* sp. and *Stylophora pistillata* seems rather resistant in terms of extension when placed in acidified conditions, (Bernardet et al. 2019; A. A. Venn et al. 2019; Comeau et al. 2019; Mollica et al. 2018) while calcification of *Acropora* sp. decrease with acidification (Comeau et al. 2019). Nevertheless, the impact of ocean acidification on telomere length regulation is until now undocumented. Here we analyzed the *Stylophora pistillata* samples from a 2 years acidification experiments performed to unravel the epigenetic mechanism and footprints of acidification (Liew et al. 2018) and samples from *Acropora* sp. coral transplantation on a naturally acidified environment in Palau Rock islands.

## 1.2. Results

### Impact of 2 years in acidified controlled conditions on *Stylophora pistillata* TL

Taking advantages of previous experiments conducted in the Centre Scientifique de Monaco (CSM) by (Liew et al. 2018) we measured the telomere length by TRF of *S.pistillata* samples that were held for 2 years in a control condition (pH 8) and acidified conditions (pH 7.8, pH 7.4, pH 7.2). Three independent experiment were conducted, the first one took place from (2012-2014) under 4 pH conditions, the second one was performed between (2014-2016) under two pH conditions (control and pH 7.2), both of those experiments were analyzed in the study from (Liew et al. 2018), a third experiment (2016-2018) testing two pH conditions (control and pH 7.2) was added to this study to confirm the results

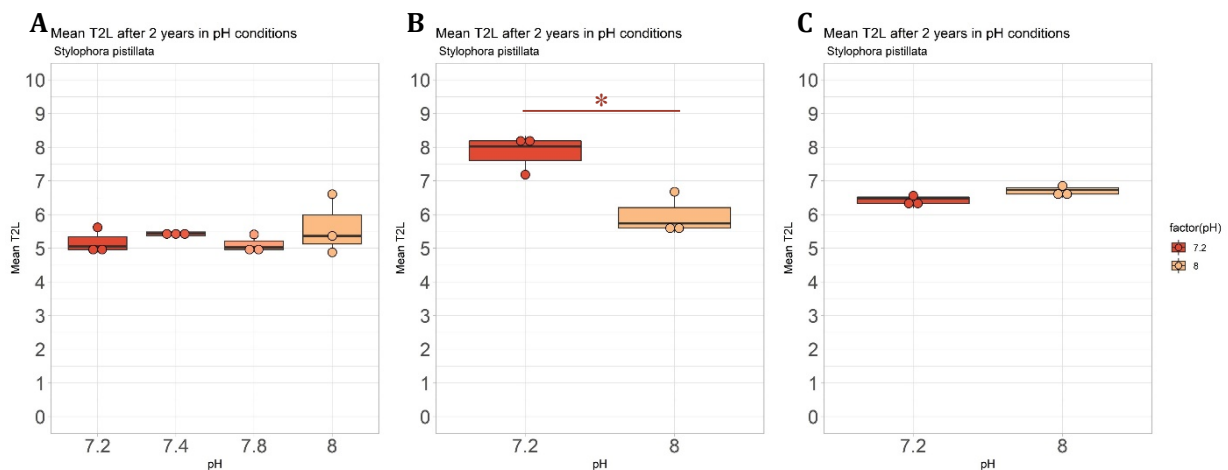


**Figure 22 : *Stylophora pistillata* TRF measurements in pH conditions.** Left panel shows TFR results for samples extracted from the first experiment (E1) after a 2 years exposure to 4 pH conditions (pH8, pH7.8, pH7.4 and pH7.2) between 2012 and 2014, (E2) experiment 2 was carried in 2 pH conditions (pH8 and pH7.2) from (2014-2016) and (E3) experiment 3 from (2016-2018). High panel shows the DNA staining with Ethidium bromide. Two first and last lanes are molecular weight ladders. Low panel shows the membrane stained with host telomere (T2) radioactive probe.

obtained from (Liew et al. 2018) the experiments DNA samples (Fig22).

The analysis of the host mean telomere length (T2L) of the first experiment (2012-2014), (Fig23:A), didn't show a difference between acidic and control condition (pH8) nor for the third experiment (2016-2018), (Fig23:C). We were able to find a significantly longer mean T2L in *S. pistillata* after 2 years exposed to pH7.2 in the second experiment. Taking together these results suggests that exposure to lower pH over 2 years does not significantly impact mean T2L in *Stylophora pistillata* (ANOVA,  $p=0.114$ ) and the lengthening effect observed in the second experiment might be due to undocumented parameters.

Using the RNA sequencing produced from the first experiment samples in the study from (Liew et al. 2018) we analyzed the data to compare the shelterin protein gene expression and telomerase protein subunit (TERT). We found that none of these genes were significantly differentially expressed in any acidified condition compared to the control condition (pH8). This result is in line with the absence of significant telomere length variation between the different pH conditions in the first experiment.

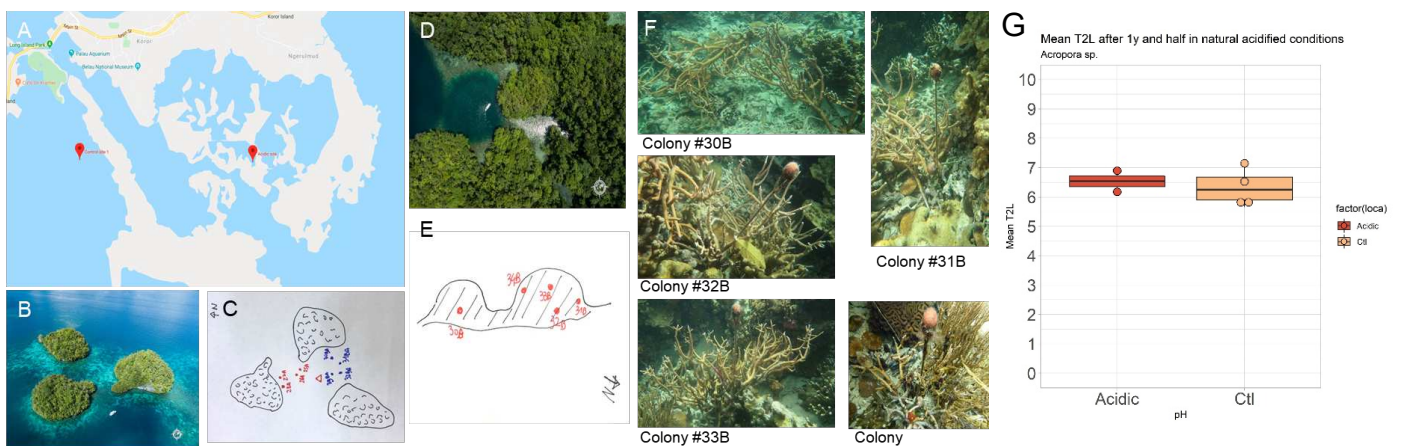


**Figure 23 : *Stylophora pistillata* mean T2L measurements in pH conditions.** (A) Experiment 1, no significant differences were observed after 2 year exposure in the different pH conditions (pH8, 7.8, 7.4 and 7.2), (ANOVA,  $p=0.616$ ). (B) Experiment 2, significant differences were observed after 2 year exposure between (pH8 and 7.2), (student test,  $p=0.0201$ ). (C) Experiment 3, no significant differences were observed after 2 year exposure between (pH8 and 7.2), (student test,  $p=0.1459$ ).

## Impact of 1 year and half in natural acidified conditions on *Acropora* sp. TL

During the TARA-Pacific expedition (Planes et al. 2019) a transplantation experiment was carried out in the Republic of Palau in January 2018. Palau Rock islands are forming small mazes that trap seawater in bays where the local pH can vary between (pH8.1 and pH7.8). This local acidification is an additive effect of water circulation, dilution by rain freshwater, organisms respiration and calcification that all together level down the pH (Shamberger et al. 2014). Six *Acropora* sp. colonies from a site with a control pH (~pH8.1) (Fig24:A,B) were tagged, and half of the colony was transplanted to a naturally acidified site (pH~7.8), Ngermid Bay. Only 5 of those 6 colonies were retrieved 1 year and half later in Ngermid bay (Fig24:C:F), and four in the control site. DNA was extracted on the field and host T2L was measured on the acidified colonies and the mother colonies retrieved from the control site.

No significant differences were observed between *Acropora* sp. colonies from the acidified transplanted site and their control colonies (Fig24:G).



**Figure 24 : Palau sampling.** (A) Google map view of the two sampling sites, control site on the left, acidic site on the right. (B) UAV picture of the control site. (C) Drawing of the control site sampling, control colonies retrieved are in red, newly transplanted colonies in blue. (D) UAV picture of the acidified site of Ngermid Bay. (credits : Richard Brooke). (E) Drawing of the acidified site sampling. (F) Pictures of the transplanted *Acropora* sp. colonies with their identification number (#). (G) *Acropora* sp. mean T2L after 1 year and half in natural acidified conditions of Ngermid Bay, Palau Republic. No significant difference was observed between the control colonies (Ctl) and their transplanted half in an acidified bay after 1 year and a half

### 1.3. Discussion

Here we reported for the first time the monitoring of telomere length in two coral species (*Stylophora pistillata* and *Acropora sp.*) in long term experiments (2 years and 1 year and half) in controlled and natural acidified conditions. We were not able to detect significant differences in mean T2L in either of the conditions nor differences in shelterin proteins and telomerase expression in *Stylophora pistillata*.

In the *Stylophora pistillata* samples it was shown in (Liew et al. 2018) that long term exposure to low pH was increasing the skeleton porosity and impairing coral growth but surprisingly it was also increasing the polyp cell sizes as well as the size of the calyxes. In acidic conditions they observed that *S. pistillata* was growing less polyps but bigger ones due to bigger cell size. Indeed, they identified differences in genome methylation and gene expression linked to cell growth and proliferation and more specifically to the MAPK pathways towards a delay in cell division in acidified conditions. This decrease in cell proliferation over cell growth could have favored an lesser decrease of TL than in control conditions. Mostly knowing that a decrease in extracellular pH can favor short TL lengthening (Ge et al. 2016). Yet we could not see a significant difference in TL in low pH conditions in two of the three experiments. Investigating short TL with more sensitive methods than TRF could allow us to maybe see an effect of acidification on a class of telomeres.

## 2. Temperature

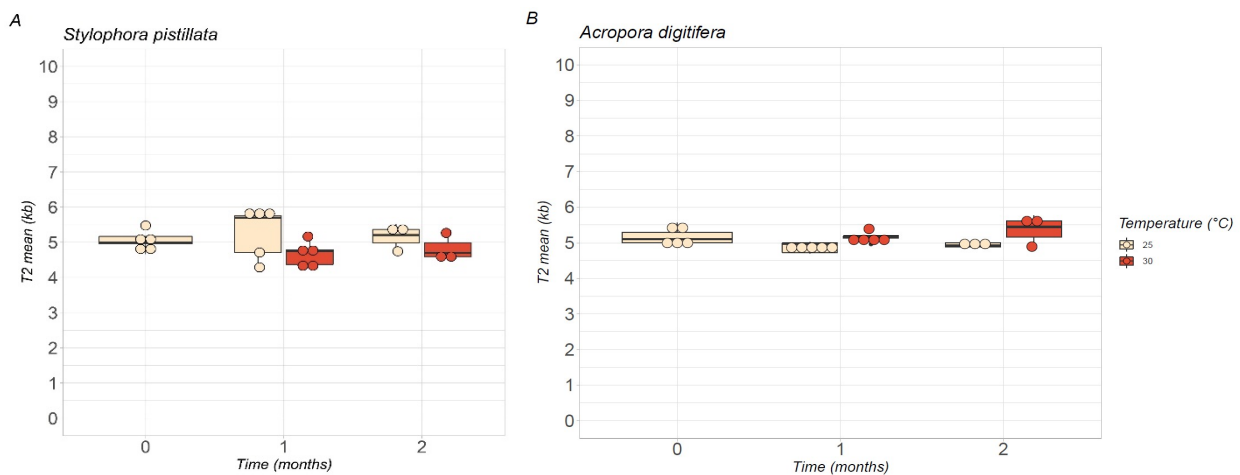
### 2.1. Introduction

Not only age can affect TL but also stress from the environment can accelerate telomeres attrition or sometimes lengthen them (Epel et al. 2004; G. H. Romano et al. 2013). Coral reefs are worldwide under the threat of global climate change (+0.74°C and -0.1pH in the last centuries) that is predicted to induce heat stress, pH stress, eventually leading to bleaching. Massive bleaching events are happening threatening the ecosystem built by coral reefs (Hughes et al. 2018). To better understand how future environmental change will affect coral physiology and ageing I induced different stress in control condition to *S. pistillata*.

## 2.2. Results

To test whether temperature could have an effect on coral TL in controlled conditions we measured before the experiment (T0) the telomere length from 5 branches of a colony that was then split in 2 groups, a control one which remains at 25°C all along the experiment, and a heat stress one that was put at 30°C, in two corals *S.pistillata* and *Acropora digitifera*. Branches were sampled after 1 month (5 branches per condition) and 2 months (3 branches per condition) in treatment or control condition. DNA was extracted and telomere length measured with TRF.

For *S. pistillata*, (Fig25:A) we were able to observe a significant decrease of mean T2L in increased temperature condition (30°C), (ANOVA,  $p=0.0498$ ), while neither time nor the interaction of time and temperature on T2L was significant. The same result was found for *Acropora digitifera*, (Fig25:B) (ANOVA,  $p=0.0121$ ) with only a linear relationship between mean T2L and temperature rather than time or mixed effect of time and temperature. Although in both cases differences between control group and treated group seemed to increase with time.



**Figure 25 : Impact of temperature on coral mean T2L. (A) *Stylophora pistillata* mean T2L before experiment (t0), after a month (t1) in control (25°C) and heat stress condition (30°C) or 2 months (t2). (B) *Acropora digitifera* mean T2L in the same settings.**

## 2.3. Discussion

Increased temperature exposure (30°C) affected mean T2L in opposite ways for *S. pistillata* and *A. digitifera*, inducing a decrease in the first one and a slight increase in the second species. As discussed in (ChapterI.2.4) *S. pistillata* strain from the CSM optimal calcification temperature is 29°C (Bernardet et al. 2019) but the calcification performance at 30°C remains high and noteworthy, higher than at 25°C. Therefore a higher calcification rate could be responsible for a faster growth and a decrease in TL. On the other hand *Acropora digitifera* have most likely a lower temperature threshold range, sampled at 30°C after 3 months were beginning to bleach, but the high temperature exposure is more likely to trigger a lengthening of telomeres, as they are longer than at the beginning (t0), rather than not decreasing them in lowering cell proliferation and growth.

Nevertheless the lack of duplication as well as the small absolute difference in T2L between control groups and treated groups encourage us to take this result with caution. It could be interesting to apply temperature variations to see if the amplitude of temperature variation could be more impacting than a constant temperature.

## 3. Bleaching

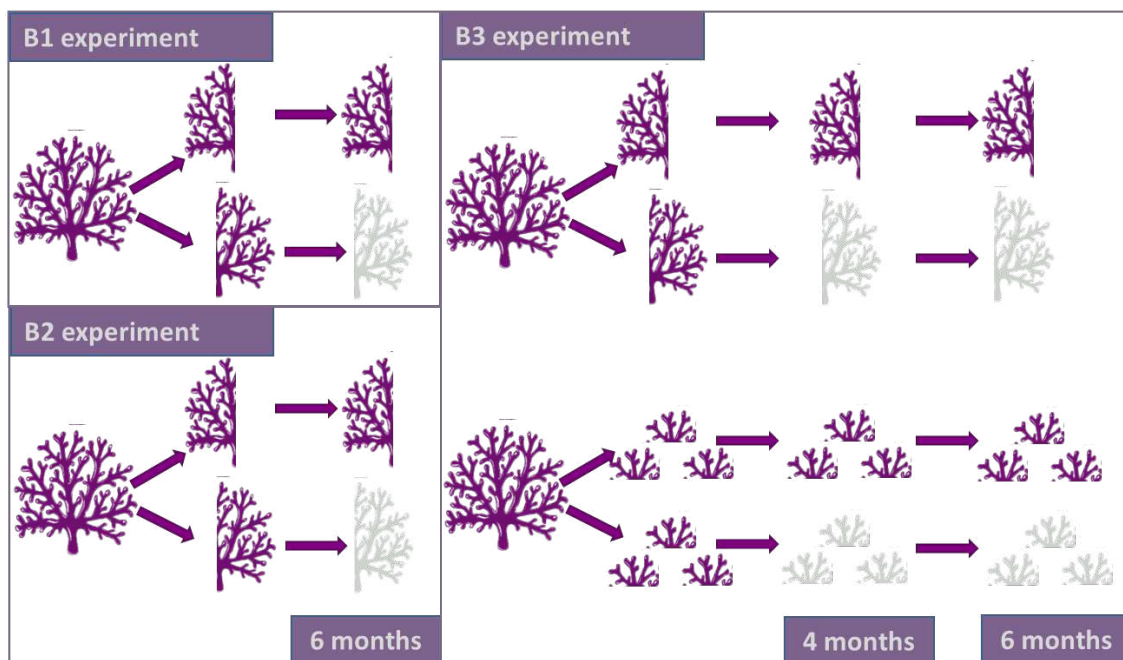
### 3.1. Introduction

Coral bleaching results from the breakage of the symbiosis and can be triggered by environmental parameters or pathogens infection. Coral bleaching is often induced by a heat stress and most of the study had been focusing on heat stress coral bleaching reporting transcriptomic signature for *Acropora sp.*, *Orbicella sp.* (Seneca and Palumbi 2015; Traylor-Knowles et al. 2017; Zhou et al. 2017; Pinzón et al. 2015). They show a positive expression of Tumor Necrosis Factor Receptor-associated Factor 3 gene (TRAF3), stress related genes and immune system related pathways with bleaching. But coral exposure to stress is having an impact on TL maintenance as shown (ChapterIV-2), therefore to separate the effect of heat stress by itself and symbiosis breakage we performed a dark induced bleaching on *S. pistillata*.

### 3.2. Results

#### Dark induced bleaching settings

To dissociate temperature stress, that has an effect on TL in *S. pistillata*, from the loss of symbionts *per se*, we induced bleaching by putting the colony in constant dark condition. Therefore, we will be considering the following results in dark-induced bleaching cases. Three experiments were carried out (Fig26), referred to as (B1, B2, B3), the first one that was meant to be a test was only performed on one branch of a bleached colony and a control colony, time since bleaching was unfortunately unrecorded. Second experiment (B2) was carried out in the same colony cut in two and 5 branches were sampled after 6 months in dark condition for the bleaching half and control conditions for the control half. The RNAsequencing was performed on samples from experiment B2. Experiment 3 (B3) was performed in parallel on a colony cut in half and sampled after 4 months and 6 months in dark-induced bleaching or control conditions and in branches.

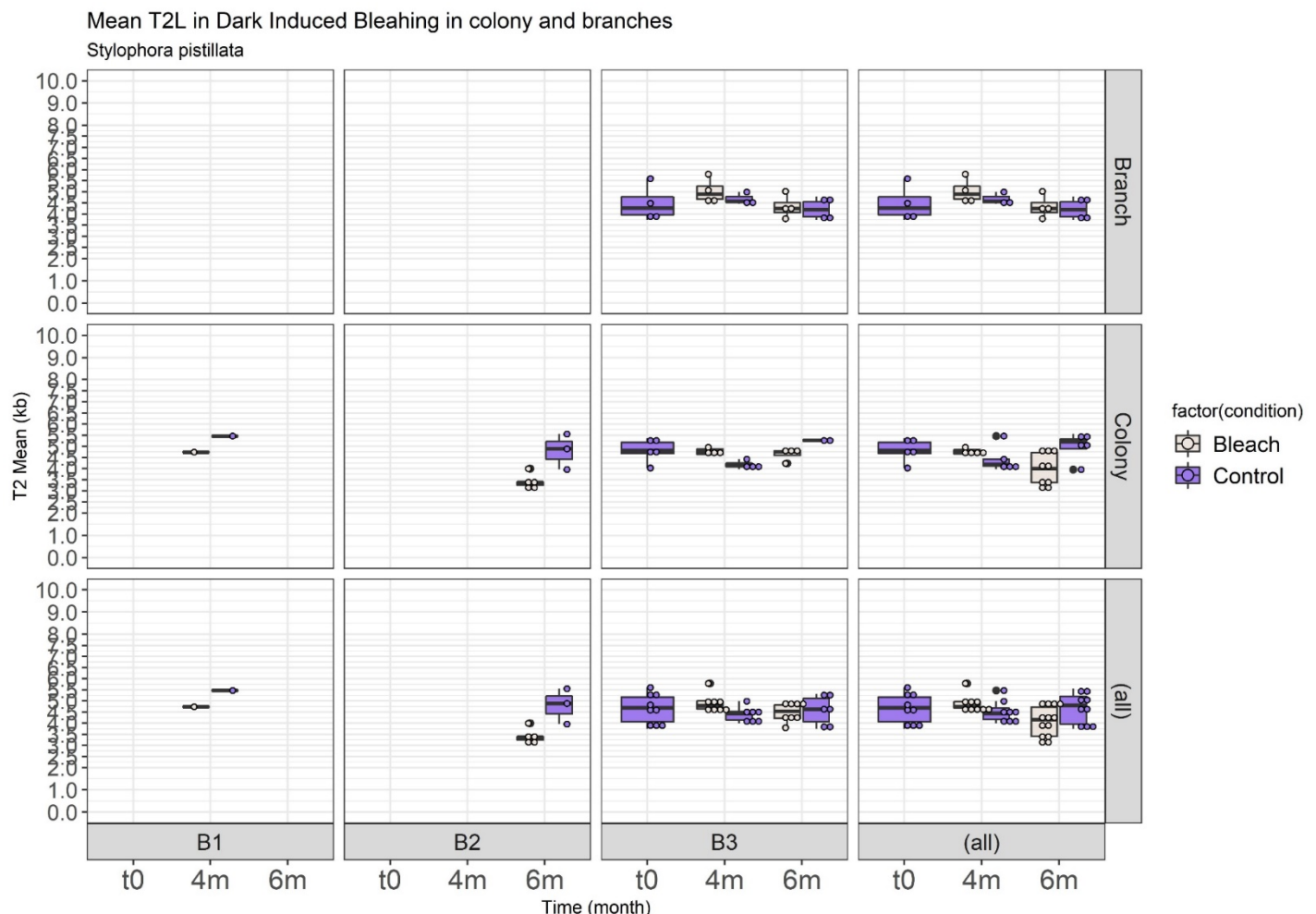


**Figure 26: *Stylophora pistillata* bleaching experiment set up.** Bleaching experiment 1 (B1) was performed on one branches of a bleached colony and a control one. Bleaching experiment 2 (B2) sampling was performed on 5 branches of a control dark-induced bleached colony since 6 months and its clonal control colony. Bleaching experiment 3 (B3) sampling was performed after 4 months and 6 months of bleaching on dark-induced bleached colony and its clonal control half, and in dark induced bleached branches and their clonal control branches.

## Telomere length in bleached samples

The first experiment (B1) showed a decrease of TL in bleached samples compared to control colonies, however only one branch per colony was tested (Fig27).

The B2 experiment showed a 2kb decrease in the average of the 6 branches mean TL in the colony that was bleached since 6 months compared to the control colony. In the B3 experiment in the 'Colony' setting one colony was cut in half and isolated in dark conditions. It seems that the cut of the colony had an impact on the control colonies as it had shorter TL 4 months in control conditions after the injury, yet after 6 months in control condition its TL was not different than at t0. For the bleached half of the colony the TL was slowly decreasing from t0 to 4 months and 6 months after.



**Figure 27 : Boxplot of *Stylophora pistillata* bleaching experiment mean T2L results.** Results are displayed at the vertical by experiments (B1,B2, B3) and horizontal by experimental set up (separated colonies or branches). Mean T2L is displayed in kb on the y axis why samples are dissociated depending on the time spend in dark condition (t0, 4 months, 6 months). Bleached (white) and control (purple) branches are represented as dots displayed over the boxplots. The last panel is representing all the results together, upper panel is all the branches results together, the middle panel is all the colonies results together, down panel is all the experiment and experimental setup together. Overall the combination of time and condition (bleached control) as well as the experiment were significantly explaining the samples variance (ANOVA,  $p=0.0159$  and  $p=0.0479$ )

After 4 months TL were shorter in the control condition than in the bleached one, but after 6 months the bleached half colony had a shorter TL than the control half. In the 'branch' setting, where branches were cut at t0 and hung in the different conditions, TL was not different between the treatments and both control and bleached branches had longer TL after 4 months and the same TL than at the beginning after 6 months (Fig27).

Finally taking all together the experiment (ANOVA,  $p = 0.00658$ ) and the mixed effect of time and treatment (ANOVA,  $p = 0.01587$ ) were significantly explaining the mean TL variance observed (Fig27).

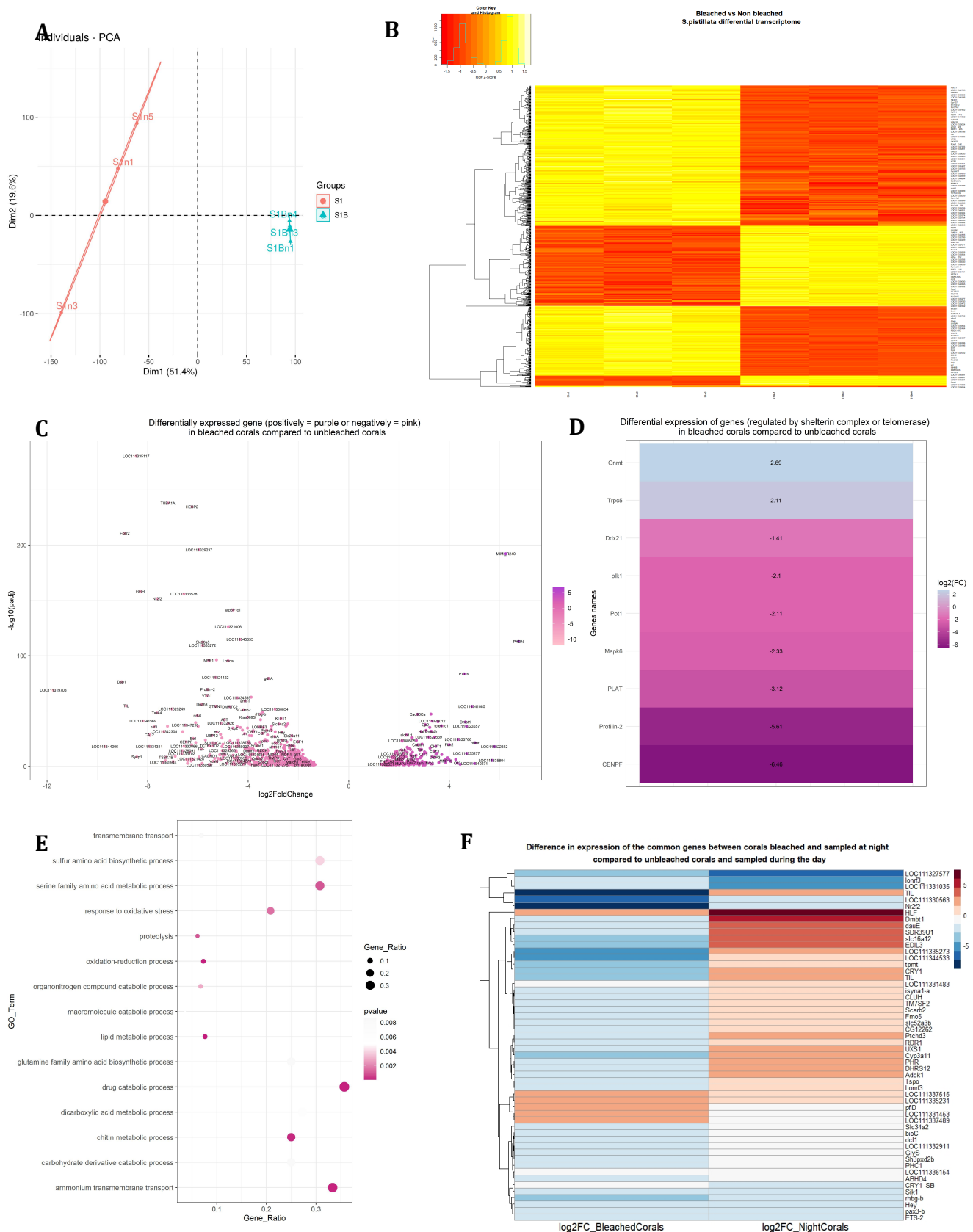
#### RNAseq analysis of dark induced bleached *S. pistillata*

The transcriptomic results were consistent between branches of bleached samples (Fig28:A), in the control colony (the other half kept in control condition), one branch was showing a slightly different transcriptome (S1n3). Nevertheless, we were able to identify significantly differentially expressed genes ( $p < 0.05$ ) in B2 experiment bleached samples (3 branches) (Fig28:B). We found more significantly downregulated genes in bleached samples than upregulated ones (Fig26:C), among them we isolated genes that were encoding for shelterin proteins, telomerase TERT or that were reported to interact with some of these proteins.

