The biodiversity and biotechnological potential of marine fungi associated with sponges

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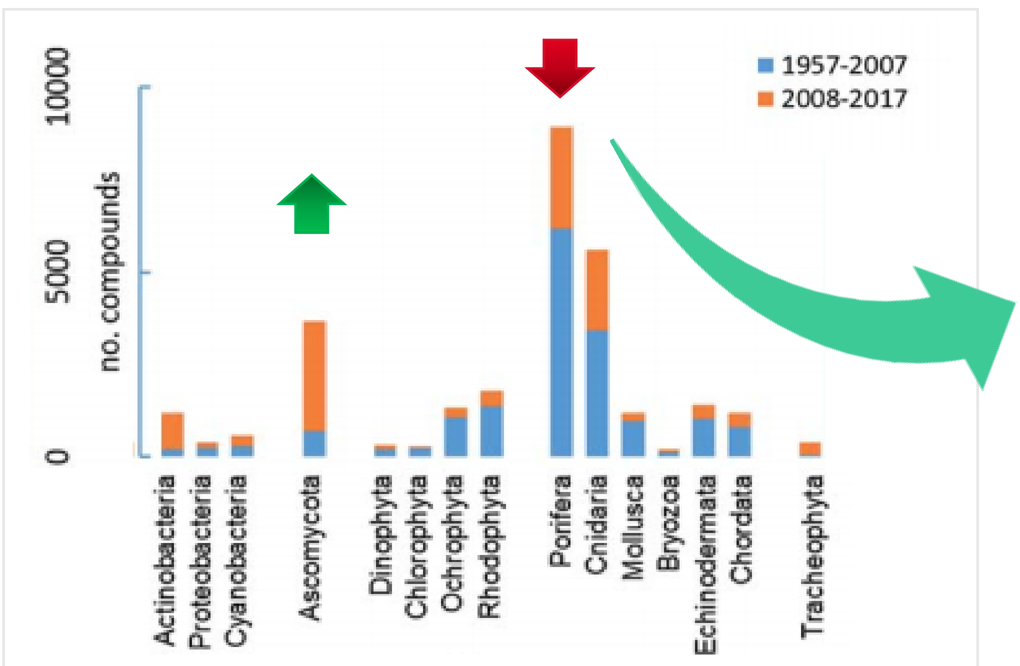
Sponges are relevant for secondary metabolites production

In 1951 the chemistry of marine natural products began with the isolation from *Cryptotethya crypta* of spongouridin and spongothymidin used as base structure for the new antiviral Ara-A (Anjum *et al.*, 2016)

Ecological roles of secondary metabolites

Defense

- Predators
- Pathogens
- Fouling organisms



Carroll *et al.* (2019) modified

Pharmacological properties

- Antibacterial
- Antifungal
- Anti-inflammatory
- Antiviral
- Anticancer
- Antitubercolosis
- Anti-Alzheimer's
- Anti-HIV
- Antimalarial

Environmental properties

- Antifouling

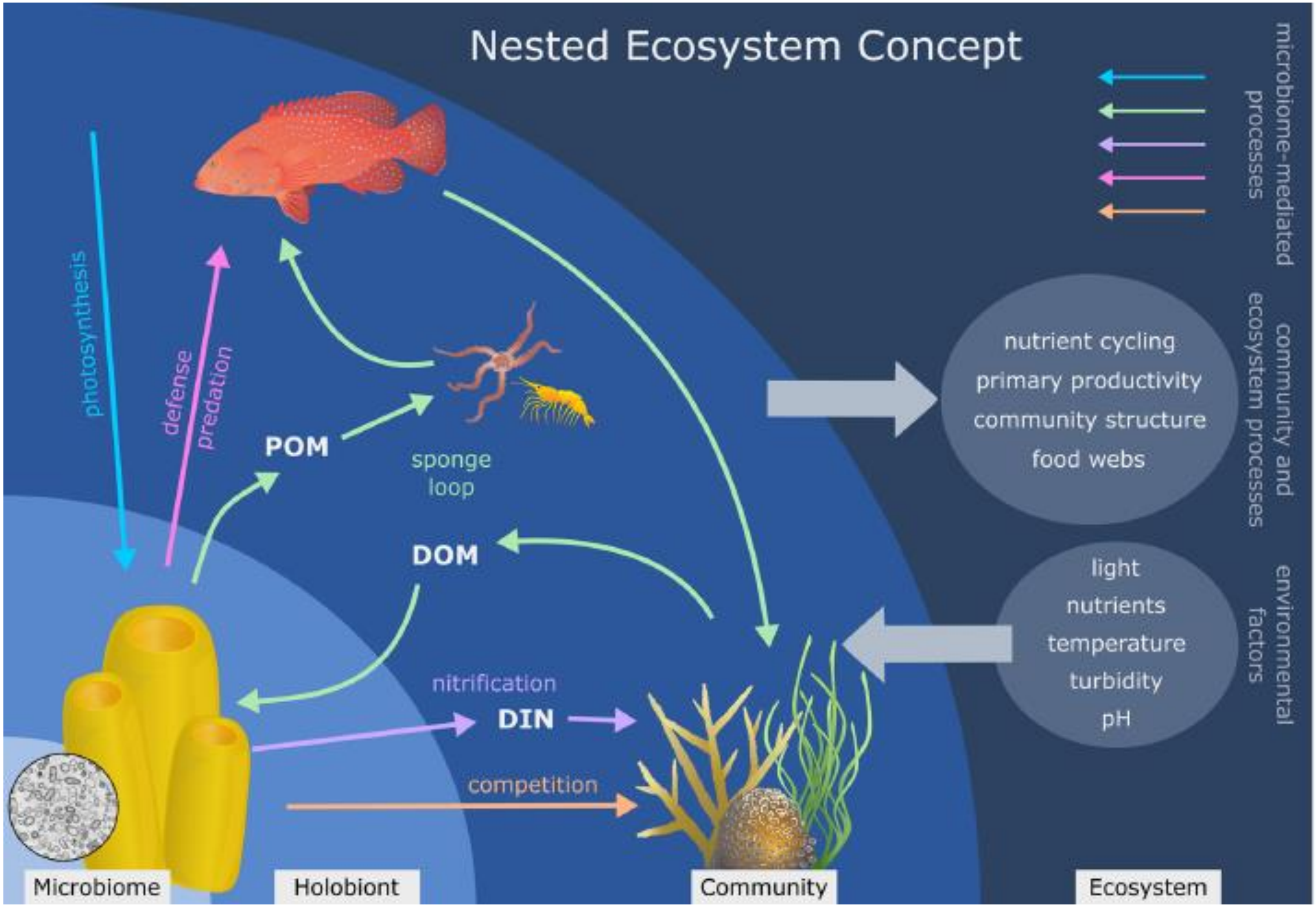
Are sponges the real producer of secondary bioactive metabolites?

