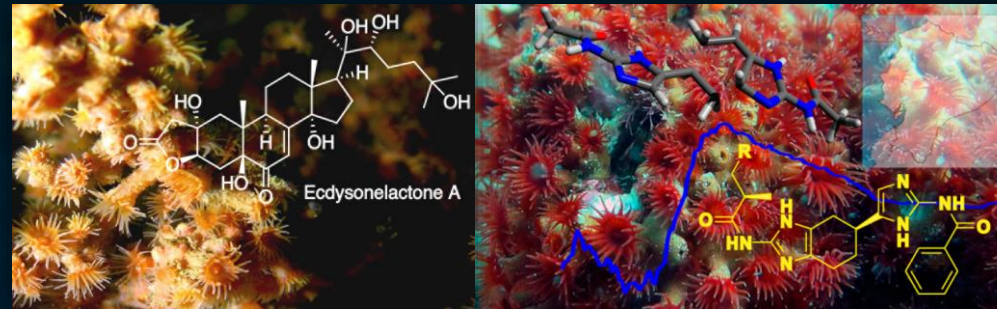


Specialized metabolites as biomarkers in Zoantharian taxonomy, a case study in the Tropical Eastern Pacific.



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Zoology, School of Natural Sciences and Ryan Institute

Zoantharians and their taxonomy

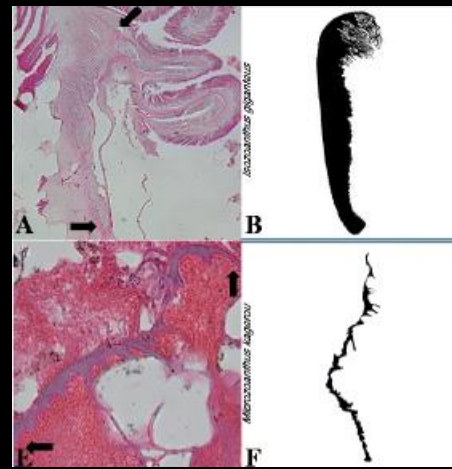
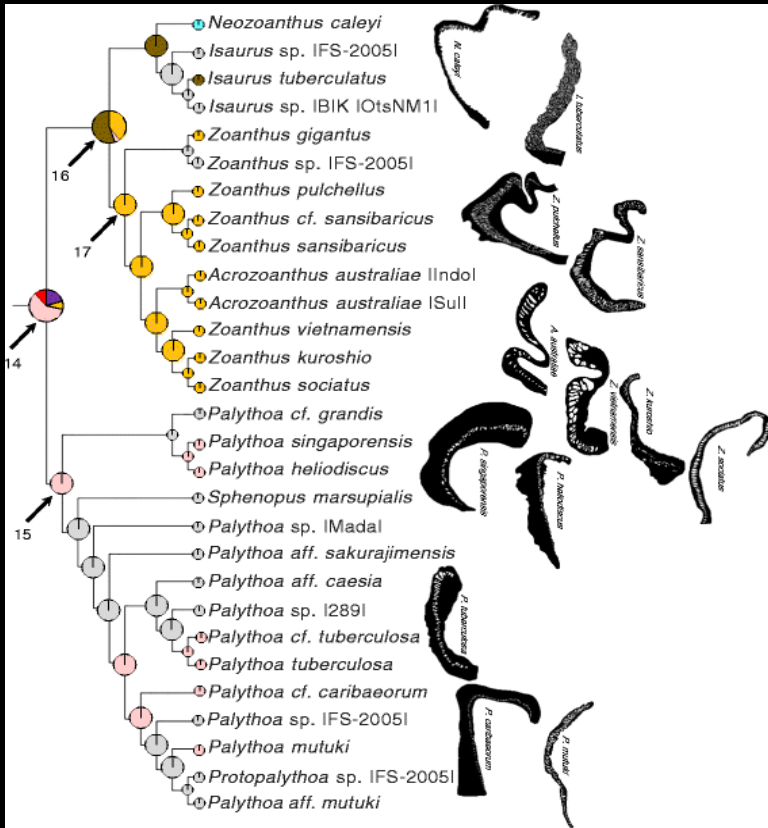
➤ The taxonomy of zoantharians is highly challenging due to a lack of clear morphological characters.

(Sinniger et al.2005)

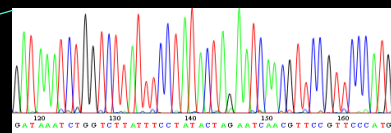
➤ Exploration of the microanatomy such as the sphincter muscle showed promising results. (Swain T.D. et al.2015)

➤ Molecular techniques have helped to clarify high level-taxonomic relationships. (Sinniger et al. 2010, Sinniger et al.2013)

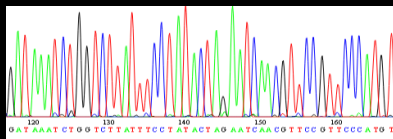
Many issues remain at species level.



