The Marine Environment: An Unchartered Resource for Drugs

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39th NAST Annual Scientific Meeting
The Manila Hotel | July 13, 2017

Drug pipeline is running dry…

for

cancer

infections

pain

neurodegeneration

aging

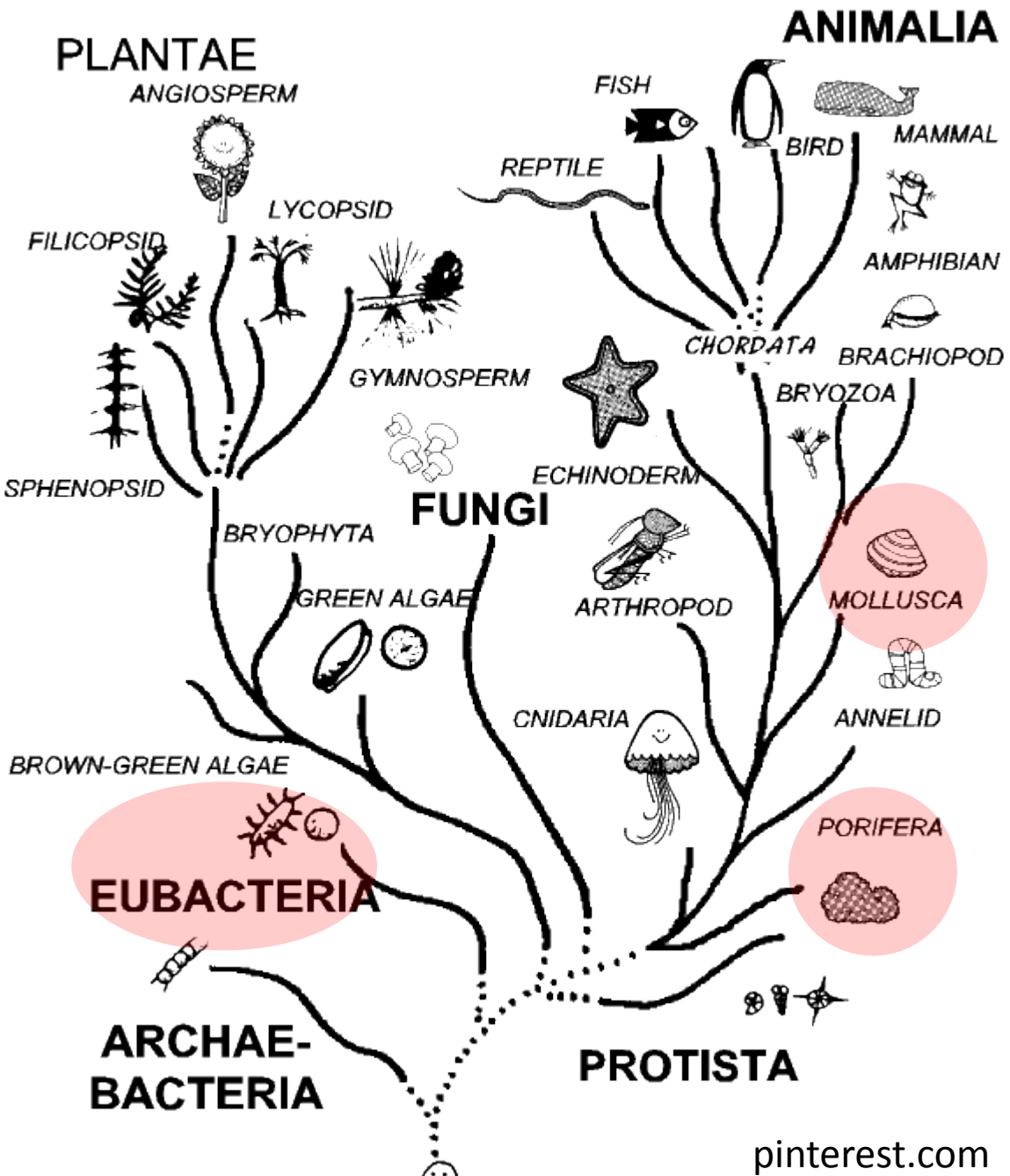
↓

COMBINATORIAL CHEMISTRY LIBRARIES

versus

BIODIVERSITY LIBRARIES

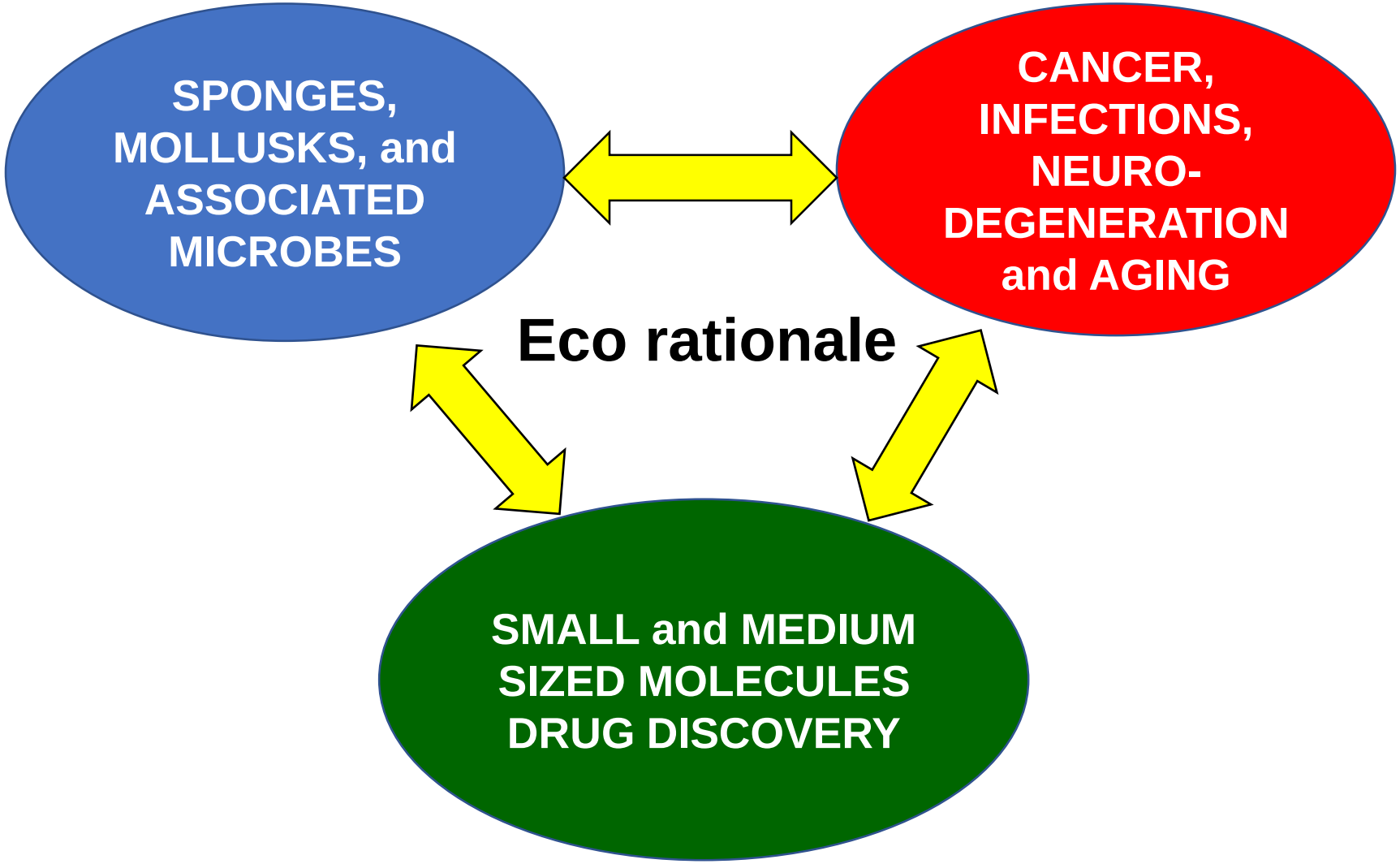
The organisms that we study



pinterest.com

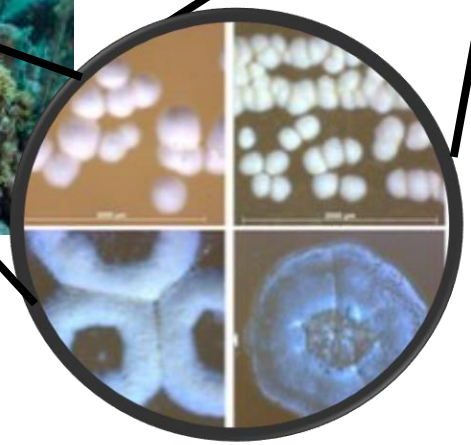
Our basis for studying these organisms

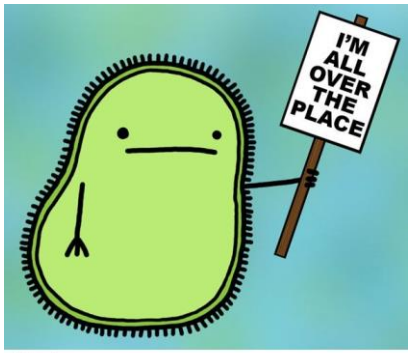
Ecological, Biological and Chemical Leads



Marine Ecosystem 1: Coral Reefs

Balancing of competitive and cooperative interactions among organisms leads to bio-chemo-diversity



