

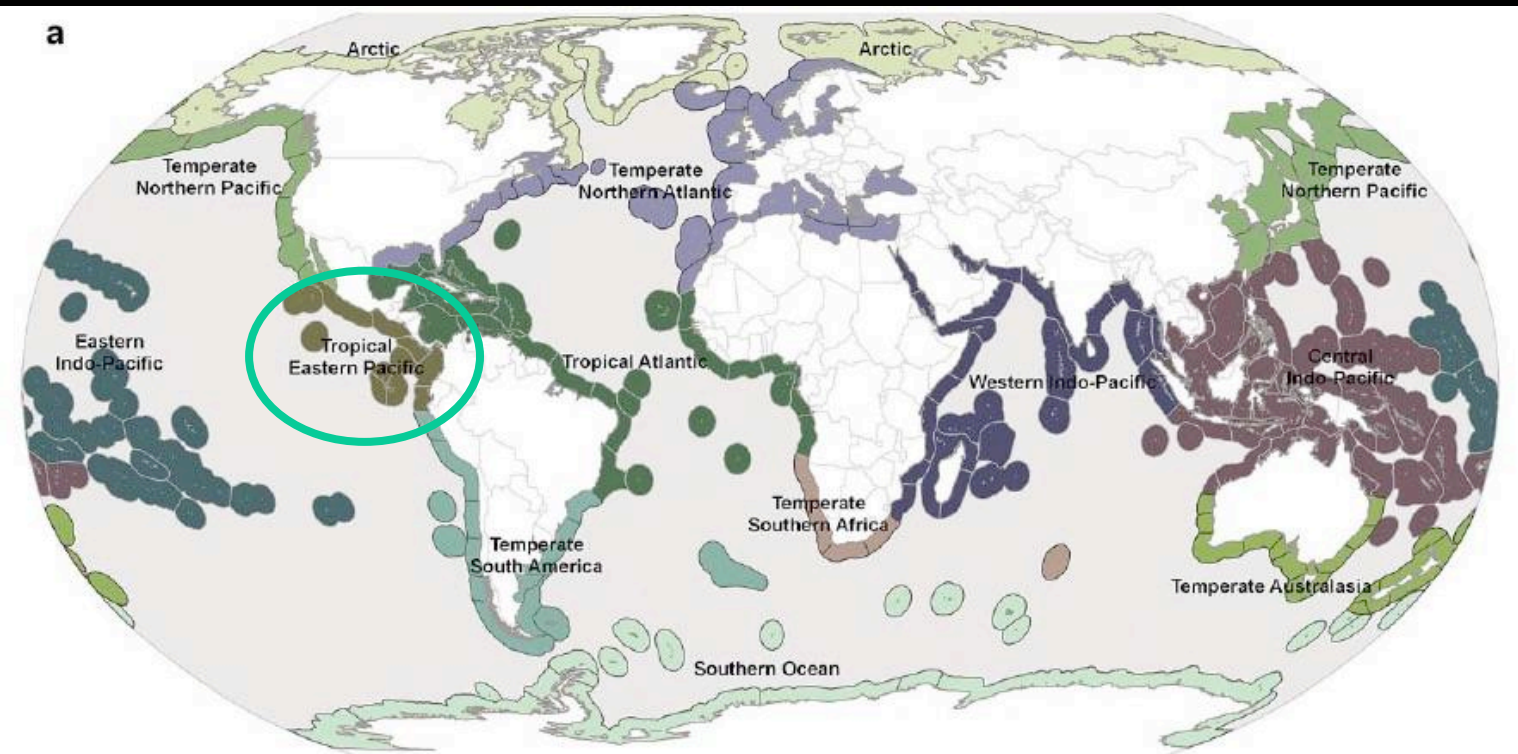


OÉ Gaillimh
NUI Galway



Zoanthids for sale... beyond their use in reef aquaria

Karla B. Jaramillo, Paul O. Guillen, Miriam Reverter, Jenny Rodriguez,
and Olivier P. Thomas

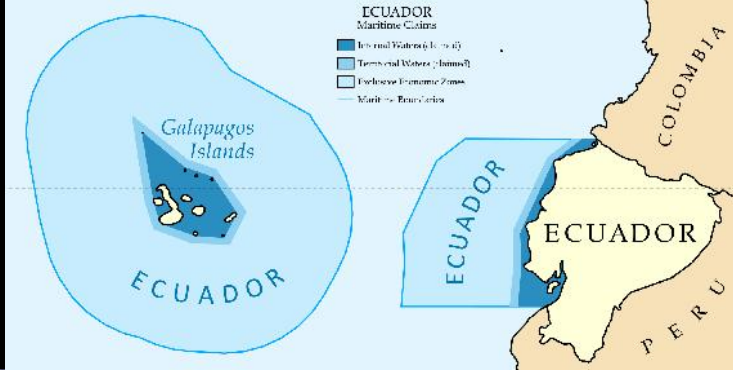


Novelty in Marine Biodiscovery

A large part of our oceans has been underexplored:

- Microbes
- Polar regions
- Deep-sea
- Some ecoregions have been less explored, mainly due to low levels of scientific support/interest.

The Tropical Eastern Pacific has been largely ignored for marine biodiscovery. Low involvement of Latin American countries on the coasts.



Bioprospecting the TEP

Only few marine stations on the Tropical Eastern Pacific coasts.

- Some geographical areas with largely undescribed biodiversity and chemodiversity
- Training students and researchers for a sustainable use of their marine biodiversity



Refurbishment of a marine centre in the mainland coast of Ecuador (10,000,000 euros) in 2015 benefiting from strong political support.
 Aquaculture and Marine Biodiversity (Jenny Rodriguez)

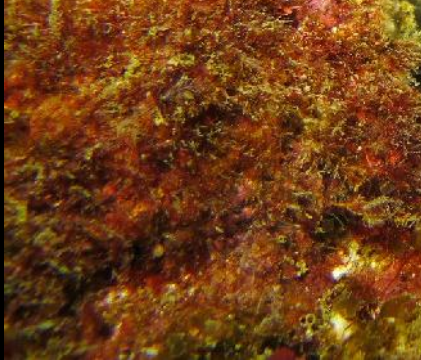


Scarce scientific knowledge

No detailed inventory of the marine biodiversity in this region. No real taxonomic expertise in marine invertebrates: only Photo Based Taxonomy. **Building a marine repository through training.**

Development of a biodiscovery workflow: preparation of fractions, first chemical and biological screenings and purification of compounds.

Structure analysis NMR MS at NUIG



Main groups

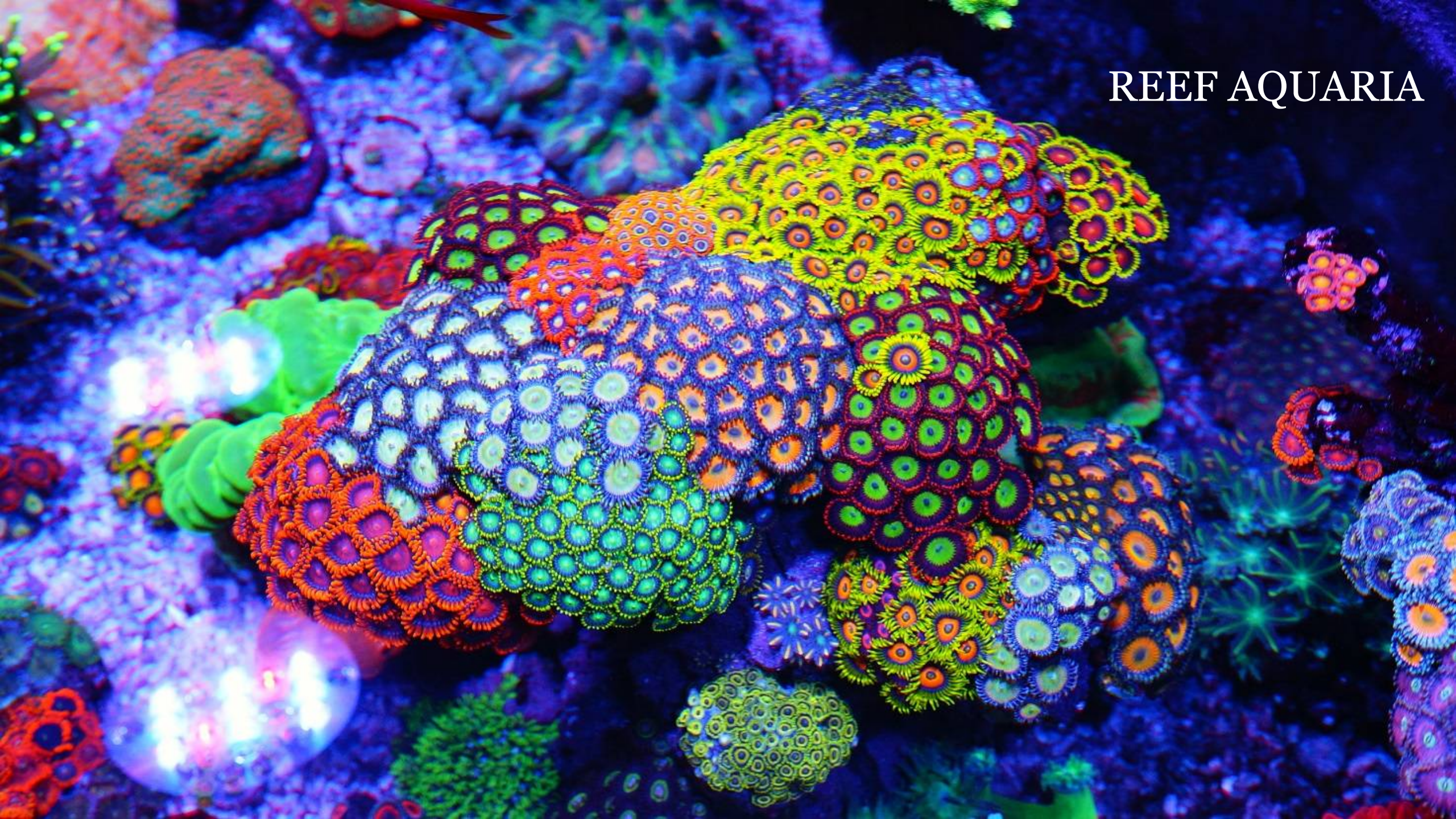
Unfortunately low sponge cover and diversity

Cnidarians highly present in this region. Especially from the class Anthozoans.