28% of novel compounds are produced by sponges associated fungi (Imhoff, 2016)

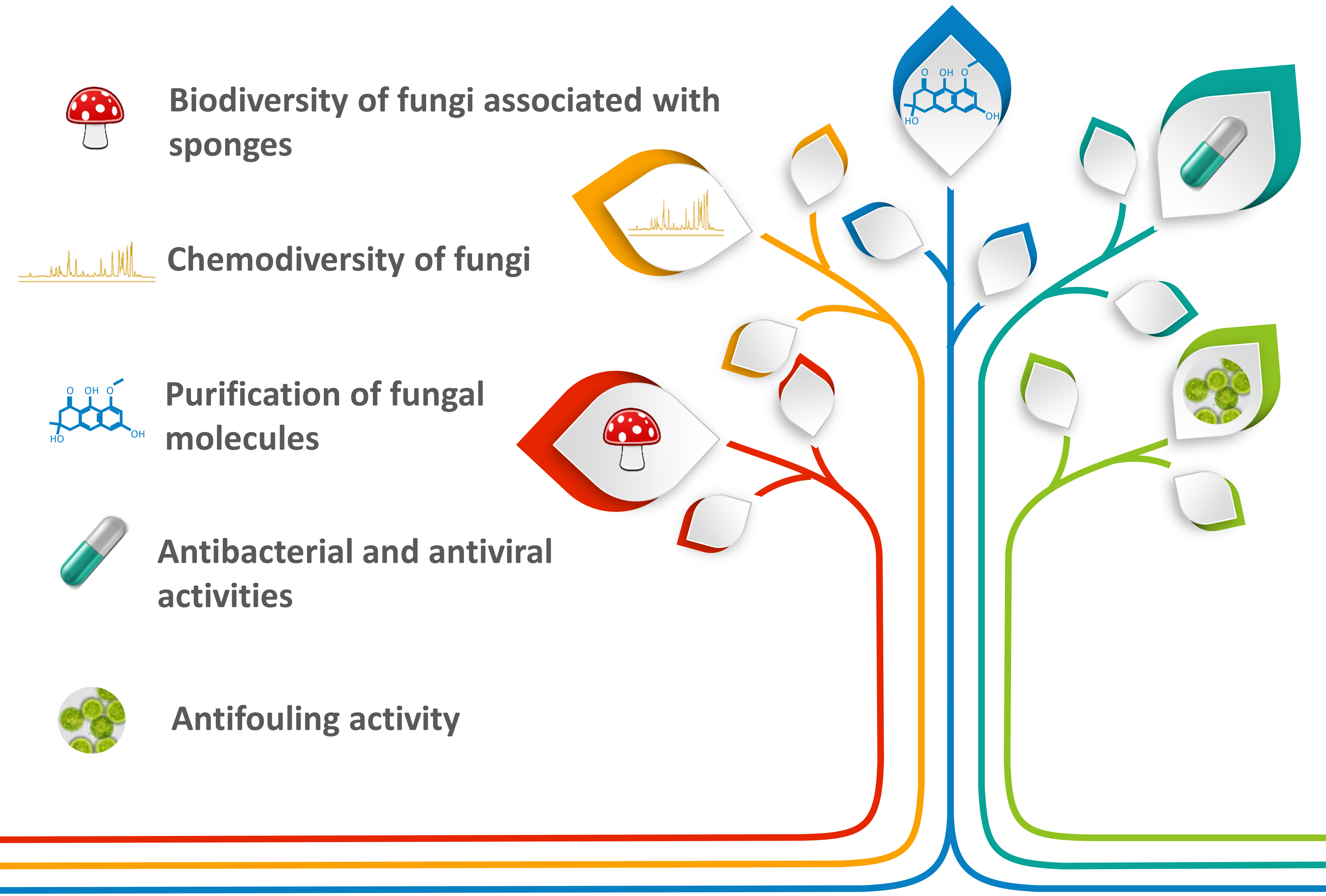
Over their 660–635 M years evolution sponges formed a close association with microorganisms including bacteria, archaea, fungi, and algae (Zumberge et al., 2018)

40-60% of sponges biomass is composed by microorganisms (Yarden, 2014)

The role of microorganisms within sponges: the "Sponge loop"



Pita *et al.* (2018)



Biodiversity of fungi associated with sponges
Chemodiversity of fungi
Purification of fungal molecules
Antibacterial and antiviral activities
Antifouling activity