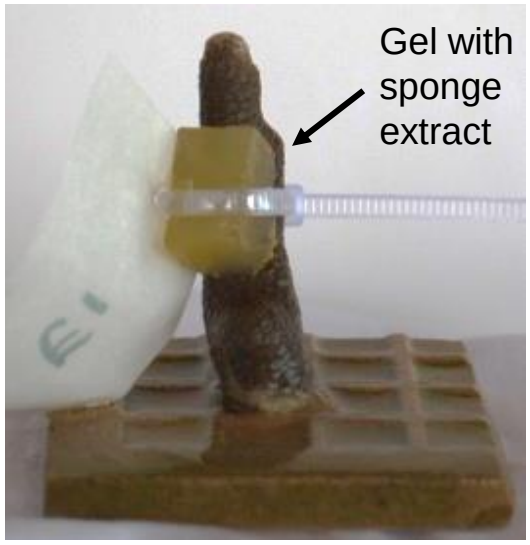


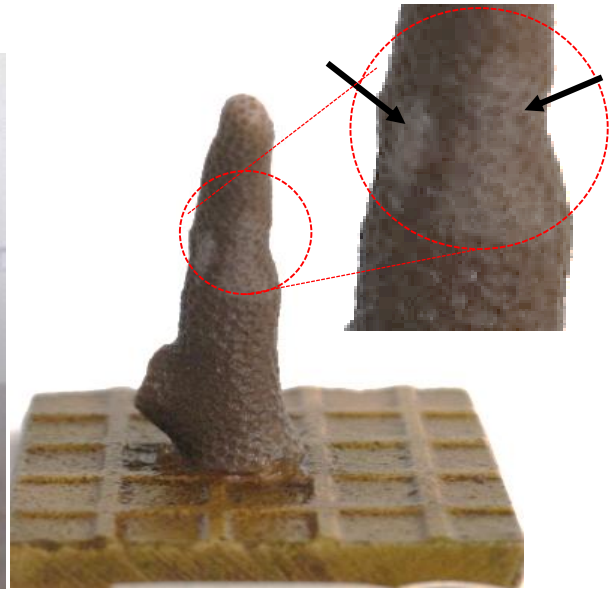
theecologist.org; underwaterkwaj.com

Chemical Ecology Lead for Anticancer Compounds

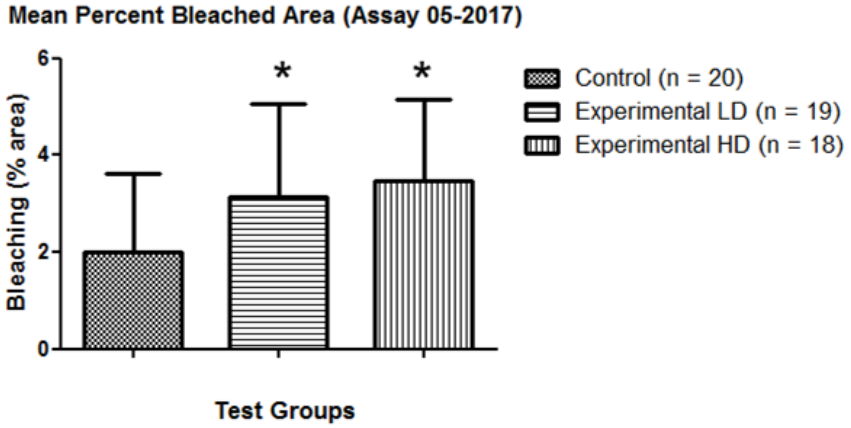
Sponge-coral competition

Sponge *Callyspongia samarensis* overgrows hard coral *Porites cylindrica*



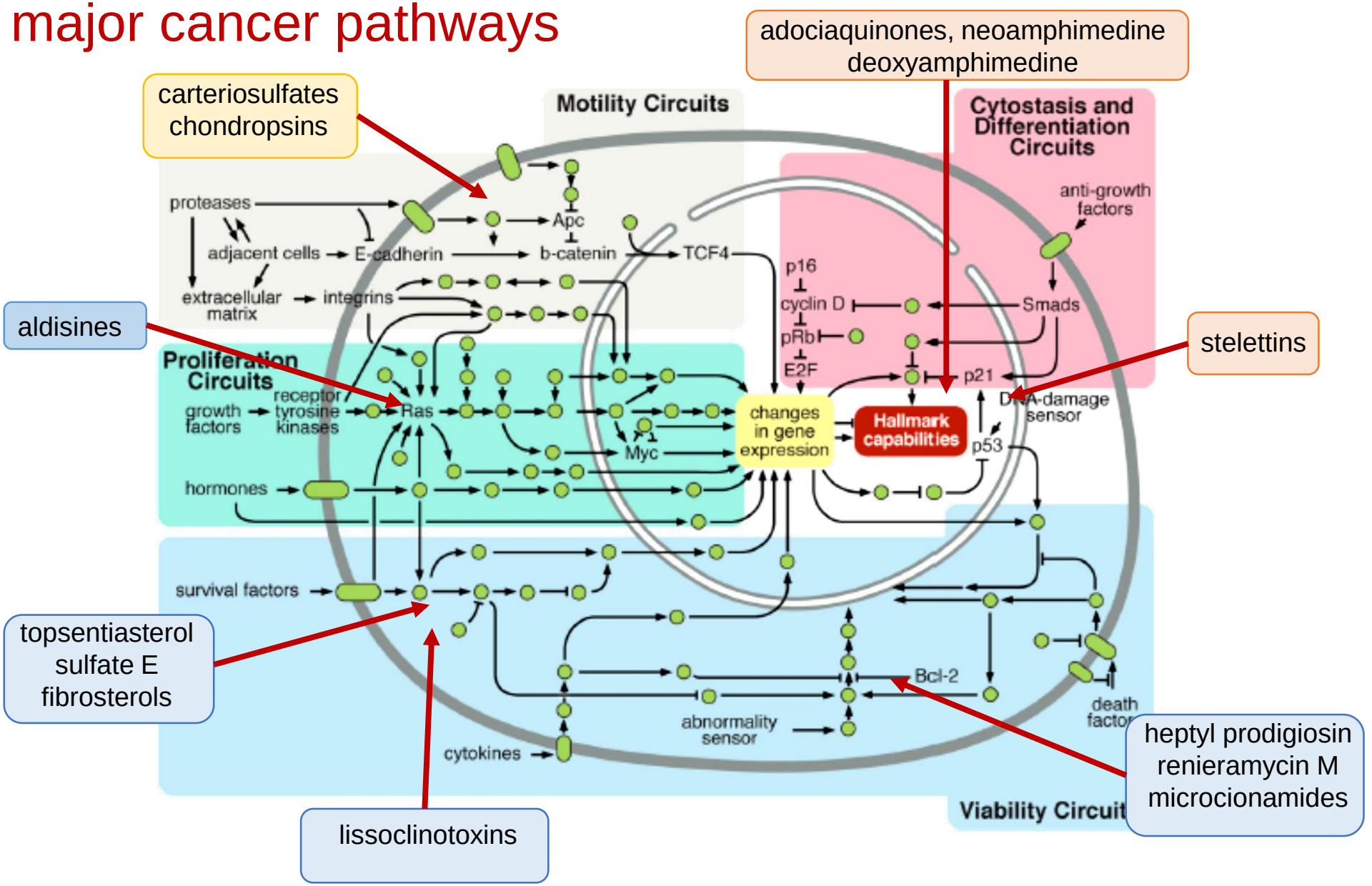


Compounds from sponge induced bleaching in corals and were cytotoxic to HCT116 (human colon cancer cell line).



PhD dissertation, MA Azcuna

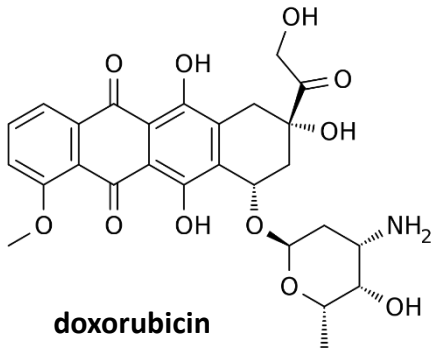
Compounds from Philippine marine organisms act on major cancer pathways



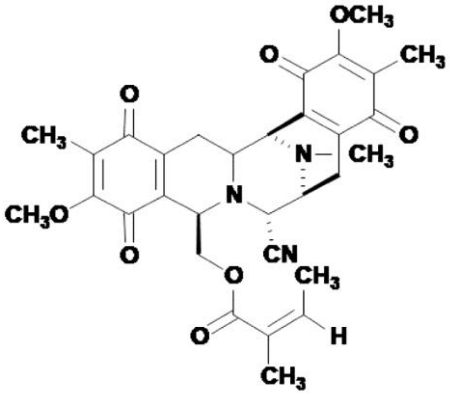
Hanahan D and Weinberg RA. *Cell*. 2011;144(5):646-74; Concepcion GP, et al. *Phil Sci Lett.* 2014; 7(1):207-227.