Swain T.D. et al.2015

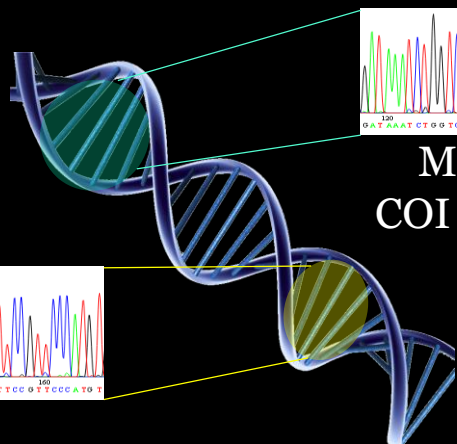


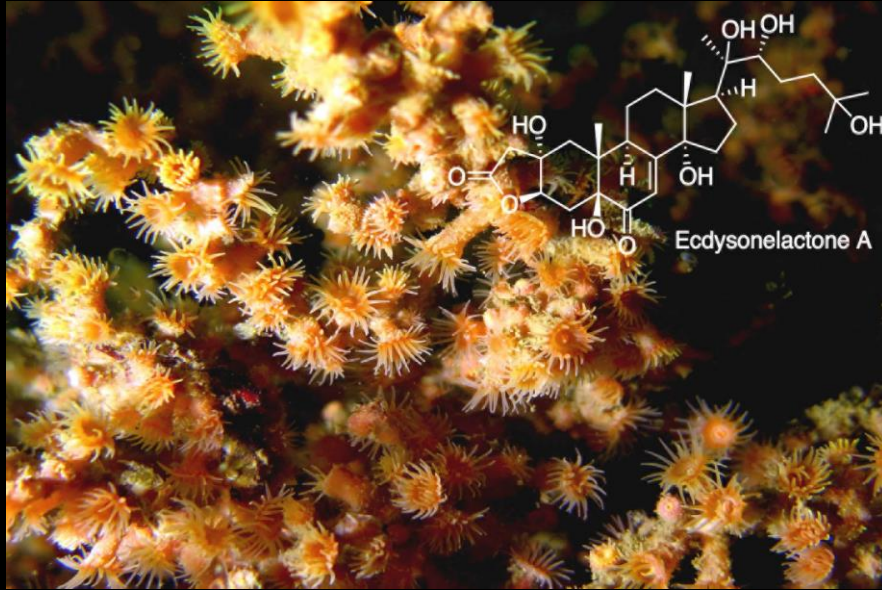
Mitochondrial COI and rDNA 16S



Nuclear ITS and rDNA 18S

Common molecular markers used





See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/271910253>

Metabolomic profiling reveals deep chemical divergence between two morphotypes of the zoanthid *Parazoanthus axinellae*

ARTICLE in SCIENTIFIC REPORTS · FEBRUARY 2015

Impact Factor: 5.58 · DOI: 10.1038/srep06262



Metabolomics
DOI 10.1007/s11306-010-0239-2

ORIGINAL ARTICLE

Metabolic fingerprinting as an indicator of biodiversity: towards understanding inter-specific relationships among Homoscleromorpha sponges

Julijana Ivanišević · Olivier P. Thomas · Christophe Lejeune · Pierre Chevaldonné · Thierry Pérez

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Abstract Sponges are an important source of secondary metabolites showing a great diversity of structures and biological activities. Secondary metabolites can display specificity on different taxonomic levels, from species to phylum, which can make them good taxonomic biomarkers. However, the knowledge available on the metabolome of non-model organisms is often poor. In this study, we demonstrate that sponge chemical diversity may be useful for fundamental issues in systematics or evolutionary biology, by using metabolic fingerprints as indicators of metabolomic

inferred from the alignment of their metabolic fingerprints. The resulting classification is congruent with phylogenetic trees obtained for a DNA marker (mitochondrial COI) and demonstrates the existence of two distinct groups within Homoscleromorpha. Metabolic fingerprinting proves a useful complementary tool in sponge systematics. Our case study calls for a revision of Homoscleromorpha with further phylogenetic studies and identification of additional chemical synapomorphic characters.

Specialized Metabolites as a complementary tool for systematics

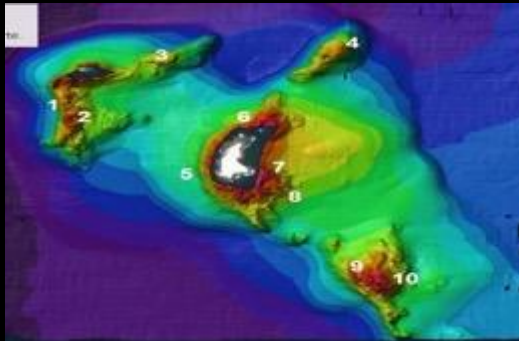
- Specialized metabolites showed promise to distinguish closely related marine invertebrates like sponges or morphotypes for zoantharians.
- Metabolomic approaches were recently applied as a useful complementary tool in integrative taxonomy of some marine invertebrates. (Ivanišević, J. et al. 2011, Cachet et al. 2015)

Metabolites from Zoantharians were largely overlooked despite the production of important families of compounds.