Four Atlantic species of sponges

Samples Collection

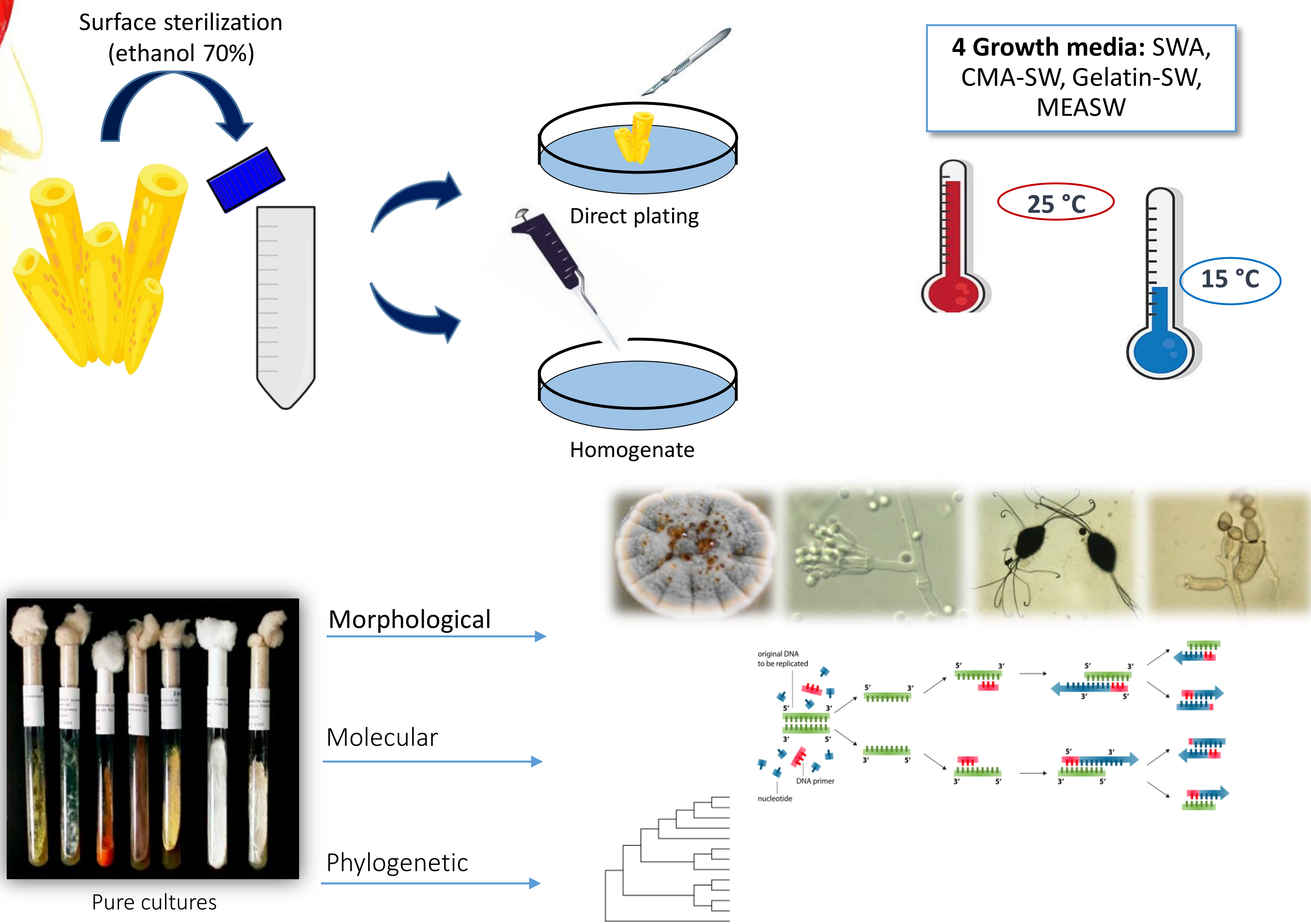
Dysidea fragilis
Demospongiae

Pachymatisma johnstonia
Demospongiae

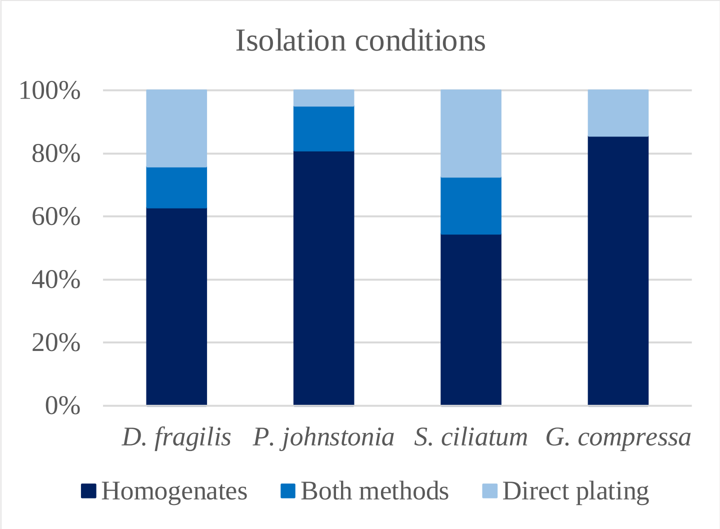
Grantia compressa
Calcarea

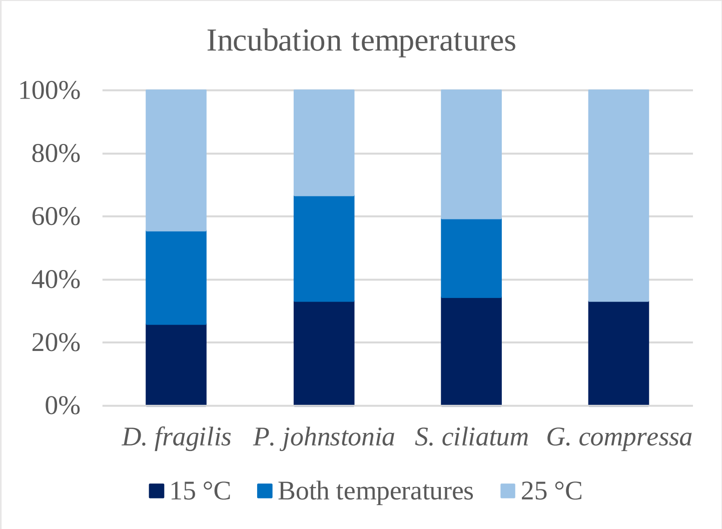
Sycon ciliatum
Calcarea

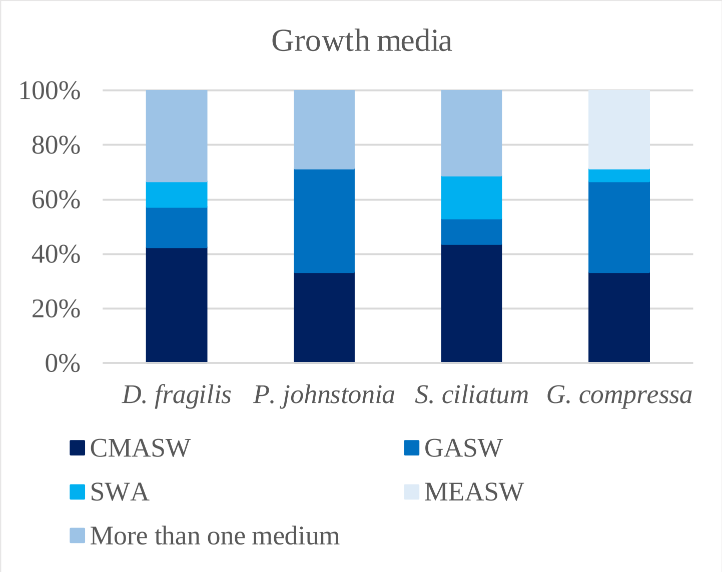
Isolation and identification of fungi



Influence of the isolation techniques on fungal diversity







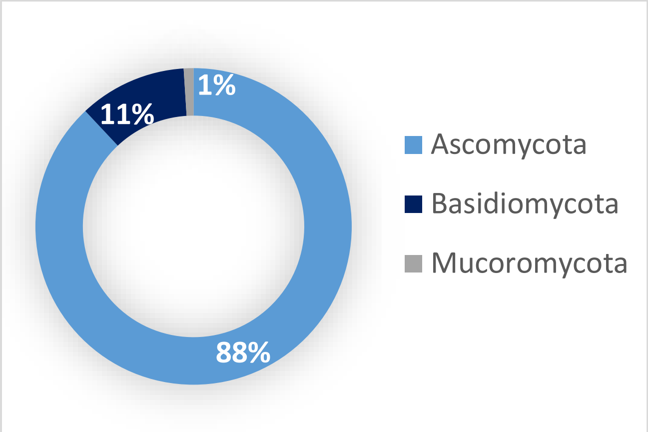
38% - 66% fungi were retrieved in only one of the conditions