Anticancer therapy

Renieramycin M acts synergistically with doxorubicin

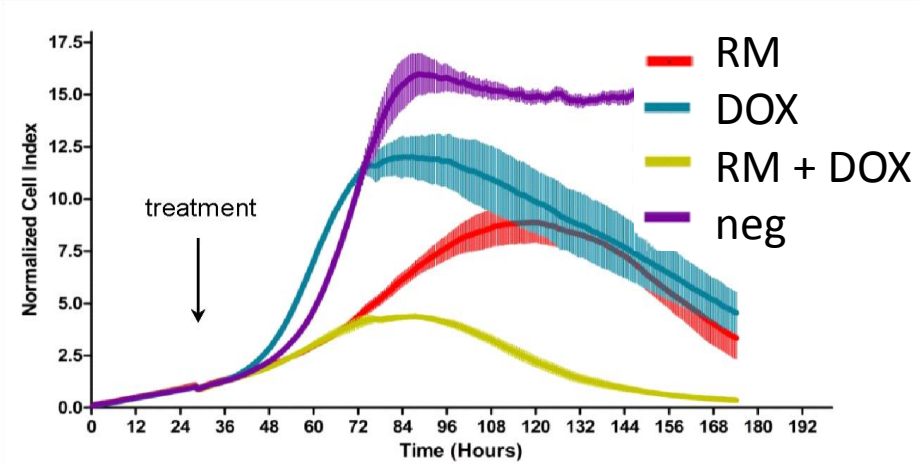


doxorubicin



renieramycin M

from ***Xestospongia* sp.** sponge
Photo by Melchor Deocadez



increase in cytotoxicity based on real-time cell analyzer (RTCA) which measures cell impedance.

increase in apoptosis after drug combination based on cell cycle analysis by flow cytometry

Masters thesis, JO Tun
Patent Pending, GP Concepcion and JO Tun

Growth and Proliferation Modulators from Organisms in Coral Reefs (Marine Ecosystem 1) as Anticancer Drug Leads:

Strategies:

1. **synergistic combination of compounds**
- acting on different molecular targets
- improves chance of 100% kill
- reduces toxicity to normal cells
- reduces drug resistance

2. **combination therapy with existing drugs**
- extends life of existing drug
- delays development of drug resistance

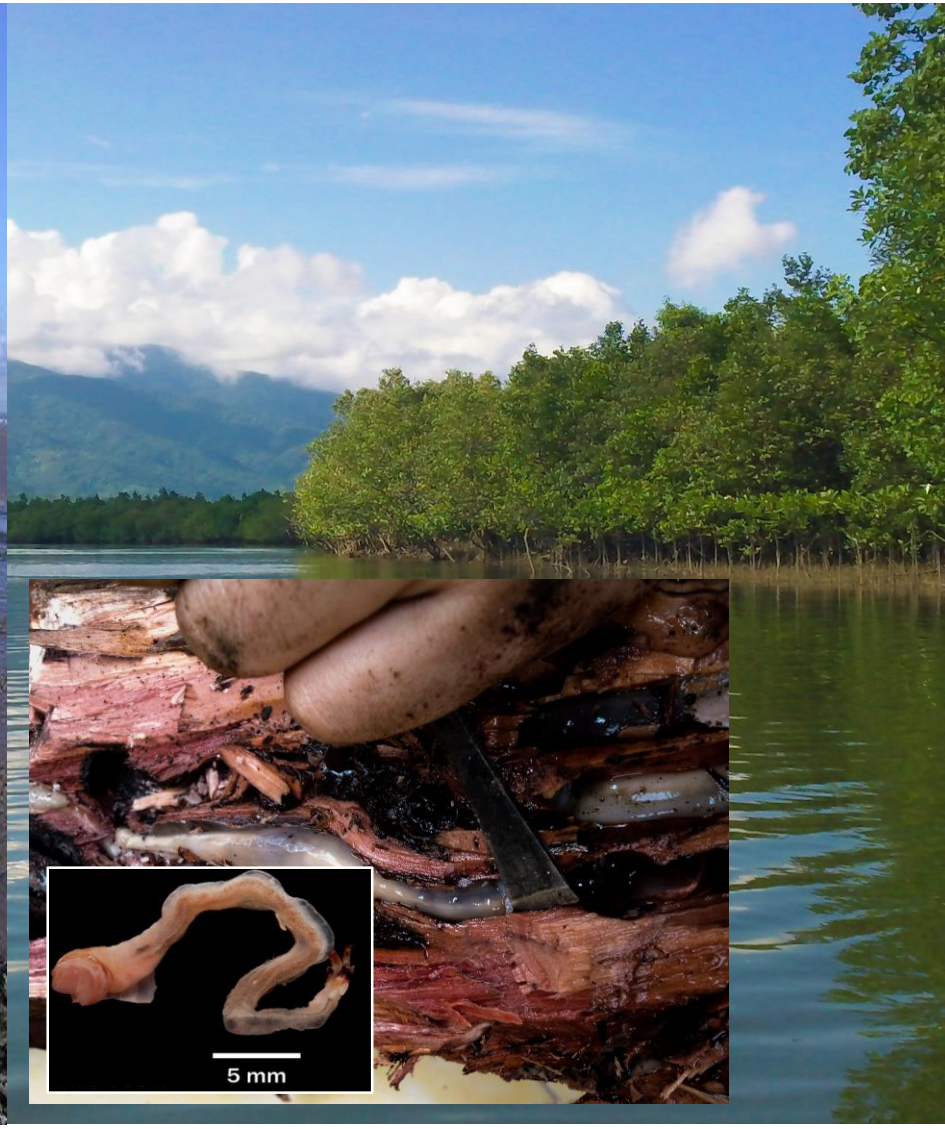
3. **use of bacterial compounds**
- intermittently released
- at low doses
- modulatory or regulatory

SYNTHETIC LETHALITY SYNTHETIC BIOLOGY

Marine Ecosystems 2 and 3

Splash Zone

Mangroves

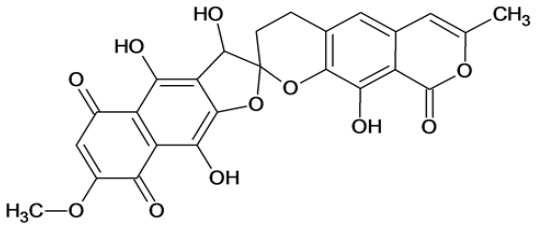


Truncatella guerinii (gastropod)

Lyrodus pedicellatus (shipworm)

Combating antibiotic resistance synergistic anti-MRSA activity

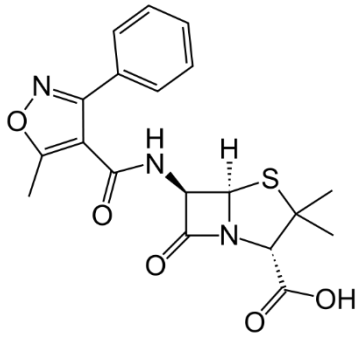
Bringing oxacillin back to the treatment armamentarium



7,8-dideoxygriseorhodin C
from ***Streptomyces* sp.**
from ***Truncatella guerinii***

7,8-dideoxygriseorhodin C works synergistically with oxacillin against methicillin-resistant *Staphylococcus aureus* (MRSA) based on antimicrobial broth microdilution assay.

7,8-dideoxygriseorhodin C is not cytotoxic to mammalian kidney and ovarian cell lines based on MTT cytotoxicity assay.



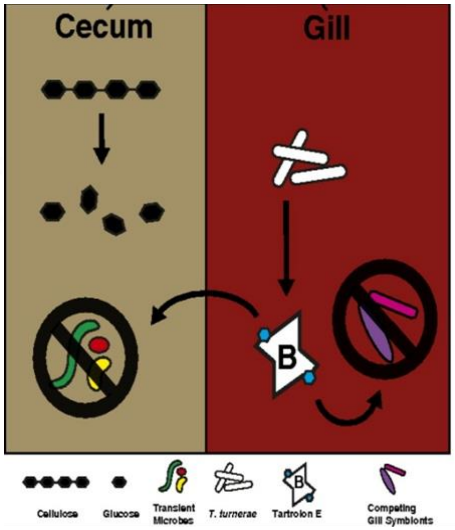
oxacillin

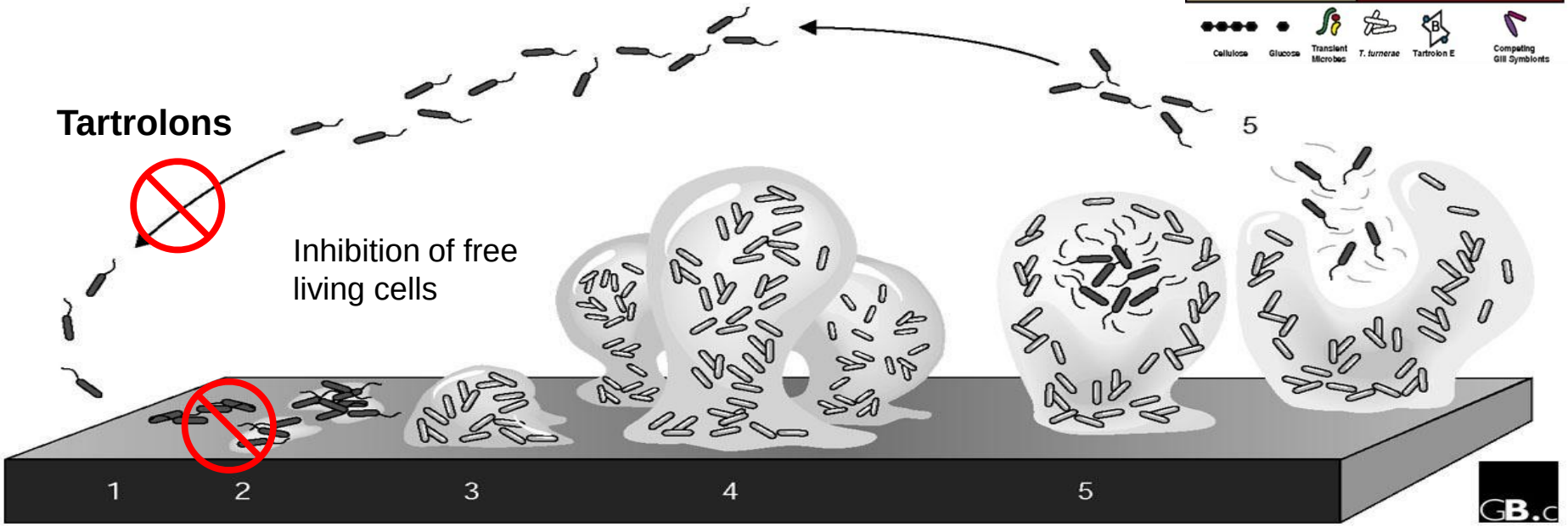
Masters thesis, JP Torres
Patent Pending, GP Concepcion, JP Torres and JO Tun