Importance of the taxonomy for this group as the diversity has been found to be very high.

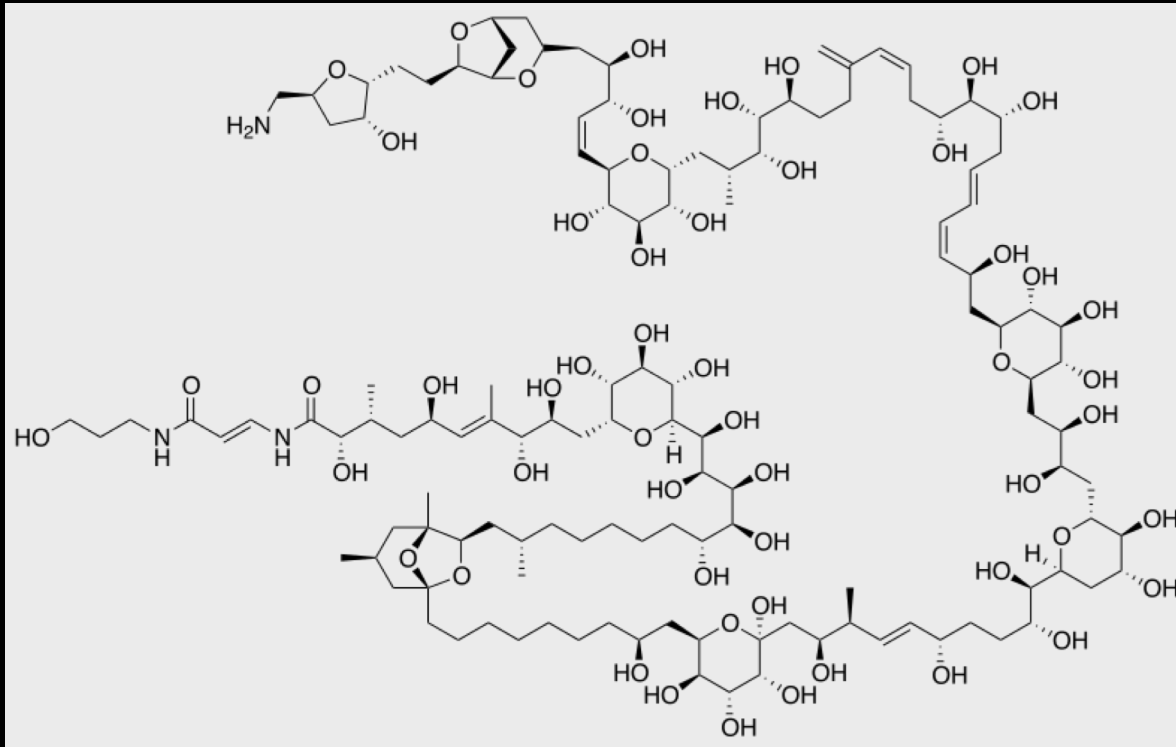
Octocorallia but also hexacorallia like zoantharians

REEF AQUARIA



Classification: Biota

> [Animalia](#) (Kingdom) > [Cnidaria](#) (Phylum) > [Anthozoa](#) (Class) > [Hexacorallia](#) (Subclass) > [Zoantharia](#) (Order)



Palytoxin, the most toxic non-protein natural substance

isolated first from *Palythoa toxica*

limu-make-o-Hana in Maui Hawaii

(Seaweed of Death from Hana)

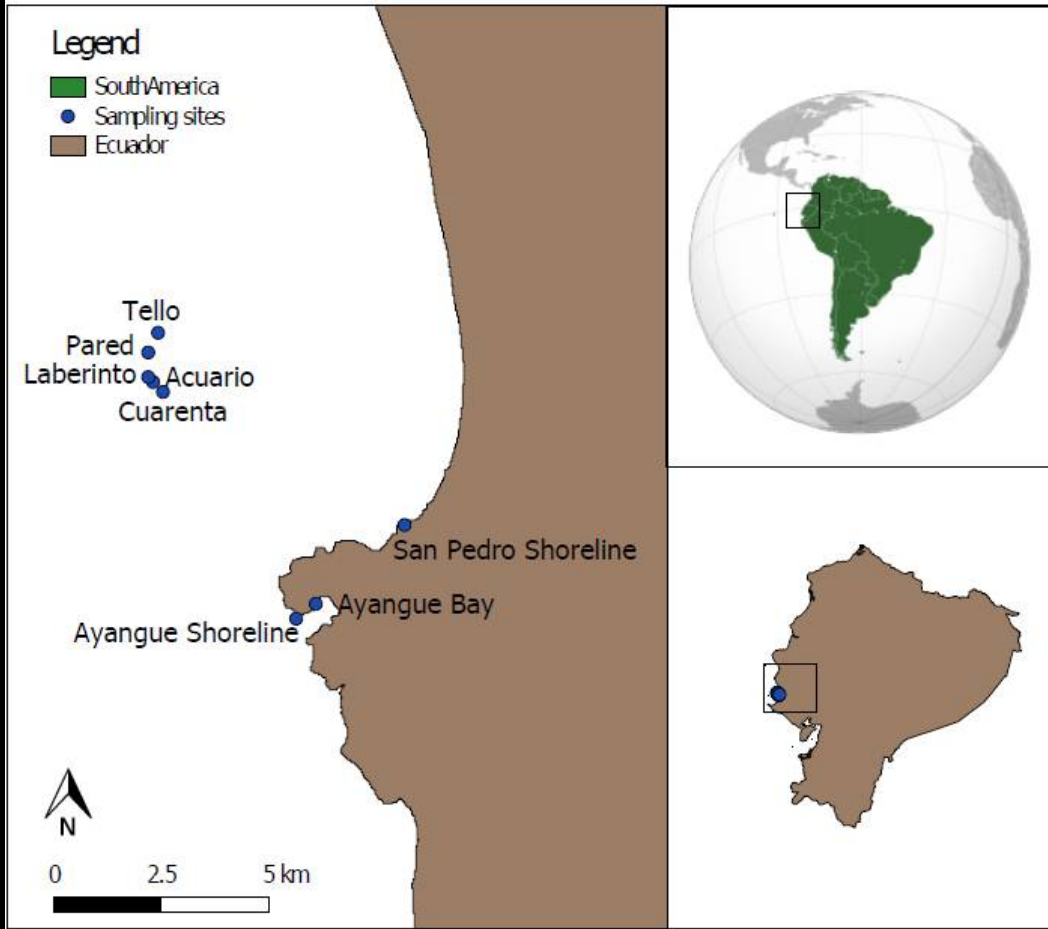
Then produced by dinoflagellate



Zoantharians in Marine Biodiscovery

Cnidarians of the order Zoantharia are largely present in some marine ecosystems like the Mediterranean, the Caribbean and spread over the Indo-Pacific. Also present in the deep sea. High cover in the TEP of Ecuador

Largely overlooked despite production of important families of compounds.



Zoantharians in the TEP

At least 7 species present in a small Marine Protected Area called El Pelado.