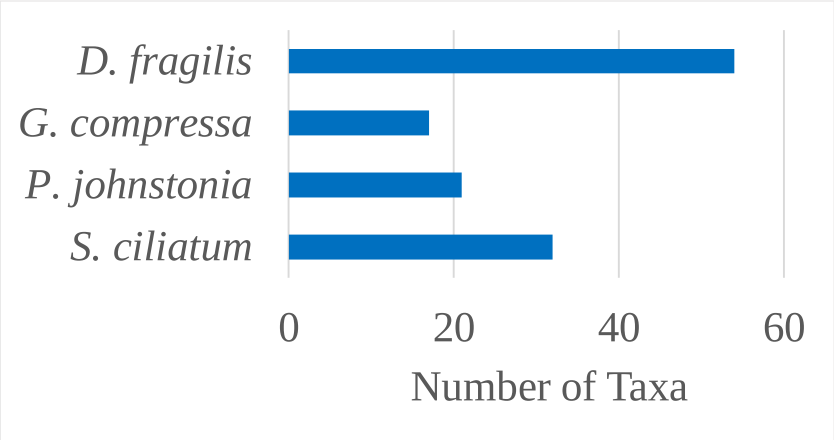
Fungal diversity

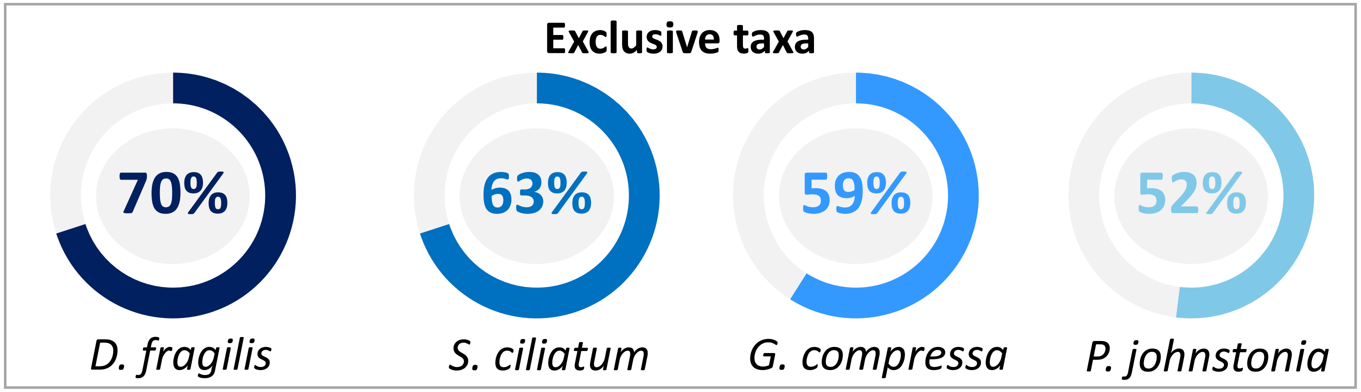
97 taxa

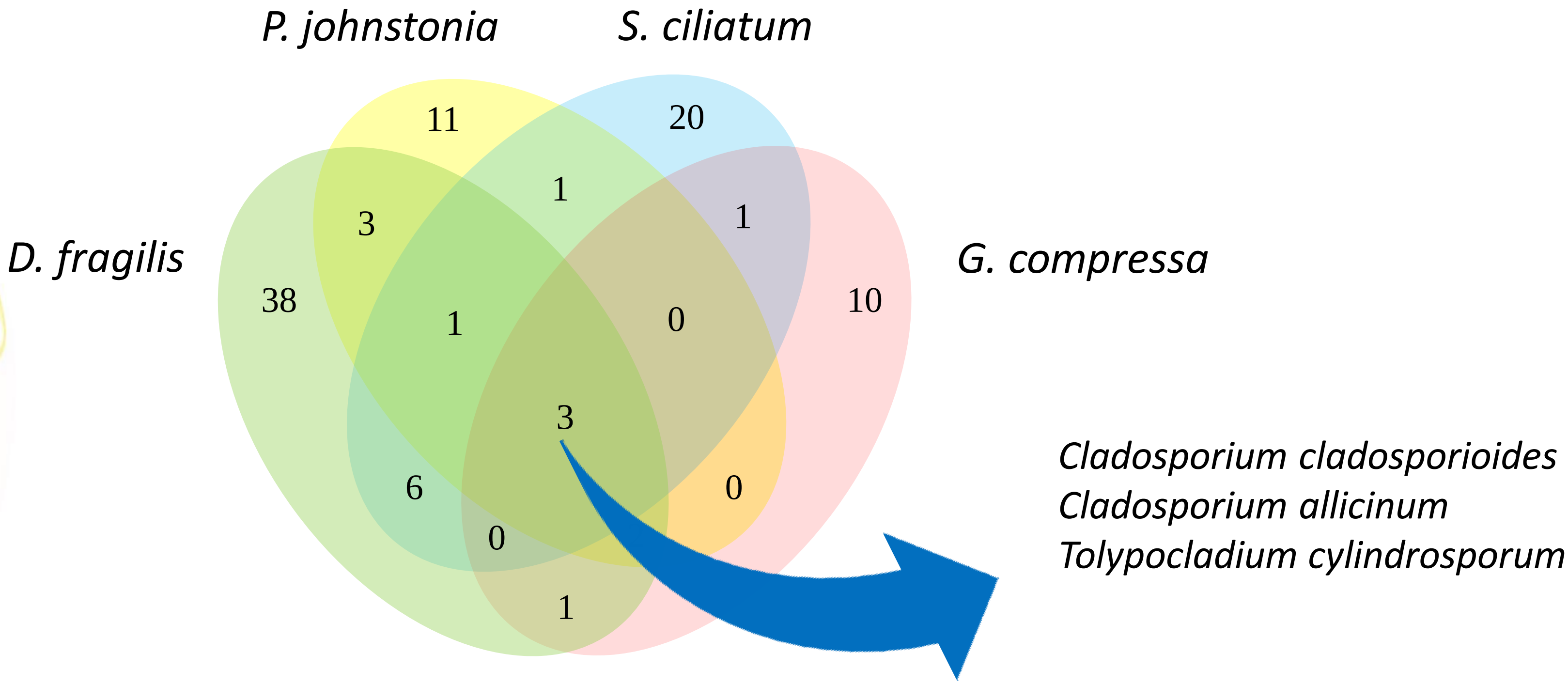
84% of taxa identified at species level

57 species → first report in sponges
21 species → first report in marine environment