Antibiotic combinations in nature

Inhibiting multiple points in bacterial biofilm development

tartrolons and **oxylipins** from ***Teredinobacter turnerae,*** gill symbiont of shipworm ***Lyrodus pedicellatus***





Oxilipins

Quorum Sensing Inhibition prevents differentiation of planktonic into biofilm cells

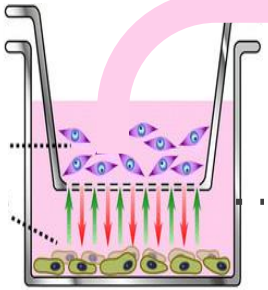
Elshahawi, et al. PNAS. *2013,*110: 295-304;
Masters thesis, NM Lacerna II, JMD Robes

Competitive interactions induce antimicrobial compound production

Shipworm bacteria challenged with environmental opportunist microbe leads to production of antimicrobials

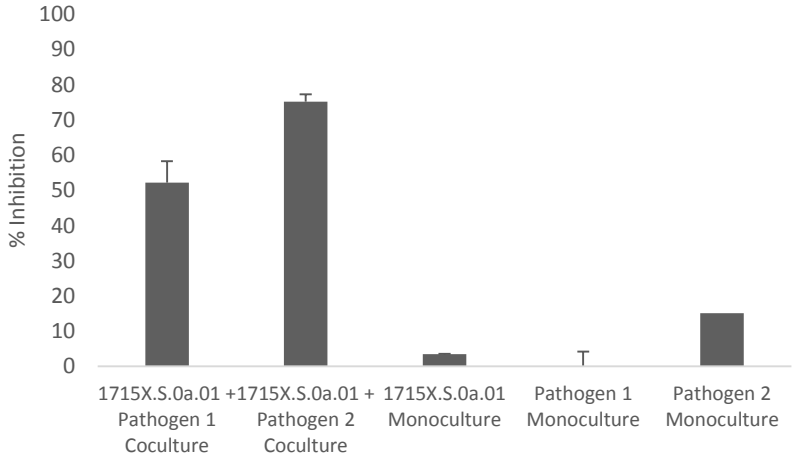
Elicited (*Shipworm isolate 1715X.S.0a.01*)

Elicitor (Opportunist)

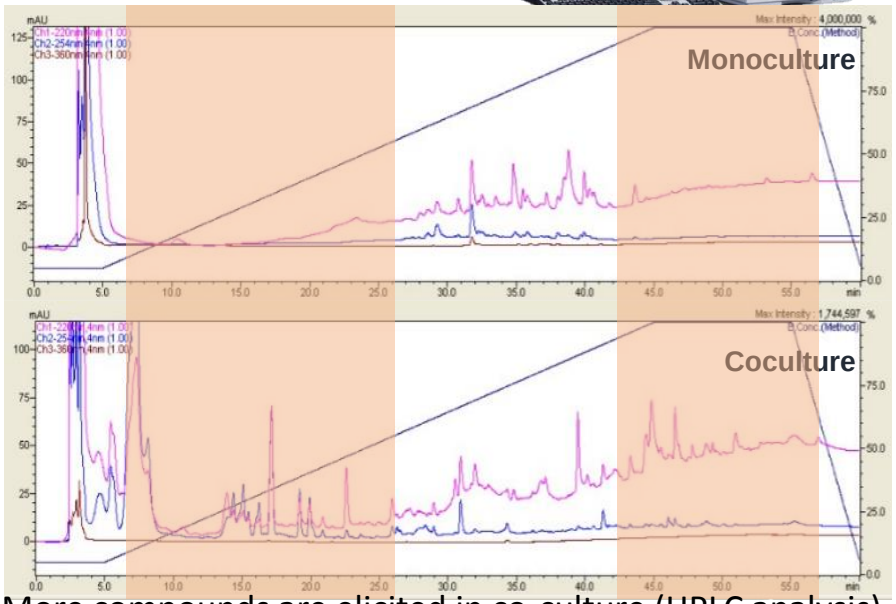


SBM+N+ Sucrose

Co-cultivation setup using a well-insert



Antimicrobial activity of co-cultures of Shipworm Isolate 1715X.S.0a.01 and environmental opportunists, *Pathogen 1* and *Pathogen 2* (100 µg/ml)



More compounds are elicited in co-culture (HPLC analysis).

Tyc et al. J. Nat. Prod., 2015, 78(3), pp 381-387; JMD Robes, JA Ibana

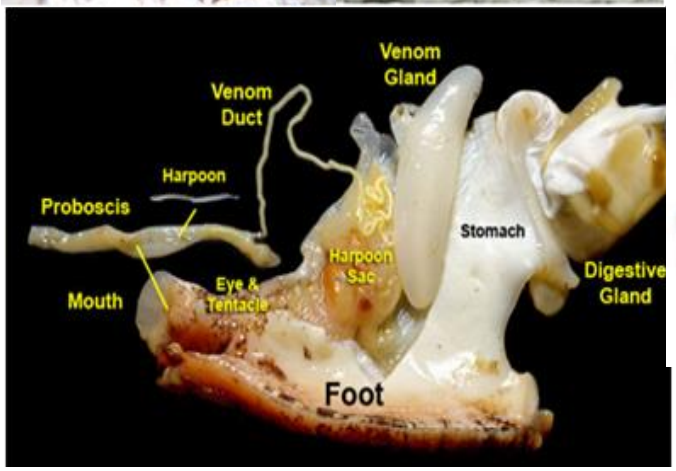
Antimicrobial Compounds Protect Mollusks in Splash Zones and Mangroves (Marine Ecosystems 2 &3)

Strategies:

1. **synergistic combination of compounds**
- targets different life stages or forms of the pathogen
- addresses drug resistance
2. **combination of new drug with drug no longer in use**
- revives use of “retired” drugs
- delays development of drug resistance
3. **compounds from bacterial communities**
- **elicitation** by test pathogen
- modulatory or regulatory

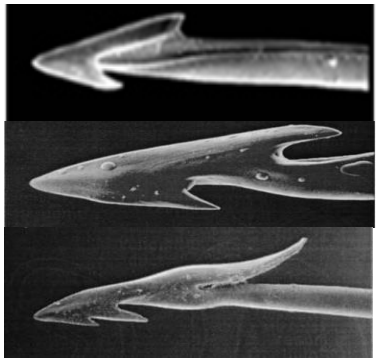
SYNTHETIC LETHALITY **SYNTHETIC BIOLOGY**

Marine Ecosystem 4: Conoideans in Sediment



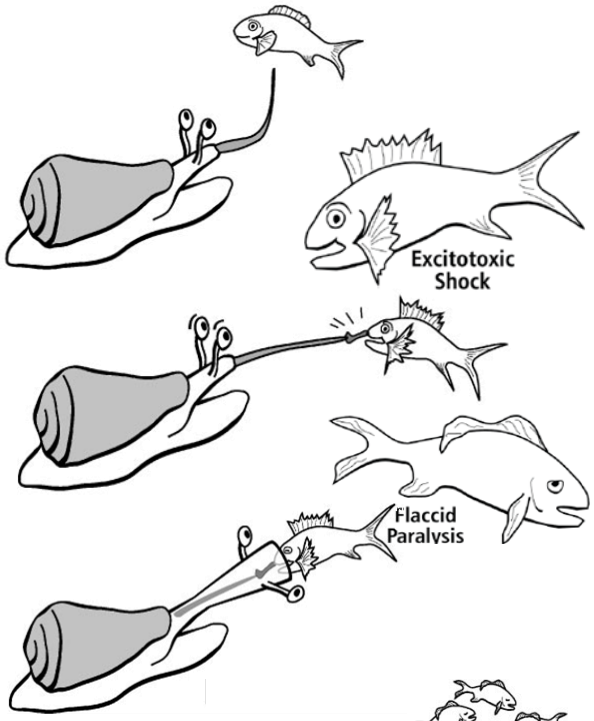
Anatomy of cone snail

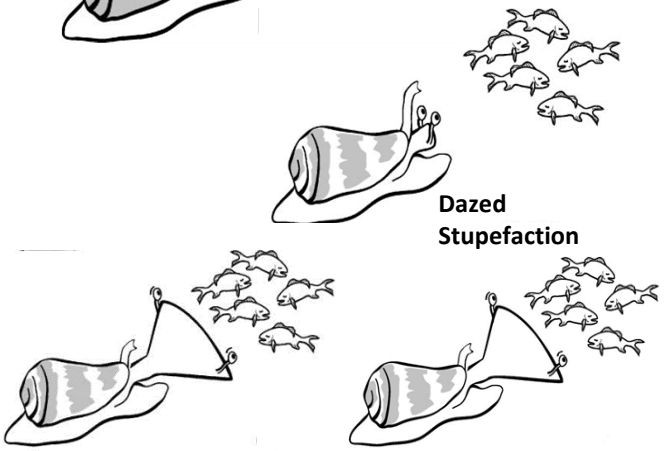
~800 *Conus* species



Toxoglossate radula

Prey capture strategies





Current Opinion in Neurobiology

Class	Mode of action	example
ω-conotoxin	$Ca_v2.2$ inhibitor	MVIIA
μ-conotoxin	Na_v inhibitor	SIIIA
μO-conotoxin	$Na_v1.8$ inhibitor	MrVIB
δ-conotoxin	Na_v enhancer	EVIA
κ-conotoxin	K_v inhibitor	PVIIA
χ-conopeptide	NET inhibitor	Xen2174
α-conotoxin	nAChR inhibitor	Vc1.1
σ-conotoxin	$5HT_3$ R antagonist	GVIIIA
ρ-conopeptide	α_1-adrenoceptor inhibitor	TIA
Conantokin	NMDAR antagonist	conantokin-G
Conopressin	Vasopressin agonist	conopressin-G
Contulakin	neurotensinR agonist	contulakin-G