We found 9 genes significantly differentially expressed, 2 upregulated and 7 downregulated. One of the two upregulated gene (LOC111338256) was predicted to be Glycine-N methyltransferase involved in DNA methylation, changes of methylation state upon dark-induced bleaching stress could be consistent with what was observed in methylome changes in *S. pistillata* upon acidification stress (Liew et al. 2018). Among the downregulated genes some are known to be involved in cellular growth process and tissue remodeling (CENPF, PLK1, Profilin-2, MAPK6, PLAT) as well as immunity (MAPK6, DDX21). Similarly to what they observed in (Liew et al. 2018) with the inhibition of MAPK pathways genes by gene methylation, we found a downregulation of a specific MAPK6. Yet this specific MAPK6 wasn't downregulated in the acidified transcriptome. Most of the gene ontology term (GO-Term) significantly ( $p < 0.01$ ) enriched in the bleached samples were related to metabolism, protein homeostasis and biosynthesis and oxidative stress (GO:0006979, GO:0055114) (Fig26E). Interestingly oxidative stress has been shown to trigger bleaching in heat stress conditions due to symbionts ROS production, however in this case the coral colony was in dark condition for 6 months even in the case of some cryptic symbionts remaining which is unlikely after such a long time in darkness, the absence of light prevents photosynthesis and O<sub>2</sub> production. Therefore, the ROS stress has to be produced by another mechanism.

Moreover POT1, a shelterin protein protecting telomeres, is downregulated in bleached samples that are also samples exhibiting significantly shorter TL (Fig28:B). Finally we compared our results to previous transcriptome study performed on *S. pistillata* colonies during day and night cycles (Ottaviani et al. 2020) to find

differentially expressed genes that could be more influenced by the dark condition rather than the bleaching condition (Fig28:F). We found 54 genes in common between the two differentially expressed sets of genes, some were downregulated in both transcriptomic analysis like the gene encoding for Hey1 that was shown to have an anti-senescence when expressed in human fibroblast (Ottaviani et al. 2020). However most of the genes were differentially regulated between the two dataset but the associated functions of some common genes to light sensing, like CRY1, involved in the circadian clock, made us considered these genes were controlled by the dark condition rather than the bleaching state of coral.



**Figure 28 : *Stylophora pistillata* bleaching RNA sequencing results.** (A) Individual Principal Component Analysis (PCA) of the RNA sequencing of 3 branches (n=nubbins) of control *S. pistillata* colony S1 and dark induced bleached colony (S1B). (B) Heatmap of differentially expressed genes in control conditions samples (S1n1, n3, n5) and dark induced bleach condition (S1Bn1, n3, n4). (C) Volcano plot of significantly differentially expressed genes in bleached condition ( $p\text{-value} < 0.05$ ). (B1) was performed on one branches of a bleached colony and a control one. (D) Significant differential expression expressed in Log2(FoldChange) of telomere related genes (Gene names) in bleached condition ( $p < 0.05$ ). (E) Significant enriched Gene Ontology term (GO Term) ( $p < 0.01$ ) in bleached condition, the gene ratio correspond to the number of significant genes divided by the annotated ones. (F) Common differentially expressed gene, log2(fold change) is displayed with colors, in bleached transcriptome and in night and day transcriptome from (Ottaviani et al., 2020)

### 3.3. Discussion

We have noted that dark-induced bleaching was having an impact on colonies of *S. pistillata* mean TL after 6 months but not when the experiment was carried out on previously cut and separated branches. We also witnessed a change in gene expression linked to metabolism and oxidative stress response. Moreover we reported a significant underexpression of protection of telomere gene (POT1) in the bleached samples that were exhibiting shorter TL than the controls.

All together it suggests a synergic effect at the colony level of bleaching that could slowly decrease TL maintenance with the first impact being measurable after 6 months by decreasing POT1 expression.

However *in situ* bleached colonies would never reach this point to face the long term effects on TL. We tried to rescue the phenotype of the experiment 3 (B3) by putting back branches and the half colony in control conditions. Unfortunately the bleached colony didn't survive the change of treatment nor did the branches. It would be of interest to research if switching between bleach and control treatment would have an effect on TL in a shorter time scale to mimic natural conditions.

## 4. Discussion and conclusion

Overall we have seen that corals are able to maintain their TL over a range of stressors and time scale. Impressively the cross study of acidification impact on TL in a 2 years treatment in 2 species showed no significant differences in TL even though acidification condition was impacting the cellular proliferation and cellular growth regulation both at the epigenetic and transcriptional level.

Temperature seems to have an overall significant impact after a 3 months treatment but the short time scale and the absence of duplication requires further investigation to conclude.

The dark-induced bleaching was from far the treatment that showed the most impact on TL, however the duplication experiment conducted in two parallel settings revealed partially reproducible results.

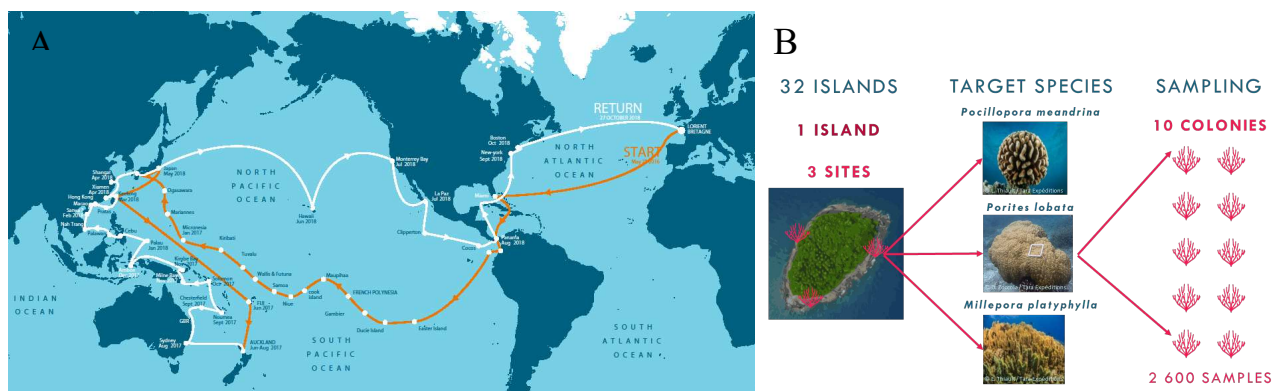
Nonetheless the DEG observed in the bleached samples with significantly shorter TL revealed an overexpression of oxidative stress response genes and a downregulation of POT1 expression that would explain the TL shortening of those samples.

# Chapter V-Telomere length dynamics in wild populations of 3 coral genera

## 1. Introduction

The TARA-Pacific expedition aimed to unravel the still largely unknown world of coral associated bacteria, viruses, Symbiodiniaceae and other protists, forming a complex symbiocosm refer to as ‘holobiont’ and to generate a nearly exhaustive morpho-molecular and environmental inventory to describe the coral interactome at an unprecedented ecological scale in 3 different coral genera (Planes et al. 2019).

The sampling that targeted 3 coral species (*Porites lobata*, *Pocillopora meandrina*, *Millepora platyphylla*), over 32 island systems, aimed to cover a large diversity of environments within the Pacific Ocean as well as within coral reef building species. Around each of the 32 islands, three sites were sampled, collecting 10 colonies of each target species and five to ten individuals of each fish species, as well as surrounding water samples (Fig29:A,B).



**Figure 29 : TARA-Pacific expedition.** (A) Road map of the TARA-Pacific expedition (2016-2018). In blue the first year sailing road, second year in orange. (B) Sampling protocol, with 3 sampling sites per islands. In each sampling site 3 target species were collected (*Porites lobata*, *Pocillopora meandrina* and *Millepora platyphylla*). For each species 10 colonies were sampled per site. The expedition sampled 32 islands, the number of sampled for the coral colonies was (~2600 samples).

The TARA Pacific Consortium aims to describe the morpho-molecular and environmental inventory of the complex coral symbiocosm sampled during the TARA-Pacific expedition. To do so the Consortium gathers multiple French and international laboratories with a broad range of expertises. Among the data generated, metatranscriptomics and metagenomics are performed for 30% of the samples to determine SNPs and differentially expressed genes (Genoscope, CRILOBE, IRCAN) while metabarcoding (18S, 16S) will be performed for 100% of the sampling (Genoscope, ETHZ, Sorbonne Université, Oregon State University, Konstanz University). Size and morphology peculiarities will be extracted from *in situ* pictures and classical biomarkers of stress response (oxidative defense, proteostasis, cell division, apoptosis and DNA damage) are currently evaluated at IRCAN and at Shimoda Marine Station (Tsukuba University). The environmental and contextual data, composed of *in situ* measurements as well as satellites data and models, are used to build a complete description of the past and present conditions at each site of each island (IMEV, University of Maine, MARUM...).

Here we will present our results on the telomere length measurements of the TARA-Pacific expeditions coral samples.

## 2. Paper

**Reef corals with different life-history strategies  
exhibit distinct patterns of telomere length variations  
across the Pacific Ocean**

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## Abstract

The linearity of eukaryotic chromosomes requires the presence of a particular terminal chromatin structure, named telomere, to control genome stability and function. Changes in telomere structure during life can determine longevity, stress resistance and disease susceptibility. Even though the use of telomere length measurement as a method of assessing stress accumulation and predicting how this will influence survival is currently being studied in numerous human cohort studies, the importance of telomere length for stress resilience and life history in ecological studies remains at its infancy. Here, we study the possible links between telomere DNA length variation and environmental stress using natural populations of reef-building coral sampled across the Pacific Ocean at an unprecedented scale. This study is of particular importance in the context of the repeated bleaching events devastating numerous coral reef ecosystems. Specifically we report here the telomere DNA length variations among 1100 colonies of corals belonging to three genera: two scleratinians, *Pocillopora* sp., and *Porites* sp. as well as one hydrozoa *Millepora* sp.. We observed an absence of TL relationship with colony size in the two scleratinian corals harbouring different morphology and life history traits. We found that *Pocillopora* sp. mean TL is constrained both by genetic and environment, identifying key genes underlying this process. While *Porites* sp. has a remarkable ability to maintain its TL regardless of size, genetic and some environment disturbance, its symbionts TL is more variable and sensitive to environmental factors. Importantly, these results uncover important differences in telomere DNA length maintenance pathways between the short-lived and stress-sensitive *Pocillopora* and the long-lived stress resistant *Porites* genera.

## Introduction

Telomeres are nucleoprotein structures, composed of short tandemly repeated DNA sequences and a protective protein complex, the shelterin, capping the ends of linear chromosomes and maintaining genome stability (E. H. Blackburn 2001; Palm and de Lange 2008; Gilson and Géli 2007). In most metazoans, the telomeres are composed of (TTAGGG) $_n$  DNA repeats (Traut et al. 2007) and are shortened at each cell division due to the inability of the conventional replication machinery to fully replicate DNA extremities (Lingner, Cooper, and Cech 1995). This continuous telomere length (TL) shortening can be restored by telomerase, a ribonucleotide reverse transcriptase, or recombination-based processes (Gilson and Géli 2007). In many, but not all, vertebrates, TL erosion occurs in adult somatic cells as a result of the down-regulation of telomerase programmed at the end of embryogenesis (Wright et al. 1996). When they reach critically short lengths, telomeres become uncapped leading to senescence and/or apoptosis. Thus, somatic TL shortening is a key driver of aging although it may also limit cancer progression (Artandi et al. 2000; Aviv and Shay 2018).

Recent reports have identified telomeres as cellular integrators of various stresses leading to modulations of the TL shortening rate during life (Ye et al. 2014b; Elizabeth H. Blackburn, Epel, and Lin 2015; Schultner et al. 2014; G. H. Romano et al. 2013; Epel et al. 2004; Chatelain, Drobniak, and Szulkin 2020). The extent to which stress-dependent TL variations affects the ability of organisms to adapt to new environments and to age has become a central question (Monaghan and Haussmann 2006). This generated a great interest for telomere studies in the fields of ecology and comparative biology of aging (Gilson and Bosch 2016; Haussmann and Vleck 2002; Quesada et al. 2019; Jebb et al. 2018; Foley et al. 2018).

In this regard, reef building corals are rare models to compare related animals sharing similar habitats and environmental stresses while exhibiting different life history traits, resilience capabilities and lifespan, some of them having extreme longevity (Darling et al. 2012; Bythell, Brown, and Kirkwood 2018). Moreover, the sessile life mode of corals prevents them from escaping adverse conditions is an interesting trait to study the effects of past stresses on nowadays telomere structure.

Reef building corals, belonging to Anthozoa, a class of Cnidaria, are usually colonial organisms formed by many individual polyps connected by a stolon (Zapata et al. 2015; Kayal et al., n.d.). Reef building corals are able to secrete a calcareous skeleton through a specialized epithelium, a process that requires a great amount of energy uneasily provided by predation effort in oligotrophic tropical and sub-tropical waters (S. Tambutté et al. 2011). This nutrient resource is provided by a mandatory symbiosis based on unicellular photosynthetic algae belonging to the Symbiodiniaceae family and hosted inside the animal endodermal cells (S. Tambutté et al. 2011; T. C. LaJeunesse et al. 2018). This photosynthetic activity places the animal cells under a massive oxidative stress on a daily basis (M. P. Lesser et al. 1990; Shick et al. 1996) that is overcome by robust anti-oxidant defenses developed during coral evolution (Furla et al. 2005). Noteworthy, since oxidative stress can lead to TL shortening (Furla et al. 2005; Barnes, Fouquerel, and Opresko 2019), coral may have developed efficient mechanisms of telomere resistance to oxidative stress although this was not explored in coral yet. Nevertheless, upon acute stress, such as temperature increase, symbionts Reactive Oxygen Species (ROS) production reaches a level that overcomes host defense leading to symbiosis breakage, a phenomenon known as bleaching (Michael P. Lesser and Farrell 2004; Courtial et al. 2017). Extended time in a bleaching state will result in coral death and the repeated massive bleaching events are a threat to the whole coral reef ecosystem (van Woesik et al. 2018; Hughes et al. 2018).

Few studies investigated telomeres in corals. The telomeres of the animal cells have the same DNA repeat sequence than in human (TTAGGG)<sub>n</sub>, while the telomeres of the unicellular symbiotic algae are composed of the DNA repeats of higher plants (TTTAGGG)<sub>n</sub> (Zielke and Bodnar 2010). In the studied species, the coral animal TLs ranged from 4 kb to 20 kb (H. Tsuta and Hidaka 2013; Sinclair, Richmond, and Ostrander 2007; Bythell, Brown, and Kirkwood 2018). The lack of correlation between TL and the weight of adult individuals of the solitary coral *Ctenactis echinata* was previously described (Ojimi et al., 2012) and besides (Hiroki Tsuta et al. 2014) that reported TL shortening decrease in *Acropora digitifera* during development from sperm, planula and larvae to adult colonies, no clear evidence exists for TL shortening during coral ageing.

In order to investigate whether peculiarities in telomere maintenance mechanisms play a role in coral life, aging and stress response, we studied natural TL variations between corals with different life history strategies. To this purpose, we took advantage of the Tara-Pacific expedition that sampled three corals genera around 32 islands across the Pacific Oceans and of the Tara Pacific consortium that generated a nearly exhaustive eco-morpho-molecular inventory of the collected corals (Planes et al. 2019). This allowed us to measure TL of the sampled corals and to correlate their variations with a large set of morphology, genomic, transcriptomic, geographical and environmental parameters. The three targeted coral genera are two scleractinian anthozoans, *Porites* sp. and *Pocillopora* sp. and one hydrozoa, *Millepora* sp. that shares with the scleractinian corals the ability to grow a calcareous skeleton that make them reef builders (Tissier and Le Tissier 1991). *Porites* sp. are massive long lived and stress resistant corals (Stafford-Smith 1993; Darling et al. 2012; Levas et al. 2013; Roff, Zhao, and Mumby 2015), while *Pocillopora* sp. are short lived (Bythell, Brown, and Kirkwood 2018) and quite sensitive to thermal stress, acidification and bleaching (Darling et al. 2012; Courtial et al. 2017; Pogoreutz et al. 2017, 2018; Dias et al. 2019). Our results unveil for the two scleractinian corals an absence of TL shortening with colony size taken as a proxy for aging. We also describe distinct patterns of TL variation among the three targeted genera across the Pacific Ocean revealing that, in contrast to *Pocillopora* sp, the TLs of animal cells of *Porites* sp. are remarkably stable facing different environments. We discuss these results in terms of age-independent telomere maintenance mechanisms allowing the corals to adapt their lifestyle to environmental disturbances.

## Results

In order to measure the telomere DNA length (TL) simultaneously for the coral host and the symbiont, we adapted the Southern blotting procedure (considered as the gold standard technique in telomere field, (Aubert, Hills, and Lansdorp 2012)) to coral tissue samples and performed sequential hybridizations, first with a probe recognizing the symbiont telomeres sequence (TTTAGGG)<sub>n</sub> (named T3, the symbiont TL being abbreviated as T3L) and then with a probe recognizing the host telomeres sequence (TTAGGG)<sub>n</sub> (named the T2 probe, the host TL being abbreviated as T2L) (SupFig4). Beyond mean TL, we calculated several parameters describing the shape of the TL distribution (in particular an eventual skewness toward short or long TLs): median (med), 1st quartile TL limit (Q1), 3rd quartile TL limit (Q3) and interquartile distance (IQ). The terminal localization of the radiolabeled fragments was checked by Bal31 digestion (SupFig1).

We measured T2L and T3L of 1095 coral colonies collected in 32 islands during the Tara-Pacific expedition belonging to three genera: *Pocillopora* (n=444) *Porites* (n=409) and *Millepora* (n=242). For the three genera and whether for T2L or T3L, there are good correlations between the different parameters (mean, median, Q1, Q3 and IQ), which was expected for some of them (e.g. IQ = Q3-Q1) (SupFig2). These measurements reveal the existence of significant TL variations between the colonies of the three genera both for the hosts and the symbionts. Noteworthy, the mean T2L distribution appears tightened up while the T3L variation is widely spread (Fig1F). A different distribution pattern is observed for the hydrozoan *Millepora* sp population with mean T2Ls higher and more widely distributed than the mean T3Ls (Fig1E-G). The range of means T2Ls ( $5.8 \pm 2.4$  kb for *Porites* sp.;  $5.1 \pm 1.0$  kb for *Pocillopora* sp. and  $12.2 \pm 8.1$  kb for *Millepora* sp.) (Fig1G) is consistent with previous reports on coral TL ranging from (~4kb) in *Acropora surculosa* (Sinclair, Richmond, and Ostrander 2007) to (~19.5kb) in *Favia pallida* and *Leptoria phrygia* (Zielke and Bodnar 2010) and (~20kb) in the hydrozoan *Hydra vulgaris* (Traut et al. 2007).

## Telomere DNA length does not correlate with colony size

First, we asked whether the mean T2L variations depend upon colony size, which can be used as a proxy for colony age. This was tested by a dedicated sampling of colonies according to four diameter classes: *Pocillopora* sp and *Porites* sp at Clipperton island (Fig.1 A-C); *Porites* sp at Palau North (Fig.1 B,D). No difference of mean T2L was detected between colonies of different sizes. Moreover, we were unable to detect any global correlation between the T2Ls of the *Pocillopora* sp. samples collected in the 32 islands of the Pacific-Pacific expedition and the different size parameters estimated for the corresponding colonies (Sylvain Agostini unpublished data) (SupFig3).

We also addressed this question by taking advantage of a repeated sampling at roughly two years difference (2016 and 2018) performed at the same diving sites of Coiba island (Fig1: E). Because the colonies were not tagged in 2016, the 2018 sampling was not necessarily performed on the same colonies. Nevertheless, we did not observe differences for the mean T2L of *Pocillopora* sp. ( $p>0.5$ ) nor *Porites* sp. ( $p>0.1$ ) colonies collected in 2016 and in 2018.

We conclude that colony size, and very likely their age, cannot resolve the T2L variation observed for the two populations of scleractinian corals collected during the Tara-Pacific expedition.

## Island effect

The averages of the mean T2Ls and T3Ls calculated per island show significant differences (Fig1H, SupFig6). Variation between sampling sites of a given island appears to be moderate compared to the differences between islands, strengthening the observed island effect (Fig1H, each site being represented by a different shape). The relative average mean TLs between islands appears different for the three genera, suggesting genus-specific determinants of TL regulation. Noteworthy, the similarity between the diving sites around the same island is less frequently observed for T3L than for T2L. This suggests more local variations for the symbiont T3L than for the host T2Ls.

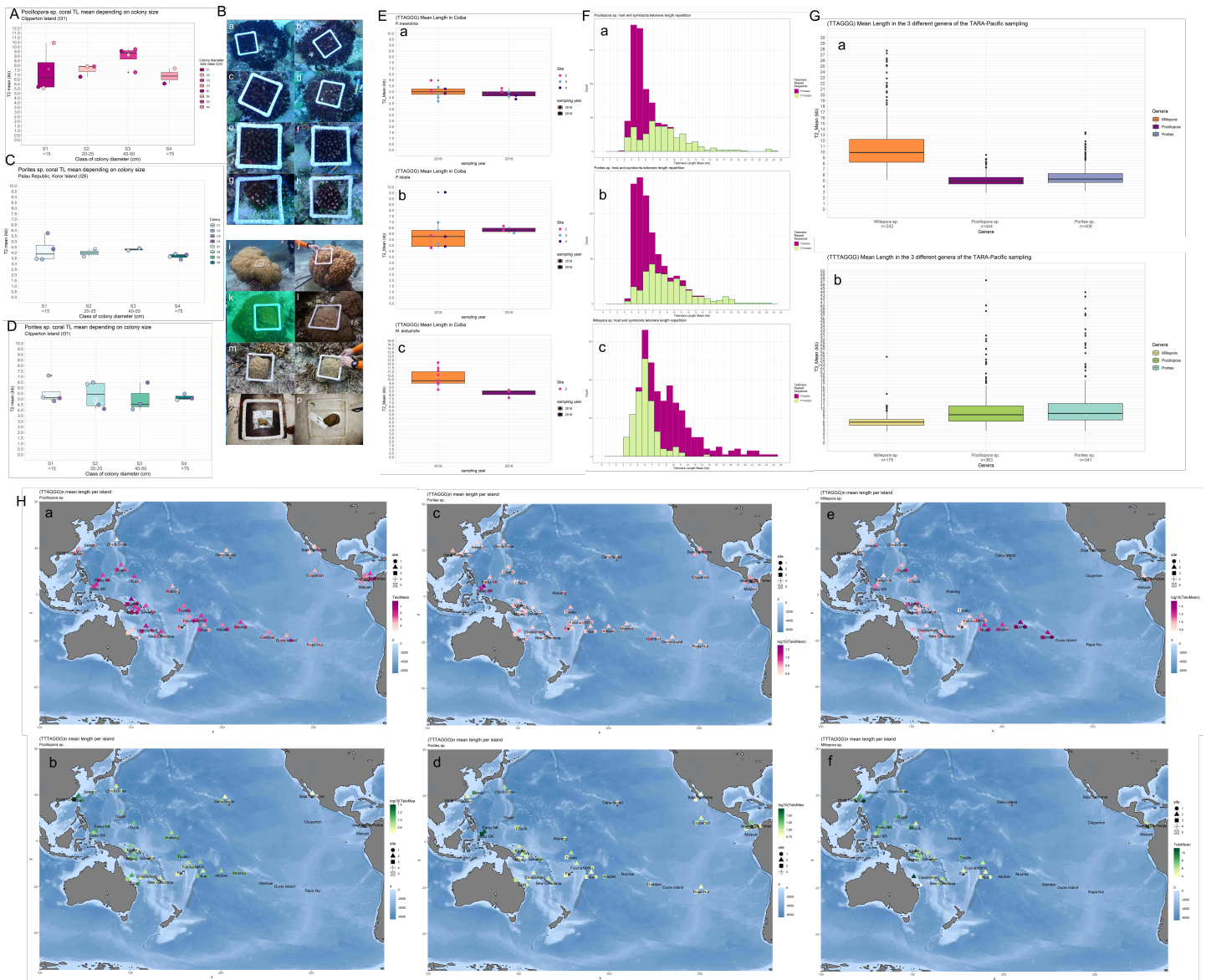
For *Pocillopora* sp, there is no clear geographical characteristic, except a trend toward long T2L for islands within or close to the Coral Triangle (Palau South and North, Kimbe, Milne and Solomon), a bioregion of high marine biodiversity with contrasted short T2Ls in the Great Barrier Reef. Yet Northern islands like Hawaii or Japan (Chichi Jima) exhibit short T2Ls as well as the South-East Easter island.

For *Porites* sp., the variation pattern for T2L appears more uniform between islands than for *Pocillopora* sp except at Palau South and in the Great Barrier Reef (GBR) where the telomeres are particularly long (Fig. 1H). Noteworthy, the mean T2Ls and

mean T3Ls exhibit a weak correlation (FigSup2) as well as a trend toward a similar variation pattern between islands as illustrated by the long telomeres found in Palau South and the contrasting short telomeres found in Easter Island (Rapa Nui) and Taiwan (Fig. 1H).

For *Millepora* sp telomeres, a T2L border can be drawn close to the anti-meridian, with a trend for longer telomeres in the eastern islands and for shorter telomeres in the western islands (Fig1He). The pattern of T3L variation is different with a trend for longer telomeres in the islands of the Northern Hemisphere than in the Southern Hemisphere.

Overall, the pattern of TL variation between islands is different for the three coral genera showing that the coral strategies of telomere maintenance can be genus-specific. To confirm this genus effect, we performed a Multivariate Correspondence Analysis (MCA) (Fig2: A) from which dimensions were mostly driven by coral genus, T2L and T3L. Intriguingly, there are no clear geographical characteristics allowing to explain the different TL variation patterns, suggesting that a complex interplay between environmental and genetic factors shapes coral telomere length variations across the Pacific.



**Figure 1 : Telomere Length (TL) of 3 cnidaria genera across the Pacific Ocean.** (A) Mean TL differences between Pocillopora sp. of 4 different class of size from Clipperton Island (Colony diameter : S1< 15 cm, cm 20 <S2< 25 cm, cm 40 <S3< 50 cm, S4> 75 cm), no significant differences ( $P>0.05$ ). (B) Pictures of colonies of different sizes of Pocillopora sp. from Clipperton (a-h) and Porites sp. from Koror Island, Palau Republic (i-p). Two colonies per size class is displayed ((a-b;i-j)=S4, (c-d;k-l)=S3, (e-f;m-n)=S2, (g-h;o-p)=S1). (C-D) Mean TL differences between Porites sp. of 4 different class of size from Koror Island, Palau Republic and Clipperton (Colony diameter : S1< 15 cm, cm 20 <S2< 25 cm, cm 40 <S3< 50 cm, S4> 75 cm), no significant differences ( $P>0.05$ ). (E) Mean TL in P. meandrina (a), P. lobata (b) and M. platyphylla (c) sampled in Coiba island in 2016 and 2018 (dots: individuals, color: sampling site), significant differences in M. platyphylla ( $P>$ ). (F) Barplots of P. meandrina (a), P. lobata (b) and M. platyphylla (c) mean TL distribution of the host (pink) and their symbionts (green). (G) Boxplots of mean TL in the host (top) and their symbionts (bottom) depending on the genera M. platyphylla (yellow) and the two scleractinians (purple) P. meandrina and P. lobata, significant differences were observed between each groups (Kruskal-Wallis test and post-hoc pairwise Wilcoxon test,  $P<0.001$ ). (H) Maps of mean TL averaged at the sampling site level for P. meandrina's host (a) and symbionts (b), P. lobata's host (c) and symbionts (d) and M. platyphylla's host (e) and symbionts (f).

## Environmental determinants

To unravel the biogeochemical parameters correlated with TL variation of the host and the symbionts we crossed our T2L and T3L measurements with contextual parameters measured during the sampling (Fig2:B, Guillaume Bourdin unpublished data) and historical record of past environment (Fig2:C, Guillaume Bourdin unpublished data). We found that *Pocillopora sp.* T2Ls are positively correlated with higher sea surface temperature (SST) (mean, IQ and Q3) and negatively correlated with dissolved oxygen (O2\_c) (mean, IQ and Q3) as well as with aerosol concentration (X\_aero) (Q1). For *Porites sp.*, among the TL measures only T3L show correlations with biogeochemical variables. Indeed, T3Ls positively vary along with aerosol concentration (med and Q1), manganese concentration (Mn) (Med and Q1), colored dissolved organic matter (CDOM) (Mean, Med, Q1 and Q3) and current speed (current spd) (mean, Q3) (Fig2B).

Since telomere length can be considered as a marker integrating past stress and injuries, we integrated past records of environmental parameters as well as index of heat anomalies for 7 islands (from Easter to Upolu). For *Porites sp.*, T2L (Med and Mean) is negatively correlated with the “minimum of recovery day between heating events” while T3L is negatively correlated with the last maximum of degree heating week (DHW) record, two indicators of heat stress. For *Pocillopora sp.* only T3L (Q1) is positively correlated to the maximum length of days in heating stress state. Interestingly, *Porites sp.* and *Pocillopora sp.* mean T2L similarly correlate with seasonal parameters : their T2L are shorter in regions with high standard deviation in seasonal variation, such as Easter island (I04).

## Interplay between species and environmental determinants for *Pocillopora sp* and *Porites sp*

To be able to catch the synergic effects of environmental parameters, islands were clustered according to their biogeochemical properties into 9 environmental clusters (SupFig5, Guillaume Bourdin unpublished data). Moreover, this analysis was performed at the species level in a subset of 11 islands (Las Perlas, Coiba, Malpelo, Easter, Ducie, Gambier, Moorea, Aitutaki, Niue, Samoa, Guam) by using the corresponding sequencing data to cluster the colonies in 5 clades for *Pocillopora sp.* (SVD1-5) and 3 clades for *Porites sp.* (SVD1-3) (Fig2:Db,Eb) (Didier Forcioli unpublished data).