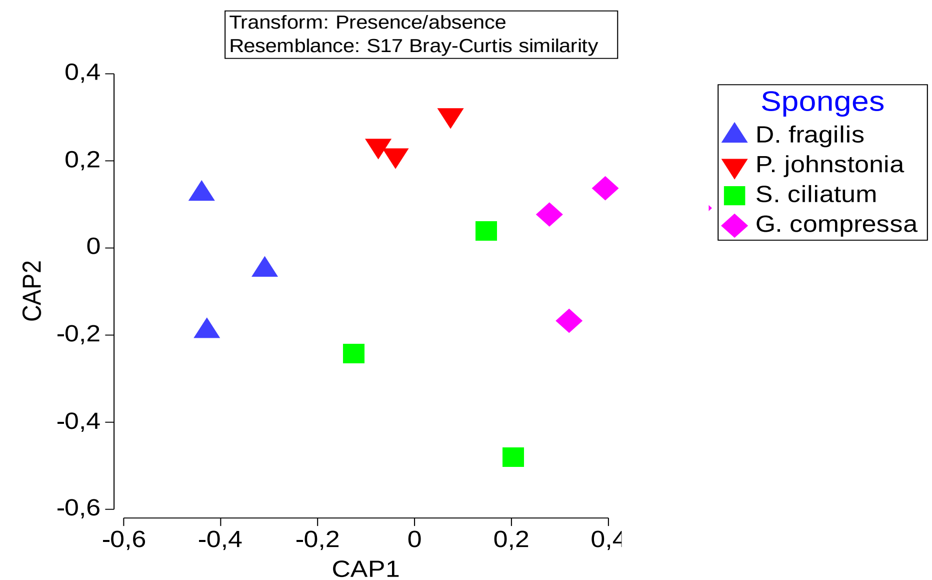






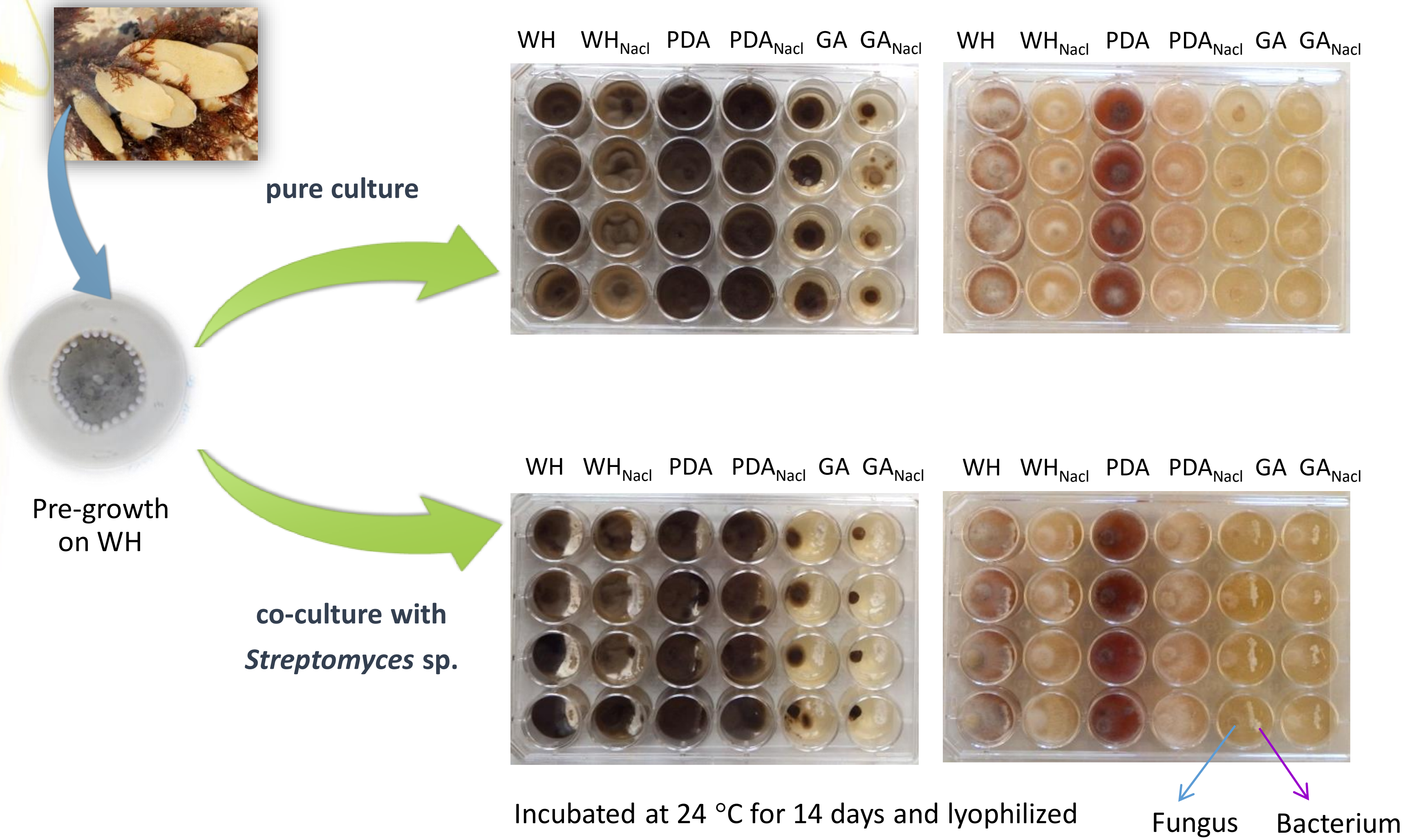
The fungal community was significantly different (*p*<0.01)

Constrained Analysis of Principal Coordinates (CAP)



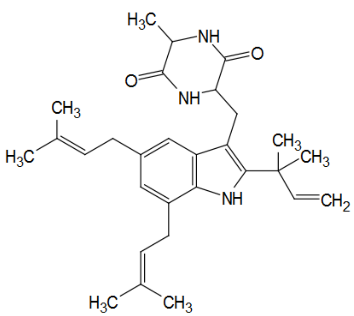
OSMAC approach (One Strain – Many Compounds)