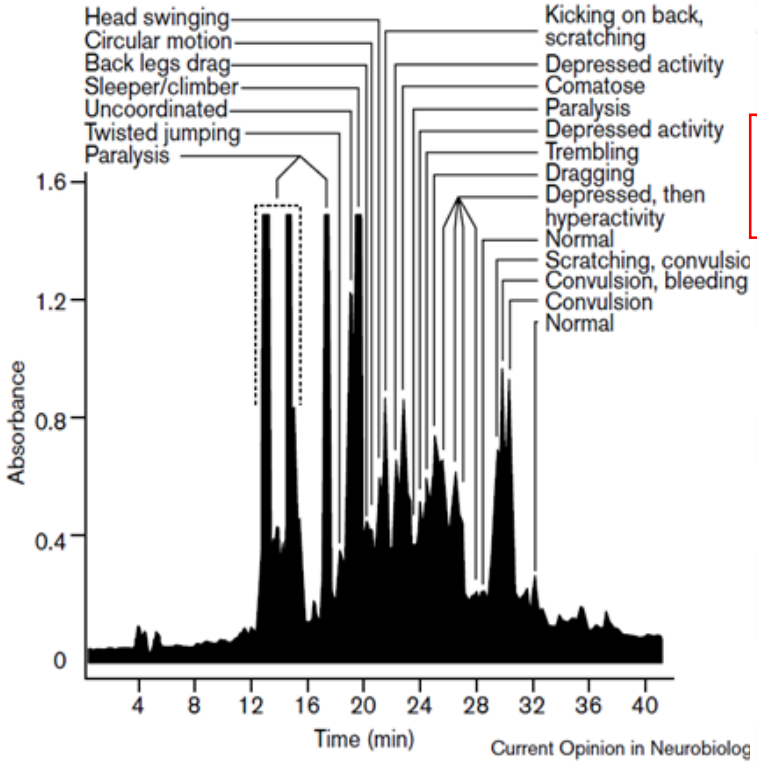


Table 3. Therapeutic Applications of Conopeptides

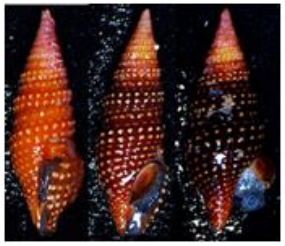
Clinical application	Conopeptide	Sequence	Target	Clinical status
Pain	ω-MVIIA (Ziconitide, Prialt®)	CKGKGAKCSRLMYDCCTGSCRSGKC*	Ca^{2+} channel ($Ca_V2.2$)	FDA approved
Pain	ω-CVID (AM336)	CKSKGAKCSKLMYDCCSGSCSGTVGRC*	Ca^{2+} channel ($Ca_V2.2$)	Phase I
Pain	Contulakin-G (CGX-1160)	ZSEEGGSNATKKPYIL	Neurotensin receptor	Phase I
Pain	α-Vc1.1 (ACV1)	GCCSDPRCNYDHPEIC*	nAChR (α9α10)	Phase I
Pain	χ-MrIA (Xen2174)	NGVCCGYKLCHOC	Norepinephrine transporter	Phase I
Pain/Neuro-protection	Conantokin-G (CGX-1007)	GEγγLQγNQγLIRγKSN*	NMDA receptor (NR2B)	Preclinical
Epilepsy	Conantokin-G (CGX-1007)	GEγγLQγNQγLIRγKSN*	NMDA receptor (NR2B)	Phase I
Pain	μ-conotoxins	Various	Na^+ channels	Preclinical
Myocardial infarction	κ-PVIIA (CGX-1051)	CRIONQKCFQHLDDCCSRKCNRFNKCV	K^+ channel (K_V1)	Preclinical

Turrids: Megadiverse group of mollusks

Lumun-lumun net being lifted

Turrids collected using *lumun-lumun* net

Crassispira cerithina



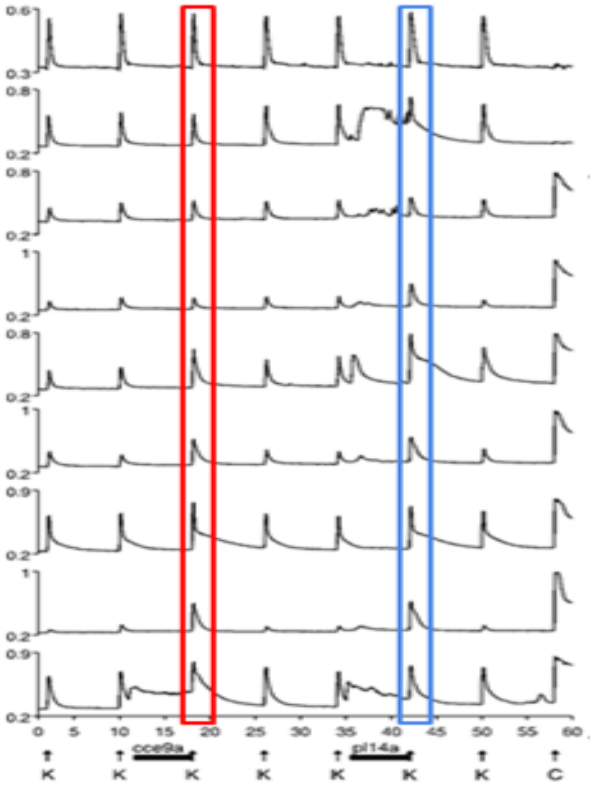
Age-dependent effects of cce9a:

Intracranial mouse bioassay:
12 - 14 d old: lethargy and delayed response to stimuli
16 d old: hyperactivity

P-conotoxin-like crassipeptide

cce9a	GSCGLPCHEN-RRCGWACYCDDGICKPLRV
iqi9a	GSCGPPCHEN-RRCGWACYCDDGFCKPLRV
cce9b	HSCRRHCHEN-RRCGWACYCDDGICKPLRV
iqi9b	DVCSGSCYYH-YQCSRSCYCHYSHCRDKYEK
cce9c	RFCGQSCHGQPSLCHWTCPCNGHFCSRL

Cabang AB et al. Toxicon 2011, 58(8):672-80;
Imperial JS et al. Toxicon 2014, 0:45-54.