Main objectives

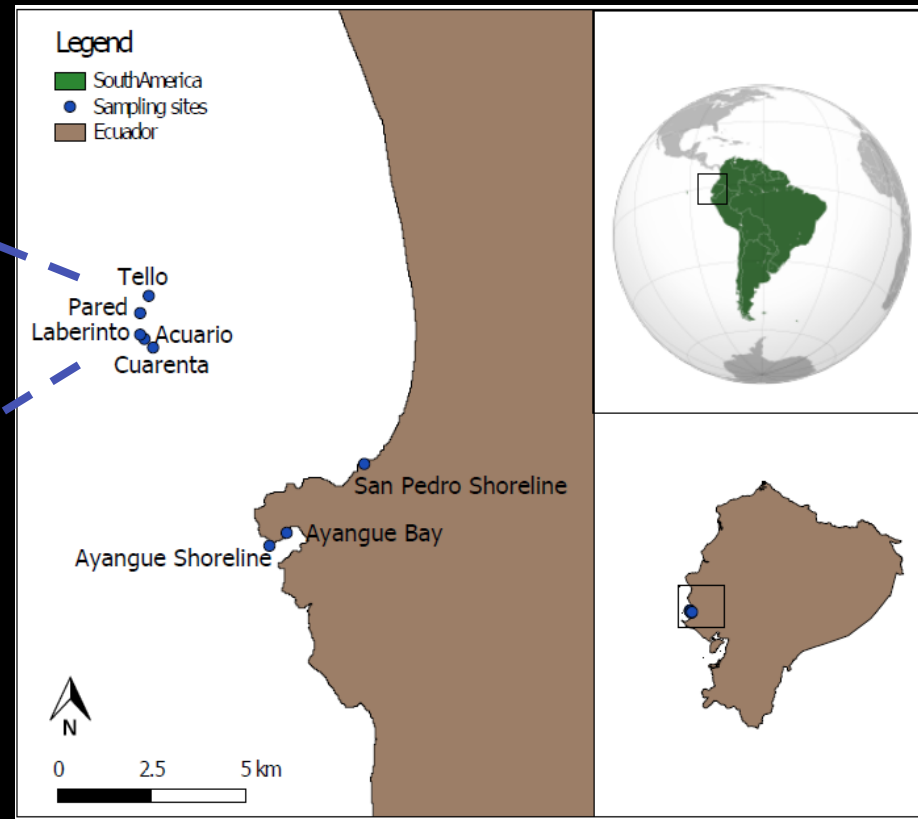
- Use a combined morphological and molecular approach to describe and classify zoantharian species of El Pelado Marine Protected Area
- Assess the potential of metabolomics to help in the identification of zoantharian species.

Study area



Map of El Pelado Marine Protected Area with ten sampling locations zoantharians

Marine area : 13004,75 ha
Land area: 96,6 ha
TOTAL area: 13101,35 ha



Map of South America and Ecuador highlighting Ecuadorian coast

METHODS

Morphology

Sampling

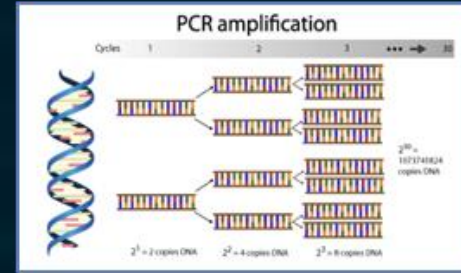


Fixation in 95% EtOH

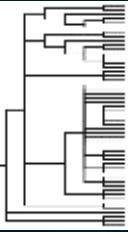
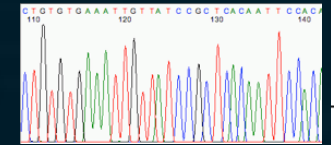
Molecular biology



DNA Extraction of small subsamples of fixed tissues



Amplification/sequencing of mitochondrial 16S, COI and nuclear 18S, ITS



Sequences and Phylogenetic analyses (BioEdit, Geneious-GTR Model, PhyML software's)

