

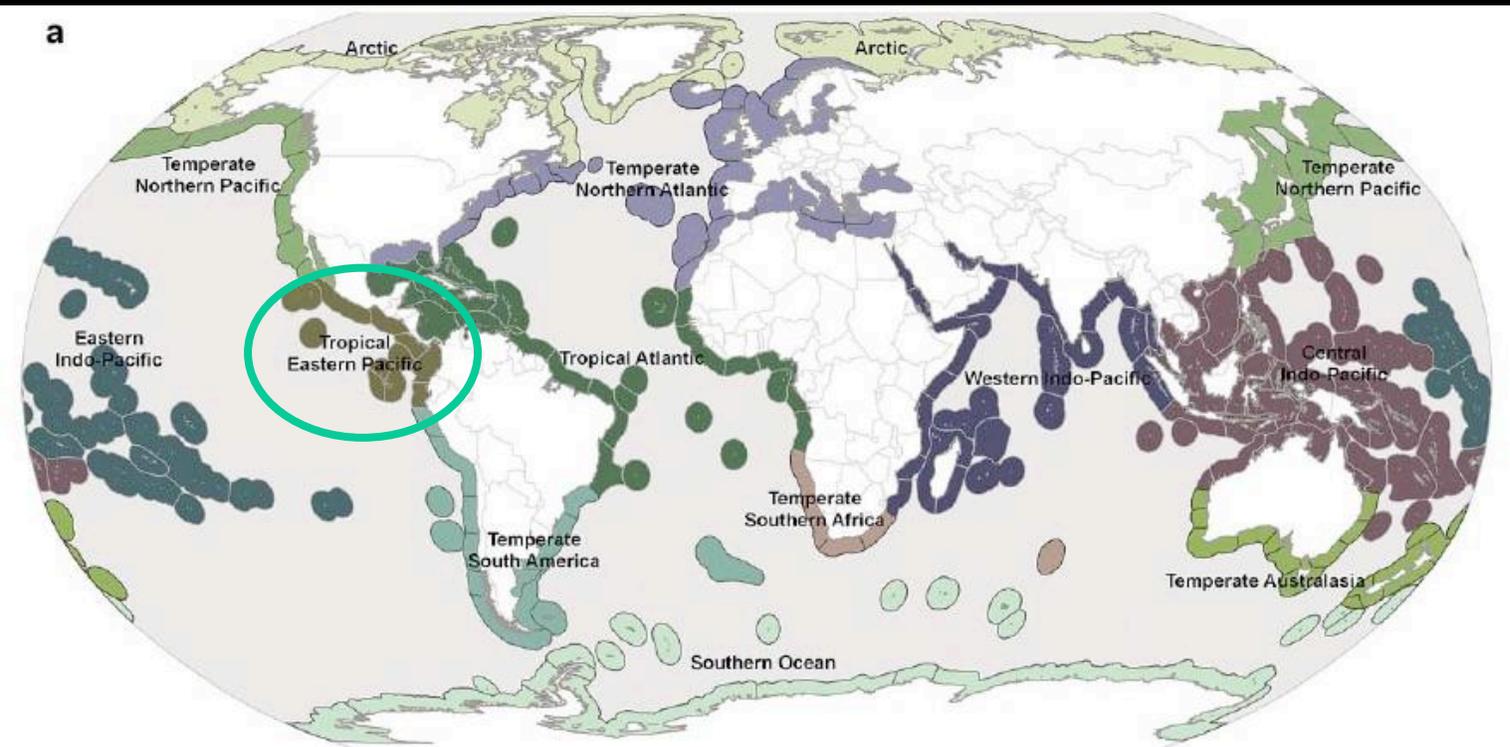


OÉ Gaillimh  
NUI Galway



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

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

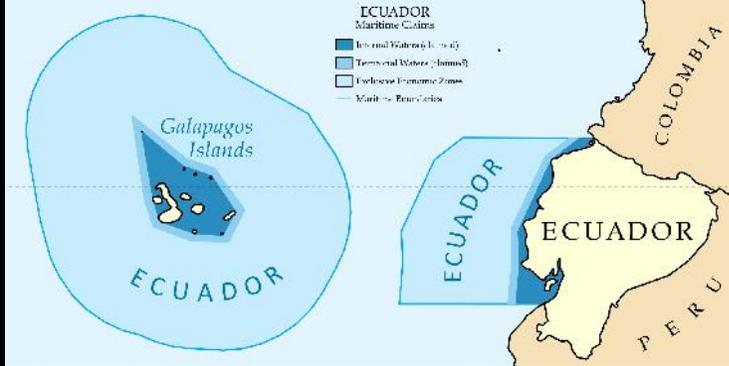


# Novelty in Marine Biodiscovery

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.

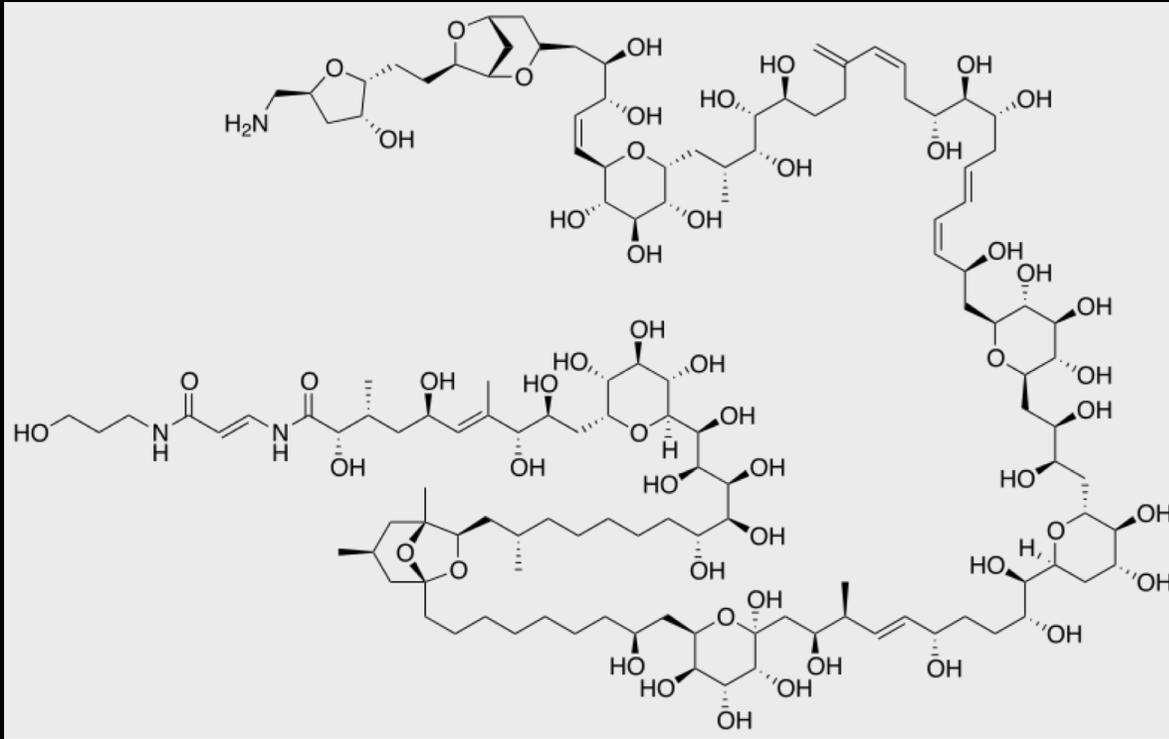
Octocorallia but also hexacorallia like zoantharians

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)