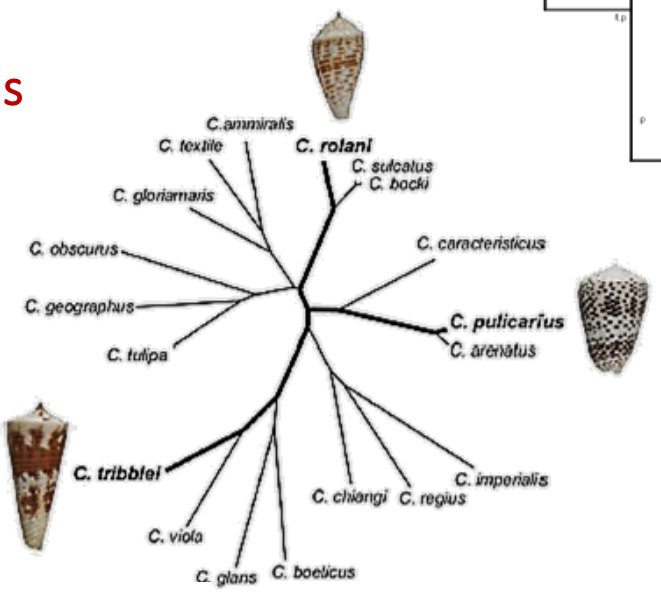


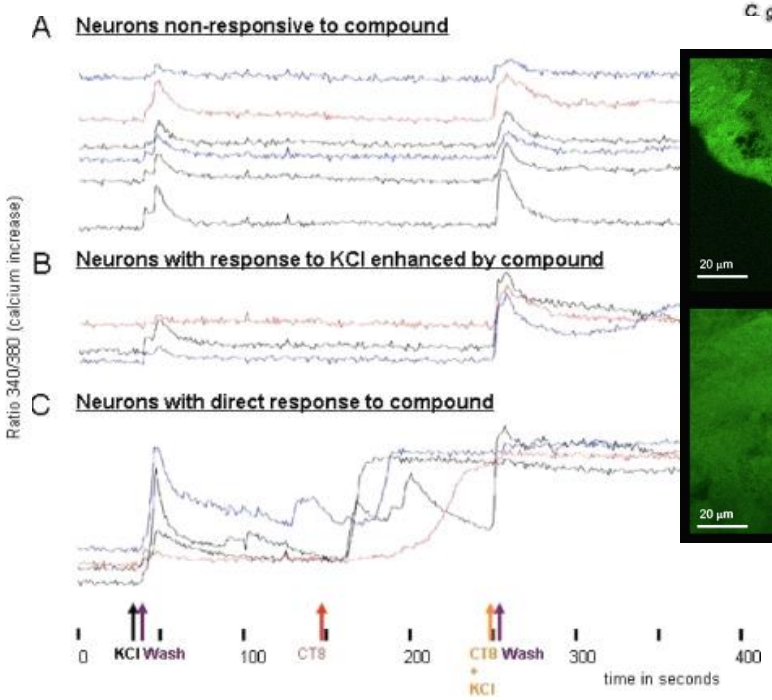
Masters thesis, AB Cabang

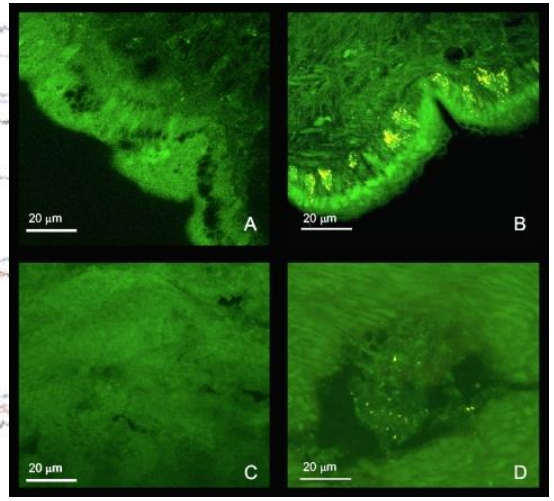
cce9a elicited /amplified responses in a subset of small-diameter capsaicin-sensitive DRG neurons also affected by κJ-conotoxin pl14a, a known $K_V1.6$ channel-blocker

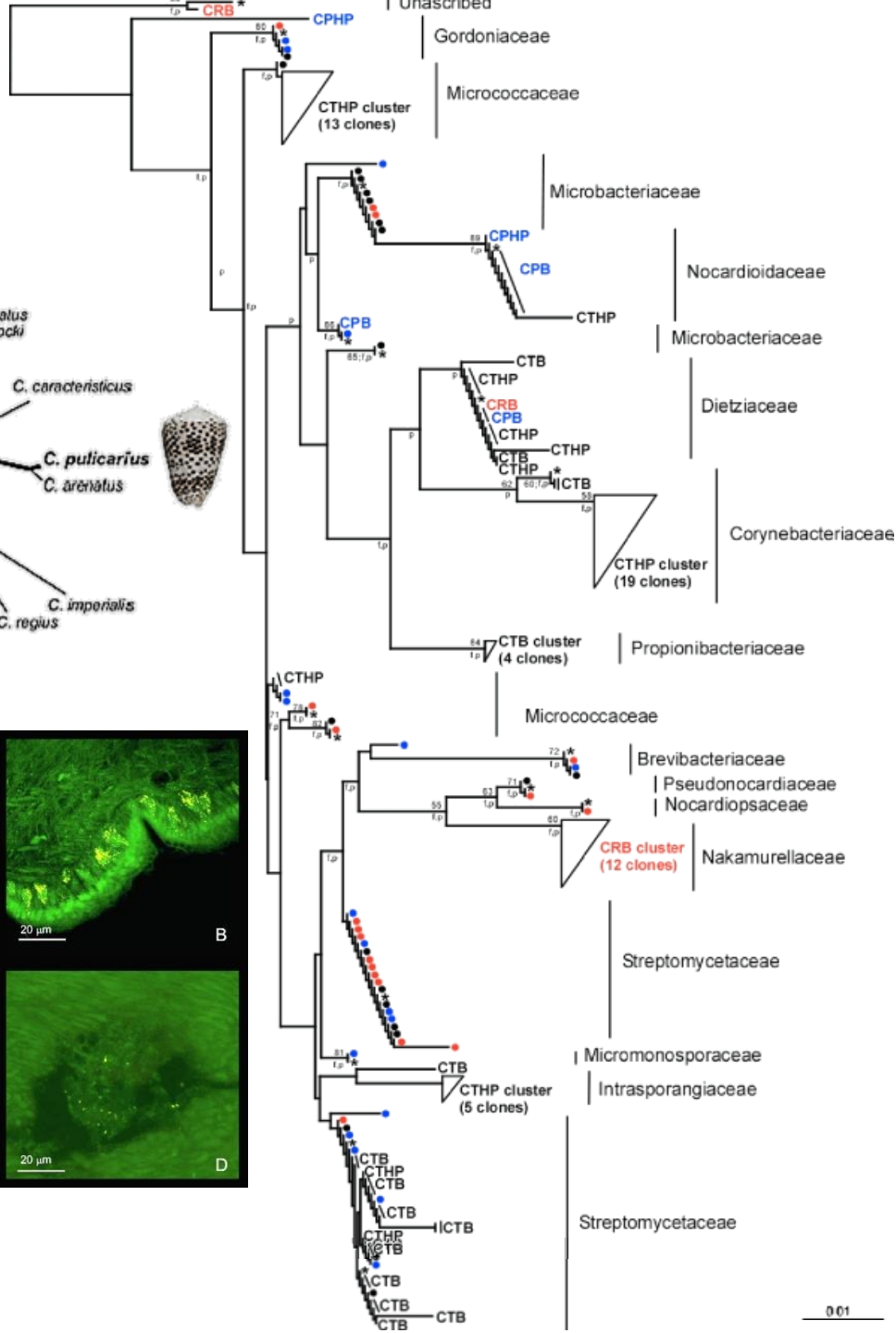
Diverse Actinobacteria within Venomous Cone Snails Producing Neuroactive Compounds

Peraud O et al. Appl. Environ. Microbiol. 2009 75(21): 6820–6826.





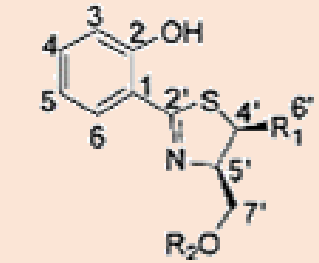




Neuroactive compounds from mollusk-associated bacteria

Pulicatins

from ***Streptomyces sp.*** CP 32
from ***Conus pulicarius***



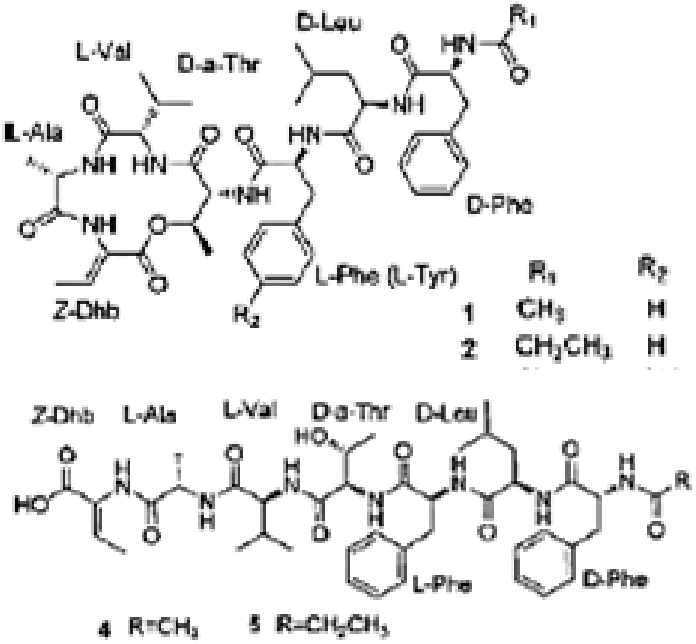
6 R_1=CH_3 R_2=H

Compound 6 inhibits 5-HT_{2B} serotonin receptor at 505 nM

Lin Z, et al. J. Nat. Prod., 2010, 73 (11), 1922-1926

Nobilamides

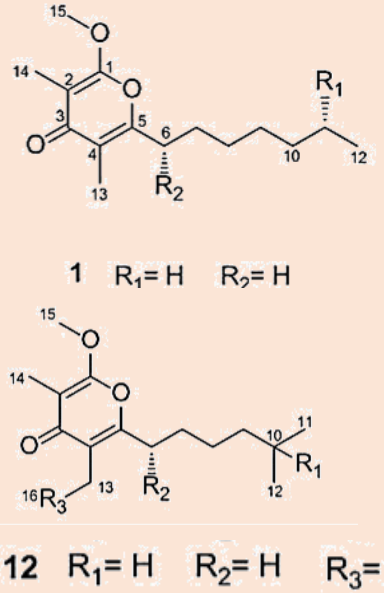
from bacteria from
Chicoreus nobilis and ***Conus tribblei***



Compounds 2 and 5 are long-acting antagonists (vs. capsaicin) of mouse and human TRPV1 channels
Lin Z et al. J Med Chem, 2011, 54, 3746-3755

Nocapyrones

from ***Nocardiopsis alba*** CR167
from ***Conus rolani***



1 R_1= H R_2= H

12 R_1= H R_2= H R_3= H

Nocapyrones B (**12**) and H (**1**) were active against nearly all DRG neuronal cell types at 50 µM
Lin Z et al. Chem Biol, 2013, 20(3):73-81

Marine Ecosystem 5:

Eutrophic Marine Habitat

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Giant shipworms discovered hiding in sulfurous lagoons

By Ryan Cross | Apr. 17, 2017 , 3:00 PM

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Live, long and black giant shipworm found in Philippines

MANILA BULLETIN

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Home Category / Environment & Nature / Sulphur-powered giant shipworm uneart

Sulphur-powered giant shipworm unearthed in Philippines

Published April 19, 2017, 3:30 PM

By Agence France-Presse

The New York Times

SCIENCE

This Is a Giant Shipworm. You May Wish It Had Stayed In Its Tube.