Metabolomics



6 replicates of each species



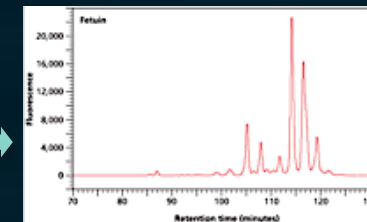
Freeze dried samples



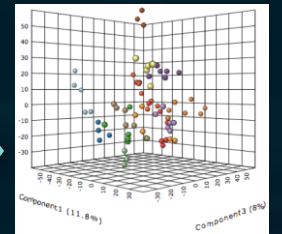
Metabolites extraction DCM/MeOH 1:1



Fractionation by C¹⁸ SPE with H₂O / MeOH/DCM



MeOH fractions were analyzed by UHPLC-Q ToF



Data analyses (msconvert, R/XMS, MetaboAnalyst softwares)

RESULTS

Morphological observations

3 Families (Hydrozoanthidae, Parazoanthidae, and Zoanthidae) and **4 Genera** (*Terrazoanthus*, *Parazoanthus*, *Antipathozoanthus*, and *Zoanthus*).

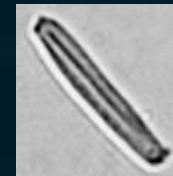
*Polyp of
Terrazoanthus patagonichus*



length and cross section of the polyp



observation of cnidocytes



Nomenclature by England, K. W., & Robson, E. A. (1991).

- a) *Antipathozoanthus hickmani* (Reimer, J. D. & Fujii. 2010)
- b) *Parazoanthus darwini* (Reimer, J. D. & Fujii. 2010)
- c) *Terrazoanthus patagonichus* (Carlgren, O. 1899)
- d) *Terrazoanthus* sp.
- e) *Zoanthus* cf. *pulchellus* (Carlgren, O. 1951)
- f) *Zoanthus* cf. *sociatus* (Ellis, J. & Solander, D. C. 1786)

a



b



c



d



e



f



RESULTS

Phylogenetic analyses

➤ The four molecular markers (mitochondrial 16S, COI and nuclear ITS-2, 18S) applied they showed a good separation at genus level.

➤ Mitochondrial markers COI and 16S confirm the presence of six zoantharian species.

Molecular markers did not allow clear distinctions between closely related species of the genera *Terrazoanthus* and *Zoanthus*.

