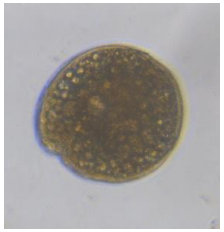
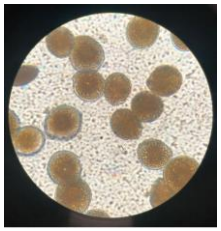
15-YEAR RETROSPECTIVE REVIEW OF CIGUATERA IN MADEIRA ISLANDS (NE ATLANTIC, PORTUGAL)



IPMA

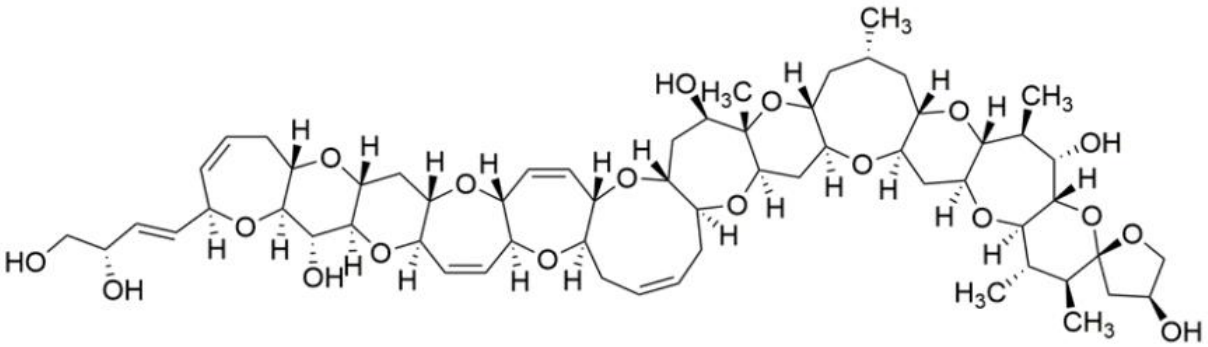
Região Autónoma da Madeira
Governo Regional

Susana Margarida Rodrigues*, Pedro Reis Costa, Catarina Churro, Lia Godinho, Lucía Solino, Bárbara Frazão, Miguel Barbosa

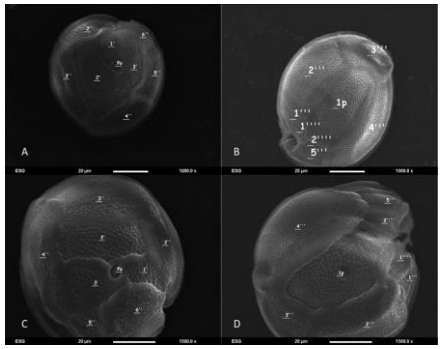
Neide Gouveia, Viriato Timóteo

* ✉ **srodrigues@ipma.pt**

Ciguatoxins - CTX



World distribution of ciguatera

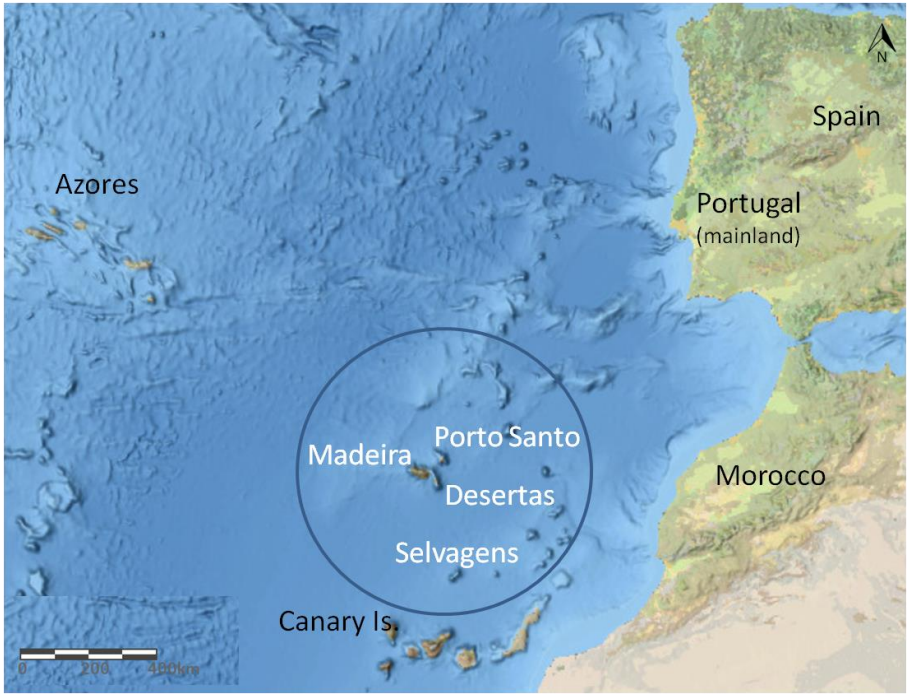


Gambierdiscus and *Fukuyoa*

Ciguatera Fish Poisoning in Portugal

First suspected case of CFP : Selvagens Islands (Madeira Archipelago), 2007

A cluster of mild neurological and gastrointestinal symptoms characteristic of CFP was first experienced among 6 vigilant keepers in charge of Madeira Natural Park at Selvagens Islands, who had consumed fish caught locally, starting in mid 2007 and lasting until mid 2008.



