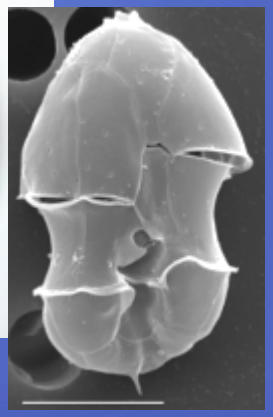
Seattle 2016

Amphidomataceae,

the dinophycean source of azaspiracid toxins:

new records, new species, new toxins



Urban Tillmann, Bernd Krock

Nicole Trefault, Haifeng Gu, Dave Clarke, Marc Gottschling, Elisabeth Nézan, David Jaen, Lourdes Fernandez, Jane Kilkoyne, Rut Akselman, Kirsty Smith, Sonia Sanchez, Valeria Guinder, Clémence Gatti, Melanie Roué, Manfred Kaufmann, Nihayet Biszel, Consuelo Carbonell-Moore

The Intergovernmental Oceanographic Commission of UNESCO *1996*

HARMFUL ALGAE NEWS

An IOC Newsletter on toxic algae and algal blooms *No. 14*

◆ WEST COAST OF IRELAND

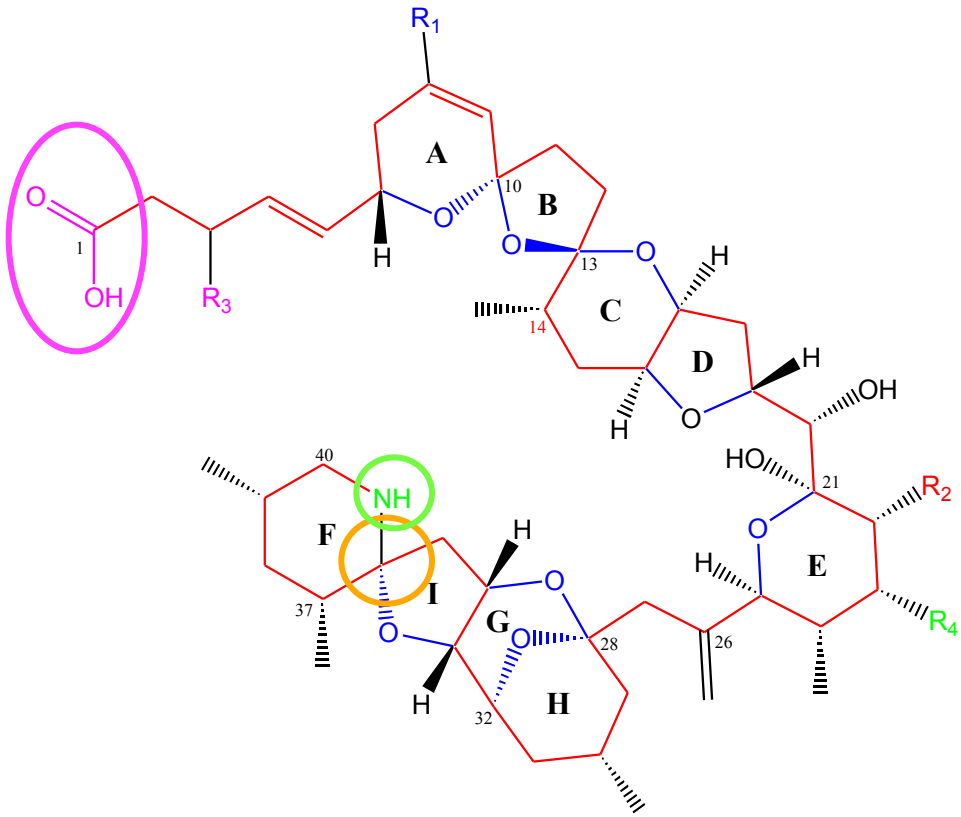
Winter toxicity of unknown aetiology in mussels

Terry McMahon and Joe Silke, Marine Institute, Fisheries Research Centre, Abbotstown, Dublin 15, Ireland.

In November 1995, a shellfish poisoning event in The Netherlands, in which at least eight people became ill after eating mussels, was traced to a consignment of mussels from a site on the west coast of Ireland. The consignment of mussels was dispatched from Ireland on 10 November, 1995. The symptoms displayed by those who became ill included nausea, vomiting, severe diarrhoea and stomach cramps. • • •

1998: Satake et al. identified azapiracid-1 (AZA-1) as the causative compound in shellfish (J. Am. Chem. Soc., 120, 9967-9968)

1. Azaspiracids: structure



azaspiracid

Polyketide:
linear carbon skeleton
with cyclic ether
bridges

amino function
chemical nomenclature:
aza = secondary amine

spiro function

acid

Azaspirazids: potent marine toxins accumulating in mussels

PERGAMON

Toxicon 38 (2000) 917–930

TOXICON

www.elsevier.com/locate/toxicon

Multiple organ damage caused by a new toxin azaspiracid, isolated from mussels produced in Ireland

Emiko Ito[a,*], Masayuki Satake[b], Katsuya
Nobuyuki Kurita[a], Terry McMahon[c], Kevi
Takeshi Yasumoto[e]

ELSEVIER

Toxicon 45 (2005) 891–900

TOXICON

www.elsevier.com/locate/toxicon

Cytotoxic and cytoskeletal effects of azaspiracid-1 on mammalian cell lines

Michael J. Twiner[a], Philipp Hess[b], Marie-Yasmine Bottein Dechraoui[a], Terry McMahon[b], Melissa S. Samons[a,c], Masayuki Satake[d], Takeshi Yasumoto[e], John S. Ramsdell[a], Gregory J. Doucette[a,*]

ELSEVIER

Toxicon 40 (2002) 193–203

TOXICON

www.elsevier.com/locate/toxicon

Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussels

Emiko Ito[a,*], Masayuki Satake[b], Katsuya Ofuji[b], Morihiro Higashi[c], Kenichi Harigaya[c], Terry McMahon[d], Takeshi Yasumoto[e]

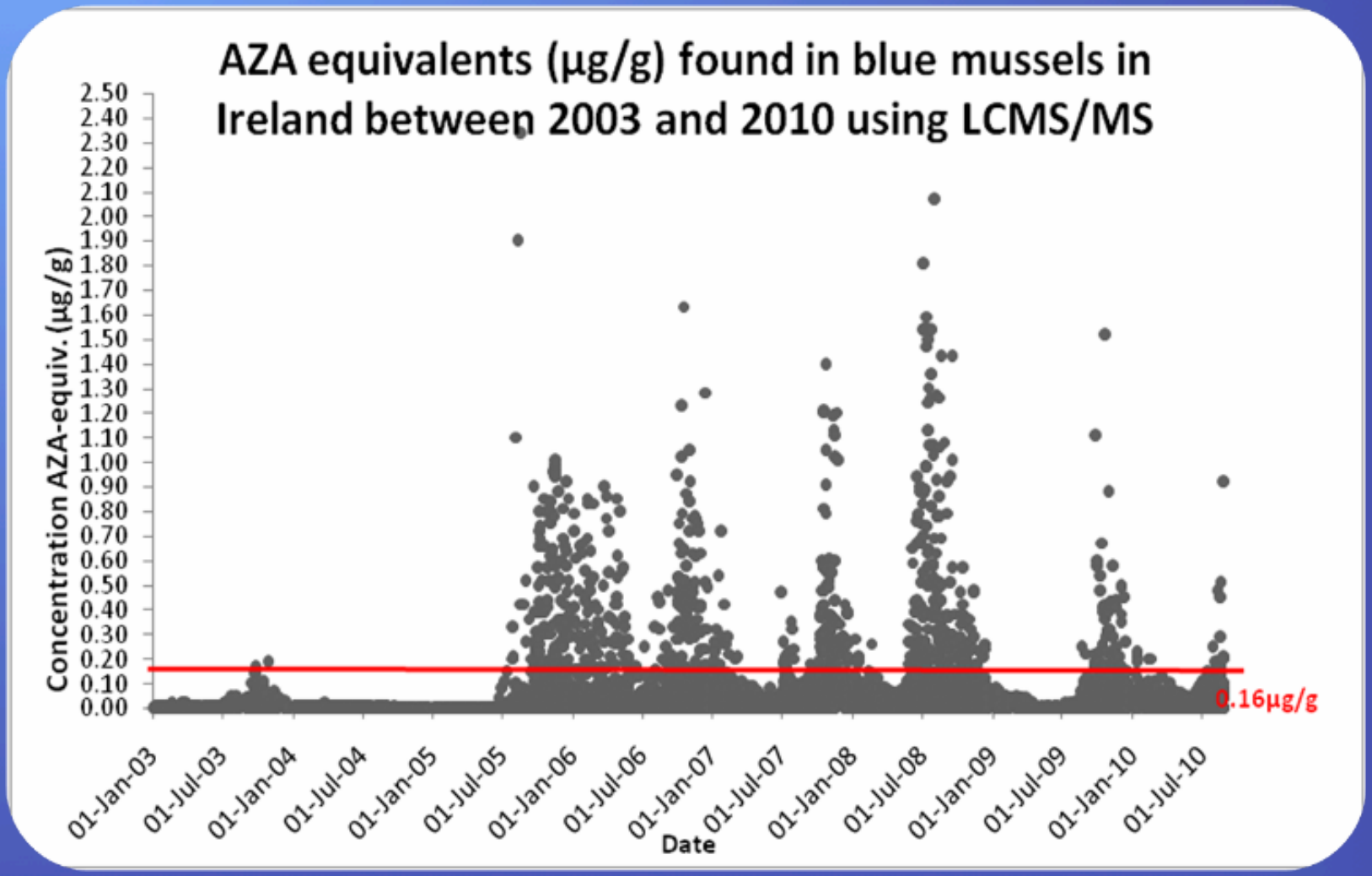
TABLE 28.1

Confirmed Cases of AZP

Event	Location of AZP	Date	Implicated Food Source	Amount Consumed	Area of Production	Number of Illnesses Recorded	[AZA$_{total}$] (μg/g)	[AZA1] (μg/g)	[AZA2] (μg/g)	[AZA3] (μg/g)	Comments	References
Confirmed AZP	The Netherlands	Nov-95	Mussels (*M. edulis*)	Not recorded	Killary Harbour, Ireland	8	1.43[a,b]	1.14[a,b]	0.23[a,b]	0.06[a,b]		[17,37,39]
Confirmed AZP	Arranmore, Ireland	Sep/ Oct-97	Mussels (*M. edulis*)	"As few as 10–12 mussels"	Arranmore Island, Ireland	Estimated 20–24 (8 seen by a doctor)	30[c]	Present	Present	Present	Equivalent to ~6 μg/g whole mussel meat; AZA4,5 were also present.	[17,37]
Confirmed AZP	Ravenna, Italy	Sep-98	Mussels (*M. edulis*)	Not recorded	Clew Bay, Ireland	10	1.3[c]	0.5[c]	0.06[c]	0.44[c]		[17,37]
Confirmed AZP	France	Sep-98	Mussels (*M. edulis*)	Not recorded	Bantry Bay, Ireland	Estimated 20–30	1.1–1.5[a]	Present	Present	Present	Were tested for DSP toxins using MBA and deemed "safe."	[17,37]
Confirmed AZP	United Kingdom	Aug-00	Frozen mussels (*M. edulis*)	Not recorded	Bantry Bay, Ireland	12–16	0.35[a]	Present	Present	Present		[17,37]
Confirmed AZP	France	Apr-08	Frozen mussels (*M. edulis*)	Not recorded	Ireland	"Large outbreak"	>0.16[a]					[37,40]
Confirmed AZP	United States	Jul-08	Frozen mussels (*M. edulis*)	*ca.* 113 and 340 grams	Bantry Bay, Ireland	2	0.086–0.244[a]	Present	Present	Present	~150 tonnes were voluntarily destroyed; AZA6 was also present.	[41]

[a] Whole mussel meat.
[b] Sampled in April 1996 (5 months after event).
[c] Digestive glands/hepatopancreas.

Twiner,M., Hess, P. Doucette, G. 2014



Salas, Tillmann et al. 2012

Shellfish species		Concentration
Common name	**Scientific name**	**AZA equiv. (µg g^{-1})**
Common Cockle	*Cerastoderma edule*	**0.08**
Pacific oyster	*Crassostrea gigas*	**0.31**
Razor clam	*Ensis arcuatus*	**0.05**
Razor clam	*Ensis siliqua*	**<0.01**
Dog cockle	*Glycymeris glycymeris*	**0.01**
Abalone	*Haliotis discus hannai*	**<LOQ**
Blue mussel	*Mytilus edulis*	**8.97**
Native oyster	*Ostrea edulis*	**0.07**
Common limpet	*Patella vulgata*	**<LOQ**
Surf Clam	*Spisula solida*	**0.15**
Manila Clam	*Tapes philippinarum*	**0.10**
Clam	*Tapes semidecussatus*	**0.01**
Pullet carpet shell	*Venerupis senegalensis*	**<LOQ**
Venus Clam	*Venus verrucosa*	**<LOQ**

Maximum concentration of AZA equivalents (µg g-1) found in shellfish species cultured in Ireland between 2003 and 2010 analysed using LC-MS/MS. LOQ = Limit of Quantification

ELSEVIER

Toxicon 41 (2003) 145–151

TOXICON

www.elsevier.com/locate/toxicon

Ubiquitous 'benign' alga emerges as the cause of shellfish contamination responsible for the human toxic syndrome, azaspiracid poisoning

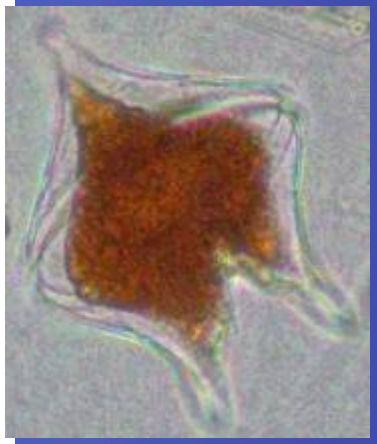
Kevin J. James[a,*], Cian Moroney[a], Cilian Roden[a], Masayuki Satake[b], Takeshi Yasumoto[c], Mary Lehane[a], Ambrose Furey[a]

[a]*Proteobio, Department of Chemistry, Mass Spectrometry Centre for Protcomics and Biotoxin Research, Cork Institute of Technology, Bishopstown, Cork, Ireland*

[b]*Faculty of Agriculture, Tohoku University, Tsutsumidori-Amamiya, Aoba-ku, Sendai, Japan*

[c]*Japan Food Laboratories, Tama Laboratory, Nagayama, Tama-shi, Tokyo, Japan*

Received 5 June 2002; accepted 5 August 2002



Protoperidinium crassipes

Abstract

A new human toxic syndrome, azaspiracid poisoning (AZP), was identified following illness from the consumption of contaminated mussels (*Mytilus edulis*). To discover the aetiology of AZP, sensitive analytical protocols involving liquid chromatography–mass spectrometry (LC–MS) were used to screen marine phytoplankton for azaspiracids. Collections of single species were prepared by manually separating phytoplankton for LC–MS analysis. A dinoflagellate species of the genus, *Protoperidinium*, has been identified as the progenitor of azaspiracids. Azaspiracid-1, and its analogues, AZA2 and AZA3, were identified in extracts of 200 cells using electrospray multiple tandem MS. This discovery has significant implications for both human health and the aquaculture industry since this phytoplankton genus was previously considered to be toxicologically benign. The average toxin content was 1.8 fmol of total AZA toxins per cell with AZA1 as the predominant toxin, accounting for 82% of the total.

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Keywords: Marine toxins; LC–MS; AZP; *Protoperidinium*; Shellfish poisoning

Eur. J. Phycol., (2009), 44(1): 63–79

Taylor & Francis
Taylor & Francis Group

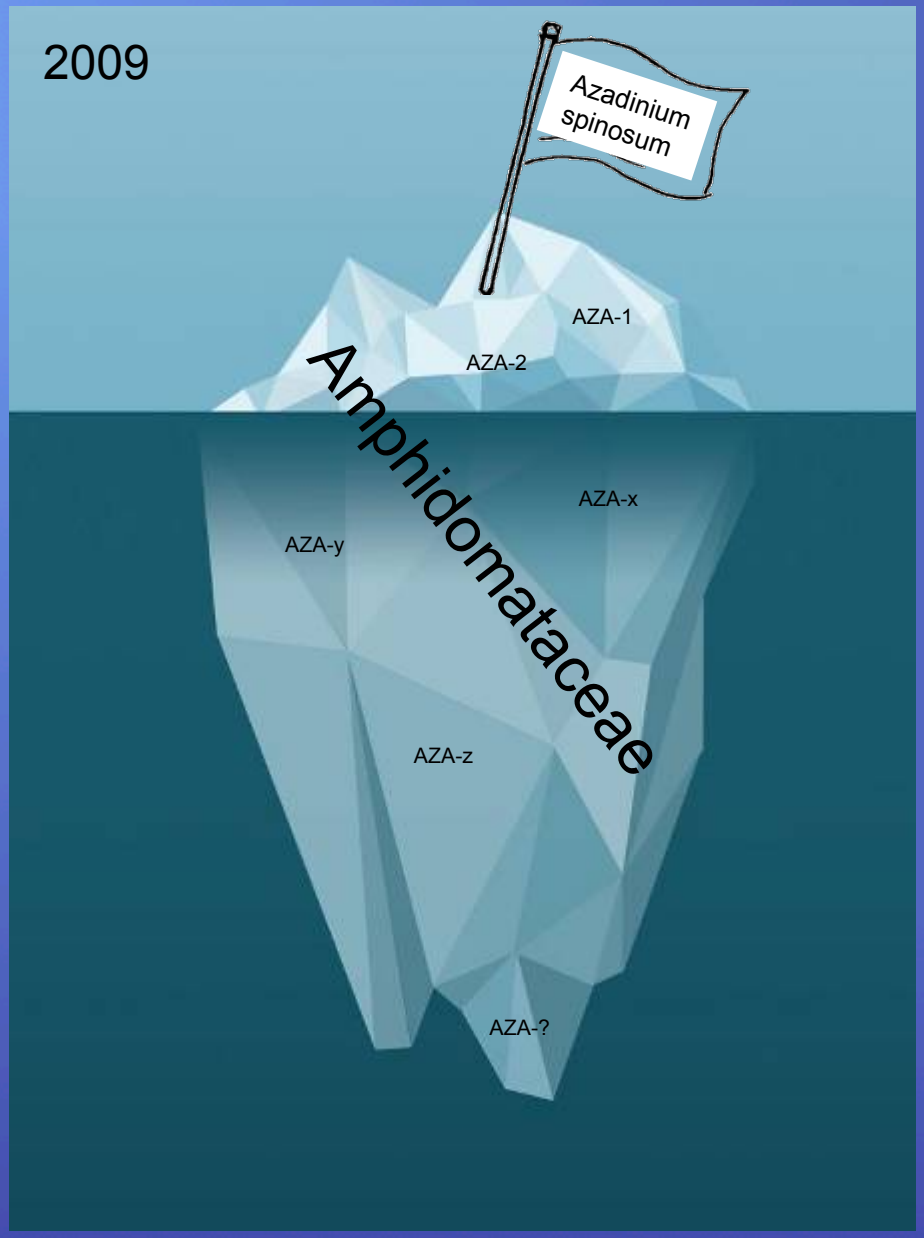
Azadinium spinosum gen. et sp. nov. (Dinophyceae) identified as a primary producer of azaspiracid toxins

URBAN TILLMANN[1], MALTE ELBRÄCHTER[2], BERND KROCK[1], UWE JOHN[1] AND ALLAN CEMBELLA[1]

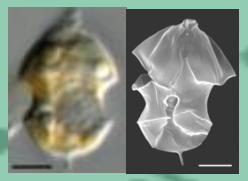
[1]*Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany*
[2]*Deutsches Zentrum für Marine Biodiversitätsforschung, Forschungsinstitut Senckenberg, Wattenmeerstation Sylt, Hafenstr. 43, D- 25992 List/Sylt, Germany*

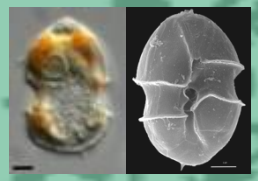
(Received 19 June 2008; revised 27 August 2008; accepted 5 September 2008)

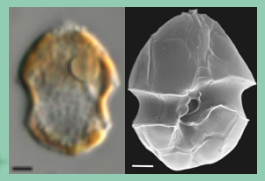
Azaspiracids (AZAs) are a group of lipophilic marine biotoxins associated with human incidents of shellfish poisoning. During a research cruise to the North Sea, we analysed size-fractionated plankton for AZA by mass spectrometry and successfully isolated an AZA-producin coast of Scotland. As shown previously, an axenic culture of this dinoflagellate produces A f AZA 2. Here we give a taxonomic description of this new taxon *Azadinium spinosum* gen. c cer of AZAs. *Azadinium spinosum* is a small (12–16 µm length and 7–11 µm width) peridinin- oflagellate with a superficial resemblance under light microscopy to gymnodinioids, but with a is spherical and st is parietal, lobed, and typical d hyposome. T , 6C,


2009
Azadinium spinosum
AZA-1
AZA-2
Amphidomataceae
AZA-x
AZA-y
AZA-z
AZA-?

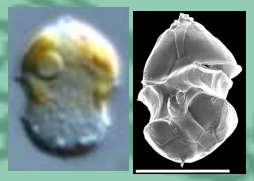
2014

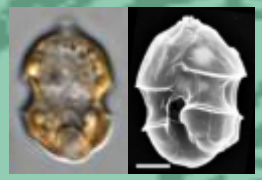




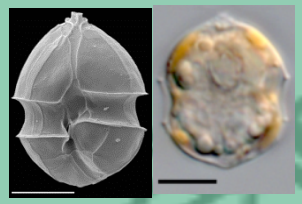


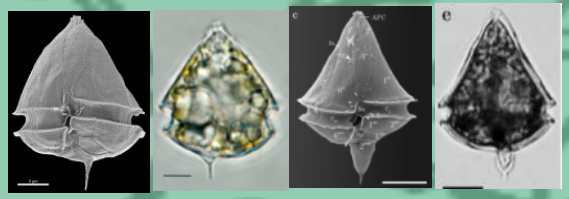
2013

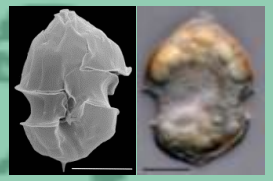




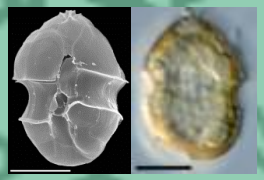
2012



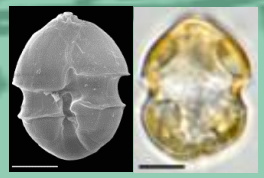




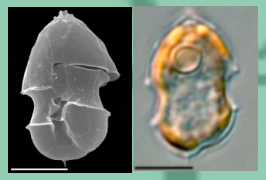
2011

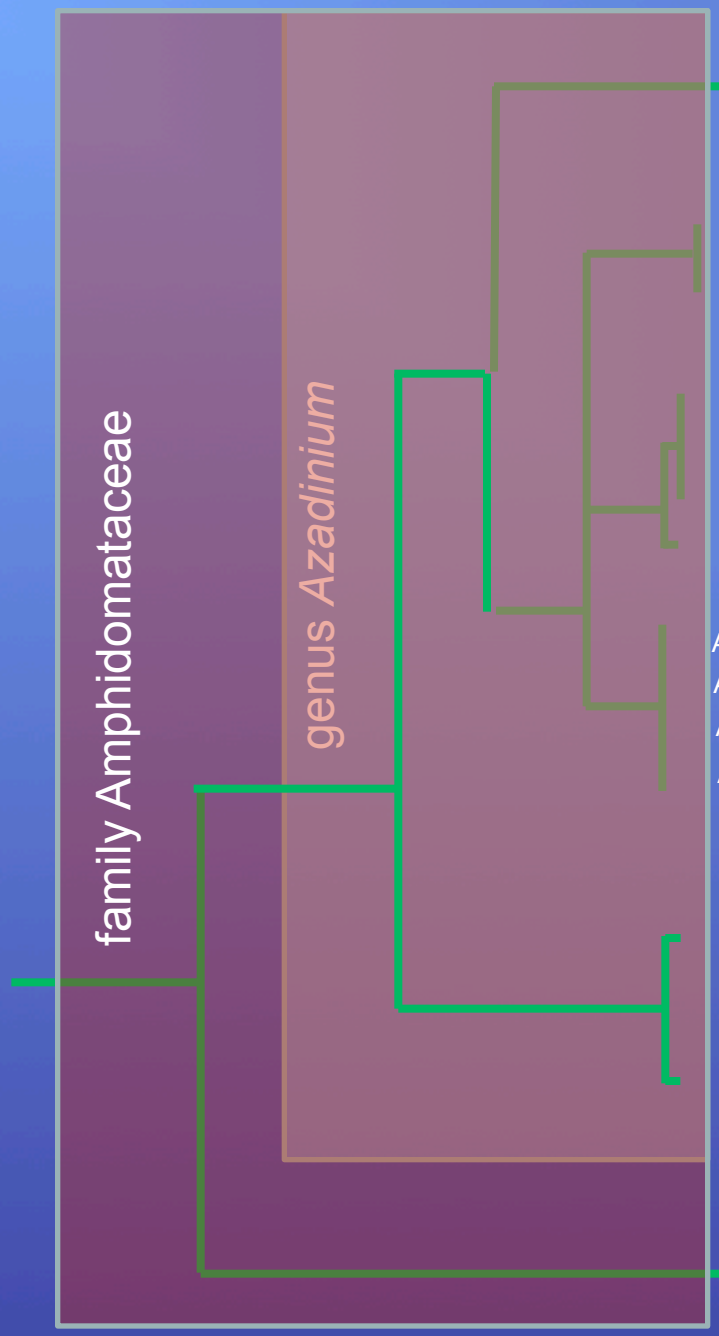


2010

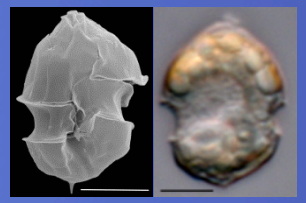


2009



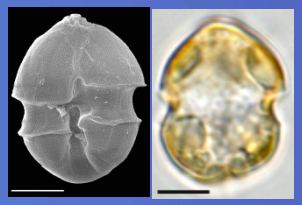


A. polongum
Shet B2



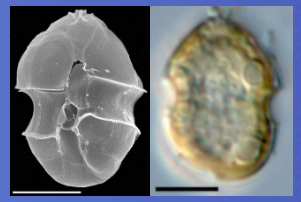
A. polongum 2012

A. obesum 2E10



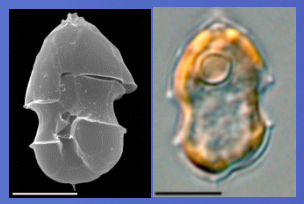
A. obesum 2010

A. poporum UTHC5
A. poporum UTHC8
A. poporum UTHD4
A. poporum HF2011



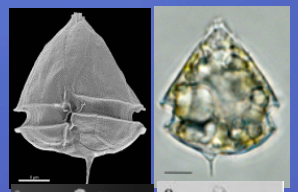
A. poporum 2011

A. spinosum Shet F6
A. spinosum 3D9
A. spinosum UTH E2
A. spinosum SM2

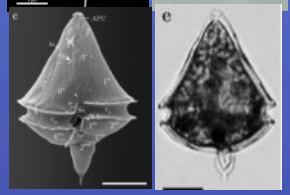


A. spinosum 2009

A. caudatum
var margalefii

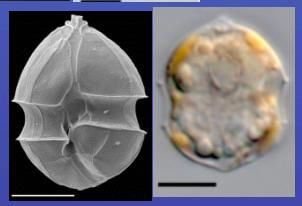


A. caudatum
var caudatum

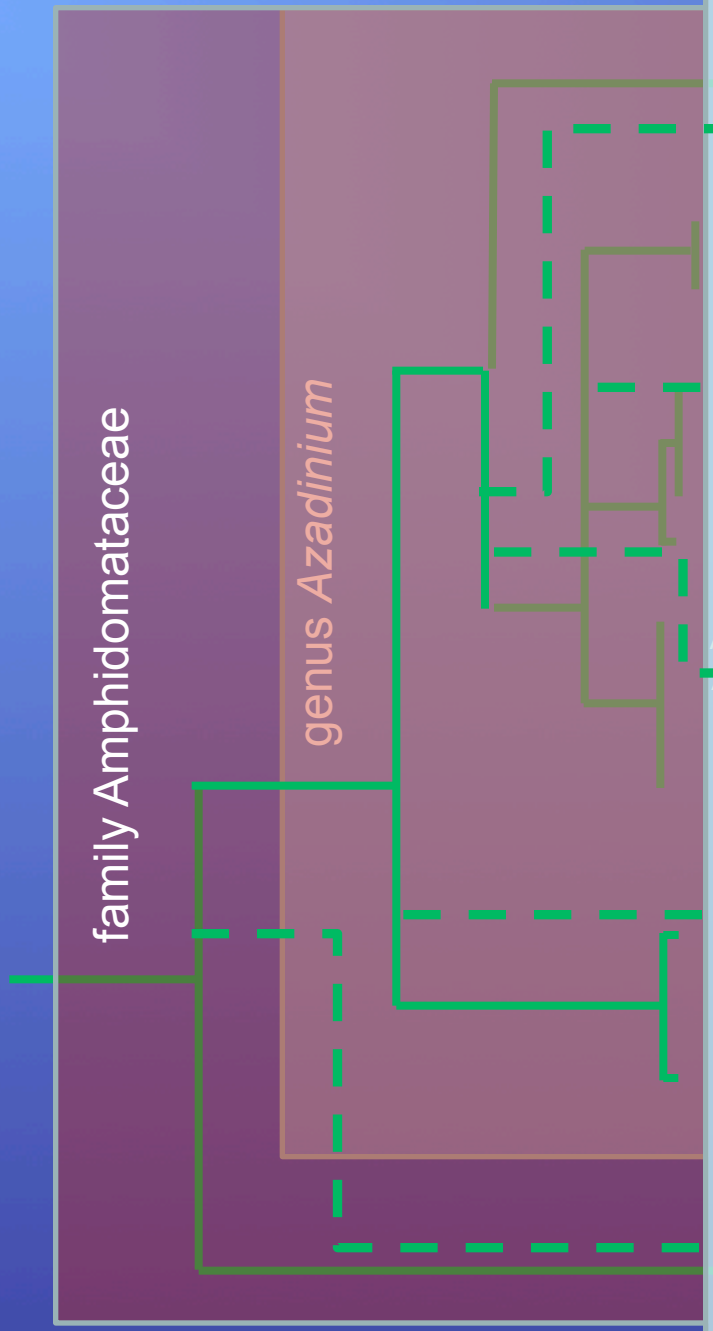


A. caudatum 2012

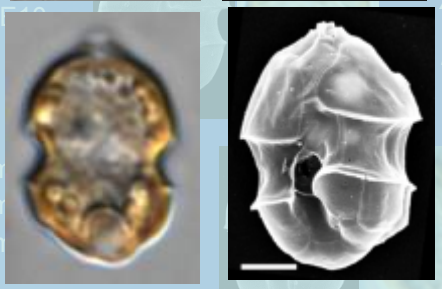
Amphidoma
languida SM1



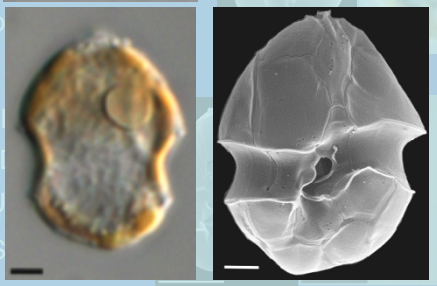
Amphidoma languida 2012



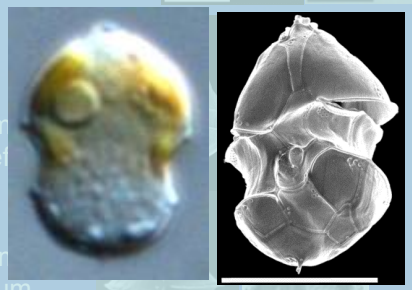
Az. trinitatum
2013/14



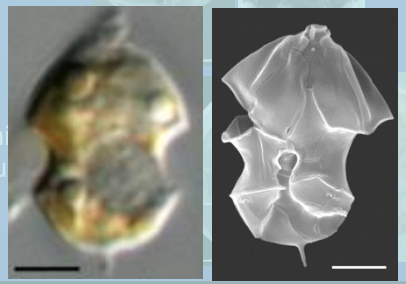
Az. dalianense
2013



Az. cunneatum
2013/14



Az. dexteroporum
2013



Az. concinnum
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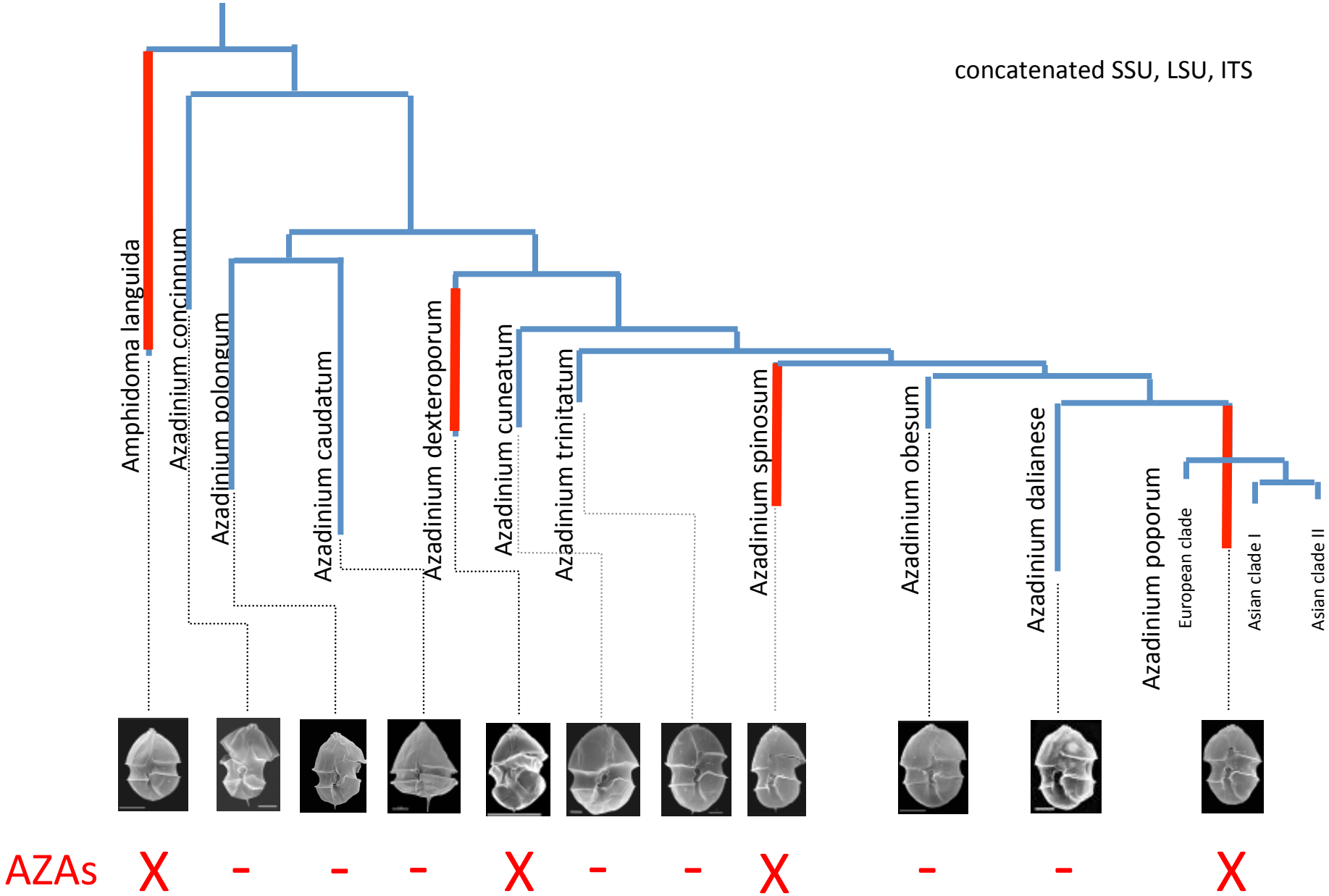
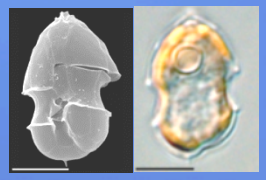


Tillmann et al. 2014, Protists

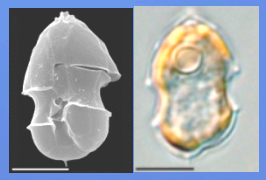
Azadinium spinosum



AZA-1
AZA-2

Krock et al. 2009

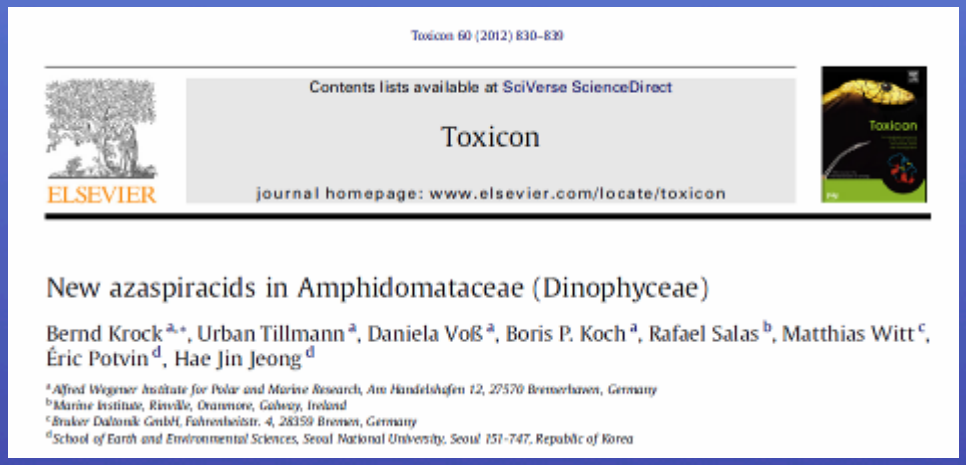
Azadinium spinosum



AZA-1 AZA-2	Krock et al. 2009
AZA-716 = AZA-33	Tillmann et al. 2012
AZA-34 AZA-35 (traces…)	Kilkoyne et al. 2014

A. obesum and *A. poporum* have been described not to contain known AZAs

2012: Identifiction of a new group of AZAs (348 fragment type) in other species of Amphidomataceae (Krock, Tillmann et al.Toxicon 2012)



Toxicon 60 (2012) 830–839

Contents lists available at SciVerse ScienceDirect

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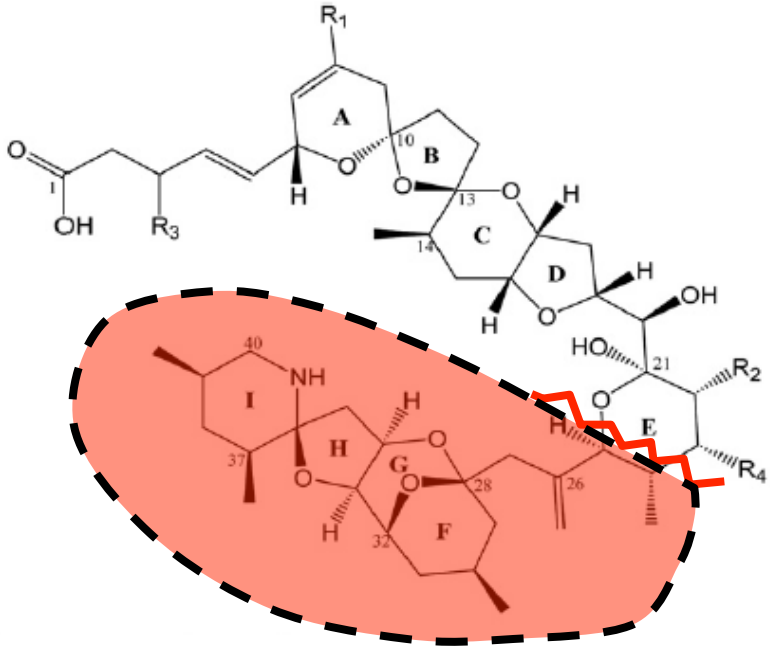
journal homepage: www.elsevier.com/locate/toxicon

New azaspiracids in Amphidomataceae (Dinophyceae)

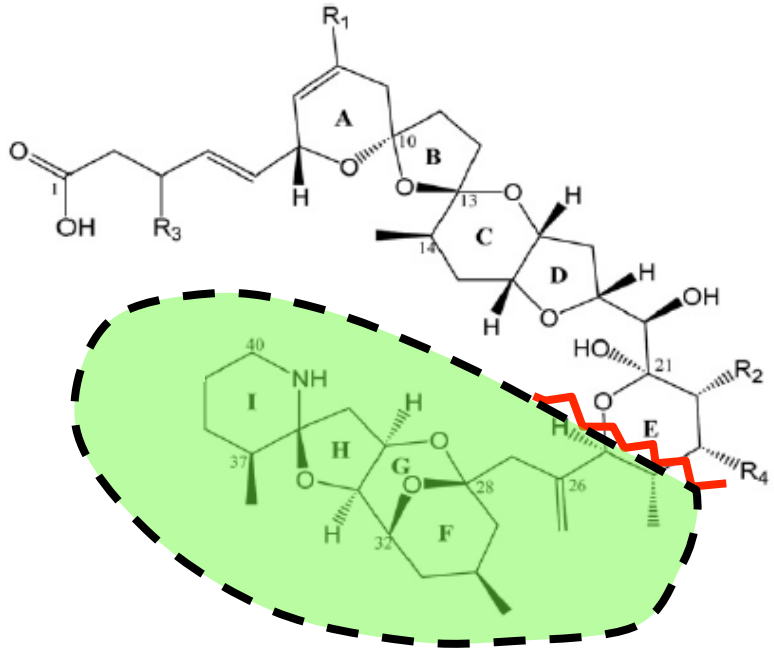
Bernd Krock [a,*], Urban Tillmann [a], Daniela Voß [a], Boris P. Koch [a], Rafael Salas [b], Matthias Witt [c], Éric Potvin [d], Hae Jin Jeong [d]

[a] Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[b] Marine Institute, Rinville, Oranmore, Galway, Ireland
[c] Bruker Daltonik GmbH, Fahrenheitstr. 4, 28359 Bremen, Germany
[d] School of Earth and Environmental Sciences, Seoul National University, Seoul 151-747, Republic of Korea



m/z 362 typ

AZA-1, -2, -33
all known shelfish metabolites



m/z 362 typ

AZA-1, -2, -33
all known shellfish metabolites

m/z 348 typ

New azaspiracids

A. spinosum AZA-1 AZA-2 AZA-33

A. poporum Korea
AZA-858 (=36)

A. poporum North Sea
AZA-846 (=37)

Amphidoma languida
AZA-816 (=38)
AZA-830 (=39)