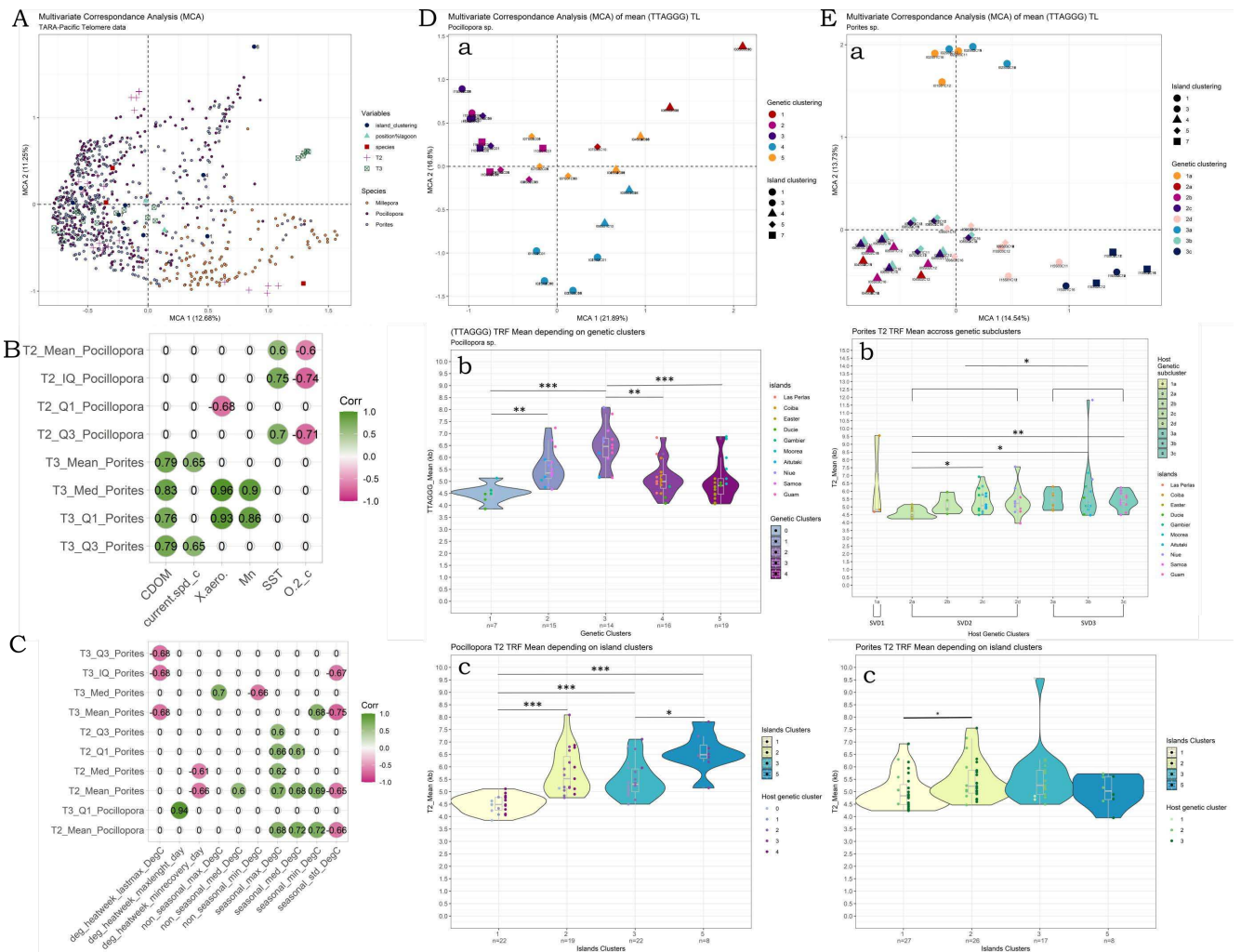
For *Pocillopora sp.*, the MCA results revealed a mixed effect of environment and genetics over mean T2L, cluster 4 and SVD account for 29.3 % of the first dimension variance while the second dimension (Fig2:Da) was mostly driven by cluster 1 and SVD4. Indeed, the mean T2Ls are significantly different between the 5 *Pocillopora* clades (Fig2 Db), with the longest telomeres being found in SVD3 and the shortest in

SVD1. They are also different between the corresponding environmental clusters (Fig 2Dc). In particular, the cluster 4 (Easter, Ducie and Gambier) groups colonies harboring the shortest telomeres belonging to three different clades (SVD1, SVD4 and SVD5). Interestingly, the SVD4 colonies with the longest T2Ls are grouped in cluster 1 (Coiba and Las Perlas). Thus, for the same clade (SVD4), the colonies with long and short T2Ls fall into different environmental clusters. Overall, *Pocillopora* sp T2L variation emerges as a complex interplay between genetic and environmental determinants.

In contrast to the *Pocillopora* clades, the 3 *Porites* clades exhibit little T2L variations between them (Fig. 2Eb). In fact, the only significant difference between SVD2 and SVD3 ( $p < 0.05$ ) is due to the colonies originated from Easter Island (SVD2a), which exhibits the shortest telomeres of the 11 islands ( $p > 0.5$  when removing the Easter samples). This together with the facts that the *Pocillopora* sp. colonies from Easter island also exhibits very short mean T2L ( $4.5 \pm 0.4$  kb), that *Porites* and *Pocillopora* T2Ls correlate with historical parameters at the level of the 32 islands and that the Easter island shows high standard deviation in seasonal variations suggest that past environmental stress had led to TL shortening in Easter island corals population.

While no difference can be detected for the *Porites* T2Ls between the environmental clusters of the 11 islands (Fig2Ec), the MCA results show that the clusters 3, 4, and 7 account for 40% of the MCA first dimension variance while the second dimension is mostly driven by cluster 1 (Fig2:E:a). These results, together with the historical impact detected at the level of the 32 islands (see above), reveal that *Porites* T2Ls can co-vary with environmental determinants while they show little variation according to their genetics.

We did not find consistent correlations between T2L and T3L even though coral host is regulating the symbiont cell cycle (Lecointe et al., 2016). The T3L variations of *Pocillopora* sp. T3Ls can be explained neither by the host genetic nor by the environmental clustering. The *Porites* sp. T3Ls are different between the symbiont clades. Contrary to T2Ls, the *Porites* sp. T3L are much more variable and exhibit strong correlations with environmental measurements as well as historical records. In particular, the *Porites* T3Ls are highly correlated to aerosol concentration, Mn and Colored Dissolved Organic Matter (CDOM). Since it was reported that these three environmental parameters can be linked (Fishwick et al, 2017), the connection between T3L and aerosol formation could reveal a daily symbionts expulsion in *Porites* sp., explained by the environmental constraints and the diurnal cycles regulating aerosol formation (Flores et al., 2020).



**Figure 2 : Impact of genetic population and environment on TL.** (A) Multivariate Component Analysis, representing the 2 major component axis (MCA1, MCA2) of all TL measurements (mean, median, 1st quartile, 3rd quartile, interquartile distance) for the host (T2) and their symbionts (T3) in the 3 genera *Pocillopora* sp. (pink), *Porites* sp. (purple) and *M. platyphylla* (yellow). Individuals are small dots colored on genera, variables are shaped as pink cross for T2, square crossed for T3, red square for genera, blue triangle stands for the position in the sampled lagoon (inside or outside) and the blue circle represents the 9 groups of islands (island\_clustering). (B) Correlogram of the significant ( $r > 0.6$  and  $P < 0.05$ ) correlation between *Pocillopora* sp. and *Porites* sp. TL measurements (mean, median, 1st quartile, 3rd quartile, interquartile distance) for the host (T2) and their symbionts (T3) and the contextual environmental parameters in the 32 sampled islands. (C) Correlogram of the significant ( $r > 0.6$  and  $P < 0.05$ ) correlation between *Pocillopora* sp. and *Porites* sp. TL measurements (mean, median, 1st quartile, 3rd quartile, interquartile distance) for the host (T2) and their symbionts (T3) and the historical environmental parameters of islands (I04 Easter Island, I05 Ducie, I06 Gambier, I07 Moorea, I08 Aitutaki, I09 Niue, I10 Samoa). Temperature historical parameters are expressed in °C ('deg\_heatweek\_lastmax\_DegC': last degree heating week event maximum, 'non\_seasonal\_min\_DegC': anomaly in comparison seasonal minimum, 'non\_seasonal\_max\_DegC': anomaly in comparison seasonal maximum, 'seasonal\_min\_DegC': seasonal trend minimum, 'seasonal\_max\_DegC': seasonal trend maximum, 'seasonal\_med\_DegC': seasonal trend median), time spend in specific environmental condition is expressed in days ('deg\_heatweek\_maxlength\_day': maximum duration of degree heating week event, 'deg\_heatweek\_minrecovery\_day': minimum duration recovery period between degree heating week events). (D) (a) Multivariate Component Analysis, representing the 2 major component axis (MCA1, MCA2) of all TL measurements (mean, median, 1st quartile, 3rd quartile, interquartile distance) for the host (T2) and their symbionts (T3) *Pocillopora* sp. individuals colored by genetic population and shaped by island clustering. (b) Mean TL of *Pocillopora* sp.'s host depending on the genetic clustering. (c) Mean TL of *Pocillopora* sp.'s host depending on the island clustering. (E) (a) Multivariate Component Analysis, representing the 2 major component axis (MCA1, MCA2) of all TL measurements (mean, median, 1st quartile, 3rd quartile, interquartile distance) for the host (T2) and their symbionts (T3) *Porites* sp. individuals colored by second order genetic population and shaped by island clustering. (b) Mean TL of *Porites* sp.'s host depending on the genetic second order clustering. (c) Mean TL of *Porites* sp.'s host depending on the island clustering. (Pairwise Wilcoxon test, '\*'  $P < 0.05$ , '\*\*'  $P < 0.01$ , '\*\*\*'  $P < 0.001$ ).

### Gene expression correlated with T2L variation in *Pocillopora* sp.

In order to explore whether T2L variation can be associated with differential gene expression in *Pocillopora* sp. we took advantage of the RNAseq data obtained from the colonies subjected to metagenomic analysis and species delimitation (see above). We considered that the set of differentially expressed genes (DEGs) associated with T2L variation reflects the physiological context that is either the cause or the consequence of T2L variations, including genes directly responsible for TL shortening or lengthening. To this purpose, we analyzed the differentially expressed genes (DEG) correlated to T2L ( $r > |0.7|$  and  $P < 0.01$ ) at the species, island and environmental cluster levels (FigSup7) (Quentin Carradec unpublished data).

At the species level, SVD1 and SVD5 exhibit the highest number of correlated DEGs with 44 in common (Fig3C). Regarding islands (Fig3A), Gambier largely shows the highest number of DEGs (1352 negatively and 589 positively) followed by Niue (308 negatively and 312 positively). Much less correlated DEGs are observed according to environmental clusters. Noteworthy, only 3 DEGs correlate with T2L for environmental cluster 4, which corresponds to colonies with the shortest TL (SupFig6).

For the Gene Ontology (GO) functional analysis, we focused on the larger sets of correlated DEGs, i.e. SVD1, SVD5, Gambier and Niue. The enriched GO terms of biological processes (BP) were grouped in 7 general biological functions (cell division, metabolism, gene expression, genome maintenance, metabolism, proteostasis, signal transduction and cellular trafficking and transport) (Fig. 3D). Notably, GO-terms of the groups related to genome maintenance and cell division are enriched in Gambier, SVD1 and SVD5. Another interesting group enriched in all four sets of DEGs is metabolism, including GO-terms related to redox metabolism. These results highlight the importance of genome maintenance, cell proliferation and metabolism in the TL variation observed for *Pocillopora* sp. across the Pacific.

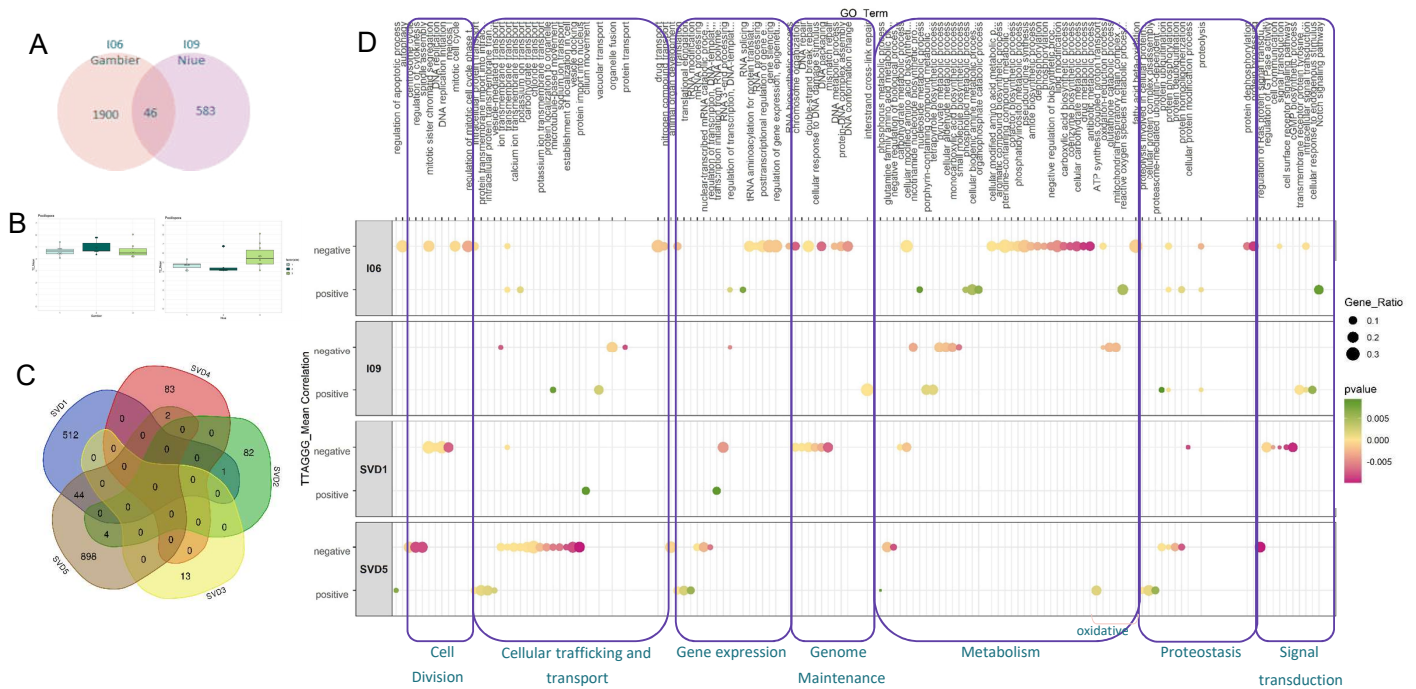


Figure 3: **Expressed genes in *P. meandrina* correlated to mean TL** (A) Venn diagram of the common genes correlated to mean TL between Gambier (I06) and Niue (I09) islands. (B) Boxplot of *P. meandrina* mean TL per site in Gambier and in Niue. (C) Venn diagram of the common genes correlated to mean TL between *P. meandrina* genetic clusters (SVD1-SVD5). (D) Biological Process (BP) Gene Ontology (GO) significant enrichment ( $P < 0.01$ ) of genes positively or negatively correlated ( $r > |0.7|$  &  $P < 0.01$ ) to mean TL of *P. meandrina* in Gambier island (I06), Niue island (I09) and in SVD1 and SVD5 genetic populations

## Discussion

By measuring the telomeric DNA length (TL) of more than 1000 coral colonies collected in 32 islands across the Pacific Ocean, this work generated a unique corpus of data allowing to investigate the role of telomeres in coral ecosystem functions. To our knowledge, no similar integrated approach was performed before to study telomere functions on a high-resolution, ecological scale. Some important conclusions regarding coral TL dynamics in the wild can be drawn from the first wave of analyses presented in this work.

We observed important natural variations among the coral samples both for the host TL (named T2L) and the symbiont TL (named T3L) both at the genus (*Pocillopora* sp, *Porites* sp and *Millepora* sp) or species level (delimited for a subset of *Pocillopora* and *Porites* colonies). This is in agreement with the natural TL variation previously reported for a large variety of animals (Monaghan and Ozanne 2018; Cook et al. 2016), including marine invertebrates (Garcia-Cisneros et al. 2015) and plants (Watson and Riha 2010; Fulcher and Riha 2015; Abdulkina et al. 2019). Interestingly, the patterns of TL variation across the Pacific Ocean differs between the three genera, suggesting different strategies of telomere maintenance between coral lineages.

We did not detect correlation between T2L and the size of the colonies of the two scleractinian coral studied here. Even though age estimation from colony size can be

affected by individual growth rate and coral fragmentation, coral branches can regenerate and grow back a colony when broken, our absence of correlation between a wide range of colony sizes and geographical locations strongly suggests a lack of age-related T2L shortening for adult colonies of *Pocillopora* sp. and *Porites* sp. For instance, the *Porites* sp. colonies used to correlate T2L and size were sampled in two different environments (2 islands separated by 12 700 km) and a repeated sampling performed at roughly two years difference at the same sites around the Coiba island did not reveal any T2L changes nor for *Pocillopora* nor for *Porites* colonies. Moreover, the differences of colony area of the analyzed *Porites* colonies (from 20 cm<sup>2</sup> to 14 256 cm<sup>2</sup>) from Palau would represent an estimated age range from 21 years to 566 years, according to the growth rate of 0.9 cm/year reported for palauan *Porites* sp. (Barkley et al. 2015). This finding is consistent with results obtained for other marine organisms, including the solitary coral *Ctenactis echinate* (Ojimi et al, 2012), sea urchins (Francis et al. 2006) and lobsters (Klapper et al. 1998). Nevertheless, it is still possible that telomere shortening occurred during the early development of *Porites* and *Pocillopora* sp as reported for the coral *Acropora digitifera* in (Tsuta et al.,2014). Noteworthy, the lack of age-related T2L shortening reported here concerns two coral genera with extremely different lifespans, estimated to only a couple of years for the branched short-lived *Pocillopora verrucosa* and > 600 years for the massive long-live *Porites lobata* (Bythell, Brown, and Kirkwood 2018). Therefore, age-related TL shortening does not appear to be a mechanism regulating longevity in coral. A similar conclusion was drawn for short- and long-lived sea urchin species (Francis et al. 2006). Whether telomeres of adult corals are maintained throughout their lifespan by a constitutive telomerase activity or recombination-based mechanisms remains to be determined. Importantly, these results show that factors, other than age, regulate telomere DNA length in coral.

In order to tackle the question of telomere length regulation in coral, we performed cross-analyses between T2L, coral species, taking advantage of metagenomics generated for one-third of the colonies from an 11 island subset, as well as a large number of environmental parameters. The T2L of *Pocillopora* sp. colonies was partly constraint by genetics while *Porites* sp. from both sides of the Eastern Pacific genetic barrier (Baums et al. 2012; Romero-Torres et al. 2018), were not significantly different, suggesting conserved mechanisms of TL regulation between *Porites* species but not between *Pocillopora* species.

Interestingly, *Pocillopora* sp. colonies sampled in cold and O<sub>2</sub> rich water show T2L shortening (Fig2B). For instance, *Pocillopora* sp. T2L is short for Chichi Jima island, which is, after Rapa Nui, the island with the lowest sea surface temperature (SST = 20.98°C) and the highest O<sub>2</sub> concentration. A pattern also found in islands such as Gambier (I06, FigSup5) where one of the few GO-terms enriched in DEGs positively correlated with T2L was related to oxidative metabolism (Fig3D).

Since TL is well known to shorten upon oxidative stress both in vitro and in the field studies (R. C. Young et al. 2017; Barnes, Fouquerel, and Opresko 2019; Chatelain, Drobniak, and Szulkin 2020), enhanced oxidative damages could cause the TL shortening observed in colonies sampled in cold and O<sub>2</sub> rich water.

In further accordance with an important role played by the environment to drive *Pocillopora* T2L variations, we observed differences of mean T2Ls between clusters of islands sharing similar environmental characteristics in a species-independent manner. For instance, the colonies from the environmental clusters 2, 4 and 8 (Easter, Ducie, Gambier, Chichi Jima, Abaiang and Baja California) (SupFig5) have shorter mean T2Ls than those from the other clusters ( $p < 0.001$ ) whereas the colonies, sampled at Moorea and belonging to the clade SVD5 also found in Easter and Ducie islands, have longer T2L. Despite the absence of global correlation between *Pocillopora* sp. T2L and historical record data, in particular the bleaching risk event (DHW) (Fig2:C), the massive bleaching events recorded in the past years in Hawaii and GBR (Hughes et al. 2018, 2019) could explain the short telomeres observed in these islands. Longitudinal telomere studies will be required to substantiate the notion that *Pocillopora* T2L shortening can be a post-event of massive bleaching events and/or a characteristic of the surviving corals.

There is no difference of mean *Porites* T2Ls between the environmental clusters. However, by a Multiple Correspondence Analysis (MCA) of T2Ls, the environmental clusters drive most of the variability over the clades, revealing a contribution of environment to *Porites* T2L variation, even if it appears less pronounced than for *Pocillopora*. Intriguingly, both *Porites* sp. and *Pocillopora* sp. exhibit shorter T2L in Easter Island (Rapa Nui) than in the other islands belonging to the same environmental cluster. This concordance for short T2L in the two coral genera suggests a conserved mechanism of telomere adaptation to a particular Rapa Nui environment. Indeed, the Rapa Nui coral populations are exposed to strong seasonal changes (Glynn et al. 2003), which could explain the short telomeres as a consequence of repeated stresses. Noteworthy, the genetic analyses show an enhanced isolation of Rapa Nui populations of *Pocillopora* and *Porites* (Fig2Db, Eb), in agreement with previous reports (e.g. (Glynn et al. 2003; Paz-García et al. 2016)). This suggests that the short T2L phenotype shared by the two genera results from a genetic adaptation favored by the geographical isolation of this island. Interestingly a longitudinal study performed on a long lived bat species *Myotis myotis* revealed that instead of decreasing TL with age, the individuals TL variation was mostly due to environmental deviation rather than inheritability (Foley et al. 2020). Altogether, these results uncover that past environmental stress can explain some T2L variations of the *Pocillopora* and *Porites* colonies.

Our results raise the possibility that extreme stresses or highly contrasted environments, such as at Rapa Nui and possibly some other islands of the Tara-

Pacific expedition, impact T2L similarly between *Pocillopora* and *Porites*, while low or moderate environmental variabilities have genus-specific consequences on T2L.

In addition to their different longevities, the two genera of scleractinian corals studied here exhibit distinct life-history strategies : the branched *Pocillopora sp* are fast growing and extremely sensitive to breakage and bleaching while the massive *Porites sp* are slow growing and stress-resistant ((Darling et al. 2012)). An hypothesis is that coral life-history is linked to specific strategies of telomere maintenance adapted to a given environment and tolerating moderate disturbances. In the case of the long-lived *Porites*, the greater stability of T2Ls in colonies facing different environments might reflect a more important investment in somatic maintenance by developing efficient mechanisms of telomere protection, thus contributing to the extreme longevity and resilience of this genus. This mechanism could be maintained genetically as suggested by the quasi-absence of T2L differences between the *Porites* clades. In contrast, the high level of T2L variations observed for *Pocillopora* colonies exposed to different environments might be due to an important sensitivity to stress and/or repeated cycles of regeneration due to branch breakages, both processes being consistent with some of the life-history traits of this genus.

Regarding the telomere relationships between the host and its symbiont, our results show that *Porites* T3Ls vary in a dissimilar manner than its host telomeres, being more sensitive to environmental parameters and regulated over a short time scale (Fig2B, C). Whether this telomere dynamics of the symbiont contributes to the longevity and resilience of *Porites* is certainly an interesting question for future studies.

As a first attempt to establish links between telomere phenotypes and coral biology, we characterized, using RNA sequencing, the transcriptional signature associated to T2L variation in *Pocillopora*. The number of genes whose expression correlates with T2L was much higher at the species and island level than according to environmental clusters of islands, suggesting species-specific transcriptomic telomere signatures associated to specific environmental niches (the island level). Two species (SVD1 and SVD5) and two islands (Gambier and Niue) exhibit a high number of correlated genes allowing us to perform gene ontology analyses. Important biological processes were found recurrently associated to the correlated genes including cell division, genome maintenance, metabolism (in particular redox homeostasis), cellular trafficking and proteostasis. Noteworthy, for the SVD1 species, several genes negatively correlated with T2L variation are involved in DNA replication, DNA repair and telomere maintenance. Overall, most of the correlated genes were overexpressed when TL were shorter which is in line with the idea that short TL triggers a cellular response and that TL regulation is a dynamic feature (Ye et al. 2014).

Whether these telomere transcriptomic signatures reflect the cause or the consequence of T2L variation remains to be determined. Interestingly, one of the correlated genes encodes the ortholog of the key telomere capping proteins TRF1/2 (TTAGGG Repeat Factor 1/2) suggesting that variations in its expression is causal to T2L variation. Nevertheless, the fact that the T2L correlated genes are involved in several pathways of cellular homeostasis uncovers important determinants of the coral biology in relation to telomere state.

The amplitude of T2L variation observed here (from 2 to 9 kb for *Pocillopora* and from 3 to 14 kb for *Porites*) is within the range of leukocyte TL variation observed with age at the human population level (Vaziri et al. 1993). In human populations, age-matched variations in telomere length are regarded as indicators of health and mortality risk ((Vaziri et al. 1993; Cawthon et al. 2003; Epel et al. 2004). Whether TL might be an interesting biomarker for coral health will require further investigations, in particular by defining a health index for coral that could be matched with TL variations. Nevertheless, our results uncover important determinants of telomere biology in coral suggesting that telomere robustness is a mechanism of stress resistance. Therefore, deciphering further the signaling, genetic, epigenetic and symbiotic mechanisms explaining the TL regulation in reef corals is expected to reveal new pathways of coral resilience and longevity.

## Material and Methods

### Sampling

One branch was collected on 10 different colonies for one species and one location in a given island. Each of the 32 sampled islands was sampled in at least 3 different sites (Planes et al. 2019). Samples were flash frozen in liquid nitrogen and stored at -20°C. Branches were collected from coral colonies of 4 different size class (S1 diameter: < 15cm, S2 diameter: 15-25cm, S3 diameter: 25-50cm, S4 diameter: > 75 cm) for *Porites* sp. in Palau Republic (n=12) and Clipperton island (n=14), for *Pocillopora* sp. in Clipperton island (n=13) during the Tara-Pacific expedition.

### DNA extraction

DNA was extracted on the first 3-4 cm of a colony branch. All steps until cell lysis were done on ice. The coral tissue was removed from the skeleton using the “air\_brush” technique in 50mL of buffer (NaCl 550mM, EDTA 0,2M (E. Tambutti et al. 2007) in a Whirl-Pak bag under the hood, then centrifuge for 10 minutes at 5 000g (Beckman Coulter, Avanti J-E centrifuge, rotor JA-18) at 4°C. The tissue pellet was kept on ice to perform high molecular weight DNA extraction following the Midi Kit Cell and Blood culture (QIAGEN). Briefly, cell lysis was carried out for 2 hours at 50°C after adding 19 mL of G2 buffer (800 mM guanidine HCl; 30 mM Tris·Cl, pH 8.0 ; 30 mM EDTA, pH 8.0 ; 5% Tween-20 ; 0,5% Triton X-100, QIAGEN) with 190 mL RNase A (ThermoFisher, ref : 12091039 ,20mg/mL) and 400 mL of protease (QIAGEN, 1 UA/mL) to the tissue pellets. Supernatant was poured in the anionic column after a 5 min centrifuge at 5 000g and 4°C, columns were washed following the kit

instructions, DNA was eluted with 5mL of warm QF buffer (1.25 M NaCl ; 50 mM Tris·Cl, pH 8.5; 15% isopropanol, QIAGEN). Precipitation was performed adding 3.5mL of 2-propanol (Sigma), after mixing by inverting, tubes were stored overnight at 4°C. Two washing steps were performed adding 1mL of 70% ethanol (Sigma) to DNA pellets after 30 min centrifuge at 15 000g and 4°C before adding 200 mL of TE buffer (Tris·Cl pH8 10mM, EDTA 1mM) for long-term storage. Pellets were resuspended at 50°C for 1 hour.

To test DNA quality and quantity both Nanodrop (ThermoFisher) and agarose gel were used, respectively 1 mL of DNA and 1,5 mL of loading buffer (New England Biolabs, 6X) in a final volume of 10 mL were loaded in a 1.2% agarose gel run for 30 minutes at 100V in Tris-Borate-EDTA (TBE) 1X. Agarose gel was incubated for 10 minutes in 4 mg/mL ethidium bromide and DNA was revealed under UV (GelDoc Transilluminator, BioRad), the average of three nanodrop measurements was kept. A diluted aliquot was prepared before storing the stock DNA at -20°C. This protocol enables to extract 15 samples every two days.

### Telomere Restriction Fragment assay:

#### Dilution and DNA digestion

To digest non telomeric DNA sequences, we added 60U HinfI (R0155M, New England Biolabs) and 60U RsaI (R4374, Promega) to 2,5 mg of DNA diluted in mQH<sub>2</sub>O and "CutSmart Buffer" 1X (New England Biolabs) in a final volume of 40mL, left overnight at 37°C.

#### Southern Blot

We measured telomere length using the Telomeric Restriction Fragment assay (TRF) using the Southern blotting procedure (Herbert, Shay, and Wright 2003) digested DNA was loaded on a 1,2% agarose gel for a 3h30 run in TBE 0.5X at 9 volts/cm in a CHEF-DR-II (BioRad). The run timing makes it suitable for 2 runs a day. After a 5 min wash in mQ H<sub>2</sub>O agarose gel was stained in Ethidium bromide bath (4 mg/mL) under agitation for 30min and imaged the gel (Typhoon, GE Healthcare, Fluorescence, Method=Alexa Fluor 555, Laser=532nm, PMT=ch.1,700V, Resolution = 50 µm). Denaturation was done in (1 M NaCl ; 0.5 M NaOH) for two incubations of 20 min followed by a neutralization step with two 20 min incubation in 1M ammonium acetate. The DNA was transferred to a Hybond N<sup>+</sup> membrane (GE Healthcare) overnight in SSC (20X). DNA was cross-linked on the membrane using the Ultraviolet Crosslinker (UVP) at 1200 x 100µ Joules. Membrane was gently washed in mQH<sub>2</sub>O before storage at room temperature for (12h up to 48h) before 1h pre-hybridization in Denhardt's buffer (10X) at 40°C. We prepared stocks of Denhardt's 100X buffer (2% Ficoll 400, 2% Bovine Serum Albumine, 2% Polyvinylpyrrolidone) filtered (0.2µm) and stored at -20°C.

