Apr 4th, 3:30 PM - 3:45 PM

First records of the genus azadinium (dinophyceae) from Puget Sound, Washington State

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First record of the genus *Azadinium* (Dinophyceae) from the Puget Sound, western Washington State

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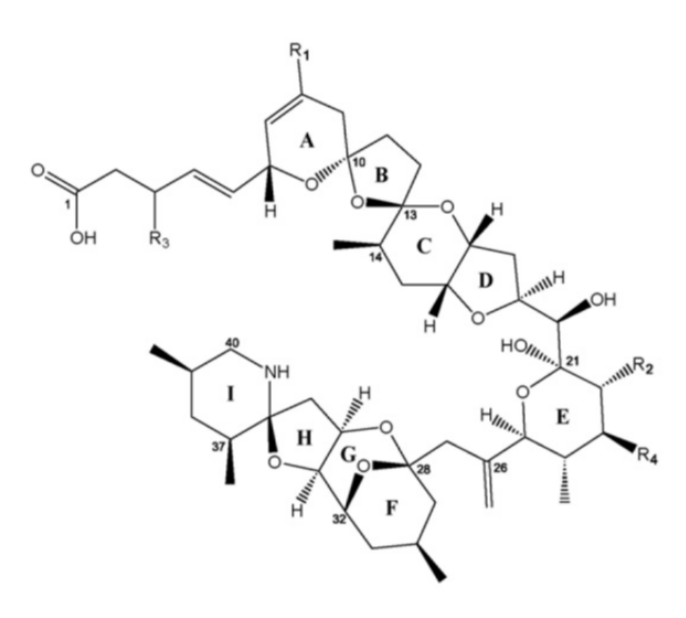
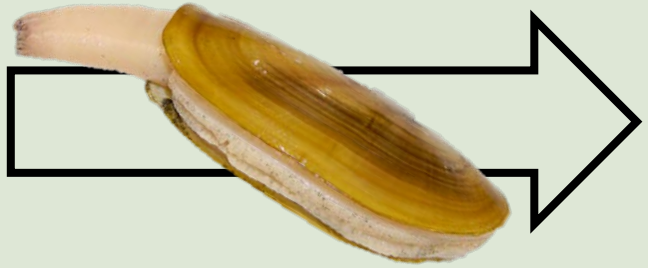
Introduction

- Azaspiracids (AZA) are a relatively new (late 90s) class of lipophilic compounds which are responsible for Azaspiracid Shellfish Poisoning (ASP)
- ASP cause several symptoms (nausea, vomiting, severe diarrhea and stomach cramps) on human health



Azaspiracid



nausea

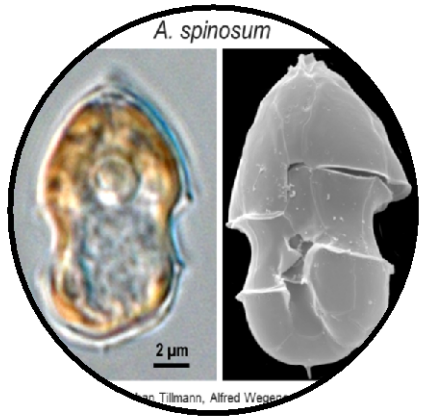
stomach cramps

Discovery timeline

1995:
Unexplained human illnesses in the Netherlands

1998:
Toxin identified and named azaspiracid (AZA)

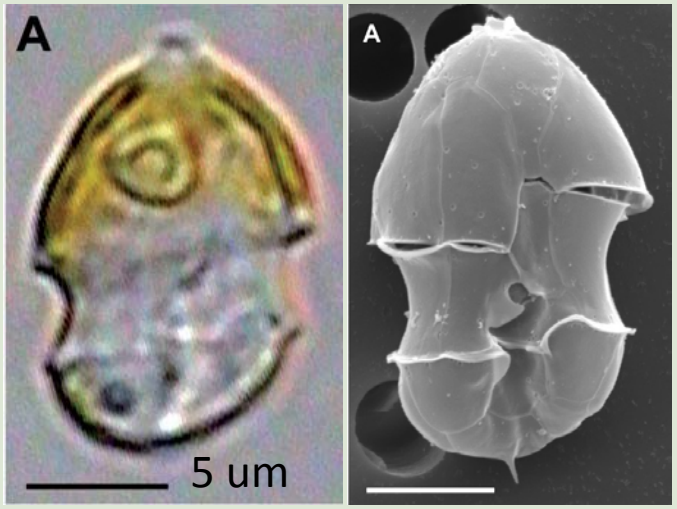
2003:
Protoperidinium crassipes thought to be AZA producer