A. obesum
not found

A. polongum
not found

A. caudatum var. *margalefii*
not found

Krock, Tillmann et al, 2012, Toxicon

A. spinosum AZA-1 AZA-2 AZA-33

A. poporum North Sea

AZA-37

A. poporum Korea

AZA-36

Az. poporum China

AZA-2

Az. poporum China

AZA-11

Az. poporum China

AZA-40

Az. poporum China

AZA-41

Az. dexteroporum Mediterranean

AZA-827

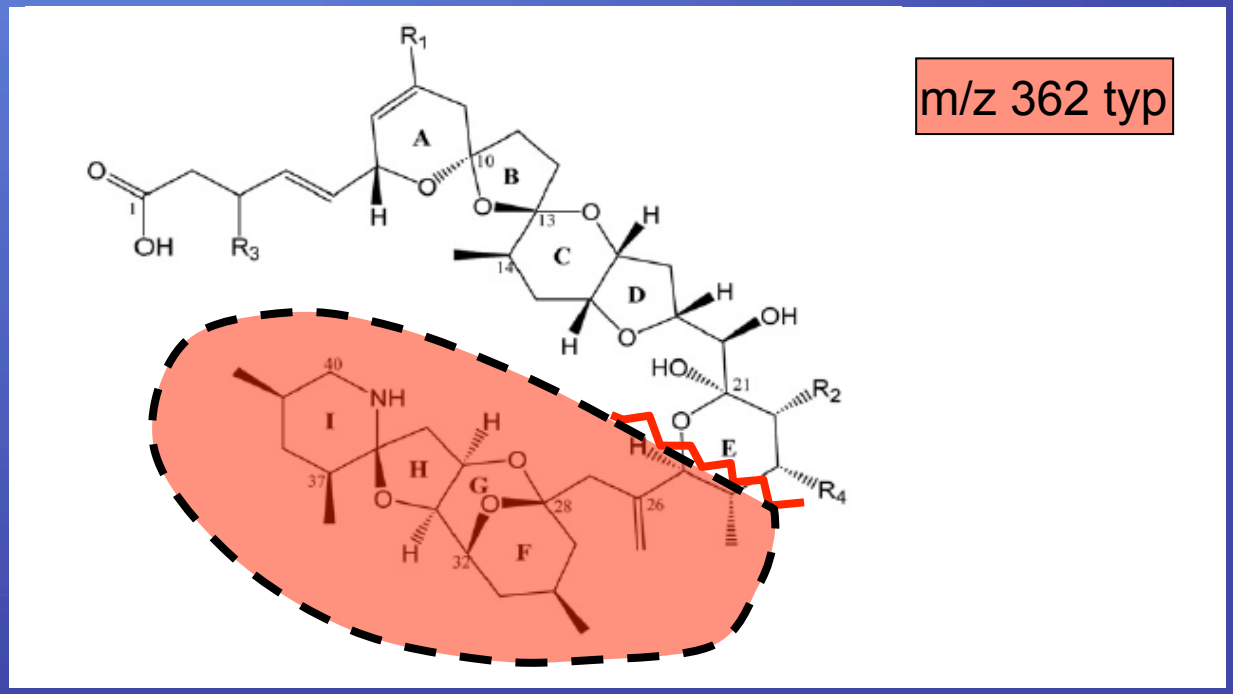
AZA-857

AZA-829

Amphidoma languida

AZA-38

AZA-39



A. spinosum AZA-1 AZA-2 AZA-33

A. poporum North Sea

AZA-37

A. poporum Korea

AZA-36

Az. poporum China

AZA-2

Az. poporum China

AZA-11

Az. poporum China

AZA-40

Az. poporum China

AZA-41

Az. dexteroporum Mediterranean

AZA-827

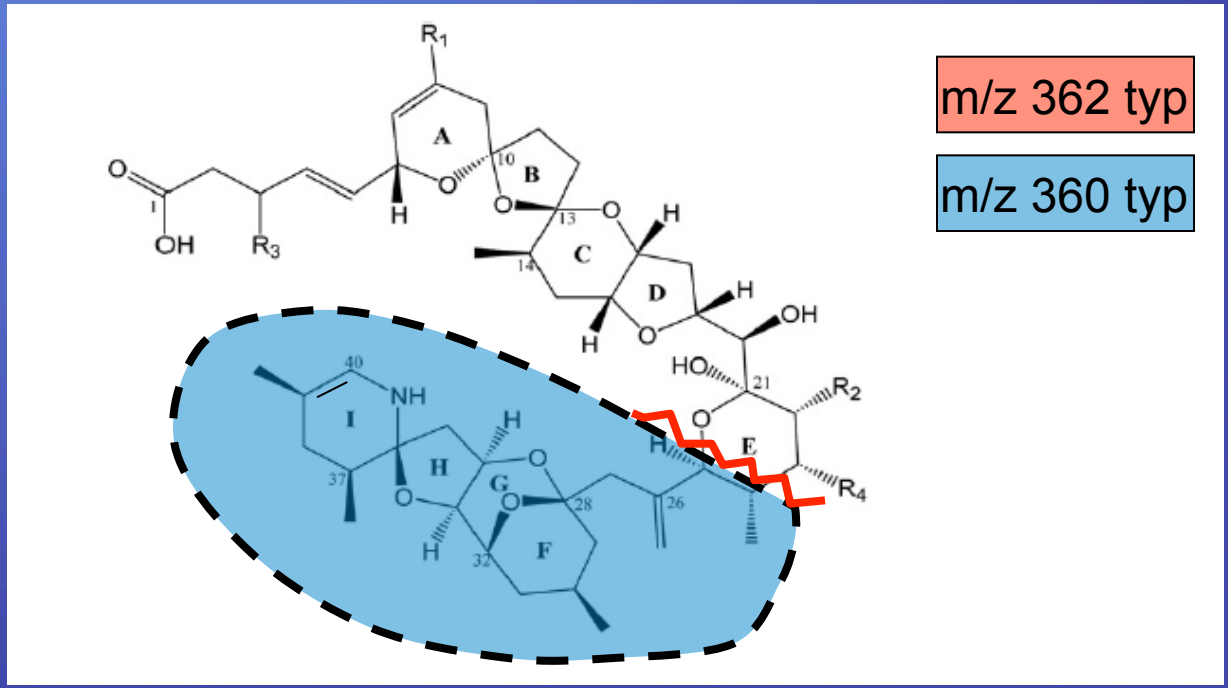
AZA-857

AZA-829

Amphidoma languida

AZA-38

AZA-39



A. spinosum

AZA-1

AZA-2

AZA-33

A. poporum North Sea

AZA-37

A. poporum Korea

AZA-36

Az. poporum China

AZA-2

Az. poporum China

AZA-11

Az. poporum China

AZA-40

Az. poporum China

AZA-41

Az. dexteroporum
Mediterranean

AZA-827

AZA-857

AZA-829

Amphidoma languida

AZA-38

AZA-39

Am. languida DJ01

AZA-2

AZA-43

m/z 362 typ

m/z 348 typ

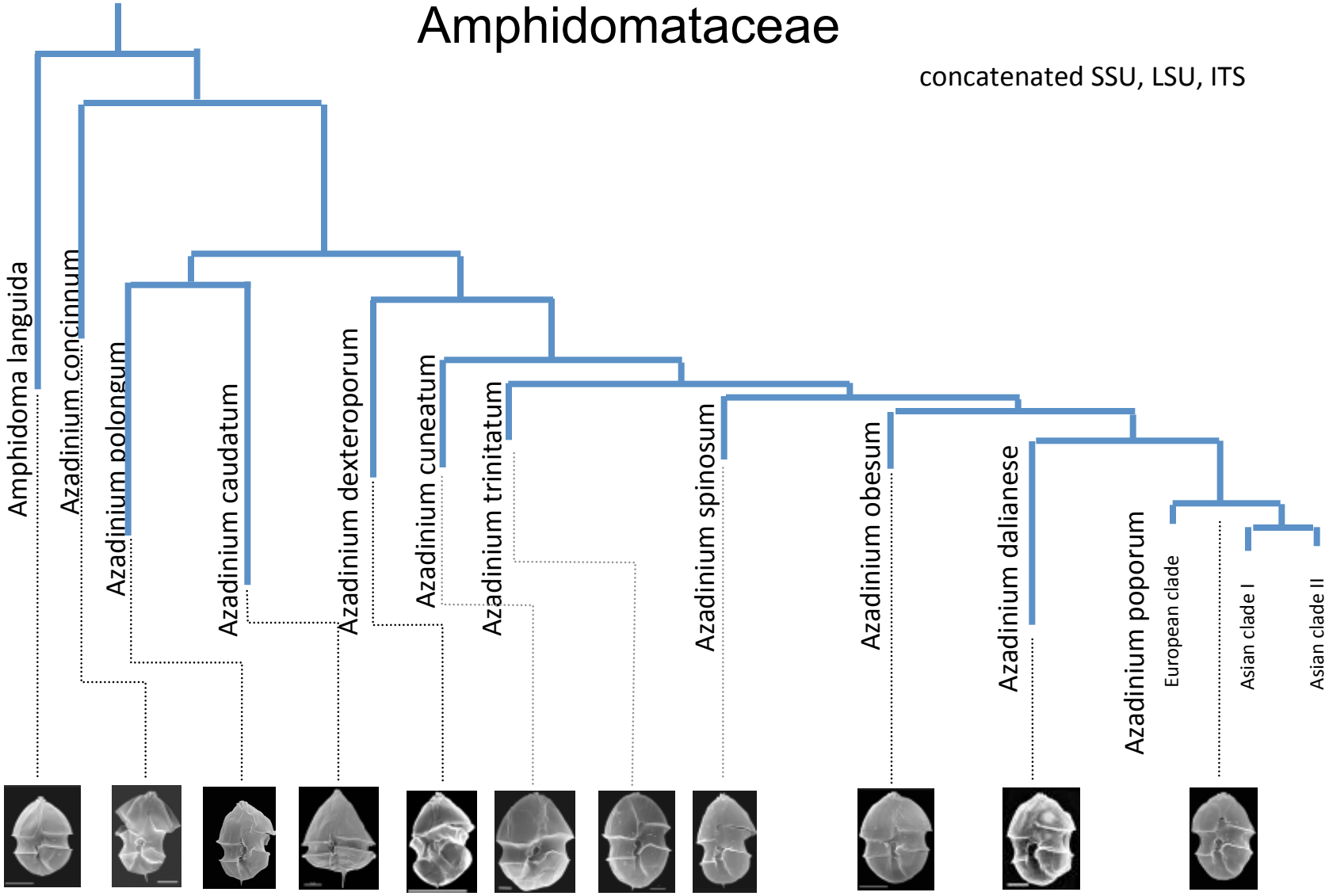
m/z 360 typ

Azaspiracids – producing organisms and toxin profile

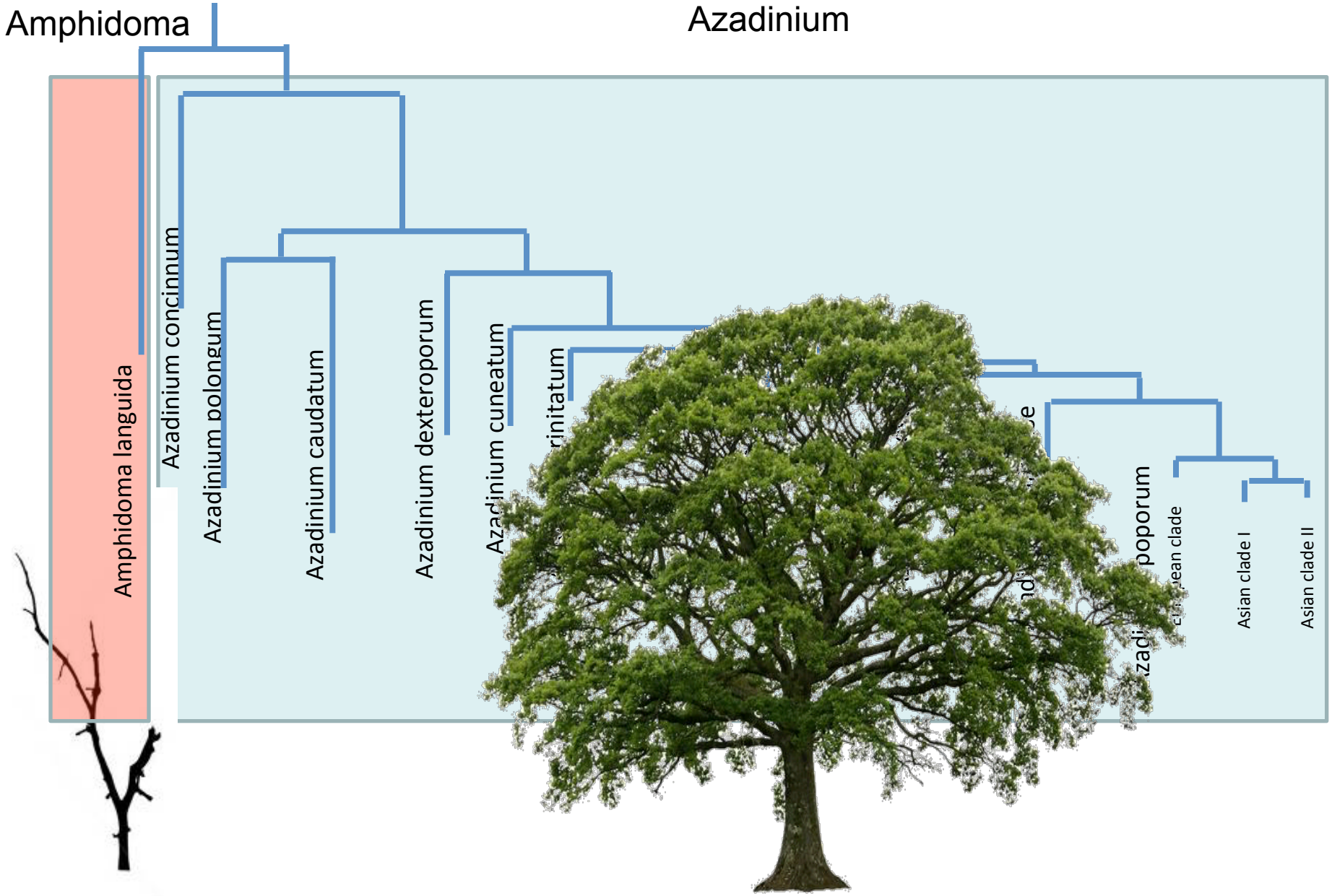
Species	AZAs	source
Azadinium spinosum	AZA-1, -2, -33, -34, -35	Tillmann et al. 2009, 2012; Kilcoyne et al. 2014
Azadinium obesum	-	Tillmann et al. 2010
Azadinium poporum	AZA-2, -11, -36, -37, -40, -41	Tillmann et al. 2011, Krock et al. 2012, 2014
Azadinium caudatum var. margalefii var. Caudatum	- Not tested	Tillmann et al. 2014, Nezan et al. 2012
Azadinium polongum	-	Tillmann et al. 2012
Azadinium dexteroporum	AZA-827, -829, -857	Percopo et al. 2013
Azadinium dalianense	-	Luo et al. 2013
Azadinium trinitatum	-	Tillmann et al. 2014
Azadinium cuneatum	-	Tillmann et al. 2014
Azadinium concinnum	-	Tillmann et al. 2014
Amphidoma languida	AZA-2, -38, -39, -43	Krock et al. 2012, Tillmann, Jaen et al. in prep.

Amphidomataceae

concatenated SSU, LSU, ITS

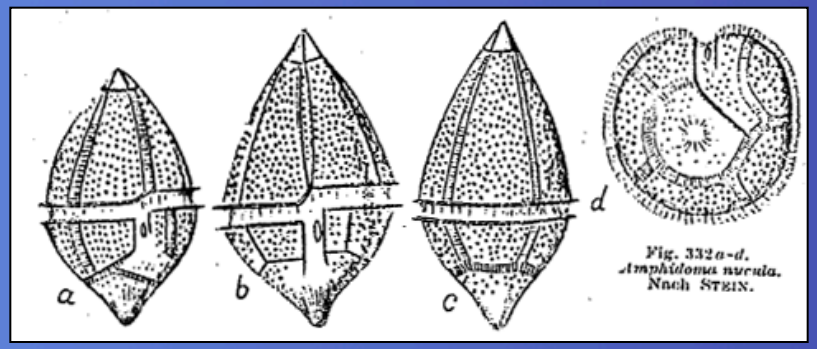


Tillmann et al. 2014, Protists

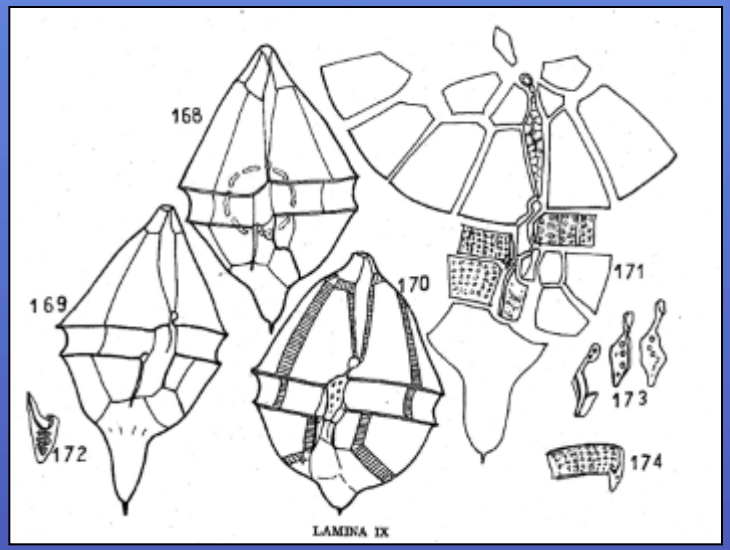

Amphidoma
Azadinium
Amphidoma languida
Azadinium concinnum
Azadinium polongum
Azadinium caudatum
Azadinium dexteroporum
Azadinium cuneatum
Asian clade I
Asian clade II

The genus Amphidoma Stein 1883

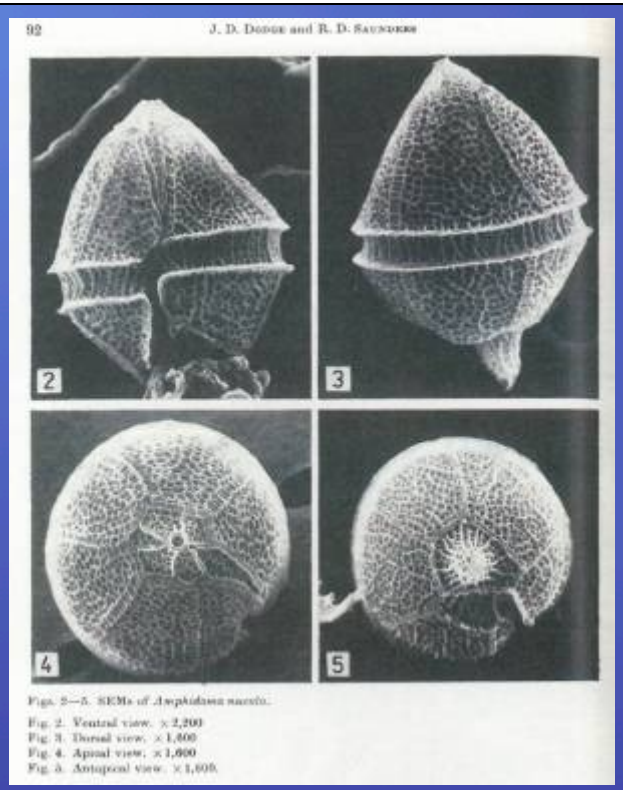
Type species: *Amphidoma nucula*



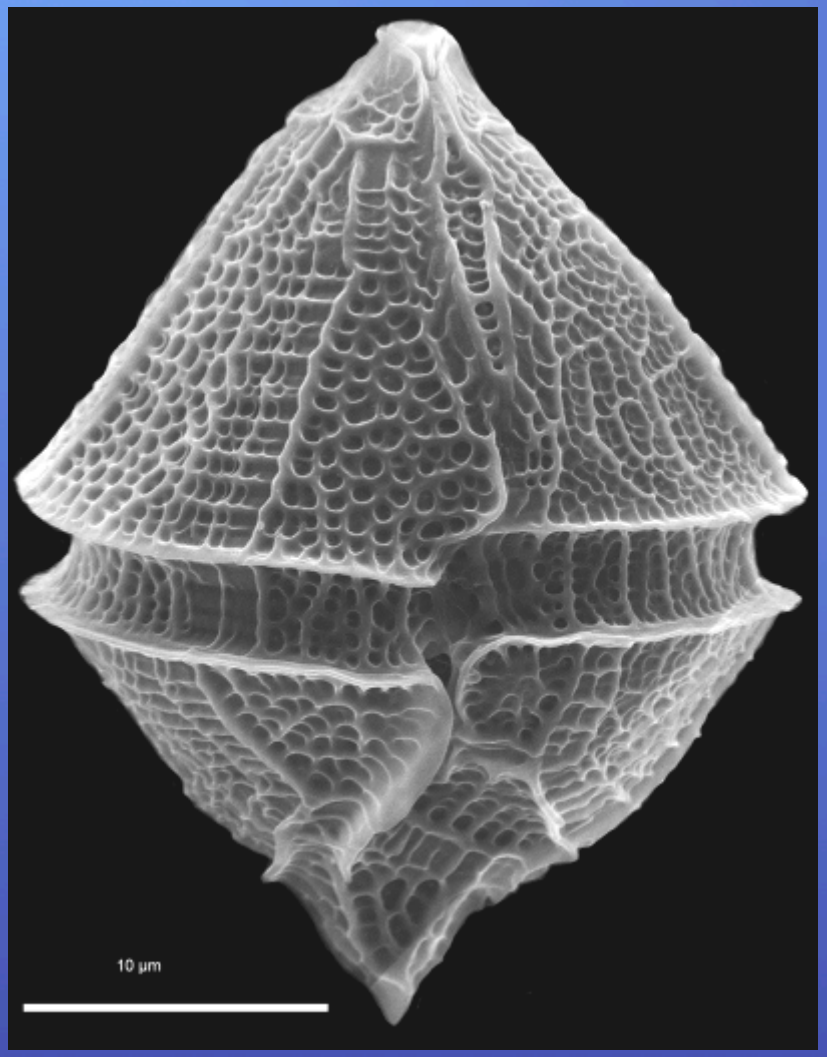
Stein 1883

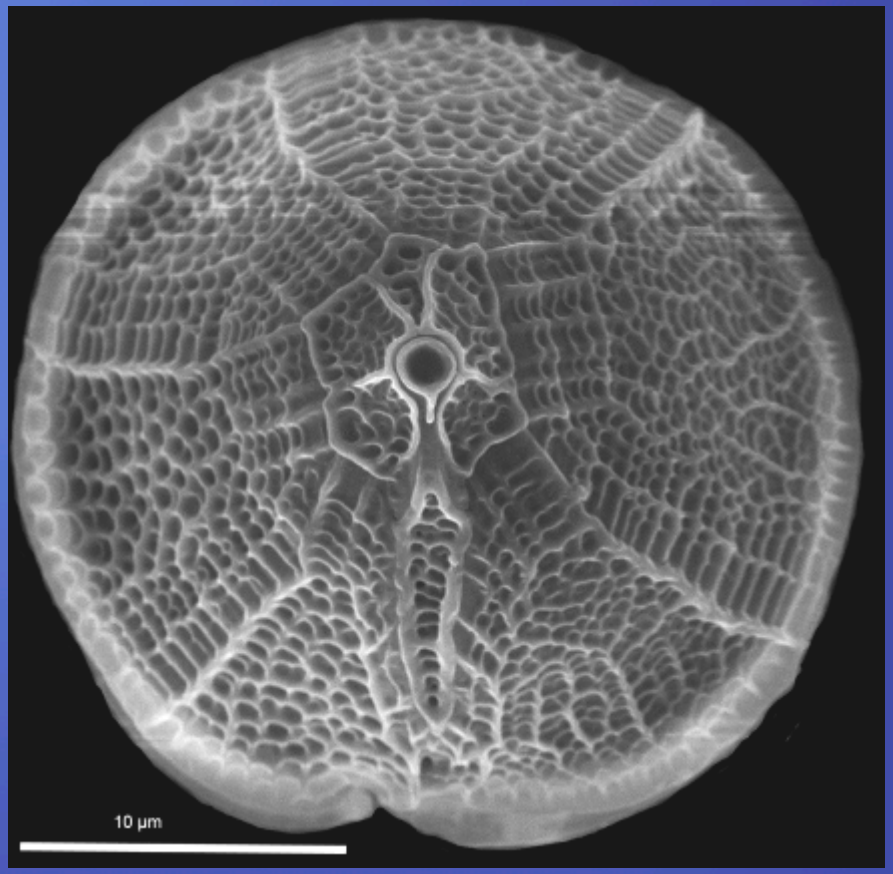


Balech 1971



Aus: Dodge & Saunders 1985





Amphidoma nucula, Indian Ocean sample, Carbonell-Moore & Tillmann unpubl.

The genus Amphidoma Stein 1883

Amphidoma nucula Stein

Amphidoma acuminata Stein

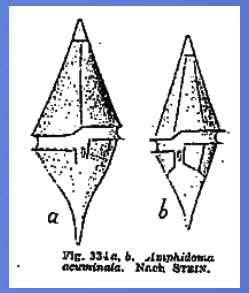


Fig. 334 a, b. Amphidoma acuminata. Nach STEIN.

Amphidoma spinosa Kofoid
(Basionym: Murayella spinosa Kofoid)

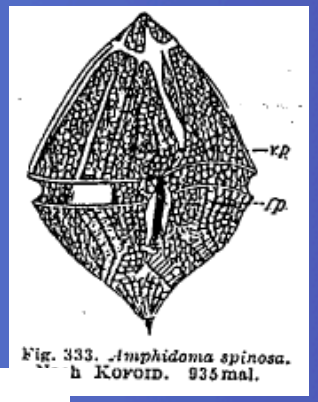
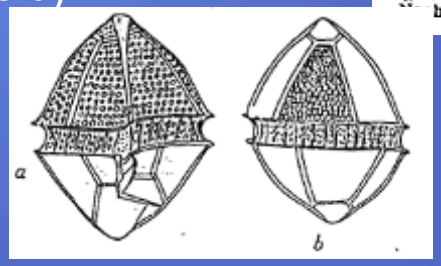


Fig. 333. Amphidoma spinosa. Nach KOFOID. 935 mal.

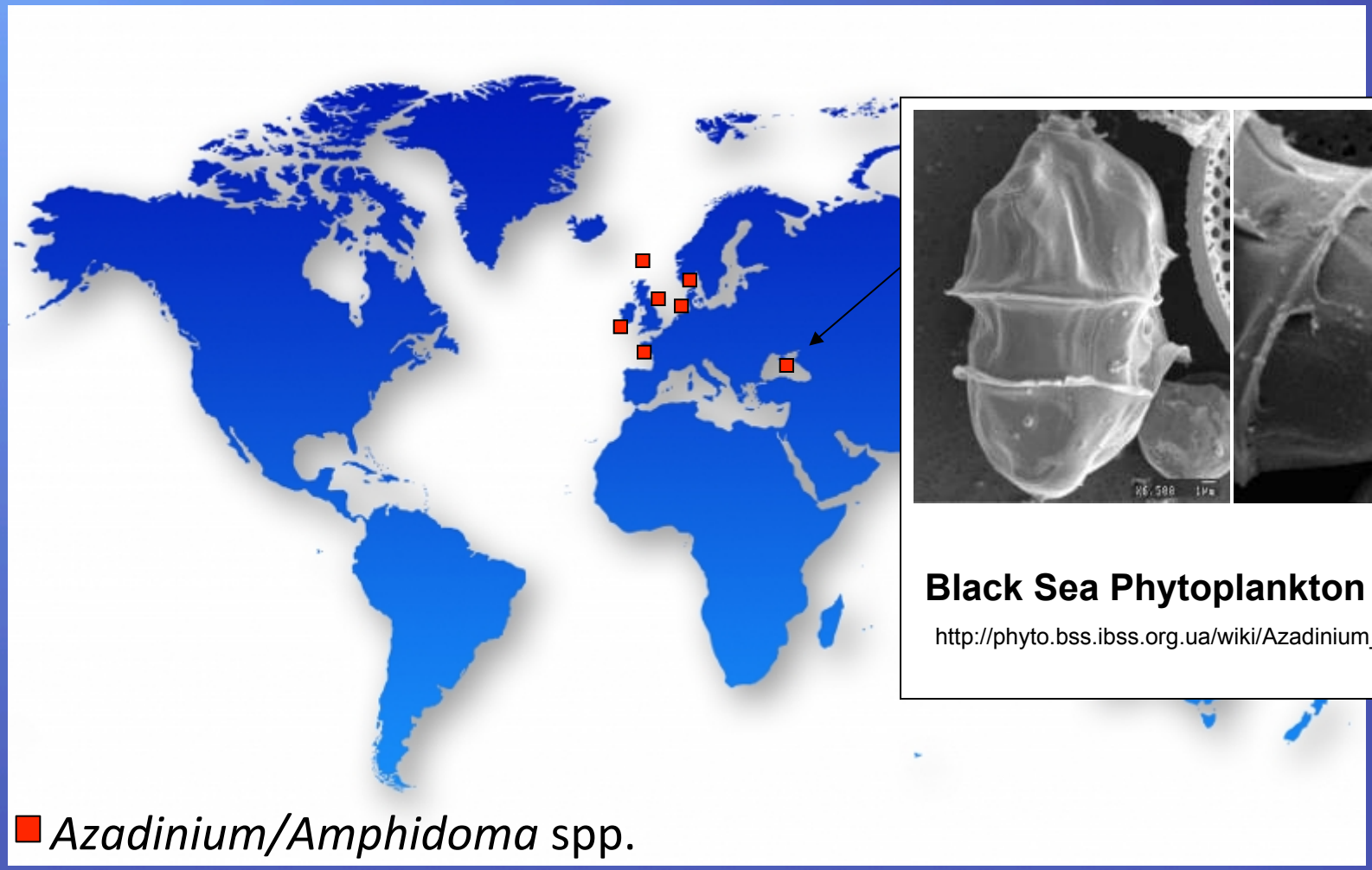
Amphidoma steinii Schiller



Amphidoma laticincta	Length: 18 µm Width: 17 µm
Amphidoma elongata	Length: 35-45 µm Width: 18-23 µm
Amphidoma curtata	Length: 30 µm Width: 23 µm
Amphidoma depressa	Length: 27 µm Width: 27 µm
Amphidoma obtusa	Length: 27 µm Width: 18 µm

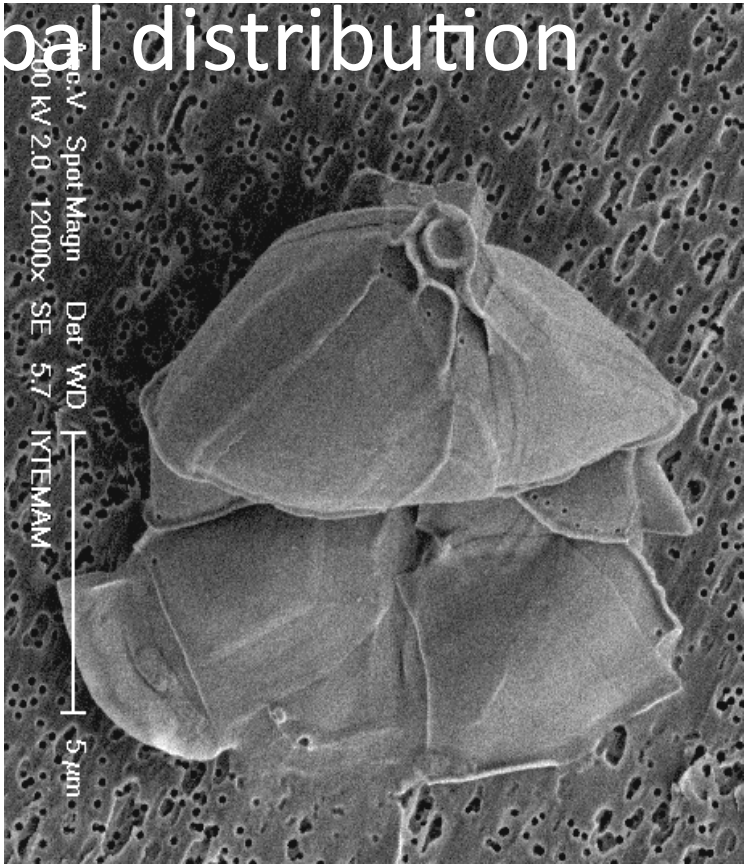
Kofoid & Michener 1911
Without illustration ☹

Amphidomataceae – global distribution



Amphidomataceae – global distribution

■ *Azadinium/Amphidoma* spp.



Prof. Dr. Nihayet BİZSEL

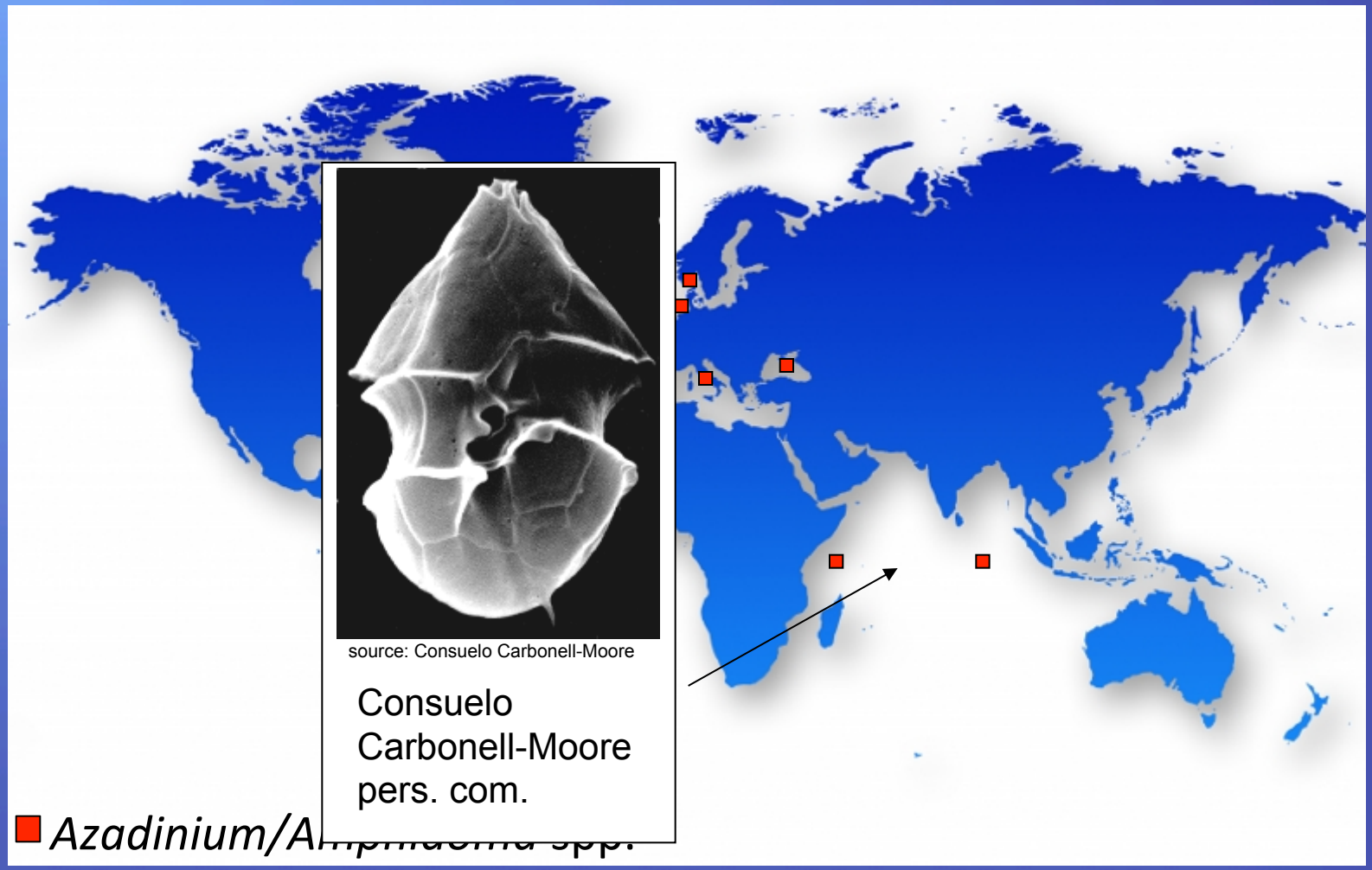
Dokuz Eylul University
Institute of Marine Science and Technology
35340 Inciralti-Izmir / TURKEY

Black Sea, May/June 2013

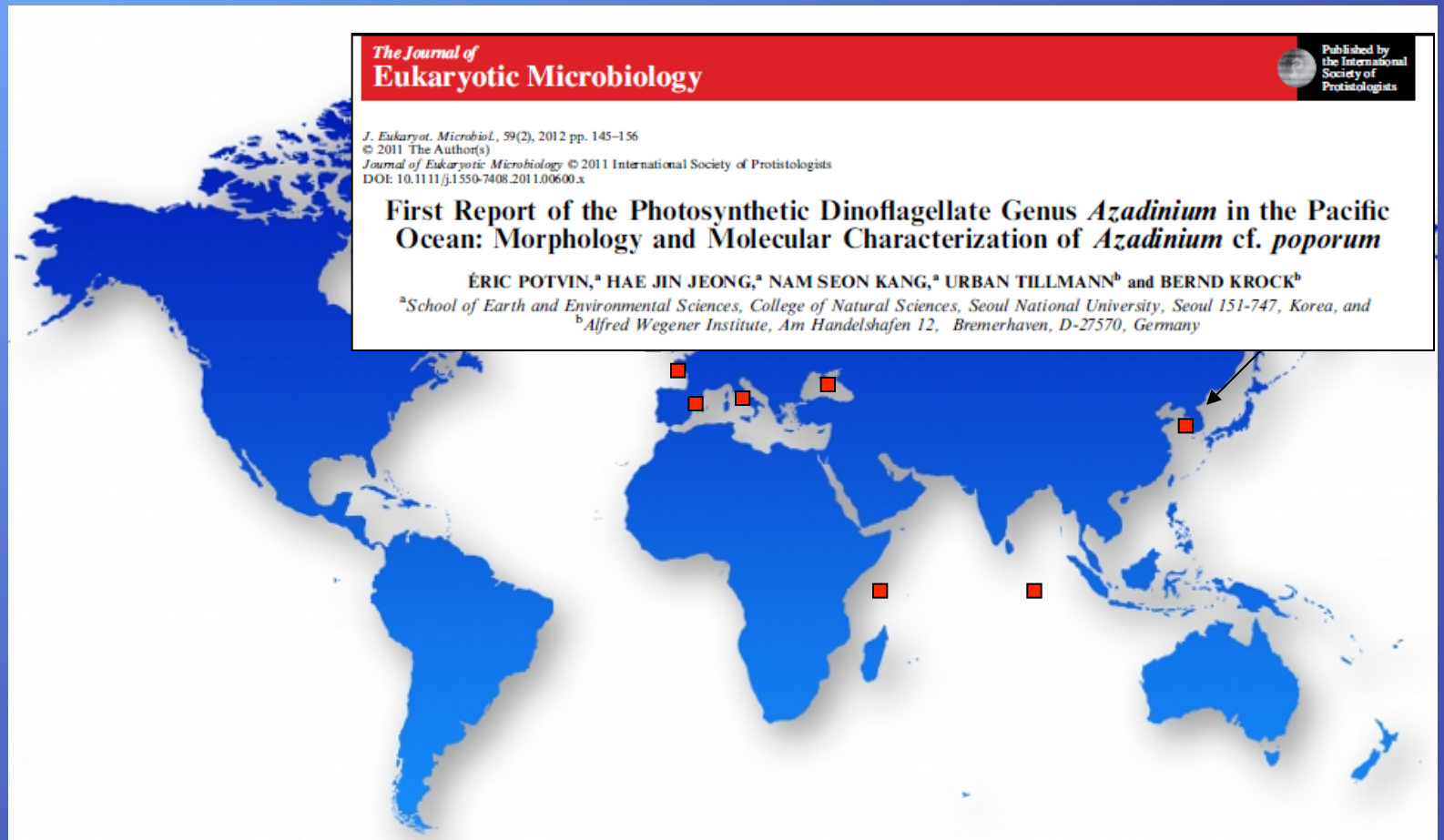
Amphidomataceae – global distribution

■ *Azadinium/Amphidoma* spp.