#### Probe synthesis

We prepared probe template 40uM stocks annealing two primers per probe. Probe targeting (TTAGGG) sequence will be called T2 and the one targeting (TTTAGGG) sequence will be referred to as T3. We mixed 25 of Primer F and 25 of Primer R (Primer **T2AG3\_F** : GGGTTAGGGTTAGGGTTAGGGAAA and **T2AG3\_R** : TTTCCCTAACCTAA, Primer **T3AG3\_F** : GGGTTTAGGGTTTAGGGTTTAGGGAAA and **T3AG3\_R** :

TTTCCCTAAACCCTAAA) with STE 5X buffer (Tris 50mM pH8, NaCl 250mM, EDTA 5mM) and heat it to 95°C to therefore gradually let it cool down to room temperature before being stored at -20°C. We mixed on ice 2.5 of dATP (10 mM) and 2.5 of dTTP (10mM, New England Biolabs, R0192) with 3 of NEB 2 buffer (10X), 15 of mQH<sub>2</sub>O, 5 of gamma-dCTP32 and 1 Klenow (3'-->5' exo-) (M0212S, NEB) incubated in a thermocycler (ThermoFisher Scientific) for a (30min 25°C, 5min 98°C, 5min 25°C) program. Then we purified the probes following the ProbeQuant G50-micro columns (GE, Healthcare) instructions. Probes were denatured at 95°C for 5 min and used or stored at -20°C.

## Hybridization

Membranes were pre hybridized for 1h at 40°C in Denhardt's buffer (10X). We added 5µL of denatured T3 probes to 15mL of hybridization buffer for an overnight hybridization at 40°C. Washing steps included a 2x20min wash in 2X SSC, a 30min step in (2X SSC, SDS 0,2%) and a final H<sub>2</sub>O washing step. Membrane was carefully wrapped in plastic sarran film and applied to a phosphor-screen (brand) for 60h. We imaged the phospho-screen on the Typhoon (GE Healthcare, Phosphorimaging, Method=[Phosphor], Laser=635nm, PMT=ch.1,1000V, Resolution = 50 µm), the screen was flashed (brand of device). Membrane was stripped with NaOH 0,4M for 30min at 42°C, then for 30min in (SDS 1%, SSC 0.1X, Tris-HCl pH7.4 1M) to remove the T3 probe. To control the stripping, membrane we exposed the membrane to a flashed phosphor-screen for 12h. The screen was imaged as described above, in the absence of signal the membrane was pre hybridized for 1h and hybridized overnight at 45°C with the T2 probe, otherwise the membrane was stripped again until no signal was imaged after 12h of exposure. Washing steps and imaging were carried out as described above with a 24h exposure of the phosphor-screen.

## Image analysis

Telomere Restriction Fragment (TRF) images signal was extracted using ImageQuant (GE Healthcare) 1D gel analysis mode and manual lane creation. Ladders signal intensity were extracted from Ethidium bromide gel images, setting the lane upper limit at the gel wells bottom. Pixel position of ladder peaks were manually reported. Host and symbiont telomere signals were extracted from the phospho screen, exposed to radioactive labelled membrane, images setting sample lane upper limit at the top of the membrane. Efficiency of stripping step between the two probes hybridization was assessed imaging phospho screens overnight exposed to stripped membranes.

## Telomere Length measurements

Single lane intensity files were fused in RStudio. Ladder peaks exact position was extracted in R, searching for the maximum intensity in a 10-pixel perimeter around the manually reported peaks. Fitted linear model coefficients (a, b) of log<sub>2</sub> ladder size (kb) against peak pixel position were calculated for high molecular weight (48.5-15kb) and low molecular weight (10-1kb) using the *lm* function of "stats" R package. Coefficients were used to transform samples intensity scale from pixel to base pair (bp) ( $bp = 2^{(a \times \text{pixel} + b)}$ ) in Excel using the high molecular weight coefficient for the upper part and the low molecular weight coefficient for the lower one. Depending on their position on the membrane samples were divided in left, right and middle to be scaled to the closest ladder. Intensity signals were

imported in R using the *read\_excel* from “readxl” package, background correction was automatically computed to level the signal by subtracting to each position the minimal intensity. Intensity was normalised by the size to avoid probe number hybridization bias, intensity above 2 kb were discarded to avoid genomic noise, interstitial telomeric sequence noise and normalisation bias (<1kb). Ponderate telomere mean was calculated as in (Li and de Lange 2003), median, quartiles, intensity signal, smoothed signal and telomere measurements were plotted for each sample using the *ggplot* function from “ggplot2” package and measurements were saved as csv files.

## Quality Control

Signal quality was manually and blindly assessed by 2 observers screening of (84 membrane labelled with (TTTAGGG) and therefore (TTAGGG) probes) to report lanes without signal or with poor quality. Overlap of the two independent selections was kept to remove lanes from analysis. Samples with critical DNA degradation were removed from the analysis. The technical standard errors for T2L (+/- 1.2 kb) and T3L (+/-2.8 kb) were estimated by running in the different gels used for the coral samples the same batch of human cell HepG2 DNA (n=79) and of *A. thaliana* DNA (n=69), respectively (Fig SupX3). Finally, the gel effect that could influence our measures was tested for coral samples by estimating twice in two different gels the TLs of 268 coral DNAs: the two estimates show a strong correlation ( $R = 0.7617388$   $p < 2.2e^{-16}$  for T2L, and  $R = 0.86123004$ ,  $p < 2.2e^{-16}$ ) (SupFig2).

## BAL 31

To control for the telomeric extremities origin of the observed signal we performed a Bal31 experiment. We had 2U of exonuclease Bal31(NEB, M0213S) to 5 ug of sample DNA that we sequentially incubated at 30°C for (0,5,10,15 and 30min). After incubation, a 10-minute step of inactivation at 65°C with EGTA (30mM). DNA was precipitated and digested overnight to be used for a Telomere restriction fragment assay (SupFig1).

## Correlation

Correlation between host and symbionts TL measurements were computed using the *ggpairs* function from the “GGally” R package, correlation of duplicated measurements and colony size with the *cor.test* function from “stats” (SupFig2,3) as well as correlation between environmental data (Fig2:B-C) that were plotted using *ggcorplot* from the “corr” R package, keeping results above ( $r > |0.6|$  and  $P < 0.05$ ) threshold, correlation with gene expression was done with *corr.test* from “psych” using ( $r > |0.7|$  and  $P < 0.001$  and TPM > 1).

## Human orthologs

Protein sequences were used to identify human orthologs. Blastp (Altschul et al. 1990) on UniProtKB/Swiss-Prot database restricted to *Homo sapiens* species was performed with a 1e-5 e-value threshold. For each protein, the best hit was selected, based on e-value and bit-score.

## Ontology

Gene Ontology (GO) analysis was performed using the “topGO” R package (Alexa, Rahnenfuhrer, and Lengauer 2006). For the cnidarian analysis, all GO terms identified in Pocillo and Porites separately were used as background. Ingenuity Pathway Analysis (Krämer et al. 2014) (QIAGEN Inc., <https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis>) was performed on human ortholog genes.

## Multivariate correspondence and principal component analysis

Multivariate correspondence analysis was carried out as in (Benedetti, Gasparini, and Ayata 2016) transforming quantitative variables (TL measurements for symbionts and coral) in categorical ones (4 levels) using the *cut2* function from the “Hmisc” package, *mca* function from “FactoMineR” package was used with the genera (Pocillopora sp., Porites sp., Millepora sp.), lagoon position (inside or outside) and island cluster (8 levels) variables for (Fig2:A) while only host meanTL, island cluster (5 levels), lagoon position and genetic cluster (5 levels) (Fig2-D) or sub genetic cluster (8 levels)(Fig2-E) (SupTable).

## Maps

The maps were generated using the function “autoplot” from ‘ggplot2’ R package, the bathymetric values were downloaded from the National Oceanic and Atmospheric Administration (NOAA) at a resolution of (15mins).

## Statistics

We tested for homoscedasticity using *fligner.test*, for normal distribution with the *shapiro.test*, differences between means were assessed with *pairwise.wilcoxon.test* for non-normal distribution, small sample data or as post-hoc test and with *aov* (anova) when residuals were normally distributed. Linear model regression was performed using *lm* function and normality of residuals was tested with the *shapiro.test* from “stats” R package. Significant differences are reported as stars ((\* P<0.05, (\*\*) P<0.01, (\*\*\*) P<0.001) or letters using *mulcompLetters* from “multcompView” package (0.05 threshold).

## Colony size measurements

Colony size was assessed using the average of 3 measurements of colony area and perimeter with ImageJ on quadrat scale picture (Fig1: B), unfortunately strong current during Porites sp. in Clipperton island didn’t allow divers to take pictures samples were therefore grouped within 4 diameter size class. Colony size measurements from Clipperton and Palau islands (Fig1: B) were analysed with ImageJ, diameter, perimeter, area were averaged on 3 measurements based on 3 drawings of each colony edge. Pictures were paneled using the ‘Make Montage’ option.

Pocillopora sp. size measurements from transects samples (SupFig3) were analysed as described in (Sylvain Agostini unpublished data).

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AR designed the project, executed and analyzed most the experiments and wrote the manuscript; ND, AO, LDM, CL, JT, LW and DZ helped for coral DNA extraction and TRF experiments; QC, EA, JLH and MP help for RNAseq analyses; GB and FB helped for environmental and historical data analyses; DF helped for genetic data analyses; EG designed and coordinated the project, analyzed the results and wrote the manuscript. All authors edited the manuscript.

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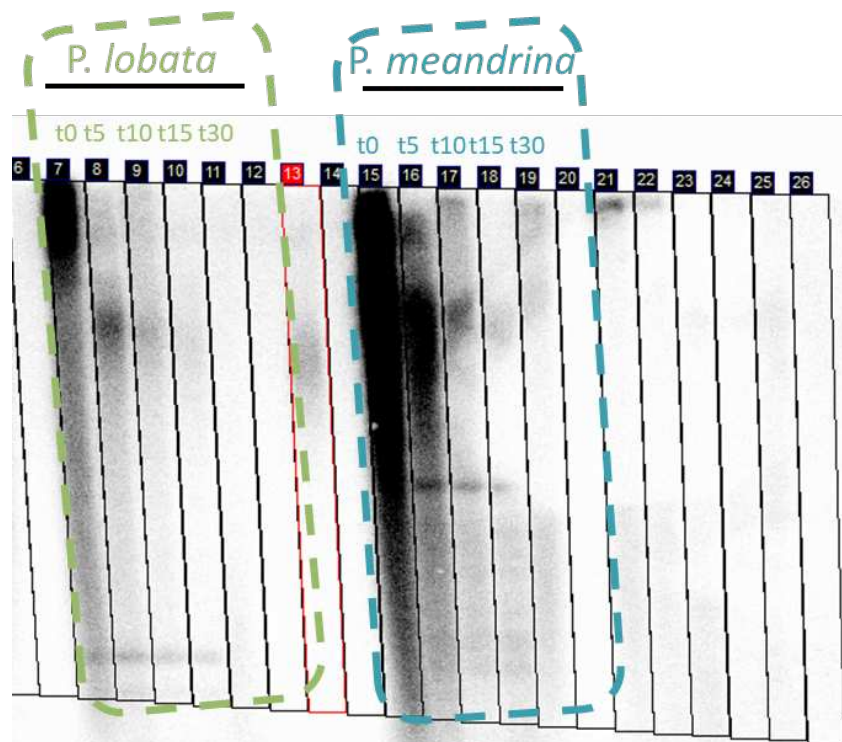
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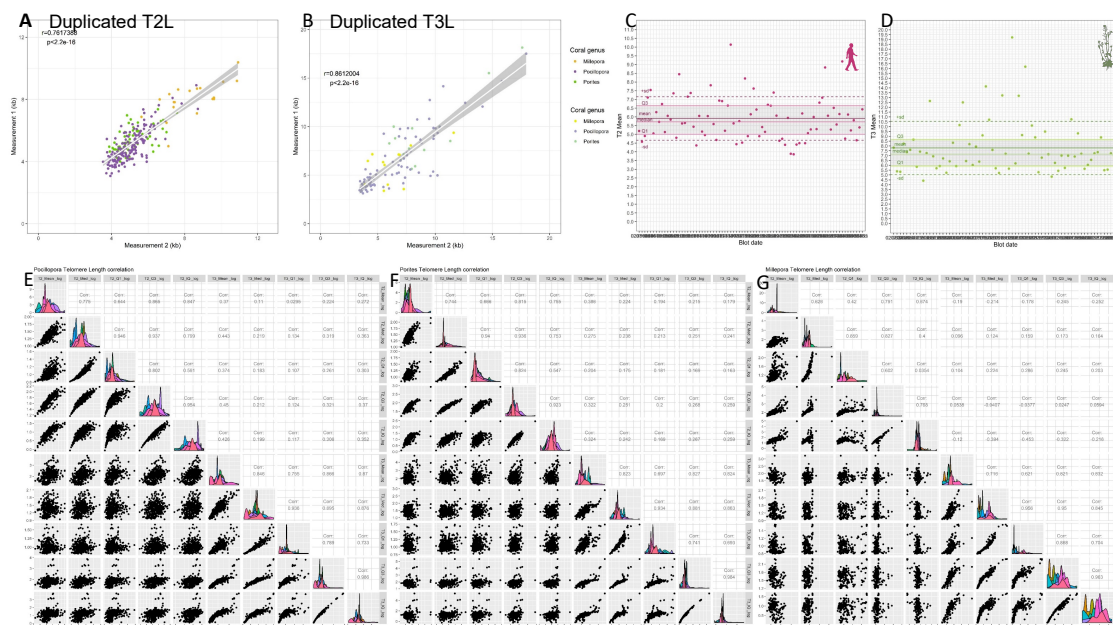
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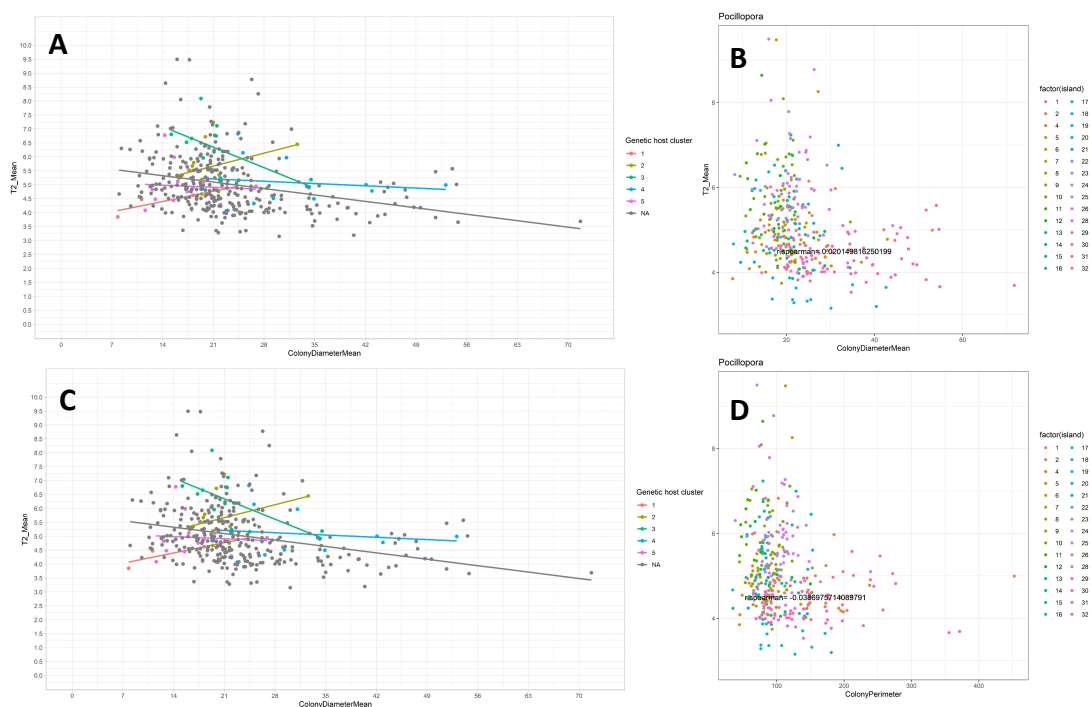
## Supplementary Figures



**Supplementary Figure 1: Bal31 Telomere restriction fragment assay** and . For each lane 5µg of DNA was digested by the exonuclease Bal31 for (t0, 5, 10, 15 and 30 minutes). Reaction was stop, DNA precipitated and samples were digested by RsaI and HinfI to be run in a TRF. Results show a decrease in the T2L smear with the increase of incubation time with Bal31, the smear signal is therefore a telomeric signal.

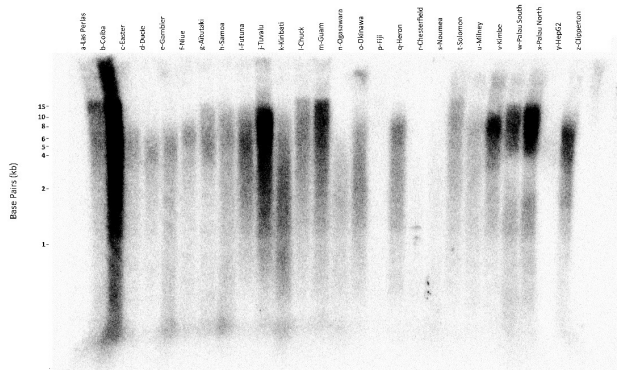


**Supplementary Figure 2: Duplicate correlation and host symbiont correlation in the 3 genera.** (A) Host duplicate mean TL measurements spearman correlation in host and symbionts (B).Correlation matrix between host (T2) and symbionts (T3) TL log(measurements) (mean,Med : median, Q1 : 1st quartile, Q3 : 3rd quartile, IQ : interquartile distance) in *Pocillopora* sp. (D), *Porites* sp. (E) and *Millepora* sp. (F) with correlation plot in the left half, correlation distribution colored by island in the middle line and correlation coefficient in the right half .

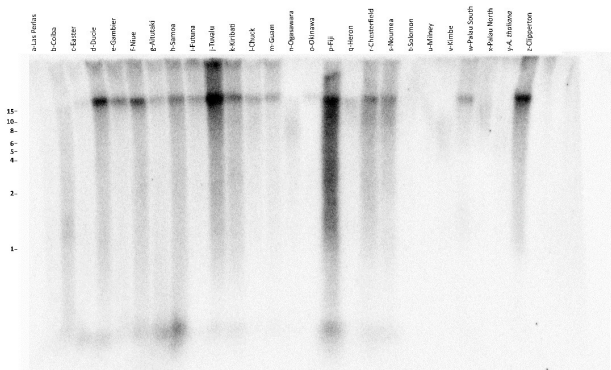


**Supplementary Figure 3: Mean TL of *P. meandrina* correlation to colony mean diameter (A,B) and colony perimeter (C,D).** (A,C) displays the mean T2L depending on the colony diameter or perimeter, colors are showing the known genetic clusters. (B,D) displays the mean T2L depending on the colony diameter or perimeter, colors are showing theislands and the correlation coefficient of Spearman is displayed on the graph.

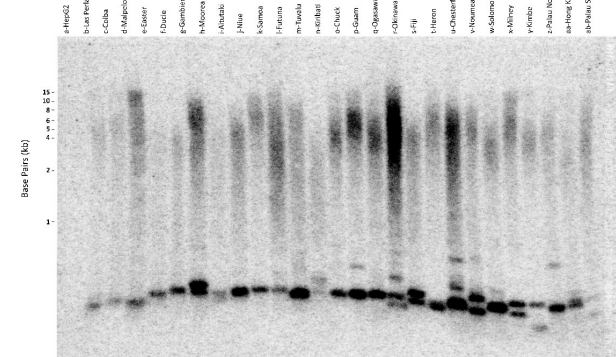
***Pocillopora* sp.**  
(TTAGGG)<sub>n</sub>



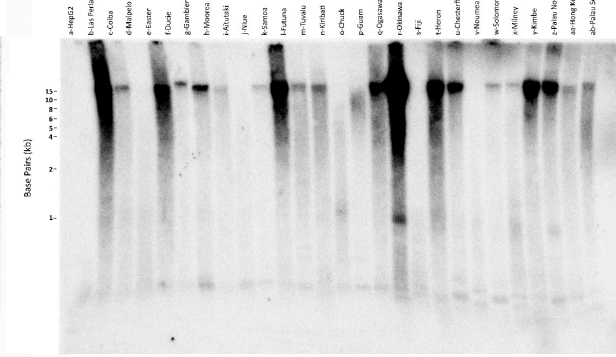
(TTTAGGG)<sub>n</sub>



***Porites* sp.**  
(TTAGGG)<sub>n</sub>

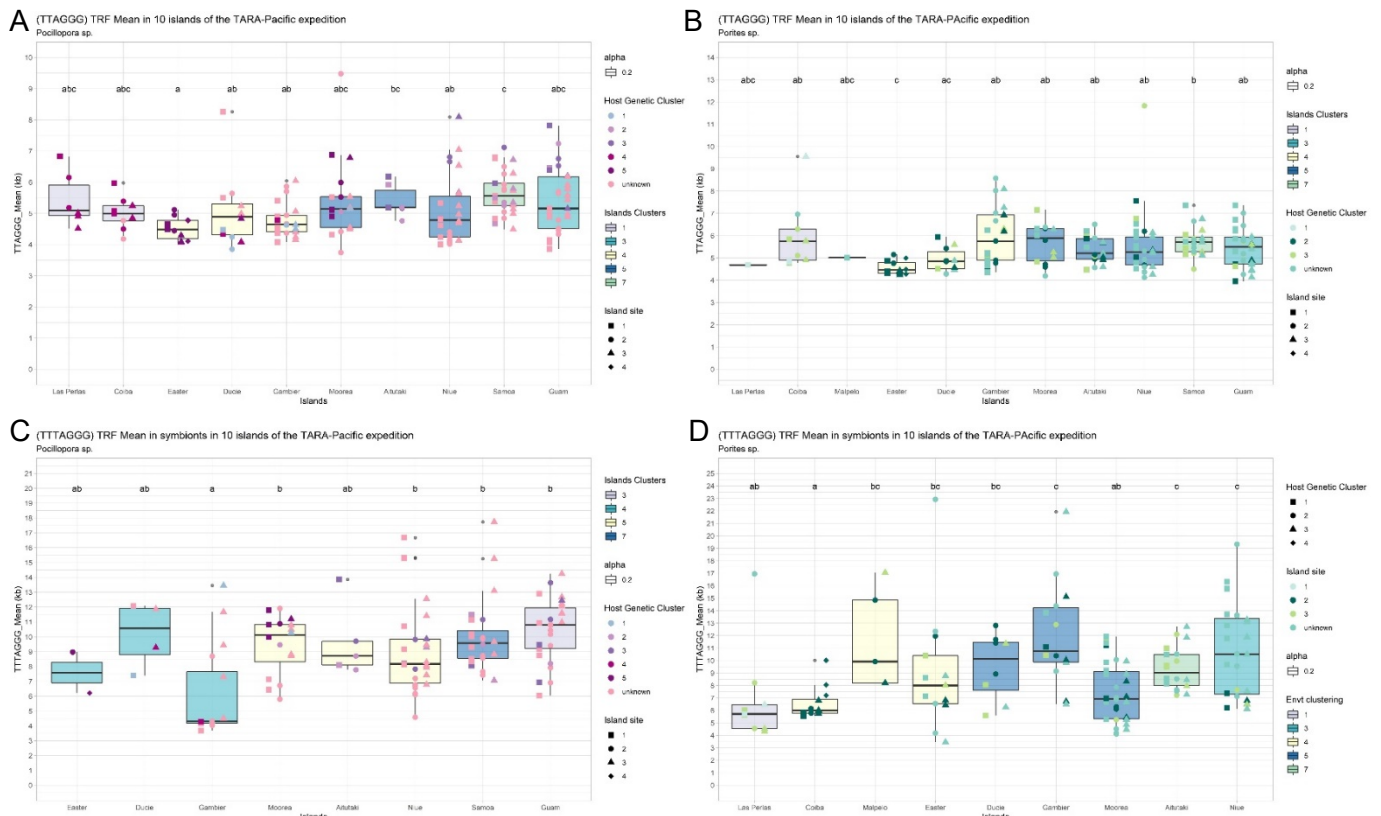


(TTTAGGG)<sub>n</sub>

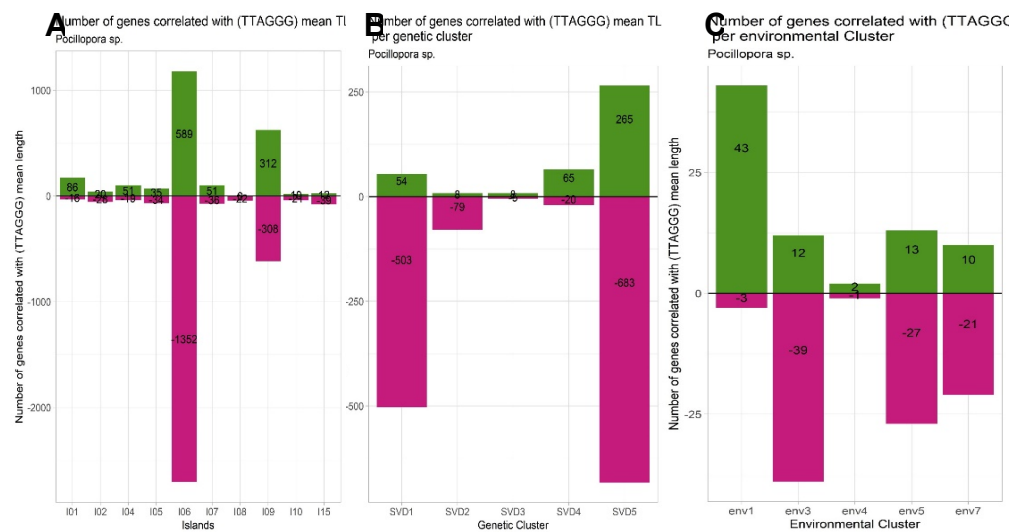


Supplementary Figure 4: **Telomere Restriction Fragment (TRF)** of (A) *Pocillopora* sp. coral samples from 25 islands of the TARA Pacific sampling (a-x,z), and two reference sample from human cells and *A. thaliana* (y), hybridized with the coral telomere sequence (TTAGGG)<sub>n</sub> (left) and the symbionts sequence (TTTAGGG)<sub>n</sub> (right). (B) of *Porites* sp. coral samples from 27 islands of the TARA Pacific sampling (b-ab), and two reference sample from human cells and *A. thaliana* (a), hybridized with the coral telomere sequence (TTAGGG)<sub>n</sub> (left) and the symbionts sequence (TTTAGGG)<sub>n</sub> (right).





**Supplementary Figure 6: Boxplot of mean T2L and mean T3L in 11 islands.** (A) Mean T2L of *Pocillopora* sp. in the 11 islands investigated for now at the genetic level. Environmental clustering is depending on the boxplot colors, sites sampled within one island are displayed with differential shapes. Each dot represent an individual and the color represents the host genetic cluster. (B) Mean T3L of *Pocillopora* sp. (C) Mean T2L of *Porites* sp. (D) Mean T3L of *Porites* sp. significant differences based on a pairwise Wilcoxon test are displayed with letters. Islands sharing at least one later are not significantly different from each other.



**Supplementary Figure 7: Number of genes correlated with T2\_Mean in Pocillopora sp.** within (A) islands, (B) genetic clusters and (C) environmental cluster. Significant spearman correlation ( $r > |0.7|$  &  $P < 0.01$ ) are displayed depending on their positive (green) or negative (pink) sign.