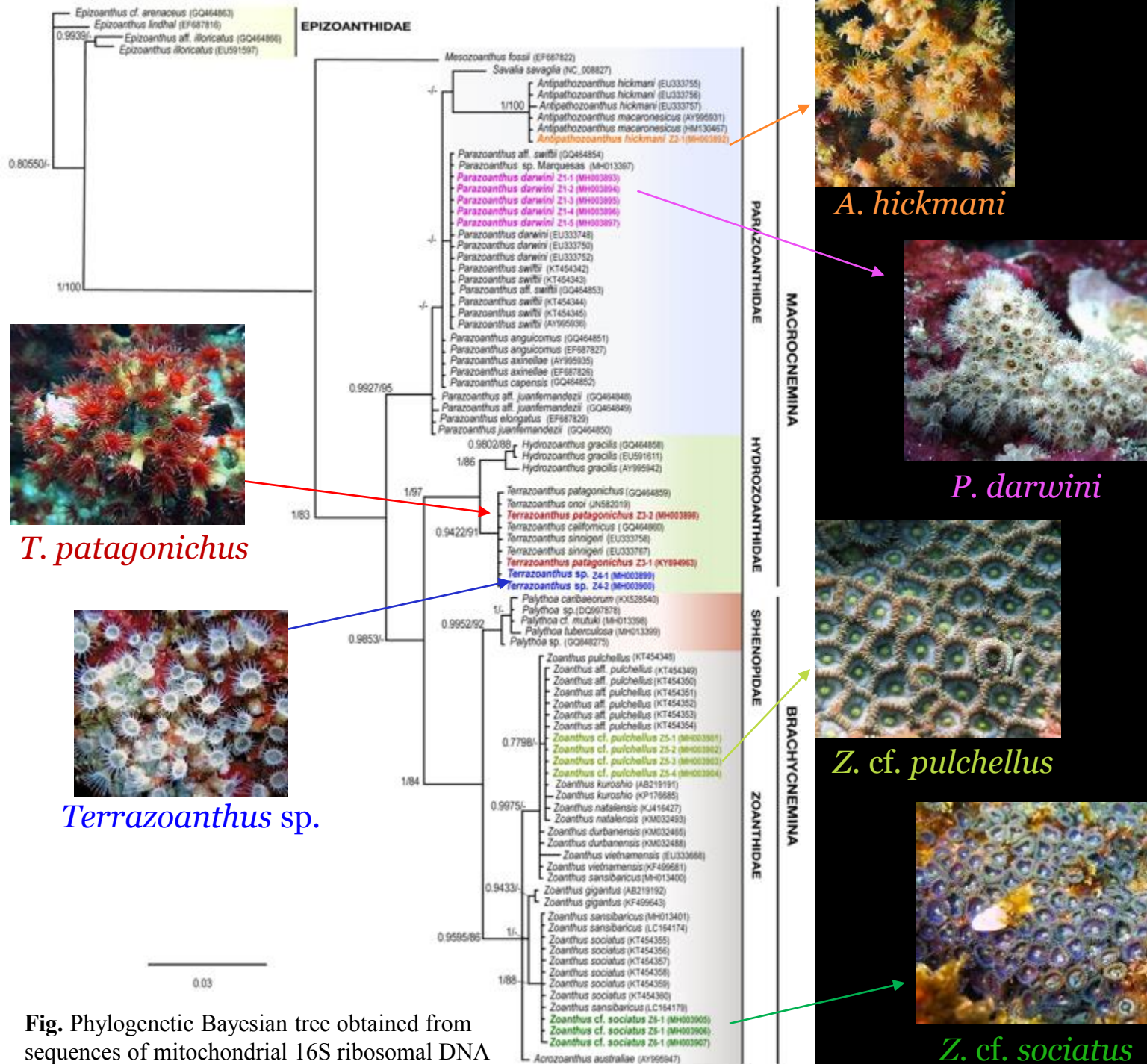
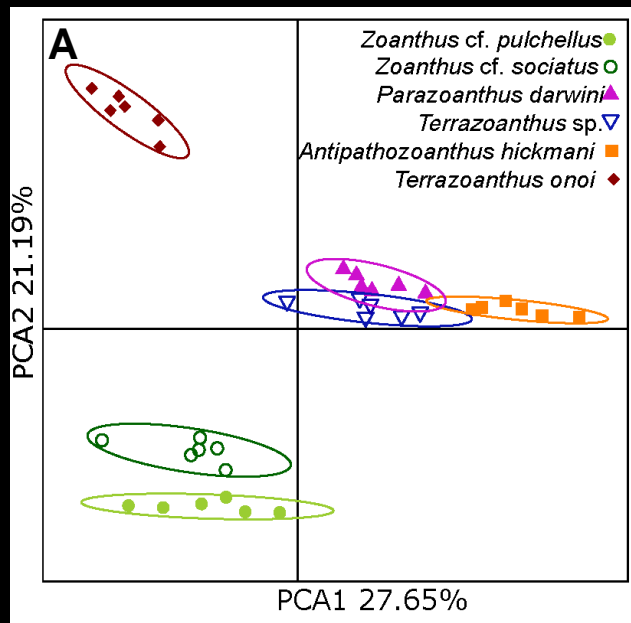
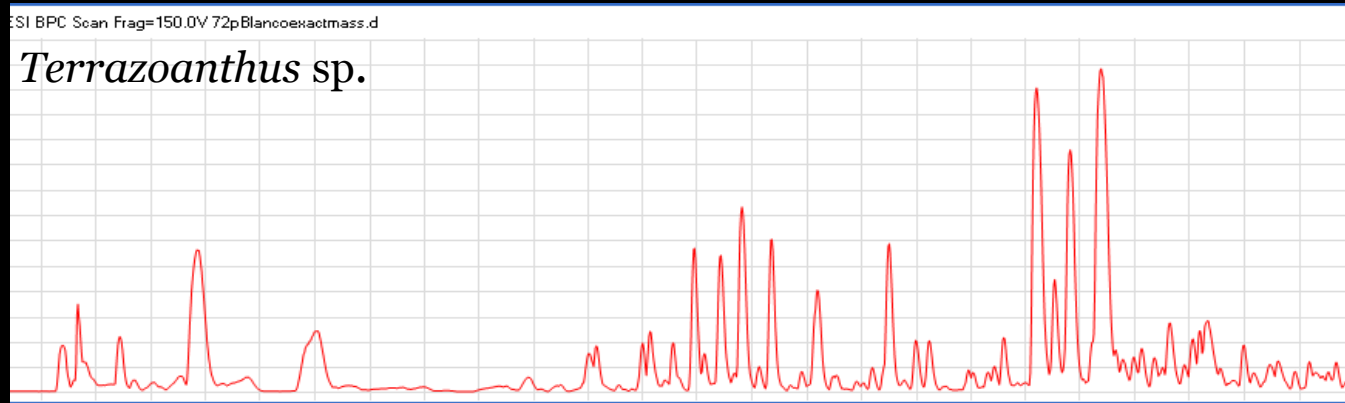