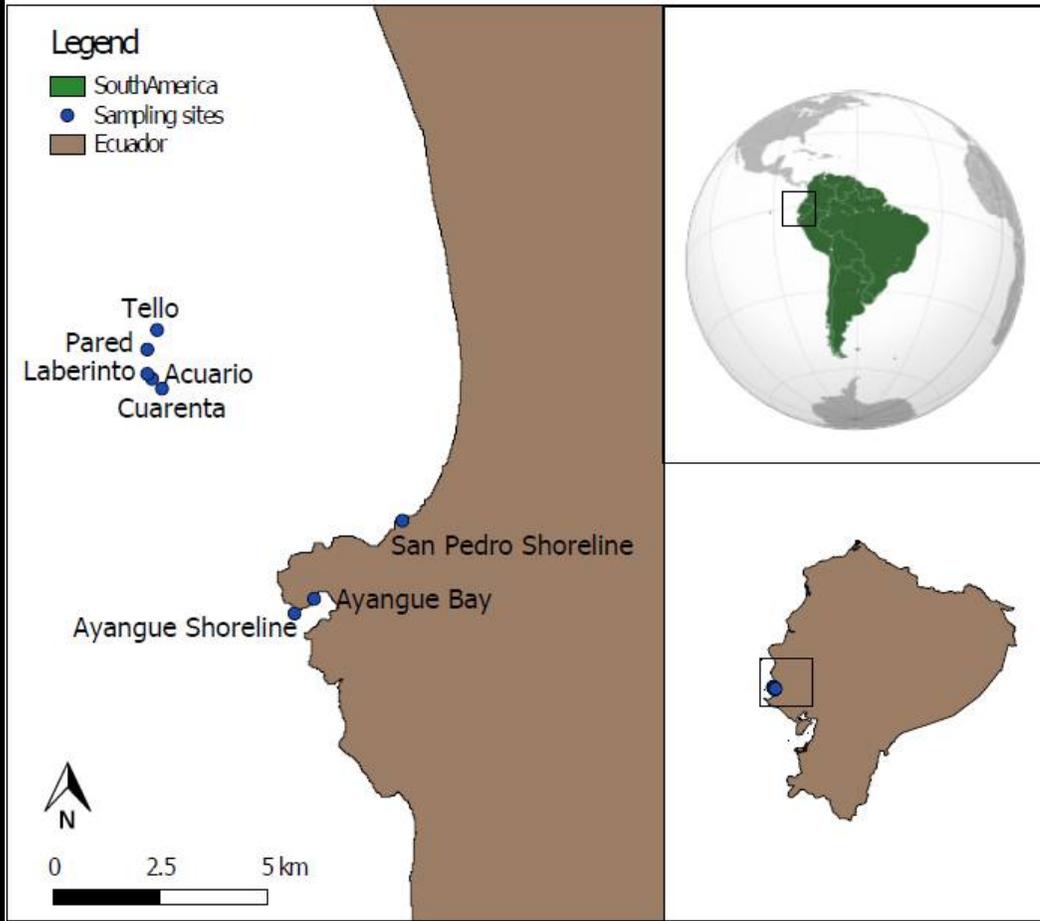
Then produced by dinoflagellate



## 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*

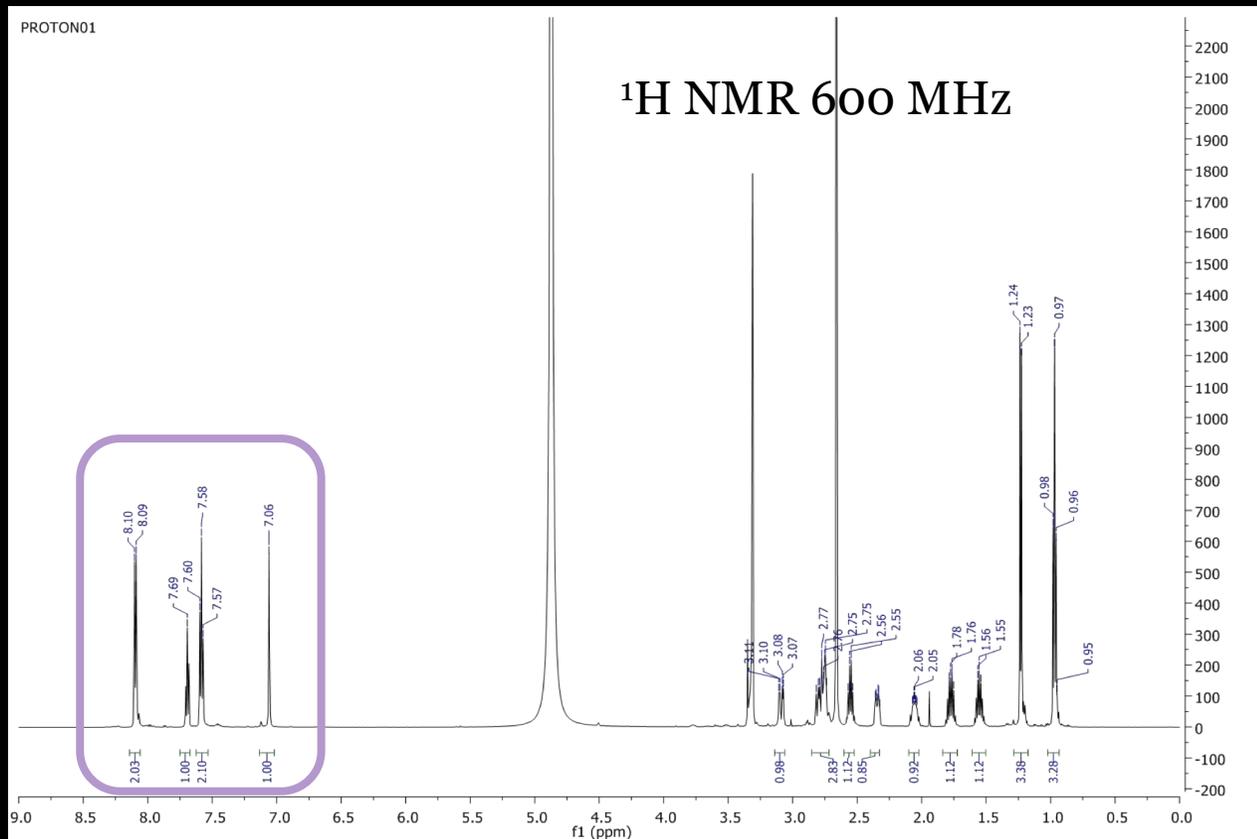
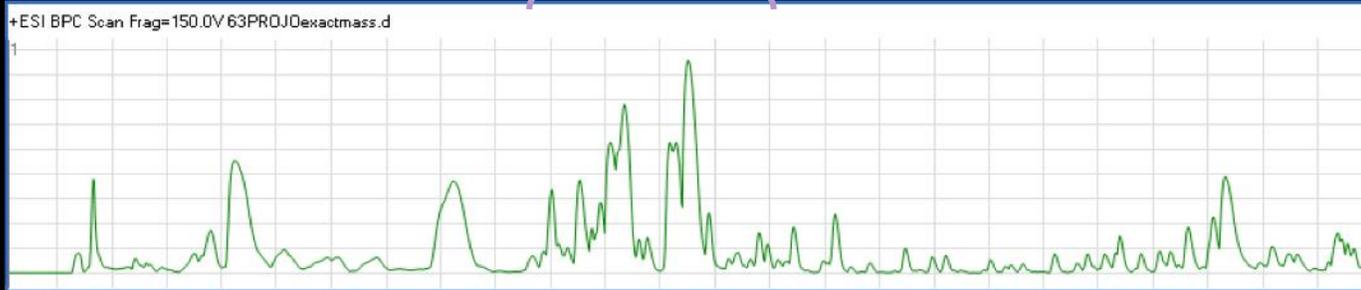
Brachycnemina: *Zoanthus*, *Palythoa*