# Supplementary Tables

## ResultsMCA

levels	variables	dataset	Figure	Dim 1	Dim 2	Dim 3	Dim 4	Dim 5	Dim 6	Dim 7	Dim 8	Dim 9	Dim 10	Dim 11	Dim 12	Dim 13
Millepora	genera	full	Fig2.A	6.38930897	3.58695968	0.01265001	1.40293821	0.61744894	0.54598647	0.3275395	1.85198361	0.29493967	0.38224604	0.47849547	0.40553636	0.35300911
Pocillopora	genera	full	Fig2.A	6.69029479	1.57336888	0.0263733	0.41660912	0.16511826	0.67072971	3.53819171	0.33382509	0.20250514	7.77076098	0.05536445	5.50411038	4.38444388
Porites	genera	full	Fig2.A	0.90894184	0.00391828	0.06229794	0.03331317	0.02063934	0.09963436	5.51302623	2.46811447	11.0483444	0.06388536	3.85919139	3.00400403	
	1 envtal cluster	full	Fig2.A	0.23495847	0.14657341	0.20627789	0.13924317	0.00782858	0.00416443	0.15285556	6.97171783	0.37994318	8.48091228	6.71092189	11.135158	1.085469
	2 envtal cluster	full	Fig2.A	0.06801083	0.00866992	0.17931368	0.30387651	0.01487527	0.00283241	0.13211551	0.13858784	0.04214507	0.00449958	32.683857	14.3023976	11.93879
	3 envtal cluster	full	Fig2.A	0.00662769	0.63795546	0.23749306	0.23785467	0.00617723	0.03503237	0.39726809	7.68666246	0.08562263	1.65209259	0.11347151	2.32032375	4.20019644
	4 envtal cluster	full	Fig2.A	0.42615997	0.25870232	0.18838613	0.0068514	0.14300242	0.13691919	0.35022641	0.08152543	0.45491229	0.73267294	6.36829809	0.57401234	9.84928382
	5 envtal cluster	full	Fig2.A	0.1455002	0.00050216	0.18319435	0.23875487	0.07580792	0.08503007	2.86237466	0.84163954	14.7116779	1.08422656	6.37004270	9.92686869	0.14846899
	6 envtal cluster	full	Fig2.A	0.1279918	0.00011783	0.20825931	0.31813458	0.29455091	0.01425697	13.849928	0.2805306	1.22222995	0.97648285	2.99656034	0.11099519	7.6406162
	7 envtal cluster	full	Fig2.A	0.19026034	0.05183926	1.21774242	0.51768386	0.00112917	1.15771735	2.68286514	3.05882765	2.99999738	2.99950627	9.4467732	13.573886	0.01047366
	8 envtal cluster	full	Fig2.A	1.02253551	4.89296485	0.00946496	0.63159406	0.35497076	0.28002251	1.38652512	0.32890637	0.55319972	2.93343559	2.12327452	2.20251456	7.75335236
	9 envtal cluster	full	Fig2.A	0.09866013	0.23288677	1.49929438	0.10491357	1.77345591	2.80376597	8.56709009	6.69384368	11.1359753	0.39798859	0.00349991	0.96460885	1.47708229
inside	position%lagoon	full	Fig2.A	0.04242001	0.22469382	0.74916666	0.03691967	0.66148716	1.81668504	23.3240413	2.67098477	10.4722163	0.99893212	1.45831567	1.51806769	0.57978665
outside	position%lagoon	full	Fig2.A	0.00506086	0.02982405	0.09948336	0.00490042	0.08780045	0.24113216	3.09584569	0.35452504	1.38999778	0.13250022	0.1935651	0.2014961	0.07695622
[2.26,2.66]	T2_Q1	full	Fig2.A	0.04279791	7.95310468	0.61325544	3.3199373	0.00176017	0.89611688	0.465360	0.38603853	0.46709853	0.09644331	2.42550733	0.2916556	2.97600655
[2.66,2.87]	T2_Q1	full	Fig2.A	0.59908585	0.21458913	0.36756078	2.71771156	1.65542491	4.54547564	2.69914514	0.01485448	17.2115023	2.55978544	0.08120296	0.31605404	2.43681989
[2.87,3.20]	T2_Q1	full	Fig2.A	0.28399618	0.97205633	1.29519272	1.39543642	1.08874404	2.23254237	0.45508529	10.9517952	7.89404817	1.66626328	0.14628322	0.45611807	1.28295701
[3.20,3.43]	T2_Q1	full	Fig2.A	2.09291348	4.63592545	0.88591496	0.93723978	0.04691264	0.14385194	0.04948865	7.73266476	0.21506328	0.00492259	2.54683114	1.68952393	0.86641061
[3.21, 5.09]	T2_Q3	full	Fig2.A	0.02711184	9.47366051	0.39174581	5.71687325	0.01699501	1.0901357	0.00121745	0.03158255	0.06834322	0.3388495	0.11742919	0.08020391	1.09404921
[5.09, 5.89]	T2_Q3	full	Fig2.A	1.48102623	0.33897072	0.32730466	5.08392672	3.58253775	10.2002591	0.56914846	0.00698526	0.95742773	0.07495969	0.22659936	0.06152466	1.53801886
[5.89, 6.94]	T2_Q3	full	Fig2.A	0.70801684	1.57402325	0.61634904	3.51472545	2.35277026	7.68274006	0.20024397	0.01190519	1.04468427	0.20303851	0.05168124	0.17532049	0.6785591
[6.94,149.29]	T2_Q3	full	Fig2.A	4.77051572	5.73589716	6.5018128	2.70512804	0.63265918	0.63155656	0.05455371	0.0001238	0.12912403	0.60924498	0.01423763	0.22219103	0.48924687
[3.34, 6.01]	T3_Mean	full	Fig2.A	7.48411113	1.54858299	0.05620876	1.7284194	0.08231523	0.0280369	0.14993931	0.02631143	0.00818527	0.04214014	0.00342587	0.89591216	0.39781674
[6.01, 7.65]	T3_Mean	full	Fig2.A	0.01197897	0.00634256	3.72728389	1.86216291	3.3118732	0.02881279	0.44185462	0.04501077	0.07787797	2.33379546	3.37796692	4.12983863	5.67600228
[7.65, 10.42]	T3_Mean	full	Fig2.A	1.73560351	0.03356671	0.52707658	0.10271997	1.10713135	1.206605	0.007921	0.12917026	1.04036464	3.74862087	10.7021243	5.37544014	1.264908
[10.42,121.11]	T3_Mean	full	Fig2.A	2.34463407	0.96492075	6.69647095	0.26022281	0.00995568	0.5814818	0.03569506	0.54087414	0.42500916	0.82746762	1.95246911	1.2474772	0.39353234
[2.45, 3.45]	T3_Med	full	Fig2.A	8.54370751	2.00898685	0.20347597	3.48194812	0.84372003	0.00717801	0.00814506	0.00305299	0.01713652	0.21475927	0.00271699	0.03814407	
[3.45, 3.97]	T3_Med	full	Fig2.A	0.19040401	0.02324374	0.96817801	0.03799503	0.99371565	2.53919806	0.57012094	2.53537422	0.12682935	2.49348986	0.36251877	0.24755008	0.09436946
[3.97, 4.58]	T3_Med	full	Fig2.A	0.86389816	0.24505172	0.43179968	0.09320937	7.24667874	2.66023541	0.69387038	4.02584495	0.49632378	5.9388435	0.10490266	1.41122597	1.45076908
[4.58,18.19]	T3_Med	full	Fig2.A	2.43573006	0.59475218	10.2914681	0.12453581	1.90180186	0.01501571	0.00015256	0.11294955	0.1992173	1.82314308	0.10936416	2.32355426	0.49502272
[0.838, 2.50]	T3_Q1	full	Fig2.A	8.10022654	1.9082523	0.21061487	2.81183684	7.0681155	0.0737105	0.33872722	0.07836152	0.06861468	0.07171394	0.06156355	0.00239069	0.13555336
[2.504, 3.22]	T3_Q1	full	Fig2.A	0.01751945	0.19599528	0.677148293	0.59123986	2.71445879	3.16113107	1.84677636	0.01543664	0.00778061	2.74727465	0.00082404	0.0320927	0.35345286
[3.220, 4.02]	T3_Q1	full	Fig2.A	1.63388056	0.04196819	0.65973743	2.32216696	6.73614855	3.6890914	4.87707991	1.71535815	0.74113793	5.84933557	0.07133669	0.0062686	1.13127409
[4.024,205.51]	T3_Q1	full	Fig2.A	2.90393891	0.51410936	8.77499354	0.38110675	5.6530287	0.17060796	0.05841251	0.82374937	0.47431317	0.35723169	9.52E-05	0.86729767	0.68346441
[2.182,54]	T3_Q1	full	Fig2.A	7.99376663	1.70821188	0.1363413	3.39735284	0.89453467	0.00176381	0.00284386	0.00575567	0.06136262	0.15185766	0.02316407	0.06997787	0.0045964
[2.542,74]	T3_Q1	full	Fig2.A	0.33347455	0.05499308	7.28261766	0.02052167	0.39807003	1.97292662	1.14013931	3.88953973	0.02551753	4.86731096	0.20478625	1.53899555	0.09944609
[2.742,98]	T3_Q1	full	Fig2.A	0.98402455	0.02589476	0.3895102	3.78103261	3.98142867	2.775257	1.38842486	6.02305137	0.08754448	9.81424332	0.02841932	0.19486713	2.63189164
[2.985,575]	T3_Q1	full	Fig2.A	1.5894472	0.82957347	8.76236939	0.00176841	1.94655531	0.04831361	0.0269758	0.16569706	0.01250644	1.74045193	0.22086612	4.10873039	1.89858533
[3.08, 5.13]	T3_Q3	full	Fig2.A	8.38610702	2.05481629	0.20266182	4.04324998	0.95625355	0.03943431	0.19005337	0.01082975	0.00793751	0.07466804	0.05475134	0.01751156	0.18192095
[5.13, 6.00]	T3_Q3	full	Fig2.A	3.70E-05	0.12744074	8.33361236	0.77213281	8.27914763	3.48899425	0.07139485	0.11424874	0.01394086	1.53040634	0.03205125	0.04522337	0.64461113
[6.00, 7.03]	T3_Q3	full	Fig2.A	1.40047092	0.20279995	0.83305153	2.7648163	9.71910064	5.42630457	2.1440684	8.82212604	0.43411056	2.78467982	0.36574884	0.06732399	0.51340145
[7.03,21.18]	T3_Q3	full	Fig2.A	2.92503933	0.39384331	11.2602789	0.28292974	0.49049041	0.4376962	1.11865877	0.45468723	0.1981018	1.36E-06	0.03693461	0.00734416	0.11624009
[2.88, 6.47]	T2_Mean	full	Fig2.A	0.09506906	8.33078598	0.65708637	3.16185767	0.28511681	0.08348959	0.54414988	0.22665588	0.01432793	0.00505162	0.45440761	0.01248298	0.78346441
[4.67, 5.46]	T2_Mean	full	Fig2.A	1.37656848	0.1121305	0.152759139	4.85744538	0.514105	3.13046283	2.03580746	1.02557961	2.19386696	0.69590041	0.07614447	0.96962226	0.23234452
[5.46, 7.22]	T2_Mean	full	Fig2.A	0.7963074	1.11853983	0.01903646	0.72934547	1.18957351	4.51363262	0.40193312	6.16588945	1.14593335	2.4628422	0.82363588	0.18948207	1.68632143
[7.22,96.65]	T2_Mean	full	Fig2.A	5.62358826	4.75167121	0.11552688	1.55279288	0.66888454	0.47117351	0.00920666	1.04898864	0.0692546	0.30882179	0.00138434	0.41845187	0.13689778
[2.61, 3.63]	T2_Med	full	Fig2.A	0.02751231	0.22235359	0.53186474	0.9354008	0.00423983	1.18509445	0.30832635	0.14976382	0.18467465	0.02479496	0.87066825	0.25100588	0.88536267
[3.63, 4.10]	T2_Med	full	Fig2.A	1.12255516	0.2916403	0.42276092	4.27937288	2.58428443	8.77386073	0.13655531	0.25071049	5.66602321	2.05264377	0.29205407	0.47317456	1.72746322
[4.10, 4.78]	T2_Med	full	Fig2.A	0.540064	1.32447966	1.13571729	2.87444338	2.82136887	5.25801408	0.14695959	7.56018175	2.93124102	1.46459608	0.4426452	2.18851982	8.00277079
[4.78,73.87]	T2_Med	full	Fig2.A	3.54470966	5.5329738	0.86614652	2.05167839	0.08248816	0.13536522	0.00086868	3.49770251	0.01326581	8.06E-05	1.14128904	1.46206867	
[0.681, 2.32]	T2_Q1	full	Fig2.A	0.06405636	6.5941441	0.40162739	5.01778225	0.02309073	0.75060007	0.15144828	0.05564868	0.00902644	0.09841328	1.83251543	0.0553187	2.44990291
[2.324, 2.87]	T2_Q1	full	Fig2.A	0.8101625	0.57438064	0.20544732	3.01387038	2.23744483	7.1042817	2.67480651	0.09331787	3.27602866	0.07555033	1.12450205	0.02883654	4.63567207
[2.874, 3.65]	T2_Q1	full	Fig2.A	1.00526137	1.3888448											

## Chapter VII-Discussion and Perspectives

Since the discovery of its shortening with cell doubling in culture, telomere length and moreover TL attrition rate had been highly investigated as a predictor of lifespan and a hallmark of ageing (Hayflick and Moorhead 1961; López-Otín et al. 2013). Indeed the highly conserved mechanism of linear genome replication is unable to replicate DNA extremities (Lingner, Cooper, and Cech 1995) leading to chromosome extremities shortening at each round of the cell's division. This unalterable but highly conserved process that eventually leads to cell death is tickling our understanding of the benefits of such a mechanism in terms of evolution. Among the several hypotheses discussed in the field one is based on the tradeoff between cell proliferation and increasing lifespan and cancer development. Telomere attrition, in humans, would result in organismal ageing but at the same time prevent cancer (Aviv and Shay 2018). Another theory based on the idea that telomere attrition is an adaptive strategy is based on the life-history regulation with telomeres accumulating loss under external stressors enabling the organism to adapt based on his resources (A. J. Young 2018). The contribution of environmental changes, lifestyle and genetics to TL dynamics remains debated but is of importance to tackle the ancestral function of this process and its evolution. Most of the research effort in that field had been focused on mammals and vertebrates, were some organism are already challenging the dogma of telomere length being the predictor of the time remaining to a cell, but to understand evolution of telomere in animals including more basal metazoan is needed.

In this work we presented the first extensive study on natural TL variation in corals which is also the first extensive study of natural TL variation in non bilaterian metazoan, as well as results on TL dynamics with age and different environmental conditions in corals.

We confirmed that additional coral species have the same telomeric DNA sequence as humans (TTAGGG)<sub>n</sub> which was reported in previous works for other coral and cnidarian species (Traut et al. 2007; Sinclair, Richmond, and Ostrander 2007; Hiroki Tsuta et al. 2014; H. Tsuta and Hidaka 2013; Zielke and Bodnar 2010).

We found that the presence of interstitial telomeric sequence was conserved in 4 coral genera. Such interstitial sequence with telomeric repeats had been hypothesized to be the result of ancient chromosome fusion. However, the number of short ITSs and their position within a coding sequence are less likely to have arisen from such an event. Some are more likely found at fragile but their repetitive structure is increasing the susceptibility to breakage and it is not clear if ITSs themselves do not participate to the frailty of the site they are found in (Ruiz-Herrera et al. 2002). Finally, other ITSs are known to result from insertion during DNA double strand repair (Ruiz-Herrera et al. 2008). The high occurrence of mosaicism in corals could explain the higher proportion of short ITSs compared to humans, and the fact that *Porites lobata* genome is proportionally containing more ITSs than *Pocillopora meandrina* or *Stylophora pistillata*. Which is questioning about a greater compliance to DNA double strand events and mosaicism in complex corals compared to robust (Maier et al. 2012; Devlin-Durante et al. 2016).

But the finding that, in 3 coral genera, ITSs are associated with genes sharing common GO-Term as humans ITSs associated genes is puzzling. Indeed, the associated genes turned out to be different in each species which, if the synteny is not conserved, suggests that some ITSs might be conserved because of their function related to their associated genes and that their insertion within the genome is meaningful.

In corals it would be interesting to see if ITSs are conserved within polyps of the same colony and if mosaicism concerns also the length, positions and numbers of those sequences and be therefore associated to a broader and shorter term adaptation. Somatic mutations and genome insertions is often conceptualized as a detrimental event and indeed it can be with for example, in population of short lived killifish, the relaxation of pressure selection that is increasing the genome size by being more permissive to insertion and mutation in a deleterious manner (Cui et al. 2019). However in corals, because they don't invest extensively in sexual reproduction and that success rate are low, because they release their gametes in the water columns but they can't mate to increase the fertilization rate, their high level of mosaicism had been proposed to be a compensatory mechanism for enhanced diversity, adaptability and fitness (van Oppen et al. 2015). Whether such a mechanism implying a correct balance between the rates of deleterious and beneficial mutations requires specific controls of DNA repair mechanisms is unknown.

An interesting observation is the fact that the telomere associated proteins, the shelterin subunits, are conserved among a huge variety of animals, plants, fungi, suggesting an ancient function and that DNA repair protein evolution is often linked to lifespan characteristics. For example long lived tortoises have specific SNPs within XRCC6 (Quesada et al. 2019), long lived bats have evolved a XRCC5 (KU80) (Foley et al. 2018) which encode an ATP\_dependent helicase II involved in DNA repair. We found a highly conserved sequence corresponding to Pif1 helicase family in *S. pistillata* and in (Bhattacharya et al. 2016) they stressed out the specific conservation between choanoflagellate, sponge and anthozoa of a class of DNA mismatch repair protein mutS from which one of them is overexpressed in older adult of *S. pistillata* exhibiting long telomeres. In long lived bats *Myotis myotis*, an overexpression compared to other species of 2 DNA repair protein ATM and the helicase SETX was found (Foley et al. 2018) as well as a high expression of the shelterin POT1. In this work we observed that *S. pistillata* POT1 was downregulated in bleached samples which had significantly shorter TL than the controls. We were not able to find a TL correlated expression of POT1 in *Pocillopora sp.* in the TARA-Pacific 10 first islands which can be a piece of evidence for a differential telomere maintenance strategy in 2 closely related coral species. It can also be due to a lack of islands with short TL population and the full transect comparison will allow to answer this issue. An interesting island to investigate in terms of transcriptomic outcome and telomeres for *Pocillopora sp.* would be the Fiji island for which telomere smear was not observed for several samples, explaining the low number of observations, recalling the stress measurements problem experienced in the coral population from this island in (Foley et al. 2018; Mayfield, Chen, and Dempsey 2017).

Overall this results suggests that corals might have evolved complex DNA repair machinery to balance the need of an efficient asexual reproduction, or of a intracolony phenotype diversity, to improve colony's survival in case of a changing environment, and a long lifespan for a highly proliferative organism (Lecointe et al. 2013, 2016) involving gens from the mutS family and the Pif1 family.

Another function usually found to evolve along with longevity is the innate immune system like in bats, to cope with high viral infection (big up SARS-CoV1,2 and all the others) due to promiscuity (Gorbunova, Seluanov, and Kennedy 2020), but downregulation of innate immune system has also been reported in long lived bowhead whales (Keane et al. 2015). Coral innate immune system evolved by untighting to allow the setting up of symbiosis (S. K. Davy, Allemand, and Weis 2012). Another side of that coin could be a higher sensitivity of corals to pathogens (Weynberg et al. 2015) and coral microbiota, either at stress (Zaneveld, McMinds, and Thurber 2017) or during aging, gets out of their immune system control. However long lived and resilient species such as *Porites porites* were reported to have a higher immune response which leave the question of a positive effect of lower innate immune system in coral unsolved (Pinzón C et al. 2014).

## Coloniality and clonality

Most corals are colonial individuals and it was suggested that the budding formation of new polyps, considered as asexual reproduction, would grow a younger polyp as well in biological age as in chronological age. Here we showed that in 3 coral genera no significant differences were found between the growing area of a branch, the tip or apex, and a chronologically older part located basally in the branch. In (Hiroki Tsuta et al. 2014) they found a decreasing trends of TL, with the borders of *Acropora hyacinthus* colonies being shorter than the center of the colonies. This result recall the experiment on *Pocillopora* sp. branches, with a non significant increasing trend from the apex to the base of the branch. It would be worth investigating the trend of TL as it would be of great interest to understand molecular coral growth. Indeed, if it appears that newly formed polyps have shorter TL because of high proliferation required for their growth, as it is suggested by (Lecointe et al. 2016) where they highlighted that primary polyp pharynx and gastrodermis proliferation was higher than in adult colony or than at early metamorphosis or larval stage in *Pocillopora damicornis*, it would mean that a telomere lengthening mechanism would have to take place to counterbalance this loss. We have not found in any of the transcriptomic analysis performed a significantly differential expression of telomerase, TERT, linked to telomere length and even if the ribonucleotide reverse transcriptase activity cannot be excluded based on those clues it could be possible that TL lengthening in corals happen through an alternative mechanism (ALT) involving maybe POT1 or specific DNA repair proteins.

## Symbiodiniacea Telomere length

Symbionts TL was weakly correlated to host TL (ChapterV, SupFig2) whereas symbionts cell proliferation is proportional to the host one, suggesting a control of the host over symbionts proliferation (Lecointe et al. 2016). It was not significantly different between host species but some evidence in *Porites* sp. suggests that TL can be different between *Symbiodiniaceae* genus which is in line with previous reports of chromosome number variation (Blank and Trench 1985). Moreover some environmental parameters were specifically correlated to symbionts TL in *Porites* sp. coupling variables linked to aerosols dynamic (Flores et al. 2020). TL was increased when sea water Manganese concentration was higher, Mn cluster is known to be required for the function of the oxygen-evolving complex of photosystem II and possibly stored in cellular vacuole (Lanquar et al. 2010). This increase of Mn in the surrounding water could be due to an increase of free-living photosynthetic organisms, but the correlation with symbionts TL raises the question of a putative link. If it exists, it could be either because of a low photosynthetic activity of symbionts which would decrease the sea water Mn uptake by the host and by producing less ROS would leave the symbionts telomeres undamaged, it could also be due to a daily turnover to replace exhausted symbionts by new ones with longer TL, releasing symbionts that would degrade in the surrounding water.

It is noteworthy that those correlations were only observed for *Porites* sp. symbionts TL, and not in *Pocillopora* sp. ones.

On one hand *Porites* sp. are slow growing species compared to *Pocillopora* sp. which could comply with the first hypothesis of a lower photosynthesis rate, and indeed *Porites* sp. symbionts have longer TL than *Pocillopora* sp. or *Millepora* sp. one (ChapterV-Fig1Gb). On the other hand, *Porites* sp. are more resilient to bleaching than *Pocillopora* sp. which can be due to an easier reestablishment of the symbiosis and could be an argument to investigate the hypothesis of a daily turnover of symbiotic populations in *Porites* sp.

#### Natural TL variation and environment

Because corals are fixed animals and therefore cannot escape predation, or stressing conditions, they are specifically sensitive to environmental change. We were interested in knowing to which extent the environment could leave a print on coral telomeres. We found that acidification is unlikely to have effects on coral telomeres as we couldn't observe TL differences in two species from different lineages, *S. pistillata* and *Acropora* sp., over a period of 2 years in various triplicate controlled experiments and in natural conditions. But the results from (Liew et al. 2018) showing that calyxes were bigger hosting polyps with bigger cells in acidified conditions, linked to one experiment where we saw significant differences between control and pH 7.2 condition, raised the question of a slight impact of acidification. If the cells are proliferating less in acidified condition it could lead to less telomere attrition in those coral leading to longer TL, like we observed in the second experiment. This question could be tackled by sampling the colonies prior the experiment to be able to compare the TL dynamics of controls over the experiment timescale.

A first attempt to address heat stress impact on TL on *S. pistillata* and *A. digitifera* was showing an opposite significant linear relationship between the 2 species. But the small absolute length differences between the treatments at each time point as well as the lack of experiment replication pushes us to be cautious with this conclusion. Nevertheless, it would be worth repeating it including longer time points (at least 6 months rather than 3), different settings (including branch and colony settings) and testing the impact of fluctuation around the species heat tolerance threshold.

Dark induced bleaching was having a significant effect on TL in the bleached colony compared to control one's after 6 months. However, the introduction in the duplicate experiment of a time point at the beginning of it, raised the question of the effect of the cut of the colony as bleaching could prevent a proper response of corals to the stress induced by the colony split and breakage. Indeed, the observation of an important lengthening in older adult TL of *S. pistillata* and the huge deviation between their branches made us wonder if repeated breakage is not impacting TL.

Indeed, the adult colonies of *S. pistillata* are the colonies used to set up experiments which implies that they are frequently sampled and cut. We were wondering if breakage would induce a change in mean T2L and we tested it by cutting branches anchored to the same part of the colony, in 5 different areas. We extracted DNA before the experiment, after 10 days of recovery, 20 days, 30 days and 50 days' post breakage. Unfortunately, the DNA quality from this experiment was not good enough to ensure some proper TL measurements but it could be of importance to reproduce these settings. Addressing this question is a central point, on one hand to take note of the impact of breakage on the branches (nubbins) that are common in experimental settings to describe coral physiological response, on the other hand because coral fragmentation is already used in restoration programs to multiply colonies and restore damaged reefs. Finally, the incidence of storms, hurricanes and typhoons is predicted to increase and be more violent in the decades to come and could damage the surviving corals at the chromosome level. If coral fragmentation is impacting TL dynamics it could influence its asexual reproduction strategies and its long term survival. Altogether it makes this topic an unanswered burning issue.

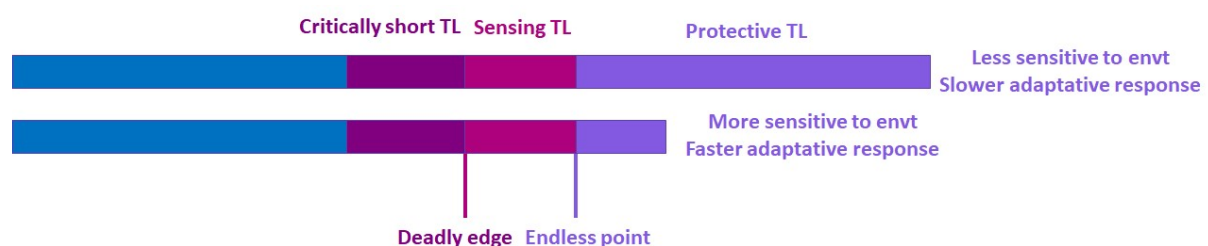
Studying the natural TL dynamics of 3 coral genera we discovered different dynamics of TL variations depending on the coral species and that could be linked to their life strategies. Yet the only common behavior between their TL variation was the negative correlation with high past seasonal change. The same results were found in a longitudinal study in bats (Foley et al. 2020) where unexpected significant TL change from one recapture to another was more correlated to seasonal deviation than inheritability. Field longitudinal study would be priceless to tackle some unanswered questions and even if sessile life mode could be detrimental for coral it makes them easier to trace.

At the crossroads between short and long: the endless point

Altogether our results suggest that TL are more sensitive to environment than age in corals and that corals developed mechanisms to maintain TL around a short size (4-5kb), like in the annelid *Aeolosoma viride* in which TL do not vary with age nor regeneration and is around (3kb) (C.-F. Chen et al. 2018). Some studies in mice reported that recently introduced wild strains had shorter TL than laboratory established mice from the same species (Hemann and Greider 2000) and that inbred mice population had longer telomeres than outbred ones (Manning et al. 2002). This results are not only underlying that an established and inbred laboratory population can mislead some assumptions on general biology and that input from wild populations is mandatory to properly understand the evolutionary role of mechanisms but they also raise the question of a positive selection of short TL in wild populations.

Telomere field had been focusing on long term effects on telomere and its link to lifespan, representing telomere as the level of time left to an organism. Telomere decrease over a lifetime has been extensively described as linear but recent development and studies are revealing that telomere length might fluctuate in a broader range than expected and over a short period of time. It is debate to assess if some TL variations over a short time is due to measurements methods variability or cell population turnover, like in leukocyte cell poulations that are used to assess TL in a lot of studies. But it is not to exclude that maybe TL could change in a shorter timescale than what is admitted nowadays. Adding that short telomeres seem to be more selected in wild populations and that between human and corals, ITSs are associated with extracellular matrix and signal sensing genes it could be possible that TL dynamics is under two regulation mechanisms. A long term one that has been described extensively and a short term one. Here I would like to propose a hypothesis on an ancestral functions of telomeres linked to a short term regulation and an environmental sensing function. Because telomeres are conserved in unicellular organisms that are very sensitive to environment, not being protected inside a multicellular structure, the ancestral telomere function could be to act as an environment sensor that would depending on its size trigger a positive adaptative response by inducing changes in the chromatin structure, like the Telomere Position Effect, or by releasing shelterins that would act as transcriptional effectors or upstream regulators of signaling pathways.

This size dependent theory needs the introduction of an equilibrium size, an 'endless point'. It was demonstrated that a short size of telomeres, called 'critically short' was triggering the DNA damage response and leading to the cell growth arrest (Abdallah et al. 2009). It could be that upon a change in the environment telomeres shortens to a size that would be longer than the critically short but shorter than the 'mute' telomere size, triggerering a positive adaptative response, before being elongated to come back to the 'mute' size. This theory involved that having short telomeres with a size that is close to the positive sensing length is better to sense faster the environmental changes but also because it would require a short lengthening, or by a low level of telomerase or by some ALT mechanisms. On top of that the well established long term effect of TL with a shortening upon cell division would take place and lead to cell growth arrest.



## Methods to measure telomere length in corals

Telomere restriction fragment assay remains the gold standard method in the field of telomere but is nonetheless a long and complex method. It requires a lot of DNA and specialized machine devices and radioactivity equipments. The correlation between measurements and duplicate is correct (0.76 for T2L and 0. for T3L) but it is not optimal either. Quantitative PCR methods adapted to telomere length measurement (Cawthon 2002) have the advantages of requiring less DNA, being faster (few hours against 2 weeks for T2 and T3 hybridization), requiring classical laboratory equipment and being easily reproducible. However, this technique that is amplifying telomere sequences is giving an amount of telomere sequence that needs to be related to the copy number of the genome. To do so one could use the DNA quantity as a normalisation, with all the variation problems in measuring precisely DNA quantity, or find a gene with a fixed and known number of copies to perform in parallel a quantitative PCR on genomic DNA to quantify the number of genome copy in the sample. In coral measuring DNA quantity was not an option as we extracted symbionts and coral DNA at the same time which results in a mixture of both DNAs in variables and unknown proportions from one sample to another. This method is way more used in large scale studies but remains nevertheless criticized for its lack of reproducibility and repeatability (Nussey et al. 2014). We successfully adapted it to measuring T2L and T3L but we faced two major problems that prevented us from going forward with this technique. When I started my PhD we didn't had a single copy number unique gene for corals neither for the symbionts and we were looking for a gene that was specific to scleractinian, to avoid measuring symbionts gene depending on the primers specificity, that was common to different genera (*Porites sp.*, *Pocillopora sp.*) to have the same set of primers for both genera and be able to easily compare them. The idea was the same for the symbionts but the genomic data available at that time were limited. This issue will probably be easier to solve nowadays but the second problem we faced isn't easy to overcome and makes this technique unsuitable for coral study in my point of view. Indeed, we realised that on one hand we had a huge variability in the primer efficiency depending on coral DNA samples and on the other hand comparing T2L HepG2 human cells to coral samples' we had an opposite result to the one we observed using TRF. Some difficulties aside in making some classical PCR works lead us to the conclusion that some PCR inhibitors might be dragged during the DNA extraction, it is true that DNA samples resuspended in TE buffer could also impair PCR efficiency but it wasn't enough to explain the variability between samples.