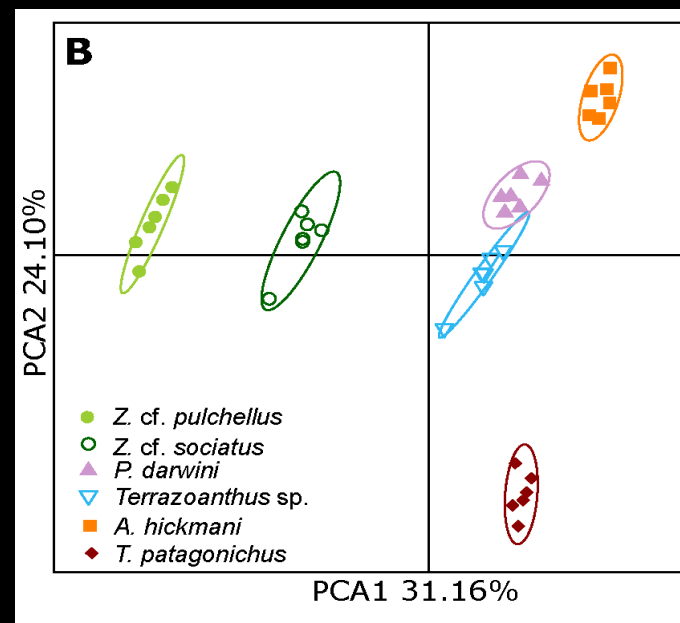
Fig. Phylogenetic Bayesian tree obtained from sequences of mitochondrial 16S ribosomal DNA

A non-targeted metabolomic approach using UHPLC-HRMS was applied to 36 zoantharian specimens, collected in different habitats and time. Injection in UHPLC-QToF (ESI+)

Untargeted Metabolomics



All metabolites



Focusing major (Area > 10⁶)

RESULTS

Phylometabolomic analysis

Why appropriate for Zoantharia?

- Ecdysteroids and zoanthoxanthins families are common to most zoantharians. (Cachet *et al.* 2015, Costa-Lotufo, L. V. *et al.* 2018)
- These major metabolites are easily ionized and then detected in mass spectrometry

Metabolomic profiles

- different for the six-studied species.
- consistent between all replicates within a species

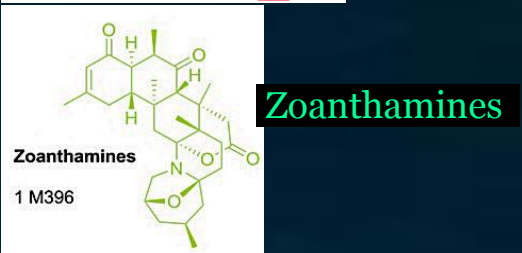
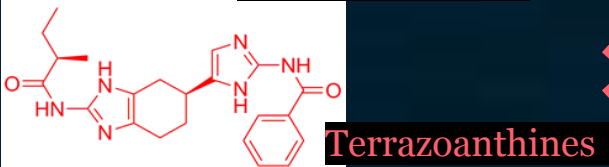
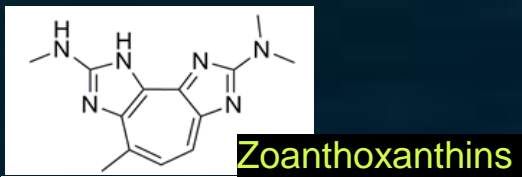
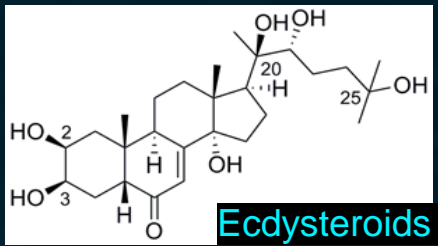
Differences between species →

Higher if the major metabolites (area $\geq 10^6$) are only considered

RESULTS

Major metabolites as biomarkers

89 major metabolites identified as markers



Brominated families

unique and unknown metabolites

	<i>P. darwini</i>	<i>A. hickmani</i>	<i>T. patagonichus</i>	<i>Terrazoanthus</i> sp	<i>Z. cf. pulchellus</i>	<i>Z. cf. sociatus</i>
Ecdysteroids	✓	✓	✓	✓	✓	✓
Zoanthoxanthins	✓ Present as minor metabolites	✓ Present as minor metabolites	✗	✓ Present as minor metabolites	✓ Present as minor metabolites	✓ Present as minor metabolites
Terrazoanthines	✗	✗	✓	✗	✗	✗
Zoanthamines	✗	✗	✗	✗	✓	✗
Brominated families	✓	✓	✗	✗	✗	✗
unique and unknown metabolites	✗	✗	✗	✓	✗	✗

TEP ecoregion Distribution and origin.



Discussion

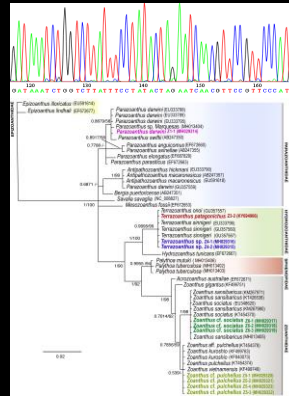
- Strong affinities between the Caribbean and the Eastern Pacific zoantharians (Reimer *et al.* 2012).
- Some Atlantic species were introduced to the Eastern Pacific through the Panama isthmus (Forsman, Z. H. *et al.* 2005; Sánchez, J. A., & Ballesteros, D. 2014; LaJeunesse, T. C. *et al.* 2016)
- Inconclusive results for *Terrazoanthus* sp.
- Systematics confirms the high level of conservatism in zoantharian DNA.