Frederic Sinniger

Karla Jaramillo  
PhD Student



# UHPLC-HRMS evidenced unusual masses



## *Terrazoanthus onoi*

Genetics close to *T. patagonichus*

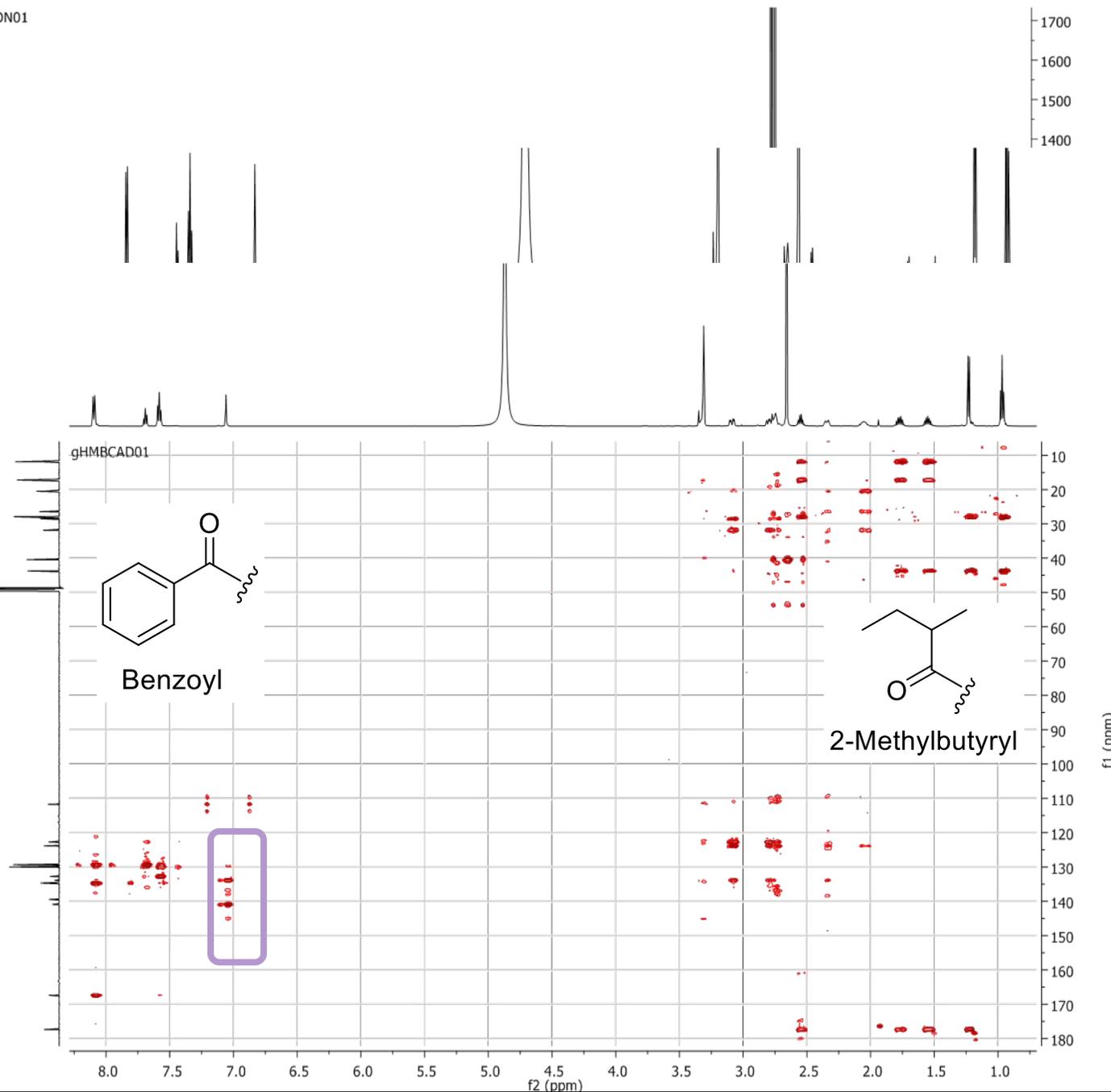
Alkaloids and ecdysteroids found as major compounds by classical NPC.

Major compound  $m/z$  497. Rule of N?

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

Paul Guillen (PhD Student)

CARBON01

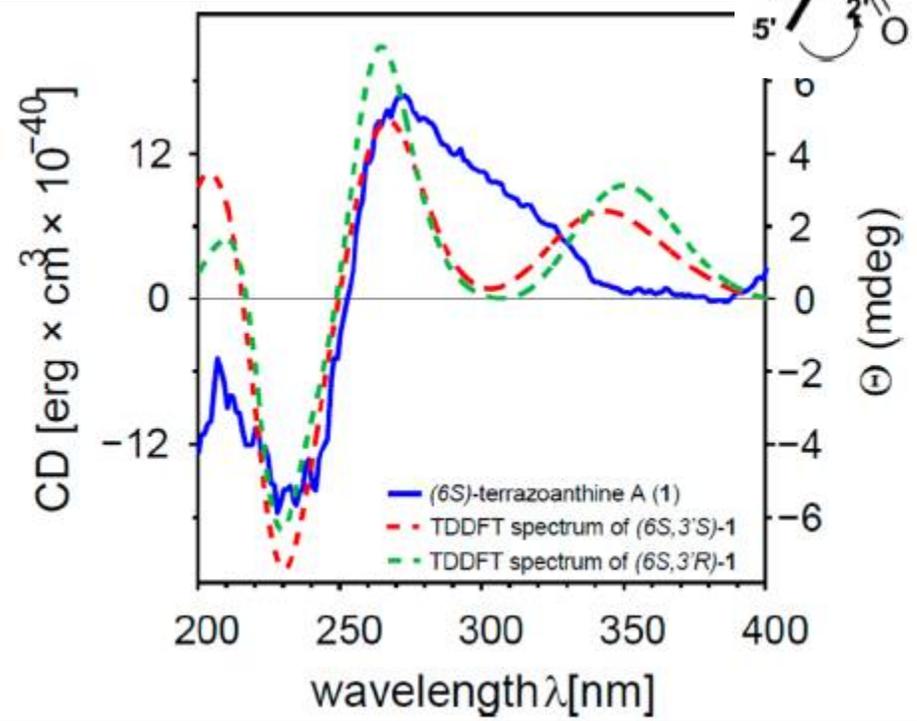
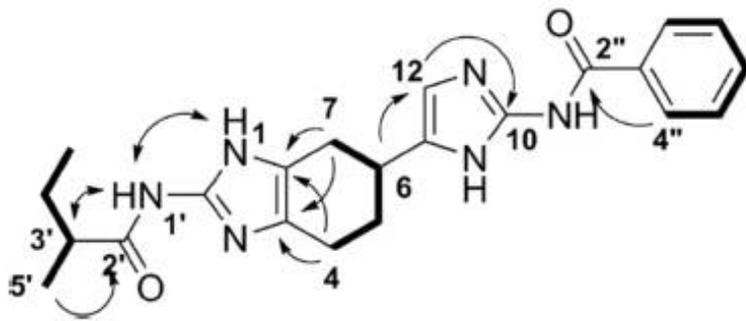


# *Terrazoanthus onoii*

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 onoi

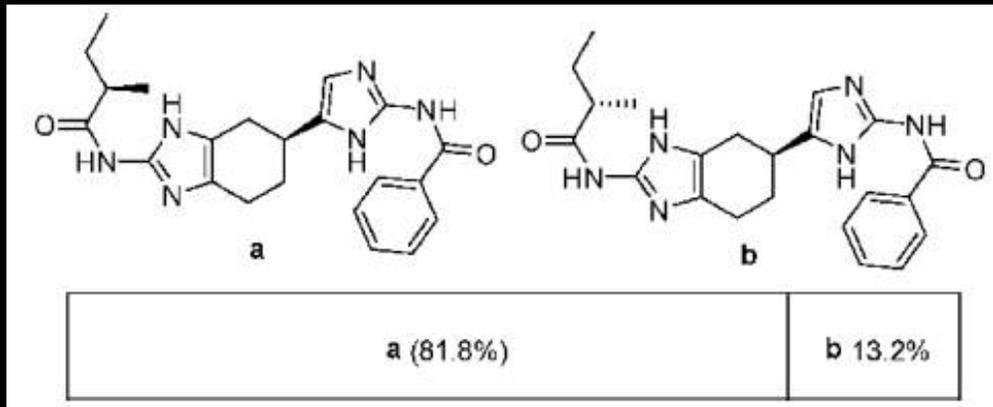
NMR performed in DMSO-*d*<sub>6</sub> for  
 exchangeable protons.