Amberjack (*Seriola* sp)
Parrotfish (*Sparisoma cretense*)
Blacktail comber (*Serranus atricauda*)
Barred hogfish (*Bodianus scrofa*)
Grey triggerfish (*Balistes capriscus*)
Red porgy (*Pagrus pagrus*)

Ciguatera Fish Poisoning in Portugal

First reported outbreak of CFP : Selvagens Islands, July 2008

- Severe outbreak affected 11 crew members of a fishing boat who reported CP symptoms after consumption of 30 kg amberjack (*Seriola* spp.) caught around Selvagens Islands.

- Symptoms onset at 4 h after consumption and included:

Diarrhea, aching of muscles and joints, headaches, sensitivity in hands and feet, prostration, reversal of hot/cold temperature sensation, itchiness, numbness of the tongue and mouth, and numbness of the extremities.

Anal. Chem. 2010, *82*, 6032–6039

First Toxin Profile of Ciguateric Fish in Madeira Arquipelago (Europe)

Paz Otero,† Sheila Pérez,† Amparo Alfonso,† Carmen Vale,† Paula Rodríguez,† Neide N. Gouveia,‡ Nuno Gouveia,‡ João Delgado,‡ Paulo Vale,§ Masahiro Hirama,|| Yuuki Ishihara,|| Jordi Molgó,⊥ and Luis M. Botana*,†

Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain., Direcção Regional das Pescas, Estrada da Pontinha, 9000-017 Funchal, Portugal, Instituto Nacional dos Recursos Biológicos, IPIMAR (INRB-IPIMAR), Av. Brasília, s/n, 1449-006, Lisboa, Portugal, Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan., CNRS, Institut de Neurobiologie Alfred Fessard, FRC2118, Laboratoire de Neurobiologie Cellulaire et Moléculaire, FRE3295, 1 Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

Ciguatera Fish Poisoning

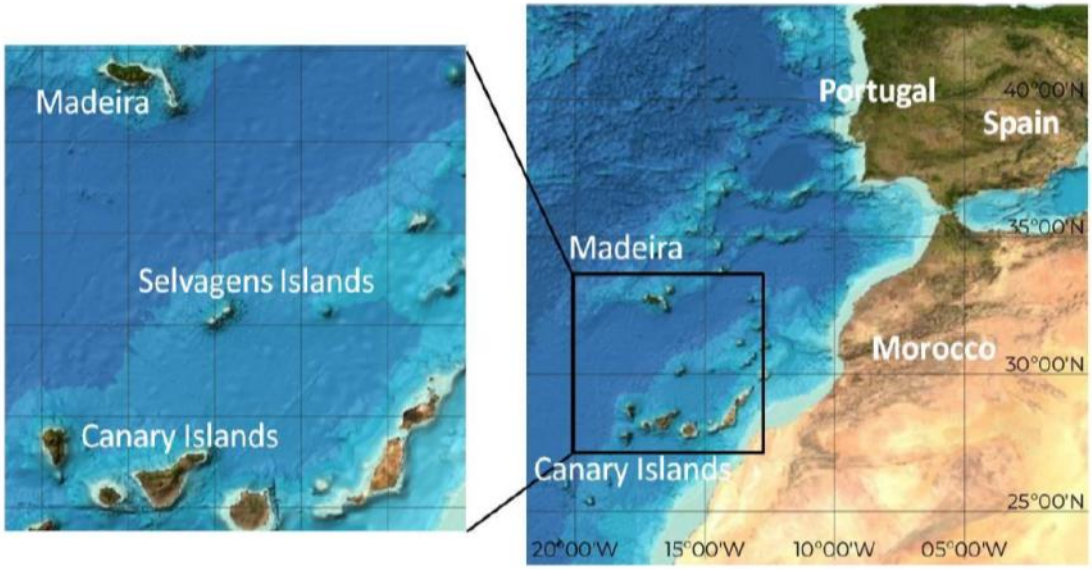
- The symptoms reported by the crew members matched with symptoms previously reported by nature wardens of the natural park of the Selvagens Islands.

- The duration of the neurological symptoms lasted between 0.5-1.5 months.

- Diagnosis is mostly done by the symptoms of the patients: Cold Allodynia

- Also in 2008, 20–30 people reported CFP symptoms after consuming amberjack purchased in the markets of the Canary Islands but caught close to Selvagens Islands.