The most suitable technique that is worth spending time troubleshooting would be in my opinion a flow-FISH adaptation to coral. Moreover, nowadays when FACS are being coupled with microscopes. Flow cytometry of coral cells has been documented (Rosental et al. 2017), telomere staining with PNA probes was successful in paraffin cut and some reference probes are under study. This method would allow to gain a tremendous overview of TL at the cellular level while TRF can only be performed at the colony level. To work on the colonial question, it would be easier to use a non calcifying model because the calcareous skeleton of corals requires long decalcifying steps, paraffin embedding and cut which results in slides in which the polyp morphology isn't always easy to reconstruct. Moreover, it would remove a highly time consuming, in coral samples, step that is the DNA extraction as the air-brush step is critical and allows only an average of 15 samples extraction every two days.

As we discussed the critically short telomeres are of interest as they trigger DNA damage pathways and are not detectable in classical TRF assay. In collaboration with Vincent Géli and Dmitri Churikov we successfully adapted the TeSLa (Lai et al. 2017) protocol to measure short telomeres in coral. Unfortunately, until now the results only concerned a few samples from the *S. pistillata* acidification and are too preliminary to be presented here.

Finally, some functional studies could be performed either in more suited models, such as the symbiotic sea anemone *Aiptasia* or the aposymbiotic one *Nematostella*. New reports from establishment of coral CRISPR line are raising hopes on using them to directly address functional questions in corals, however it would mainly concern early life stage and setting of long term genetic lineage might take some more years due to the coral growth rate (Cleves et al. 2020).

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# ANNEXE

## Material and methods

### ITS

First, the motif (TTAGGG)<sub>4</sub> was searched in the coral genomes using blastn. The blast result was filtered to keep hits with an identity percentage above 75%, a minimum coverage of 75% and two mismatches maximum were allowed. Distant hits of less than 400 bp were gathered to form a single ITS.

ITS-associated genes are the closest genes or genes containing an ITS defined using *bedtools closest* tool. Telomere prediction was carried on for *P. lobata* and *P. meandrina* based on the ITS position to contig extremities calculating the distance to each of the contig extremities based on the contig length and the ITSs coordinates (< 10 bp). ITS clustering was performed using the 'distance to upstream ITS' to group (n) ITS and the (n+1) ITS, with a (<10kb) and (<50kb) threshold, and annotate ITSs clusters.

### Probabilities

Significativity of overlapping genes was computed using a hypergeometric test implemented in R with the *phyper* function.

### PIF1 gene identification

Genome of *Stylophora pistillata* (Voolstra et al., 2017), *Pocillopora meandrina* and *Porites lobata* were blasted against *Symbiodinium kawugatii*, *goreaui* and *minutum* to identify a coral specific conserved sequence. The longest was a 7kb sequence corresponding to *S. pistillata* transcript (LOC111331544) predicted to be coding for a Pif1 helicase family (30% of identity with hPIF1 and a evalue of 7e-21), based on a P\_loop\_NTPase motif (InterPro IPR027417). Another similar transcript was found in *S. pistillata* (LOC111339554 ) being a probable TDG. *S. pistillata* had several highly conserved matches (80% to 99% identity) when blasted on *P. lobata* and *P. meandrina*.

### Human orthologs

Protein sequences were used to identify human orthologs. Blastp [**blast**] on UniProtKB/Swiss-Prot database restricted to *Homo sapiens* species was performed with a 1e-5 e-value threshold. For each protein, the best hit was selected, based on e-value and bit-score.

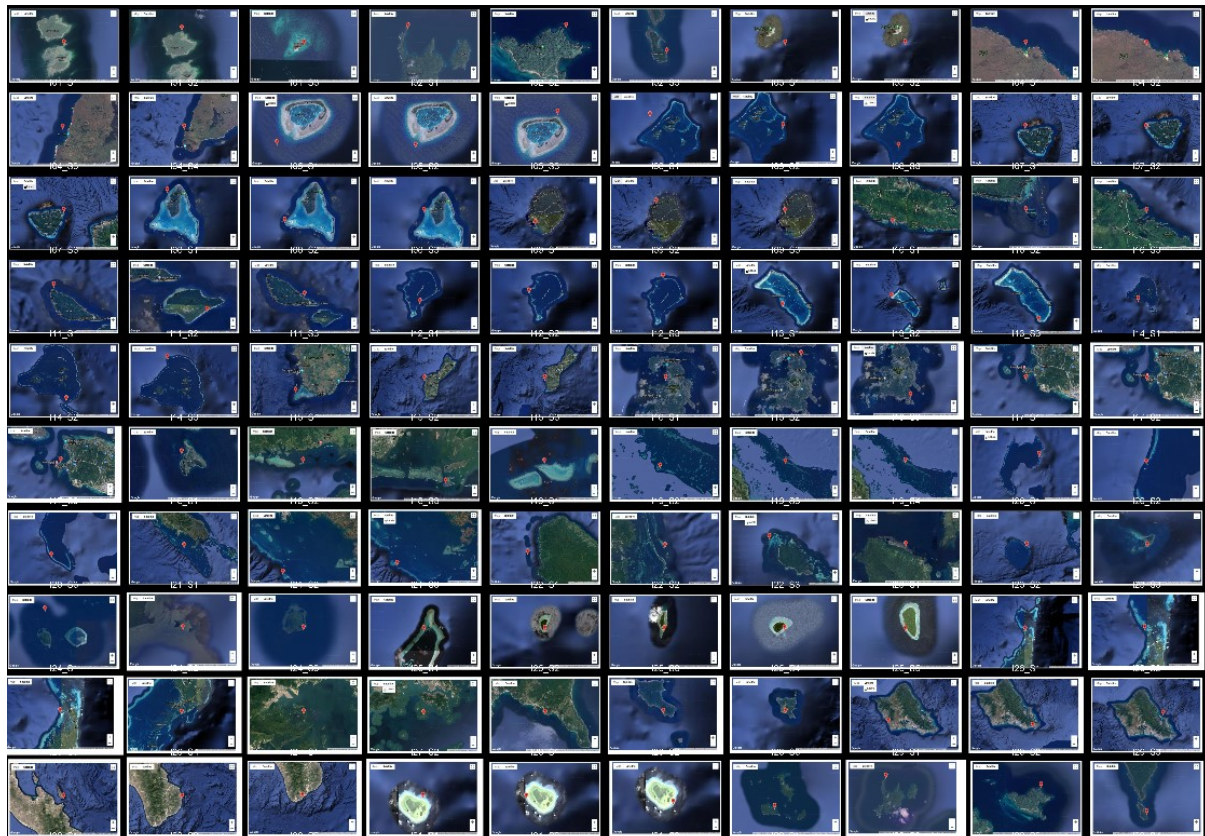
## Ontology

Gene Ontology (GO) analysis was performed using “topGO” R package [topGO]. For the cnidarian analysis, all GO terms identified in *Pocillopora meandrina* and *Porites lobata* separately were used as background. A metascape analysis was performed using human orthologs. Briefly, terms were filtered with a p-value < 0.01, a minimum genes count of 3 and an enrichment factor > 1.5.

## Palindromic ITS

The dna folding structure was generated using mfold with default parameters. The 188 bp satellite sequence was searched in the different coral genomes using Blastn. All the hits had an evalule < 1e-13 and an identity percentage > 80%. Then, the matching sequences were used to build a HMM profile, using hmmbuild from HMMER suite (v3.3). Finally, additional matching regions were searched using nhmmer with an e-value cutoff of 0.01.

## Supplementary Figure



GPS view from Google maps of the islands and the sampled sites.

## Supplementary table

### Age transcriptome, Young vs adult significantly DEG

GeneId	ext_gene	baseMean	log2FoldCha	lfcSE	stat	pvalue	padj	absFC
LOC111319665	un	86.98731748	4.890197579	0.745093980	5.221083089	1.778797032	0.000842331	4.890197579
LOC111320764	un	946.8029527	3.941149161	0.654665419	4.492598926	7.035921681	0.009799383	3.941149161
LOC111320872	SYCE2	76.77540995	-2.791394997	0.400862211	-4.468854744	7.863950819	0.010022081	2.791394997
LOC111320898	un	46.62435172	-5.663539707	0.858498595	-5.432204234	5.566216288	0.000329478	5.663539707
LOC111321006	un	584.2156623	-2.540785671	0.346006357	-4.453055955	8.465668091	0.010022081	2.540785671
LOC111321477	un	14.46915741	6.265946944	1.204761653	4.370945016	1.237099451	0.013314001	6.265946944
LOC111322394	un	154.7183089	-2.555202349	0.366980061	-4.23783881	2.256816891	0.021964301	2.555202349
LOC111322886	un	35.39033164	22.60851571	2.951551866	7.321069287	2.460027728	1.941535884	22.60851571
LOC111323053	CKMT2	415.5940775	-2.866383764	0.410115717	-4.550871095	5.342427233	0.007905790	2.866383764
LOC111323796	hblA	163.7962872	10.35028616	2.304688421	4.057071697	4.969183093	0.036767296	10.35028616
LOC111326887	ABT	249.8993320	-3.22498779	0.473512921	-4.698895625	2.615720826	0.004764032	3.224987796
LOC111328456	MDN1	183.7146354	-2.75671640	0.428432439	-4.100334707	4.125530166	0.031509734	2.756716400
LOC111328694	Dunh8	306.8191730	-3.03236059	0.416803371	-4.87606563	1.082226106	0.002507638	3.032360598
LOC111331152	un	116.9420664	8.759792582	1.769745835	4.384693228	1.161493435	0.013095561	8.759792582
LOC111332001	un	168.8467013	-2.484592411	0.480624198	-4.26235802	2.022810127	0.020823511	2.484592411
LOC111332660	un	185.9484296	-2.48445932	0.306437545	-4.844247541	1.270924013	0.002507638	2.484459326
LOC111333239	un	301.5813285	25.84648844	2.452351359	10.13170007	3.996397849	9.462271187	25.84648844
LOC111334180	un	59.14700249	7.697941263	1.688238177	3.967414877	7.265644979	0.049151050	7.697941263
LOC111334563	un	891.5565332	6.897202192	1.215001234	4.853659424	1.212038188	0.002507638	6.897202192
LOC111335102	un	27.48287667	-5.244442321	0.865342775	-4.90492605	9.346254421	0.002458791	5.244442321
LOC111336763	APOL3	170.9560159	4.979984605	0.960173847	4.145066661	3.397148680	0.028726531	4.979984605
LOC111336809	un	189.2804256	1.916451672	0.178211120	5.142505523	2.710984691	0.001069799	1.916451672
LOC111336929	un	62.21374917	-3.11469366	0.523687578	-4.038082542	5.388989216	0.038665181	3.114693669
LOC111339345	un	69.68980392	3.981000673	0.668400426	4.459902410	8.199698063	0.010022081	3.981000673
LOC111339772	un	1740.197432	5.126994682	1.004438170	4.108759309	3.977903871	0.031394943	5.126994682
LOC111340586	un	314.2172468	-3.609672202	0.564135501	-4.625966983	3.728546955	0.006305771	3.609672202
LOC111340691	Wdr33	566.7762058	3.992498865	0.710464281	4.212032811	2.530827709	0.022609007	3.992498865
LOC111341782	un	39.06528415	-4.034974923	0.717197357	-4.231715147	2.319160073	0.021964301	4.034974923
LOC111343643	un	682.6649613	8.808804901	1.589292842	4.913383294	8.951802714	0.002458791	8.808804901
LOC111343690	RECK	165.7051710	-2.947379565	0.472993470	-4.117138365	3.836057875	0.031319428	2.947379565
LOC111345559	Ryncolin-4	851.8907489	8.510016955	1.866429208	4.023735227	5.728231461	0.039890393	8.510016955
LOC111345813	un	134.8618155	8.321900713	1.482246759	4.939731301	7.823029647	0.002458791	8.321900713
LOC111346134	un	111.1290862	-2.245869195	0.270880447	-4.599332322	4.238471864	0.006690286	2.245869195
LOC111346485	KO2A.2.6	548.9688894	26.64433302	2.554755531	10.03788139	1.038812831	1.229798570	26.64433302
LOC111346794	un	223.2799780	6.487377332	1.304083542	4.207841871	2.578211736	0.022609007	6.487377332

## Co-author Manuscript

Longevity strategies in response to light in the reef coral *Stylophora pistillata*




The Tara Pacific expedition—A pan-ecosystemic approach of the “-omics” complexity of coral reef holobionts across the Pacific Ocean





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# Longevity strategies in response to light in the reef coral *Stylophora pistillata*

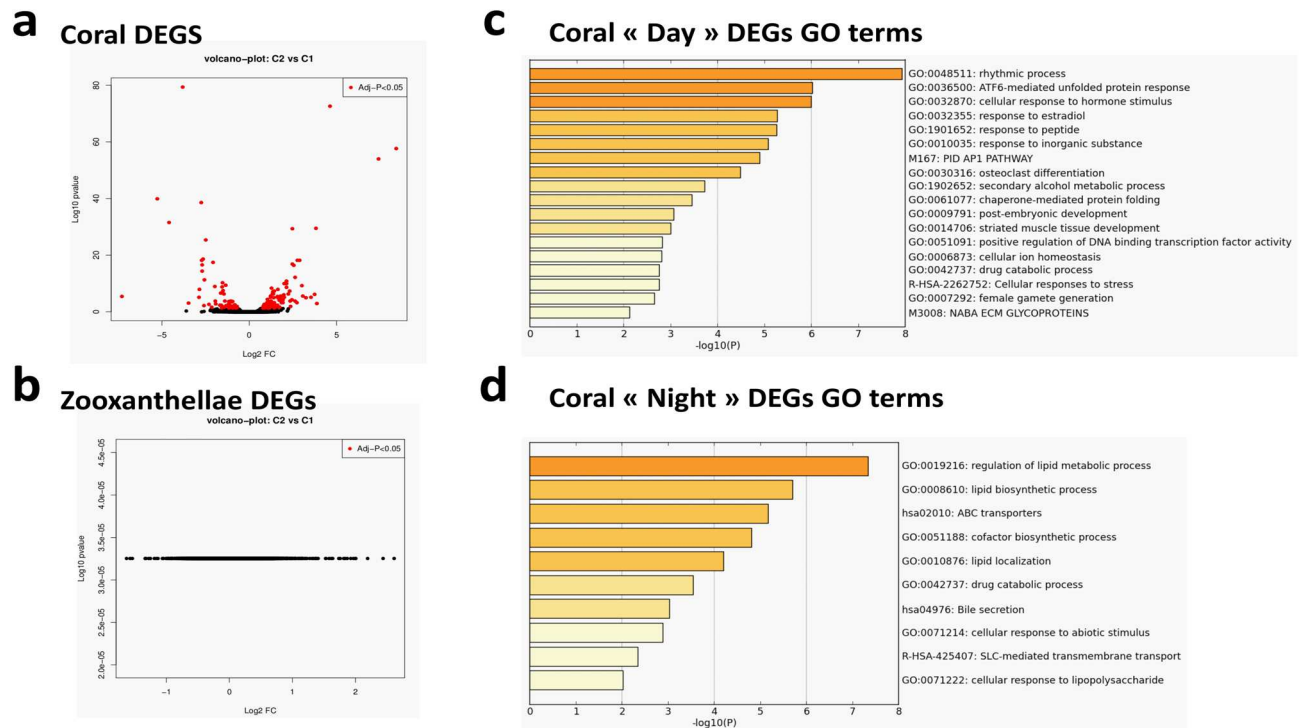
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Aging is a multifactorial process that results in progressive loss of regenerative capacity and tissue function while simultaneously favoring the development of a large array of age-related diseases. Evidence suggests that the accumulation of senescent cells in tissue promotes both normal and pathological aging. Oxidative stress is a key driver of cellular senescence. Because symbiotic long-lived reef corals experience daily hyperoxic and hypoxic transitions, we hypothesized that these long-lived animals have developed specific longevity strategies in response to light. We analyzed transcriptome variation in the reef coral *Stylophora pistillata* during the day–night cycle and revealed a signature of the FoxO longevity pathway. We confirmed this pathway by immunofluorescence using antibodies against coral FoxO to demonstrate its nuclear translocation. Through qPCR analysis of nycthemeral variations of candidate genes under different light regimens, we found that, among genes that were specifically up- or downregulated upon exposure to light, human orthologs of two “light-up” genes (HEY1 and LONF3) exhibited anti-senescence properties in primary human fibroblasts. Therefore, these genes are interesting candidates for counteracting skin aging. We propose a large screen for other light-up genes and an investigation of the biological response of reef corals to light (e.g., metabolic switching) to elucidate these processes and identify effective interventions for promoting healthy aging in humans.

Aging is classically defined as a progressive loss of organ and organismal function, renewal potential, and stress resistance. At the cellular level, aging represents the accumulation of unrepaired or irreparable damage leading to cellular senescence<sup>1</sup>. Although aging affects all organisms, its mechanisms have mainly been studied in a limited number of model organisms (yeasts, nematodes, mice, and *Drosophila*)<sup>2,3</sup>. Exploration of the aging process of non-model organisms is crucial for developing a more generalized understanding of aging. Organisms with unique habitats or life histories are particularly likely to have distinct biological processes useful in the development of novel environmental or medical applications.

In this context, cnidarians such as hydra and corals emerge as potent models of aging as their genetic individuals exhibit extreme lifespan, regeneration capacity, and resistance to ultraviolet (UV) light or oxidative stress<sup>4–7</sup>. Reef-building corals host photosynthetic endosymbiotic dinoflagellate algae (family Symbiodinaceae), commonly called zooxanthellae, in their endodermal cells. As a result of the photosynthetic activity of these algae, coral cells undergo drastic and rapid nycthemeral variation in exposure to oxygen and/or reactive oxygen species (ROS); the extreme limits of this variation correspond to hypoxia (i.e., < 5% O<sub>2</sub>) at night and hyperoxia (i.e., > 60% O<sub>2</sub>) during the day<sup>8–10</sup>. However, at the same time these animals can live and expand clonally for decades in aquaria and in the field, and the ages of some wild genetic individuals have been estimated to be more than several hundreds of years<sup>11,12</sup>. As previously discussed<sup>5</sup>, we consider in this work that a coral individual refers to the syngenic colony as a whole and not to an isolated polyp. Thus, reef building corals represent a paradox in terms of current theories of aging, which characterize high ROS levels as an accelerant of aging<sup>13</sup>. Some specific mechanisms of oxidative stress resistance have been identified in corals<sup>6,7,14–16</sup>; however, their contribution to the

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**Figure 1.** Day vs. night transcriptomic signatures. **(a,b)** Volcano plots of significantly differentially expressed genes (DEGs) in *Stylophora pistillata* **(a)** or *S. microadriaticum* **(b)** transcriptomes, generated with the R software package. **(c,d)** Bar charts representing significantly enriched terms identified in Metascape annotation analyses among significantly upregulated **(c)** or downregulated **(d)** genes in Day or Night transcriptomic signatures of *S. pistillata*. Color gradient is scaled to the represented  $-\log_{10}(P\text{-value})$ .

extreme longevity of these animals remains unknown. Obviously, as exposure to daylight is the main driver of symbiotic corals physiology, these resistance mechanisms are linked to circadian rhythms<sup>6,17–19</sup>, but, interestingly, even in mammals, circadian molecular clocks are crucial for stress management and their dysfunctions can result in aging phenotypes<sup>20</sup>. This suggest very ancient evolutionary links between these processes, echoing with the common origin of cryptochrome proteins from the core molecular clocks and light-induced UV damage-repairing photolyases<sup>21</sup>.

In this study, we investigated nycthemeral transcriptional variation in the coral *Stylophora pistillata* and the expression of genes involved in the nycthemeral oxic transition as well as those related to anti-senescence properties in humans. Our results help elucidate the biological response of corals to light and contribute to the identification of new genes and biological pathways involved in human aging.

## Results

**A transcriptomic signature of the nycthemeral oxic transition.** Isogenic samples of *Stylophora pistillata* were collected 1 h before the end of each 12 h:12 h photoperiod of light:dark conditions, and we will respectively refer to them as “day” and “night” samples. Total RNA was extracted and used to prepare cDNA libraries that were sequenced with SOLiD technology (Fig. S1). Reads were mapped against the *Stylophora pistillata* gene models<sup>22</sup> to obtain relative expression data. Using an adjusted P-value cutoff of 0.05, we identified 151 genes that were significantly modulated between these two conditions (Fig. 1a, Table S3) and contained 122 nonredundant annotations. When reads were mapped against the *Symbiodinium microadriaticum* gene models<sup>23</sup>, no significantly up- or downregulated genes were obtained given our P-value threshold (Fig. 1b) although these mapped reads represented about a third (31.33%) of total mapped reads (Table S1). This inability to identify significantly modulated *Symbiodinium* genes from our RNA sequencing (RNA-seq) samples was surprising. The relatively low sequencing depth of our RNAseq study (Table S1) precludes the analysis of low expressing DEGs. However, this is unlikely to explain the absence of algal DEGs since large and similar numbers of reads were observed for key photosynthesis genes in both “day” and “night” conditions (Table S2). Moreover, similarly very limited transcriptional changes have been reported in previous studies in dinoflagellates for both axenic<sup>24</sup> and coral-hosted<sup>25</sup> cultures upon exposure to thermal or other stresses. Thus, it appears that dinoflagellates mainly regulate daily or short-term expression through posttranslational mechanisms that may consist of regulating small RNA<sup>26</sup> or trans-splicing with spliced leader (SL) sequences<sup>24</sup>. Regulation involving these mechanisms might be complex<sup>24</sup>, and their effects may be invisible to polyadenylated mRNA-based RNA-seq differential expression analyses, such as the present study.

Gene ontology (GO) and pathway analyses were performed with 102 orthologous human genes among the 122 *S. pistillata* nonredundant differentially expressed genes (DEGs), determined by identifying the best BLAST

hits against the UniProtKB/Swiss-Prot database. GO terms related to lipid metabolism and small-molecule transport were overrepresented among downregulated genes (i.e., more present in night samples; Fig. 1d), and those associated with circadian rhythms, cellular responses to stimuli, and stress and protein stability were overrepresented among upregulated genes (i.e., more present in day samples; Fig. 1c). These results are consistent with previous studies of cnidarian transcriptomics that have reported similarly enriched terms among DEGs between day and night samples in *Acropora cervicornis*<sup>15</sup> and *A. millepora*<sup>18</sup> and heat or light stress samples in *A. hyacinthus*<sup>27</sup> and *S. pistillata*<sup>28–30</sup>. Noteworthy, we did not find significant enrichment for biological processes such as DNA repair and cell cycle<sup>29</sup>, although some of our DEGs had corresponding annotations: XPC and PHR involved in DNA repair while HSP68, CRY1, CALR, COT2, PHC1, ECT2, ATF4, ETS2, XPC; CB042, CLOCK and ANPRA are linked to cell cycle.

**FOXO is a master regulator of the nycthemeral oxic transition transcriptional signature.** To determine the upstream factors regulating the transcriptomic signature of day and night alternation, we compared our signature to published lists of mouse genes known to be directly regulated by stress response master transcription factors XBP1, ATF4, and ATF6 for Unfold Protein Response (UPR), NRF2, and FOXO for oxidative stress and HIF1 $\alpha$  for hypoxia (Table S4a)<sup>31–35</sup>. Published HIF1 $\alpha$  and ATF4 target genes lists were not significantly overrepresented in the *S. pistillata* transcriptomic signature, whereas all other lists were (Fig. 2a), further suggesting endoplasmic reticulum and oxidative stresses pathways are involved. We found that most DEGs were FOXO targets in one or several of four different animal species for which FOXO chromatin immunoprecipitation DNA sequencing (ChIP-Seq) data are available (Figure S4, Table S4b)<sup>35</sup>, which suggests that FOXO is a master regulator of a large proportion of transcriptional responses to coral nycthemeral oxic transitions.

In classical models, FOXO activity is regulated by reversible phosphorylation and subcellular localization; its activation triggers its translocation into the nucleus. To investigate the regulation of putative nuclear translocation of spFOXO, we raised antibodies specifically directed against the spFOXO protein (Figure S5). In immunofluorescence experiments, we observed a clear punctuated FOXO pattern in the nuclei of *S. pistillata* tissue subjected to 72 h of continuous light compared to a control sample at the end of the scotophase that exhibited only diffuse cytoplasmic staining with spFOXO antibodies (Fig. 2b). Thus, spFOXO is activated in coral tissue on exposure to light, in agreement with the FOXO transcriptomic signature observed during the nycthemeral oxic transition.

**Light-regulated coral genes.** Among all significantly modulated genes (Table 1), we selected 23 genes of interest that were representative of the GO terms and pathways. We analyzed their expression by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) in new coral samples collected at the same nycthemeron times as those used in the RNAseq analysis. Among them, 11 genes were validated since they were consistently modulated in the same direction as observed in RNA-seq analyses and with  $|\text{fold-change}| > 2$ .

We investigated whether these genes were regulated by a biological clock or directly by light. We performed an experiment in which isogenic coral microcolonies were collected in triplicate every 4 h during a 48 h period under three different conditions: a control condition similar to aquarium culture with daily alternation of 12 h of light and 12 h of darkness (ALT), continuous light (Light), and continuous darkness (Dark; Fig. 3a), and were then analyzed by qRT-PCR.

Using these results, we characterized different expression patterns (Fig. 3b). The expected oscillations were obtained in the ALT condition regarding the expression of the clock gene CRY1. Interestingly, continuous light preserved CRY1 oscillations despite a decrease in their amplitude. However, complete darkness resulted in a rather flat expression pattern, which suggests that oscillating CRY1 expression over a 24 h period may depend on the presence of light but not its alternation with darkness. We identified this pattern, also followed by catalase (CATA), carbonic anhydrase 2 (CAH2) and photolyase (PHR), as “circadian”.

Then we characterized other patterns, including a “light-up” pattern that was observed for the HEY gene; this pattern exhibited oscillations under ALT conditions but general gene expression upregulation under continuous light (Fig. 3). The same pattern was observed for the LONF3 gene.

By contrast, the “light-down” pattern exhibited by PTHD3 showed general gene expression downregulation under continuous light, with some conservation of oscillations, which suggests modulation by both light and the biological clock (Fig. 3).

Some other genes showed a relatively “flat” pattern, with no characteristic oscillations or recurrent changes and no clear effects of continuous light or darkness.

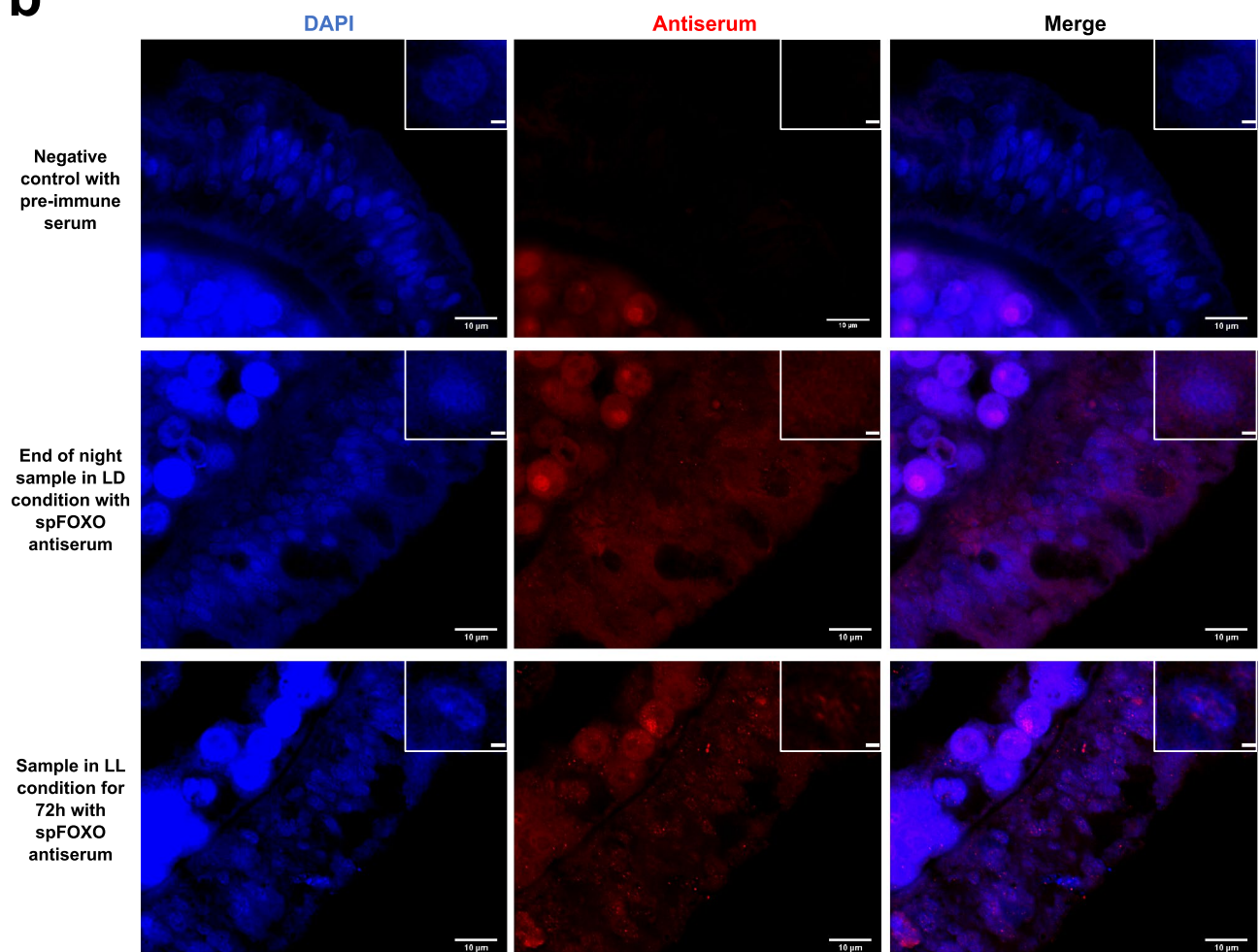
**Two light-up genes protect against senescence in human fibroblasts.** Because genes with circadian or light-dependent patterns participate in oxic transition protection mechanisms, we knocked down expression of their human orthologs in MRC5 fibroblasts cultured at 5% or 20% O<sub>2</sub> and measured the proportion of cells displaying senescence-associated  $\beta$ -galactosidase ( $\beta$ -gal) staining (Fig. 4). We tested genes orthologous to the light-up genes including all three spHEY human orthologs (HEY1, HEY2, and HEYL). PHR has no human ortholog, and the KD of PTHD3 produced unsatisfactory results; therefore, these genes are not discussed further here. Although catalase KD had no significant impact, KD of genes orthologous to the light-up genes HEY and LONF3 increased the proportion of cells with  $\beta$ -gal staining at 5% and 20% O<sub>2</sub>, which indicates that these genes have longevity properties in human cells.