All 20 strains isolated from *G. compressa* were tested in 12 conditions

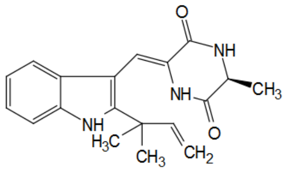


Incubated at 24 °C for 14 days and lyophilized

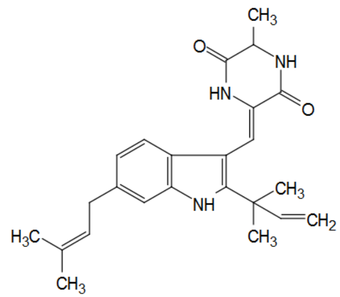
10 compounds isolated from *Eurotium chevalieri* MUT 2316



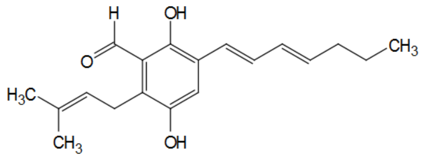
Echinulin
(1) 🟢



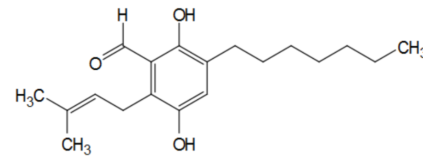
Neoechinulin A
(2) 🟢



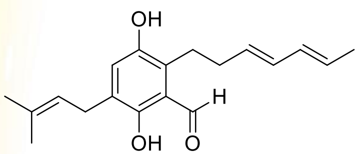
Neoechinulin D
(3) 🟣



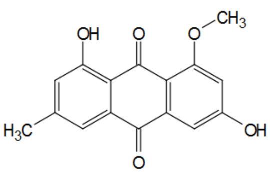
Dihydroauroglaucin
(4) 🟢



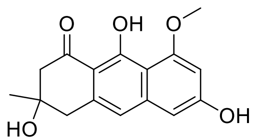
Flavoglaucin
(5) 🟢



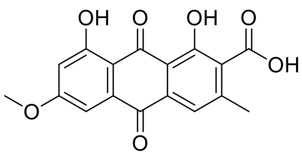
Isodihydroauroglaucin
(6) 🟢



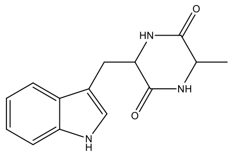
Physcion
(7) 🟢



Asperflavin
(8) 🟢



Cinnalutein
(9) 🟣



Cyclo-L-Trp- L-Ala
(10) 🟢

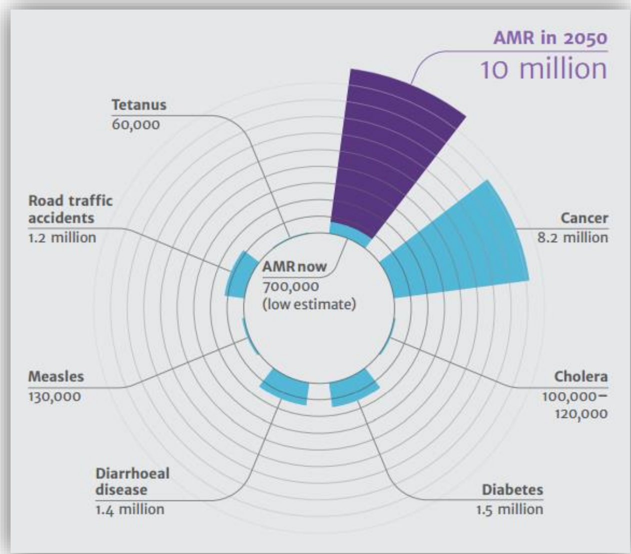
- 🟣 **Isolated for the first time from a marine fungus and in *E. chevalieri***
- 🟢 **Already recorded in *E. chevalieri* or its anamorph *Aspergillus chevalieri*** isolated from soil, air or plants

(Tawfik *et al.,* 2017; Zin *et al.,* 2017; Micheluz *et al.,* 2016; Kanokmedhakul *et al.,* 2011; Fraga *et al.,* 2008; Wu *et al.,* 2014; Ishikawa *et al.,* 1984; Hamasaki *et al.,* 1981)

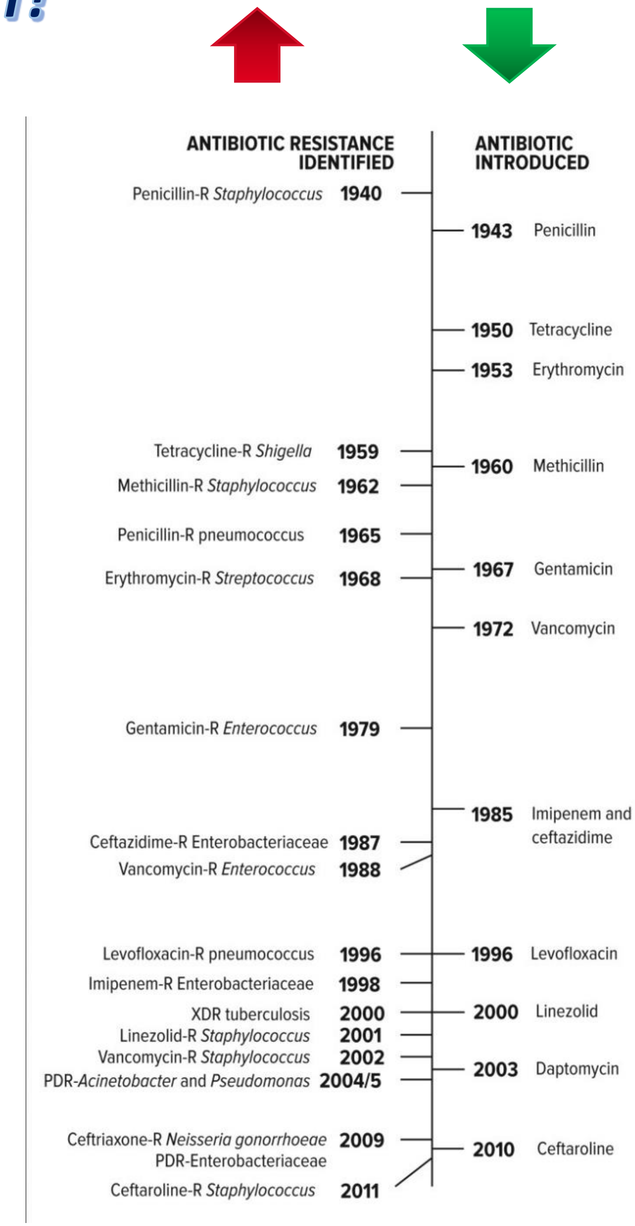
Which target for the 10 compounds produced by *E. chevalieri?*