Selvagens Islands - Ciguatera hotspot



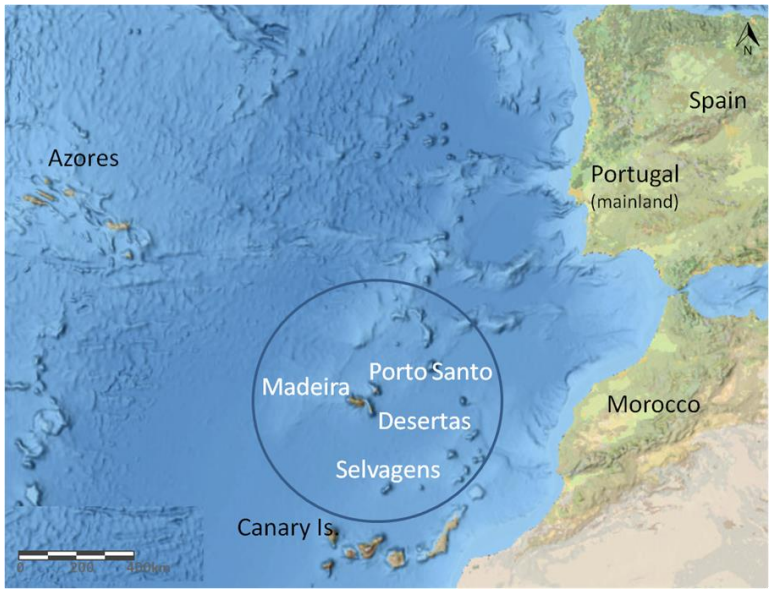
Total number of CFP affected individuals: **49**

This number may be regarded as a low value suggesting CFP is not a common intoxication, but ciguatera is not a mandatory notifiable disease in Madeira which certainly leads to several underreported cases.

Ciguatera - Preventive Measures

To minimize the risk of ciguatera, after 2008 Madeira authorities:

- interdicted fisheries in Selvagens Islands
- banned the capture of amberjacks and groupers weighing more than 10 kg in Madeira archipelago.



Ciguatera Fish Poisoning

- **Which are the ciguatera causing dinoflagellates from the Madeira & Selvagens Islands?**

- **Which are the ciguatoxins bearing fish species in Madeira & Selvagens Islands?**

- **What are the ciguatoxins occurring in the Portuguese waters?**

- **Which strategy should be used to minimize the risk of ciguatera poisoning?**

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CIGUA

efsa European Food Safety Authority
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EuroCigua

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EuroCigua II

efsa
European Food Safety Authority

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EuroCigua

Objective (s): EVALUATION OF CTXs IN SEAFOOD AND THE ENVIRONMENT for the RISK ASSESSMENT OF CIGUATERA FISH POISONING (CFP), with the consequent OBTENTION OF REFERENCE MATERIAL

Our Tasks:

- 1 - Sampling seawater for *Gambierdiscus* identification and isolation
- 2 - Sampling fish for toxicity and toxins determination

- Sampling seawater in Madeira 2016
- Sampling seawater in Madeira and Selvagens 2017
- Opportunistic fish sampling Madeira and Selvagens 2017
- Fish and Seawater sampling in Selvagens in September 2018 (scientific cruise)
- Seawater Sampling in Madeira 2019.