Macrocnemina: *Antipathozoanthus*,
Parazoanthus, *Terrazoanthus*

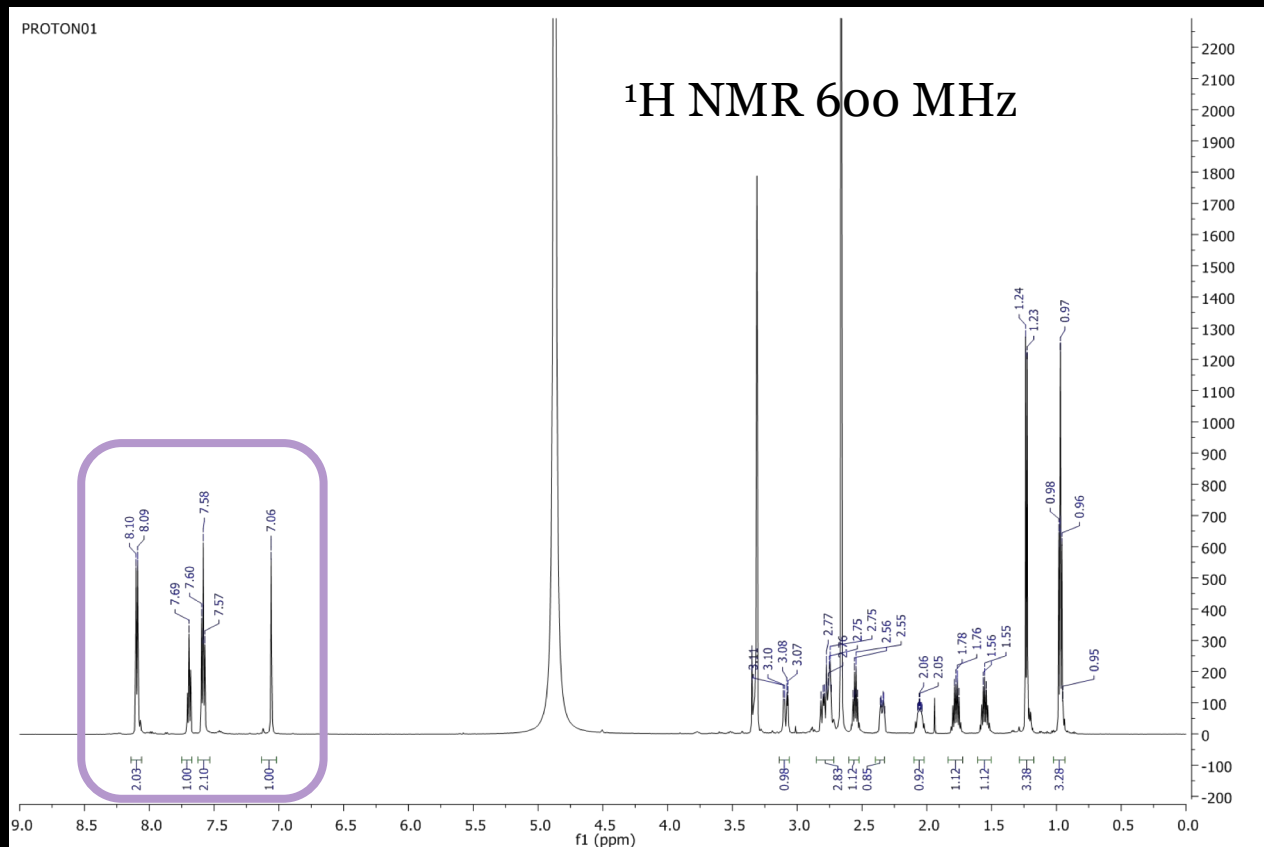
Brachycnemina: *Zoanthus*, *Palythoa*

Frederic Sinniger

Karla Jaramillo
PhD Student



UHPLC-HRMS evidenced unusual masses



Terrazoanthus onoi

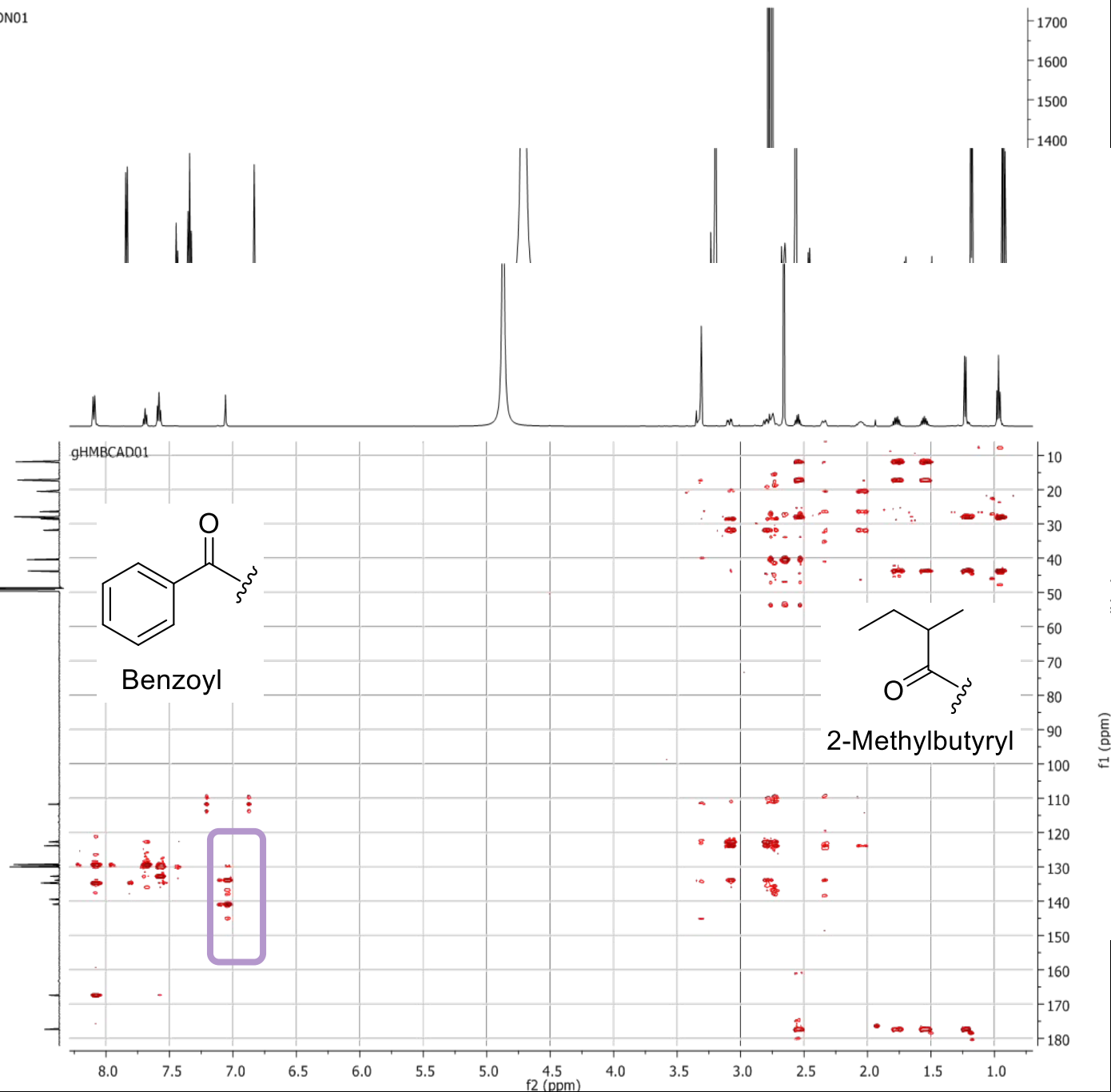
Genetics close to *T. patagonichus*

Alkaloids and ecdysteroids found as major compounds by classical NPC.

Major compound m/z 497. Rule of N?

Interesting aromatic signals, two methyls. Neither zoanthoxanthins nor ecdysteroids.

Paul Guillen (PhD Student)

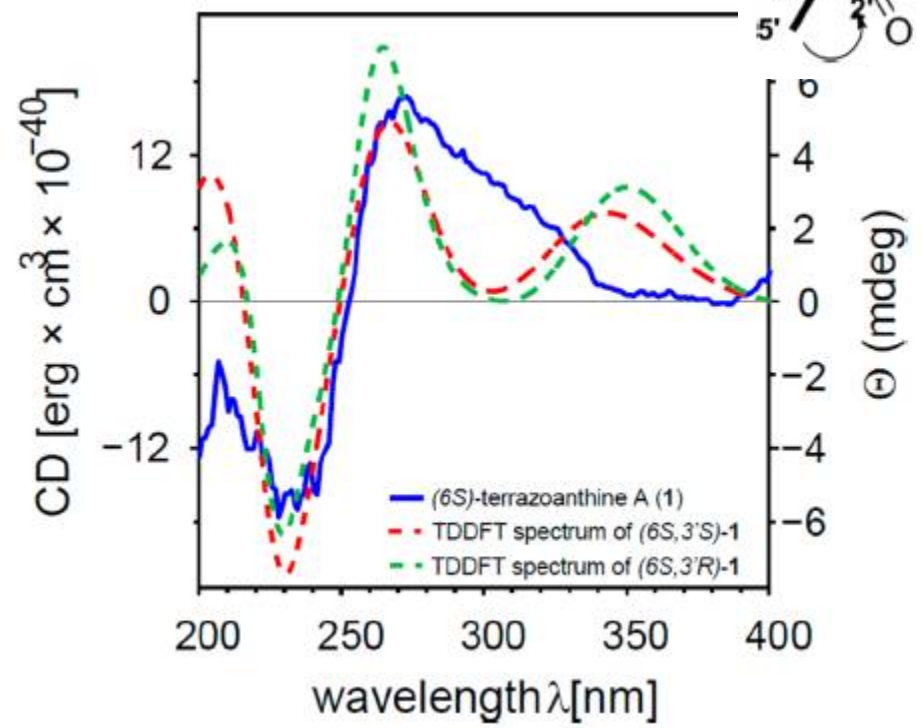
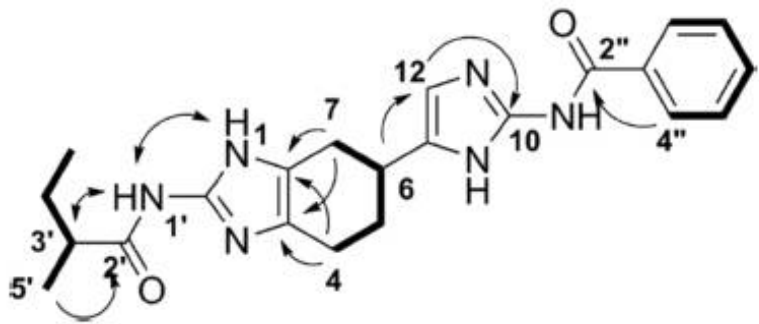
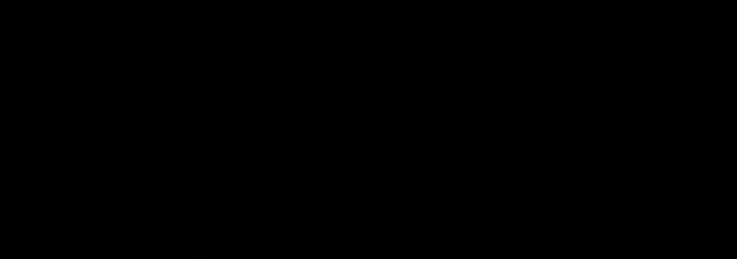


Terrazoanthus onoii

More than one phenyl and two
carbonyls: **heteroaromatics?**

Non equivalent gem protons on two
methylenes: **cycle?**

Benzoyl and 2-methylbutyryl
confirmed.