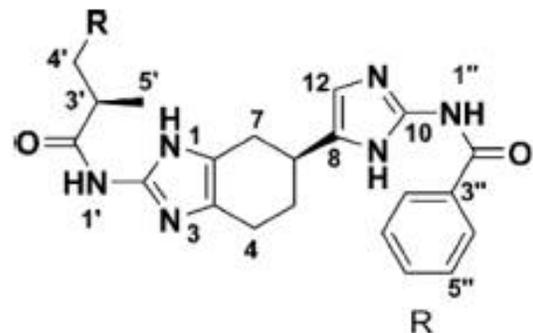
<sup>13</sup>C NMR modeling for the place of the  
 benzoyl

Stereochemistry: another challenge.

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

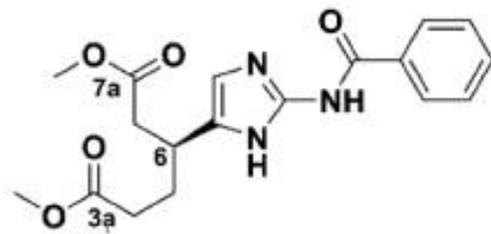
Total synthesis ongoing  
 Paul Murphy  
 NUI Galway



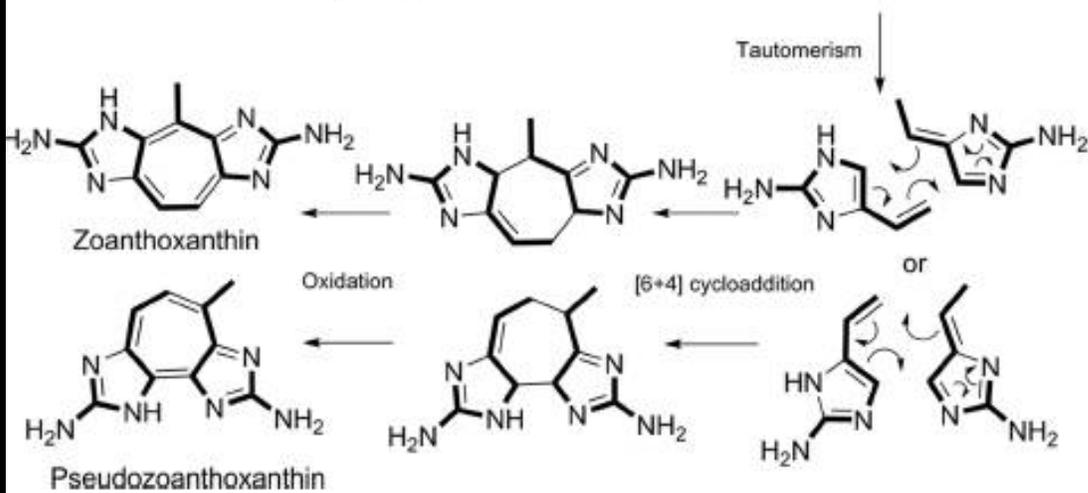
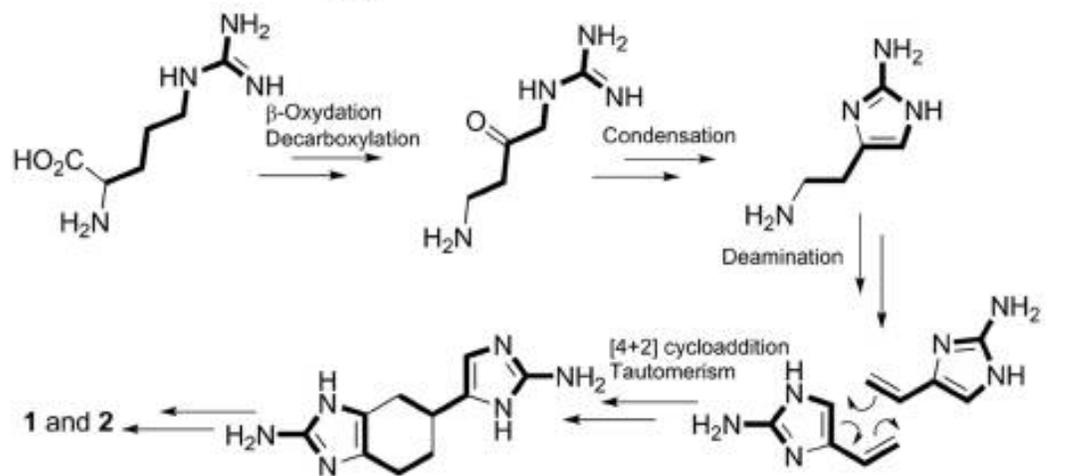


Terrazoanthine A (1) CH<sub>3</sub>

Terrazoanthine B (2) H



Terrazoanthine C (3)



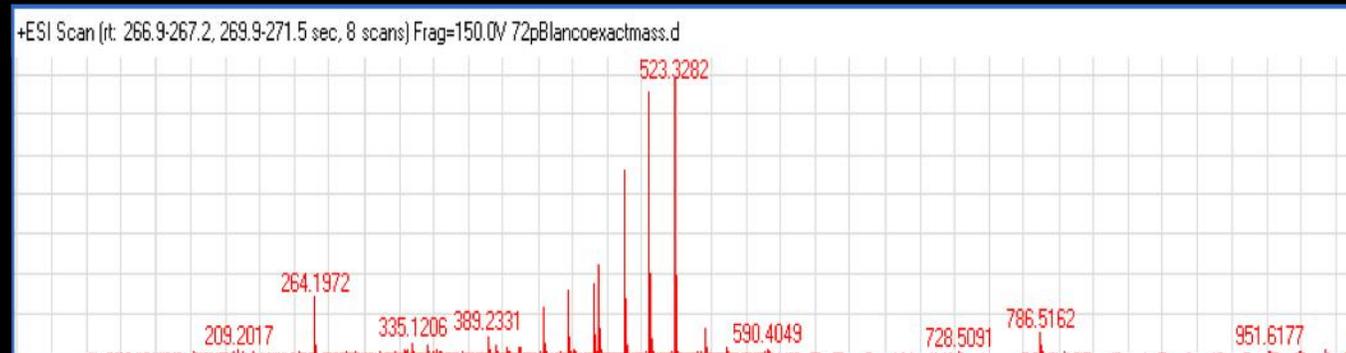
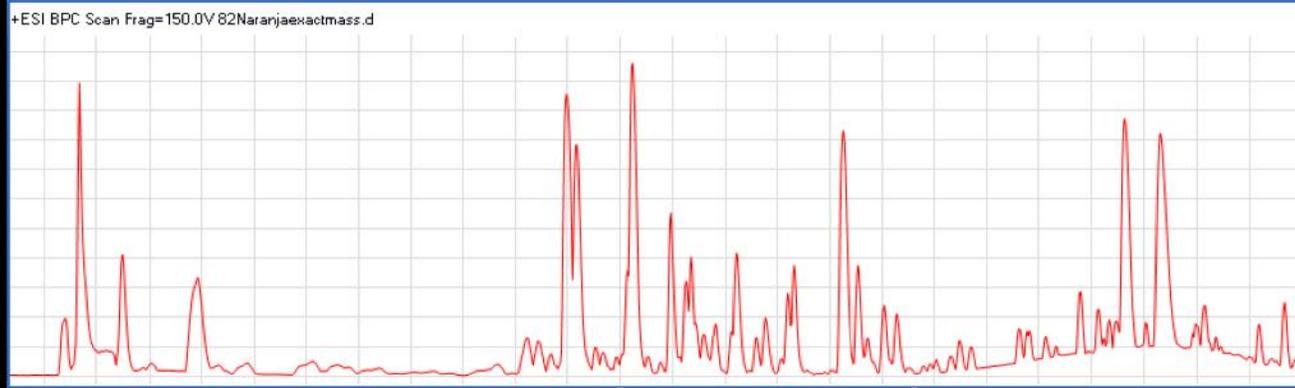
# Terrazoanthus onoii