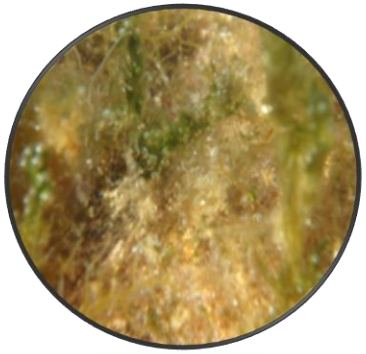
Sampling *Gambierdiscus*

Plankton net

Artificial substrate

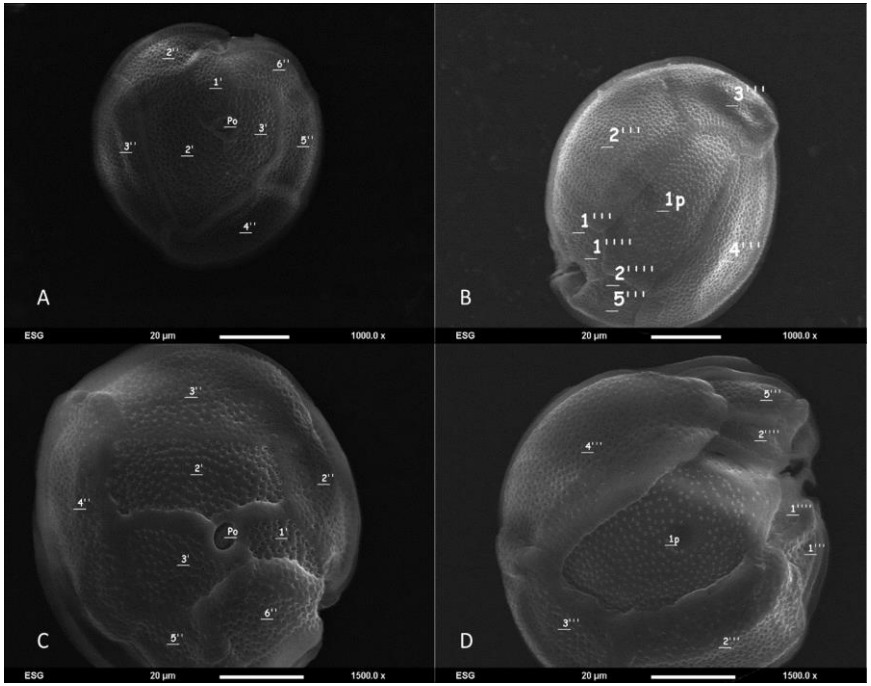
Attached macroalgae

Suspended macroalgae



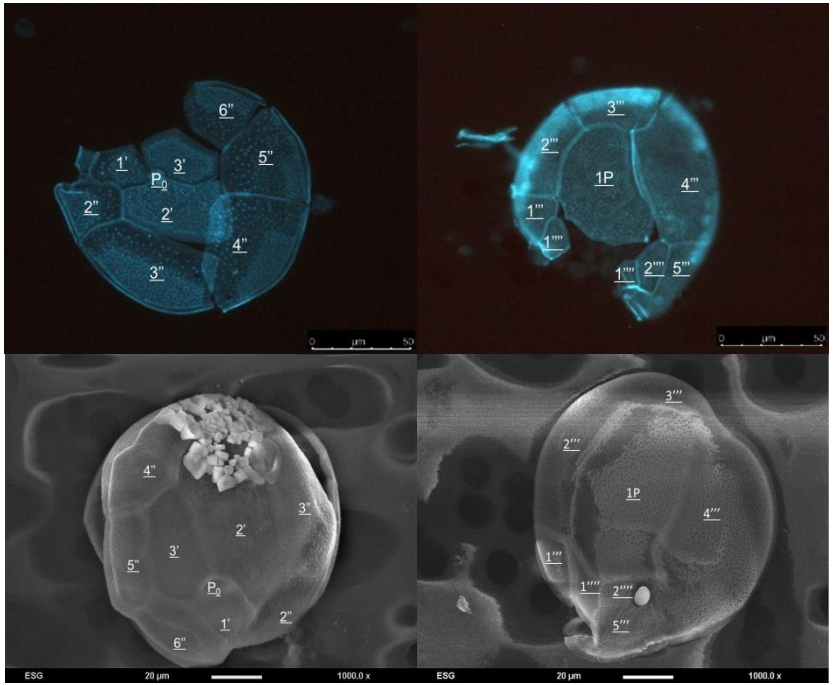
Planktonic samples

Morphological identification of *Gambierdiscus* species from the Selvagens Islands & Madeira