Gregory Genta-Jouve
University Paris Descartes

Terrazoanthus onoii

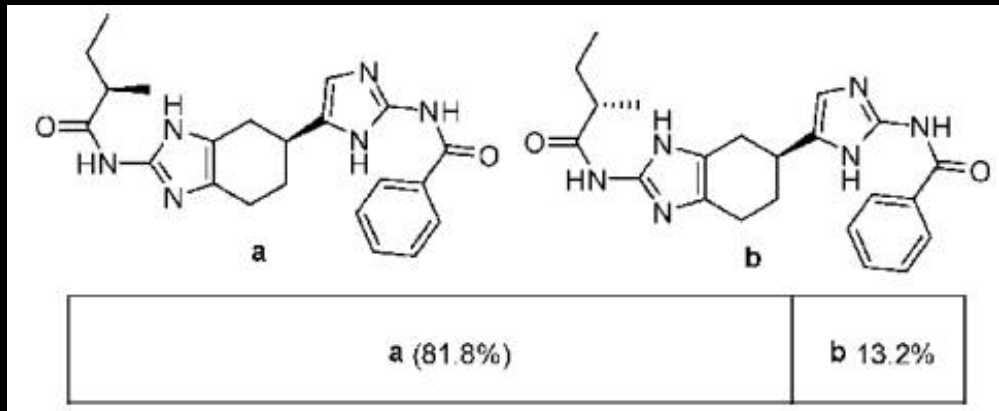
NMR performed in DMSO-*d*₆ for exchangeable protons.

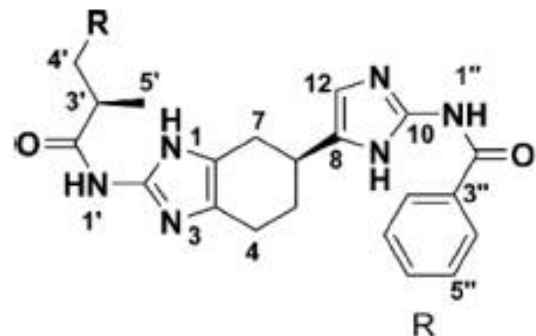
¹³C NMR modeling for the place of the benzoyl

Stereochemistry: another challenge.

- ECD for chiral center at C-6
- ¹³C NMR modelling and DP4 for the chiral center at C-3'

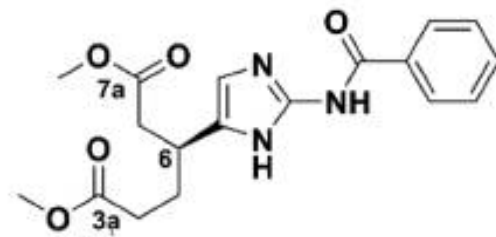
Total synthesis ongoing
Paul Murphy
NUI Galway



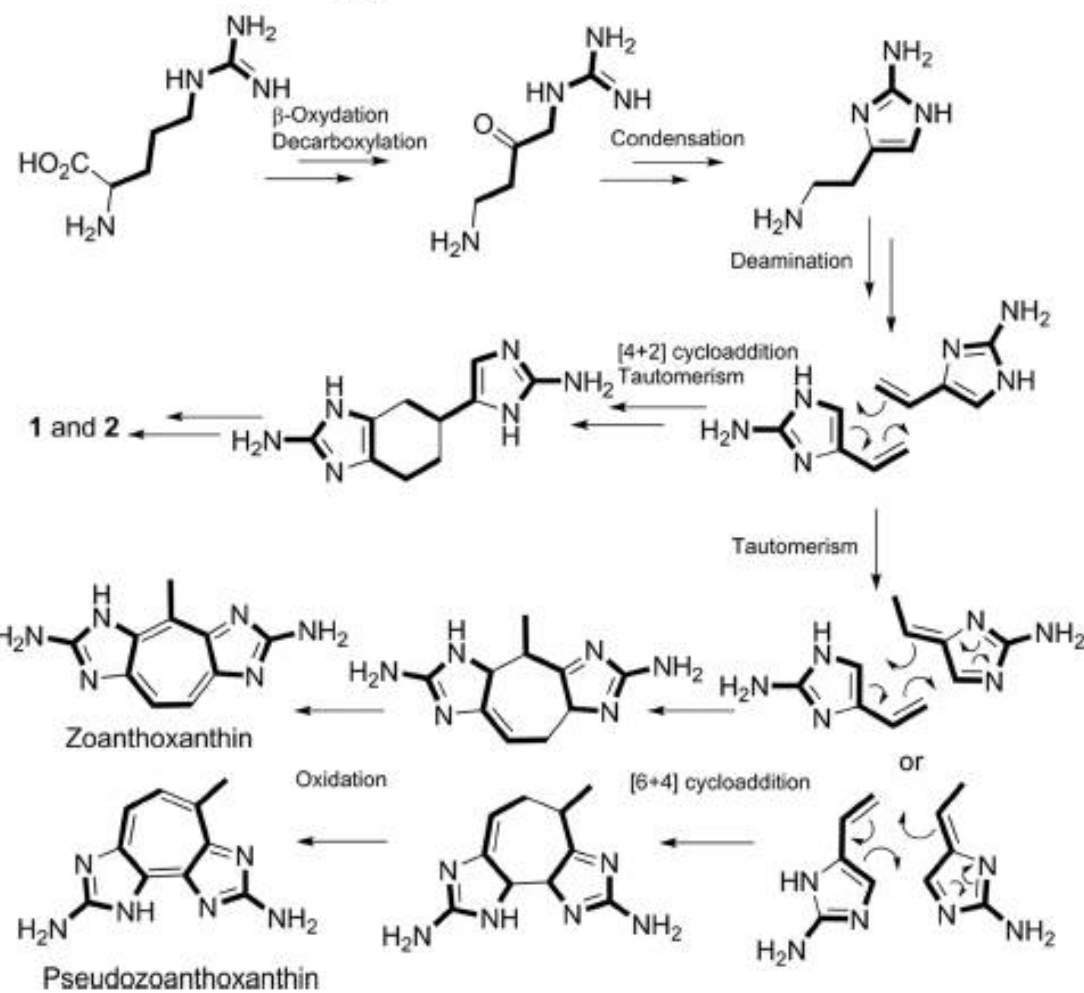


Terrazoanthine A (1) CH_3

Terrazoanthine B (2) H



Terrazoanthine C (3)



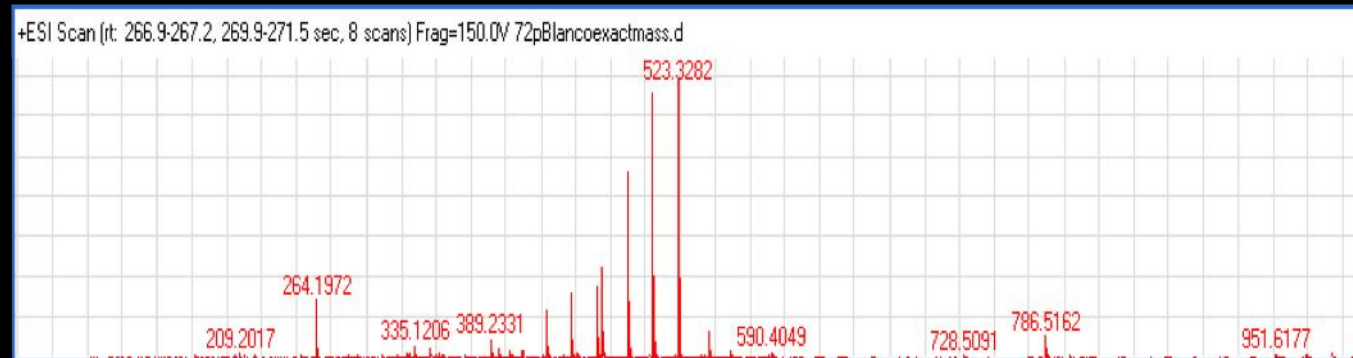
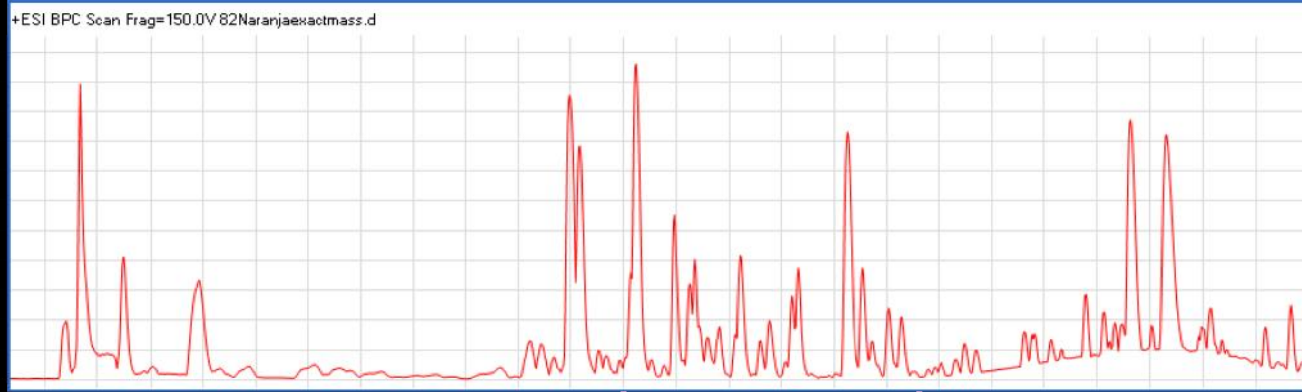
Terrazoanthus onoii

Three analogues, C may be produced by oxidative cleavage of the second 2-aminoimidazole

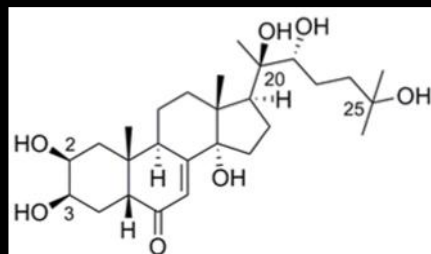
The proposed biosynthesis links the terrazoanthines to the previously known zoanthoxanthins. Key Diels Alder type reaction may be involved as proposed by Buchi.