The zoantharian diversity remains to be fully assessed over most of the Eastern Pacific and especially off the Ecuadorian coast.

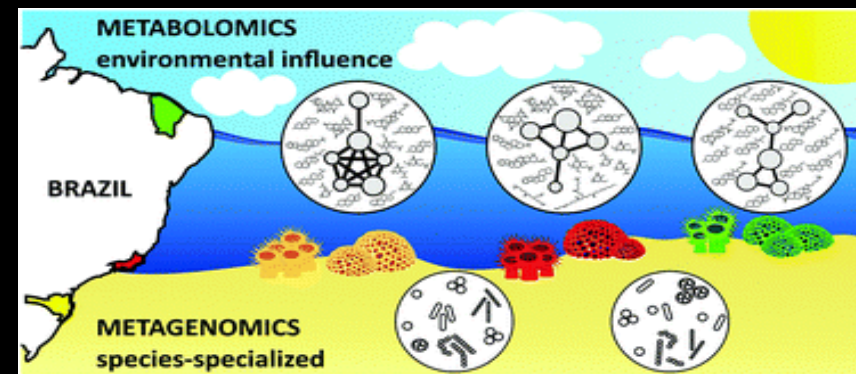
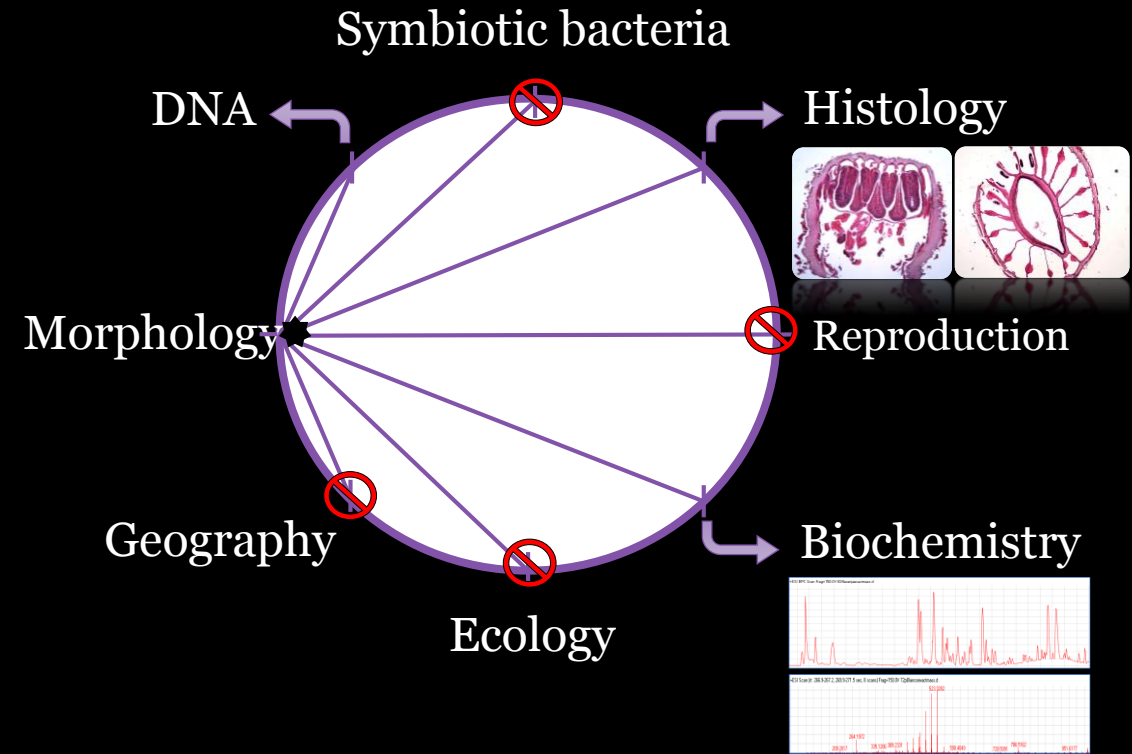
Discussion

- We recommend the inclusion of metabolomic data for future integrative taxonomy of zoantharians due to the presence of easily ionized major specialized metabolites.
- However, metabolomic analyses of two *Palythoa* species along the Brazilian coast showed high intraspecific metabolomic variability with geography (Costa-Lotufo, L. V. et al 2018).