Amphidomataceae – global distribution



Amphidomataceae – global distribution



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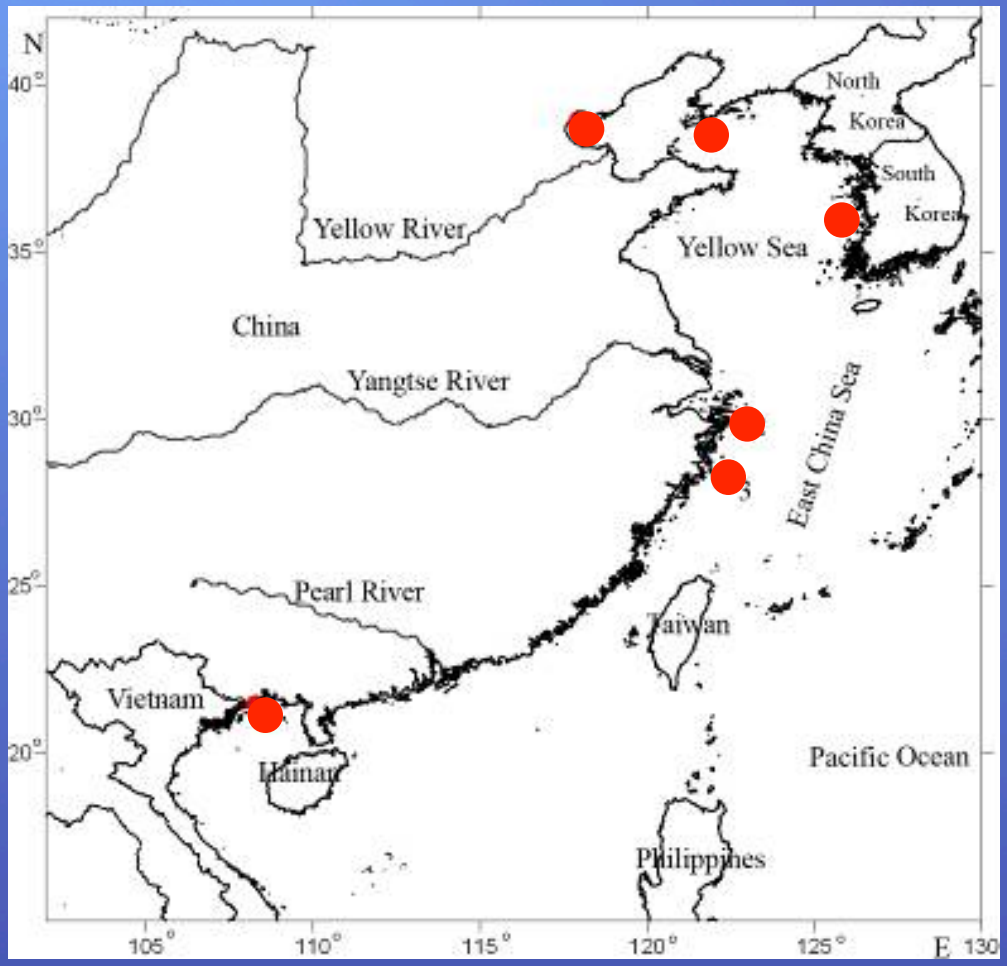
Journal of Eukaryotic Microbiology © 2011 International Society of Protistologists
DOI: 10.1111/j.1550-7408.2011.00600.x

First Report of the Photosynthetic Dinoflagellate Genus *Azadinium* in the Pacific Ocean: Morphology and Molecular Characterization of *Azadinium* cf. *poporum*

ÉRIC POTVIN,[a] HAE JIN JEONG,[a] NAM SEON KANG,[a] URBAN TILLMANN[b] and BERND KROCK[b]
[a]*School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 151-747, Korea, and*
[b]*Alfred Wegener Institute, Am Handelshafen 12, Bremerhaven, D-27570, Germany*

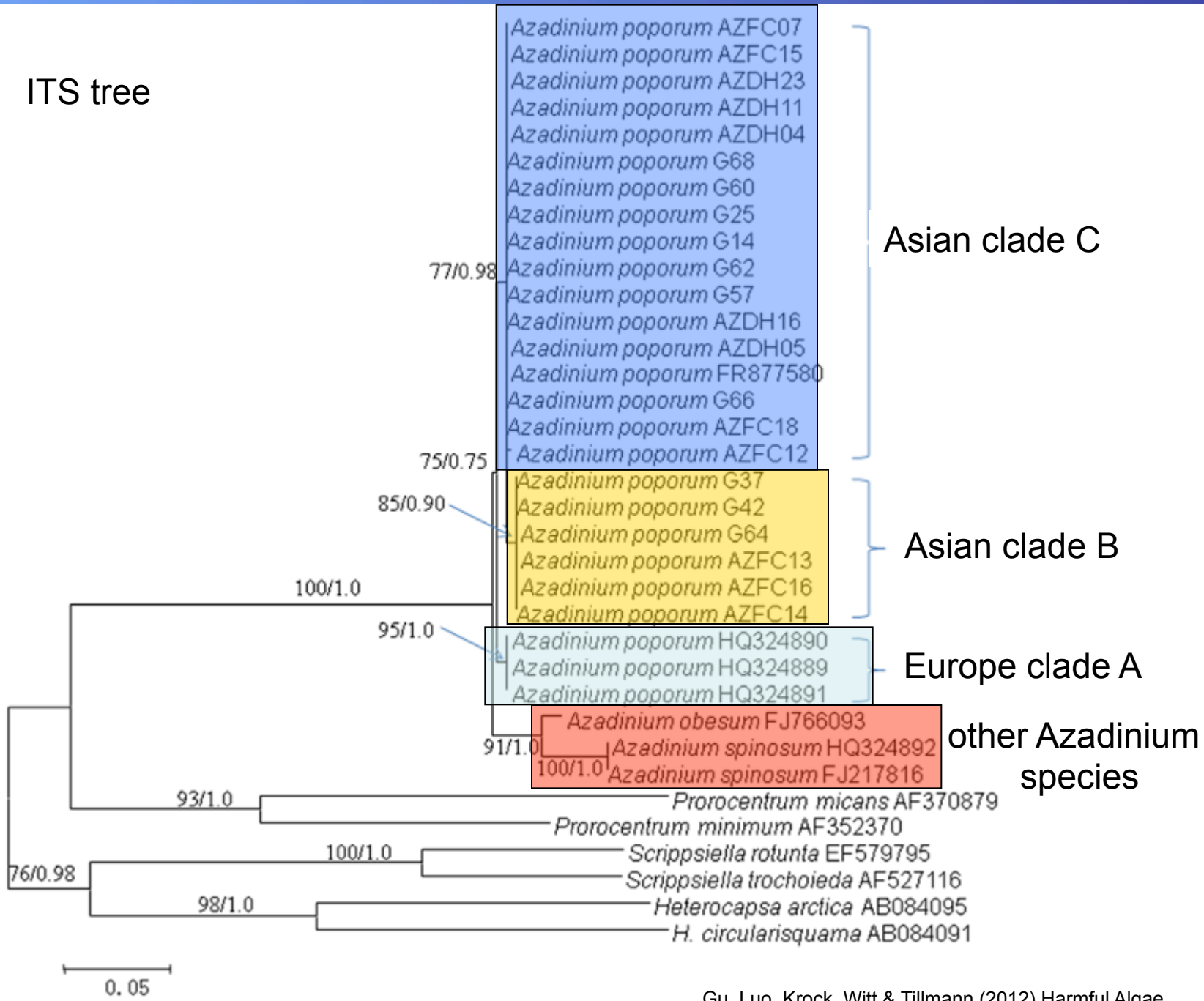
- *Azadinium/Amphidoma* spp.

Azadinium poporum from the Asian Pacific

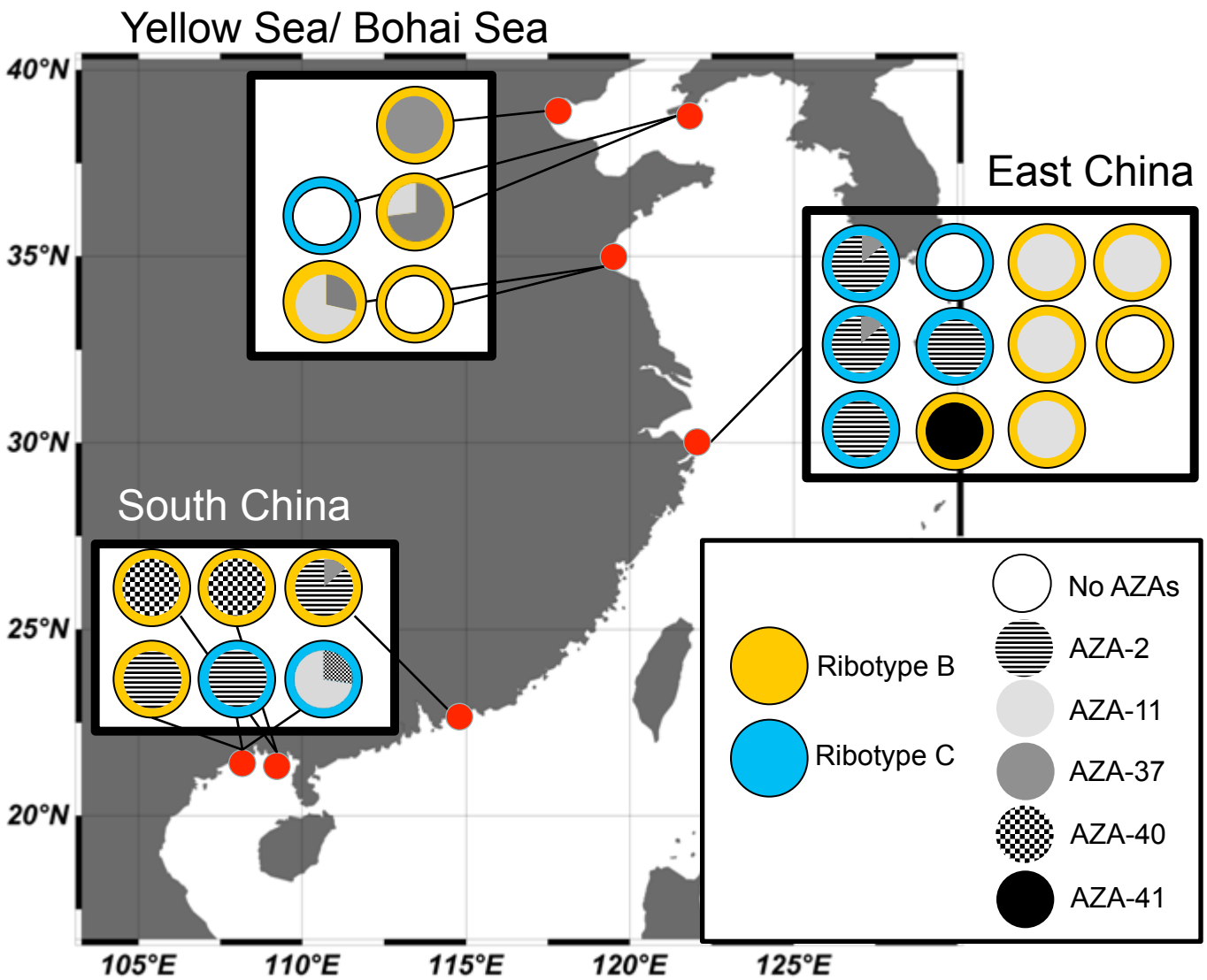


Potvin et al. 2012
Gu et al. 2013
Krock et al. 2014

ITS tree

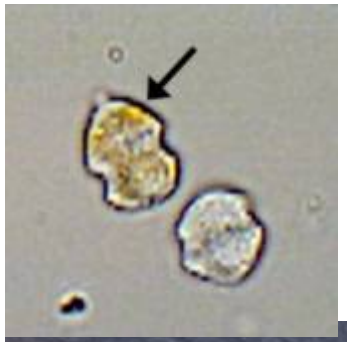


Gu, Luo, Krock, Witt & Tillmann (2012) Harmful Algae



Krock et al. 2014

Amphidomataceae – global distribution



Protist, Vol. 165, 441–444, August 2014
http://www.elsevier.de/protis
Published online date 2 May 2014

Protist

ORIGINAL PAPER

Morphological and Molecular Characterization of Three New *Azadinium* Species (Amphidomataceae, Dinophyceae) from the Irminger Sea

Urban Tillmann[a,1], Marc Gottschling[b], Elisabeth Nézan[c], Bernd Krock[a], and Gwenaël Billen[c]

Marine Biodiversity Records, page 1 of 11. © Marine Biological Association of the United Kingdom, 2015
doi:10.1017/S1755267215001128; Vol. 8; e142; 2015 Published online

First records of *Amphidoma languida* and *Azadinium dexteroporum* (Amphidomataceae, Dinophyceae) from the Irminger Sea off Iceland

URBAN TILLMANN[1], MARC GOTTSCHLING[2], ELISABETH NÉZAN[3] AND BERND KROCK[1]
[1]Alfred Wegener Institute, Am Handelshafen 12, D-27570 Bremerhaven, Germany, [2]Department Biologie, Systematische Botanik und Mykologie, GeoBio-Center, Ludwig-Maximilians-Universität München, Menzinger Straße 67, D-80638 München, Germany, [3]Ifremer, Station de Biologie Marine, Place de la Croix, BP 40537, 29185 Concarneau Cedex, France

- *Azadinium/Amphidoma* spp.

– global distribution

Nova Hedwigia Vol. 101 (2015) Issue 1–2, 205–232 Article
published online April 17, 2015; published in print August 2015

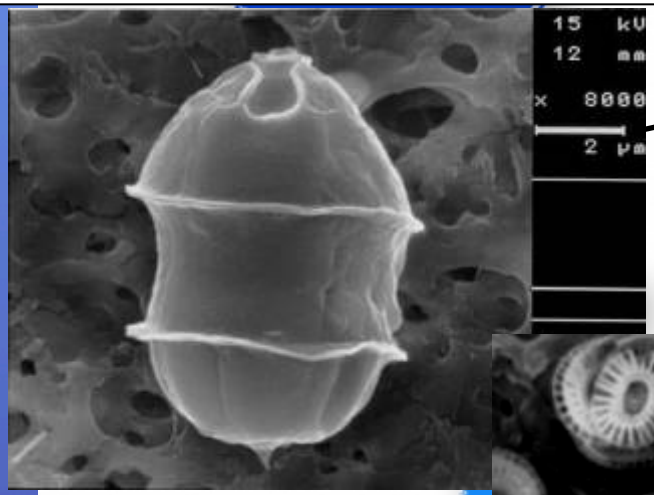
Checklist of nanno- and microphytoplankton off Madeira Island (Northeast Atlantic) with some historical notes

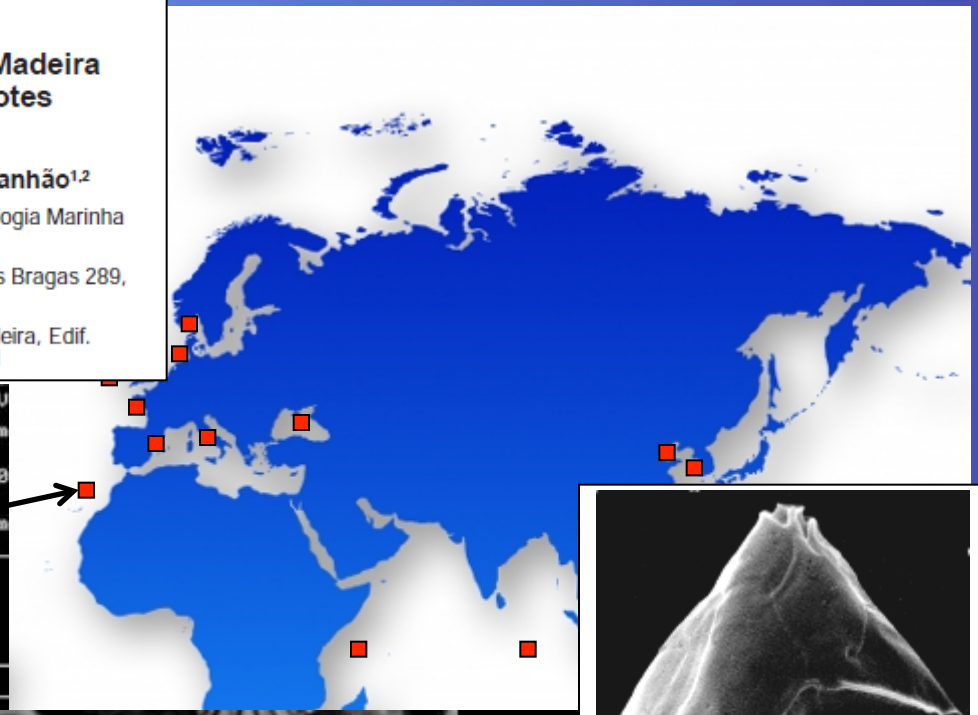
Manfred J. Kaufmann[1,2,3*], Fátima Santos[1] and Manuela Maranhão[1,2]

[1] Universidade da Madeira, Centro das Ciências da Vida, Estação de Biologia Marinha do Funchal, 9000-107 Funchal, Portugal

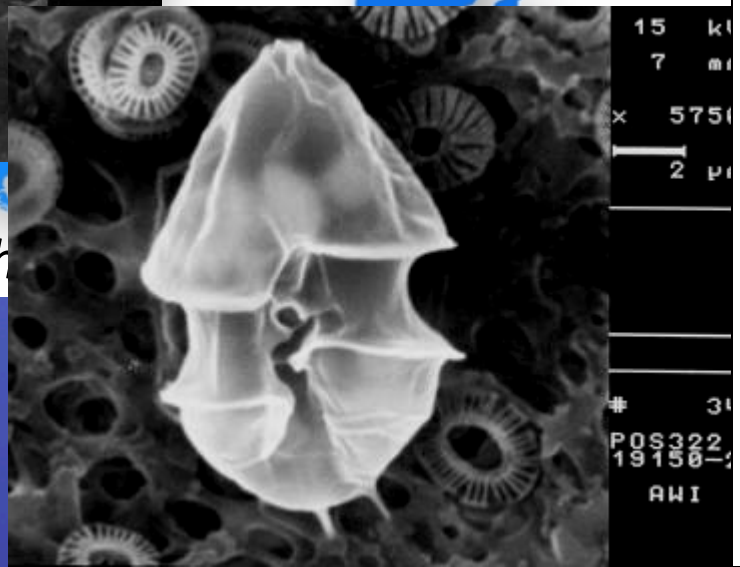
[2] CIMAR/CIIMAR – Centro de Investigação Marinha e Ambiental, Rua dos Bragas 289, 4050-123 Porto, Portugal

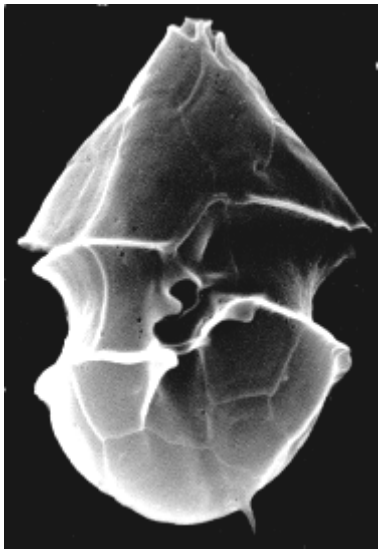
[3] CIIMAR-Madeira – Centro de Investigação Marinha e Ambiental da Madeira, Edif. Madeira Tecnopolo, Caminho da Penteada, 9020-105 Funchal, Portugal





■ *Azadinium/Amph*

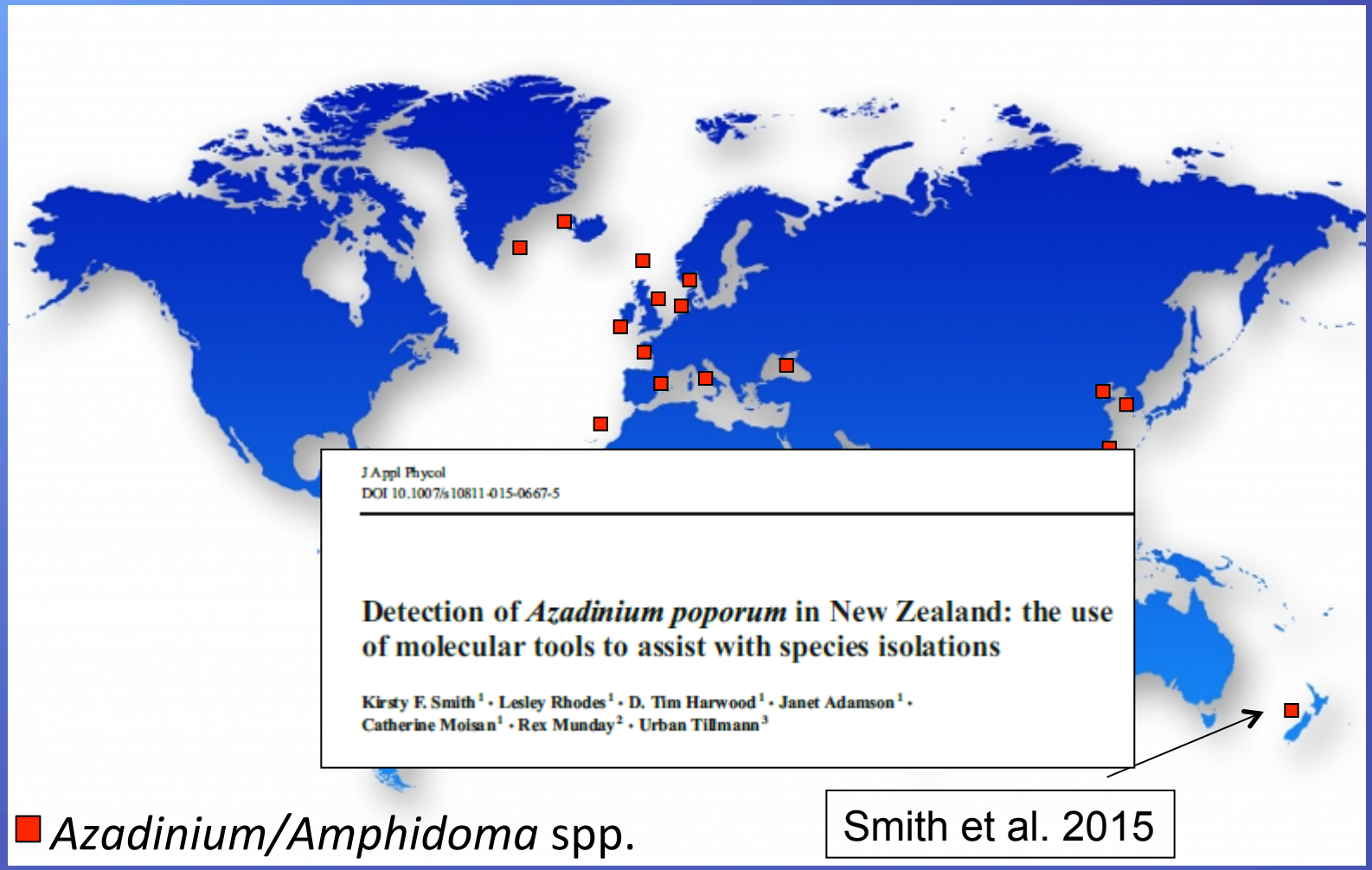




source: Consuelo Carbonell-Moore

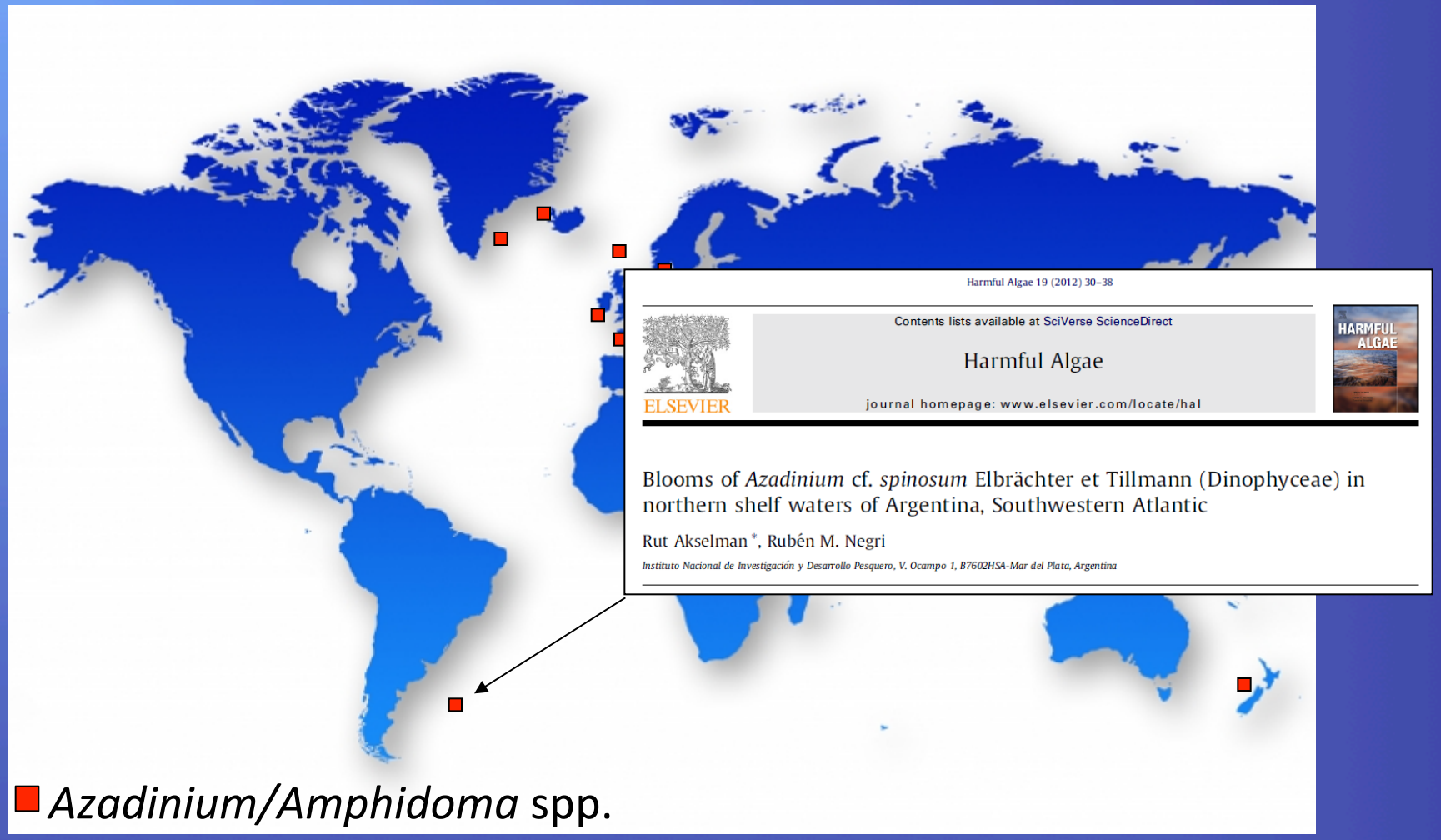
Consuelo Carbonell-Moore pers. com.

Amphidomataceae – global distribution



■ *Azadinium/Amphidoma* spp.

Amphidomataceae – global distribution



■ *Azadinium/Amphidoma* spp.

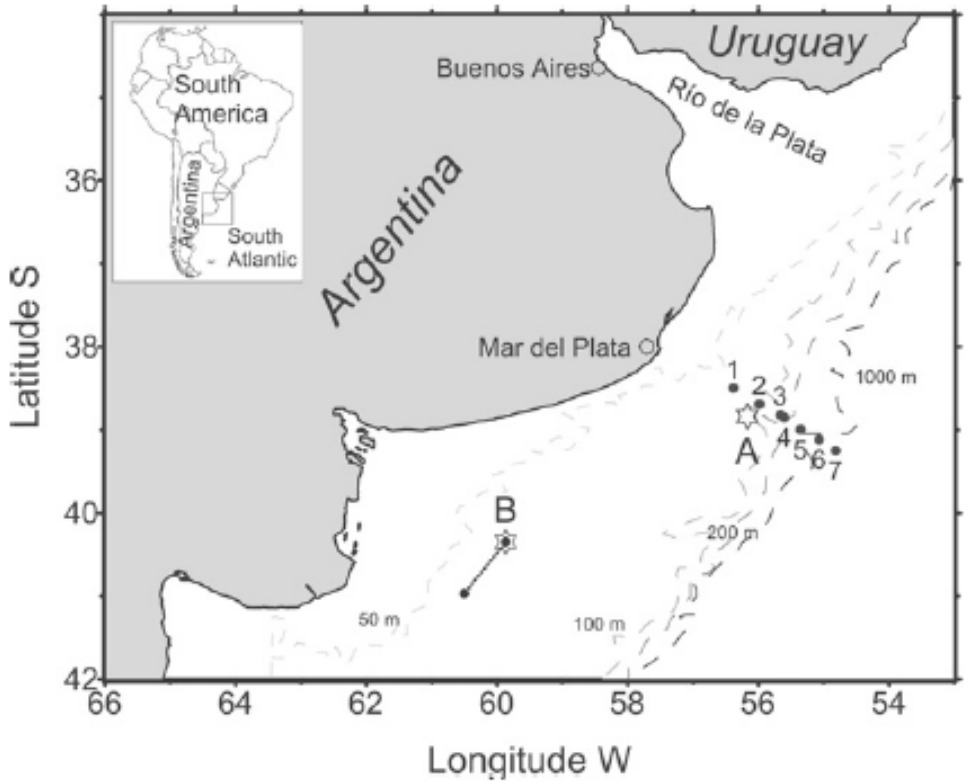


Fig. 1. *Azadinium* cf. *spinosum* blooms in the northern Argentine shelf. Areas of water discolorations recorded during the first bloom in November 1990 correspond to open star A (position of the samples S1 and S3 analyzed at "3.3. Grazing of *A. spinosum*") and open star B (straight line near B corresponds to a navigation section on brownish-red water patches). The seven stations marked along the transect with numbers 1–7 correspond to the oceanographic cruise at which the second bloom was recorded in September 1991.

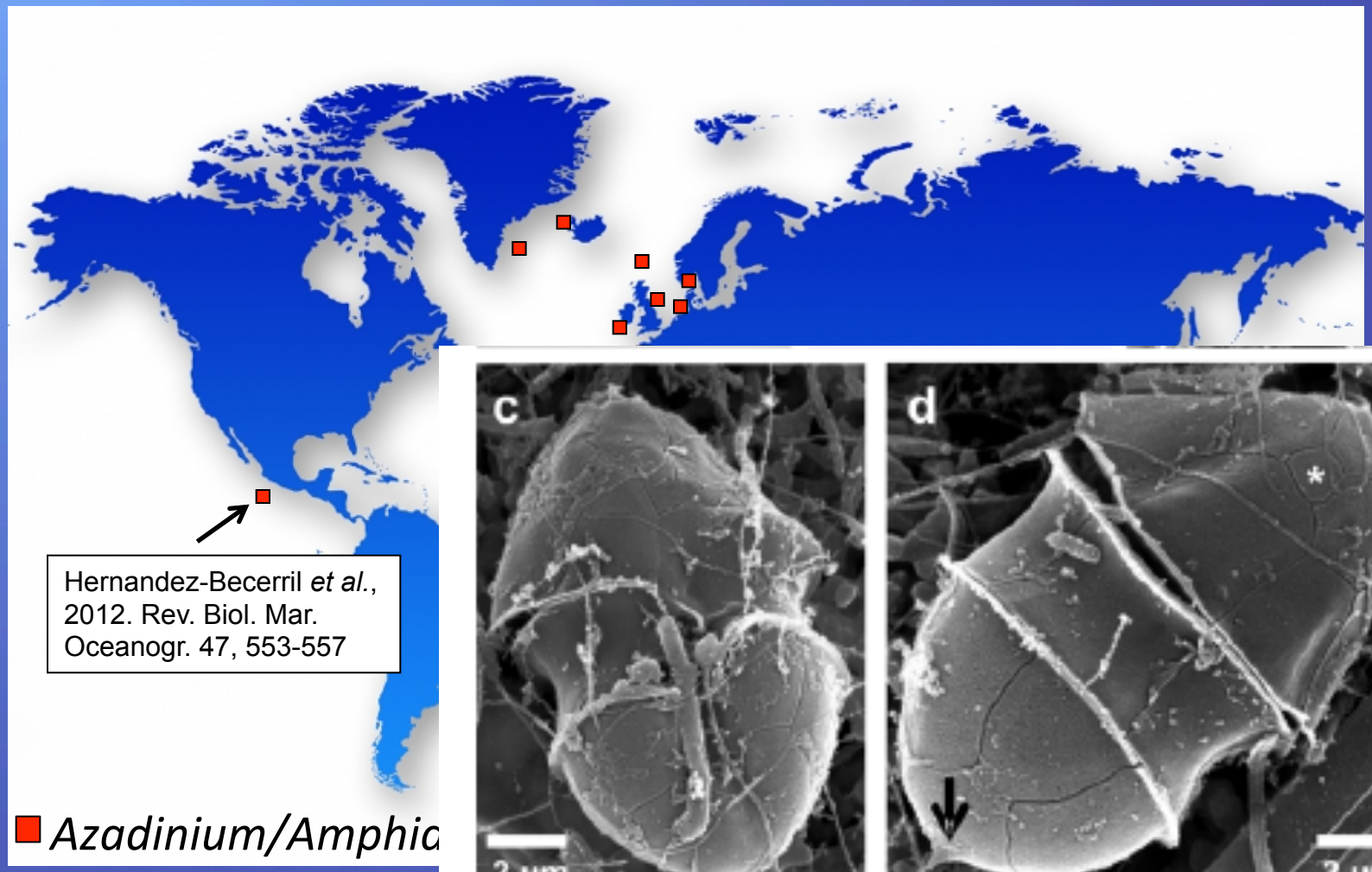
Bloom concentrations:

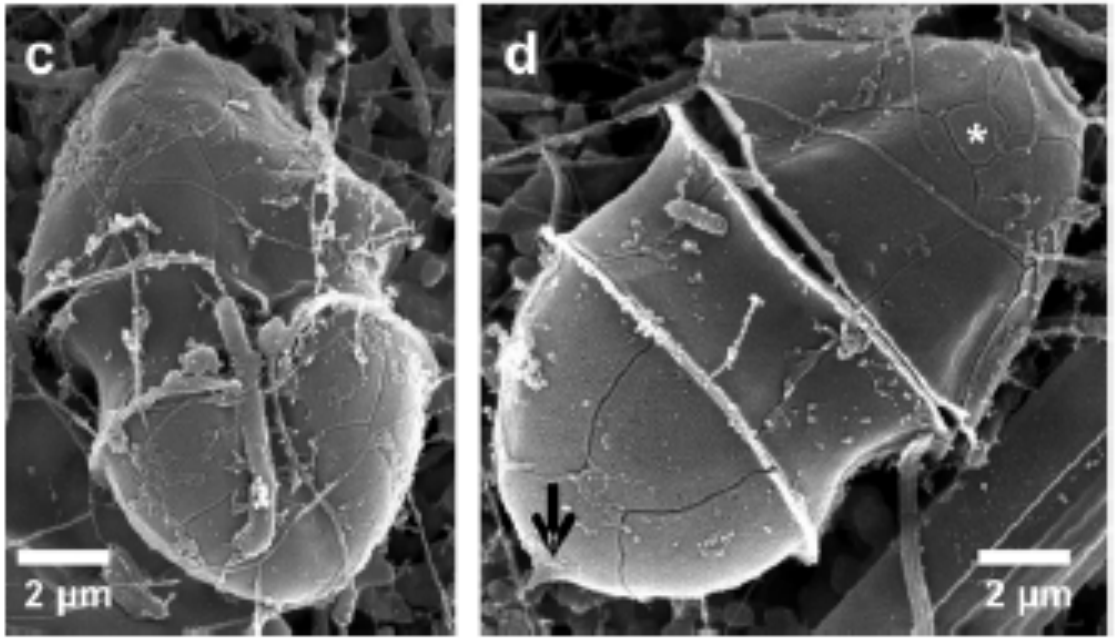
9×10^6 cell l^{-1} (1990)

$1.5 – 3 \times 10^6$ cells l^{-1} (1991)

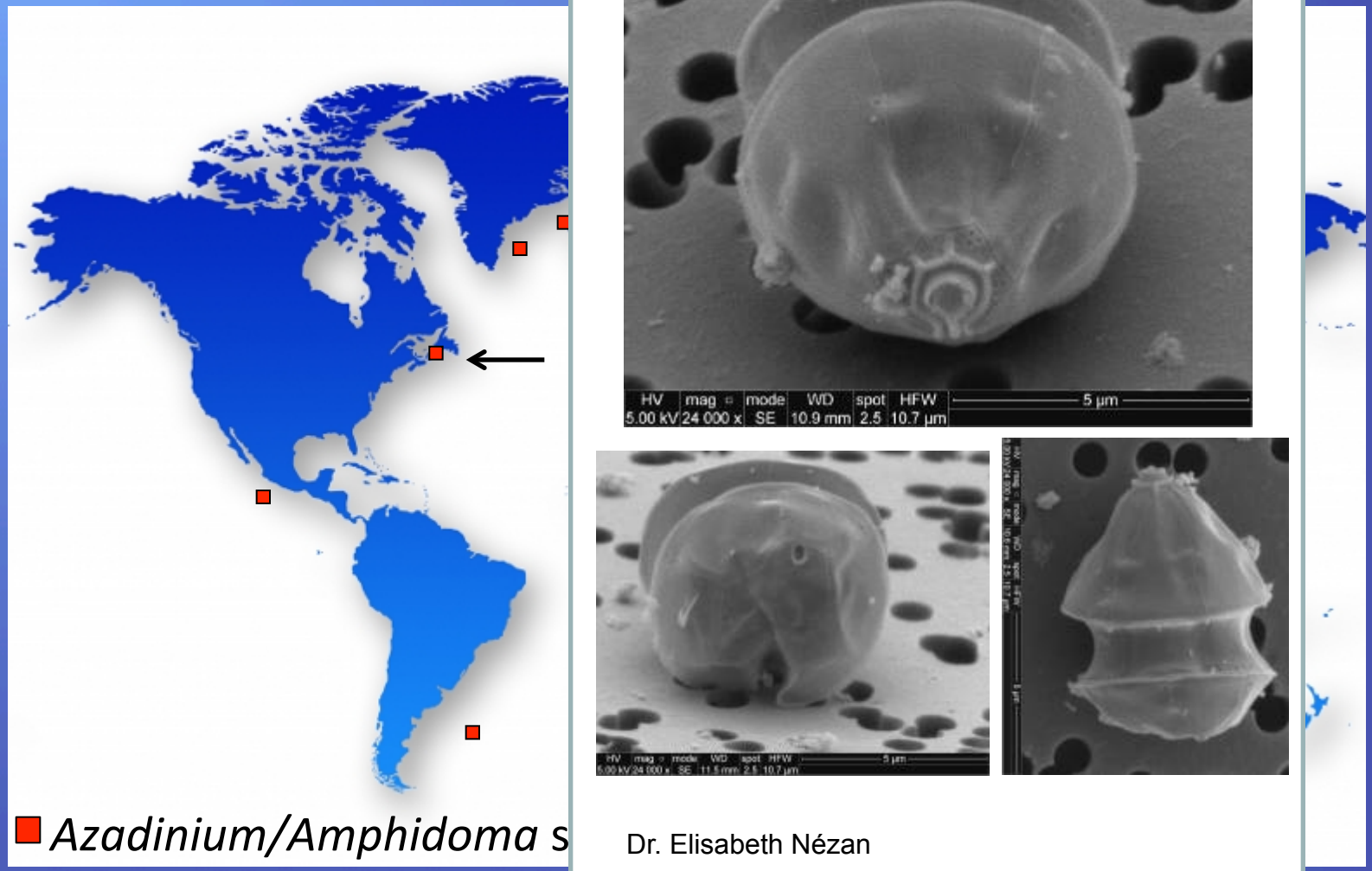
Akselman & Negri 2012

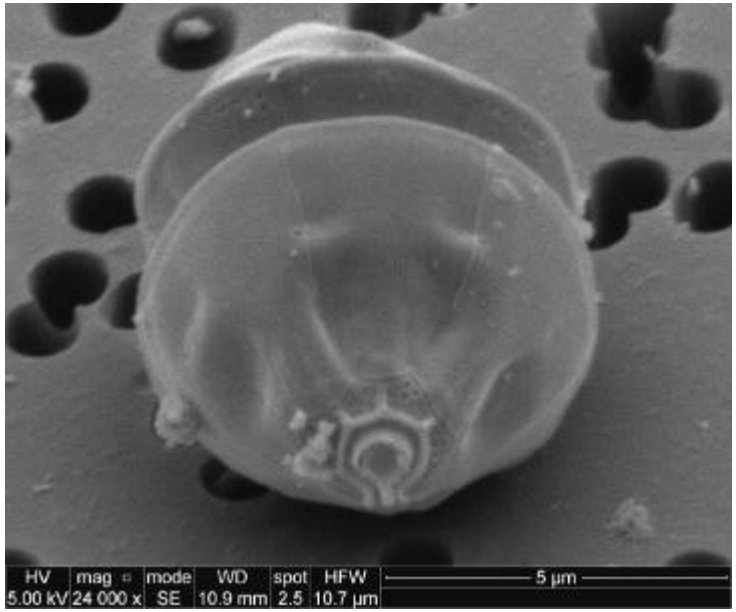
Amphidomataceae – global distribution





Amphidomatace n





Dr. Elisabeth Nézan

North/East Atlantik, Saint-Pierre and Miquelon (56°19.74'W, 47°06.66'N) in 2012

Amphidomataceae – global distribution

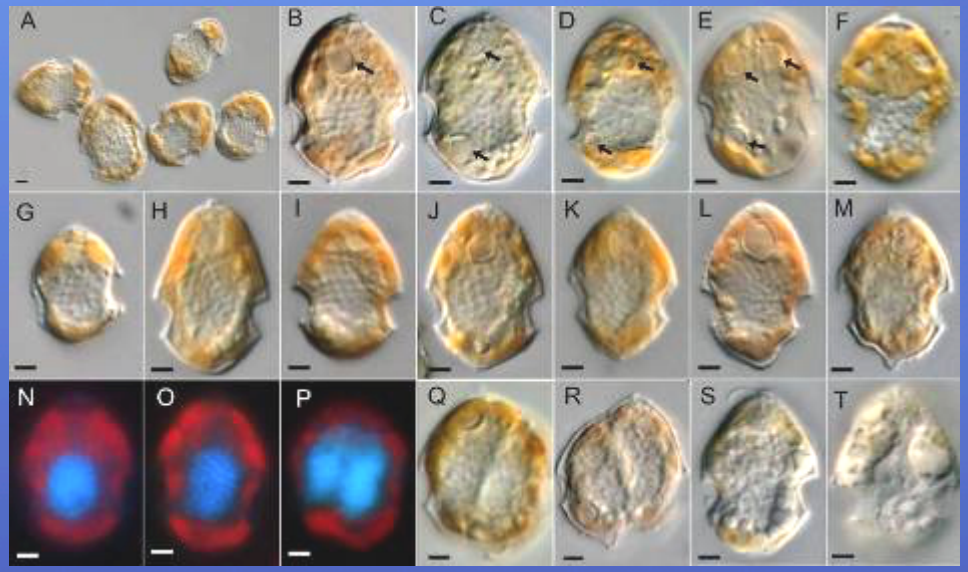
■ *Azadinium/Amphidoma* spp.

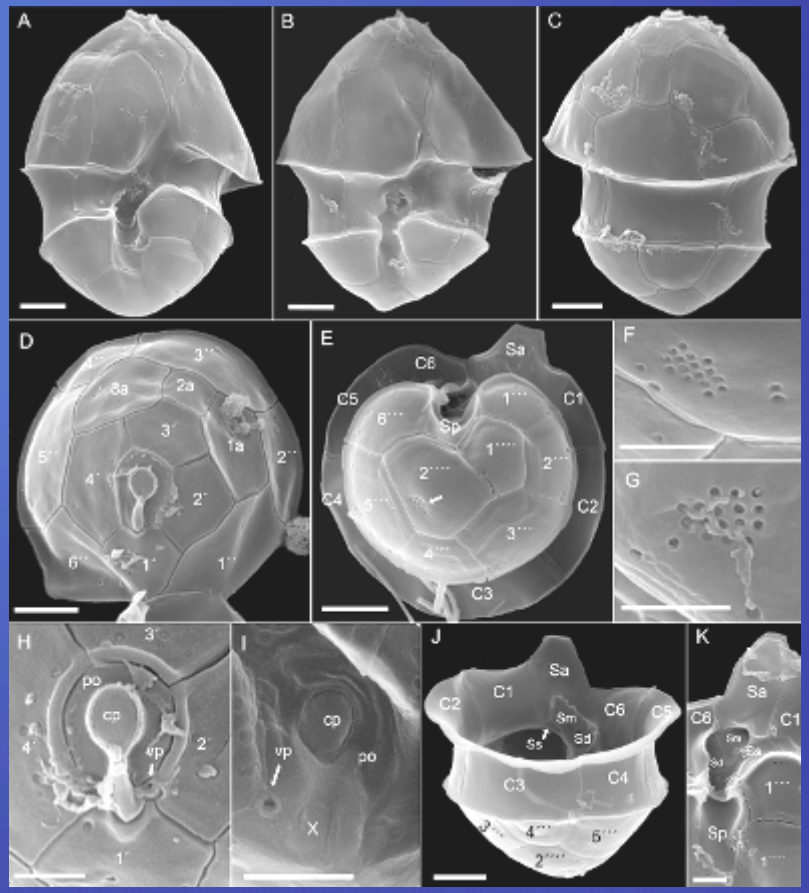
Amphidomataceae – global distribution

■ *Azadinium/Amphidoma* spp.