[4+2] and [6+4] cycloadditions may explain the diversity of alkaloids

Guillen et al. Organic Letters 2017



Typical mass fragmentation pattern of ecdysteroids



Ecdysteroids

20-Hydroxyecdysone M481

Antipathozoanthus hickmani

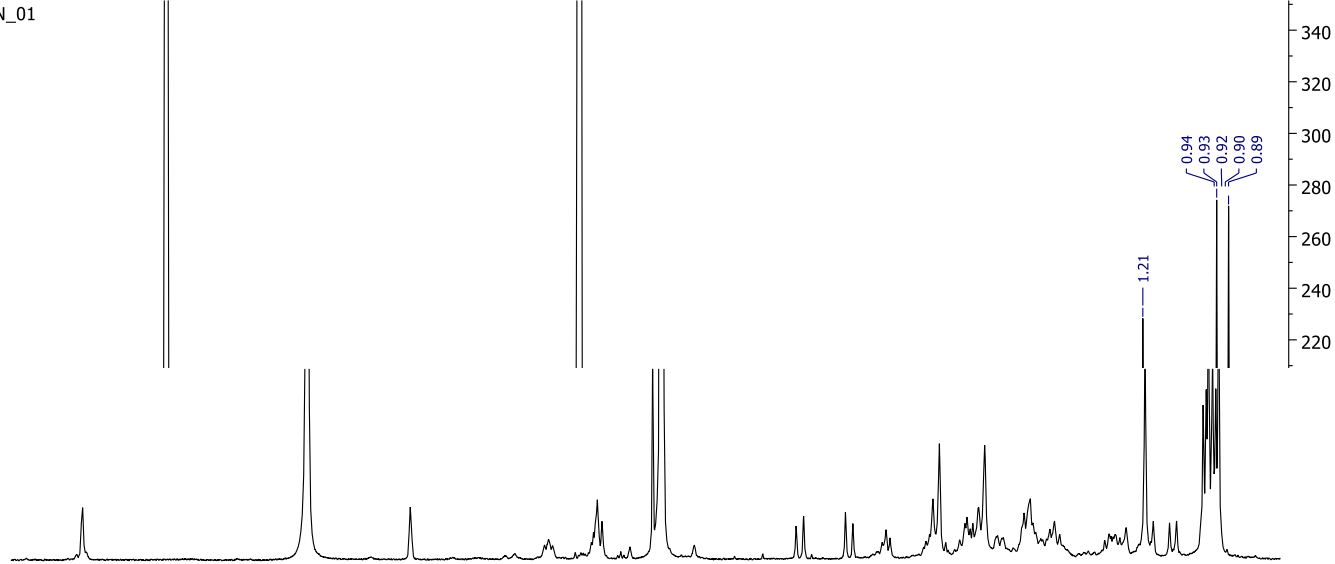
Large diversity of ecdysteroids, some of them with unknown masses!

Isolation and purification of the major compounds produced by this species targeting the ecdysteroids

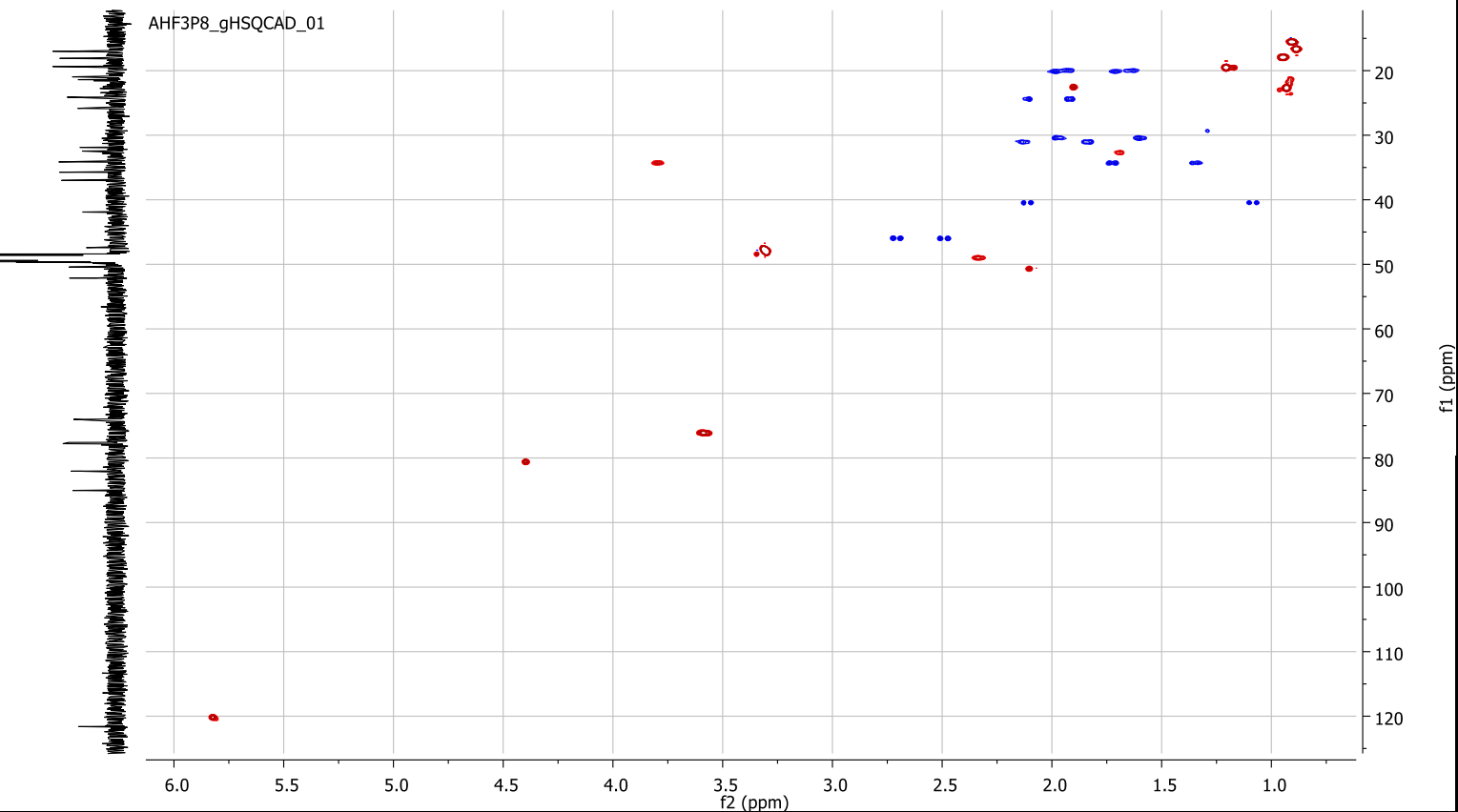
Markers of zoantarians?

Also produced by shrimps and insects as a molting hormone.

AHF3P8_PROTON_01



AHF3P8_gHSQCAD_01



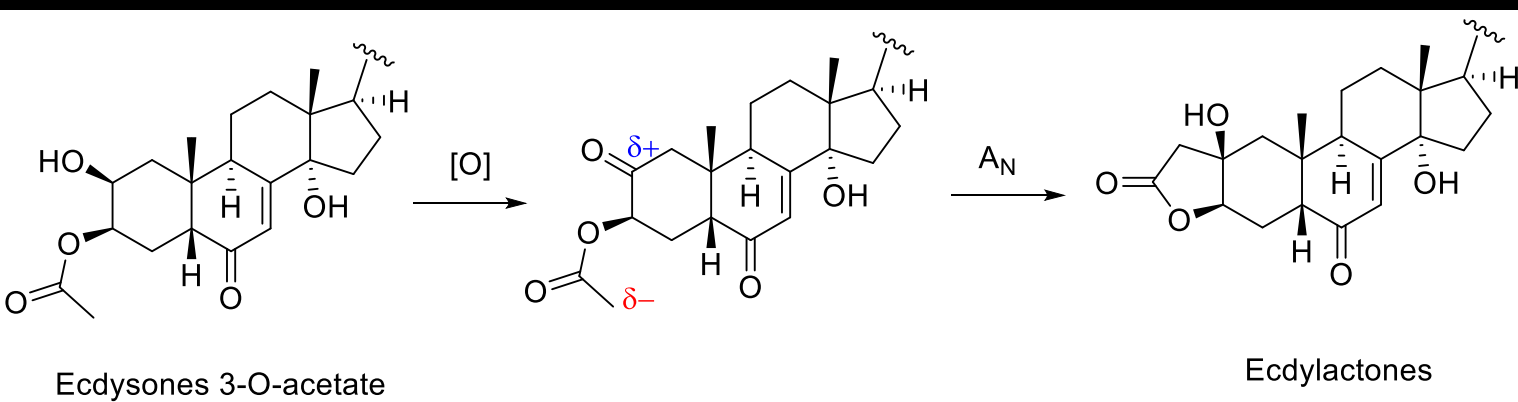
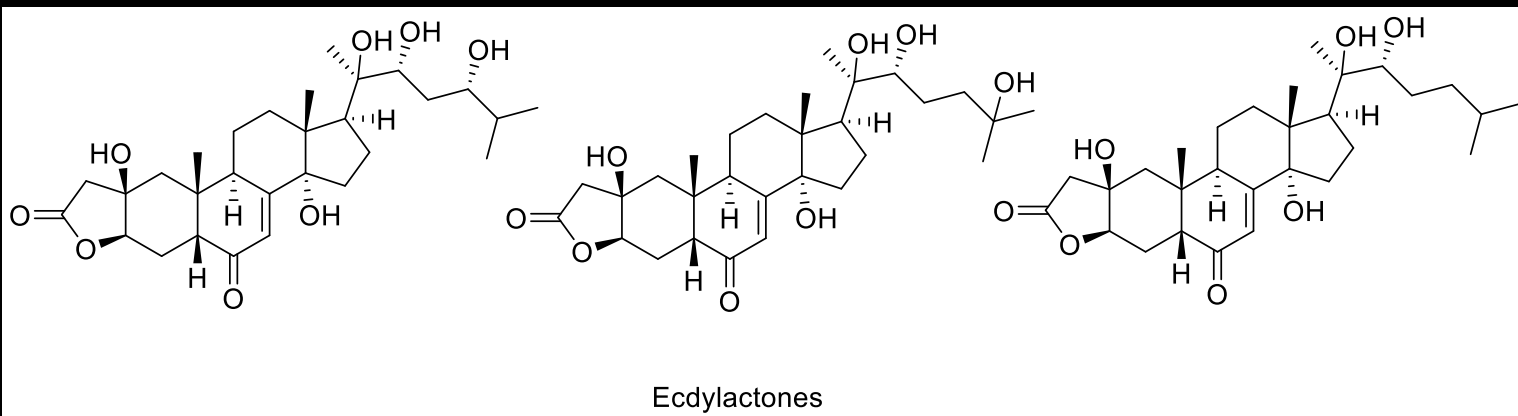
Antipathozoanthus hickmani