Jaramillo et al. 2018_Scientific Reports



INTEGRATIVE TAXONOMY



Costa-Lotufo, L. V. et al 2018

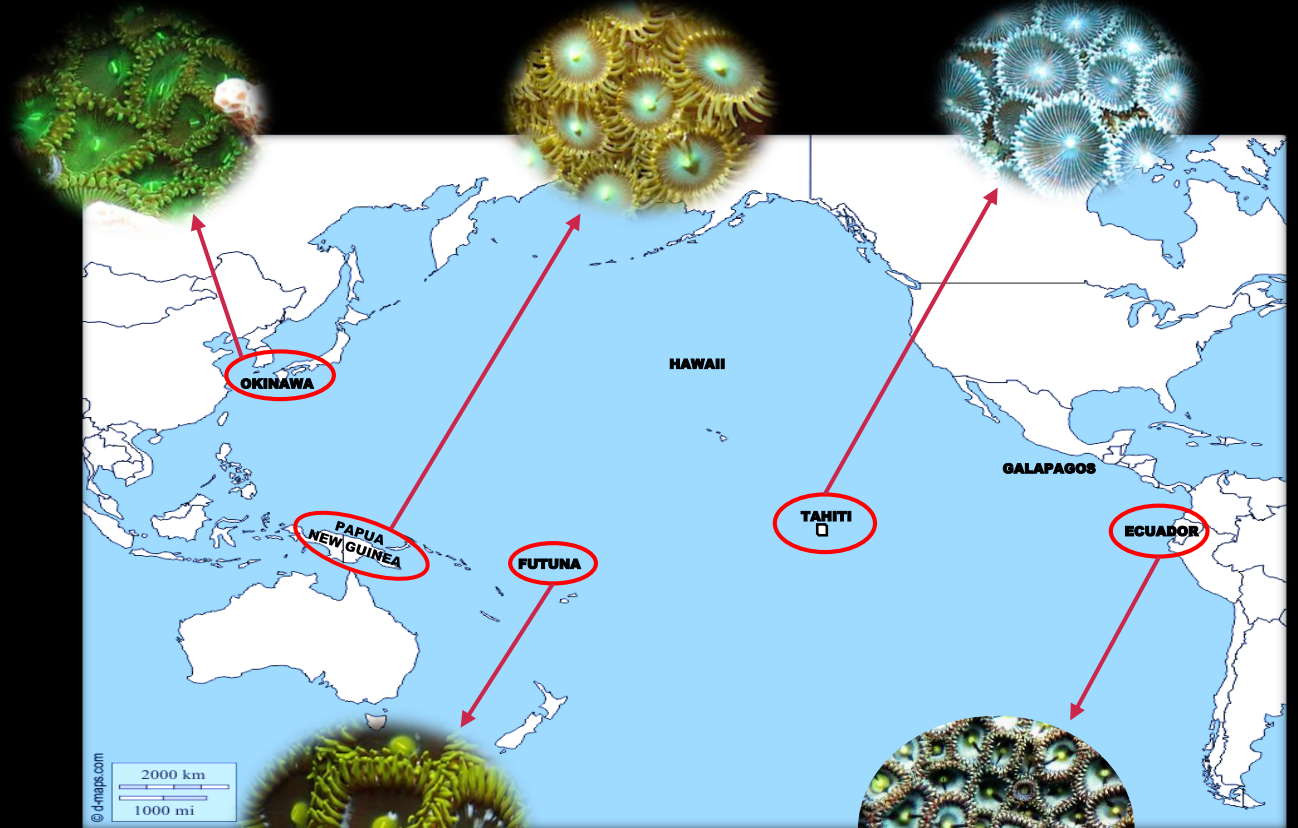
Perspectives

- Perform taxonomic comparisons between Atlantic/Pacific specimens to confirm the systematic relationships of already described species with Ecuadorian ones.
- Expand the integrative taxonomy approach to other Atlantic, Pacific and Caribbean species to investigate the classification of zoantharians at a broader scale.

OKINAWA

PAPUA NEW GUINEA

TAHITI



FUTUNA

ECUADOR

T. californicus



Carlgren O. 1951

T. sinnigeri



Reimer & Fujii. 2010

Terrazoanthus sp.



This study

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Taxonomy of
Zoantharians



Dr. M. Reverter
PostDoc
Statistics



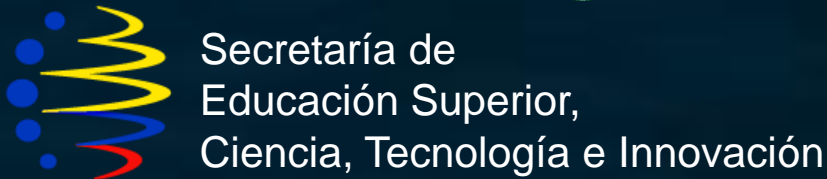
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PhD student
Chemistry



Prof. G. McCormack
Supervision



Prof. J. Rodriguez
Supervision



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