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

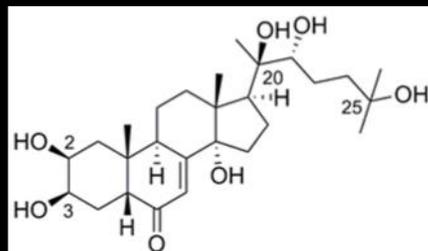
The proposed biosynthesis links the terrazoanthines to the previously known zoanthoxanthins. Key Diels Alder type reaction may be involved as proposed by Buchi.

[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*

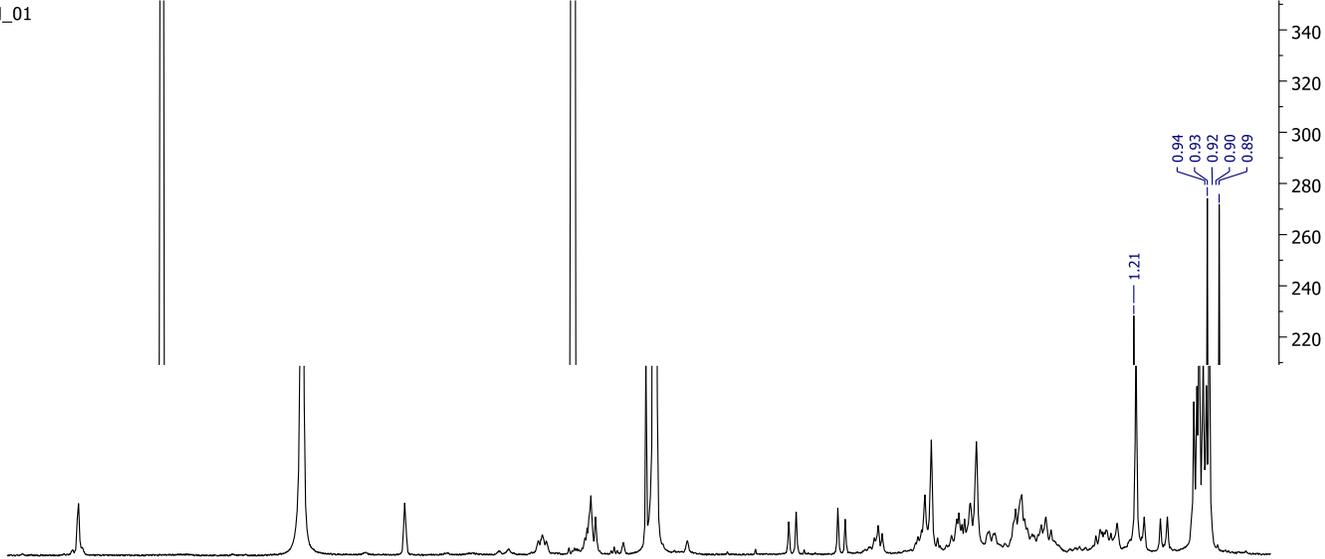
Large diversity of ecdysteroids, some of them with unknown masses!

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

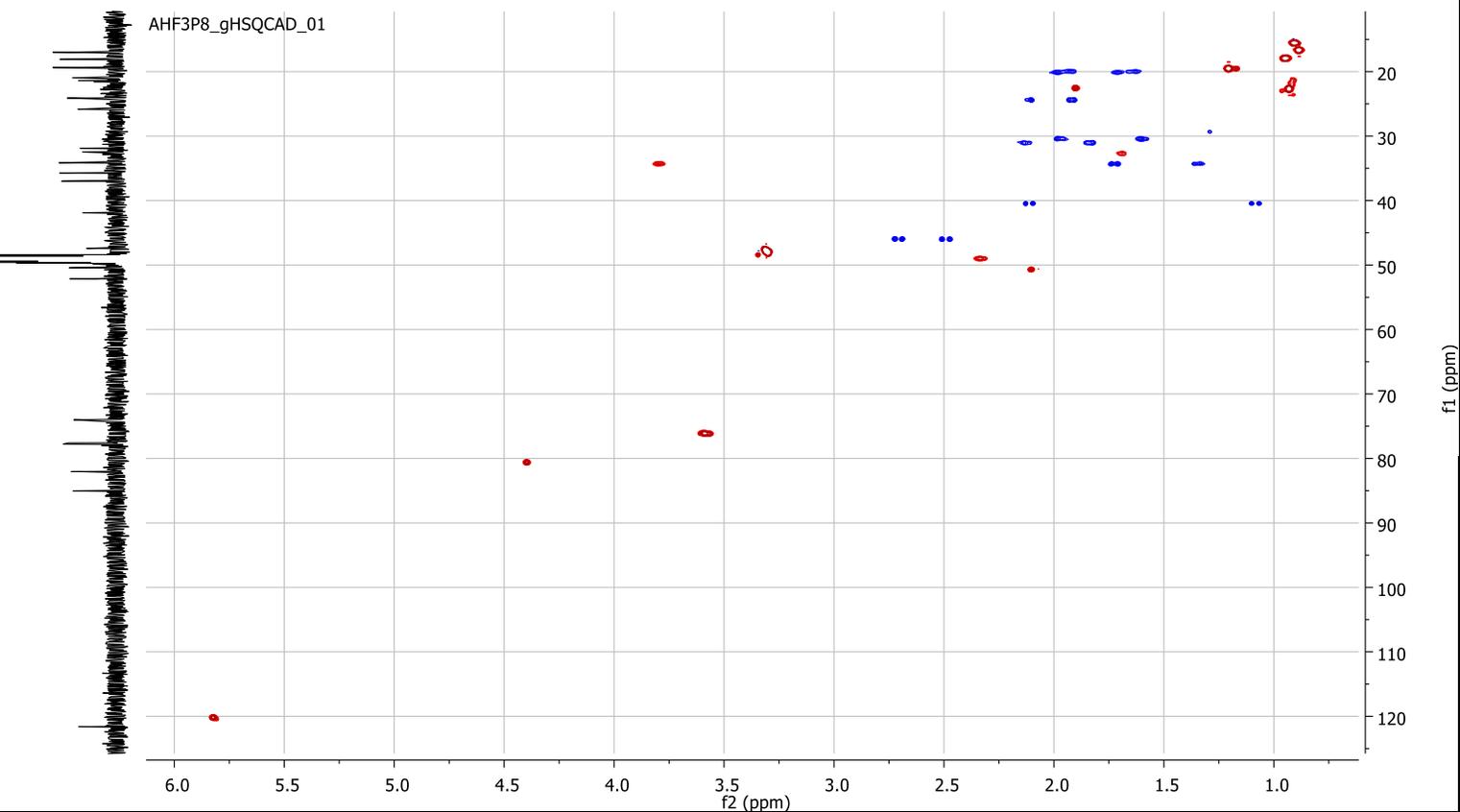
Markers of zoantarians?

Also produced by shrimps and insects as a molting hormone.