NATIONAL GEOGRAPHIC

WEIRD & WILD

Watch: Bizarre Deep-Sea 'Worm' As Long As an Arm Revealed

Scientists studying the giant shipworm for the first time made a truly strange discovery.

The Washington Post

Democracy Dies in Darkness

Speaking of Science

Scientists find giant, elusive clam known as 'the unicorn of mollusks'

GMA NEWS ONLINE

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LOOK

KMJS feature sparks discovery of ancient creature

Published April 22, 2017 5:41pm

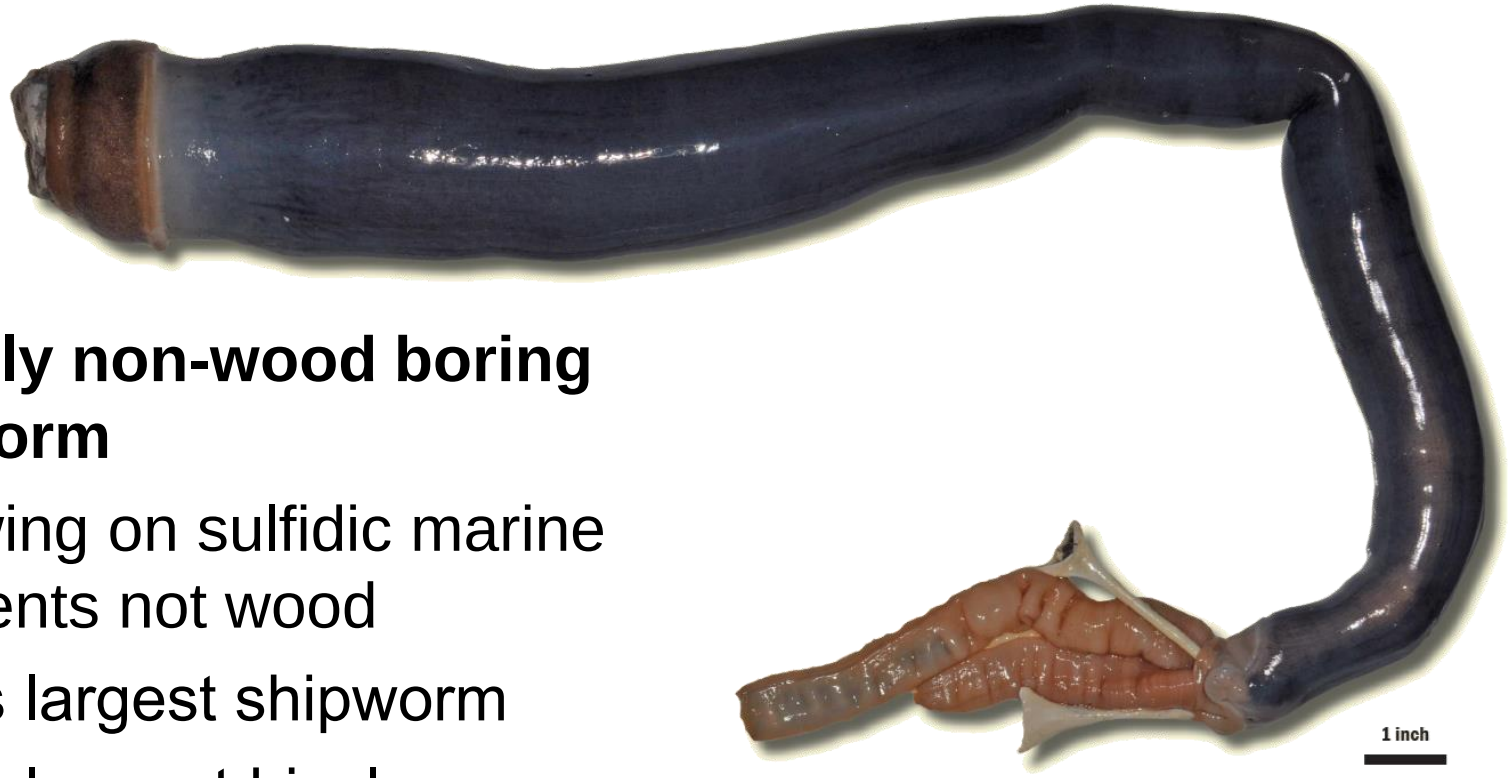
By TJ DIMACALI, GMA N

theguardian

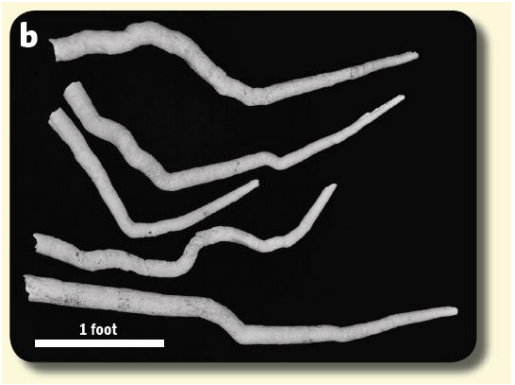
Bizarre bivalve: first living giant shipworm discovered in Philippines

Mud-dwelling organism that lives head down in a tusklike tube found alive for ce had been known of for centuries

Kuphus is a peculiar shipworm

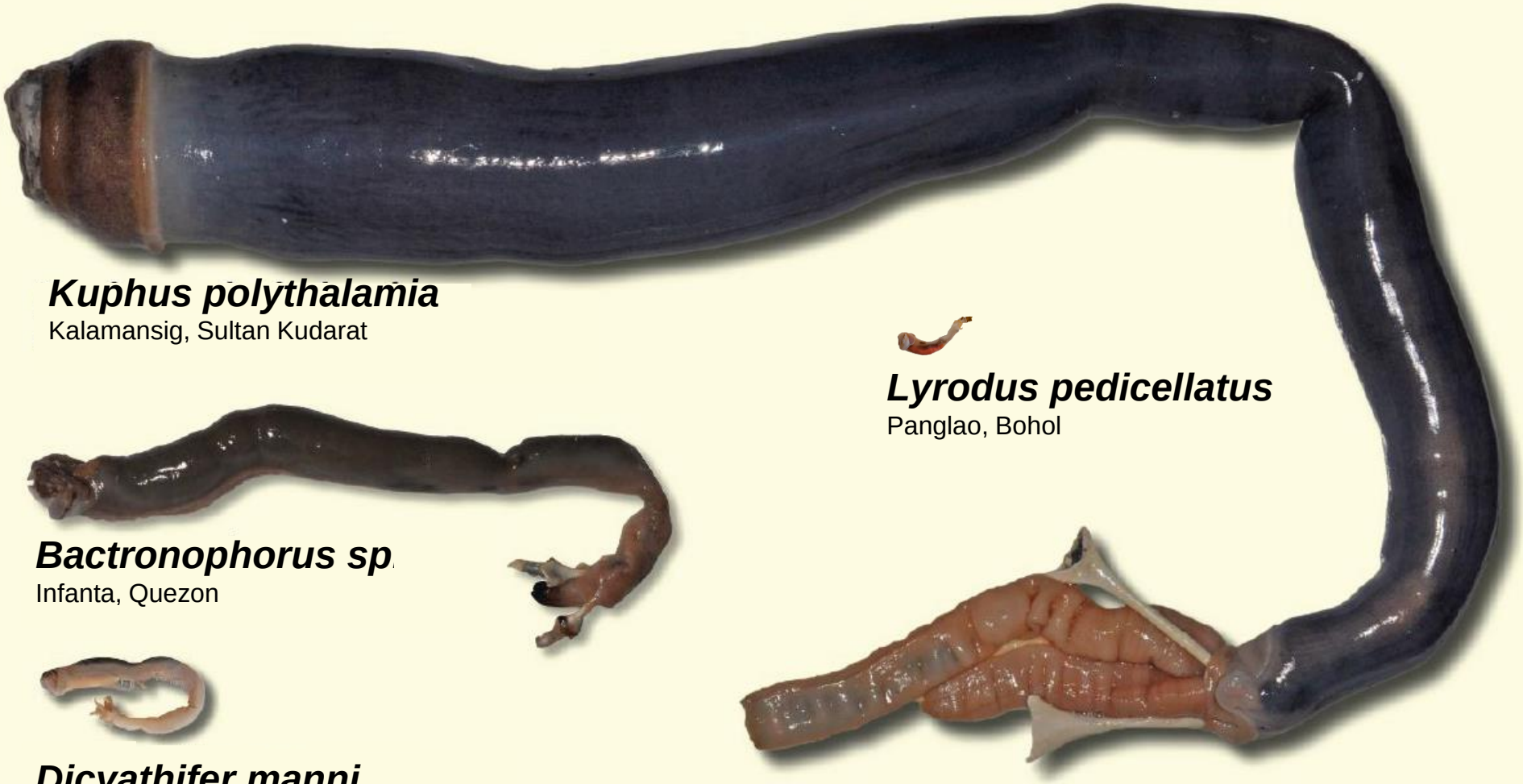


- **the only non-wood boring shipworm**
- burrowing on sulfidic marine sediments not wood
- world's largest shipworm
- world's longest bivalve
- what does it eat?
- does it have symbionts?
- very rare