1. Antibacterial activity

Antimicrobial resistance is now a serious threat to life quality and expectancy (Brown and Wright, 2016)



In 2050 multidrug resistant bacteria will be the first cause of death (O'Neill, 2016)



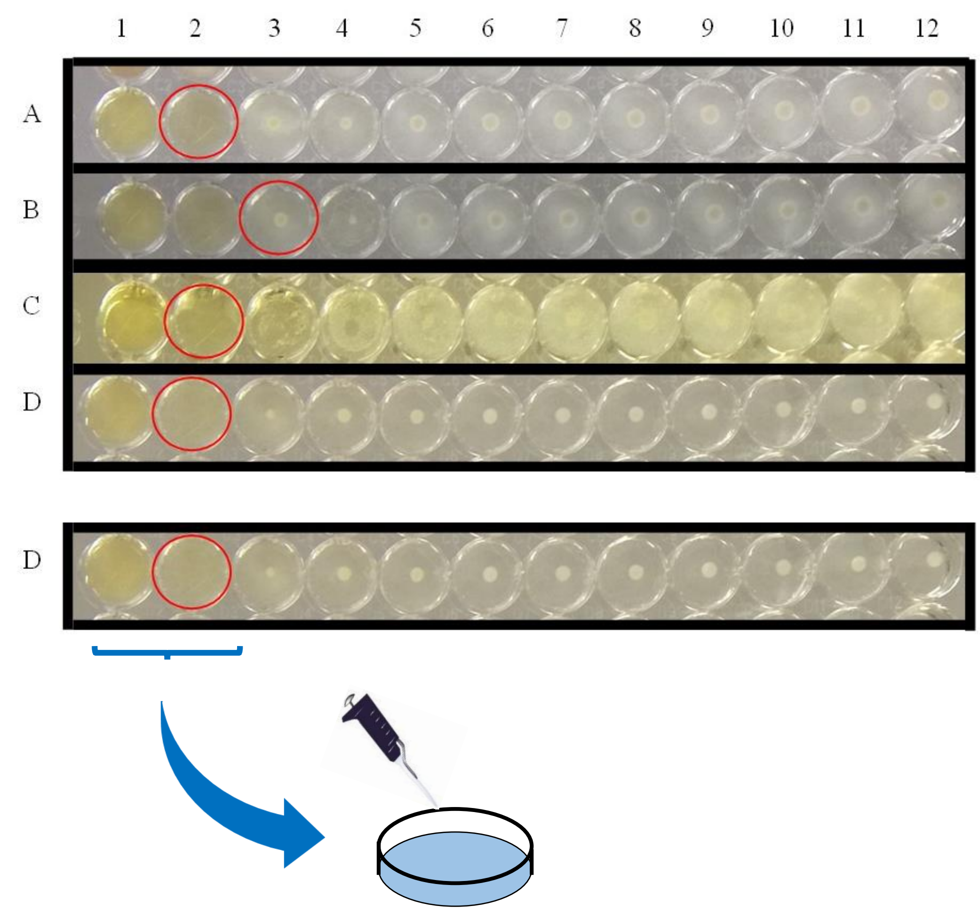
From Ventola, 2015

Antibacterial activity

Species	Code	Gram +/-	Resistance
Enterococcus fecalis	ATCC 29212	+	-
Escherichia coli	ATCC 25922	-	-
Pseudomonas aeruginosa	ATCC 27853	-	-
Staphylococcus aureus	ATCC 29213	+	-
Staphylococcus aureus	Monza-PFI	+	Methicillin resistant
Staphylococcus aureus	Monza-FD1	+	Fluoroquinolone-resistant
Streptococcus pneumoniae	ATCC 49619	+	-
Streptococcus pneumoniae	Monza-82	+	Macrolide-resistant

Minimal Inhibitory concentration (MIC µg/mL)
the lowest concentration of antimicrobial agent causing visible inhibition of bacterial growth

Minimal bactericidal concentration (MBC µg/mL)
the lowest concentration of antimicrobial agent needed to kill bacteria



The molecules were active against Gram + bacteria

Molecules	*S. aureus*		*S. aureus*		*S. aureus*		*E. faecalis*		*S. pneumoniae*		*S. pneumoniae*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Neoechinulin D **(3)** ☆	-	-	-	-	-	-	-	-	-	-	64	-
Dihydroauroglaucin **(4)** ☆	128	-	128	-	128	-	64	-	-	-	8	32
Isodihydroauroglaucin **(6)**	64	128	64	128	32	64	64	-	-	-	4	16
Physcion **(7)**	-	-	-	-	-	-	-	-	-	-	16	-
Asperflavin **(8)**	64	-	128	-	64	-	-	-	-	-	32	128
Cinnalutein **(9)** ☆		-		-	128	-	-	-	-	-	32	

"-"→ >128 µg/mL

Methicillin resistant   Fluoroquinolone resistant   Macrolide resistant

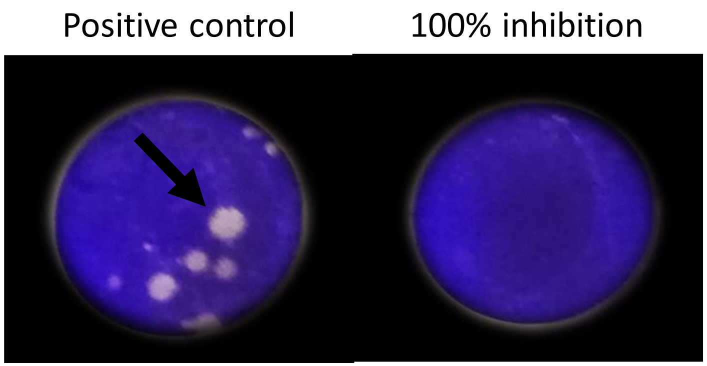
MIC values lower than that reported for Gentamicin