Gambierdiscus autrales

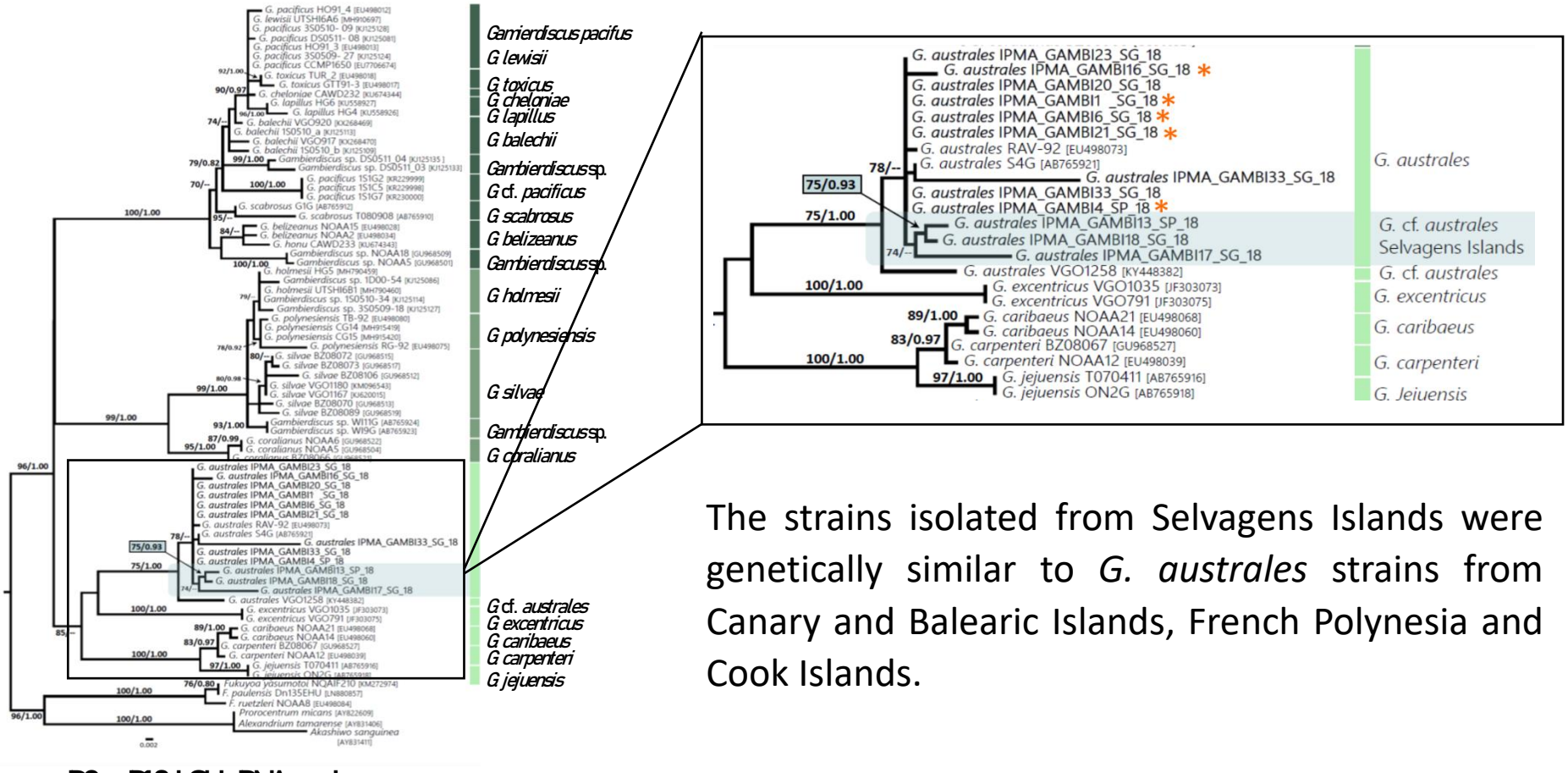
Selvagens Islands



Gambierdiscus excentricus

Madeira Islands

Molecular and phylogenetic analysis of *Gambierdiscus* from the Selvagens Islands



D8 – D10 LSU rDNA region

The strains isolated from Selvagens Islands were genetically similar to *G. australes* strains from Canary and Balearic Islands, French Polynesia and Cook Islands.

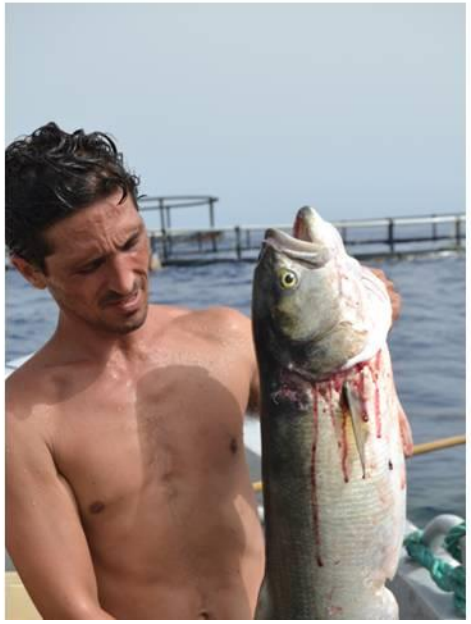
Citotoxicity of *Gambierdiscus australes* from Selvagens Islands

Strain	Species	Island	fg P-CTX1B eq. cell $^{-1}$
IPMA_GAMBI1_SP_18	*G. australes*	Selvagem Pequena	83
IPMA_GAMBI6_SP_18	*G. australes*	Selvagem Pequena	9,45
IPMA_GAMBI21_SG_18	*G. australes*	Selvagem Grande	9,21
IPMA_GAMBI4_SG_18	*G. australes*	Selvagem Grande	2,46

IRTA Institut de Recerca i Tecnologia Agroalimentàries

Jorge Diogene's Team

Sampling CTX bearing fish

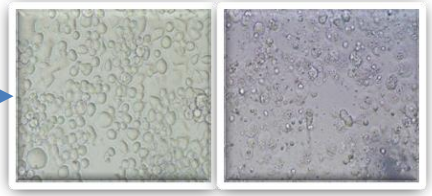


LC-MSMS

(Lack of)Toxin Standards

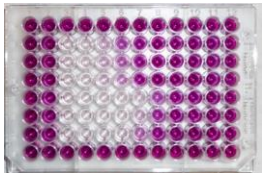
Toxicity evaluation with a Cell-Based Assay

Neuro2a cells



No toxicity Toxicity

MTT viability assay



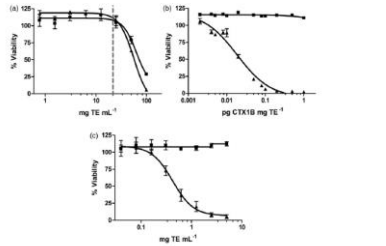


Figure 2. Dose-response curves of neuroblastoma (neuro-2a) cells exposed for 24h to a non-toxic fish sample (*S. faxiata*, sample 4) (a), non-toxic fish sample (*S. faxiata*, sample 4) spiked with CTX1B (b) and toxic fish sample (*S. faxiata*, sample 2) (c), with (▲) and without (■) O/V pretreatment. The limit of tissue equivalent (TE) exposure for matrix interferences is represented by a dotted vertical line.