Marvin Altamia

Shipworm size comparison



Kuphus polythalamia
Kalamansig, Sultan Kudarat

Lyrodus pedicellatus
Panglao, Bohol

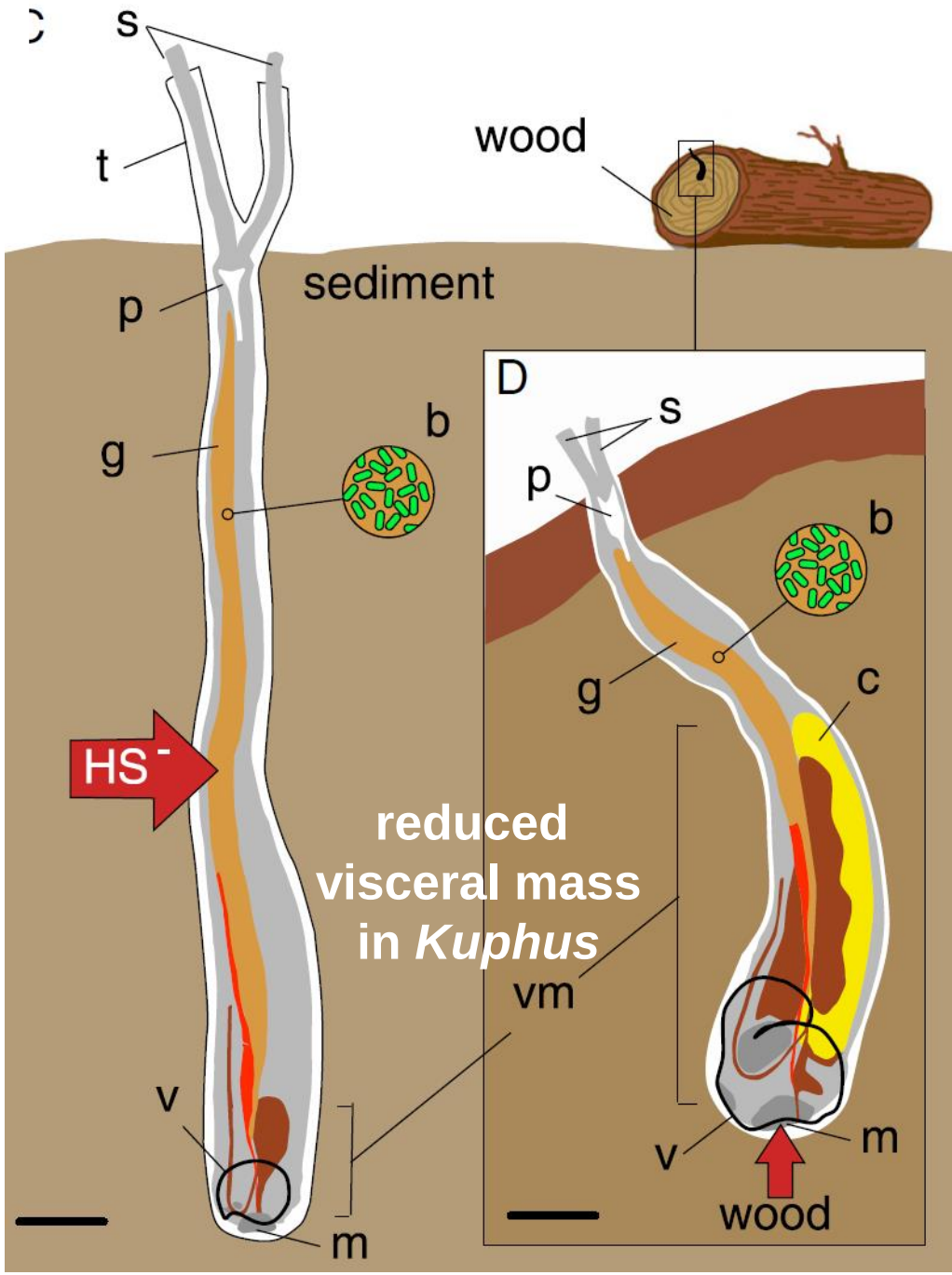
Bactronophorus sp
Infanta, Quezon

Dicyathifer manni
Infanta, Quezon

1 inch

Major findings:

- *Kuphus* does not partner with cellulolytic symbionts unlike its relatives
- relies on endosymbiotic bacteria that use H_2S sulfide to fix CO_2 into a biomass that can be utilized by the host
- first cultivatable sulfur-oxidizing endosymbiont



Kuphus polythalamia *Lyrodus pedicellatus*

Distel D, Altamia M et al.
PNAS April 2017

Kuphus polythalamia

THE ANIMAL:

- Anatomy
- Metabolism
- Mode of Nutrition
- Life History
- Reproduction
- Habits
- Habitat
- Ecosystem
- Bio-Geo-Evolution

BIOTECHNOLOGY:

- ***Nutritional value***
- ***Nutraceutical value***
- ***Antibiotics***
- ***Other Drugs***
- ***Anti-Aging***

Links between Primary Metabolism and Secondary Metabolism

(*Kuphus* in Eutrophic Marine Ecosystem 5)

Evolutionary Strategies:

1. "Big is Small!" Small is versatile, can do all! And "Big" benefits and becomes so big! (Bacteria contribute to Primary Metabolism of Host.)
2. Bacteria protect the Host (Antimicrobial Secondary Metabolites).

Link between organisms in shallow waters and deep vents

DRUG LEADS
from Big Questions driving Evolution

SCIENCE RETREAT IN DUYAN, SINAG-TALA, ORANI, BATAAN

HALLUCINOGENIC, DEADLY TRUMPET FLOWER

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Proposed NAST Resolution relating to Marine Drug Discovery and Development:

Whereas the Philippines is the center of marine biodiversity in the world, where marine invertebrate animals, other macro-organisms and their associated microorganisms evolved in unique marine ecosystems throughout the country's archipelago;

Whereas biodiverse organisms found in bio-ecological niches produce a rich array of bioactive compounds to modulate growth and development, pacify prey, ward off competitors and predators, prevent one organism's dominance over another, and thus marine organisms are a rich source of new drug leads or models for serious human diseases;

Whereas there is an urgent need worldwide to discover and develop new drugs and treatment regimens for cancer, infections, pain, lifestyle and aging-associated neurodegenerative diseases, because of increasing mortality and morbidity, and because fewer new classes of drugs are entering the market, for these conditions;

Whereas marine drug discovery and development require creating a value chain involving collection and documentation of marine organisms, use of high-level technologies requiring state-of-the-art instrumentation, cooperation of expert scientists working in various disciplines, testing of drug leads in disease models, access to clinical samples and data, support from the biotechnology and pharmaceutical industry to bring drug leads faster to the market, and conservation and protection of marine ecosystems for their sustainable use and development;

Whereas Philippine marine biodiversity is a valuable pharmacological resource with tremendous potential to provide significant economic and health benefits to Filipinos;

Resolved, that the National Academy of Science and Technology urge the national government:

through the Department of Agriculture-Bureau of Fisheries and Aquatic Resources (DA-BFAR), to facilitate access to marine samples by *bona fide* Filipino researchers and their foreign collaborators engaged in marine biodiversity and drug discovery research, while ensuring equitable sharing of benefits derived from the research, with various stakeholders in government, local communities and academe; to implement the provisions of the Nagoya Protocol (ratified by the Philippine government in 2015) by establishing streamlined and more efficient processes for access and benefit sharing;

through the Commission on Higher Education (CHED)and the University of the Philippines System (UP System), to lead a nationwide campaign and to offer local and foreign scholarships and other incentives and benefits, to encourage a great number of Filipino academics to pursue Masters and PhD degrees, and postdoctoral fellowships, to gain high-level expertise, in this research area and postgraduate education as a top priority;

through the Department of Budget and Management-Government Procurement Policy Board (DBM-GPPB), to streamline and hasten the procurement process; to pursue new protocols for ensuring the highest-quality- for-best-price of procured R&D equipment, supplies and infrastructure;

through the Department of Science and Technology (DOST), to further increase R&D funding for marine biodiversity exploration, drug discovery and development; to recruit expert scientific and technical reviewers of research proposals and output; to provide funds for technical training and updating of researchers and technicians; to provide larger funding for early, middle and late stage commercial development of marine drugs; to establish core facilities and equipment useful to a large community of researchers; to create a one-stop-shop (one coordinating agency) to serve as liason between academics and industry investors to pursue commercial applications of R&D projects;

through the Department of Health (DOH), to identify priority diseases of national importance for which large numbers of clinical samples and clinical data from hospitals countrywide can be readily accessed by researchers through a simplified and faster patient informed consent and ethics review process; to simplify and streamline the process for conducting clinical trials of new drug applications;

through the National Economic and Development Authority (NEDA), to provide tax and other economic incentives for industry to invest in R&D in academe at an earlier stage; to initiate a program to match with government funds 1:1, industry funds to be invested in R&D such as the development of new drugs.