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Usual olefinic proton and other oxygenated methines.

Uncommon AB system.



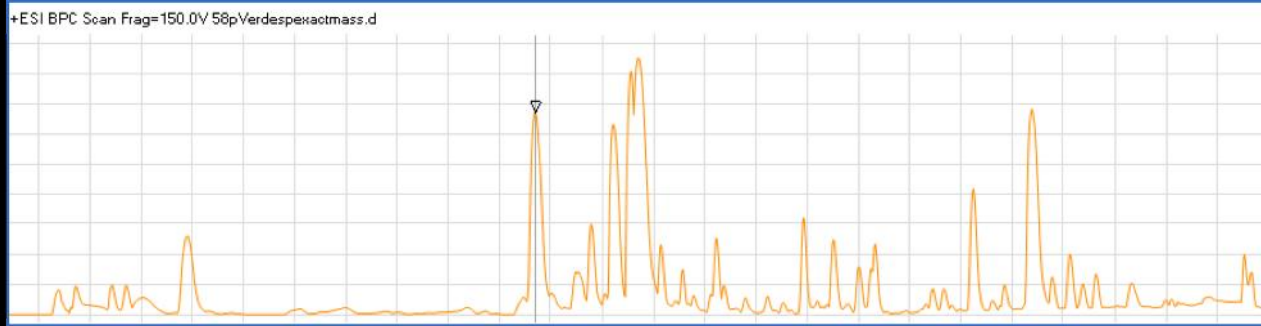
Antipathozoanthus hickmani

New lactone system fused on ring A.

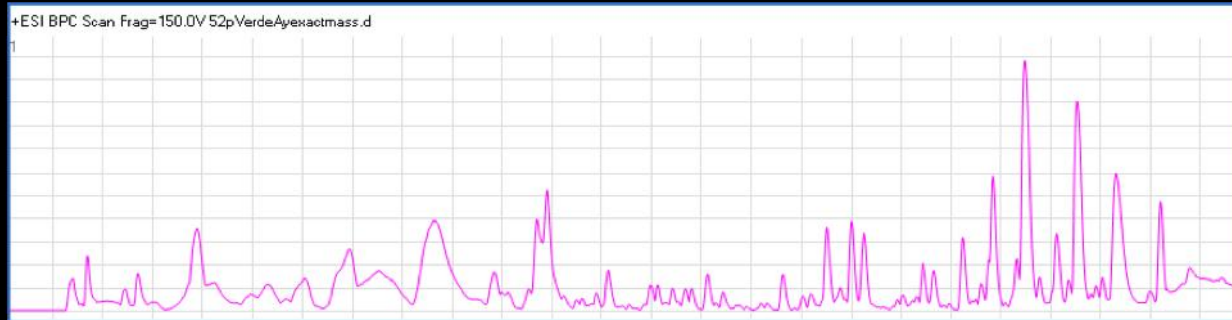
Diversity of analogues never seen in ecdysteroids.

Possible production by oxidation at C-2/cyclisation from the 3-O-acetate analogue

Zoanthus sp1.



Zoanthus sp2.



sp1



sp2

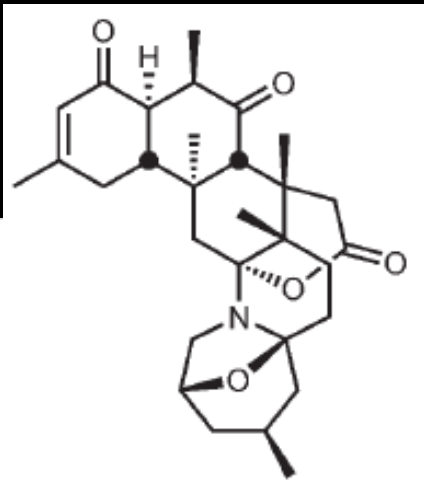


Zoanthus sp.

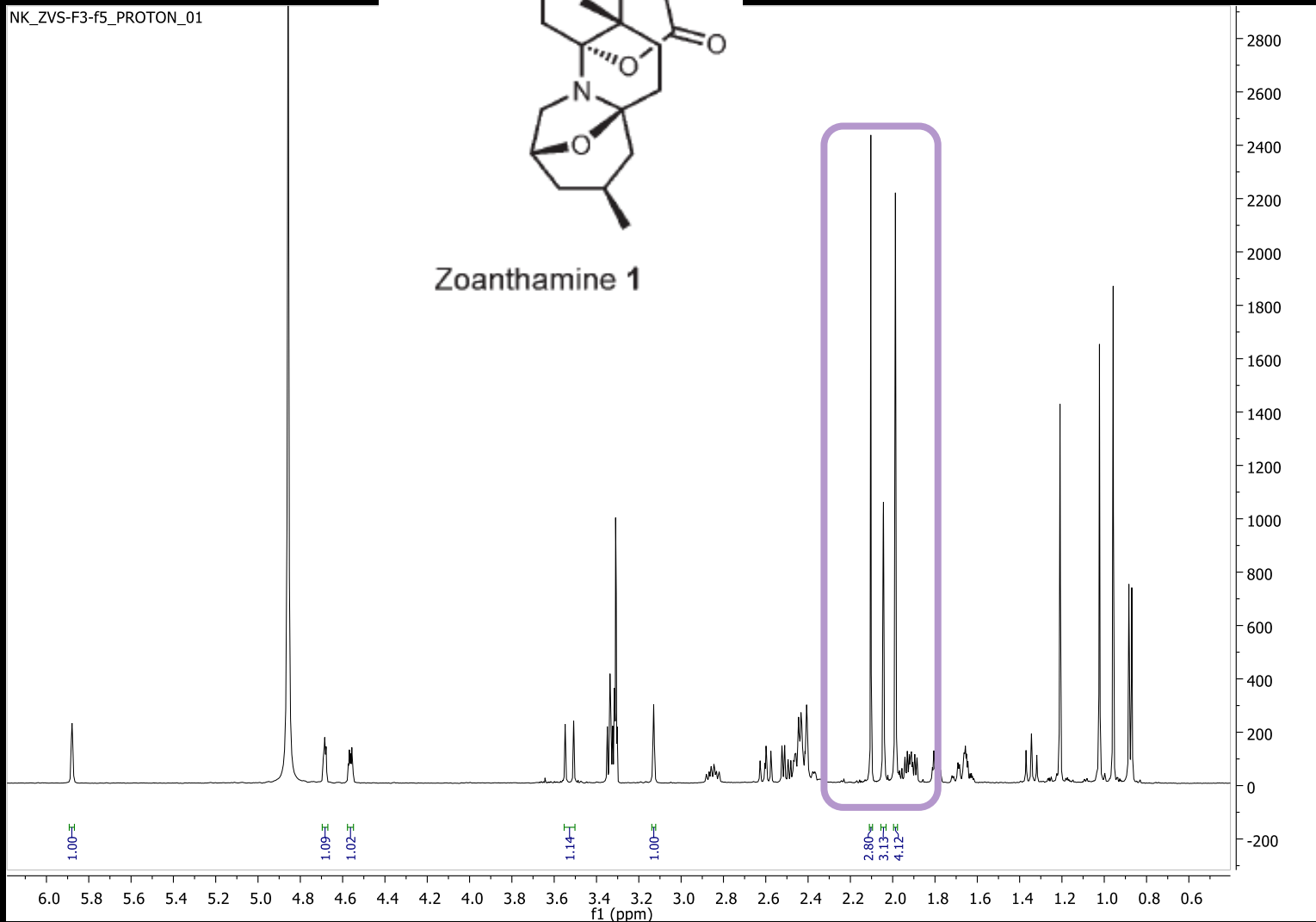
DNA shows clear differences between these two shallow water and intertidal species.

Chemical profiling confirms this difference.

Search in the literature suggests that *Zoanthus* sp1. produces non aromatic alkaloids that could belong to the zoanthamine family.



Zoanthamine 1



Zoanthus sp1.