## Discussion

Two main conclusions can be drawn from the results of this study: FOXO is a master regulator of the nycthemeral oxic transition transcriptional signature in coral, and genes modulated by light in coral (light-up/-down genes) are novel candidates for stress resistance or longevity properties that may be conserved in orthologous species.

**a**

Transcription factor	Targets list source	Ratio Present/Targets number	Enrichment <i>p</i> -value
mHIF1a	Loftus et al. Pigment Cell Melanoma Res 2017	4/591	2,92E-01
mATF4	Han et al NCB 2013	5/472	6,81E-02
mNRF2	Malhotra et al. NAR 2010	10/1055	2,42E-02
mXPB1	Diego Acosta-Alvear et al. Mol Cell 2007	8/545	3,81E-03
mFOXO	Webb et al. Aging Cell 2016	44/6199	8,32E-04
mATF6	IPA database	6/54	1,82E-07

**b**

**Figure 2.** FOXO response in light-exposed coral. **(a)** Comparison of transcriptomic signatures with published lists of selected transcription factor targets<sup>31–35</sup>. **(b)** spFOXO activated in light-exposed coral tissue. Immunofluorescence labeling (red signal) with pre-immune serum (negative control) or spFOXO antiserum and DAPI counterstain (blue signal) of paraffin-embedded coral tissue from syngenic samples collected at night from a colony grown in light/dark conditions and at noon after 72 h of continuous light. An enlarged view of an isolated nucleus is provided in the top right corner, scale bar herein is 1 µm.

The FOXO family of transcription factors has been extensively associated with biological rhythms, stress responses, stem cells, and longevity in animals<sup>36</sup>. In cnidarians, FOXO plays a key role in determining *Hydra* self-renewal capabilities and apparent immortality by maintaining stemness in all three stem cell lineages of these organisms<sup>37</sup>. In the present study, FOXO was activated in corals upon stimulation with light, which suggests the existence of FOXO functions related to defense mechanism management in corals as previously hypothesized<sup>38</sup>. Stem cell compartments have not yet been characterized in *S. pistillata*; however, management of daily hyperoxia in progenitors is likely key in its exceptional resistance and longevity, and our results further suggest a suspected role of FOXO in the underlying cellular processes.

Investigation of the nycthemeral oxyc transition mechanisms in corals is difficult as they may be mitigated by effects of the endogenous circadian clock, light stimulation independent of photosynthesis, and light stimulation

Gene symbol	qPCR	RNA seq
	LOG2 (FC)	LOG2 (FC)
ACOD	− 0.81	− 1.48
ACOX1	1.10	− 2.13
APLP	0.61	1.16
<b>CAH2</b>	<b>2.21</b>	<b>1.53</b>
<b>CATA</b>	<b>2.66</b>	<b>1.55</b>
<b>CLOCK</b>	<b>2.69</b>	<b>1.43</b>
<b>CRY1</b>	<b>1.63</b>	<b>2.48</b>
CRYD	− 1.19	− 0.90
ECT2	− 0.79	− 1.43
<b>EGR1</b>	<b>1.55</b>	<b>2.65</b>
<b>FOS</b>	<b>2.16</b>	<b>2.58</b>
HECD3	0.21	− 1.08
<b>HEY</b>	<b>4.13</b>	<b>3.82</b>
<b>LONF3</b>	<b>4.02</b>	<b>4.59</b>
MDR1	− 0.69	− 1.05
<b>MMP19</b>	<b>2.16</b>	<b>1.48</b>
MRC1	0.82	− 1.39
PAX3	0.89	2.72
<b>PHR</b>	<b>− 1.98</b>	<b>− 2.55</b>
<b>PTHD3</b>	<b>− 2.27</b>	<b>− 2.46</b>
SC5A9	2.52	− 1.28
SIK1	0.44	2.07
XPC	− 0.91	− 2.31

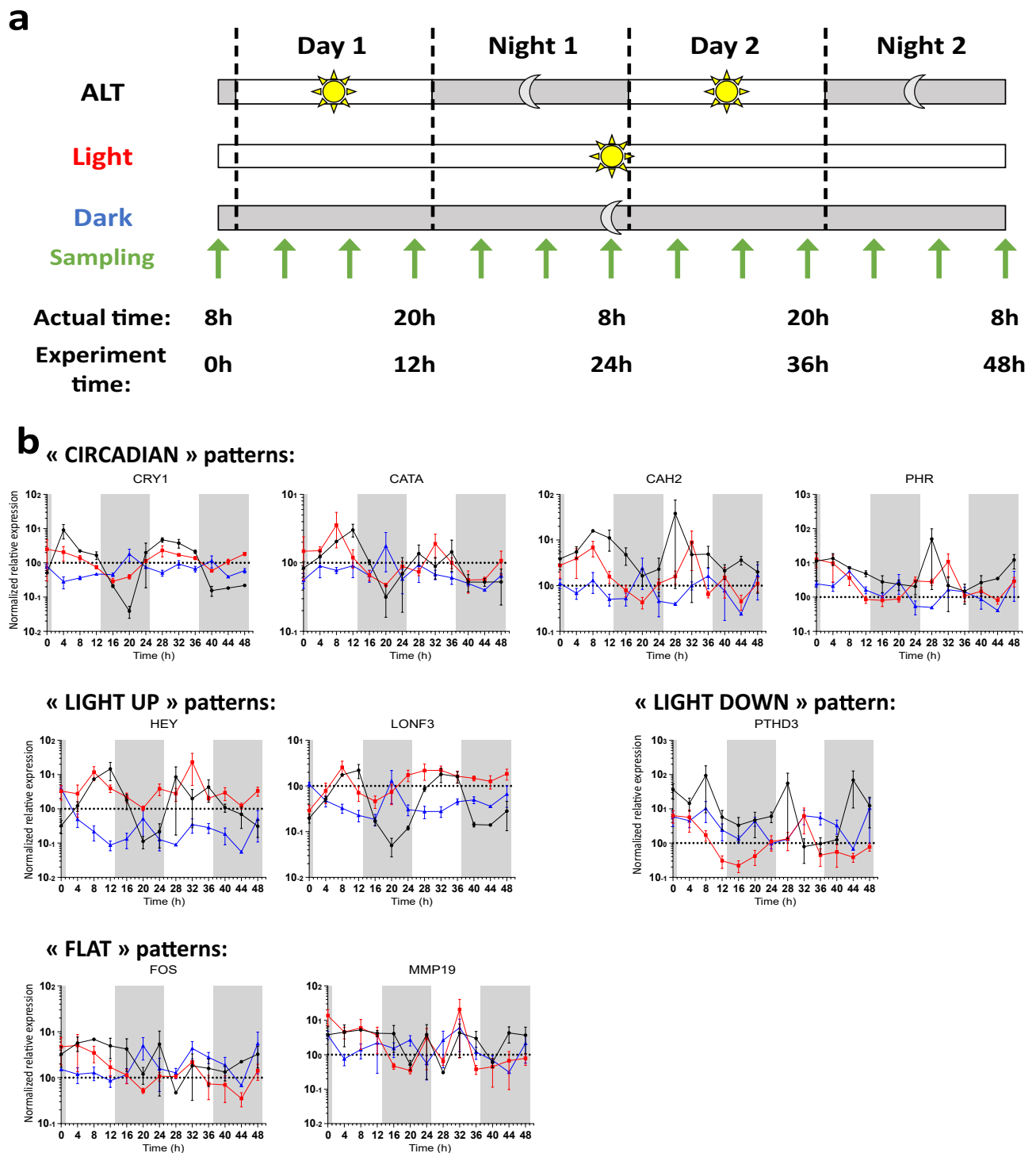
**Table 1.** Validation of selected genes of interest. We selected 23 genes of interest among differentially expressed genes (DEGs) based on main pathways, ontology, and functional terms overrepresented in the transcriptomic data as tested by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) using two reference genes (36B4 and  $\beta$ -actin) and compared them to RNA sequencing results in six independent samples collected at the exact same moments of the nycthemeron. We validated 11 genes with co-directional expression and  $|\text{fold-change}| > 2$  (bold) as candidate genes.

dependent on photosynthesis (symbiotic relationships). Our RNA-Seq experimental design did not permit us to disambiguate the effects of these parameters because we focused on exploring global responses. Among our candidate genes, we identified two clock genes, CRY1 and Clock. However, our time-course analyses of candidate genes allowed us to refine interpretations of the contribution of the biological clock to regulating the expression of these genes.

Only a few studies have examined cnidarian models under conditions of continuous light<sup>17,39</sup> or continuous darkness<sup>18,21,40</sup>, and none have explored both within the same experiment. Our results suggest that light (or its consequences, i.e. increased  $O_2$  concentration) is not directly linked to CRY1 gene expression amplitude but rather is necessary for the pattern of its oscillation over a 24 h period independent of a darkness phase. This finding suggests that light is a necessary kick-starter for endogenous clock oscillations and that oscillating expression of CRY genes is hardwired into their molecular pathways, which makes this molecular clock an intermediate between simple light-induced expression and an autonomously oscillating clock. Additional evidence, notably a more extended list of clock genes, will be required to prove this hypothesis and determine whether it represents a widespread strategy among cnidarians. As recent studies continue to reveal connections between the transcriptional clock, autonomously oscillating redox and metabolic systems, or aging, endosymbiotic cnidarians may prove to be interesting models for deciphering these relationships<sup>19,20</sup>.

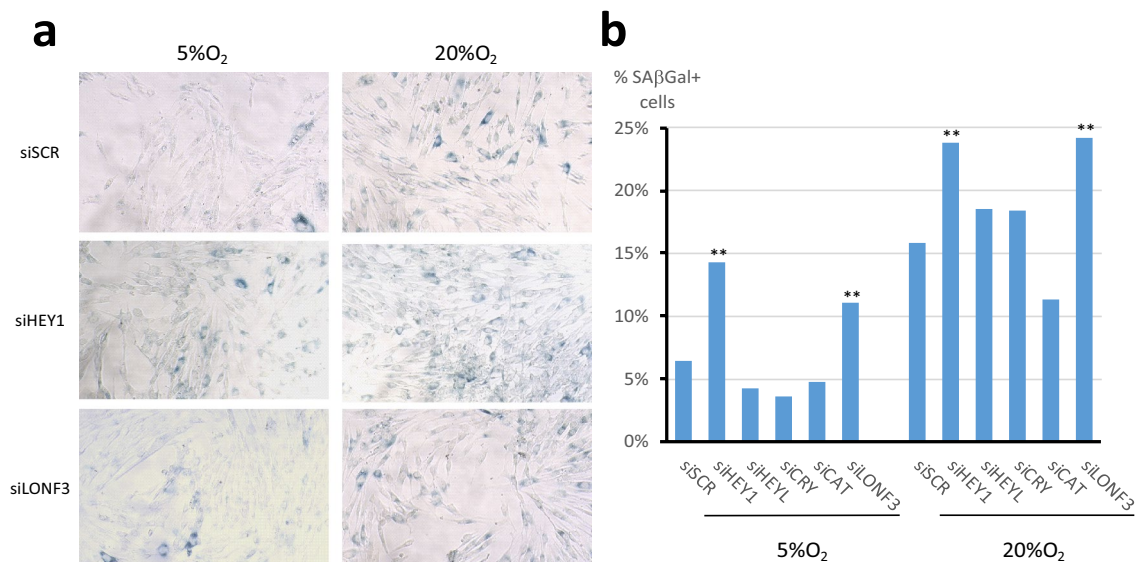
Among our candidate genes that met all selection criteria, some might have been expected based on their known functions. Examples include CRY1, CATA, PHR, and CAH2. Catalase is well known for its antioxidant defense functions and has been extensively studied in cnidarians<sup>41</sup>. However, the pattern of its expression is surprisingly similar to that of CRY1 and is unaltered by continuous light treatment, which suggests that circadian control is the main regulator of this gene. Photolyase (PHR) has a blue light-induced UV-damaged DNA repair function that seems particularly relevant in endosymbiotic cnidarians but has been poorly investigated in these organisms<sup>42</sup>. In our study, PHR expression patterns did not account for clear clock- or light-induced modulations, although its expression seems to increase around subjective dawn in ALT or continuous light conditions. Carbonic anhydrase 2 (CAH2) activity provides inorganic carbon for both photosynthesis and calcification<sup>43,44</sup>; thus, circadian control of its expression is consistent because it is expected to fuel both processes in the context of light-enhanced calcification<sup>45,46</sup>.

It is interesting that human orthologs of the two candidate genes showing light-up modulation (LONF3 and HEY1) have anti-senescence properties in human fibroblasts. LONF3 is related to LON proteases, which are



**Figure 3.** Circadian expression patterns of candidate genes. **(a)** Three samples were collected every 4 h during a 48 h period from isogenic *S. pistillata* colonies under conditions of alternating light and darkness (ALT), continuous light (Light), or continuous darkness (Dark). **(b)** Expression kinetics of candidate genes measured using RT-qPCR and ordered by the different patterns observed. White and grey backgrounds correspond to the actual/subjective photophase and scotophase, respectively.

conserved across all kingdoms and implicated in proteostasis, a critical process for the turnover of damaged proteins, the failure of which is a hallmark of aging. LON proteases are also involved in mitochondrial UPR<sup>47</sup>. LONF3 shares its N-terminal substrate binding domain but has two zinc finger motifs. However, its specific function has not been reported in any model organism, except for a single screen in which its KD increased gH2AX phosphorylation in HeLa cells<sup>48</sup>, which suggests that it may contribute to protecting against DNA damage. HEY1 belongs to a family of bHLH transcription factors related to the *Drosophila* Hair and Enhancer of Split genes.



**Figure 4.** Knockdown of candidate gene orthologs in MRC5 fibroblasts. Non-senescent MRC5 cells were cultured in either 5% or 20% O<sub>2</sub> and then transfected with indicated siRNA 96 h prior to senescence-associated β-galactosidase staining. **(a)** Representative photographs of labeled cells. **(b)** Results are expressed as percentages of labeled cells. A minimum of 200 cells were counted in each condition. P values were calculated using the binomial law, relative to the control condition, and corrected for multiple comparisons with the Bonferroni correction ( $\alpha' = 0.008333$ ; \*\*P-value < 10<sup>-5</sup>).

Hey genes are targets of the Notch and TGFβ signaling pathways and repress other TFs as dimers recruiting HDACs<sup>49</sup>. Consistent with our data, the closely related HELT in *Nematostella vectensis* exhibits light-dependent oscillating expression<sup>40</sup>. A HEY family of TFs was recently associated with the maintenance of stem cell functional pools through p53 or lamin regulation in mice and *Drosophila*<sup>50,51</sup> and with cancer cell self-renewal and differentiation in humans, which suggests that it may contribute to cell renewal homeostasis in physiological and aging contexts. PTHD3 belongs to the Patched Domain-Containing gene family, which is ancient in animal and plant evolution<sup>52</sup>. These genes are related to hedgehog signaling, sterol metabolism, and cell patterning; however, their precise functions remain elusive.

Overall, our candidate genes involved in metabolism and antioxidant defenses appear to rely mainly on circadian patterns, which suggests anticipation strategies. Transcription factors are more directly regulated by light, which suggests a role centered on defense adjustment to actual environmental conditions. This hypothesis is supported by our KD experiments in human fibroblasts, which revealed a protective role in their orthologs. Mechanisms behind both anticipation and adjustment strategies may be relevant to the aging process since molecular damages accumulate faster during time shifts between raises in damaging sources and corresponding defense mechanisms<sup>1,19,20</sup>. The precise functions and mechanisms of action of these candidate genes or their paralogs remain poorly described, which suggests that they may harbor original functions that are key to the exceptional resistance and longevity exhibited by corals and that they may have unsuspected benefits in humans.

In this study, we used an unconventional animal model with extreme properties to reveal unsuspected functions of orthologous systems. Although further study is required to fully understand their precise mechanisms and how evolution has altered their regulation, we identified candidate genes that likely contribute to stress resistance and longevity in corals and mammals. We also established a novel set of tools and techniques for this new coral model. Overall, our approach supports the idea that biodiversity can potentially help discover new mechanisms for human systems.

## Methods

Detailed methods for coral total RNA extraction, cDNA synthesis, transcriptomic data processing and spFOXO purification and antibody development are available in Supplementary data.

**Coral culture.** Experiments were conducted with the tropical symbiotic scleractinian species *Stylophora pistillata* which is kept in long-term culture at the Centre Scientifique de Monaco. Colonies were supplied with water from the Mediterranean Sea (exchange rate 2% per hour) under controlled conditions, including a semi-open circuit, a temperature of 25 °C, salinity of 38, irradiance of 175 μmol photons m<sup>-2</sup> s<sup>-1</sup> on a 12:12 photoperiod. Corals were fed daily with frozen rotifers and twice a week with *artemia nauplii*. Microcolonies were propagated by cutting terminal portions of branches (6–10 mm in length) from three syngenic parent colonies, hung by nylon thread with a specific label in a dedicated aquarium under similar conditions, and left for 10 days for recovery prior to experiments. Day coral samples for RNA-seq were harvested and flash-frozen 1 h before the end of the light period; night samples were harvested 12 h later in the same manner. For circadian expression experiments, isogenic coral microcolonies were collected in triplicate and flashfrozen in liquid nitrogen every

4 h during a 48 h period under three different conditions: a control condition similar to aquarium culture with daily alternation of 12 h of light and 12 h of darkness (ALT), continuous light (Light), and continuous darkness (Dark). For conditions involving continuous light or darkness, coral colony fragments were placed in these conditions 24 h before the first samples were collected.

**Library preparation and sequencing.** The concentration and quality of total RNA were assessed with a Bioanalyzer 2100 and RNA 6000 nano kit (5067–1511; Agilent). All total RNA samples with a RIN superior to 8 (Figure S2) were used for mRNA preparation using the MicroPoly(A) Purist kit (AM1919; Applied Biosystems). mRNA quality was assayed using 2100 Bioanalyzer and the RNA 6000 pico kit (Figure S3). mRNA with a good profile on Bioanalyzer were used for libraries preparation. mRNA was prepared with the MicroPoly(A) purist kit (AM1919; Applied Biosystems). We used 1 µg mRNA per sample to construct libraries using the SOLiD Total RNA-Seq Kit (4445374; Thermo Fisher) and they were barcoded with SOLiD RNA barcoding kit (4427046; Applied Biosystems). Library concentrations were measured with quantitative PCR with the Kapa DNA quantification kit (KK4806; Kapa). Library quality was measured with a Bioanalyzer 2100 and high-sensitivity DNA kit (5067–4626; Agilent). Fragment sequencing was achieved through emulsion PCR, bead deposition, and ligation-based sequencing performed with a SOLiD 3 sequencer and the Top Paired end sequencing kit (4459182; Applied Biosystems) according to the manufacturer's instructions. 3 samples were sequenced for each condition and each sequencing generated 30 to 55 million reads.

**qRT-PCR.** To ensure useful comparison of all quantitative PCR results and setup conditions for all primer pairs, we generated an artificial reference sample using equal quantities of cDNA from all 109 quality control-validated samples from the 48 h expression kinetics experiment. We diluted this reference sample 5, 10, 20, 40, 80, 320 and 1280 times to establish standard curves for primer pair parameters determination and the 40 times dilution was prepared in larger quantities and used as a common normalizer sample for all experiments (D40Ref).

All primer pairs were designed in silico on exon junctions whenever possible with Primer3 (<http://bioinfo.ut.ee/primer3/>) and checked for specificity with BLAST searches (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and for amplicon secondary structures with mfold (<http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form>). Primer pairs were ordered from Sigma and tested for specificity by endpoint PCR using D40Ref and the GoTaq G2 Hot Start Green Master Mix (Promega). Finally, primer pair parameters were determined by quantitative PCR using standards created from the reference sample (Table S5).

All quantitative PCR runs were performed on a StepOnePlus system (Applied Biosystems) with the FastStart SYBR Green Master ROX kit (Roche). A 40 times dilution of each sample cDNA was run in technical triplicate with each primer pair. All samples flagged by the system software for high variability or specificity issues were rerun or removed from the analysis. All run results were analyzed with a relative quantitation method and were normalized first using a triplicate of the D40Ref normalizer sample loaded in all qPCR plates and then with a geometric mean of the results from two reference genes ( $\beta$ actin and 36B4) amplified for all samples. Results are then expressed per condition and timepoint as arithmetic mean of the three biological replicates with SEM as error bars.

**Immunofluorescence on coral samples.** Apexes of *S. pistillata* were fixed at 4 °C overnight in 3% paraformaldehyde in S22 buffer (450 mM NaCl, 10 mM KCl, 58 mM MgCl<sub>2</sub>, 10 mM CaCl<sub>2</sub>, 100 mM Hepes, pH 7.8) and then decalcified using 0.5 M ethylenediaminetetraacetic acid (EDTA) in Ca-free S22 buffer at 4 °C. They were dehydrated in an ethanol series and embedded in Paraplast (Leica). 5 µm thick sections were cut from blocks of paraffin-embedded tissues, transferred on glass slides, deparaffinized with 2 × 5 min xylene baths and rehydrated with 5 min baths in decreasing concentrations of ethanol in water (100%, 95%, 70%, 40%, 0%). Antigen retrieval was achieved by incubating slides for 20 min in hot (95 °C) citrate buffer (10 mM citrate, 0.05% Tween 20, pH 6.0) in a steamer and 20 min of cooling at RT. Sections were washed with water and dried overnight. Tissue permeabilization was performed for 1 h at RT in 1× phosphate-buffered saline (PBS); 2 N HCL, 0.5% Triton X-100 followed by two washes with 1× PBS for 5 min. Tissue sections were then fixed in 0.7× PBS, 4% paraformaldehyde, 20 mM sucrose at RT for 15 min with shaking and washed twice with 1× PBS for 5 min. Treatment with 20 µg/ml RNase was performed in a humid chamber at 37 °C for 1 h in 2× saline–sodium citrate (SSC) buffer and then washed twice with 1× PBS for 5 min. Tissue sections were then incubated for 1 h in saturating buffer (1× PBS, 1% bovine serum albumin [BSA], 0.05% Tween 20, pH 7.4) at 37 °C and rinsed twice with 1× PBS at RT. Samples were incubated overnight at 4 °C in saturating buffer with anti-spFOXO antiserum (0.5 µg/mL) as a primary antibody. After rinsing with saturating buffer, samples were incubated for 2 h with 4 µg/mL Alexa 488-coupled donkey anti-rabbit antibodies (A21206; Molecular Probes) in saturating buffer. After rinsing with PBS (pH 7.4), samples were finally mounted in 4',6-diamidino-2-phenylindole (DAPI)-containing VectaShield (H-1200; Eurobio scientific) antifade medium. Immunostaining experimental controls were obtained with rabbit pre-immune serum and Alexa 488-coupled anti-rabbit antibodies as described above. Bichannel (DAPI and FITC filter sets) stacks of images were acquired on a DeltaVision elite system (GE Healthcare) epifluorescence microscope with a 60× oil-immersed objective (1.42 NA, Plan Apochromat; Olympus) and a CCD CMOS monochromatic camera. No binning was used and voxel size is 109 µm × 109 µm × 350 µm. All images stacks were then deconvolved and individual in focus optical slices from different conditions were extracted and treated similarly with Fiji software<sup>53</sup> regarding signal thresholding and amplification to reveal nuclei outlines and antibody specific signal.

**Tissue culture, siRNA transfections and SA- $\beta$ -galactosidase staining.** Human primary MRC-5 cells were obtained from the ATCC, grown at 37 °C, 5% CO<sub>2</sub> and 5% or 20% O<sub>2</sub> in DMEM supplemented with 10% fetal calf serum and 100 U/ml penicillin–streptomycin, and regularly tested for mycoplasma contamination.

siRNA On-Target Plus SMARTpools (Dharmacon) targeting specified genes were transfected with Dharmafect1 transfection reagent (Dharmacon) following manufacturer's instructions. For each condition, 150,000 MRC-5 cells in 6-well plates with coverslips were transfected 72 h prior to analyses.

The detection of SA- $\beta$ -gal-positive cells was carried out using the Senescence Detection Kit (Abcam, ab65351) following the manufacturer's instructions.

## Data availability

Raw transcriptomic data has been deposited on the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) repository under accession number GSE153706 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153706>).

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## Author contributions

A.O., R.E., M.J.G.P., G.B., C.G., K.V., E.G., D.A., P.B., D.Z. and S.T. designed and conceived the experiments. A.O., R.E., D.Z., J.M.D., E.B., K.L. and C.B. performed and analyzed the experiments. A.O., K.J., P.L., M.P. and D.Z. analyzed transcriptomic data. M.J.G.P., A.R., D.Z. and S.T. provided experimental support. A.O. and E.G. wrote the manuscript. All authors read and approved the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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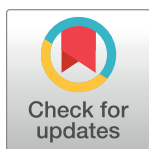
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COMMUNITY PAGE

# The *Tara* Pacific expedition—A pan-ecosystemic approach of the “-omics” complexity of coral reef holobionts across the Pacific Ocean

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## Abstract

Coral reefs are the most diverse habitats in the marine realm. Their productivity, structural complexity, and biodiversity critically depend on ecosystem services provided by corals that are threatened because of climate change effects—in particular, ocean warming and acidification. The coral holobiont is composed of the coral animal host, endosymbiotic dinoflagellates,

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**Abbreviations:** CBD, Convention on Biological Diversity; FAIR, findable, accessible, interoperable, reusable; GLODAP, Global Ocean Data Analysis Project; MODIS, Moderate Resolution Imaging Spectroradiometer; MS, mass spectrometry; NASA, National Aeronautics and Space Administration; NMR, nuclear magnetic resonance; OTU, operational taxonomic unit; SST, sea surface temperature.

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associated viruses, bacteria, and other microeukaryotes. In particular, the mandatory photosymbiosis with microalgae of the family Symbiodiniaceae and its consequences on the evolution, physiology, and stress resilience of the coral holobiont have yet to be fully elucidated. The functioning of the holobiont as a whole is largely unknown, although bacteria and viruses are presumed to play roles in metabolic interactions, immunity, and stress tolerance. In the context of climate change and anthropogenic threats on coral reef ecosystems, the *Tara Pacific* project aims to provide a baseline of the “-omics” complexity of the coral holobiont and its ecosystem across the Pacific Ocean and for various oceanographically distinct defined areas. Inspired by the previous *Tara Oceans* expeditions, the *Tara Pacific* expedition (2016–2018) has applied a pan-ecosystemic approach on coral reefs throughout the Pacific Ocean, drawing an east–west transect from Panama to Papua New Guinea and a south–north transect from Australia to Japan, sampling corals throughout 32 island systems with local replicates. *Tara Pacific* has developed and applied state-of-the-art technologies in very-high-throughput genetic sequencing and molecular analysis to reveal the entire microbial and chemical diversity as well as functional traits associated with coral holobionts, together with various measures on environmental forcing. This ambitious project aims at revealing a massive amount of novel biodiversity, shedding light on the complex links between genomes, transcriptomes, metabolomes, organisms, and ecosystem functions in coral reefs and providing a reference of the biological state of modern coral reefs in the Anthropocene.

## Introduction

The 20th century has seen the earth enter into the now widely called Anthropocene [1]. Anthropogenically induced changes are happening on both global and local scales and are altering the physiology of organisms and ecosystems by modifying the entire earth's physical, chemical, and biological processes [2]. Among marine ecosystems, coral reefs have the unfortunate privilege of being highly sensitive to these environmental modifications [3]. In particular, the thermally mediated process of coral bleaching—i.e., the loss of the obligate photosynthetic microalgal endosymbionts—is increasingly decimating corals. Projections estimate that approximately 25% of reefs have already been lost, and up to 99% will be threatened and dramatically transformed by 2050 [4]. Even though they cover only approximately 0.2% of the ocean's surface [5], coral reefs harbor approximately 30% of ocean biodiversity [6], providing ecological services (fisheries, tourism, coastal protection) to nearly 1 billion people [7], and are estimated to be worth USD 30 billion per year [8].