Citotoxicity of fish from the Selvagens Islands

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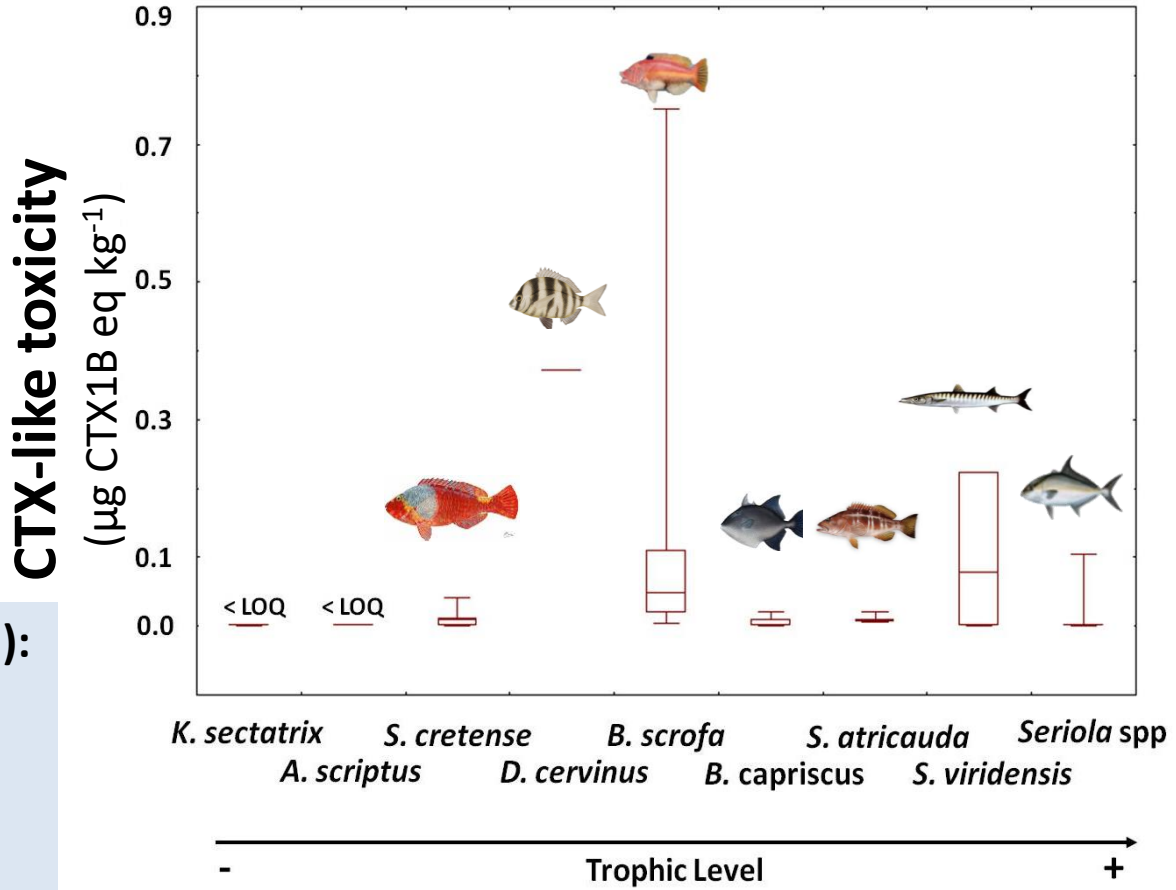
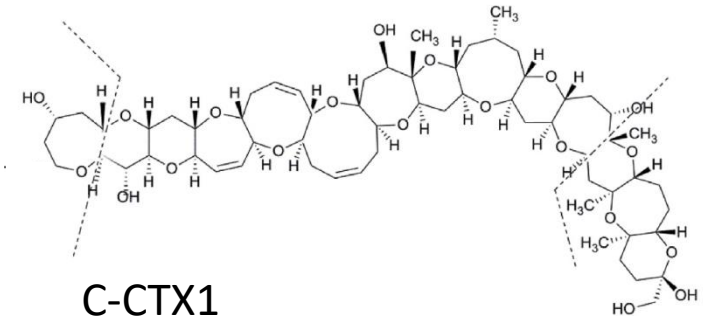
Sampling Campaigns (Eurocigua I):

- Fish sampling in Selvagens in September 2018
- 56 fish samples from different trophic levels
- 12 species within different trophic levels