Widespread in the coastline

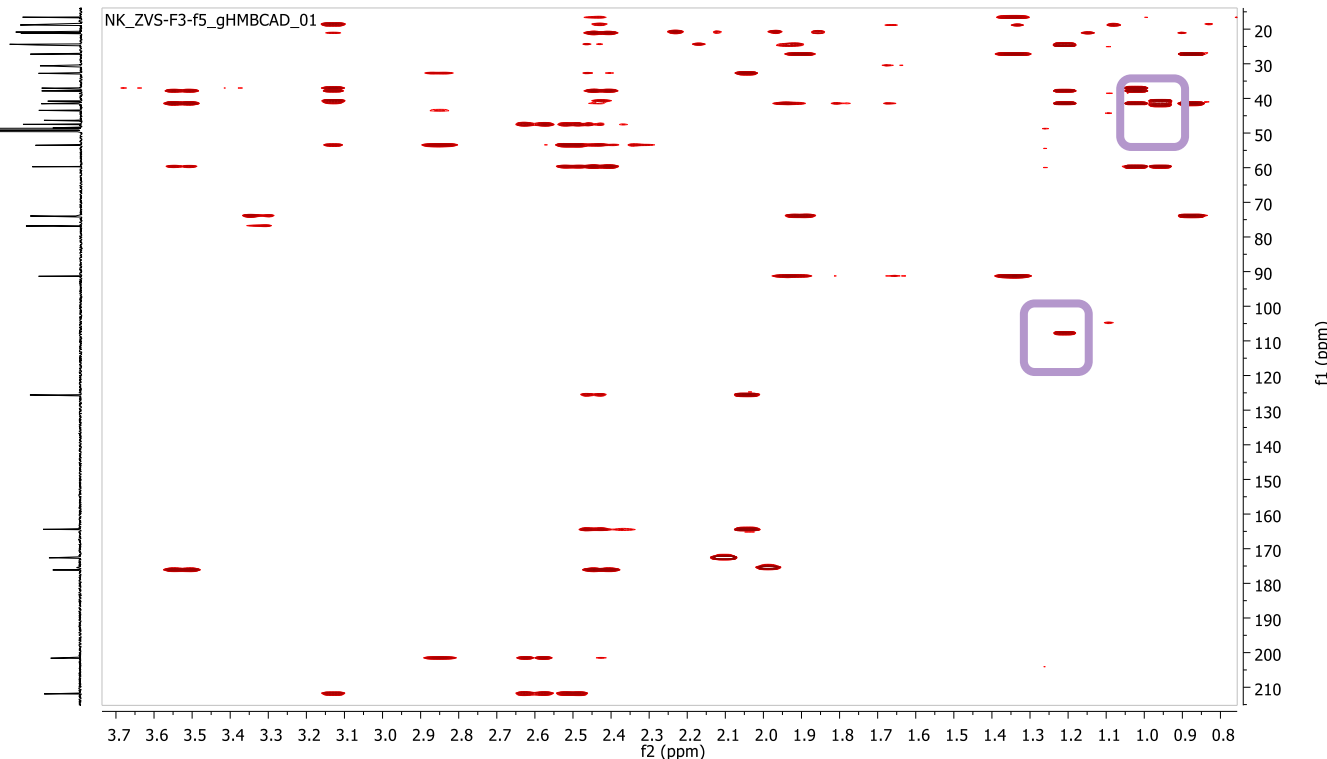
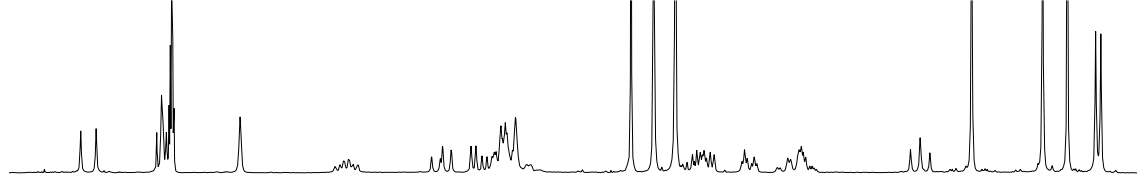
Large diversity of zoanthamine analogues with new masses.

The major compound isolated: 25 mg.

New signals in the NMR spectra.

Norzoanthamine

Three methyls instead of one for usual zoanthamines at around 2 ppm



Zoanthus sp1.

A norzoanthamine with a O-acetyl at C-3

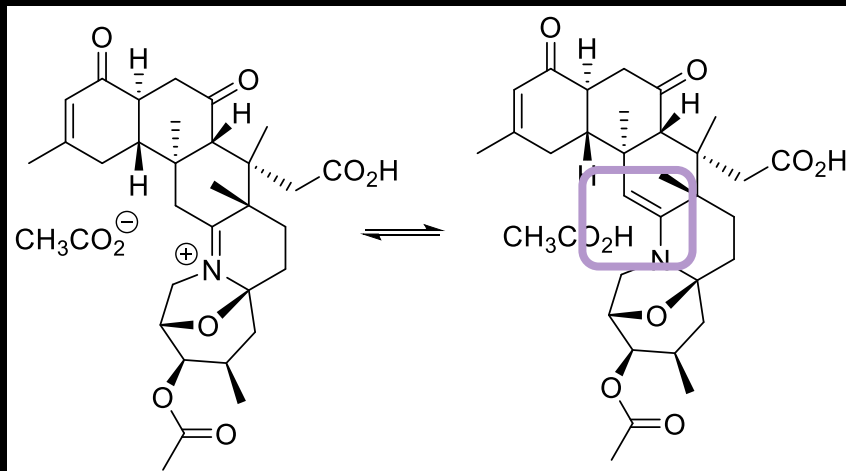
Some signals missing in HMBC and ¹³C!

Acido-basic equilibrium

iminium/enamine

New norzoanthamine. Others to come.

Iminium



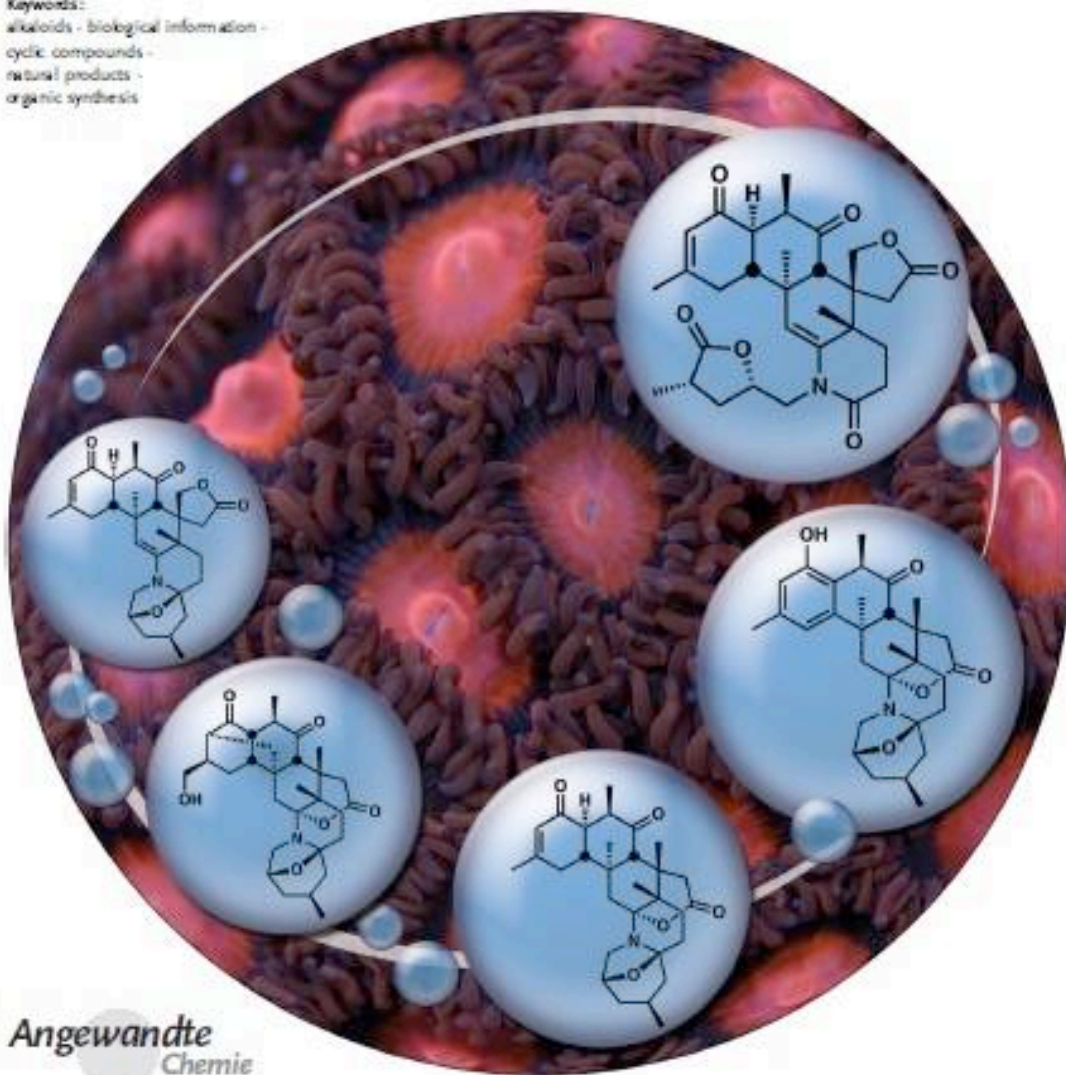
Enamine

The Biology and Chemistry of the Zoanthamine Alkaloids

Douglas C. Behenna, Jennifer L. Stockdill, and Brian M. Stoltz*

Keywords:

alkaloids · biological information ·
cyclic compounds ·
natural products ·
organic synthesis



Angewandte
Chemie

Zoanthus sp1.