AHF3P8\_PROTON\_01



AHF3P8\_gHSQCAD\_01



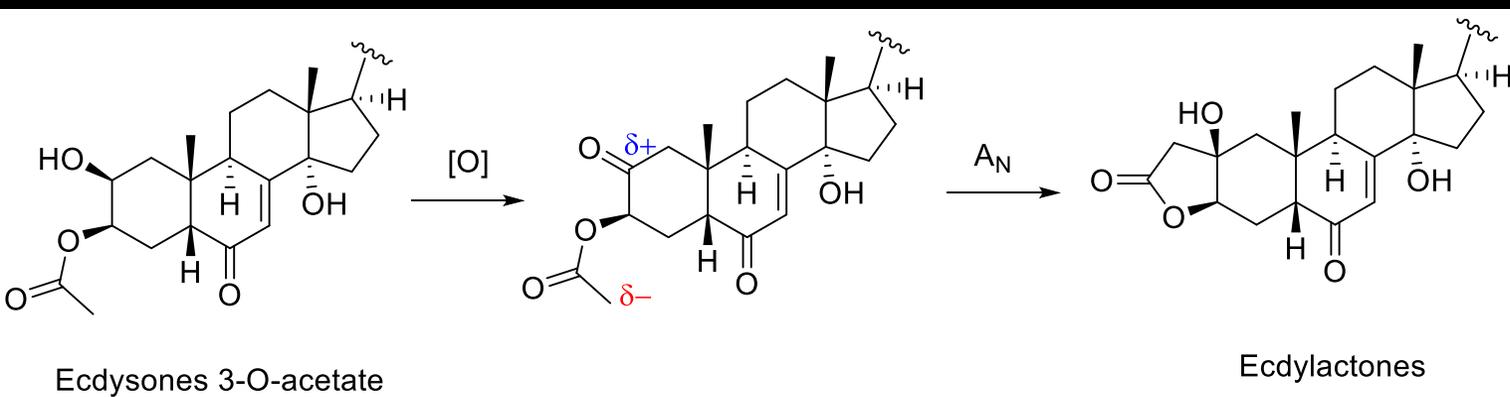
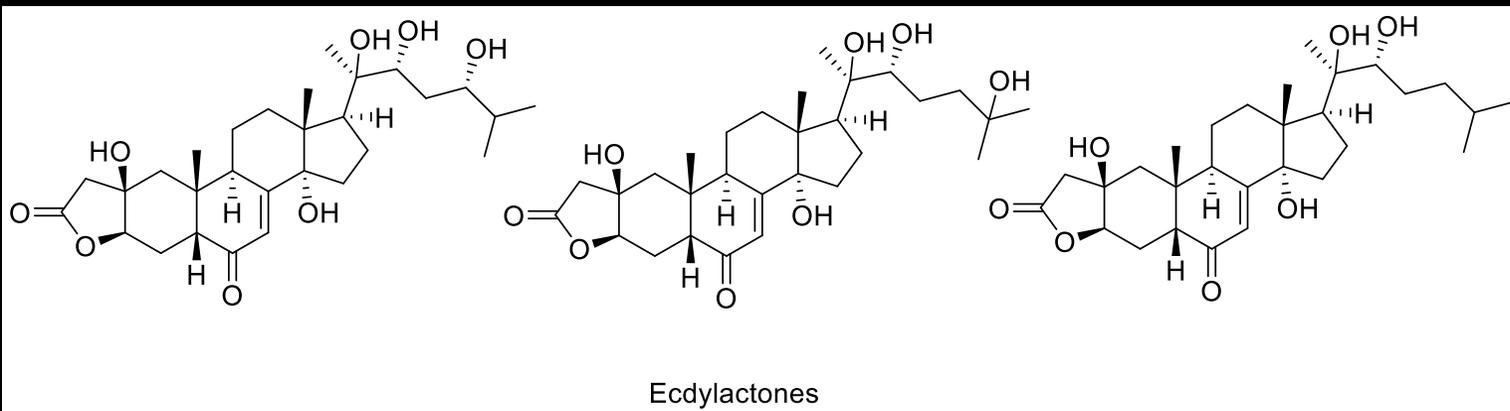
# *Antipathozoanthus hickmani*

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

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

Usual olefinic proton and other oxygenated methines.

Uncommon AB system.



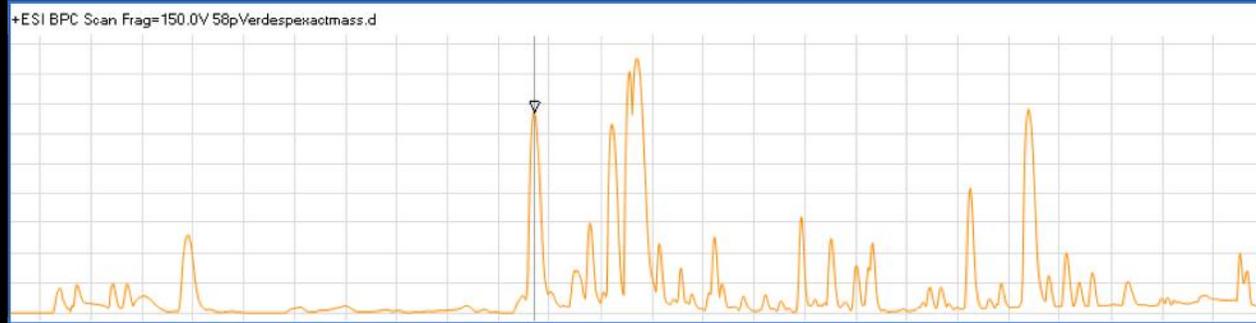
# *Antipathozoanthus hickmani*

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



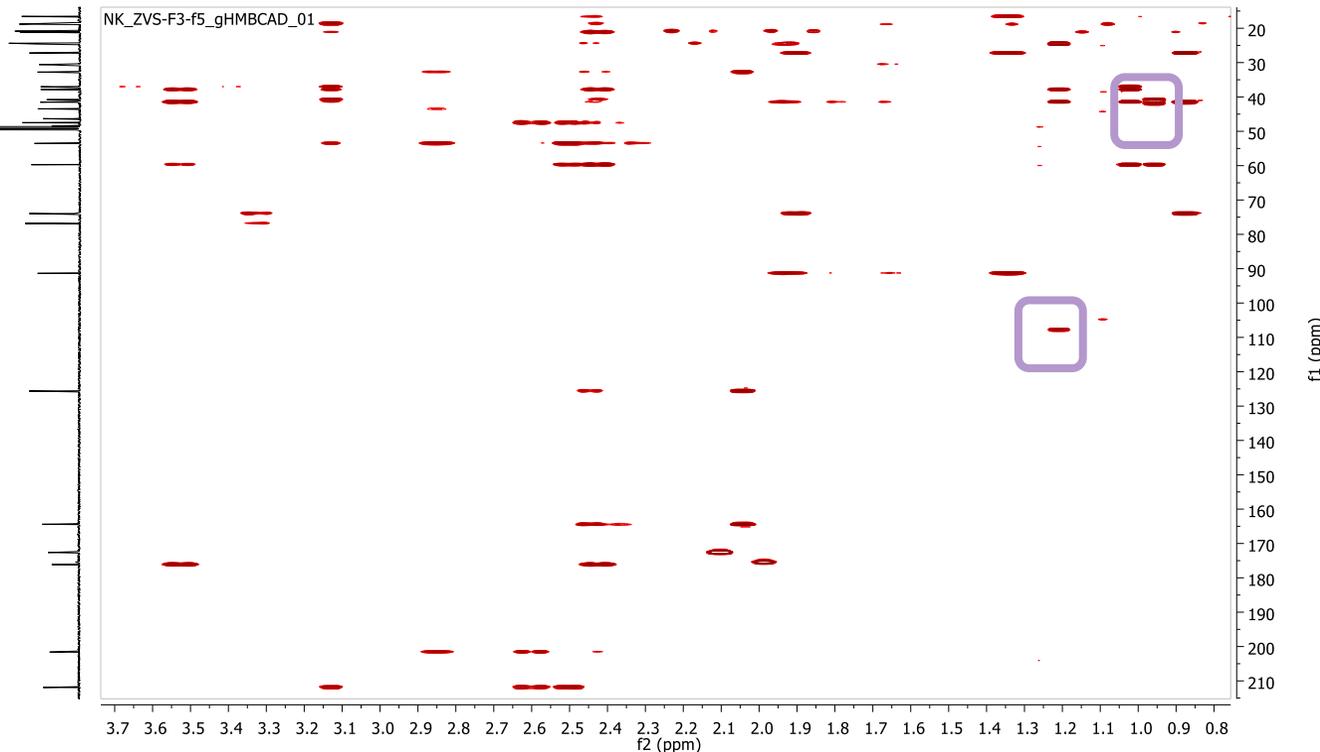
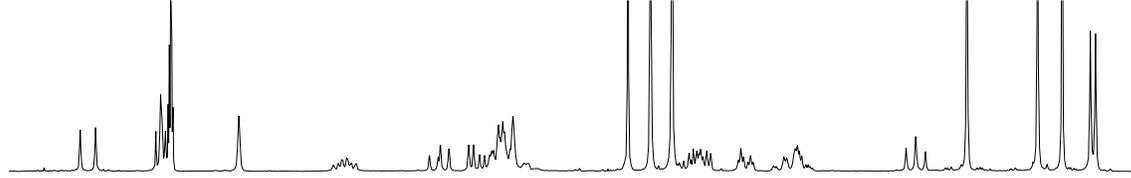
*Zoanthus* sp.