Costa et al, 2021. *Toxins* 13(8):580

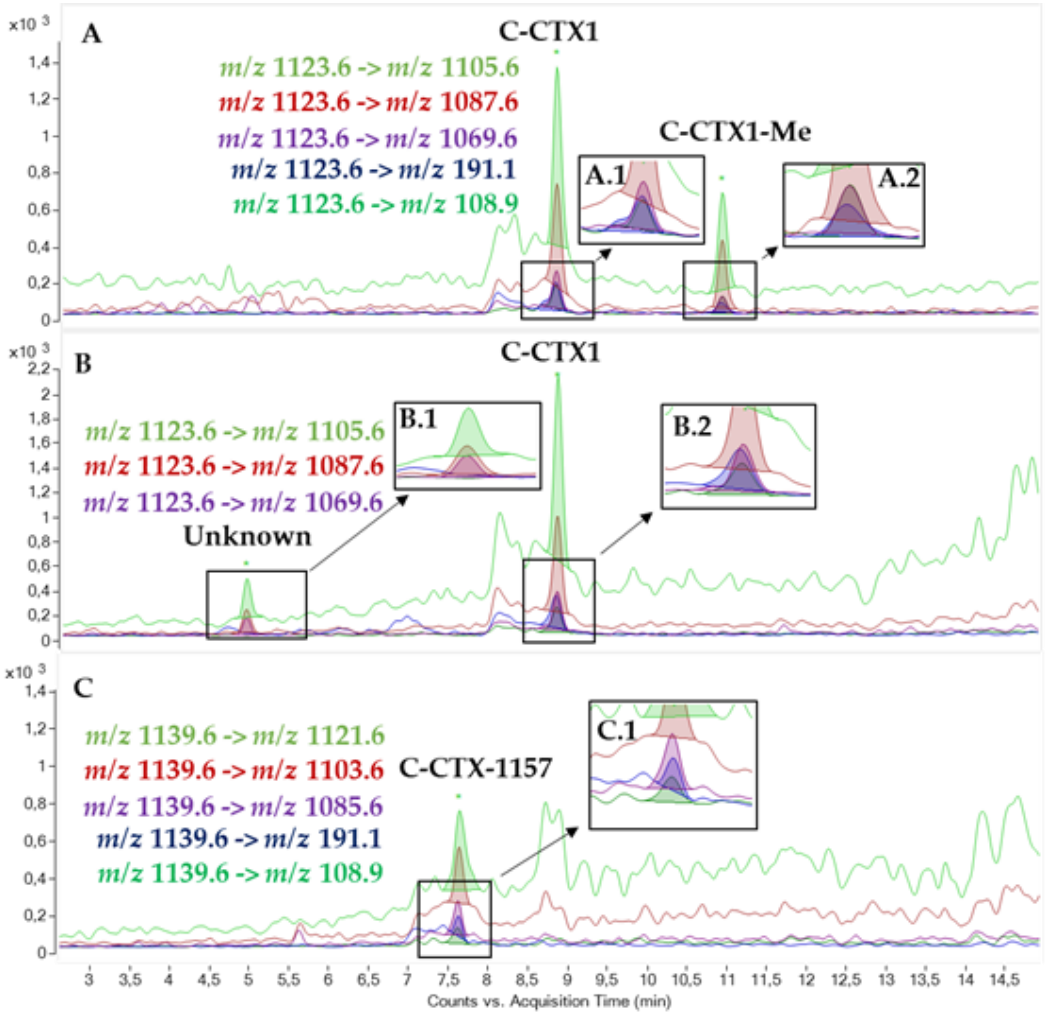
LC-MSMS toxin profile of fish from the Selvagens Islands



C-CTX1

Universidade Vigo

Ana Gago's Team



Eurocigua Outputs

toxins

MDPI

Article

New Insights into the Occurrence and Toxin Profile of Ciguatoxins in Selvagens Islands (Madeira, Portugal)

Pedro Reis Costa [1], Pablo Estevez [2], David Castro [2], Lucía Soliño [1], Neide Gouveia [3], Carolina Santos [4], Susana Margarida Rodrigues [1], José Manuel Leao [2] and Ana Gago-Martínez [2,*]

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4 Instituto das Florestas e Conservação da

toxins

MDPI

Article

An Update on Ciguatoxins and CTX-like Toxicity in Fish from Different Trophic Levels of the Selvagens Islands (NE Atlantic, Madeira, Portugal)

Pedro Reis Costa [1,2,*], Pablo Estévez [3], Lucía Soliño [1,2], David Castro [3], Susana Margarida Rodrigues [1], Viriato Timoteo [4], José Manuel Leao-Martins [3], Carolina S and Ana Gago-Martínez [3,*]

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EUROPEAN JOURNAL OF PHYCOLOGY, 2022
https://doi.org/10.1080/09670262.2022.2086710

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Distribution, identification and cytotoxicity of *Gambierdiscus* (Dinophyceae) in the Atlantic Selvagens Islands (Madeira, Portugal): a ciguatera gateway to Europe