The late 20th century has also seen our world entering into the “-omics” revolution sparked by the development of high-throughput analyses of DNA, RNA, proteins, and metabolites. This revolution has changed our approach to investigate organisms, and it is about to change the descriptions of ecosystems as genes-to-ecosystem modeling improves [9]. It will advance our capabilities to investigate the biodiversity and functioning of our oceans in a holistic way. Holistic approaches, interrogating components across the various levels of organization of an ecosystem, appear accessible today, as exemplified in the *Tara Oceans* project [10], which continues to decipher biodiversity and structural networks across all plankton organisms in the ocean [11].

*Tara Pacific* is a unique scientific expedition inspired by earlier maritime explorations that uncovered the uncharted territories of marine biodiversity. Coral reef research

started with the contribution of Charles Darwin during “the voyage of the *Beagle*” (1831–1836), when Darwin explored many reefs in the Indian and Pacific Oceans and established his biophysical [theory](#) of the formation of [coral reefs](#) and [atolls](#) [12]. He also highlighted the paradoxical high diversity of reef organisms living in a nutrient-poor system. Later, the Great Barrier Reef Expedition (1928–1929) led by Charles Yonge was a landmark in coral reef research, as it established the scientific basis of coral physiology (nutrition, symbiosis, growth, etc.). The “polypifers,” later called coral polyps, were then demonstrated to host a large population of organisms (protists, viruses, bacteria, archaea, and all sorts of unicellular and multicellular eukaryotes), which are intimately involved in the physiology of the coral animal host in a complex and still mysterious system named the holobiont [13].

The *Tara* Pacific expedition has built on these early tracks, applying the most recent technologies to map the “-omics” complexity of the coral holobiont within its ecosystem and across the Pacific Ocean. Through the exploration of marine biodiversity at scales spanning from organisms to genes to biomolecules, *Tara* Pacific is undertaking the first pan-ecosystemic study of coral reef diversity across an entire ocean basin ([Fig 1](#)). Given that a holistic approach integrating all components of the reef biota is unrealistic, the *Tara* Pacific expedition focuses its approach on widely distributed coral and fish holobionts and their contextual biological (plankton) and physicochemical environment, including modifications in the context of global changes ([Fig 2](#)).

The geographic and sequencing extent of *Tara* Pacific will bring an exceptionally comprehensive description of coral holobionts, together with the surrounding fish and plankton biota. Such unprecedented sequencing coverage and depth will place coral reefs among the first ecosystems with a comprehensive description of their hologenomic diversity (i.e., the totality of genomes making up the coral holobiont) across their natural environments. We will go beyond the mere description of species composition to investigate in detail the gene content and gene expression of these communities, as well as the interconnectedness with their hosts and habitats. Broad emerging patterns will then be accessible through large-scale data mining and network analyses.

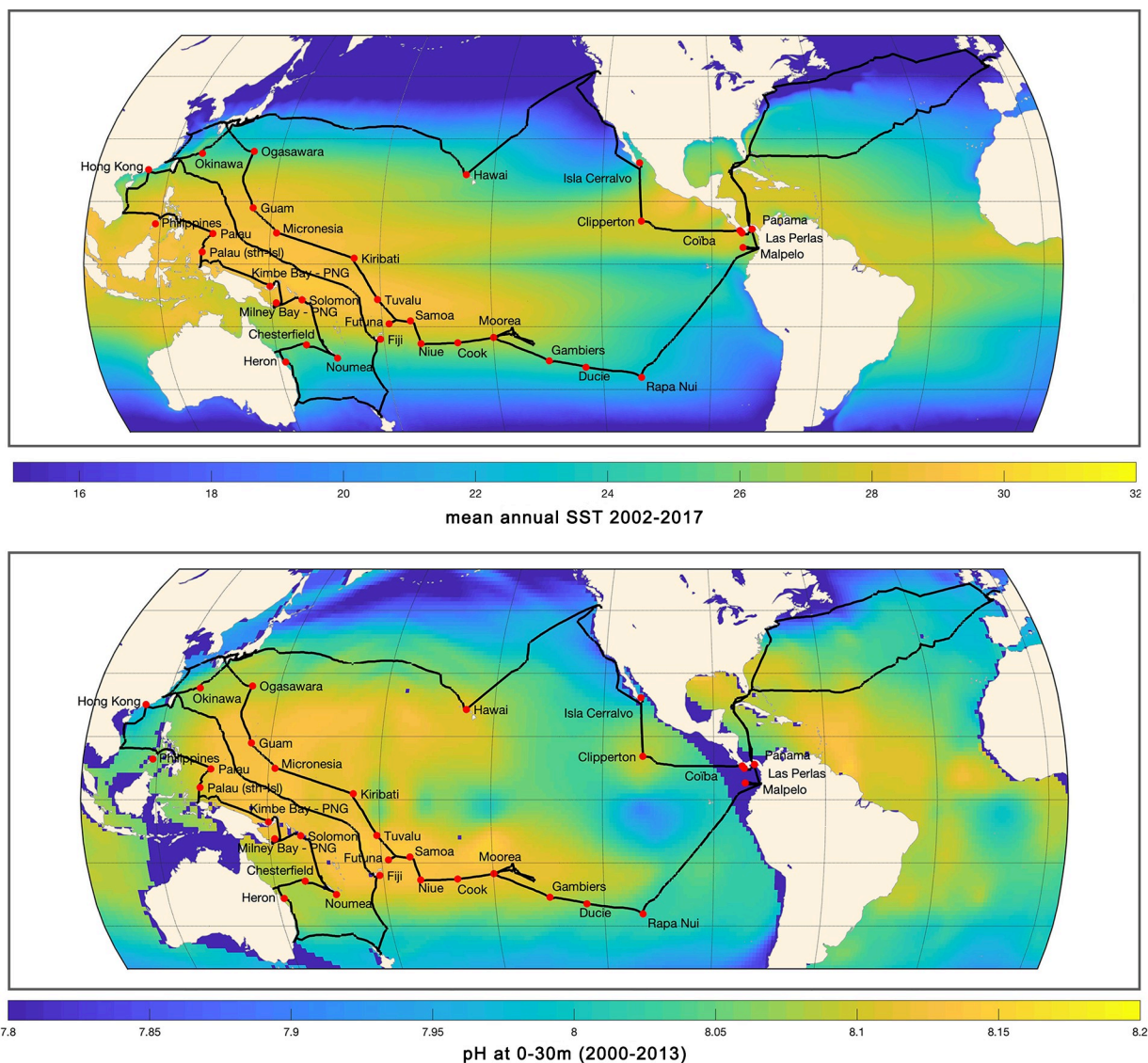
## The coral holobiont

### The coral host

Scleractinian corals evolved over 450 million years ago [14] and belong to the phylum Cnidaria, animals located close to the root of the metazoans. Because of their place in the early Eumetazoa and as a sister group of bilaterians, the cnidarians allow interesting evolutionary analyses regarding the origins of animal complexity and the coevolution of early animals with microbes. Shinzato and colleagues [15] were the first to show that the coral genome is as complex as the one of vertebrates and that it has retained many ancestral genes lost in other lineages. Subsequent studies confirmed the deep genomic differences between coral taxa [16]. Moreover, the exceptional longevity of corals, reaching several hundred to thousands of years, makes them attractive emerging models for aging studies.

### The coral endosymbiont microalgae

Reef corals host symbiotic unicellular algae of the family Symbiodiniaceae inside their endodermal cells [17], raising intriguing questions on animal–plant interactions, photosymbiosis, coevolution, and speciation [18]. The divergence and genetic variation within and between populations of coral species is also investigated, allowing us to firmly ground the exact taxonomic status of each coral host [19].



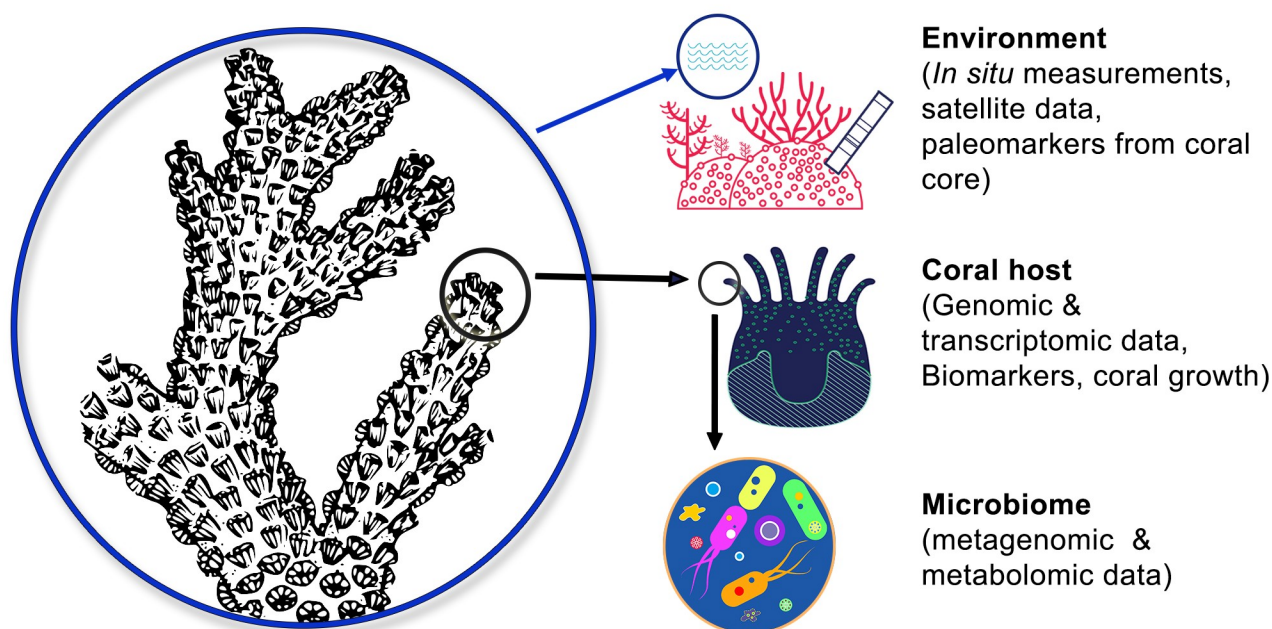
**Fig 1. Map showing the route of the *Tara* Pacific expedition and the sampling sites (red spots) throughout the Pacific Ocean (July 2016 to October 2018) as well as the mean annual SST (top) and pH (bottom).** Global annual SSTs were extracted from the MODIS-Aqua satellite and correspond to global mapped climatologies of the period from 2002 to 2018 (NASA Goddard Space Flight Center), whereas pH values originate from the GLODAPv2 database [59, 60], with mean data displayed corresponding to the 0–30 m depths between 2000 and 2013. GLODAP, Global Ocean Data Analysis Project; MODIS, Moderate Resolution Imaging Spectroradiometer; NASA, National Aeronautics and Space Administration; PNG, Papua New Guinea; SST, sea surface temperature.

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## The coral microbiome

In addition to the Symbiodiniaceae, corals host diverse groups of microorganisms, bacteria, archaea, fungi, and protists, together forming the so-called coral holobiont [13]. The importance of the microbiome in the normal steady-state functioning of corals or during environmental stress is not well understood, but recent research suggests that microbiome structure aligns with and contributes to stress resilience [20, 21, 22] (in particular, bleaching [23, 24]), as seen in humans [25]. Recent work provides evidence that corals have coevolved with certain groups of microbes [26–28], but the exact nature of these host–microbe symbioses and their involvement in coral resilience remains unclear. Therefore, *Tara* Pacific will draw, using

## Coral Health and Resilience



**Fig 2. Schematic representation of the frame and major goals of Tara Pacific, which is investigating jointly the coral, microbiome, and environment.**

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marker gene and metagenomic/metatranscriptomic approaches, a nearly exhaustive census of the microbial diversity associated with targeted coral taxa, allowing the understanding of the major drivers of the microbiome diversity and its role in coral health, resilience, and evolution.

### The coral virome

Viruses infect all known cellular organisms and are the most abundant biological entities on our planet [29]. Yet even the basic diversity of viruses associated with marine species and habitats is shockingly unknown. This is particularly true in tropical reef systems, where research on viral abundance, diversity, and dynamics is still in its infancy (for review, see [30]). Tara Pacific is using metagenomics and microscopy to interrogate the diversity of viruses across Pacific waters and within the foundation species of coral reefs.

### Research objectives of Tara Pacific

Our goal is to unveil the entire organismal diversity of eukaryotes, prokaryotes, and viruses associated with the targeted coral and fish holobionts and their surrounding waters and to assess variation across the explored ecological and geographical gradients. The main objectives of Tara Pacific are as follows:

- Draw a near-exhaustive census of the biodiversity composing and surrounding coral holobionts. This includes the coral hosts, their endosymbiotic microalgae (zooxanthellae of the family Symbiodiniaceae), and the associated microeukaryotes, bacteria, archaea, and viruses found either as endo- or exosymbionts, or in the plankton in surrounding water, using a metabarcoding approach.

- Determine the spatial patterns of the holobionts and the environmental diversity within coral reefs throughout the Pacific Ocean and derive large-scale biogeographic patterns to be compared with macrofauna patterns of diversity (i.e., the Pacific biodiversity gradient).
- Compare metatranscriptomes and metagenomes of coral reefs throughout the Pacific Ocean and elucidate the contribution of microbial diversity to local versus basin-scale adaptation in response to climate change. Importantly, the entire plankton community surrounding the corals and the islands has been sampled and integrated with physicochemical contextual data, providing critical context to advance our understanding of the environmental contribution to coral reef resilience.
- Investigate divergence and standing genetic variation within and between populations of the targeted coral species and their Symbiodiniaceae symbionts across the Pacific Ocean basin via marker gene barcoding as well as genome and transcriptome sequencing (population genomics using SNPs). Extensive metadata resources collected during *Tara* Pacific will allow the coral holobiont composition to be modeled, taking environmental and coral trait data into account (e.g., temperature, pH, bleaching, symbiont acquisition, reproduction cycle, etc.) to identify drivers of adaptation/selection or acclimatization, providing insights into coral stress resilience.
- Investigate the health status of corals by measuring growth parameters of the recent coral skeleton and stress biomarkers (antioxidant capacity, apoptosis, stress response pathways, transcriptomic signatures) as well as by determining the telomere status of each sample (telomeric DNA length and damage as potential proxies for stress). These data will be studied in relation to holobiont biodiversity, transcriptomic and metabolomics data, modern and/or historical environmental parameters, and stresses determined from a geochemical analysis of the coral skeleton.
- Generate a holistic coral metabolome for coral species as a foundation to the identification of the links between holobiont metabolism and prevailing environmental conditions.
- Identify how the environment (physical, chemical, biogeochemical, and biological) is influencing coral holobiont diversity, physiology, and evolution using an extensive compilation of environmental conditions.

## A unique, pan-ecosystemic sampling strategy

The Pacific Ocean covers approximately one-third of the earth's surface, with nearly 25,000 islands, most of which harbor coral reefs. *Tara* Pacific equipped the schooner *Tara* to explore 32 islands across the entire Pacific over a period of 2.5 years (Fig 1). The route of the *Tara* schooner was chosen to maximize the number of visited remote islands and atolls and to perform the widest possible comparative survey, from the equator to the temperate and more acidic regions, encompassing most of the environmental range where scleractinian coral species can live. It also covers a biodiversity gradient, from the low diversity present in the eastern Pacific reefs to the highly diverse western Pacific "warm pool" [14]. This sampling strategy also encompasses a wide variety of environments, from high temperature/low seasonality to low temperature/high seasonality, and also a full range of trophic status and physicochemical environmental parameters that affect coral reef ecosystems (sea surface temperature [SST], pH, nutrients, lights, pollutants, etc.).

Across its entire route, the *Tara* Pacific expedition targeted two species of scleractinian corals (Cnidaria, Anthozoa: *Pocillopora meandrina* and *Porites lobata*), one species of hydrocoral

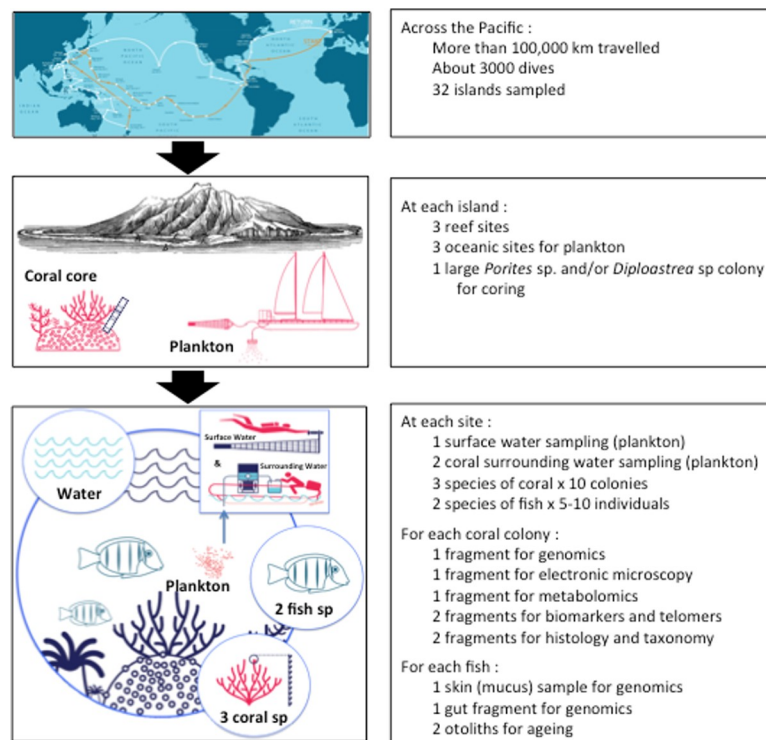
(Cnidaria, Hydrozoa: *Millepora platyphylla*), and two species of reef fish (*Acanthurus triostegus* and *Zanclus cornutus*) (Fig 3). The chosen taxa are among the few species that occur across most of the Pacific Ocean and are usually abundant on reefs. Around each of the 32 islands, three sites were sampled, collecting 10 colonies of each coral species and 5–10 individuals of each fish species, as well as water samples (see below) (Fig 3). Coral fragments and fish samples were preserved immediately on board, using specific buffers (e.g., DNA/RNA shield for genomics, glutaraldehyde for microscopy, etc.), and/or flash frozen for subsequent laboratory analyses (e.g., metabolomics, biomarkers, etc.).

In addition, seawater biogeochemistry and plankton microbiomes (viruses to zooplankton) were assessed in oceanic waters upstream and downstream of each island, as well as from water above each site (i.e., “surface water”) and surrounding two colonies of sampled *P. meandrina* corals at each site (i.e., “coral-surrounding water”). Underwater propeller-driven plankton nets (for surface water) and water pumping systems (for coral-surrounding water) were developed to collect plankton on reef sites, whereas the preparation of plankton samples into different size classes (<0.2, 0.2–3, 3–20, 20–2000 µm) and their preservation followed the *Tara* Oceans protocols [31] in order to maximize comparability and integration of data. Samples will be analyzed using high-throughput imaging and genomic techniques.

Finally, key physical, chemical, and biological parameters were measured continuously from surface water and from aerosols using specific instruments installed on board (e.g., thermosalinograph, mass- and spectrophotometers), as well as samples for imaging and genetic analysis of aerosol particles. Environmental context will also be obtained from satellite images and operational oceanographic products from the European Copernicus Marine Service and Mercator Ocean.

## **Tara Pacific samples and data resources for the immediate and long-term future**

*Tara* Pacific endorses the findable, accessible, interoperable, reusable (FAIR) principles for scientific data management [32] as well as ethical and responsible use of data. With a few exceptions, all physical samples of coral, plankton, fish, and sediments were preserved on board *Tara* and sent back to partner laboratories for subsequent analyses. Controlled vocabularies describing sampling devices and sample preparation protocols were used throughout the expedition to capture provenance metadata on customized log sheets, and samples were assigned unique identifiers to facilitate their traceability. Legal documents regulating the collection, export, and import of samples, as well as links to the Convention on Biological Diversity’s (CBD) access and benefit-sharing clearing house (<https://absch.cbd.int/>) are in the process of being provided for each sample as a first step toward ethical and responsible use of the *Tara* Pacific data. The detailed registry of all samples, including their provenance and environmental and legal context, is curated manually using simple semantics that enable machine- and human-readable data discovery services. Sequencing data will be deposited at the free, open-access European Nucleotides Archive (<https://www.ebi.ac.uk/ena>); environmental data are deposited at the free, open-access PANGAEA database (<https://www.pangaea.de/>); and both archives will be interlinked *via* the sample registry available online at BioSamples (<https://www.ebi.ac.uk/biosamples/>). Metabolomic data (mass spectrometry [MS] and nuclear magnetic resonance [NMR]) and their annotations will also be accessible through the Metabolights portal (<https://www.ebi.ac.uk/metabolights/>). The submission of data in a relational, open-access, updated, and cured database is critical and necessary for our aim to establish a reference of the biological state of coral reefs in the Anthropocene for the broader research community.



**Fig 3. Schematic representation of the sampling design allowing the comparison of various components of coral reef ecosystems.** Using the developed protocol, we collected 2,500 oceanic samples (32 islands  $\times$  3 oceanic sites  $\times$  5 size fractions  $\times$  2–5 protocols), 7,500 coral-surrounding and surface water samples (32 islands  $\times$  3 coral sites  $\times$  3 environments  $\times$  5 size fractions  $\times$  2–5 protocols), 40 coral core samples (32 islands  $\times$  1 *Porites* sp. and/or *Diploastrea* sp  $\times$  1–2 core samples), 20,160 coral fragments (32 islands  $\times$  3 coral sites  $\times$  3 species of corals  $\times$  10 colonies  $\times$  7 protocols), and 9,600 fish tissue samples (32 islands  $\times$  3 coral sites  $\times$  2 species of fish  $\times$  5–10 individuals  $\times$  5 protocols). Taken together, the dataset comprises a total of approximately 40,000 samples. Bottom figure copyright to Agence DATCHA/[taraexpeditions.org](http://taraexpeditions.org).

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## Metagenomics and metatranscriptomics to explore the diversity and physiology of coral reef holobionts

Building upon expertise gained in the previous *Tara* Oceans project [33–36], we are applying Illumina HiSeq technology to sequence a series of barcodes designed recently to explore the diversity of bacteria and archaea (V4–V5 region of the nuclear 16S rRNA gene [37]), eukaryotes (V9 region of nuclear 18S rRNA gene [33]), Symbiodiniaceae (ITS2 region of the nuclear ribosomal DNA [38–40]), and metazoan species (mitochondrial COX1 gene [41]), as well as metagenome assembly-defined viral populations [42, 43]. Barcode-specific PCRs are performed on DNA from coral tissues, coral-surrounding water, fish tissues, and surface water above the reef in order to assess interkingdom diversity associated with the holobiont at different degrees of proximity from the coral animal host, from endosymbiotic to drifting in coral-surrounding and surface waters. Biogeographic gradients will also be investigated between the studied island systems. The sequence reads are assembled into operational taxonomic units (OTUs) for bacteria, archaea, and eukaryotes and into ITS2-type profiles (using the SymPortal framework at [symportal.org](http://symportal.org) [40]) for Symbiodiniaceae and taxonomically annotated by comparison to reference databases. Classical numerical ecology methods are applied to employ OTU richness and abundance data to (1) assess and compare the total diversity of bacteria, archaea, and eukaryotes associated with various compartments of the different coral

holobionts; (2) compare biodiversity patterns across coral colonies, hosts, reefs, islands (geographic and Lagrangian oceanic circulation distances), and all contextual environmental parameters measured; (3) integrate the *Tara* Pacific data into the *Tara* Oceans global plankton dataset. In addition, co-occurrence graph techniques inspired from systems biology [44, 9] are used to reconstruct OTU interaction (sub)networks, and we will aim to disentangle the intrinsic (symbiotic auto-organization) from the extrinsic (environmental, historical contingencies) forces on holobiont biodiversity. Overall, this study is the first attempt to characterize coral reef and holobiont diversity across comprehensive taxonomic, ecological, and geographical scales over an entire oceanic basin. Comparison with *Tara* Oceans plankton data will also reveal which part of the coral holobiont biodiversity is found in the open ocean and thus bring fundamental information about reef connectivity, as recently shown for Symbiodiniaceae, which are found all over the plankton [34].

The sequencing of the holotranscriptomes of coral holobionts sampled along the transect will provide important genetic information to uncover ecological and evolutionary questions at both intraspecific and community levels. Two protocols are successively applied on total RNA from three specimens per species and per site. In a first step, a polyA+ enrichment protocol is applied to provide eukaryotic mRNA in order to study the coral and Symbiodiniaceae dual transcriptome, and in a second step, rRNA is removed from the remaining polyA- fraction to sequence microbial mRNA. We are applying different metatranscriptomics protocols to capture the gene expression of the Cnidaria, Symbiodiniaceae, bacteria and archaea, other microbes, and even viruses from the same initial tissue samples [45, 46]. In addition, we are using similar analysis procedures after filtration of coral-surrounding water samples. This may potentially reveal how microbes and viruses interact with the coral host and how the coral host adapts or acclimatizes to their presence. On top of providing deep insight on gene expression networks at the holobiont level, mRNA samples will also be used to generate transcriptome-wide SNPs for the coral host, allowing in-depth characterization of the patterns of diversity, connectivity, demographic history, and local adaptation of sampled coral species. Hence, the resulting patterns will provide a solid foundation on which to base the study of partner relationships within the coral holobiont, and possibly even allow for the identification of the selective forces acting on key genes for these symbiotic associations, plus an account on standing genetic diversity within and between reefs to highlight regions of high genetic endemism and diversity important for conservation efforts.

## A metabolomic approach

We will also assess the metabolome (i.e., the metabolic diversity) of the three targeted coral species (two stony and one fire coral, see above). Small molecules (i.e., specialized metabolites) are the end products of unique metabolic pathways. Most of these presumably possess an ecological role, and as such, they represent key phenotypic traits for the specimens. The broad geographical cover of the collected coral species will enable us to provide some insight into the relative contribution of environmental factors and the genetic information expressed in the metabolome by comparison with genomic data. Recent advances in analytical techniques and bioinformatics led to the development of global metabolomics approaches capable of providing an overview of the thousands of metabolites present in a minute amount of sample [47, 48]. A nontargeted metabolomics approach is applied to the three species of corals collected across the Pacific Ocean using both MS and NMR. The subsequent identification of the chemomarkers unveiled by these comparative approaches are performed using not only databases of experimental spectra but also comparison with in silico databases of metabolites. The results will finally contribute to the assessment of the reef health status when combined with other “-omics” data.

## Identifying the interaction among environmental stressors, the holobiont, and coral resilience

A key question to be addressed by *Tara Pacific* is how reef corals change geographically and how corals locally adapt or acclimate to environmental changes and increasing stress. This question has been rarely studied at the scale of a complete ocean and across multiple populations under consideration of phenotypic traits [49]. The physiological status of corals will be assessed by measuring the parameters of coral growth such as density, linear extension, and calcification rates of *P. lobata* (with added *Diploastrea heliophora* in few places) colonies by analyzing coral cores recovering the last 50–150 years by sclerochronology. The annual density bands and growth parameters of the massive coral sampled along the transect will be determined using X-ray radiography and 3D-computed tomography of growth rings on sliced coral cores [50–52], allowing the calculation of recent reef growth over the last decades and century. In parallel, ambient sea temperature and pH changes over the same period of time will be documented by analyzing, at an annual or monthly timescale, the most advanced geochemical tracers along the cores, such as boron isotopes or trace elements, trapped along the cores during the coral life [53–56]. Recent stress events will be identified by a series of conventional markers of physiological stress or damage (see above) [57]. In addition, telomere length measurements of coral and microalgal symbiont genomes will be conducted. Notably, although measurements of telomere length as a method of assessing stress accumulation is currently being employed in numerous human cohort studies, the importance of telomere length for stress resilience and biodiversity in ecological studies is in its infancy and remains to be determined [58]. Thus, it will be highly informative to compare telomere length variations with coral growth measurements assessed by sclerochronology.

More broadly, the integration of environmental and physicochemical with biological data will allow us to pinpoint the specific adaptations that enable corals to live and survive across environmental gradients. Comparison of current to historical data throughout the Pacific will then show the biological cost of this adaptation and the associated time span. This in turn will make for a much better understanding of the capacity of coral holobionts to adapt to adverse environmental conditions and the required time scales.

In summary, *Tara Pacific* is poised to build the most comprehensive morphomolecular inventory of the phenotypic and genotypic biodiversity of coral reef ecosystems, including a wide spectrum of life from viruses to bacteria and from unicellular eukaryotes to metazoans and covering nested spatial scales from coral holobiont colonies, their surrounding water (i.e., coral-surrounding water), reef surface water, and neighboring oceanic surface waters (upstream and downstream of the sampled islands). The project focuses on selected key species that are ubiquitous in coral reef ecosystems throughout the Pacific and can be traced from an environmental, physicochemical, biological perspective down to the gene. This ambitious project has the potential to reveal substantial uncharted biodiversity; to shed light on the complex links between genomes, transcriptomes, metabolomes, organisms, and ecosystem functions in coral reef systems; and to provide a basis for the biodiversity and biological state of modern coral reefs for the research community at large. In addition, this project will contribute significantly to other fields of research such as stress and aging biology. We expect that the unprecedented scale of this project will help to decipher the complex interactions that together ensure a healthy state of coral reefs, a quest for which advances are urgently needed.

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