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.





# Zoanthus sp1.

A norzoanthamine with a O-acetyl at C-3

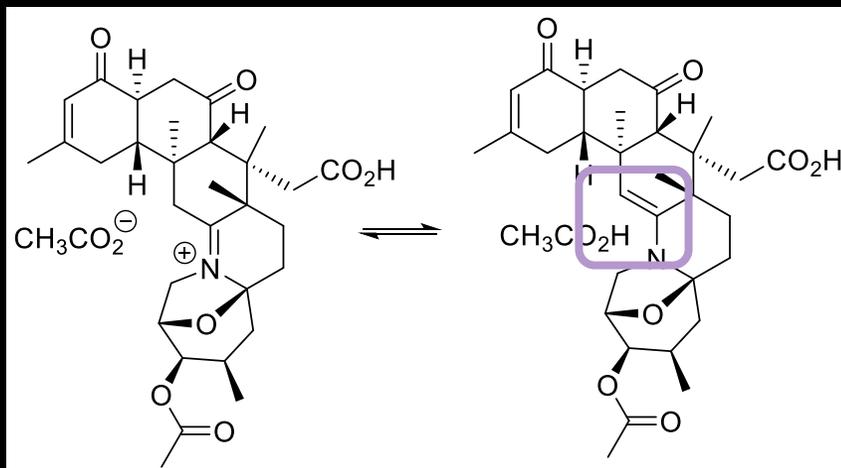
Some signals missing in HMBC and <sup>13</sup>C!

Acido-basic equilibrium

iminium/enamine

New norzoanthamine. Others to come.