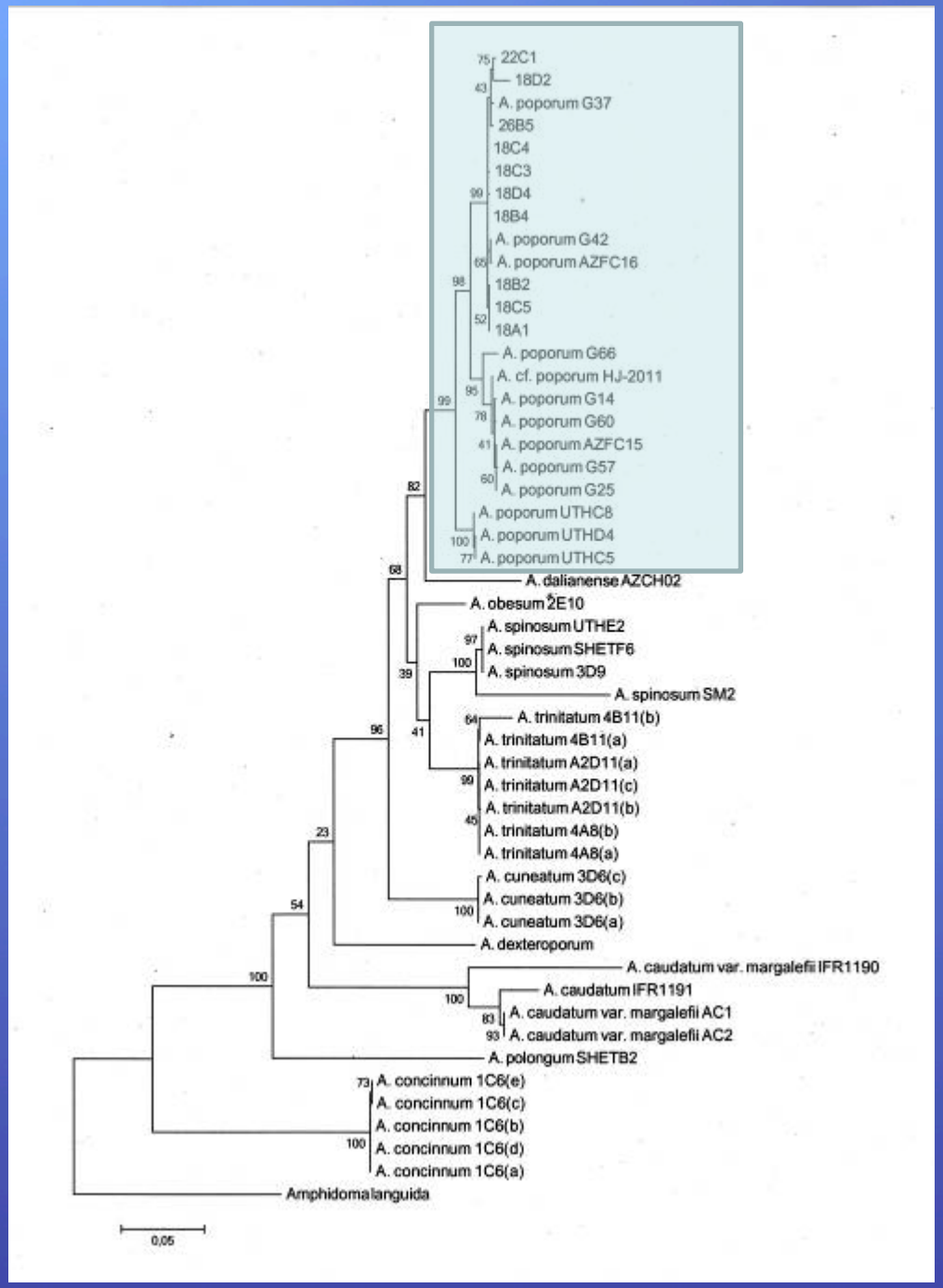
Amphidomataceae – global distribution

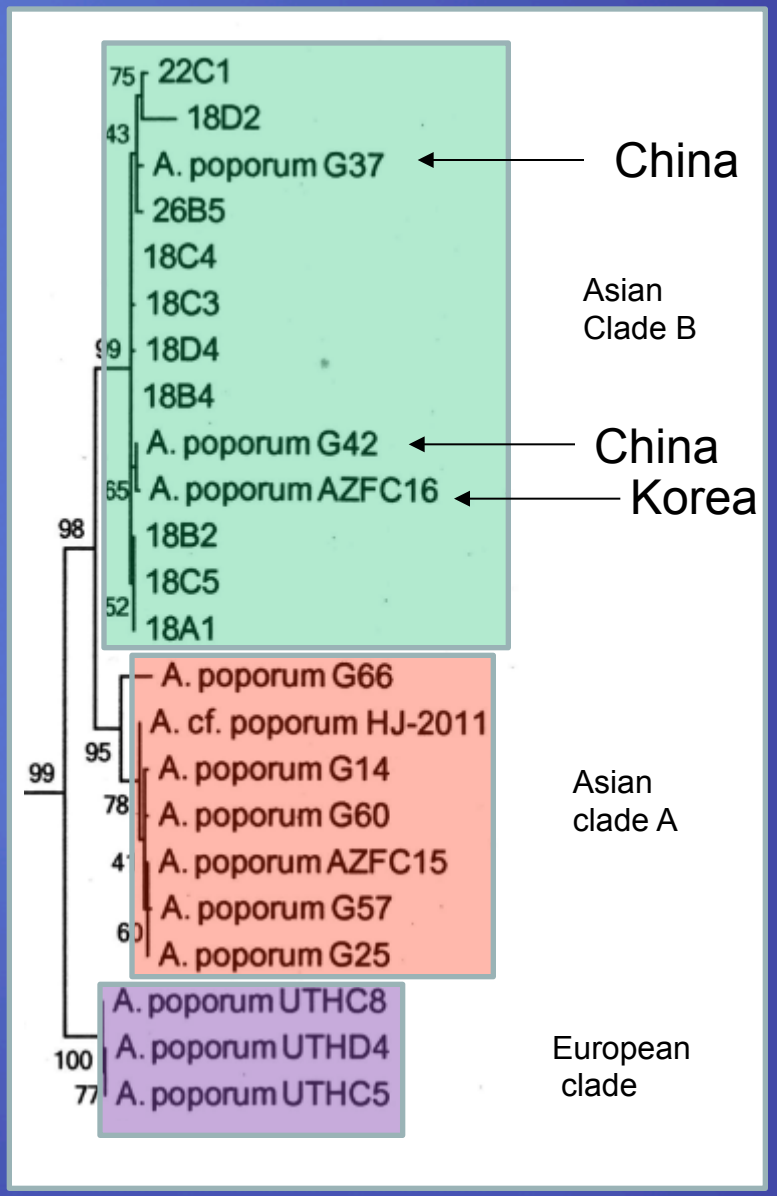
Azadinium poporum South Atlantic





Tillmann et al. 2016





Tillmann et al. 2016

Azadinium poporum, South Atlantic, Argentina

Azaspiracid profile: AZA -2

and a new AZA (MW 935)

→ AZA-2-phosphate….

AZA Cell quota: ~ 2-7 fg $cell^{-1}$

Tillmann et al. 2016

Amphidomataceae – global distribution

Harmful Algae 55 (2016) 56–65

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

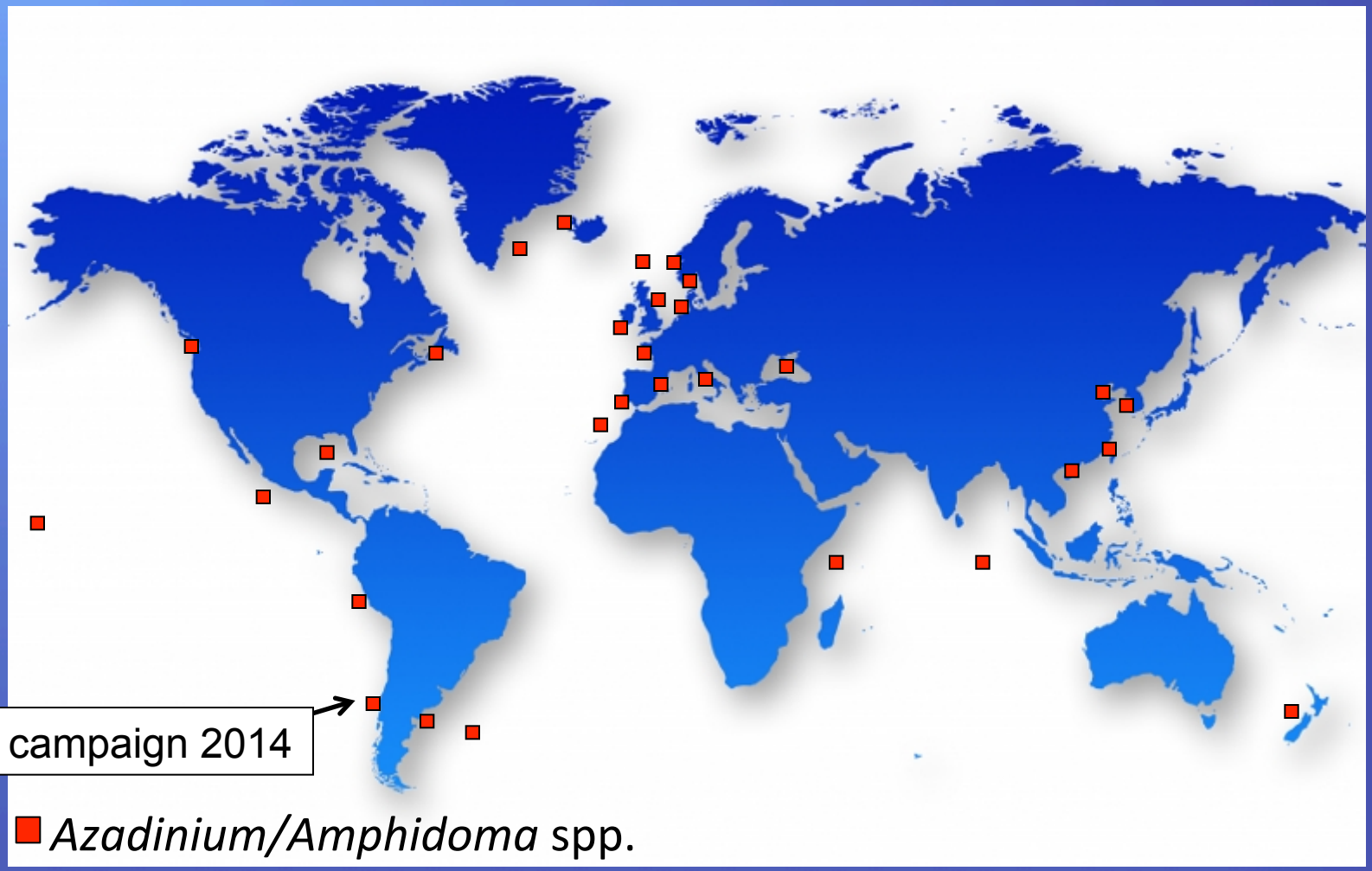
Review

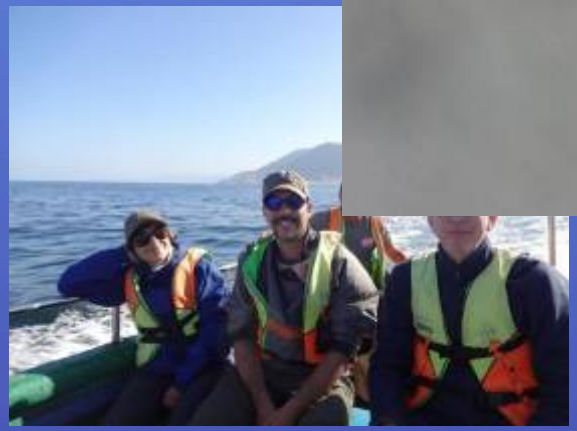
Morphology, molecular phylogeny and azaspiracid profile of *Azadinium poporum* (Dinophyceae) from the Gulf of Mexico

Zhaohe Luo [a,b], Bernd Krock [c,*], Kenneth Neil Mertens [d,1], Andrea Michelle Price [e], Robert Eugene Turner [f], Nancy N. Rabalais [g], Haifeng Gu [b,*]

■ *Azadinium/Amphidoma* spp.

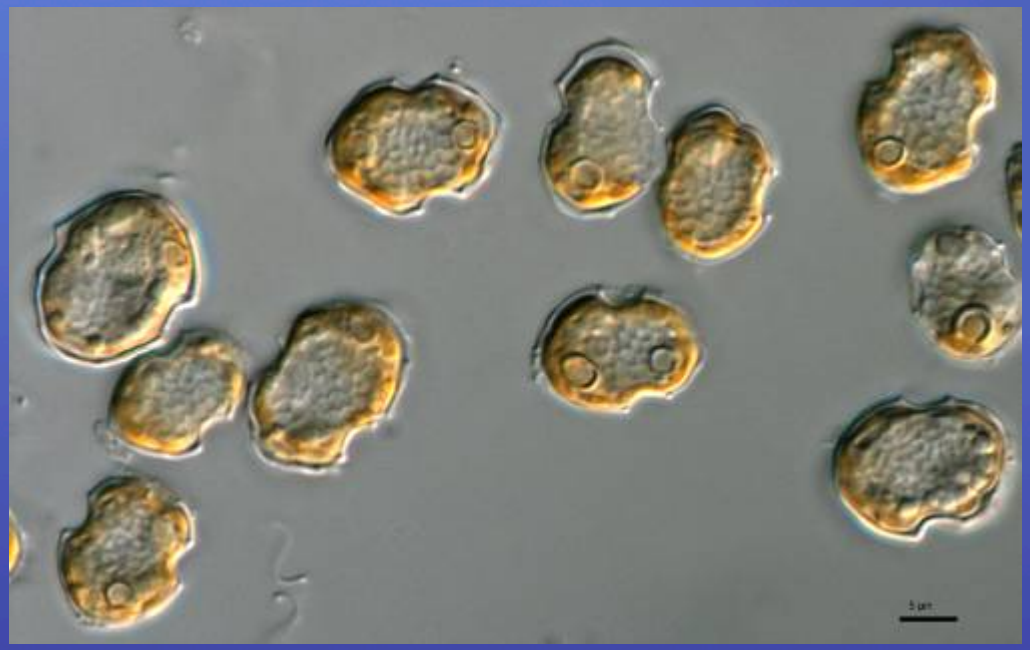
Amphidomataceae – global distribution





Azadinium poporum, South Pacific, Chile

3 clonal isolates



Tillmann, Trefault et al, in prep

Azadinium poporum, South Pacific, Chile

Azaspiracid profile: AZA -11

and a new AZA (MW 910)

→ AZA-11-phosphate….

Cell quota: ~5 fg cell-1

Tillmann, Trefault et al in prep

Molecular tree

Asian Clade A

A new clade D
A. poporum from the Gulf of Mexico
Luo et al. 2016

Asian Clade B

European Clade

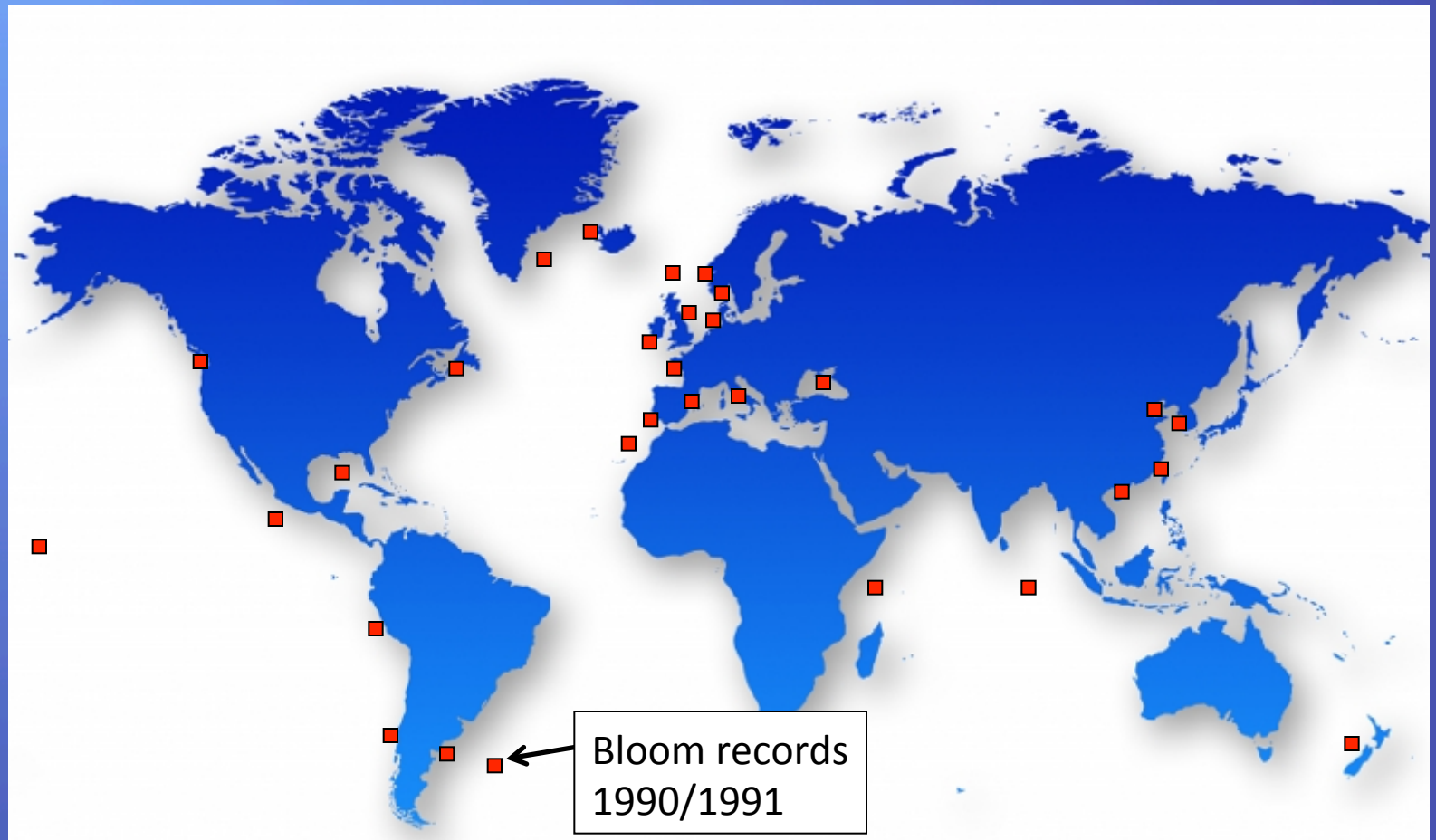
Chile isolates

ML tree using only **ITS** sequences of *A. poporum*

A. dalianense AZCH02 was used as outgroup. 1000 bootstrap replications.

Tillmann, Trefault et al in prep

Amphidomataceae – global distribution



■ *Azadinium/Amphidoma* spp.

Harmful Algae 19 (2012) 30–38

Contents lists available at SciVerse ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Blooms of *Azadinium* cf. *spinosum* Elbrächter et Tillmann (Dinophyceae) in northern shelf waters of Argentina, Southwestern Atlantic

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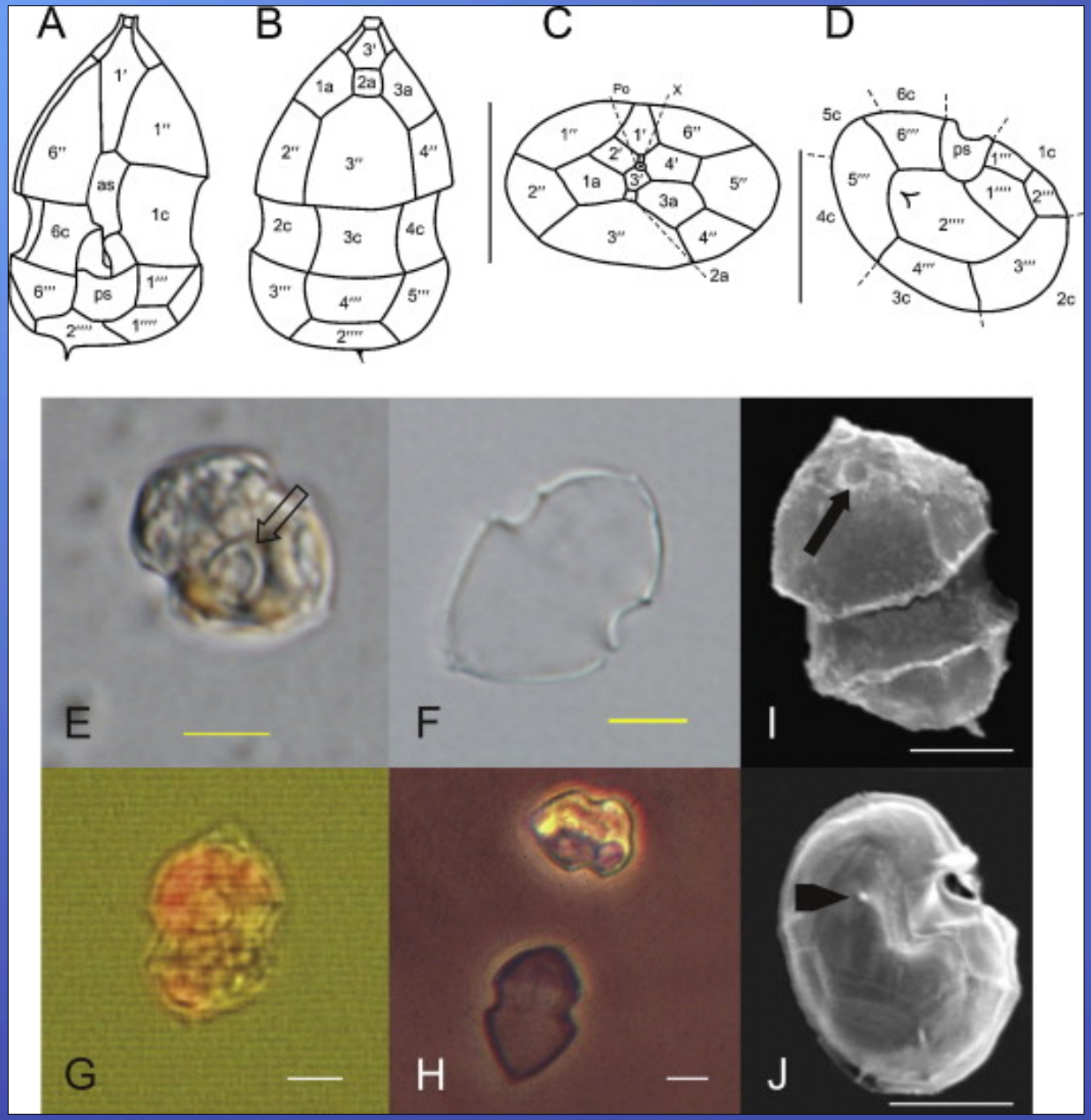
ARTICLE

Azadinium (Amphidomataceae, Dinophyceae) in the Southwest Atlantic: *In situ* and satellite observations

Azadinium (Amphidomataceae, Dinophyceae) en el Atlántico Sudoccidental: Observaciones *in situ* y satelitales

Rut Akselman[1], Rubén M. Negri[1] and Ezequiel Cozzolino[1]

[1]Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP, V. Ocampo 1, Escollera Norte, B7602HSA-Mar del Plata, Argentina. rutaks@inidep.edu.ar



Akselman & Negri 2012

The Argentinean 1991 bloom species: *Azadinium luciferelloides* sp. nov.