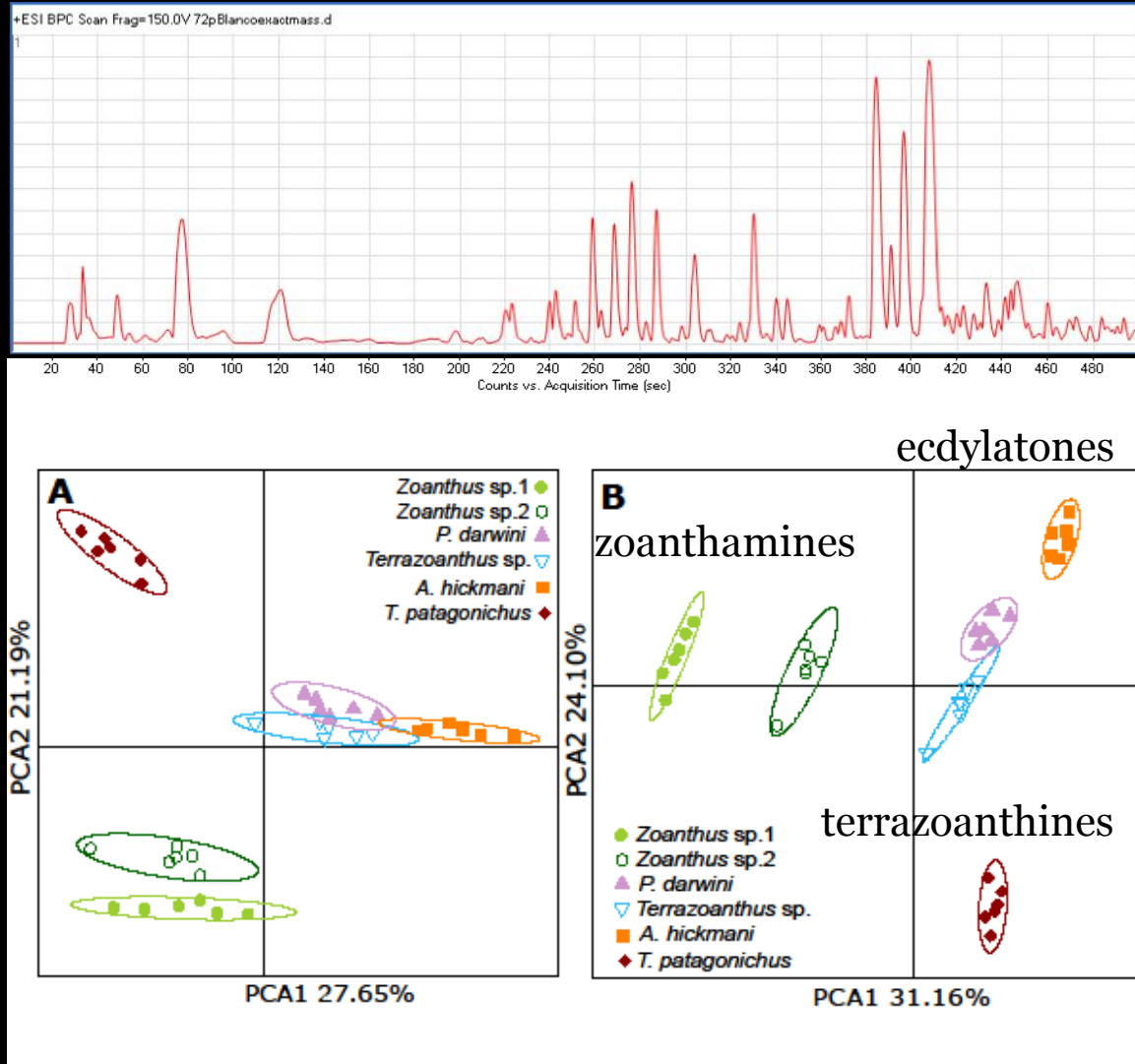
Zoanthamines are promising candidates for marine biodiscovery pipeline.

Chemical library ready for testing at
Fundacion Medina - Granada



Fundación MEDINA
Discovering the Future

6 replicates of 6 different species of zoantharians collected in different habitats and time. Simple process with 250 mg of dry material, SPE C18 elution MeOH. Injection in UHPLC-QToF



Including minor

Focusing major (Area > 10⁶)

Phylometabolomic analysis

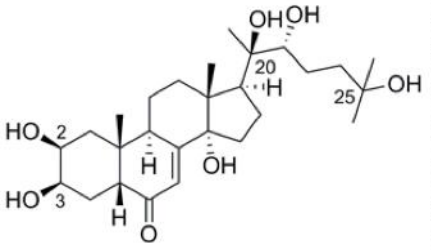
Why appropriate for Zoantharia?

Checklist:

- Morphological and genetic data ✓
- Some specialized metabolites identified ✓
- Easily ionized for detection in MS ✓

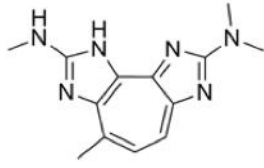
If not the environment can drive the metabolomics profiles.

Karla Jaramillo and Miriam Reverter



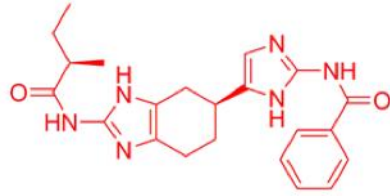
Ecdysteroids

20-Hydroxyecdysone M481



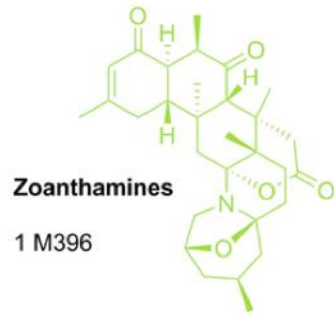
Zoanthoxanthins

Paragraine M257



Terrazoanthines

A M407



Zoanthamines

1 M396

Phylometabolomic analysis

Does not fit perfectly with the phylogenetic tree. Hypotheses.

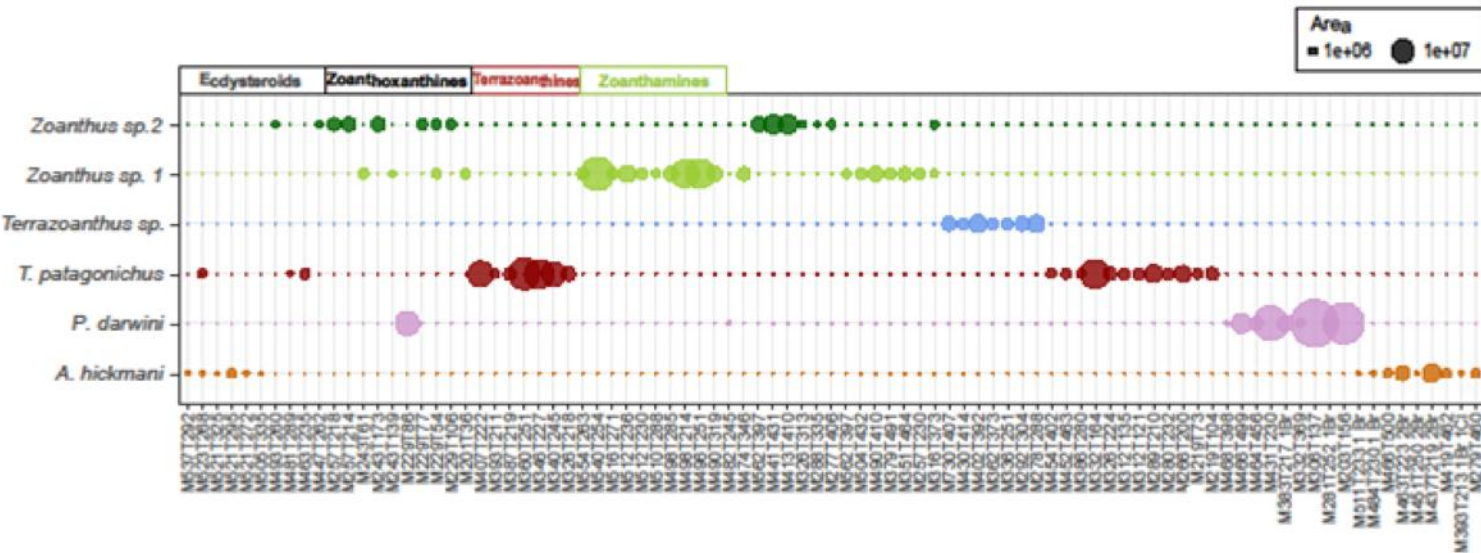
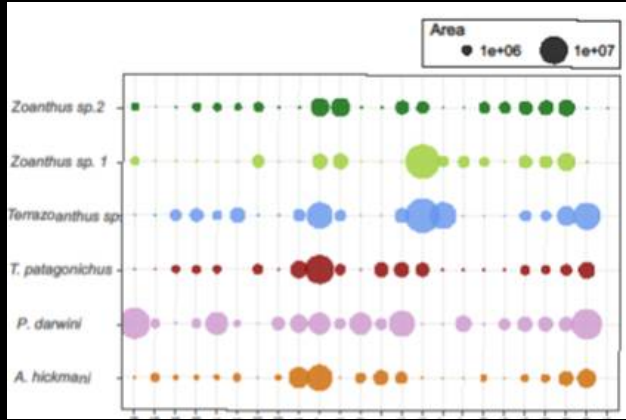
However:

- No overlap between different species replicates
- *Zoanthus* are grouped mainly due to minor compounds

Markers of the dissimilarity.

Common major metabolites

Species specific metabolites



Conclusive comments

FUNDING



Exploration of understudied ecoregions is still promising for Marine Biodiscovery.

Extending global knowledge on the marine biodiversity and chemical diversity.

Training of taxonomists and chemists in countries with high biodiversity.

Zoantharia should be considered in a usual marine biodiscovery workflow.

Construction of repositories and chemical libraries for screening.

Promotion of win/win international collaborations

Uses of the isolated metabolites: drug discovery and ecological understanding like
phylometabolomics



National Marine Biodiscovery Laboratory in Ireland





Minoan civilization