Iminium



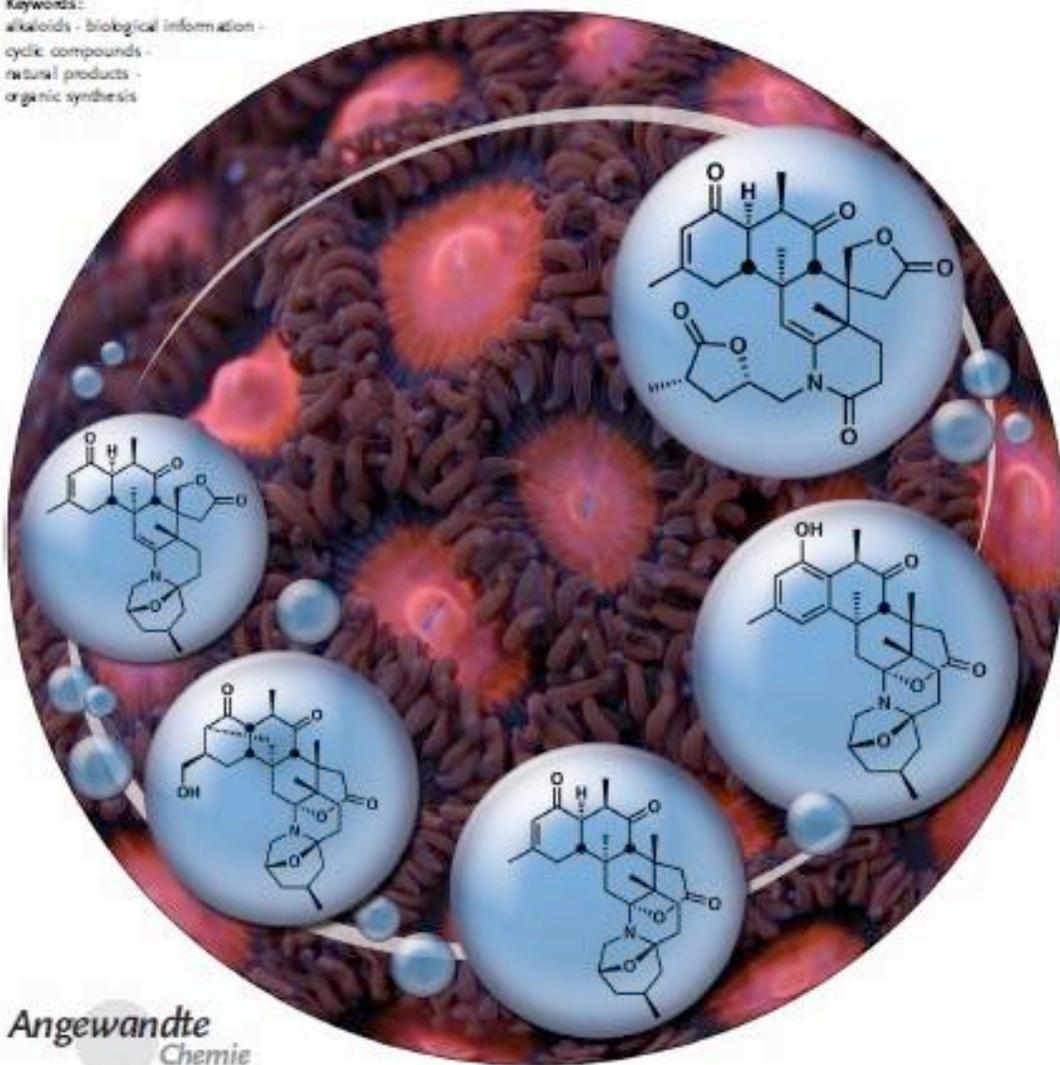
Enamine

# 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.

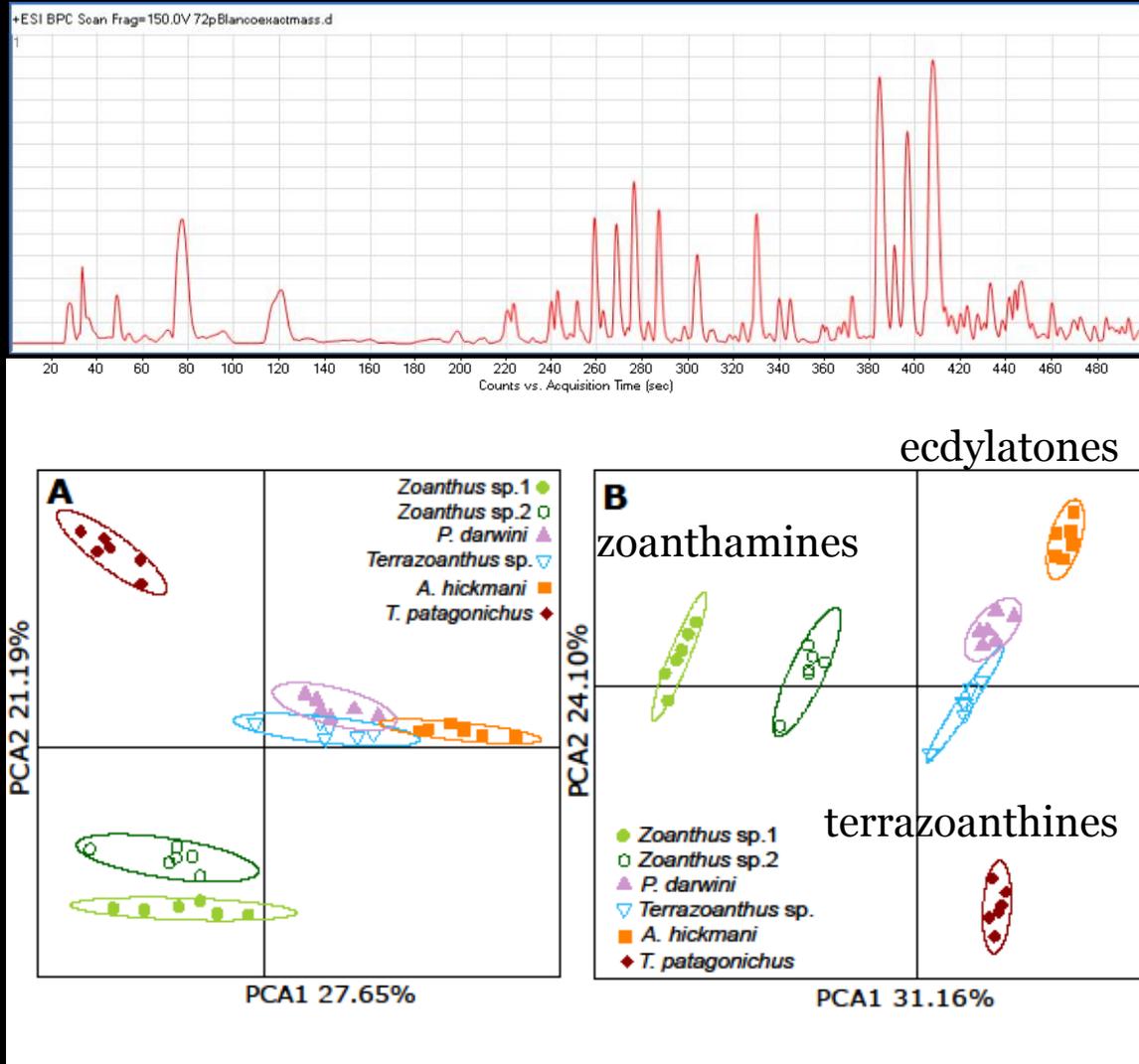
Zoanthamines are promising candidates for marine biodiscovery pipeline.

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 > 10<sup>6</sup>)

# Phylometabolomic analysis

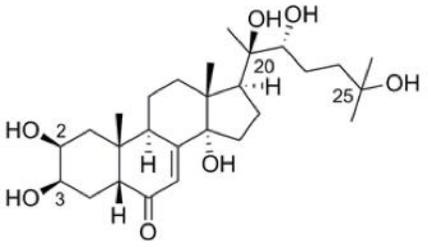
Why appropriate for Zoantharia?

Checklist:

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

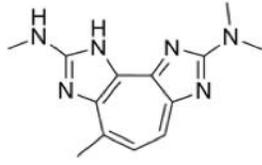
If not the environment can drive the metabolomics profiles.

Karla Jaramillo and Miriam Reverter



Ecdysteroids

20-Hydroxyecdysone M481



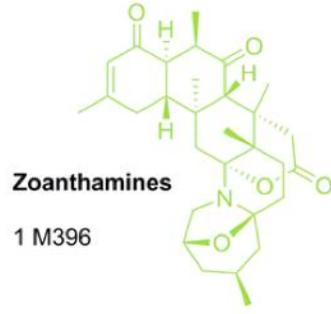
Zoanthoxanthins

Paragraine M257



Terrazoanthines

A M407



Zoanthamines

1 M396

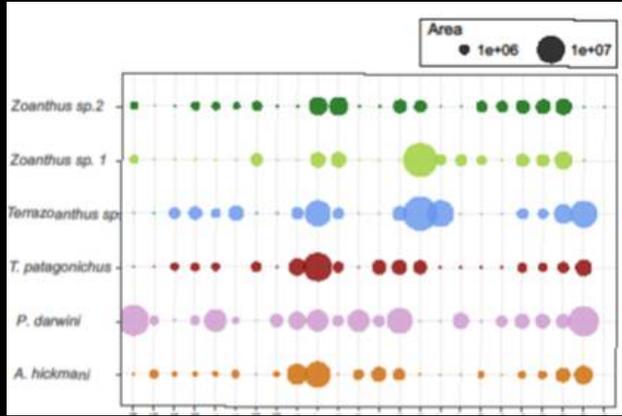
# Phylometabolomic analysis

Does not fit perfectly with the phylogenetic tree. Hypotheses.

However:

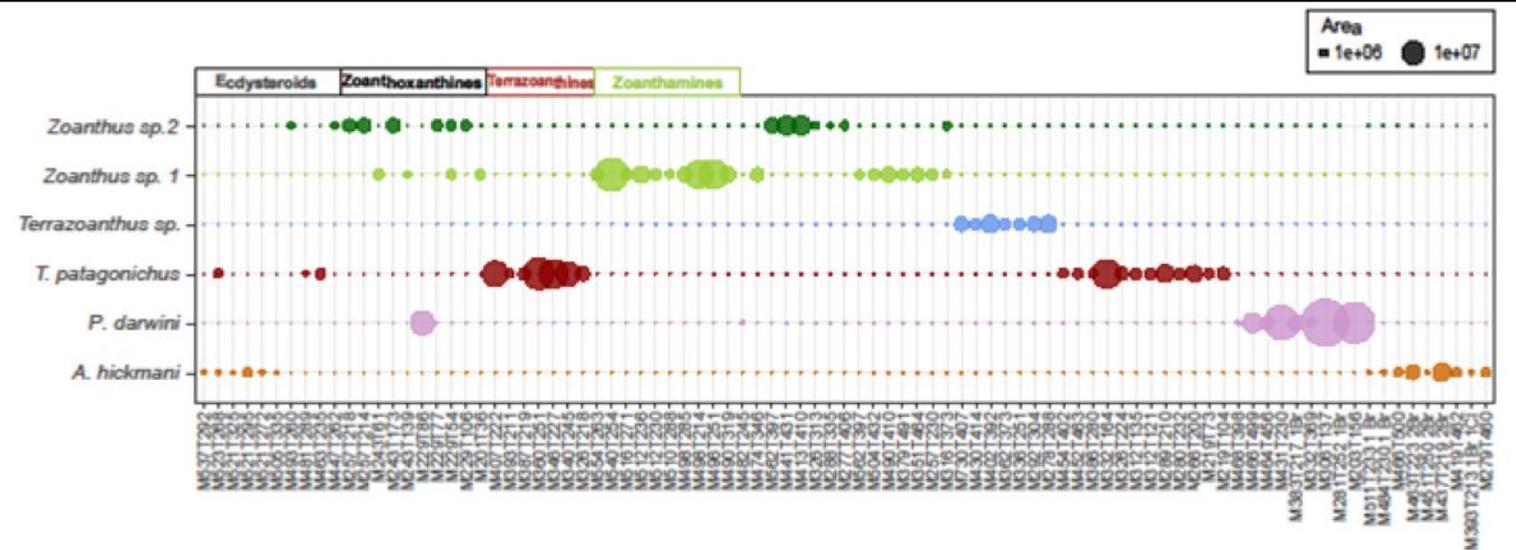
- No overlap between different species replicates
- *Zoanthus* are grouped mainly due to minor compounds

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