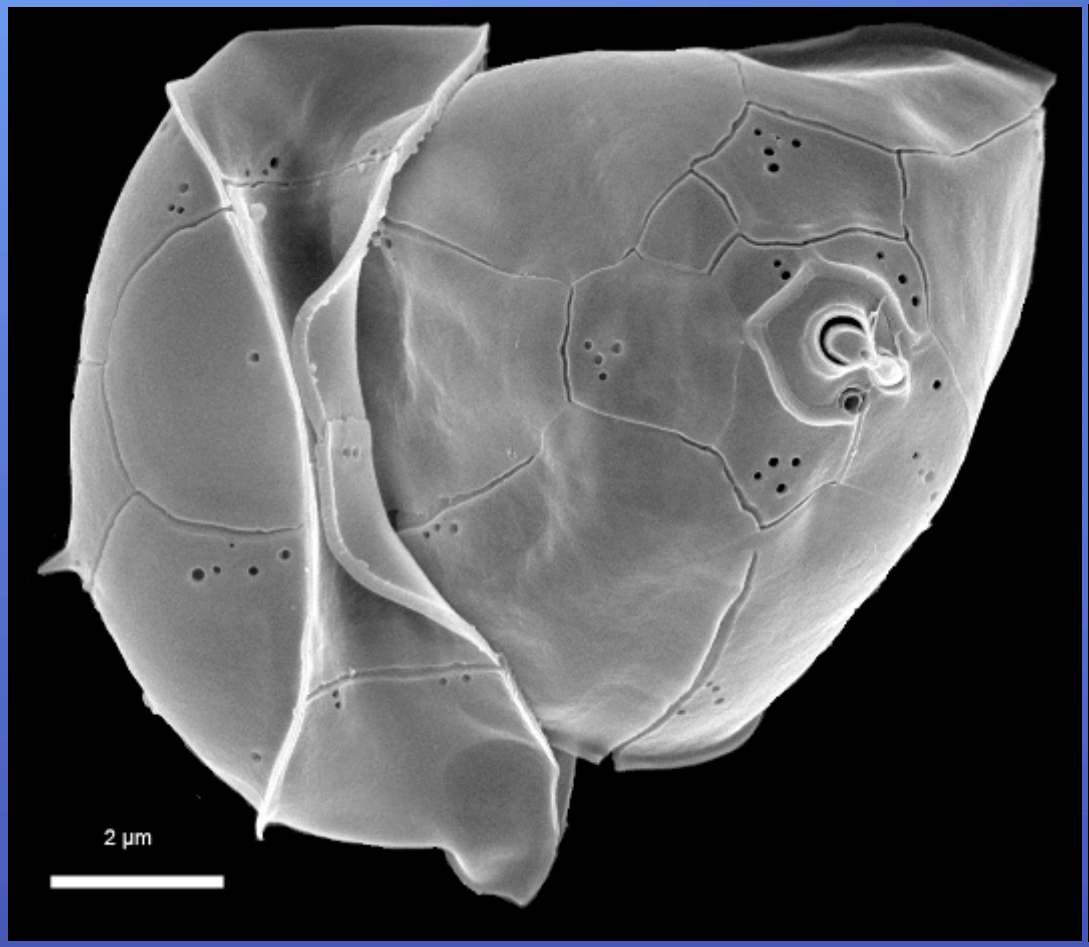
Tillmann & Akselman, (almost…) acceped

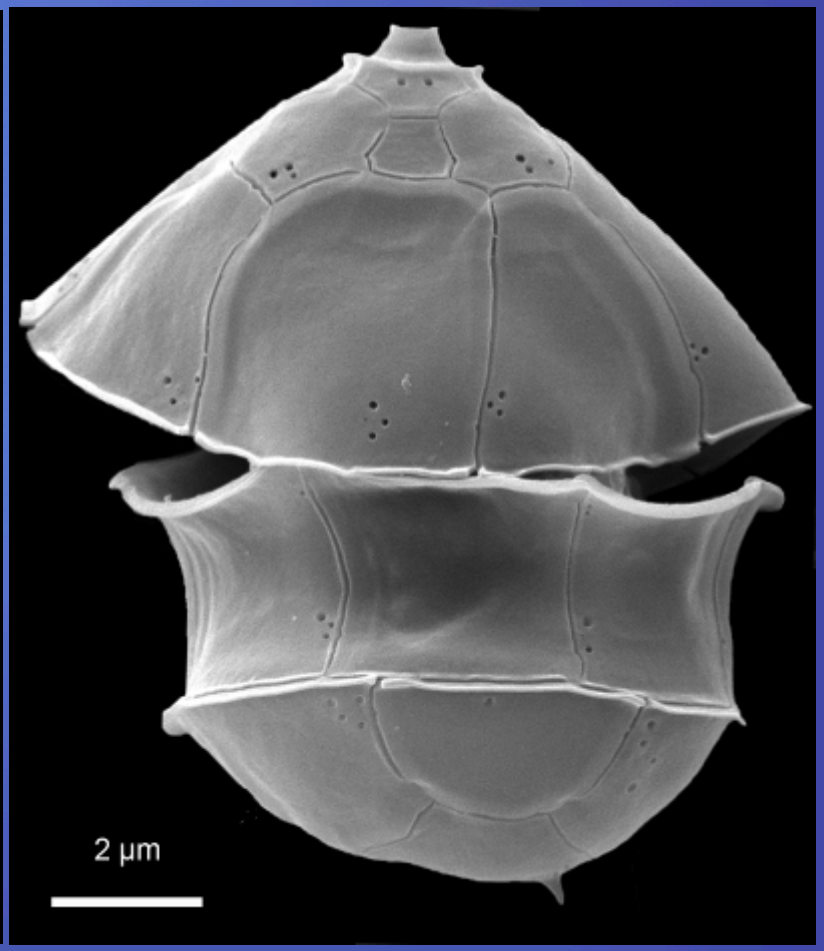
Azadinium spinosum 3D9

Azadinium luciferelloides

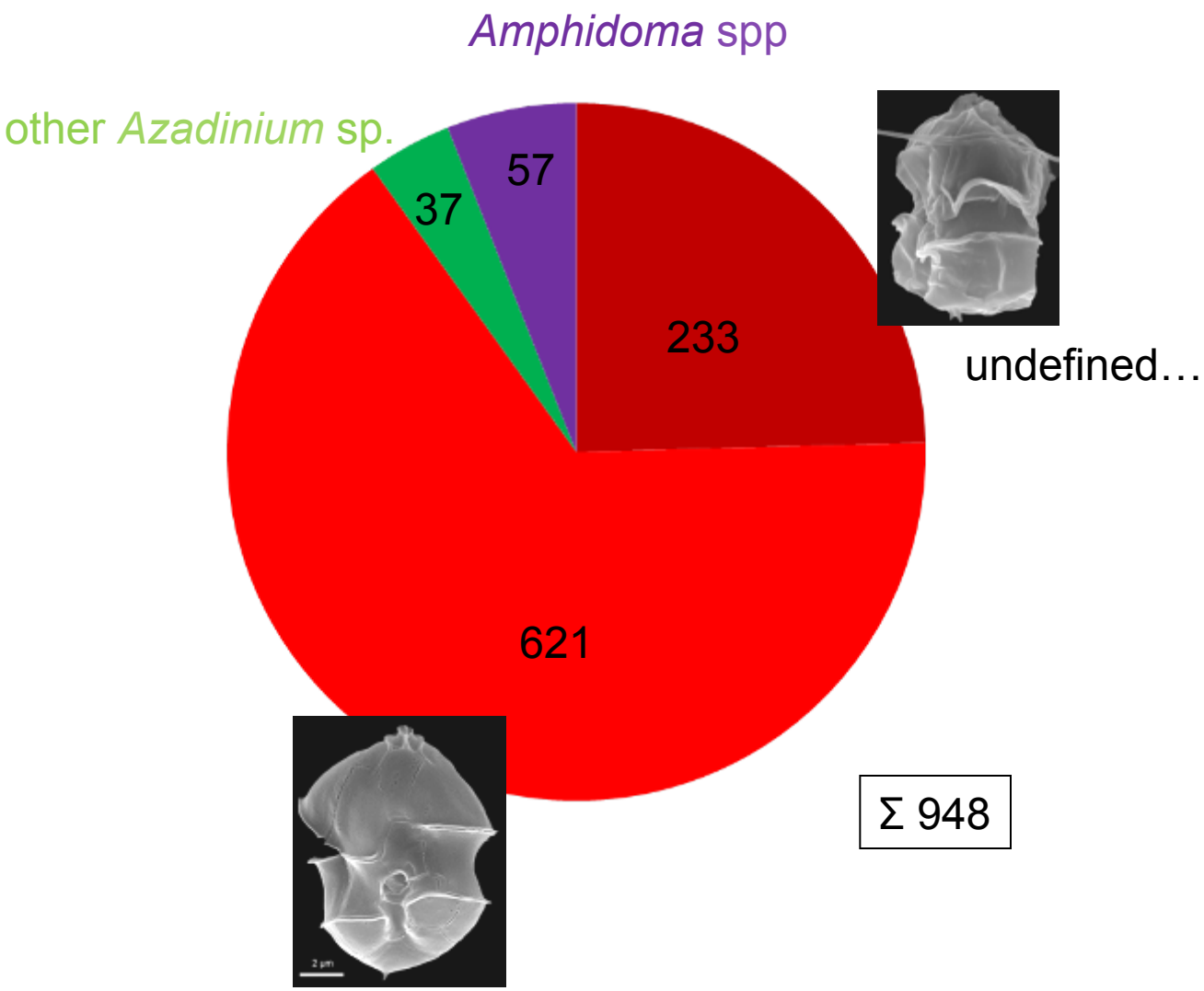
Tillmann & Akselman, (almost…) acceped

The Argentinean 1991 bloom species:
Azadinium luciferelloides





Tillmann & Akselman, (almost…) acceped

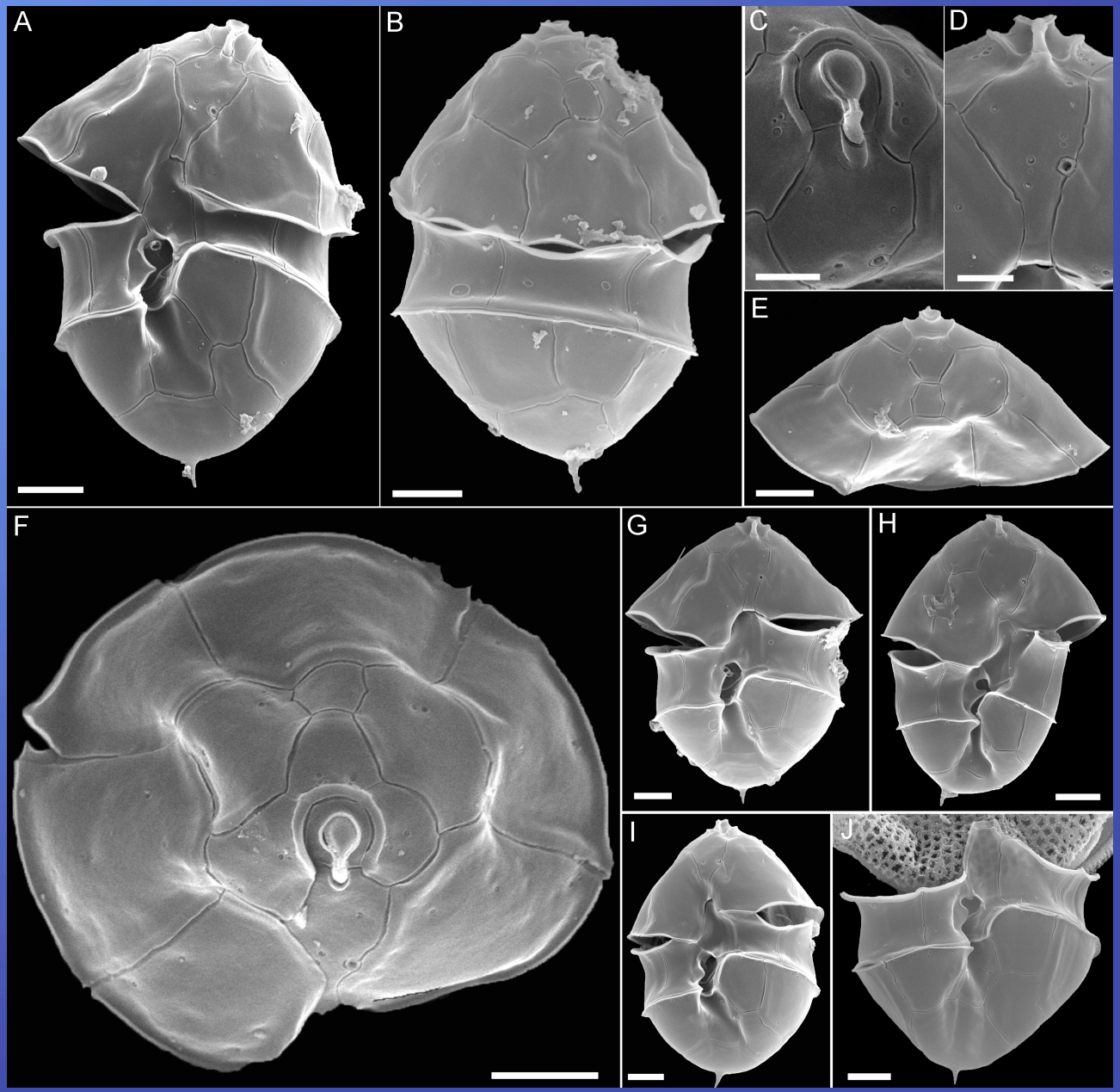


Azadinium luciferelloides
87 % of identifiable Amphidomataceae

Tillmann & Akselman, (almost…) acceped

Argentinean
bloom sample 1991

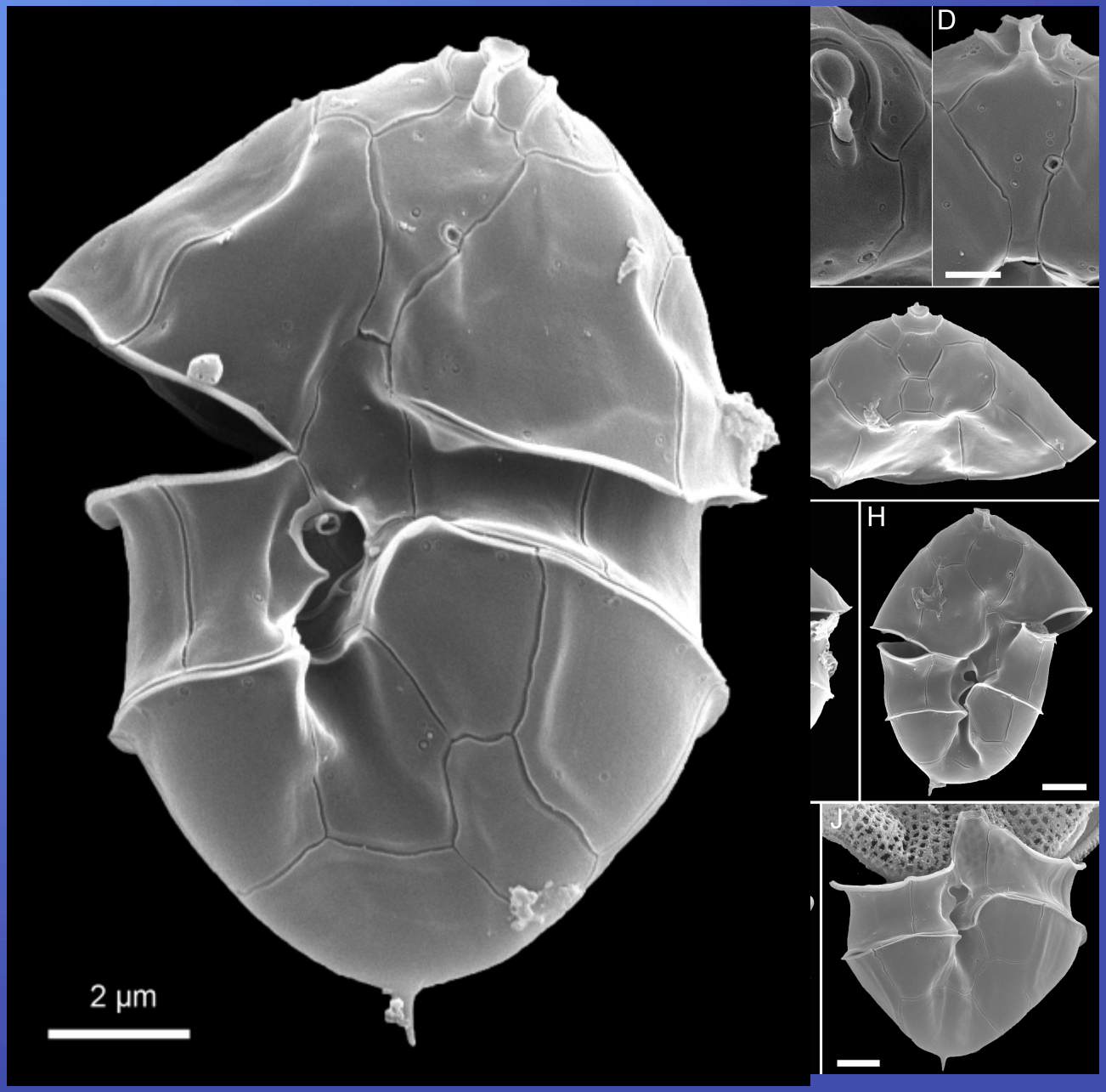
A. spinosum



Tillmann & Akselman, (almost…) acceped

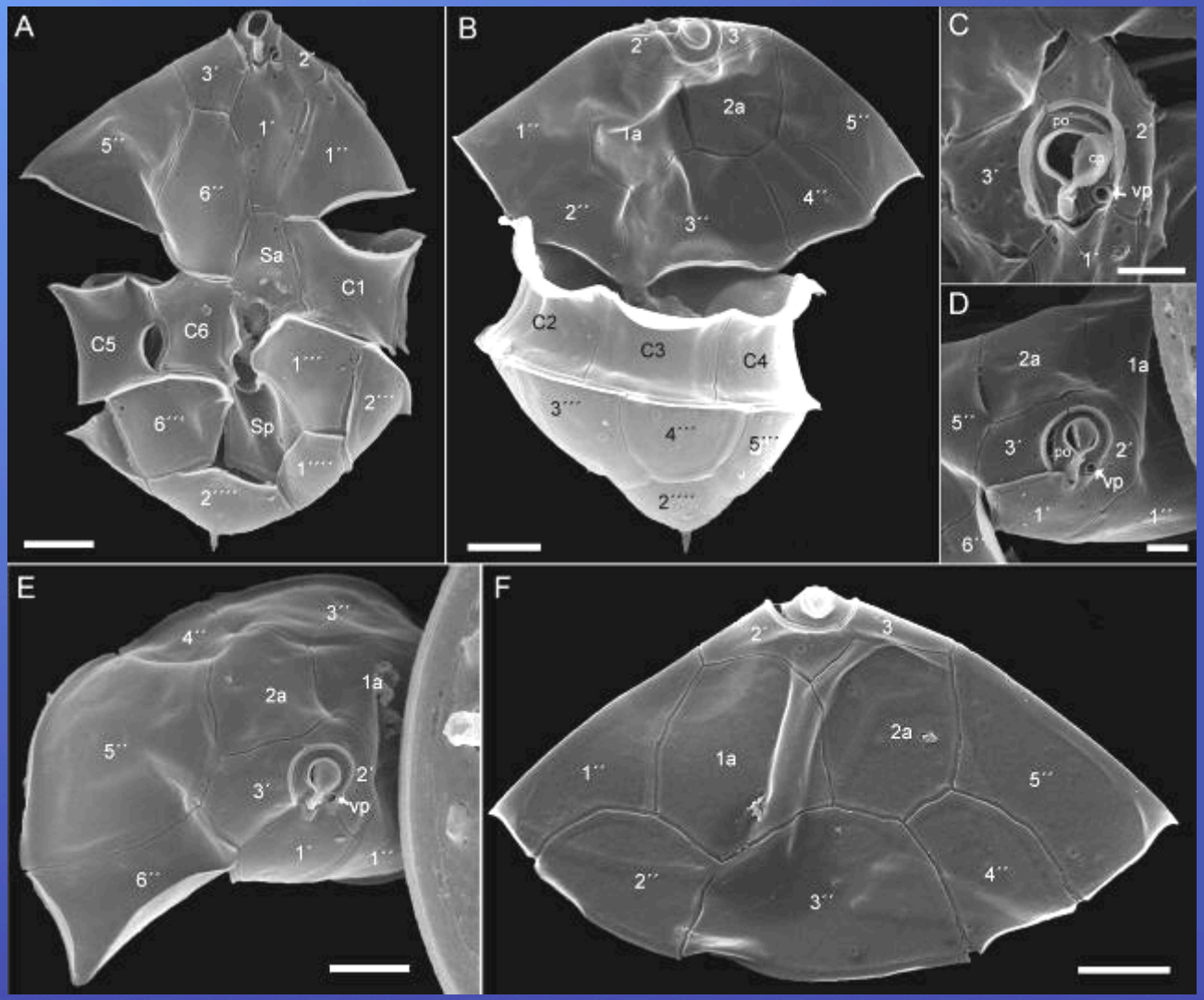
Argentinean
bloom sample 1991

A. spinosum

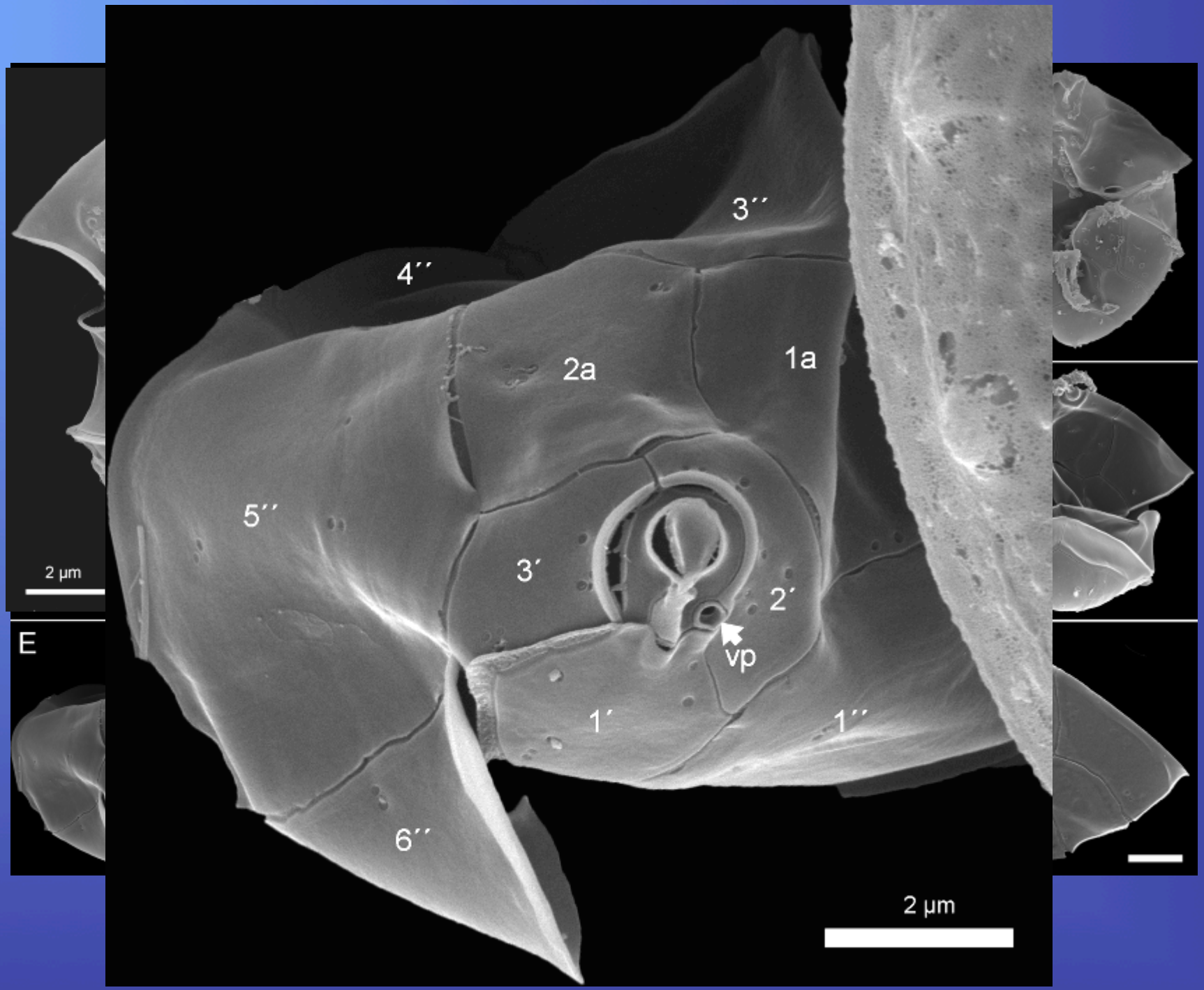


Tillmann & Akselman, (almost…) acceped

Argentinean bloom sample 1991 *A. dalianense*

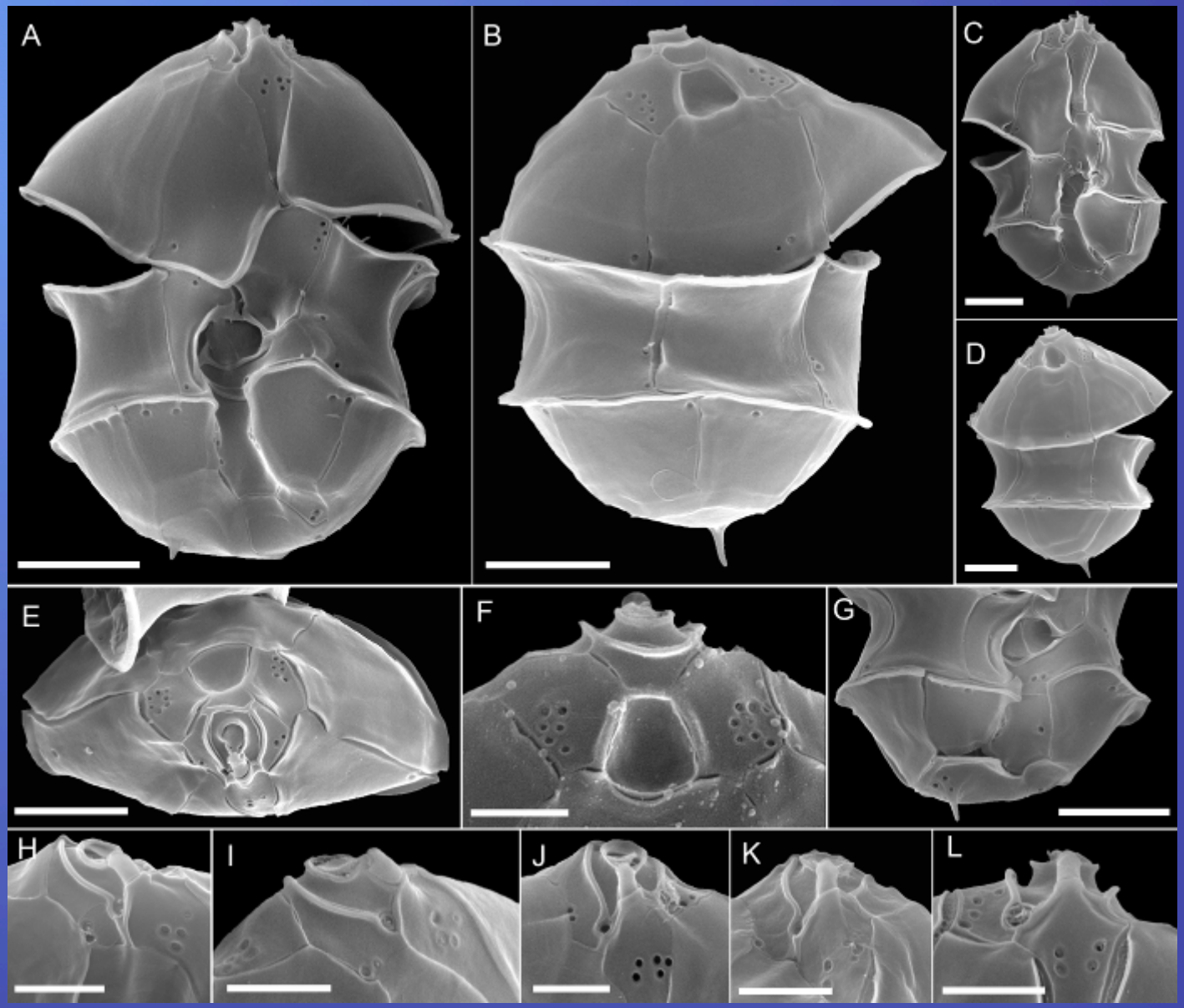


Tillmann & Akselman, (almost…) acceped


3´´
4´´
2a
1a
5´´
3´
2´
vp
1´
1´´
6´´
2 µm
2 µm
E

Argentinean
bloom sample 1991

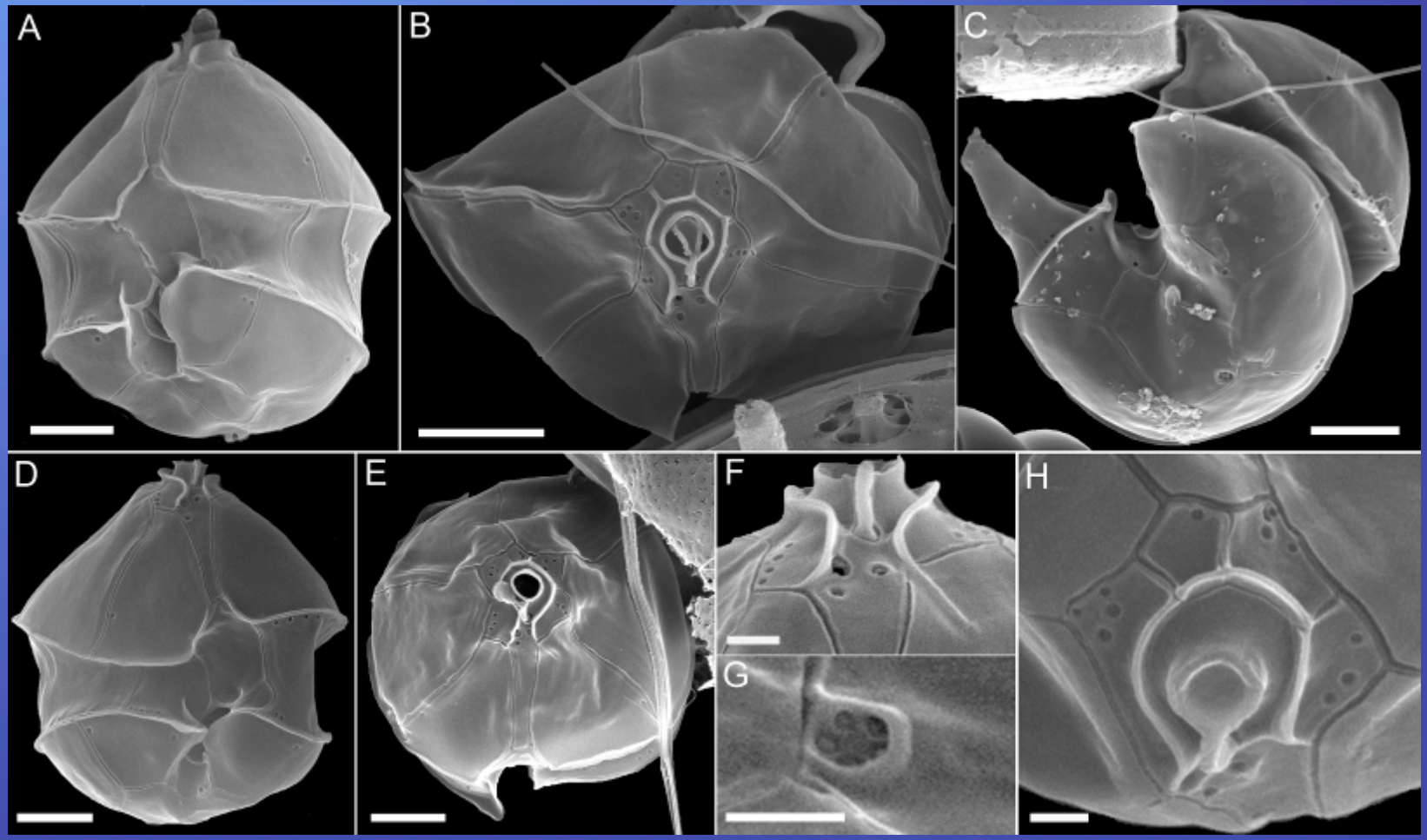
A. dexteroporum



Tillmann & Akselman, (almost…) acceped

Argentinean
bloom sample 1991

Amphidoma languida



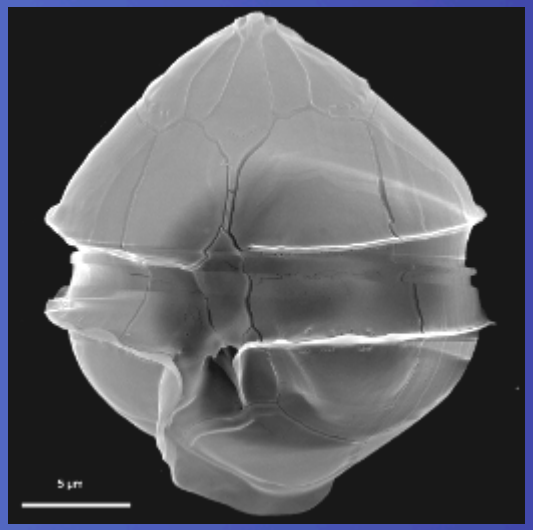
Tillmann & Akselman, (almost…) acceped

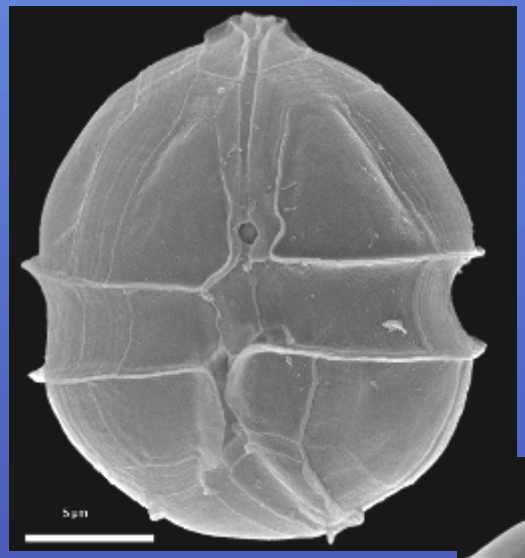
but that´s not all.....

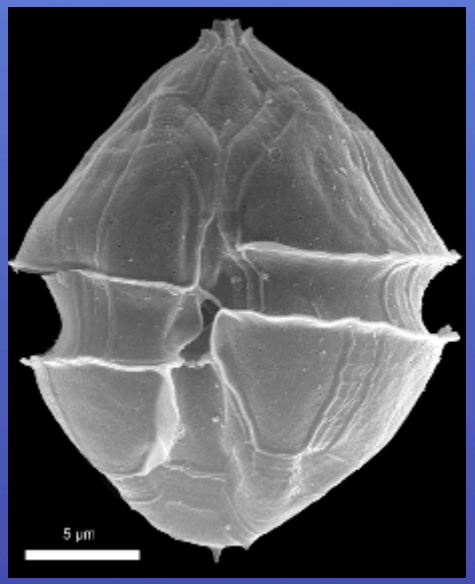
at least 4 other species of *Amphidoma*

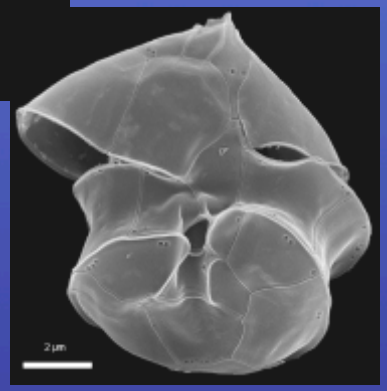
and one more of *Azadinium*

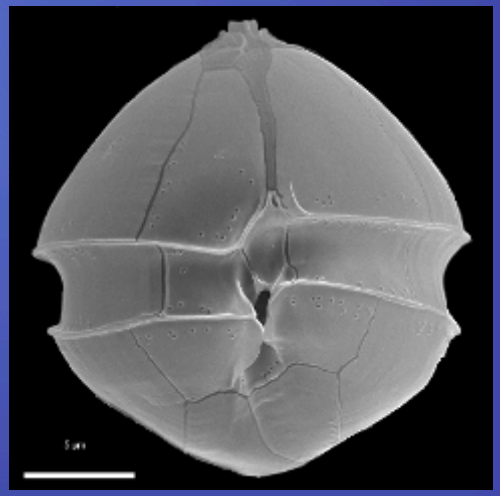
all new species waiting for their formal description



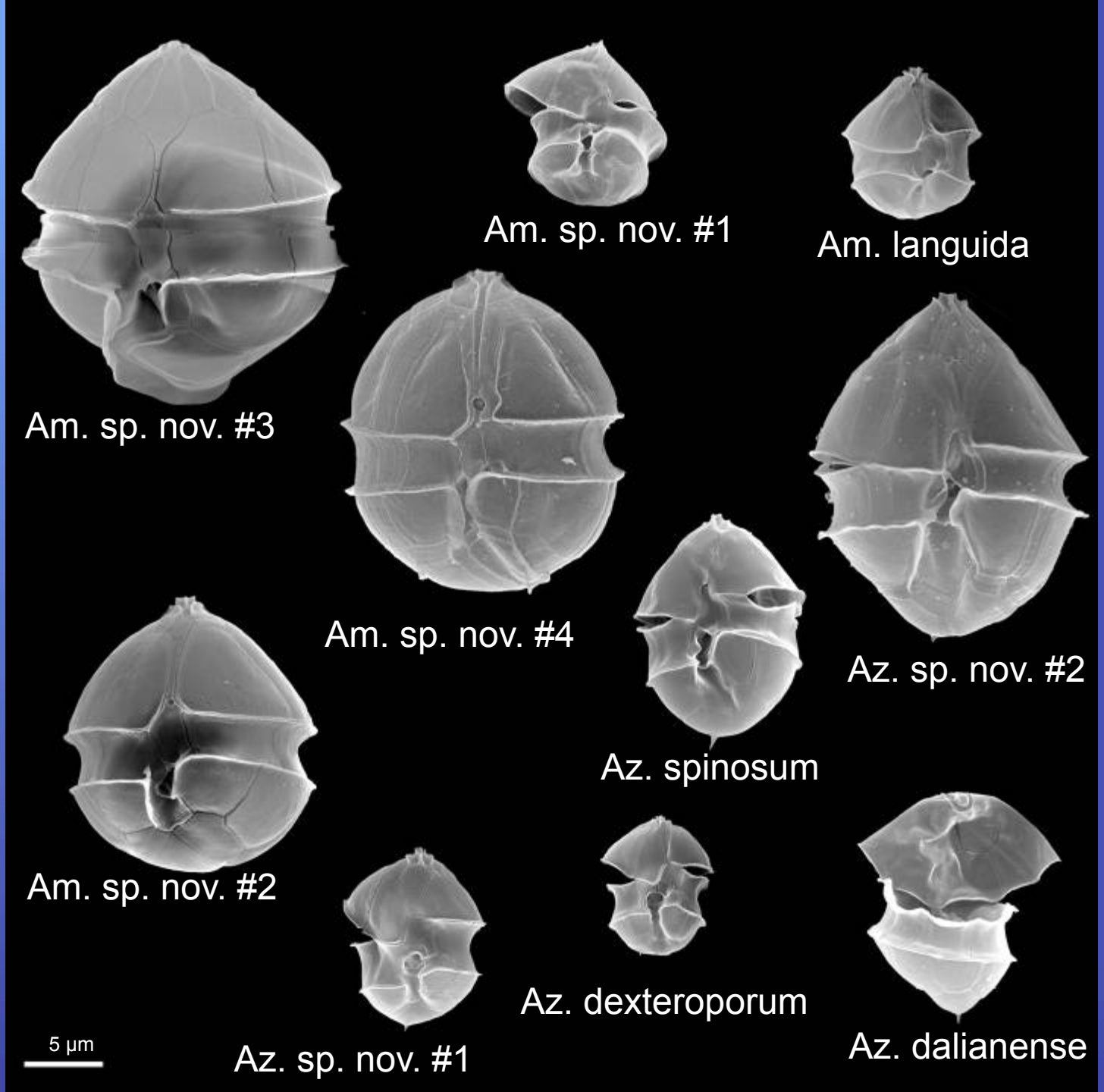




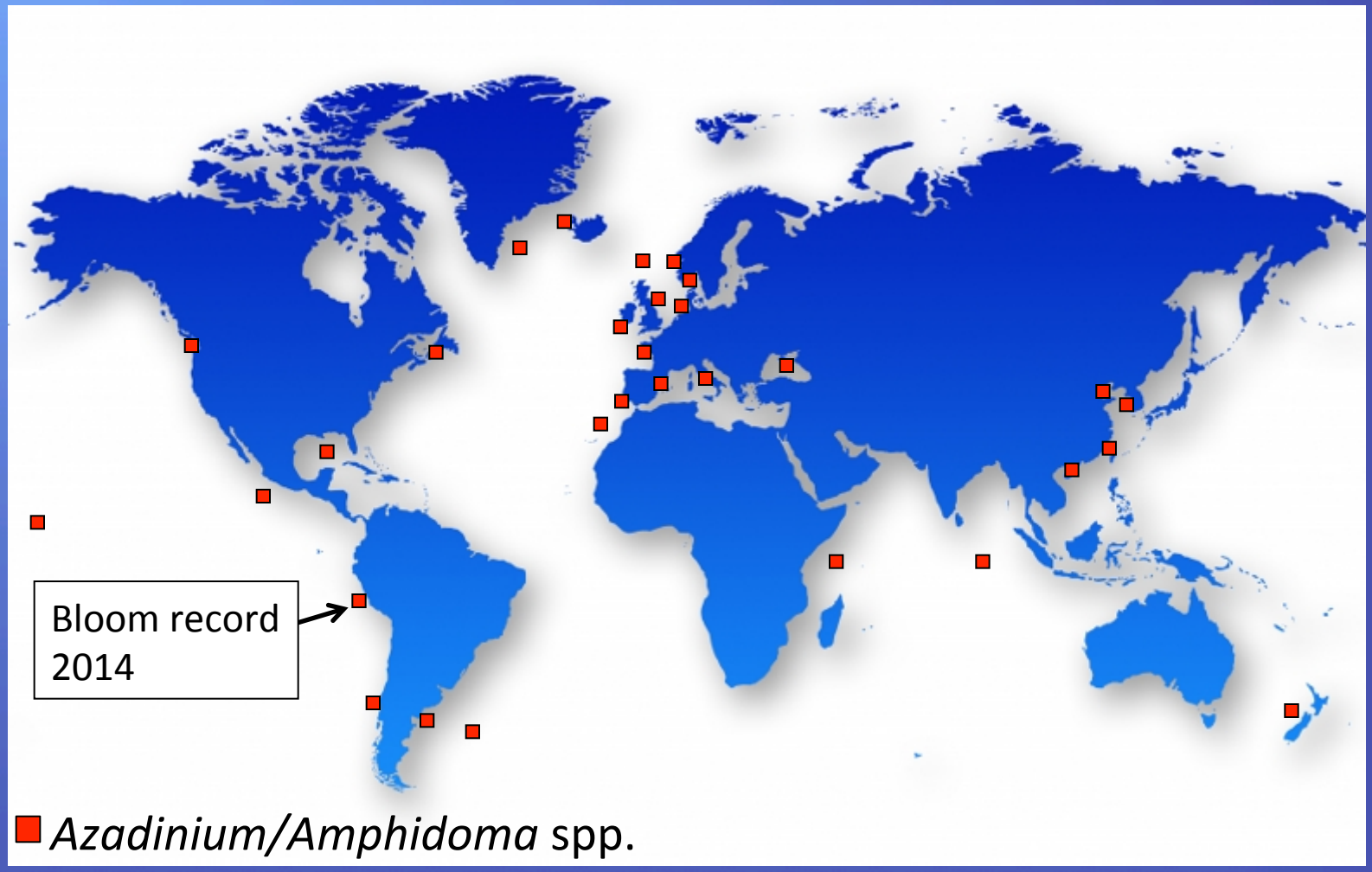




Tillmann & Akselman, (unpublished


Am. sp. nov. #3
Am. sp. nov. #1
Am. languida
Am. sp. nov. #4
Az. sp. nov. #2
Az. spinosum
Am. sp. nov. #2
Az. dexteroporum
Az. dalianense
Az. sp. nov. #1
5 µm

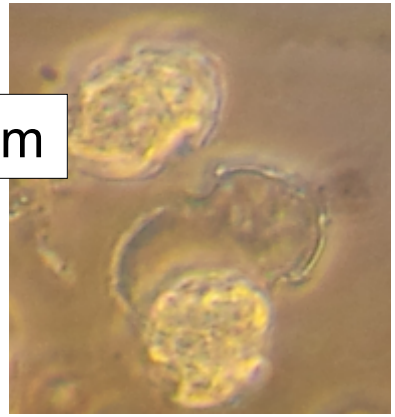
Amphidomataceae – global distribution



LABORATORIO DE FITOPLANCTON Y PRODUCCION PRIMARIA

REPORTE TECNICO FLORACION ALGAL Nº 004 - 2014	
1.- ESPECIE CAUSANTE:	cf. *Azadi* (cf = con Concentración celular: $7x10^5$ cel.L^{-1} (Estación 2-10 mn) $1x10^6$ cel.L^{-1} (Estaciòn 3- dentro de 5 mn)
2.- LUGAR Y COLORACIÓN	
3.- FECHA Y DURACION:	
4.- CARACTERISTICAS:	rgo y 7–11 µm de ancho, considerado como especie tóxica, produce toxinas de tipo azasparácidos (lipofílicas).
5.- OTRAS ESPECIES:	*Skeletonema costatum, Thalassiosira subtilis, Prorocentrum minimum, Protoperidinium depressum*, entre otros.
6.- DATOS DEL AMBIENTE:	Prof (m) 0 / TSM (°C) 20,5 - 20,8 / Transparencia (m) 1,5 – 2 / Oxígeno (mL.L^{-1}) 5,54 – 7,85
7.- DISTRIBUCIÓN	Irlanda (1997), F mexicano (2010)
8.- OBSERVACIONES	El envenenamie intoxicación diar se presentan en los seres humanos. En otras latitudes, ha causado muerte como es el caso de los mejillones azules (*Mytilus edulis*), Irlanda.
9.- REFERENCIAS:	Tillmann *et al*., 2009
10.- ANALISTA (S):	Avy Bernales, Nelly Jacobo
11.- VERIFICADOR:	Elcira Delgado, Sonia Sánchez

cf. Azadinium spinosum



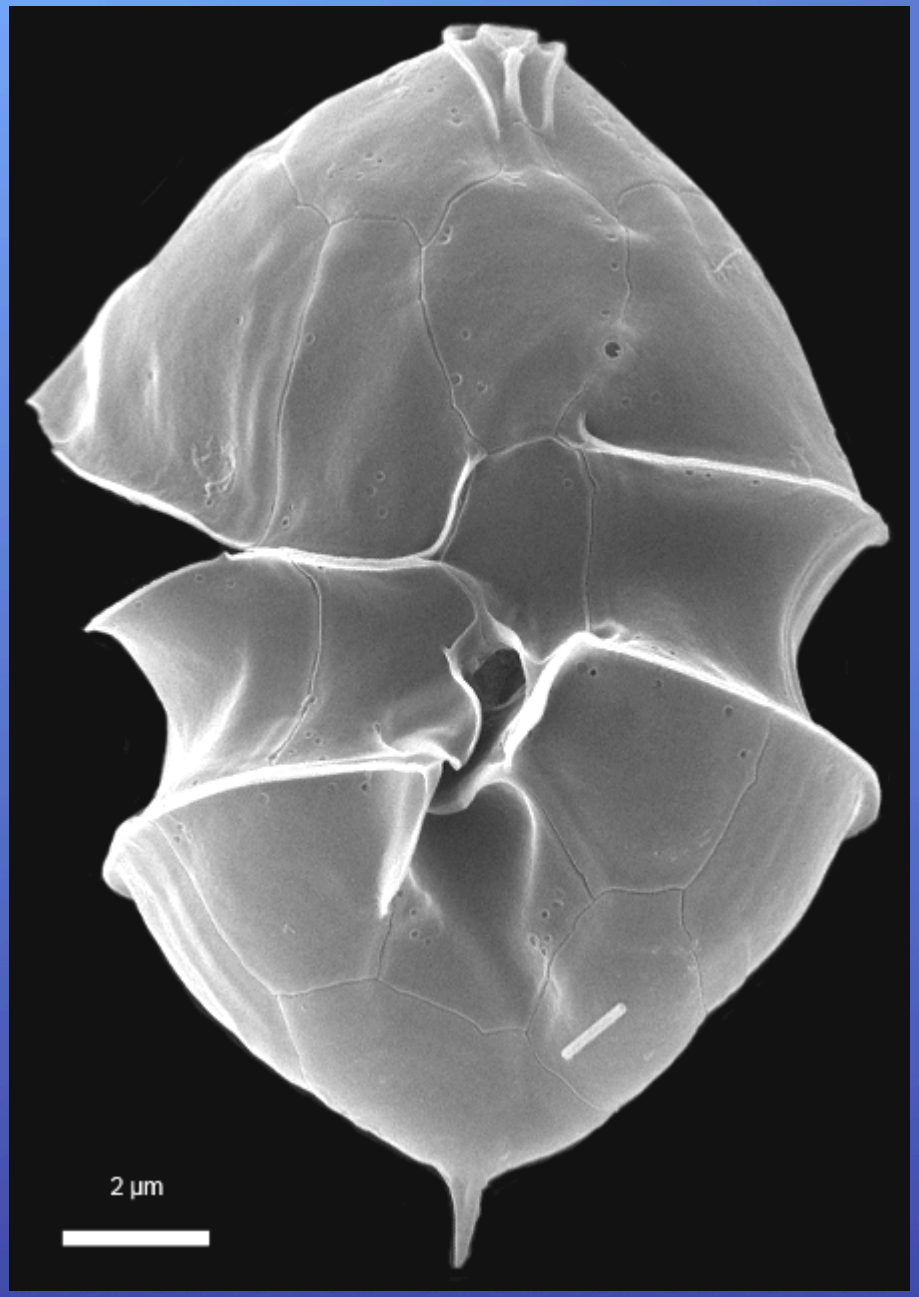
cell density
7×10^5 L^{-1} (Stat. 2)
1×10^6 L^{-1} (Stat. 3)

water temperature
20.5 – 20.8 °C

bloom sample
(2 L, GF/C filter) → no AZAs

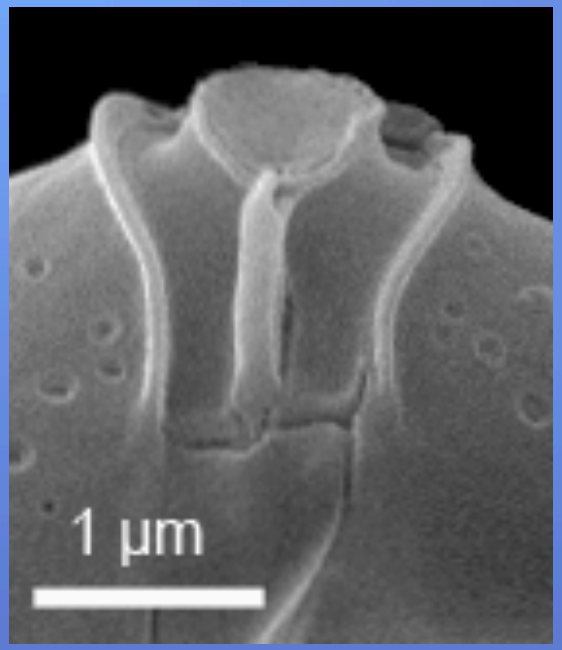
bloom sample
(formalin fixed) → SEM

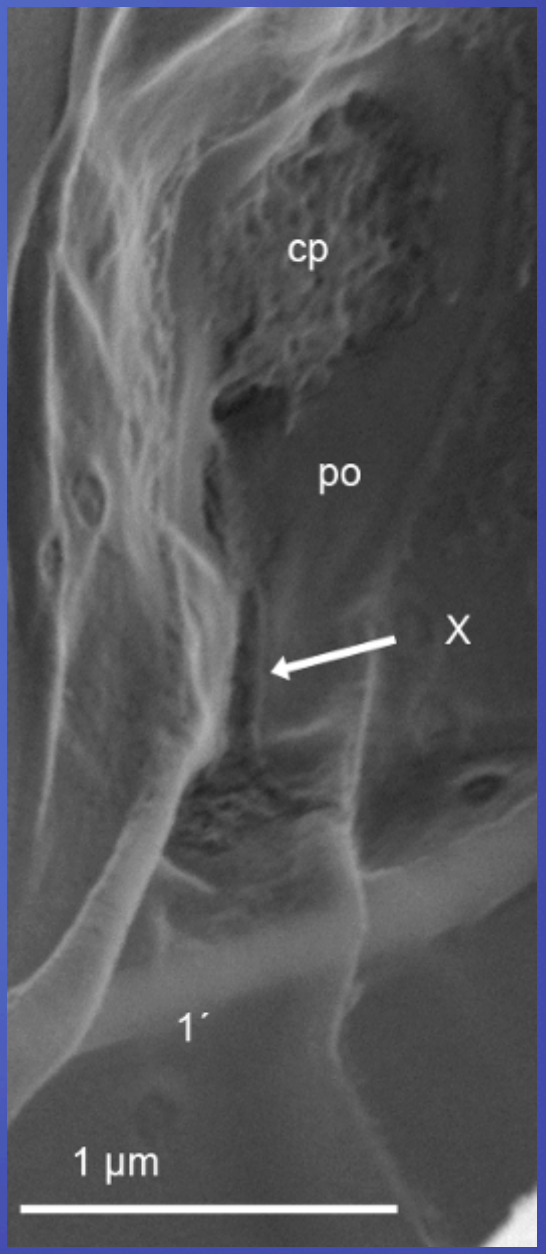
field sample, Peru 2014, *Azadinium cf. polongum*



Tillmann, Sanchez, Krock, in prep

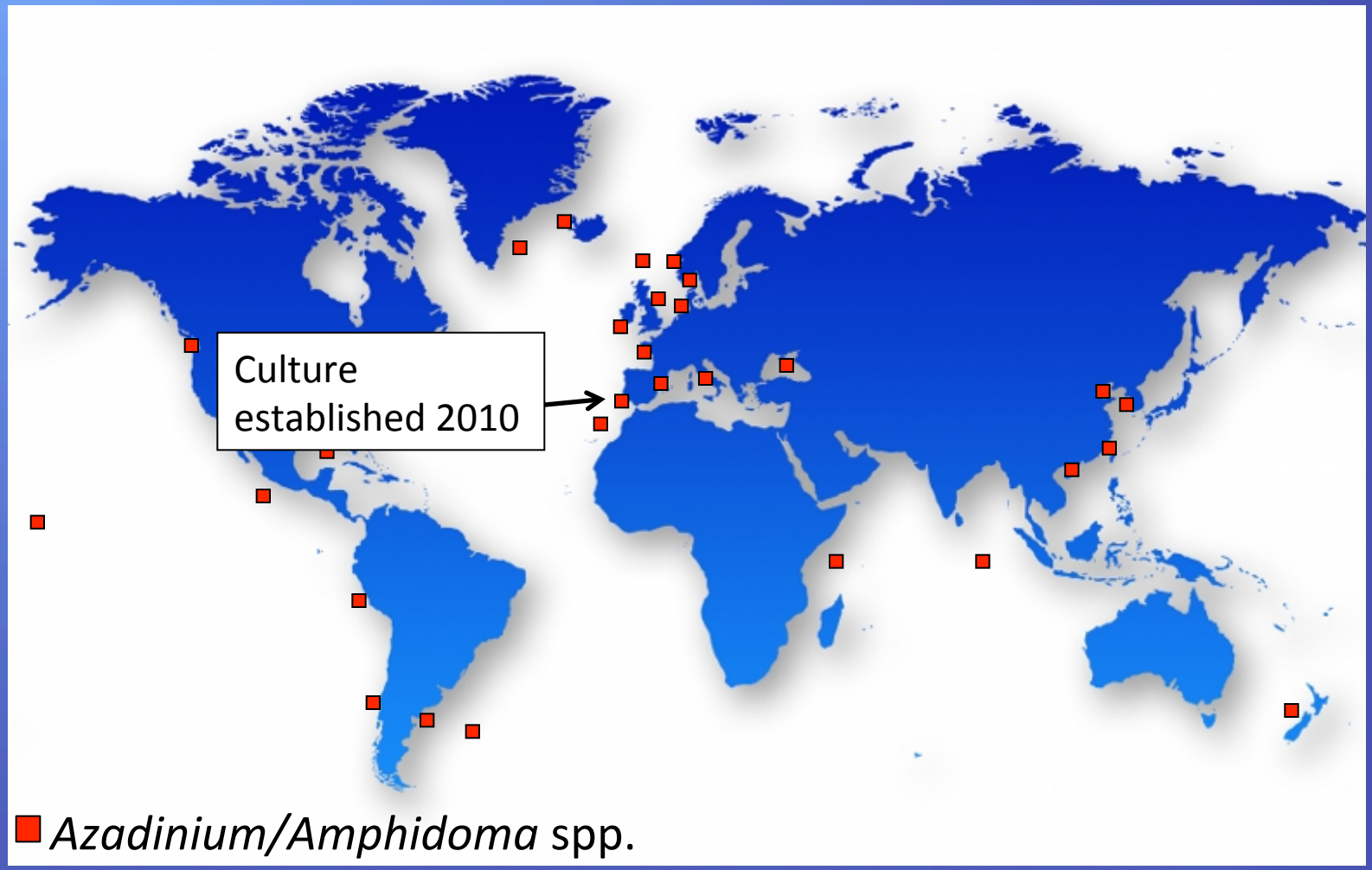
field sample, Peru 2014





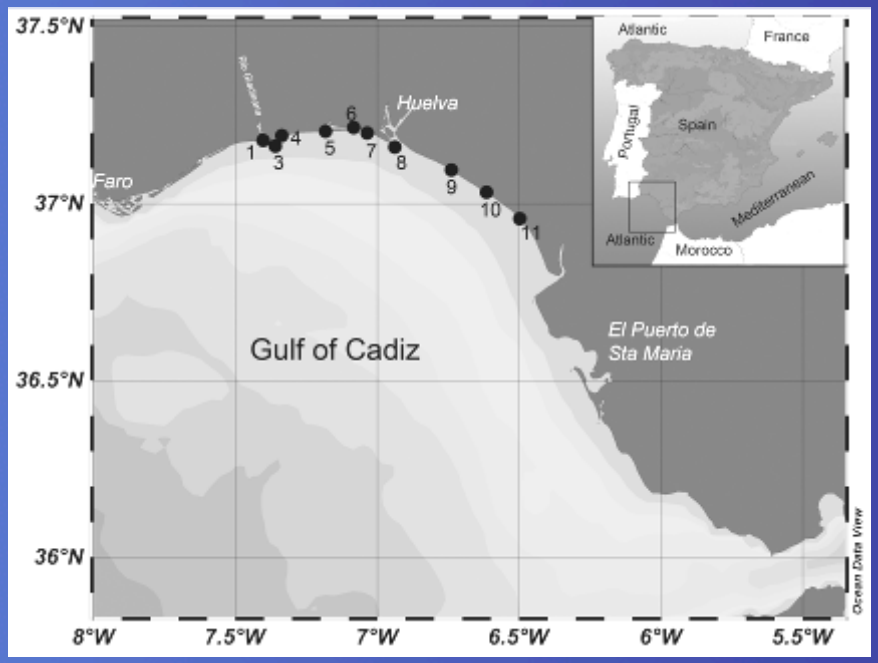
Tillmann, Sanchez, Krock, in prep

Amphidomataceae – global distribution



Donax trunculus

Week 2009	A 4	A 5	A 7	A 8	A 9	A 10	A 11
06.7.-12.7.	15.0		19.8			120.3	72.6
13.7.-19.7.	25.0		44.1	105.0	126.6		
20.7.-26.7.	21.0	34.8	100.4	123.4	108.1	284.5	109.1
27.7.-02.8.	91.7	92.7					
03.8.-09.8.	96.9	84.0	94.5	95.6	98.4	101.3	
10.8.-16.8.	87.7	71.0	85.2	96.6			81.0
17.8.23.8.		0.0	73.3	75.6	0.0 0.0	84.2 0.0	77.3 0.0
24.8.-30.8			79.0	0.0			



> 80 µg Kg^{-1} (1/2 reg. level)

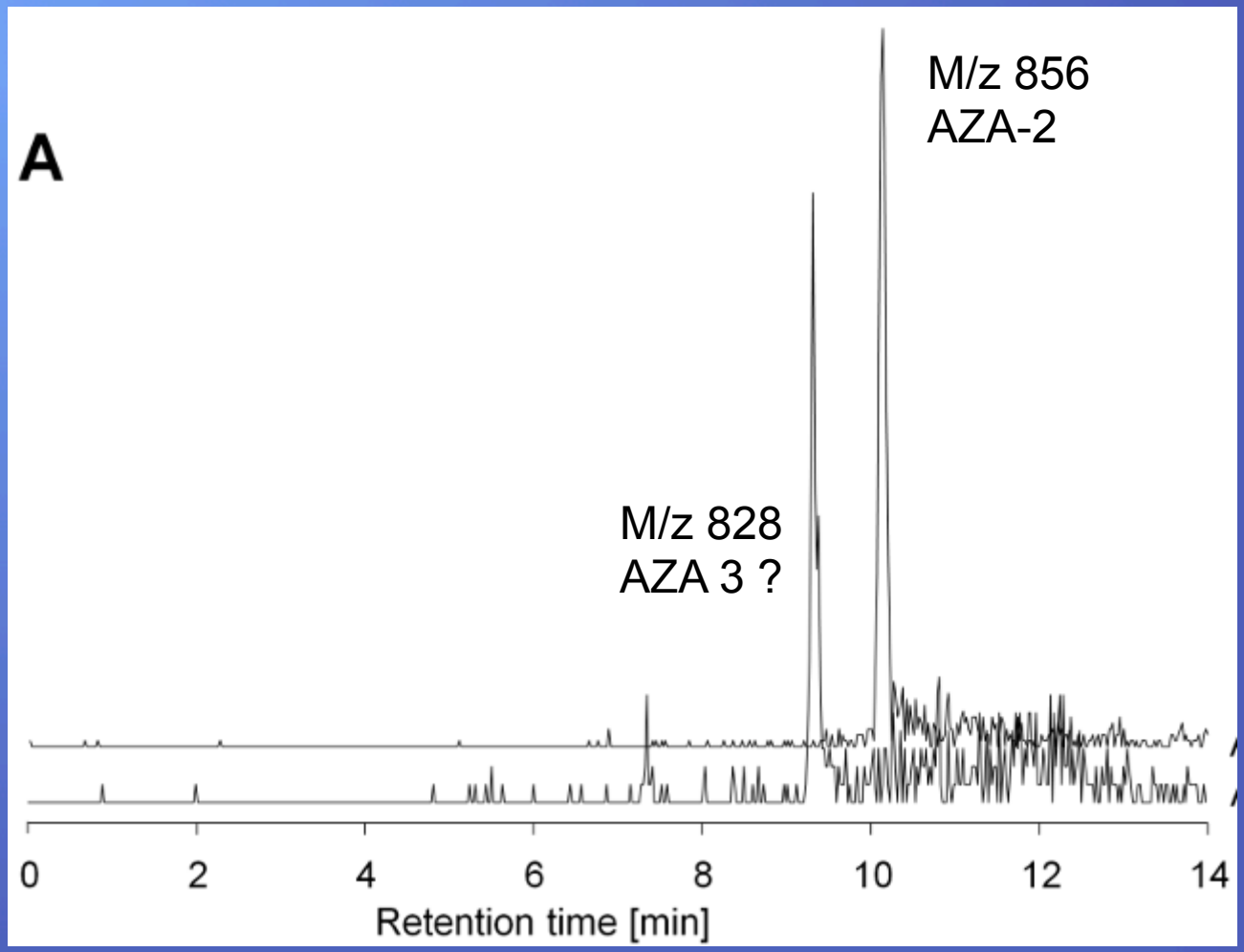
> 160 µg Kg^{-1} (reg. level)

AZA-2 concentration (µg kg^{-1}) in two different shellfish species collected in Juli/August 2009 at 10 sampling sites (A 1 – A 11) along the Huelva coast.