Lia Godinho[a], Lucía Soliño[a,b], Catarina Churro[a,c], Viriato Timoteo[d], Carolina Santos[e], Neide Gouveia[d], Jorge Diogène[f] and Pedro Reis Costa [a,b]

[a]IPMA – Portuguese Institute of the Sea and Atmosphere, Rua Alfredo Magalhães Ramalho, 6, 1495-165, Lisbon, Portugal; [b]CCMAR – Centre of Marine Sciences, University of Algarve, Campus of Gambelas, 8005-139, Faro, Portugal; [c]Blue Biotechnology and Ecotoxicology (BBE), CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450–208 Porto, Portugal; [d]Regional Fisheries Management – Madeira Government, DSI-DRP, Estrada da Pontinha, 9004-562, Funchal, Madeira, Portugal; [e]Instituto das Florestas e Conservação da Natureza, IP-RAM, Secretaria Regional do Ambiente, e Recursos Naturais e Alterações Climáticas, Regional Government of Madeira, Rua João de Deus, no. 12 E/F, R/C-C | 9050-027 Funchal, Madeira, Portugal; [f]IRTA – Institute of Agrifood Research and Technology, Carretera Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Spain

ABSTRACT

The emerging threat of ciguatera poisoning (CP) in Europe has been associated with fish captured in the Canary Islands (Spain) and Selvagens Islands (Portugal). The first are heavily populated islands where numerous scientific studies have been carried out. Conversely, the Selvagens Islands are a nature reserve with low human pressure that have been rarely surveyed in terms of the marine benthic microalgae, including the epiphytic ciguatera-causing dinoflagellate species. To investigate the harmful microalgal diversity of the Selvagens Islands, a scientific cruise to these remote islands took place in September, 2018. The *Gambierdiscus* species composition and distribution, and the associated epiphytic dinoflagellate community, were assessed using artificial substrate devices. *Gambierdiscus* cells were found in all samples, reaching concentrations of up to 725 cells 100 cm^{-2}. *G. australes* was the only species identified after morphological and molecular

Research - CFP

GP/EFSA/KNOW/2022/03

To contribute to the FULL CHARACTERIZATION OF THE CP RISK in Europe in IMPORTED AND INDIGENOUS FISH from EU Risk areas, as well as in the MICROALGAE RESPONSIBLE for the contamination of the fish in HOTSPOT AREAS OF MADEIRA ARCHIPELAGO selected for the study

Our Tasks:
1 - Field campaigns to collect naturally contaminated fish samples
2 - Cultivation of *Gambierdiscus* strains to obtain biomass for chemical analyses

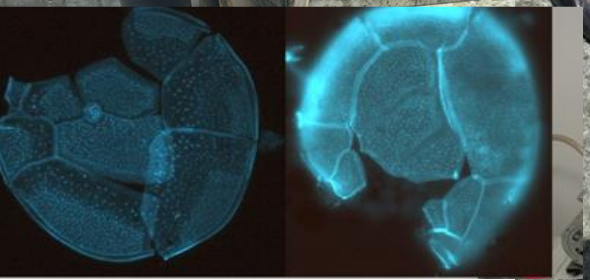
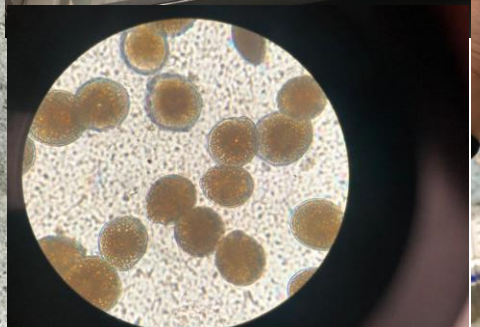
Ongoing activities :

- First sampling campaign carried out in September 2022
- Second sampling campaign carried out in July 2023
- *Gambierdiscus* strains from the Selvagens Islands and Madeira, already available at IPMA (Results of project Eurocigua I)
- Identification of *Gambierdiscus* by microscopy and molecular biology techniques
- Cultures are maintained in controlled conditions and being prepared for scale-up







900
800
700
600
500
400
300
200
100
1000 ml

HIGHLIGHTS

- Selvagens Islands are the main spot for Ciguatera in Portugal;

- High *Gambierdiscus* cell densities and fish toxicity were observed in samples from Selvagens Islands;

- *Gambierdiscus australes* was the only and single species identified in Selvagens.

- *Gambierdiscus excentricus* was the only species observed in Madeira.

- CTX-like toxicity was observed in several fish species throughout the marine food web;

- C-CTX1 appear to be the dominant compound.

PORTUGUESE TEAM

Pedro

Susana

Barbara

Catarina

Miguel

Lia

Lucía

Viriato

Neide

IRTA Institut de Recerca i Tecnologia Agroalimentàries

Jorge Diogene's Team

Universidade Vigo

Ana Gago's Team

FUNDING

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EuroCigua II

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