MIC values lower than that reported for Erythromycin

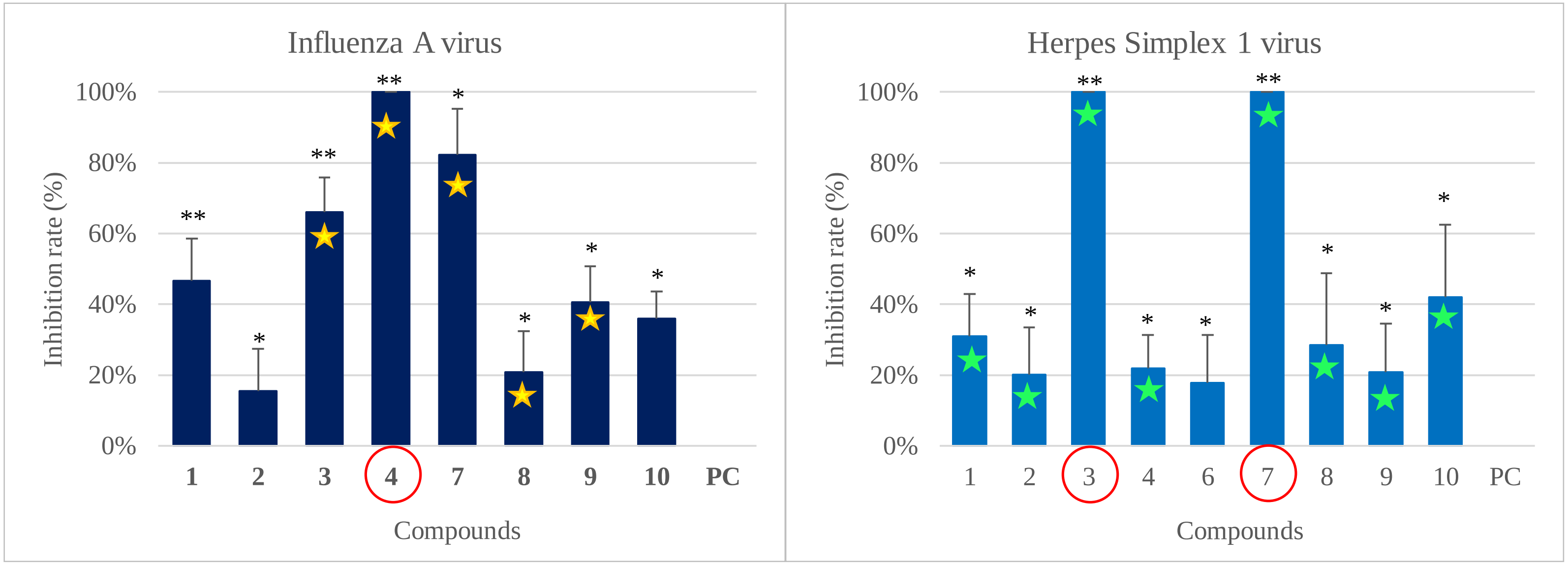
☆ First report of the antibacterial activity

2. Antiviral activity

The antiviral activity was assessed against **Influenza A virus** and **and Herpes Simplex Virus 1**



All the compounds inhibit the viral replication of Influenza A virus and Herpes Simplex 1 Virus



* p<0.05 **p<0.01

Dihydroauroglaucin (**4**) completely inhibited the Influenza A virus

Neoechinulin D (**3**) and Physcion (**7**) completely inhibited the Herpes Simplex 1 virus

★ First report of the antiviral activity against Influenza A virus

★ First report of the antiviral activity against Herpes Simplex 1 virus

3. Antifouling activity

Marine biofouling: accumulation of microorganisms, algae and aquatic animals on surfaces immersed in seawater (Amara *et al.*, 2018)

Consequences:
- Up to 40% Increased fuel consumption
- Increased carbon dioxide and sulphur dioxide emissions
- Invasive species might be transported by ships

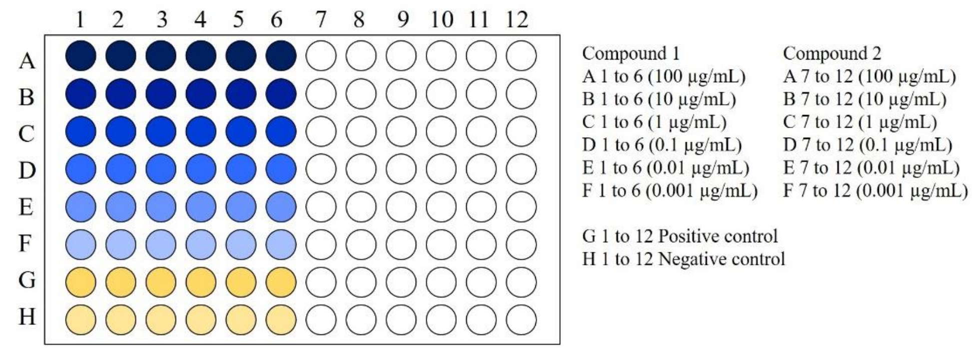
Employed antifouling:
- Tributyltin based compounds → banned in 2008
- copper based paints → not environmental friendly

New and environmental friendly formulations are needed

Seven molecules were assessed for the ability to **inhibit the growth (Gr) and adhesion (Ad) of 5 marine bacteria and 5 algae representative of fouling organisms**

Bacteria	**ATCC code**
Halomonas aquamarina	14400
Polaribacter irgensii	700398
Roseobacter litoralis	49566
Vibrio aestuarianus	35048
Pseudoalteromonas citrea	29720

Microalgae	**AC Code**
Cylindrotheca closterium	170
Exanthemachrysis gayraliae	15
Halamphora coffeaeformis	713
Porphyridium purpureum	122
Phaeodactylum tricornutum	171



Growth and adhesion were monitored spectroscopically

Bioassays against **macrofoulers**

Inhibition of blue mussel *Mytilus edulis* settlement **tyrosinase assay**

Low Observable Effect Concentration (LOEC): the lowest concentration with an average response that is significantly different from the control

Conclusions

1. Increase the knowledge of marine fungi associated with sponges. **97 taxa were isolated**

2. Significant **difference** among the **mycobiotas of the sponges**

3. The **10 molecules** produced by *E. chevalieri* demonstrated an incredible **biotechnological potential**

4. More than half of the **compounds were active against at least one bacterium**, with several molecules more efficient than already known antibiotics

5. **Neoechinulin D** and **Physcion** completely **inhibited the replication of Herpes Simplex 1 virus**. **Dihydroauroglaucin** completely **inhibited the replication of Influenza A virus**

6. **Cyclo-L-Trp- L-Ala** performed better in the antifouling tests showing **inhibition of tyronisase, antibacterial and antimicroalgal activities**

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Vinci Program and **Galileo Program**


Thanks for the attention