Chamelea galina

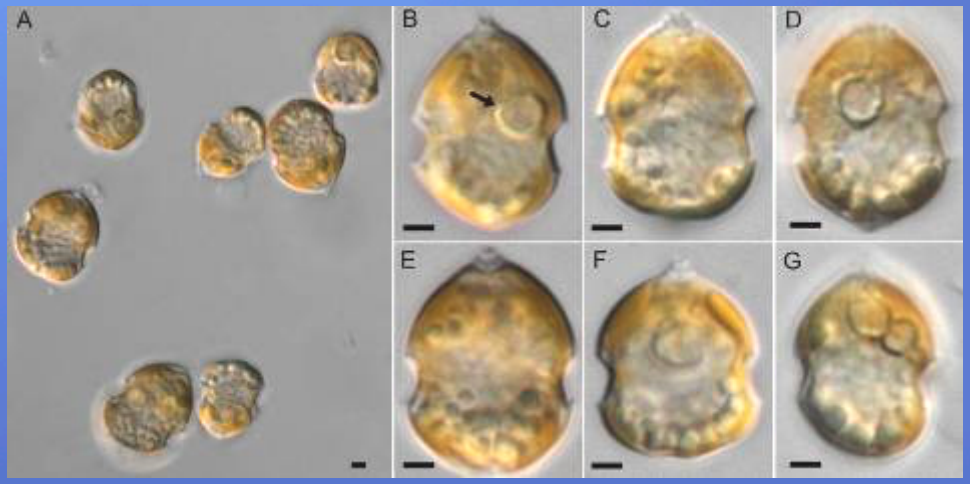
Week 2009	A 4	A 5	A 7	A 8	A 9	A 10	A 11
06.7.-12.7.							
13.7.-19.7.				176.2		0.0	150.5
20.7.-26.7.	29.8	45.2		168.0	180.4	93.5	575.4
27.7.-02.8.		109.5		101.0	92,5	97.6	100.9
03.8.-09.8.	86.9	94.0		97.2	88,0		118.5
10.8.-16.8.	107.0			94.0	77.7	90.0	
17.8.23.8.				83.0		85,8	
24.8.-30.8							

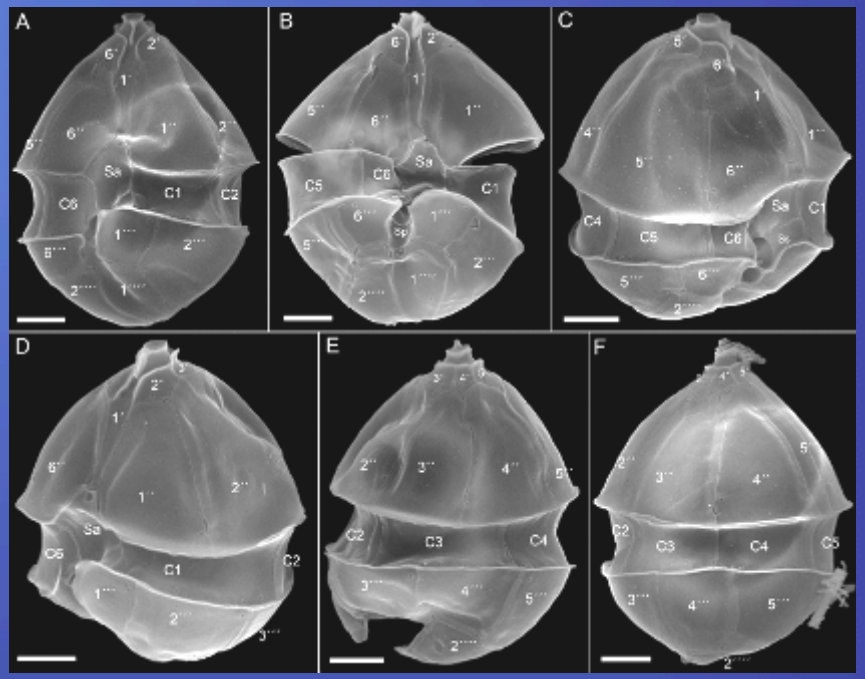
Tillmann, Jaen et al., in prep

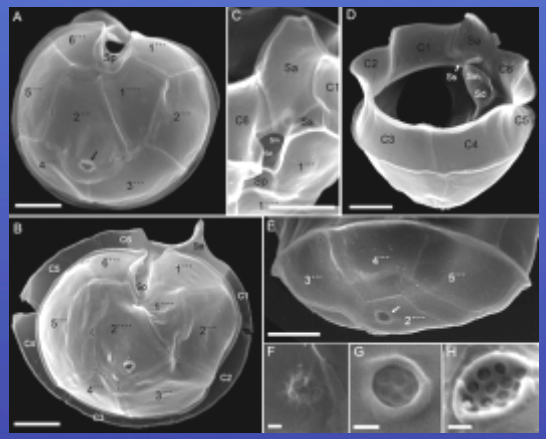


Tillmann, Jaen et al., in prep

Isolate identified as *Amphidoma languida* (LM, SEM, sequence data)





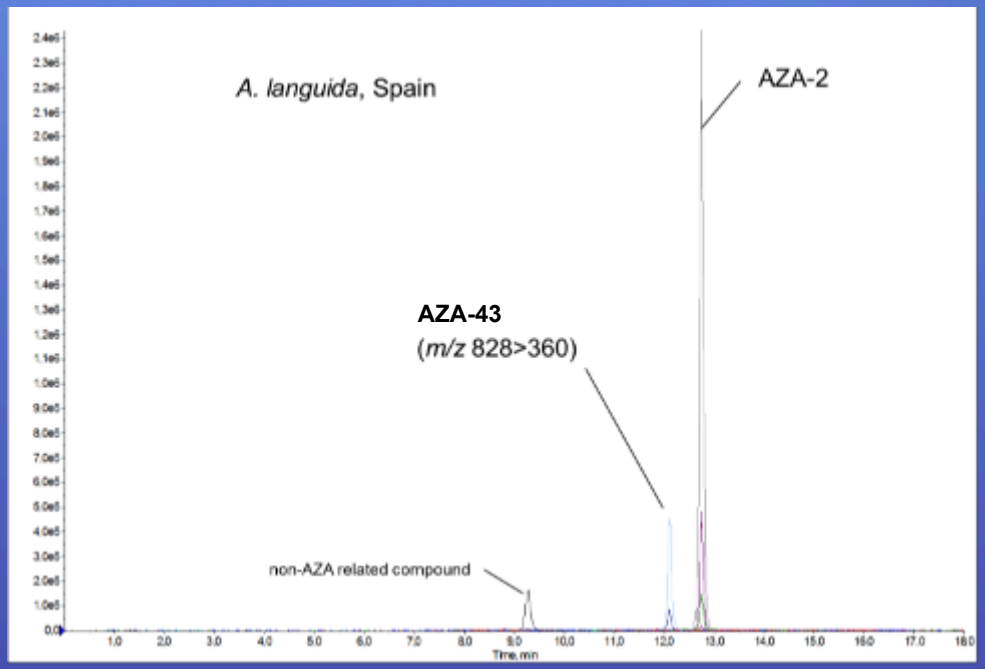


Tillmann, Jaen et al., in prep

Amphidoma languida DJ01 – AZA profile
- AZA-2
- A new compound (m/z 828, to be named AZA-43)

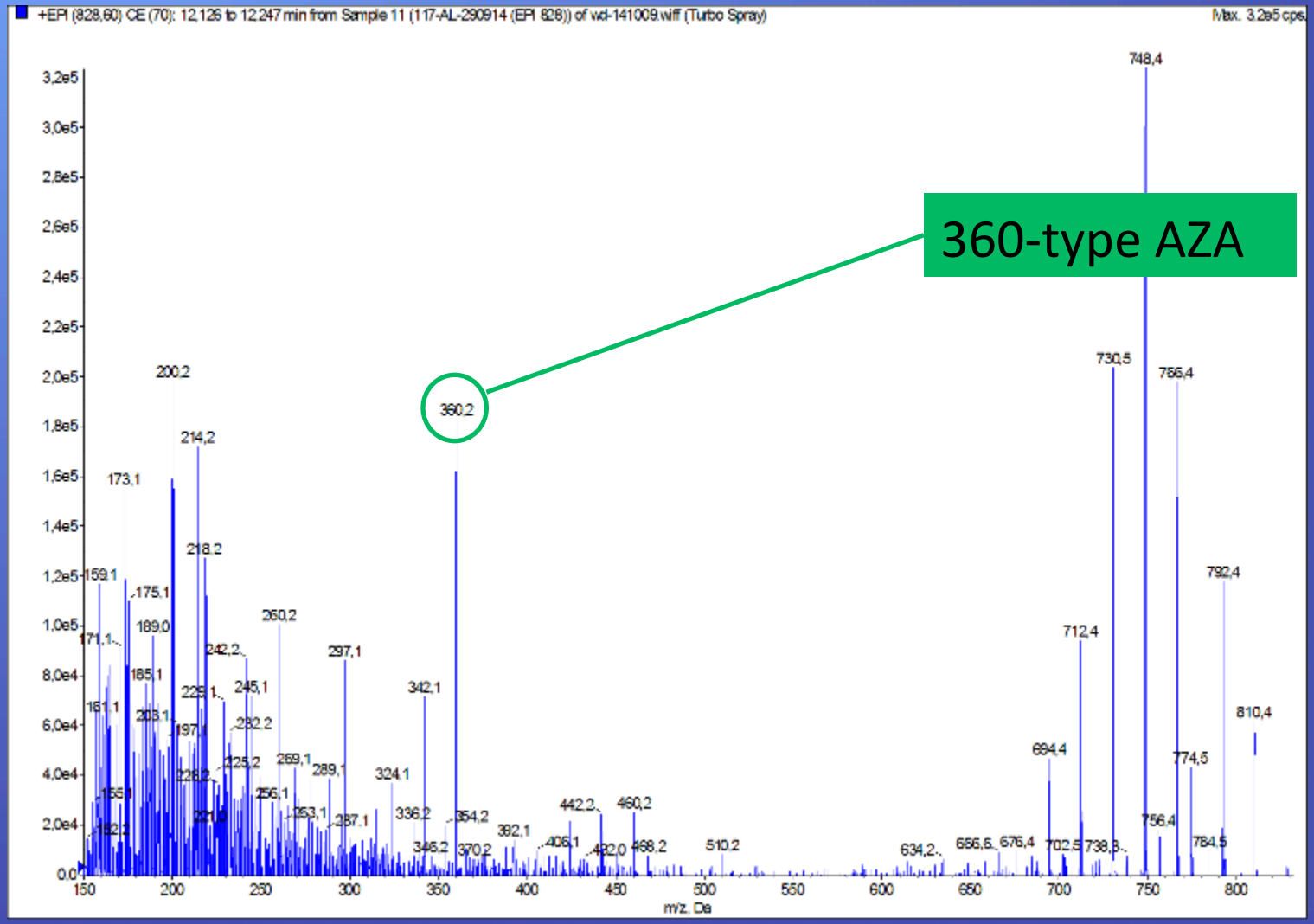
Different to other *A. languida* isolates

- SM2 (Irish isolate): AZA-38, -39
- 2A11 (Iceland isolate): AZA-38, -39



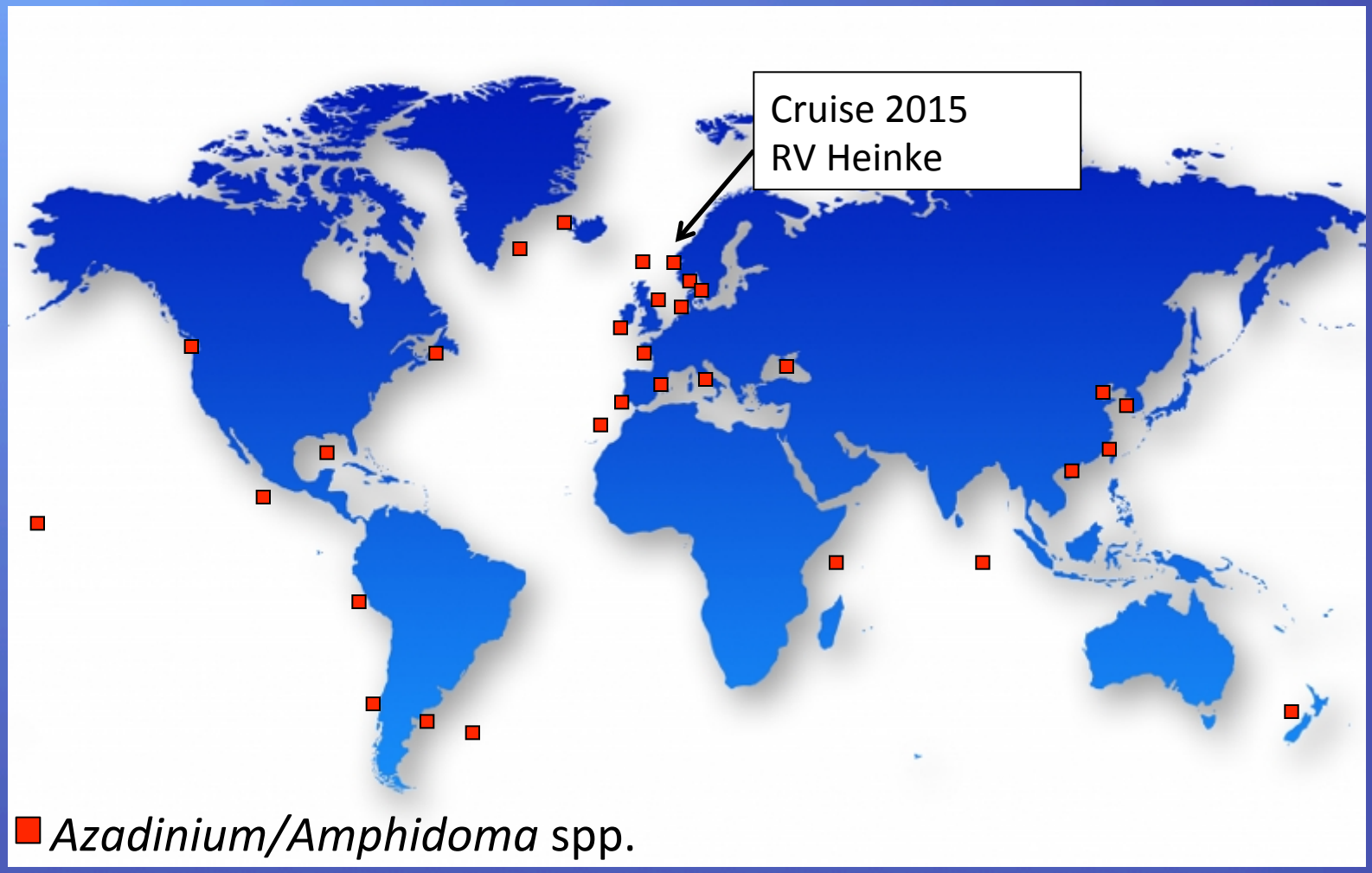
Tillmann, Jaen et al., in prep

AZA-43

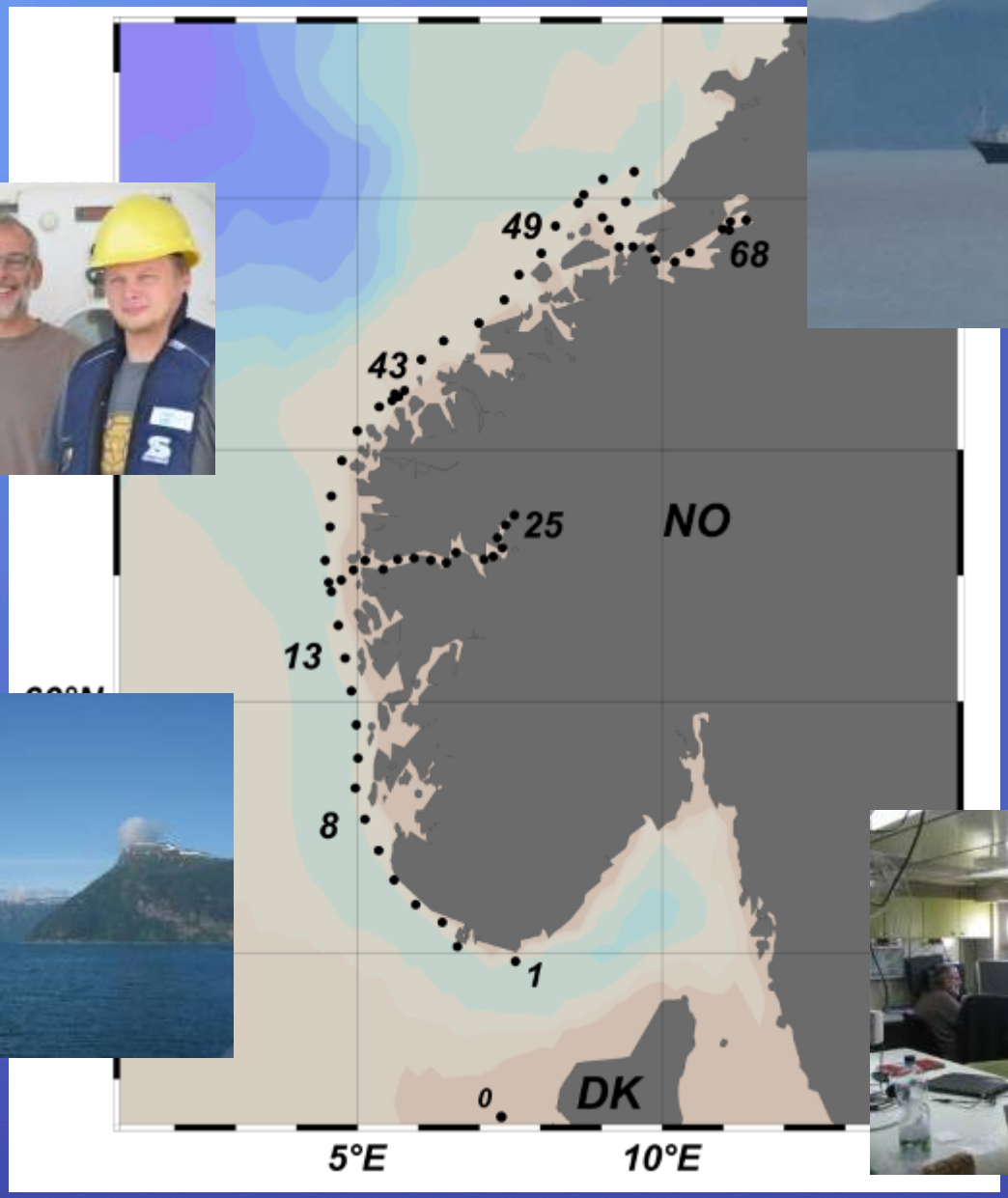


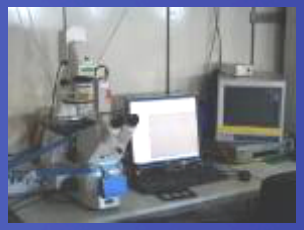
Tillmann, Jaen et al., in prep

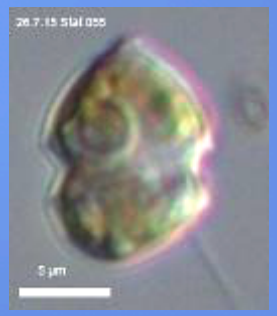
Amphidomataceae – global distribution

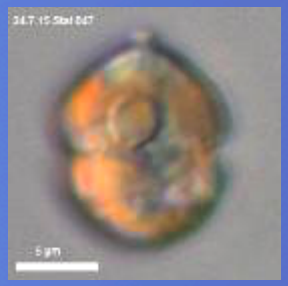


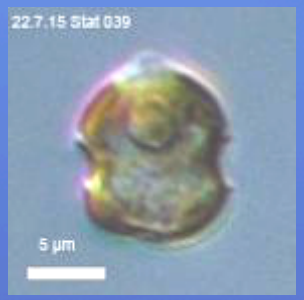
Heincke cruise HE448, July 2015

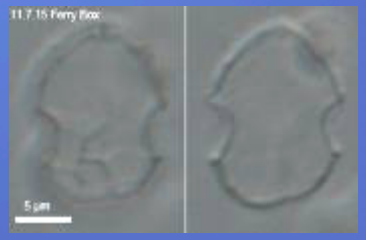


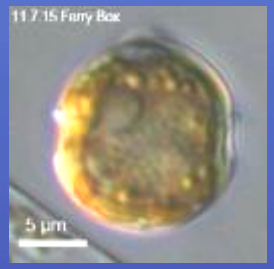


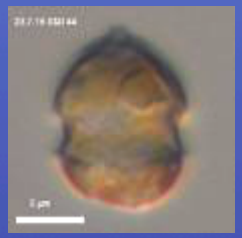

5 µm

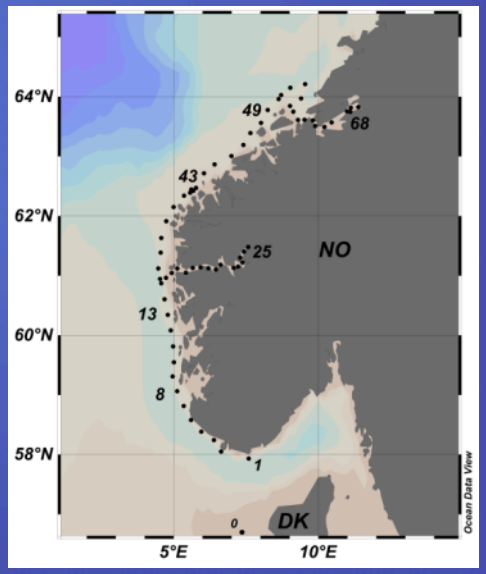



22.7.15 Stat 039
5 µm


11.7.15 Ferry Box
5 µm


11.7.15 Ferry Box
5 µm

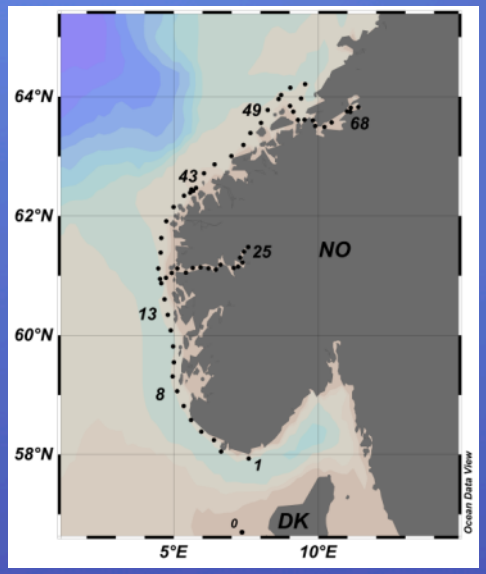



64°N
62°N
60°N
58°N
5°E
10°E
49
68
43
25
NO
13
8
1
0
DK
Ocean Data View

64°N
62°N
60°N
58°N
5°E
10°E
49
68
43
23
13
8
1
0
NO
DK
Ocean Data View

Azaspiracids……?

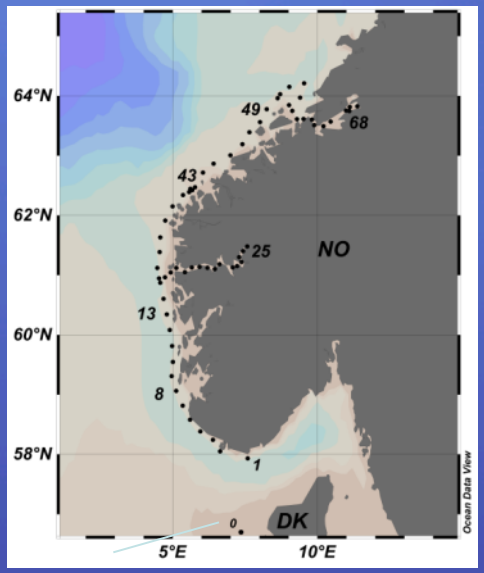
No….



All stations tested with the genus-specific qPCR Probe (Smith et al. 2015):

All samples positive

But for most stations indicating low abundance



In total 32 clonal cultures estabished representing 7 different species

13 x Am. languida
8 x Az. spinosum
5 x Az. dalianense
3 x Az. poporum
1 x Az. obesum
1 x Az. trinitatum
1 x Az. polongum

Stat 40
3 x Am. Languida N-40-03, N-40-04, N-40-06

Stat 41
1 x Az. obesumN-41-01

Stat 47
1 x Az. polongum N-47-01

Stat 38
3 x Az. dalianense N-37-02, N-37-03, N-37c-01

Stat 39
3 x Az. poporum N-39-02, N-39-03, N-39-13
3 x Am. Languida N-39-06, N-39-07, N-39-12
1 x Az. trinitatum N-39-04

Stat 37
1 x Am. languida N-37-01

Stat 33:
1 x Am. languida N-33-01

Stat 16:
2 x Az. spinosum N-16-01, N-16-02

Stat 15:
1 x Az. dalianense N-15-01

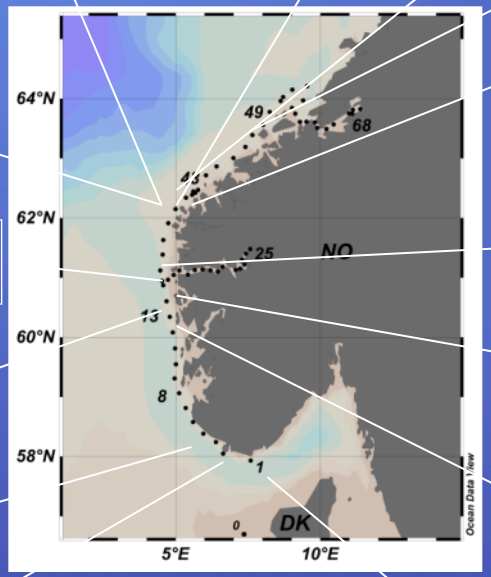
Stat 14:
1 x Az. spinosum N-14-02
1 x Am. Languida N-14-01

Stat 12:
1 x Am. languida N-12-01
1 x Az. dalianense N-12-04

Stat 5:
2 x Az. spinosum N-05-01, N-05-02

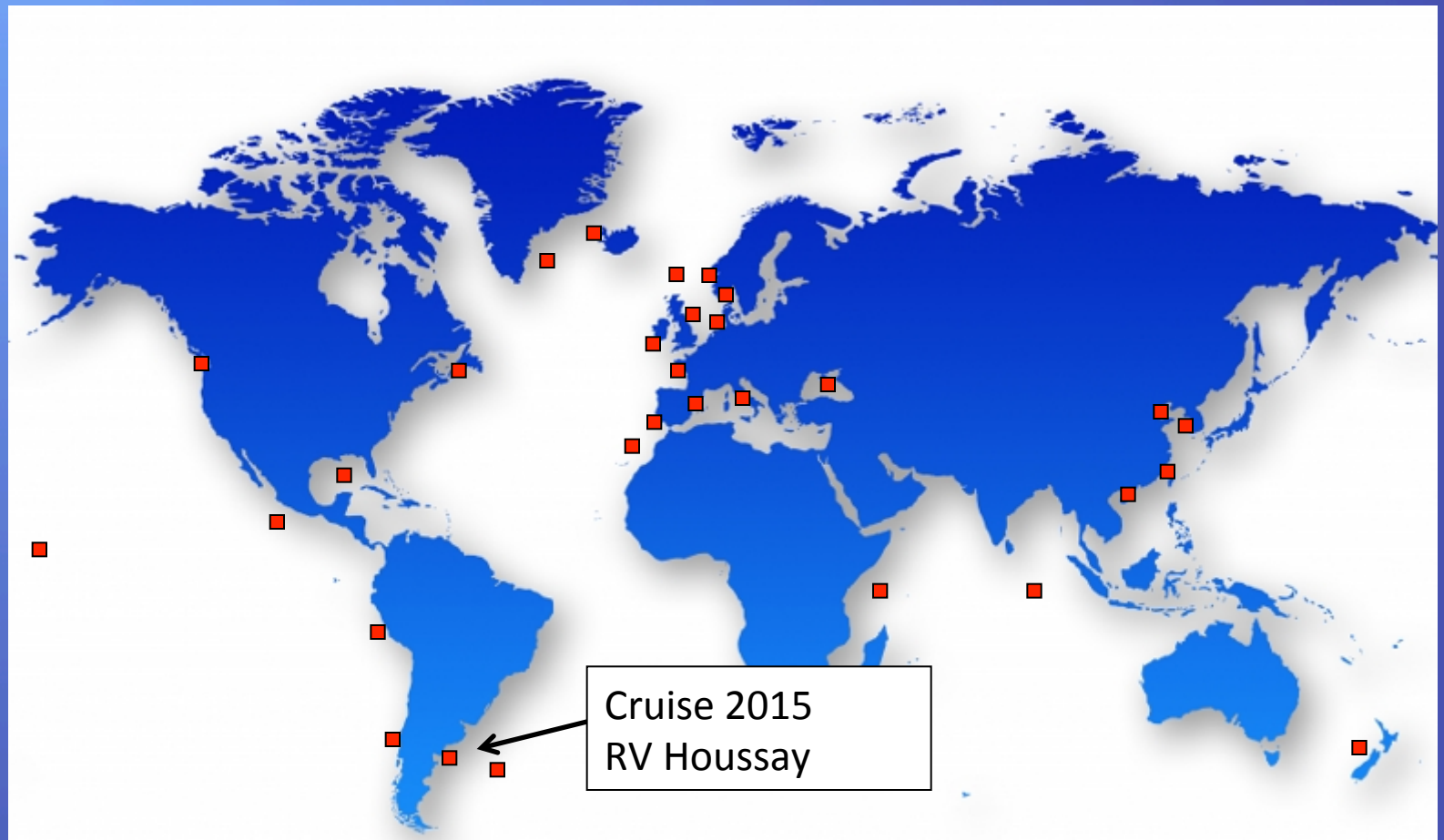
Stat 4:
3 x Az. spinosum N-04-01, N-04-02, N-04-04
1 x Am. languida N-04-03

Stat 1:
1 x Amphidoma languida N-01-01



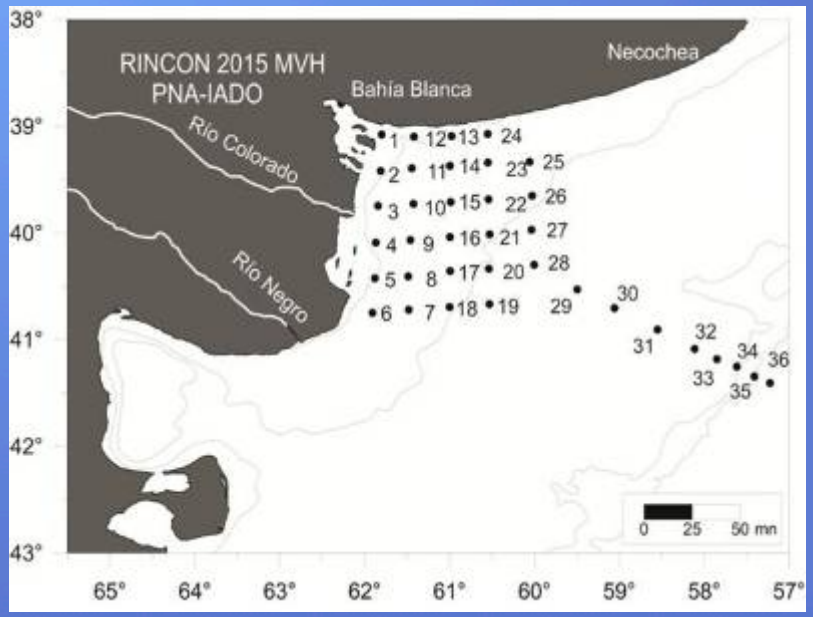
species	Strain	AZA-1	AZA-2	AZA-11	AZA-37	AZA-38	AZA-39	AZA-51	AZA-50	AZA-52	AZA-53
dalianense	N-12-04										
dalianense	N-15-01										
dalianense	N-38-02										
dalianense	N-38-03										
dalianense	N-38c-01										
obesum	N-41-01										
polongum	N-47-01										
trinitatum	N-39-04										
languida	N-01-01					x	x				
languida	N-04-03					x	x				
languida	N-12-01					x	x				
languida	N-14-01					x	x				
languida	N-33-01					x	x				
languida	N-37-01					x	x				
languida	N-39-06					x	x				
languida	N-39-07						x				x
languida	N-39-12					x	x				
languida	N-40-03						x			x	
languida	N-40-04					x	x				
languida	N-40-06						x				x
languida	N-XX-01					x	x				
poporum	N-39-01				x						
poporum	N-39-03				x						
poporum	N-39-13				x						
spinosum	N-04-01	x	x								
spinosum	N-04-02	x	x								
spinosum	N-04-04							x			
spinosum	N-05-01			x				x			
spinosum	N-05-02							x			
spinosum	N-14-02			x				x	x		
spinosum	N-16-01							x			
spinosum	N-16-02										

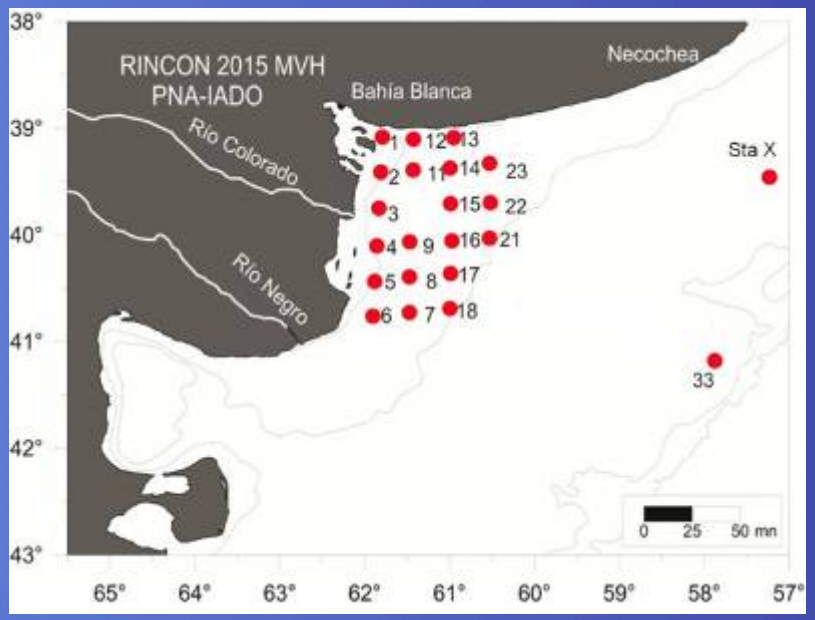
Amphidomataceae – global distribution



■ *Azadinium/Amphidoma* spp.

RV Houssay, Sept 2015


RINCON 2015 MVH
PNA-IADO
Bahia Blanca
Necochea
Río Colorado
Río Negro
38°
39°
40°
41°
42°
43°
65°
64°
63°
62°
61°
60°
59°
58°
57°
0 25 50 mn

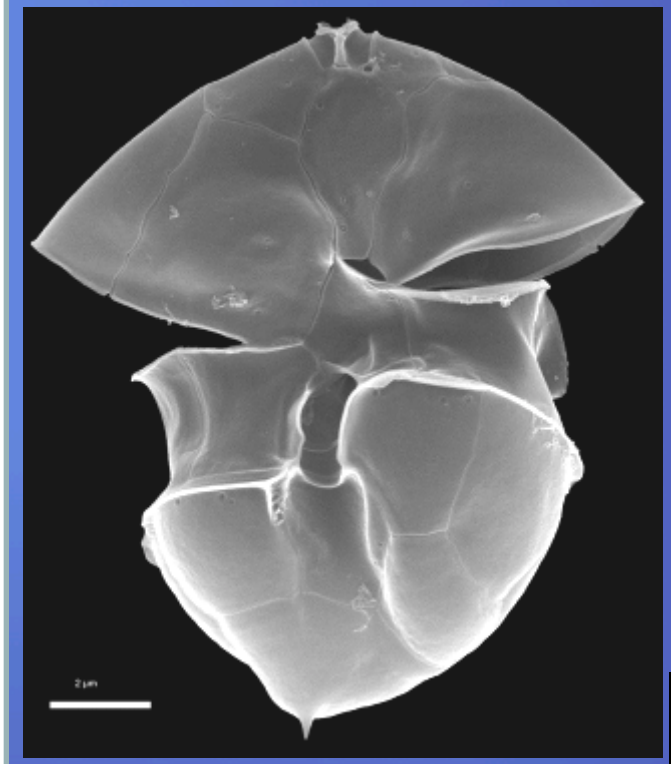

RINCON 2015 MVH
PNA-IADO
Bahia Blanca
Necochea
Río Colorado
Río Negro
Sta X
38°
39°
40°
41°
42°
43°
65°
64°
63°
62°
61°
60°
59°
58°
57°
0 25 50 mn

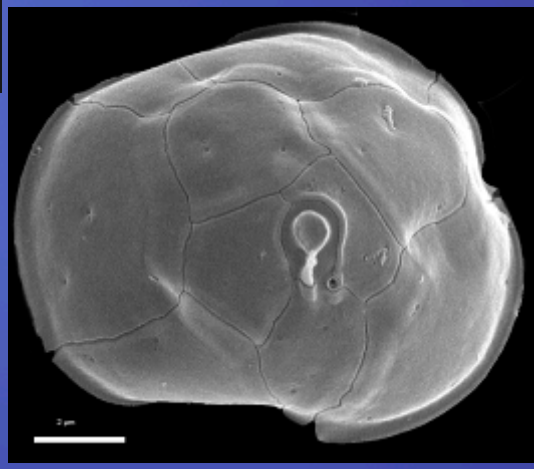
Fixed samples:
Up to 280,000 „*Azadinium*-like“ cells L^{-1}

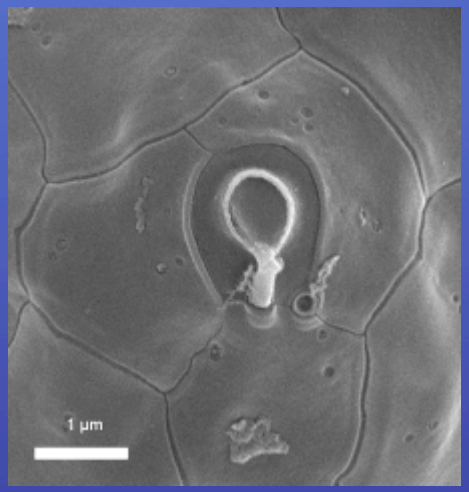
Isolate	Species	AZAs
H1-E9	Amphidoma sp. new	(neg)
H-4-E8	dalianense	neg
H-4-A4	dalianense	neg
H-4-F1	dalianense	neg
H-2-G7	dalianense	neg
H-4-A3	dalianense	neg
H-2-G9	dalianense	neg
H1-D11	cf. spinosum ??	AZA-2
H-1-B5	cf spinosum	neg
H-3-B5	cf spinosum	neg
H-4-A10	cf spinosum	neg
H-2-D8	cf spinosum	neg
H-4-D4	cf spinosum	neg
H-4-E6	cf spinosum	neg
H-4-D6	cf spinosum	neg
H-1-H6	cf spinosum	neg
H-1-D4	cf spinosum	neg
H-1-D7	cf spinosum	neg
H-4-C2	cf spinosum	neg
H-2-D7	cf spinosum	neg
H-4-E9	cf spinosum	neg
H-4-A1	cf spinosum	neg
H-1-G4	cf spinosum	neg
H-4-G9	cf spinosum	neg
H-4-F9	cf spinosum	neg
H-4-C4	cf spinosum	neg

Azadinium dalianense

Isolate	Species	AZAs
H1-E9	Amphidoma sp. new	(neg)
H-4-E8	dalianense	neg
H-4-A4	dalianense	neg
H-4-F1	dalianense	neg
H-2-G7	dalianense	neg
H-4-A3	dalianense	neg
H-2-G9	dalianense	neg
H1-D11	cf. spinosum ??	AZA-2
H-1-B5	cf spinosum	neg
H-3-B5	cf spinosum	neg
H-4-A10	cf spinosum	neg
H-2-D8	cf spinosum	neg
H-4-D4	cf spinosum	neg
H-4-E6	cf spinosum	neg
H-4-D6	cf spinosum	neg
H-1-H6	cf spinosum	neg
H-1-D4	cf spinosum	neg
H-1-D7	cf spinosum	neg
H-4-C2	cf spinosum	neg
H-2-D7	cf spinosum	neg
H-4-E9	cf spinosum	neg
H-4-A1	cf spinosum	neg
H-1-G4	cf spinosum	neg
H-4-G9	cf spinosum	neg
H-4-F9	cf spinosum	neg
H-4-C4	cf spinosum	neg

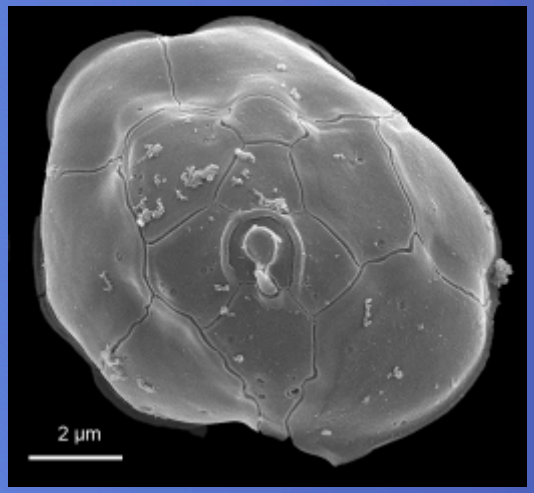


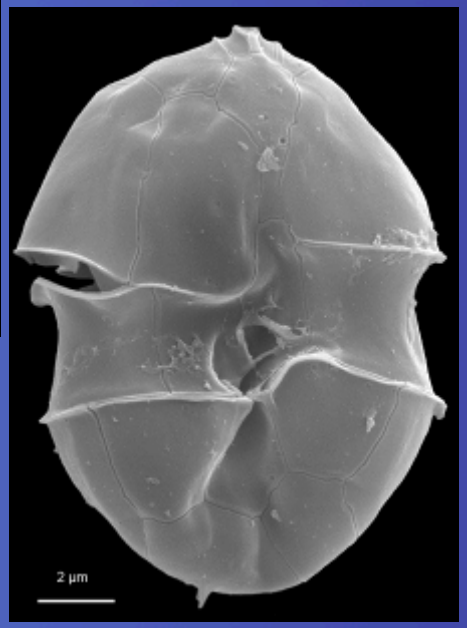


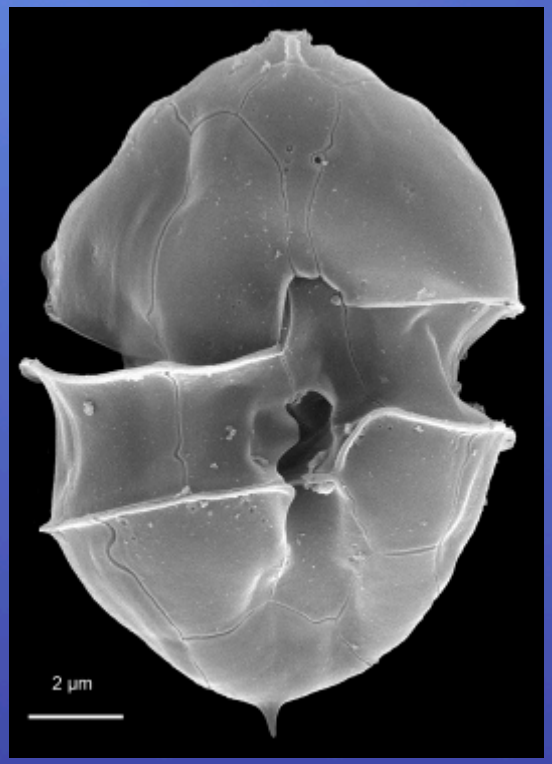


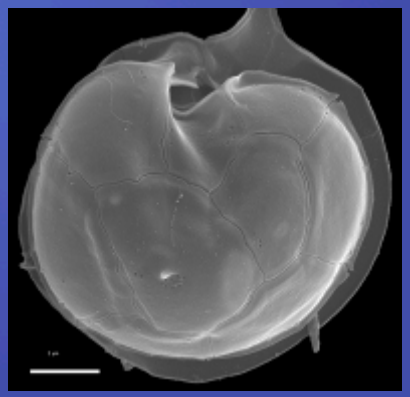
Isolate	Species	AZAs
H1-E9	Amphidoma sp. new	(neg)
H-4-E8	dalianense	neg
H-4-A4	dalianense	neg
H-4-F1	dalianense	neg
H-2-G7	dalianense	neg
H-4-A3	dalianense	neg
H-2-G9	dalianense	neg
H1-D11	cf. spinosum ??	AZA-2
H-1-B5	cf spinosum	neg
H-3-B5	cf spinosum	neg
H-4-A10	cf spinosum	neg
H-2-D8	cf spinosum	neg
H-4-D4	cf spinosum	neg
H-4-E6	cf spinosum	neg
H-4-D6	cf spinosum	neg
H-1-H6	cf spinosum	neg
H-1-D4	cf spinosum	neg
H-1-D7	cf spinosum	neg
H-4-C2	cf spinosum	neg
H-2-D7	cf spinosum	neg
H-4-E9	cf spinosum	neg
H-4-A1	cf spinosum	neg
H-1-G4	cf spinosum	neg
H-4-G9	cf spinosum	neg
H-4-F9	cf spinosum	neg
H-4-C4	cf spinosum	neg

Azadinium cf. *spinosum*



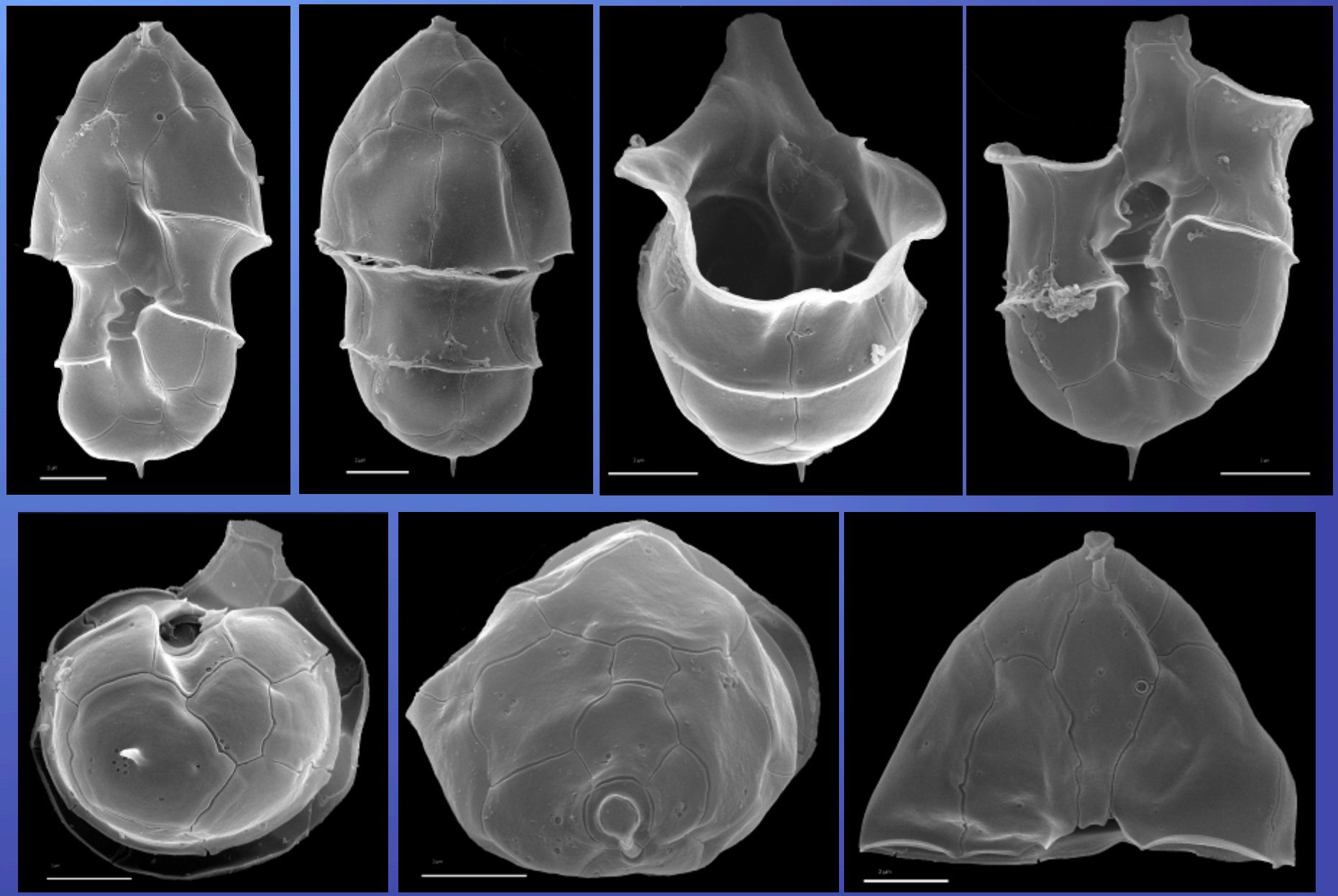


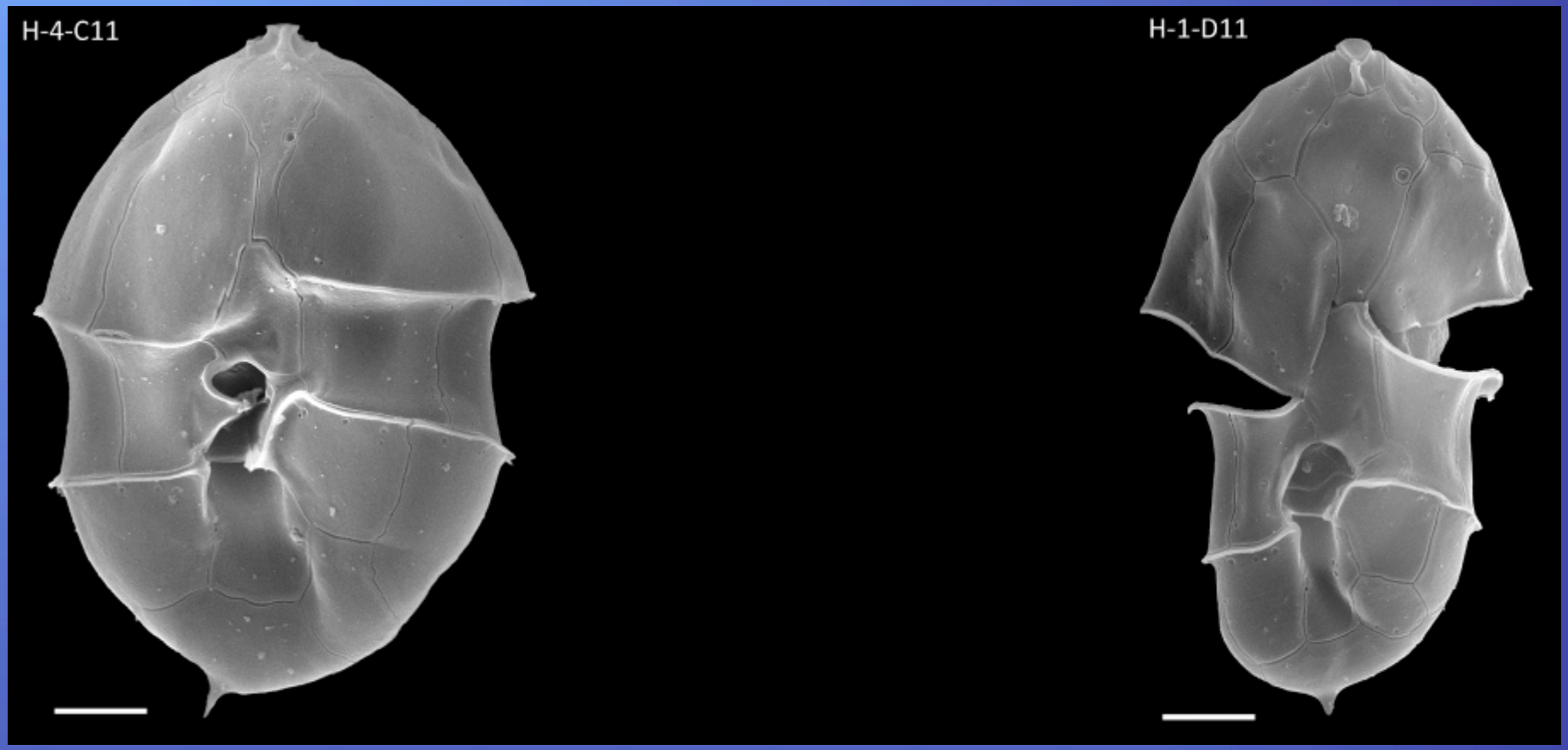


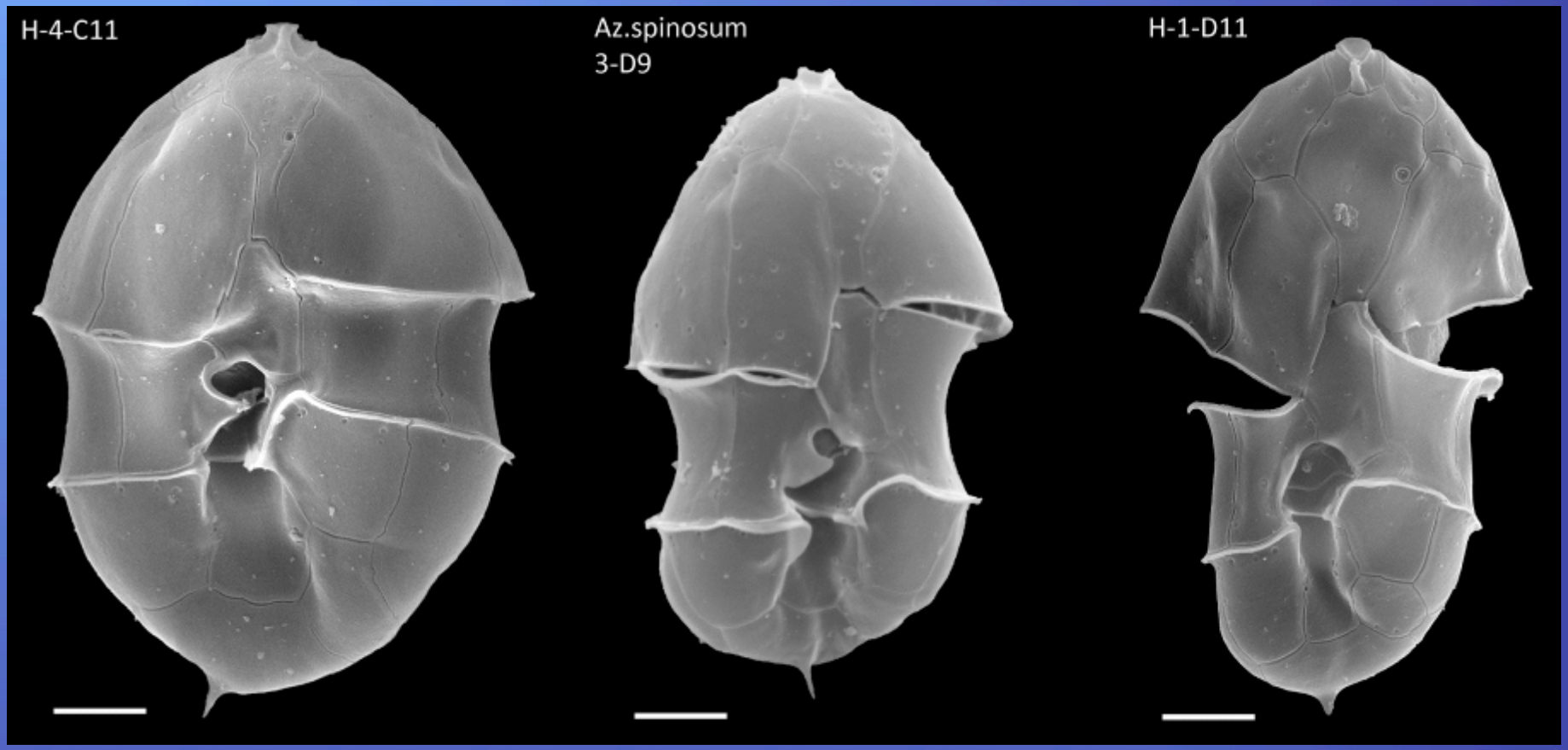


Isolate	Species	AZAs
H1-E9	Amphidoma sp. new	(neg)
H-4-E8	dalianense	neg
H-4-A4	dalianense	neg
H-4-F1	dalianense	neg
H-2-G7	dalianense	neg
H-4-A3	dalianense	neg
H-2-G9	dalianense	neg
H1-D11	cf. spinosum ??	AZA-2
H-1-B5	cf spinosum	neg
H-3-B5	cf spinosum	neg
H-4-A10	cf spinosum	neg
H-2-D8	cf spinosum	neg
H-4-D4	cf spinosum	neg
H-4-E6	cf spinosum	neg
H-4-D6	cf spinosum	neg
H-1-H6	cf spinosum	neg
H-1-D4	cf spinosum	neg
H-1-D7	cf spinosum	neg
H-4-C2	cf spinosum	neg
H-2-D7	cf spinosum	neg
H-4-E9	cf spinosum	neg
H-4-A1	cf spinosum	neg
H-1-G4	cf spinosum	neg
H-4-G9	cf spinosum	neg
H-4-F9	cf spinosum	neg
H-4-C4	cf spinosum	neg

Isolate H-1-D11



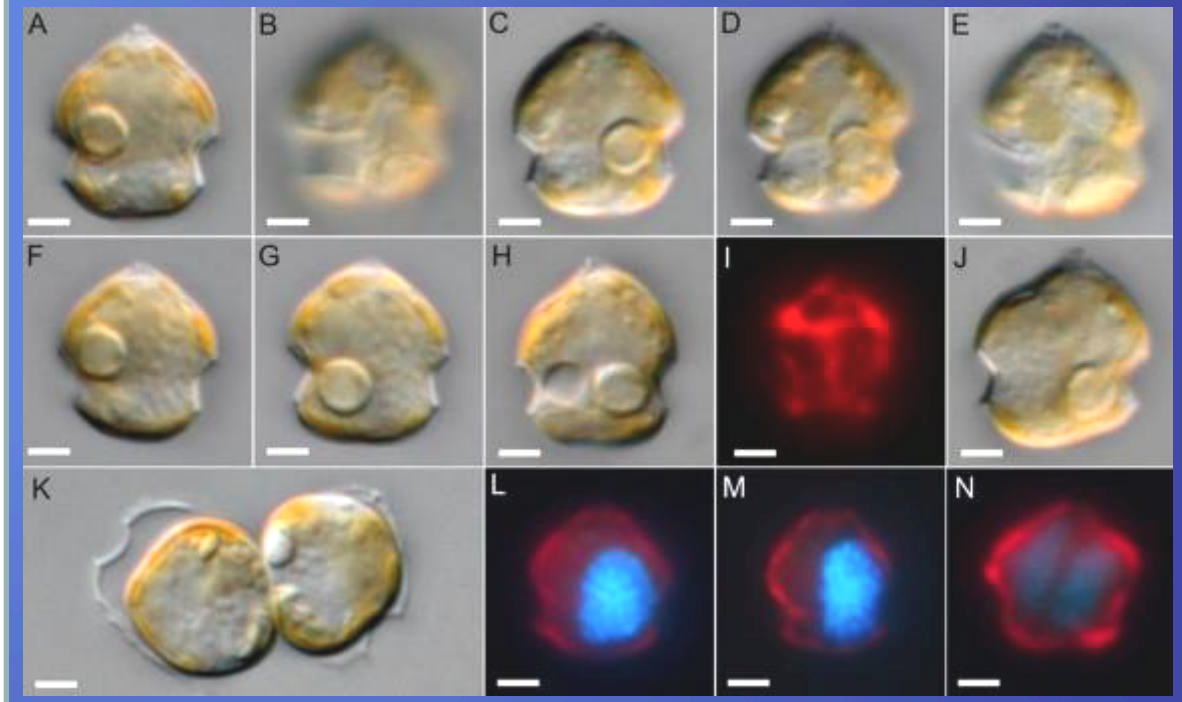

H-4-C11
H-1-D11

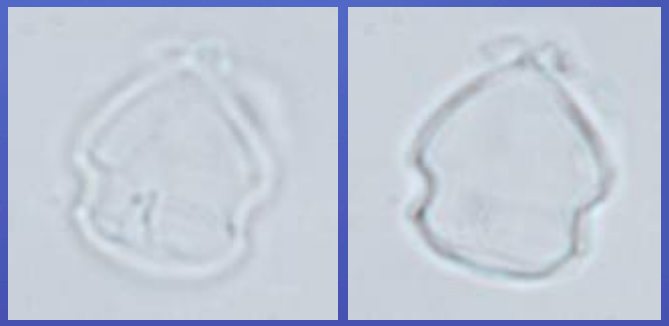


Waiting for molecular sequence data …

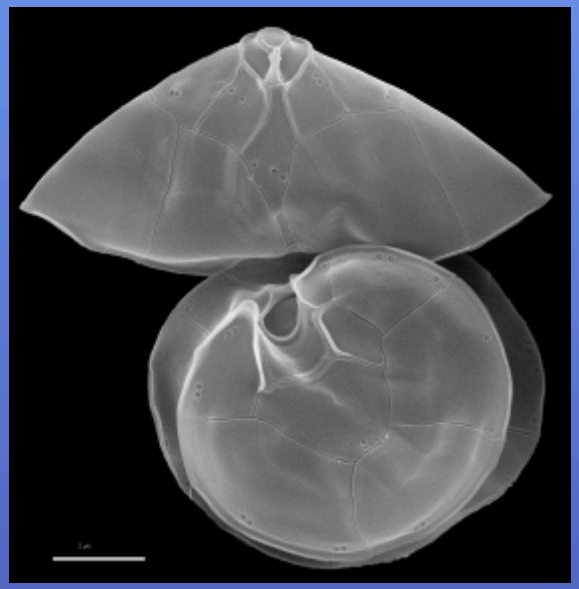
Isolate	Species	AZAs
H1-E9	Amphidoma sp. nov.	(neg)
H-4-E8	dalianense	neg
H-4-A4	dalianense	neg
H-4-F1	dalianense	neg
H-2-G7	dalianense	neg
H-4-A3	dalianense	neg
H-2-G9	dalianense	neg
H1-D11	cf. spinosum ??	AZA-2
H-1-B5	cf spinosum	neg
H-3-B5	cf spinosum	neg
H-4-A10	cf spinosum	neg
H-2-D8	cf spinosum	neg
H-4-D4	cf spinosum	neg
H-4-E6	cf spinosum	neg
H-4-D6	cf spinosum	neg
H-1-H6	cf spinosum	neg
H-1-D4	cf spinosum	neg
H-1-D7	cf spinosum	neg
H-4-C2	cf spinosum	neg
H-2-D7	cf spinosum	neg
H-4-E9	cf spinosum	neg
H-4-A1	cf spinosum	neg
H-1-G4	cf spinosum	neg
H-4-G9	cf spinosum	neg
H-4-F9	cf spinosum	neg
H-4-C4	cf spinosum	neg

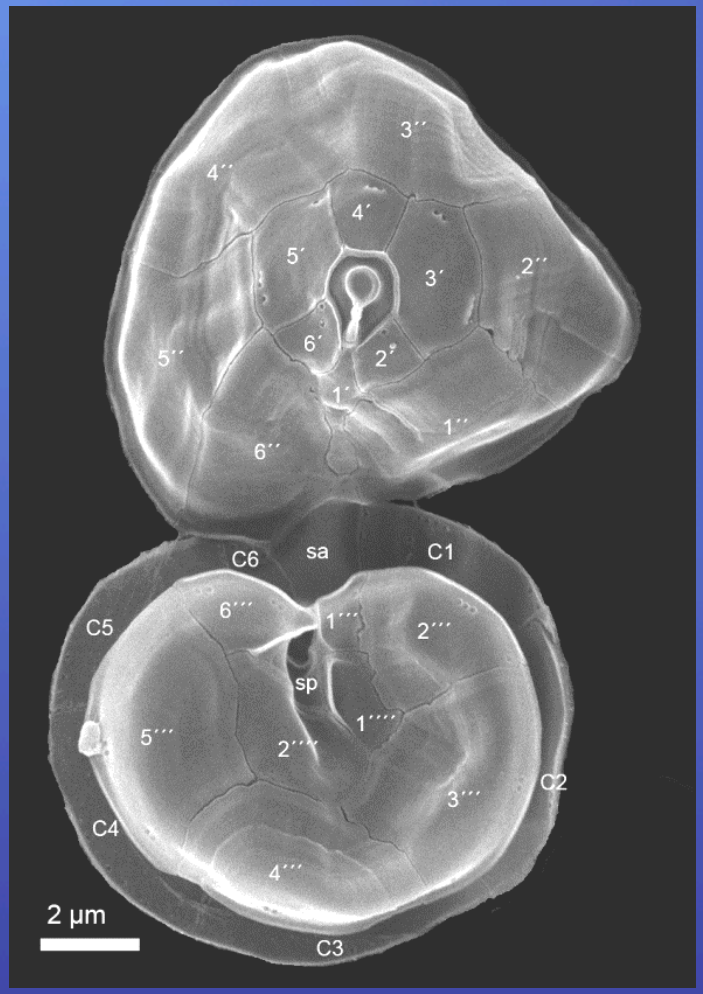
Isolate H-1-E9

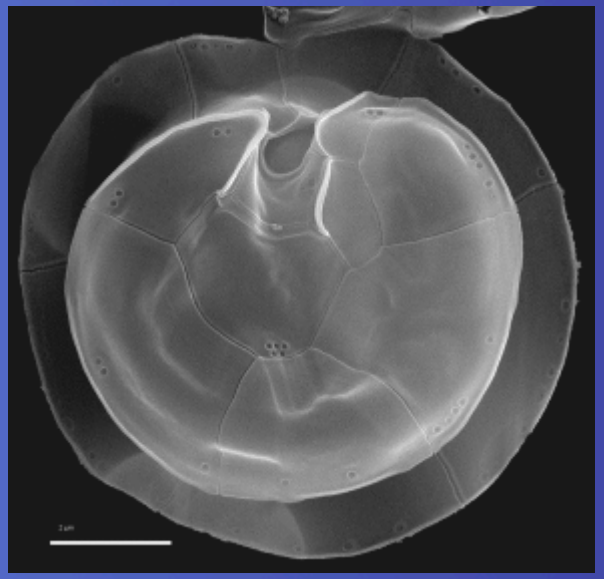


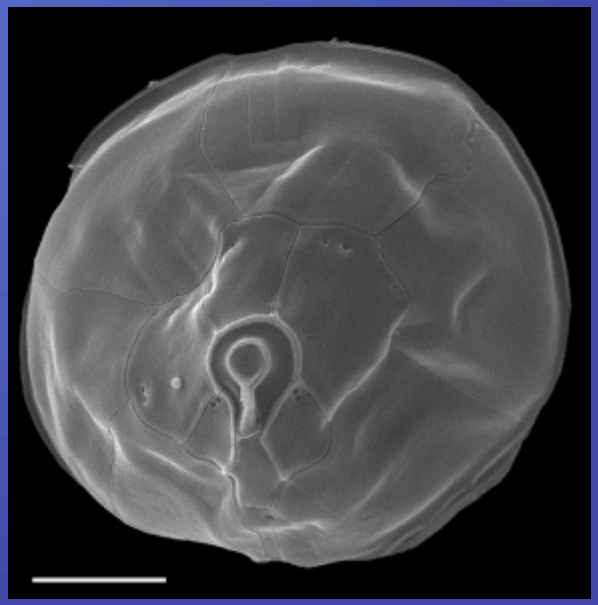


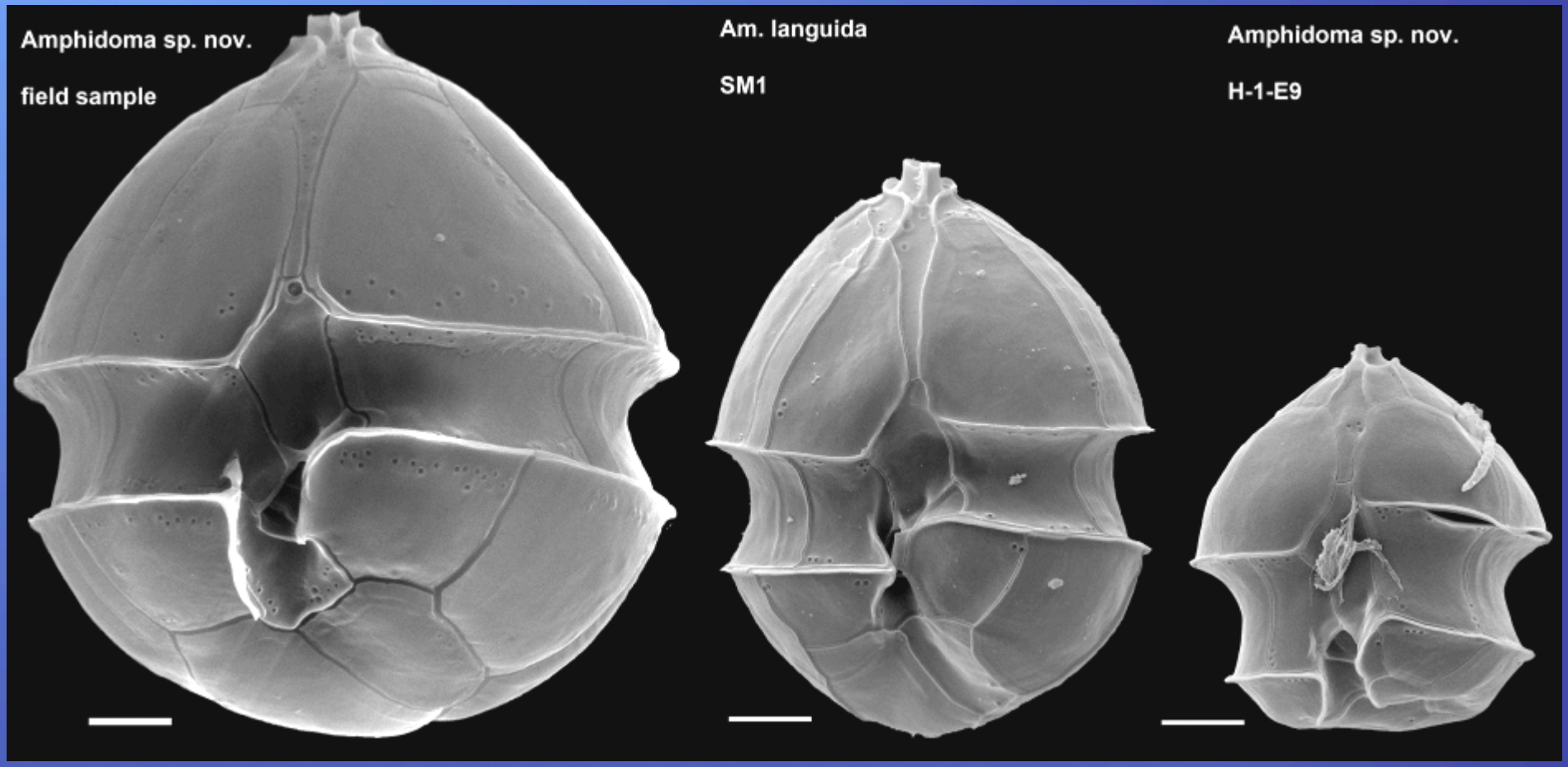
A new species of *Amphidoma*



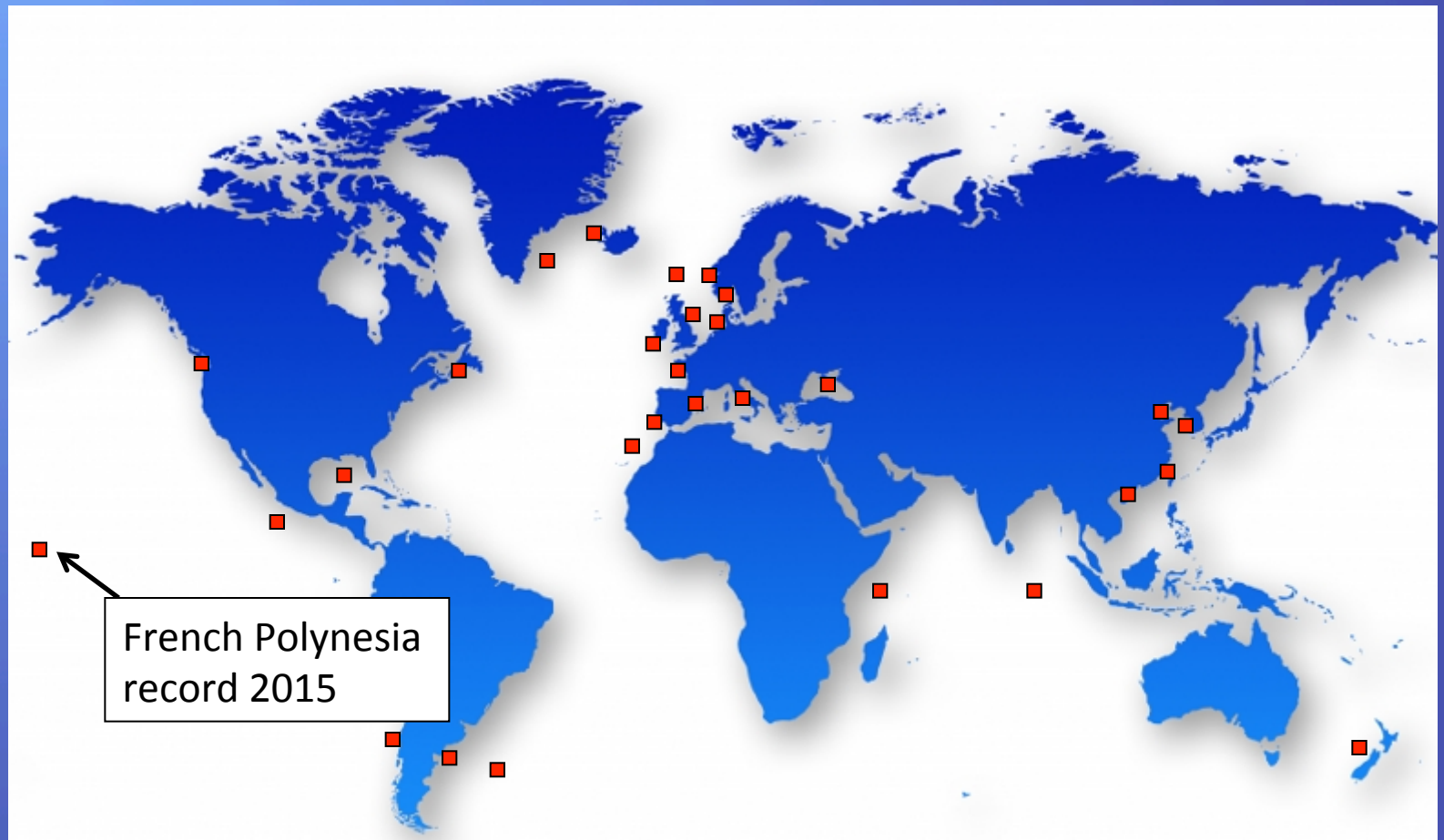




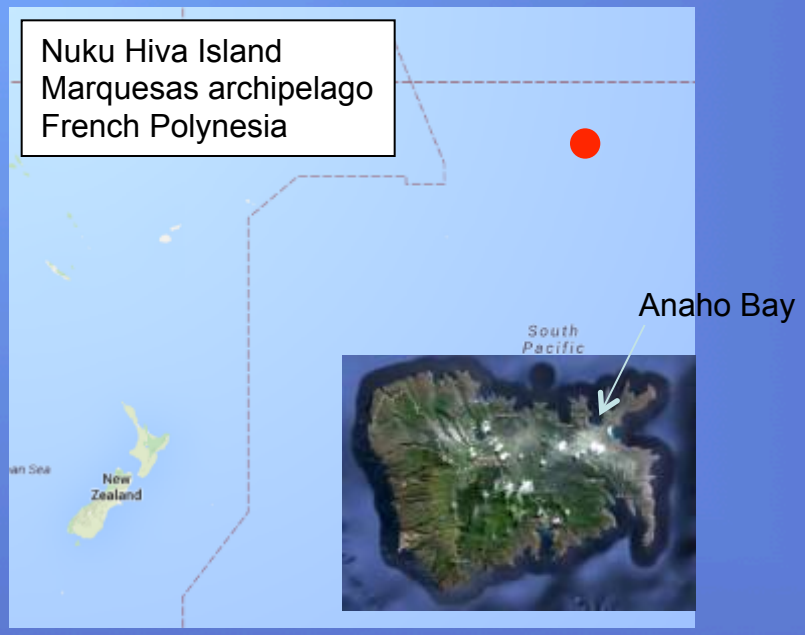



Amphidoma sp. nov.
field sample
Am. languida
SM1
Amphidoma sp. nov.
H-1-E9

Amphidomataceae – global distribution



■ *Azadinium/Amphidoma* spp.



2014
„Foodborne intoxication of nine tourists after consumption of gastropods collected in Anaho Bay
Ciguatera like syndrome

but

unusual symptoms as well (rapid onset < 2h; oropharyngeal burning sensation)

The gastropod *Tectus niloticus* collected 1 month after the toxic episode revealed giguatoxins and traces of AZA-2…

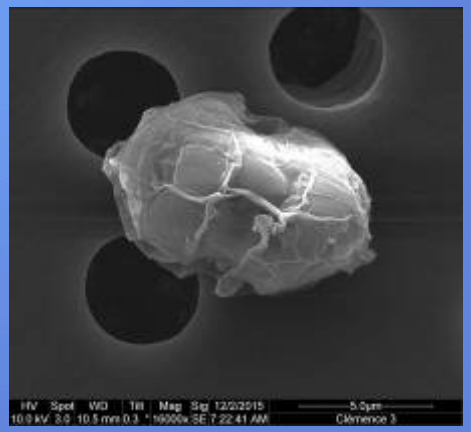
Gatti, C. et al.,
presentation French HAB conference

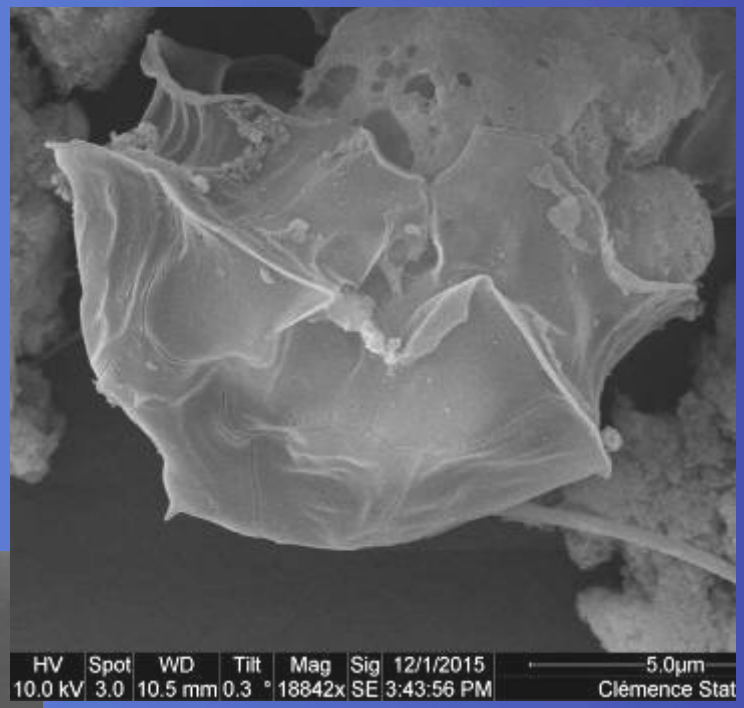
Plankton sampling in 2015:

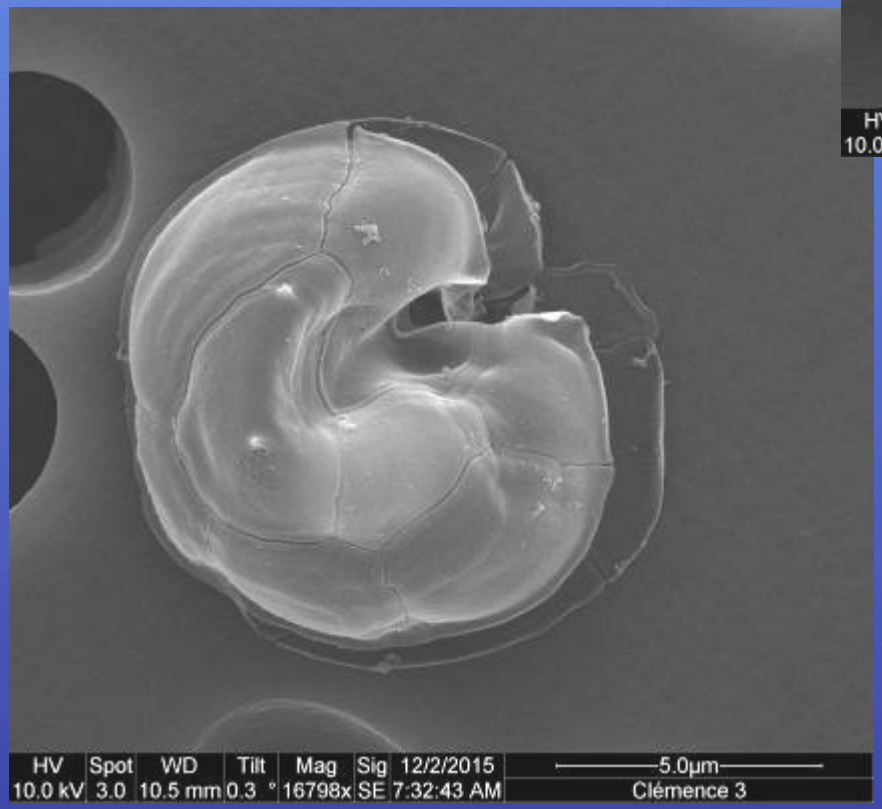
DNA samples positive for Azadinium genus-specific probe
But negative for species specific probes of *A. spinosum* and *A. poporum*

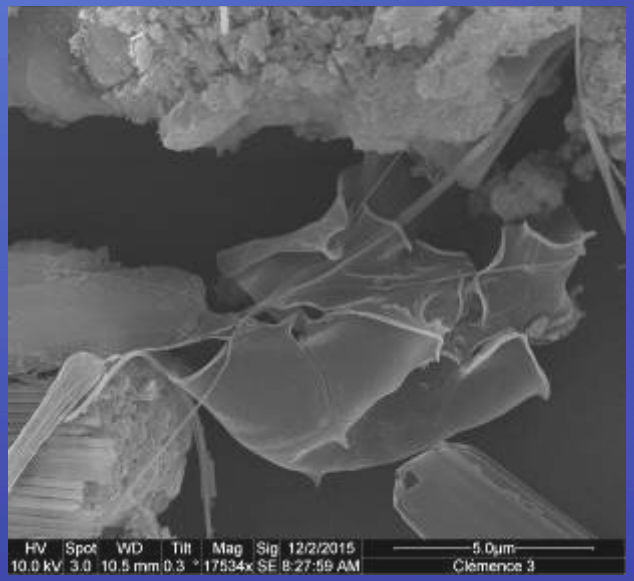
Smith, K. et al. unpublished

Azadinium sp from French Polynesia









Amphidomataceae – global distribution

■ *Azadinium/Amphidoma* spp.

Azadinium, quantitative data…

Az. poporum, 3 year time series, qPCR, Shiwa Bay, Korea

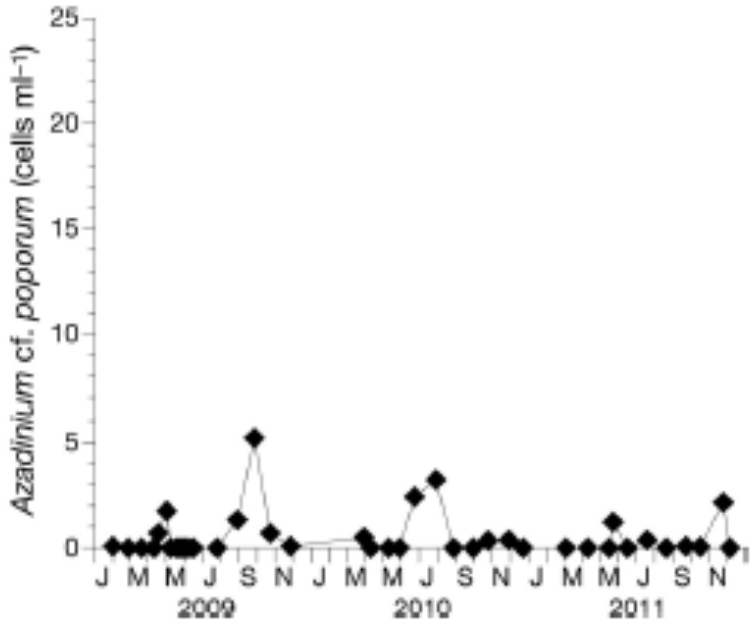


Fig. 6. *Azadinium* cf. *poporum*. Dynamics of abundances quantified by real-time PCR in Shiwha Bay, Korea, from 2009 to 2011. The highest values obtained at each sampling date are reported. The presence of ice prevented sampling in some months

Potvin et al. 2013

Az. spinosum, temporal and spatial distribution data Ireland

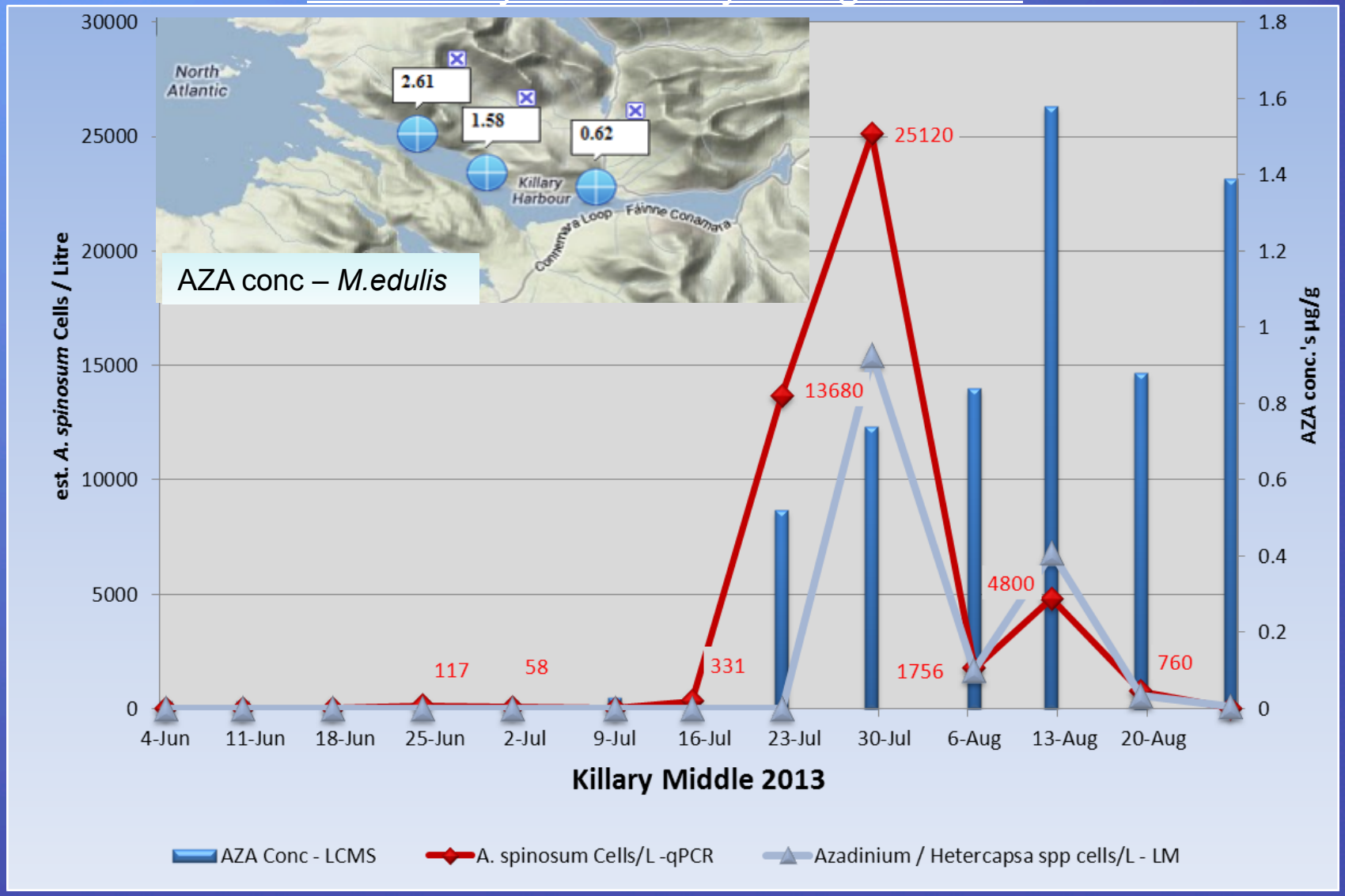
Real Time PCR methodologies as a routine monitoring tool for the detection of *Pseudo-nitzschia* , *Azadinium* & *Alexandrium* species in Irish waters

Dave Clarke, Rafael Gallardo Salas, Joe Silke
Marine Institute, Ireland

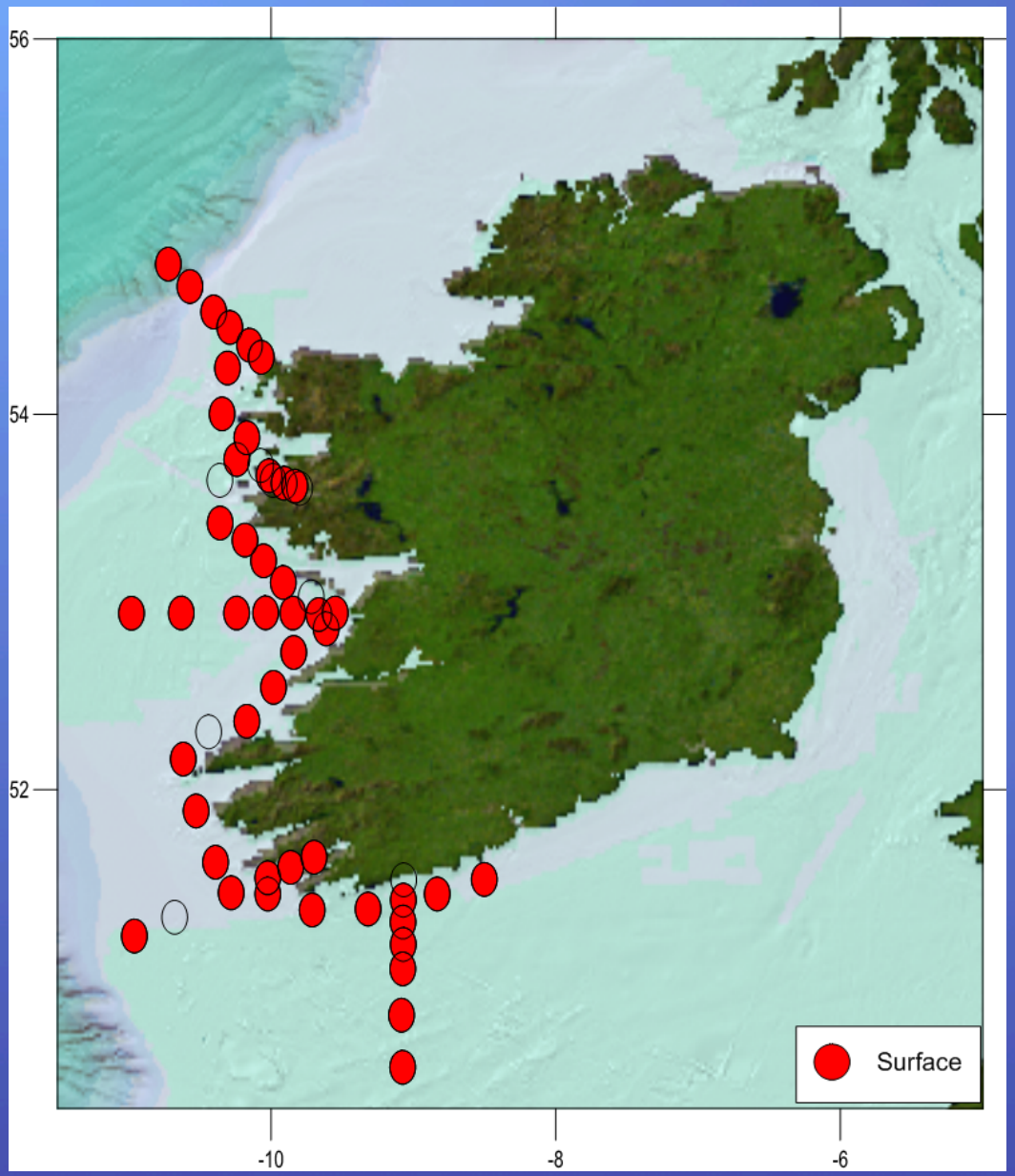
AOAC Biotoxin Taskforce, Baiona
17th June 2015

Marine Institute
Foras na Mara

AZA conc.'s in *M.edulis* & *A. spinosum* cells/litre (qPCR) from Killary Middle July – August 2013



Dave Clake, Rafael Salas, Joe Silke, MI Ireland



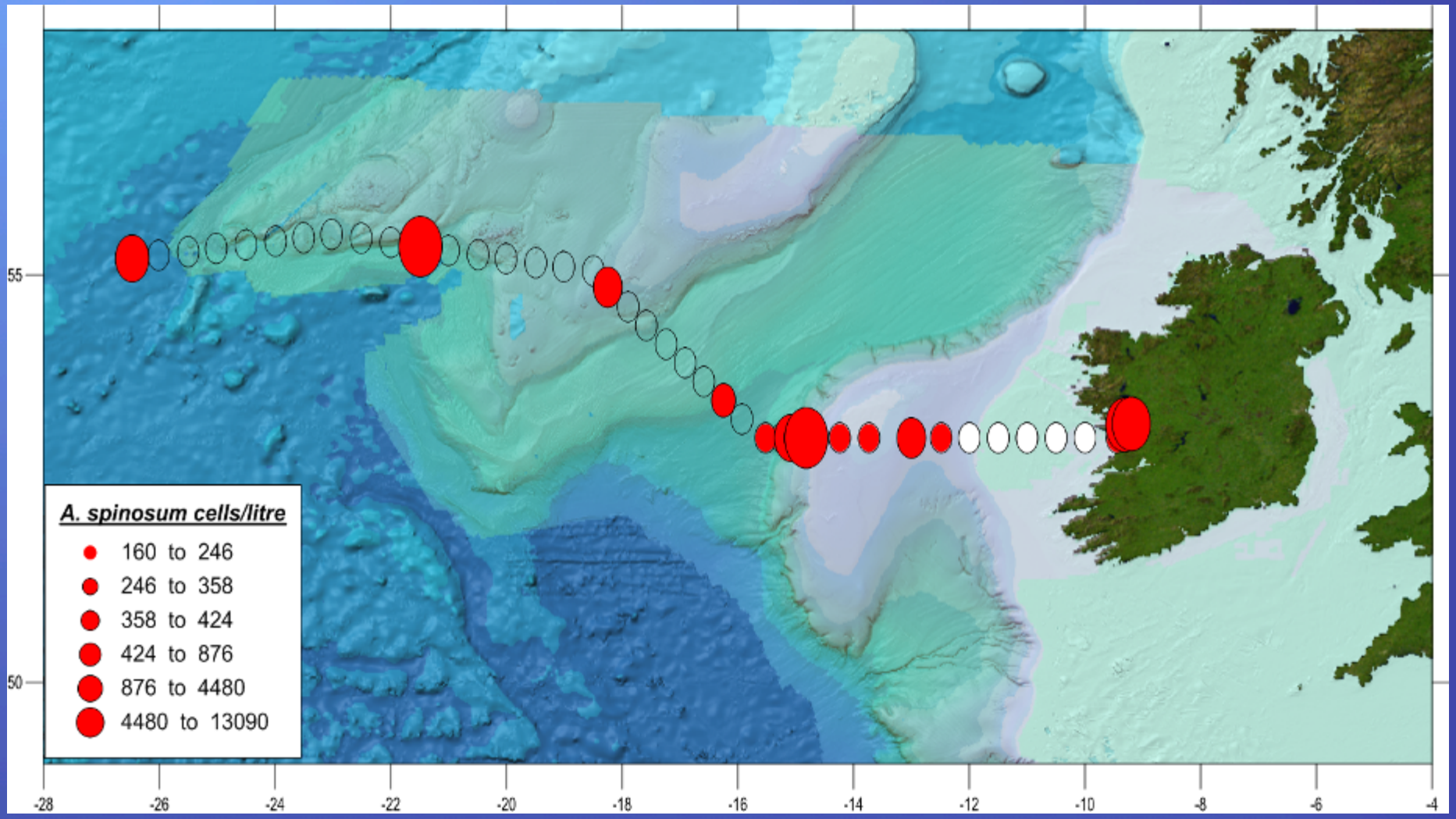
CV12016 Survey

3µm TSTP Filters
qPCR detection

A. spinosum
presence / absence

Dave Clake, Rafael Salas, Joe Silke,
MI Ireland

Newfoundland - Ireland Transatlantic Survey 2014
CE14008



A. spinosum cells counts – DNA extracted from cells filtered onto 3µm TSTP filters from 3 depths analysed by qPCR

Dave Clake, Rafael Salas, Joe Silke, MI Ireland

„Bloom“ recods

Based on light microscopy counts

Akselman & Negri: Argentinean Shelf

Azadinium spp bloom concentrations:

9×10^6 cell l^{-1} (spring 1990, temp: not reported, but probabaly <10 °C))

$1.5 – 3 \times 10^6$ cells l^{-1} (spring 1991, temp: 5–10 °C)

Sonia Sanchez et al: Peruvian coast

Azadinium polongum bloom concentrations:

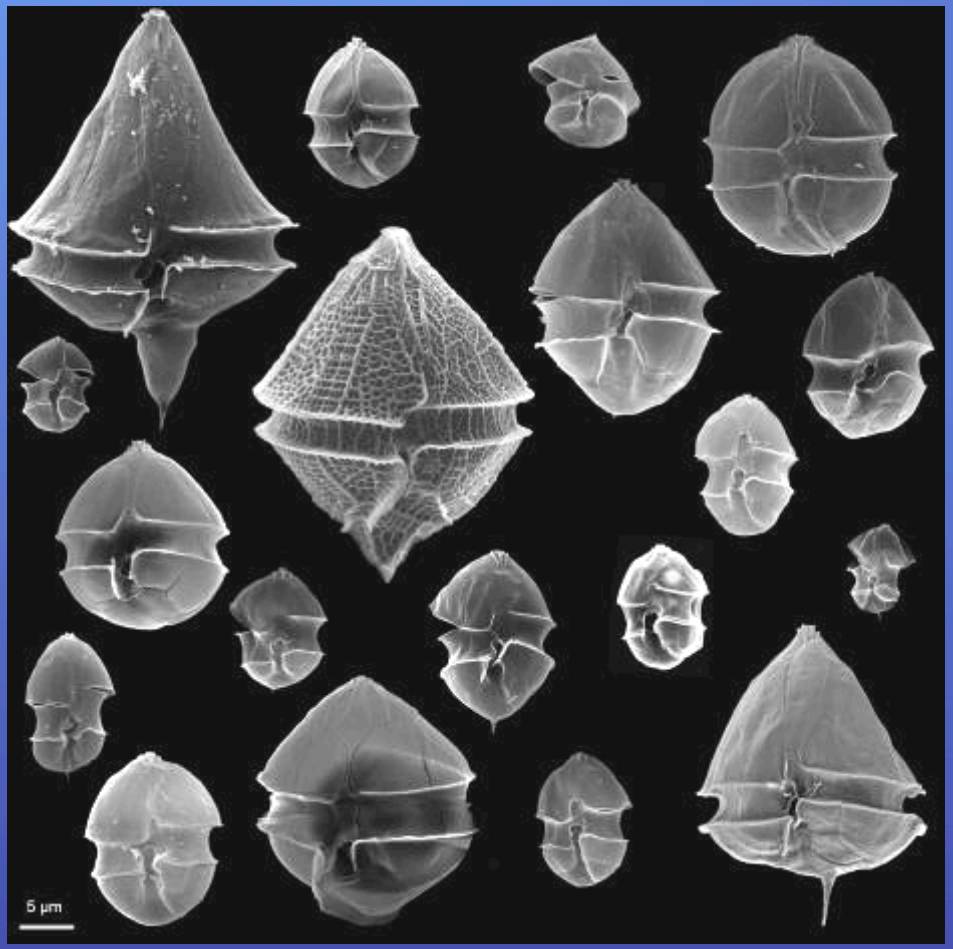
Up to 1×10^6 cells l^{-1}
(summer 2014, temp: 20–21 °C)

Guinder & Tillmann: Argentinean Shelf

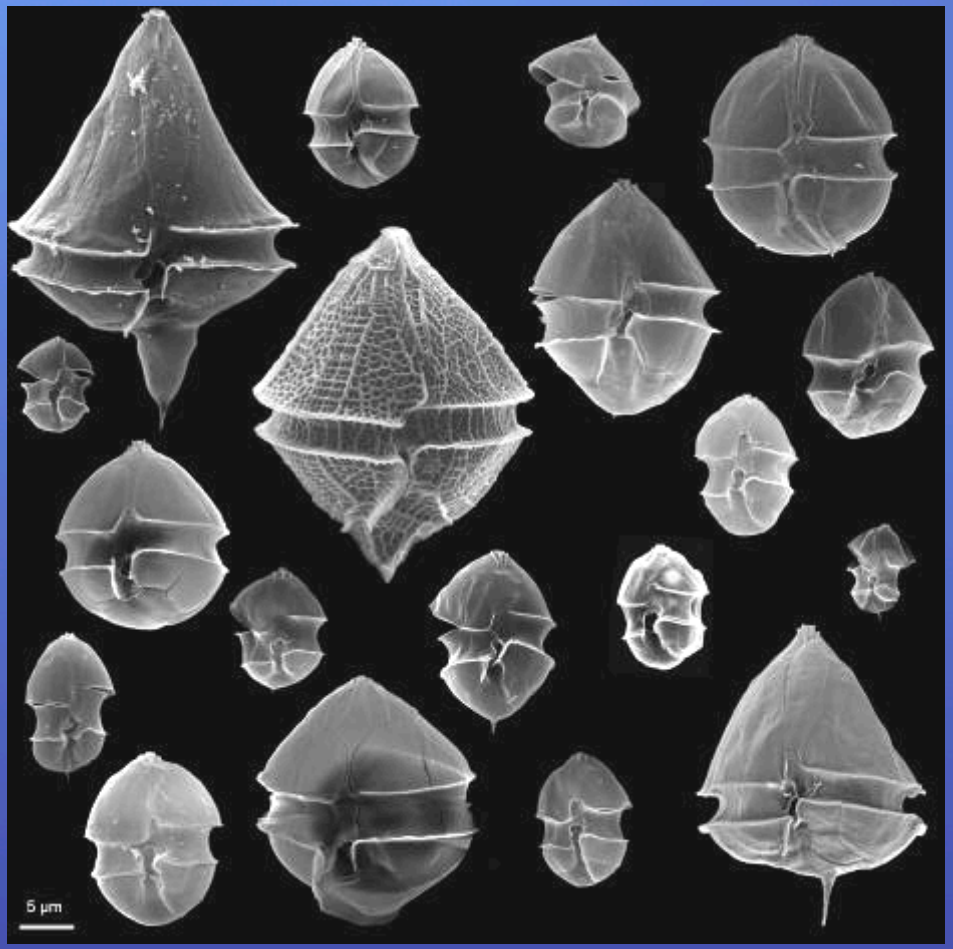
Azadinium spp concentrations:

Up to 0.28×10^6 cells l^{-1}
(Houssay cruise spring 2015, temp : around 10 °C)

Diversity of Amphidomataceae



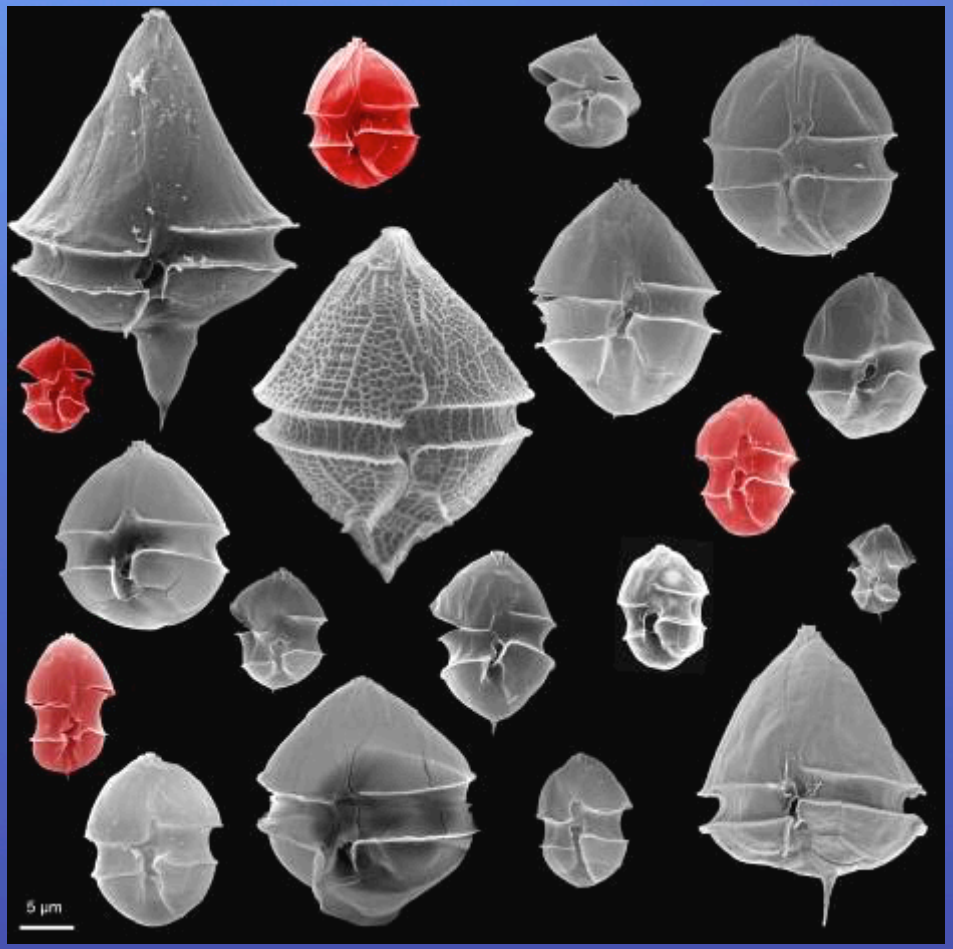
Diversity of Amphidomataceae



Az. spinosum
Az. obesum
Az. poporum
Az. polongum
Az. caudatum var *caudatum*
Az. caudatum var *margalefii*
Az. dexteroporum
Az. dalianense
Az. trinitatum
Az. cuneatum
Az. concinnum
Az. luciferelloides
Az. sp. nov. *#1*

Am. languida
Am. sp. nov. *#1*
Am. sp. nov. *#2*
Am. sp. nov. *#3*
Am. sp.nov. *#4*
Am. nucula
Am. steinii
Am. acuminata
Am. laticincta
Am. elongata
Am. curtata
Am. depressa
Am. obtusa

Diversity of Amphidomataceae



Az. spinosum
Az. obesum
Az. poporum
Az. polongum
Az. caudatum var *caudatum*
Az. caudatum var *margalefii*
Az. dexteroporum
Az. dalianense
Az. trinitatum
Az. cuneatum
Az. concinnum
Az. luciferelloides
Az. sp. nov. *#1*

Am. languida
Am. sp. nov. *#1*
Am. sp. nov. *#2*
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Am. nucula
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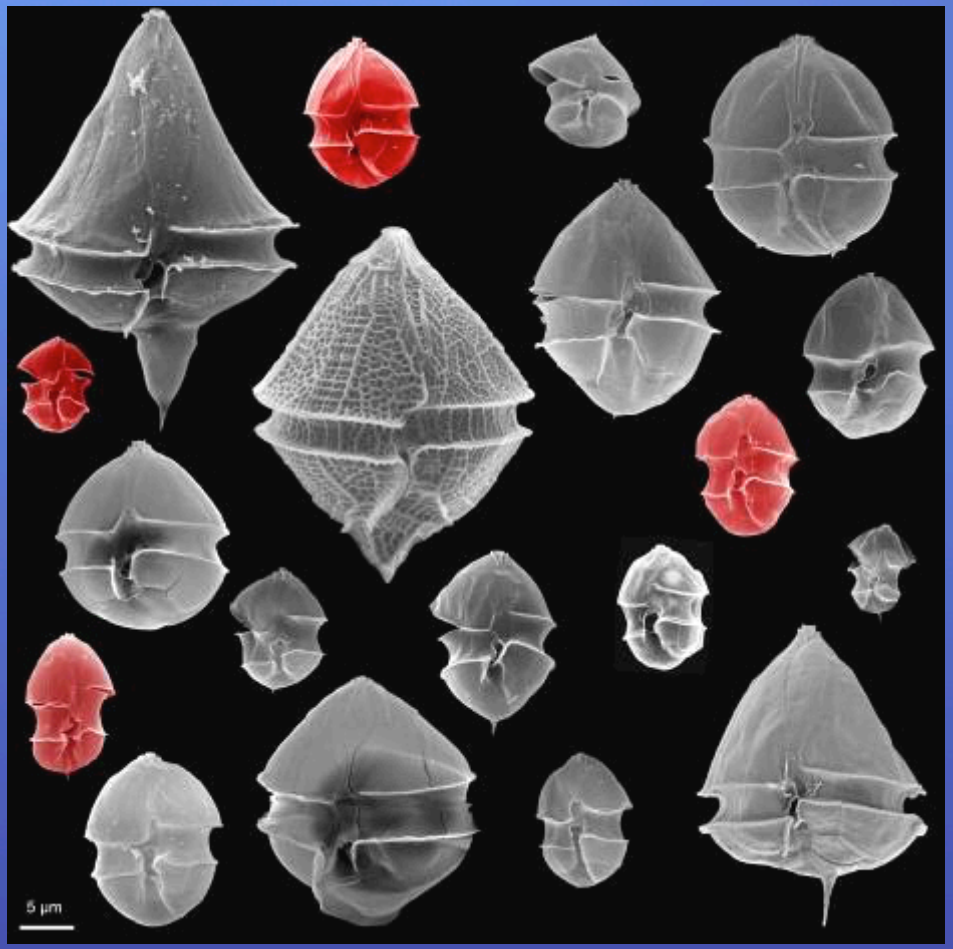
Am. laticincta
Am. elongata
Am. curtata
Am. depressa
Am. obtusa

Diversity of Amphidomataceae



Az. spinosum
Az. obesum
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Az. caudatum var *caudatum*
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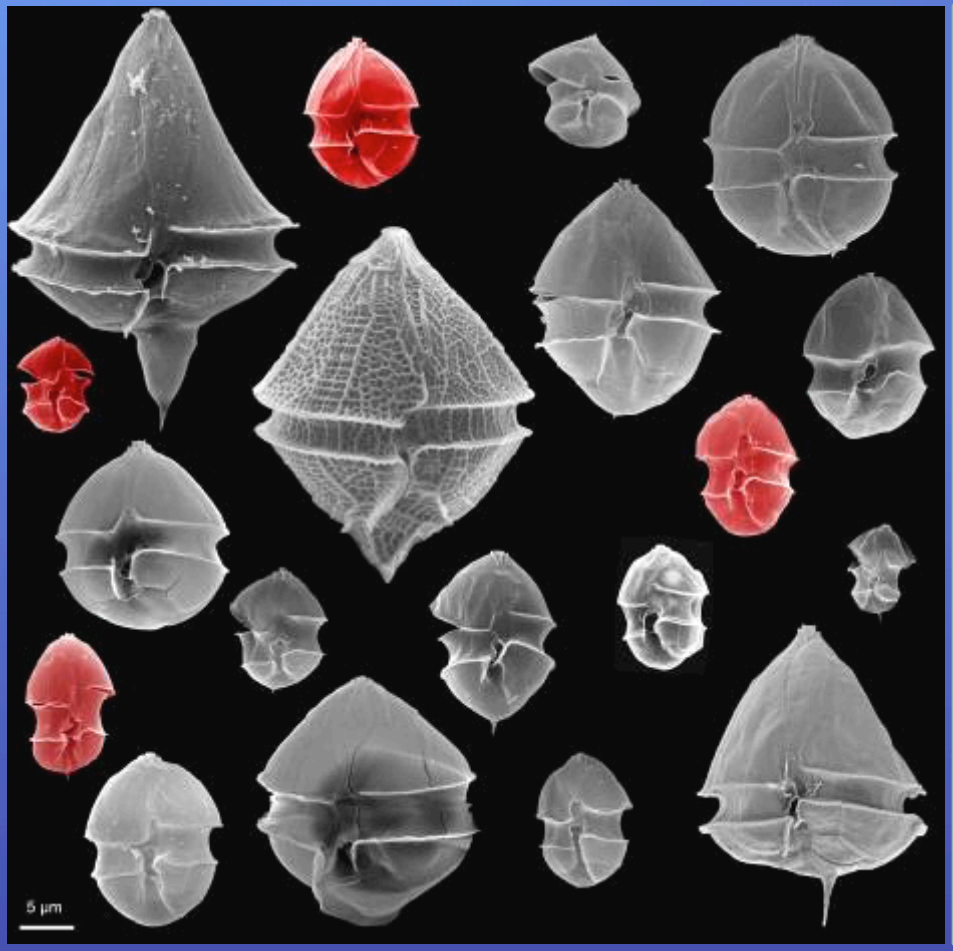
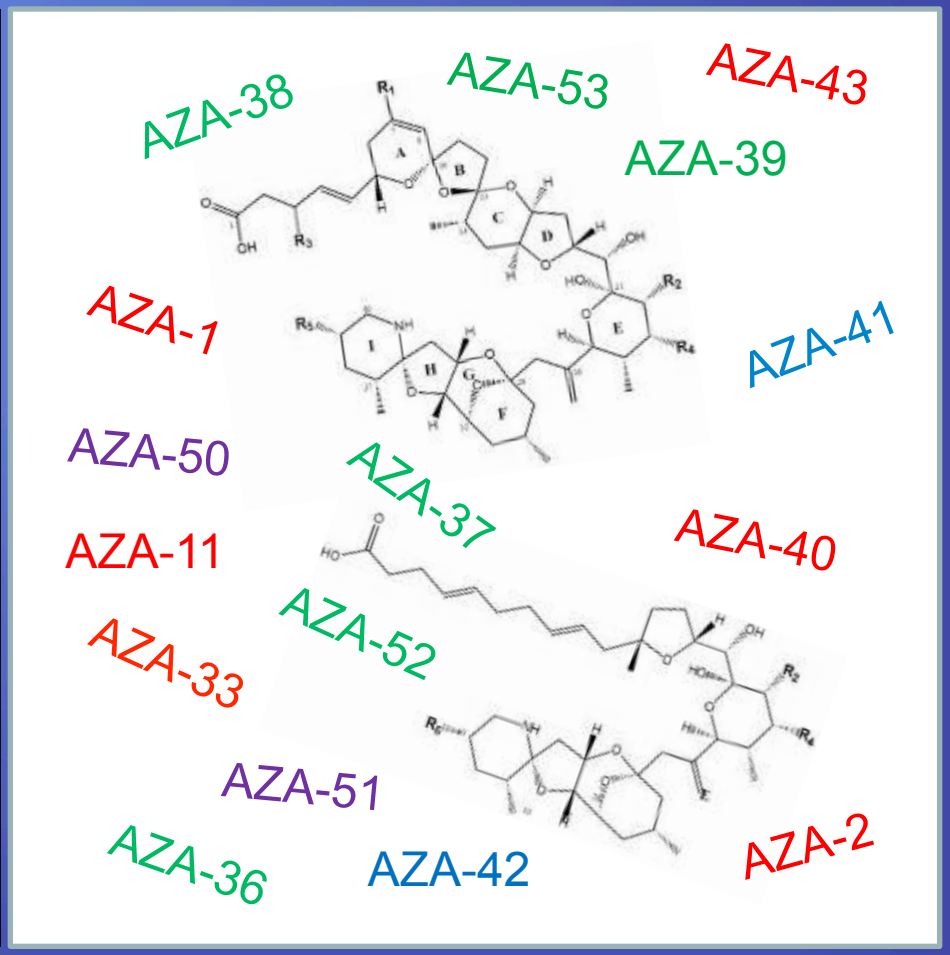
Az. sp. nov. *#1*

Am. languida
Am. sp. nov. *#1*
Am. sp. nov. *#2*
Am. sp. nov. *#3*
Am. sp.nov. *#4*
Am. nucula
Am. steinii
Am. acuminata
Am. laticincta
Am. elongata
Am. curtata
Am. depressa
Am. obtusa

Diversity of Amphidomataceae





AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	Phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.

not existent

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	Phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.

not existent

artefact

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	Phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.

not existent

artefact

metabolite

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	Phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.

not existent

artefact

metabolite

phycotoxin

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H	CH3	CH3	888,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	Phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	Phycotoxin	Krock & Tillmann, unpubl.

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	phycotoxin	Krock & Tillmann, unpubl.

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Krock et al. 2009
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Krock et al. 2009
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	A. poporum	phycotox, metabol	Krock et al. 2012
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	phycotoxin	Krock & Tillmann, unpubl.

Structure elucidated by NMR, cytotoxicity tested

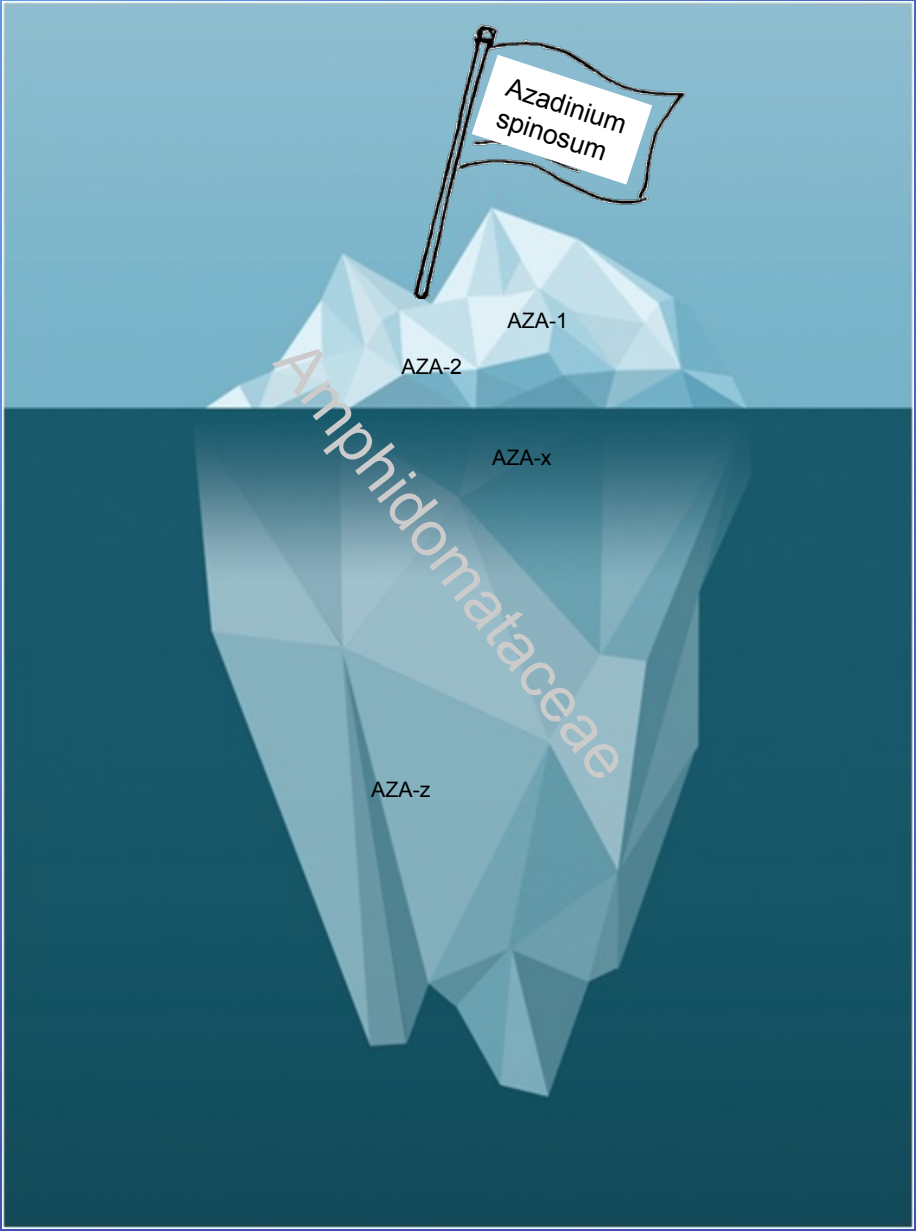
Cytotoxicity (T Lymphocyte Cytotoxicity)

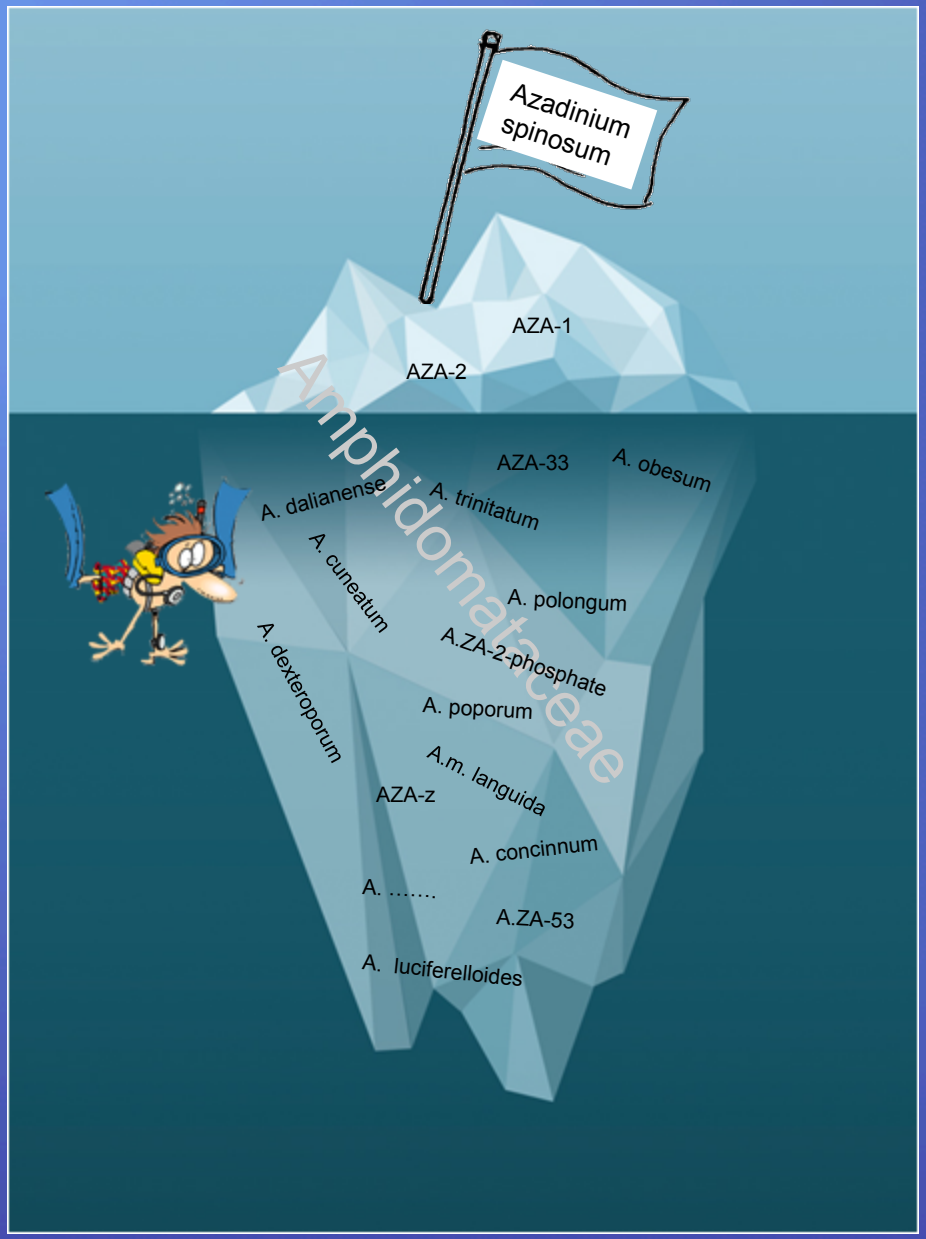
compound	rel. potency
AZA-1	1.00
AZA-2	3.67
AZA-33	0.21
AZA-34	5.50
AZA-36	0.16
AZA-37	0.33

Twiner et al. 2012
Kilkoyne et al. 2014
Krock et al. 2015

AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 - 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 - 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362-262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348-248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348-248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348-248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	phycotoxin	Krock & Tillmann, unpubl.
AZA-2-phosphate	H	CH3	H	CH3	H	CH3	CH3	936,5	362-262	A. poporum	phycotoxin	Tillmann et al. 2016
AZA-11-phosphate	OH	CH3	H	CH3	H	CH3	CH3	952,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-35-phosphate	–	H	H	CH3	H	CH3	CH3	910,5	362-262	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA-37-phosphate	OH	H	H	CH3	H	H	CH3	926,5	348-248	A. poporum	phycotoxin	Krock & Tillmann, unpubl.

Minor compound (< 10% of the most abundant AZA)


Azadinium spinosum
AZA-1
AZA-2
AZA-x
AZA-z
Amphidomataceae


Azadinium spinosum
AZA-1
AZA-2
Amphidomataceae
AZA-33
A. obesum
A. dalianense
A. trinitatum
A. cuneatum
A. polongum
A.ZA-2-phosphate
A. dexteroporum
A. poporum
A.m. languida
AZA-z
A. concinnum
A. …….
A.ZA-53
A. luciferelloides

Acknowledgment

ASTOX II

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