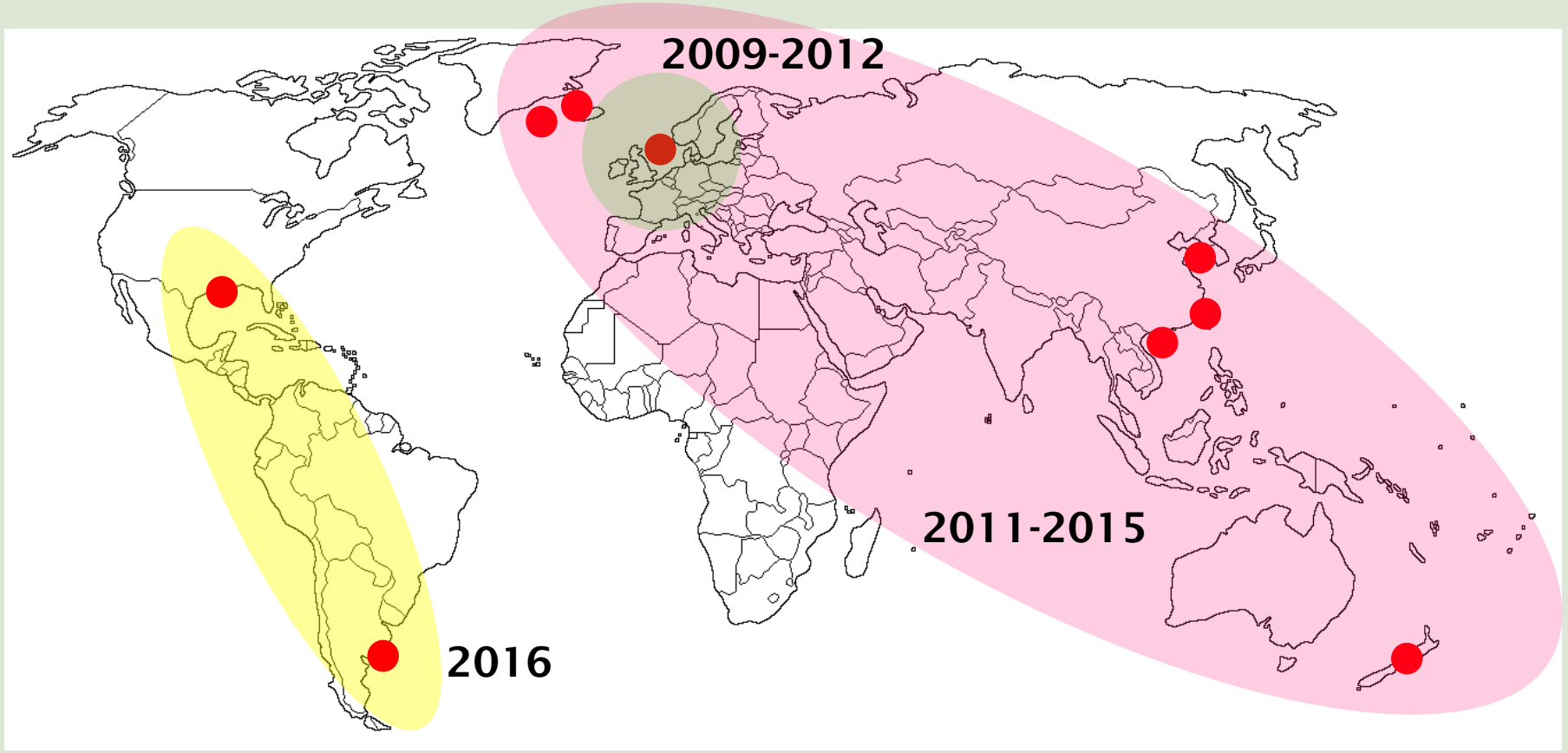
2009:
Azadinium spinosum identified as the toxin producer

- *Azadinium spinosum* has been known as a producer responsible for azaspiracids in 2009 (Tillmann et al., 2009).
- Some species of the genus *Azadinium* were described as azaspiracid producers (*A. dexteroporum*, *A. poporum*, *A. languida*) and this genus has a global distribution.

Our motivation: Undiagnosed illnesses with DSP symptoms in Washington State



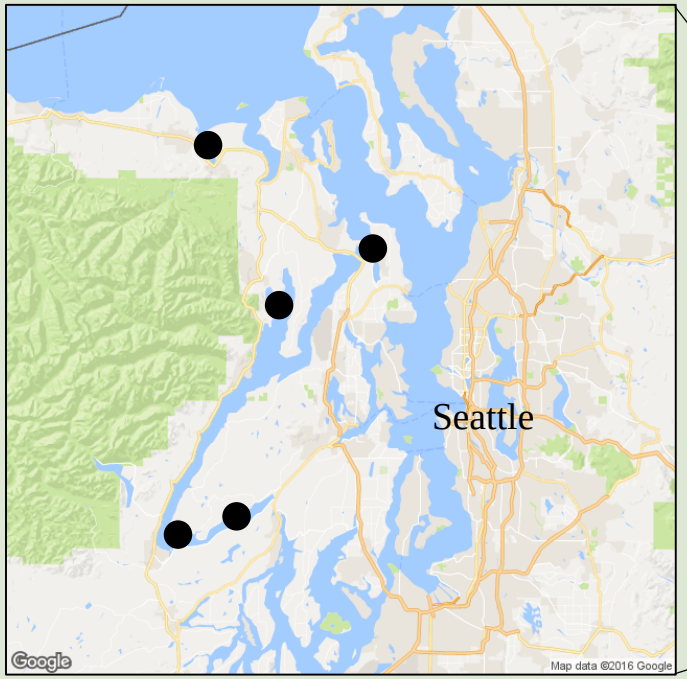
Azadinium spinosum
(Tillmann, 2009)



Materials & Methods

Sampling

- Sampling was performed during Jan-Feb 2016
- Sediment samples were collected from Puget Sound using Van Veen grab



Detection of *Azadinium* using molecular tools

- To assist *Azadinium* isolation, qPCR assay was conducted on sediment samples
- DNA extraction of sediment was conducted (Kim et al., 2016)
- Relative quantity of *Azadinium* cysts was estimated using Amphidomataceae family specific primer (Smith et al. 2015)

Sediment suspension (0.1g / mL)

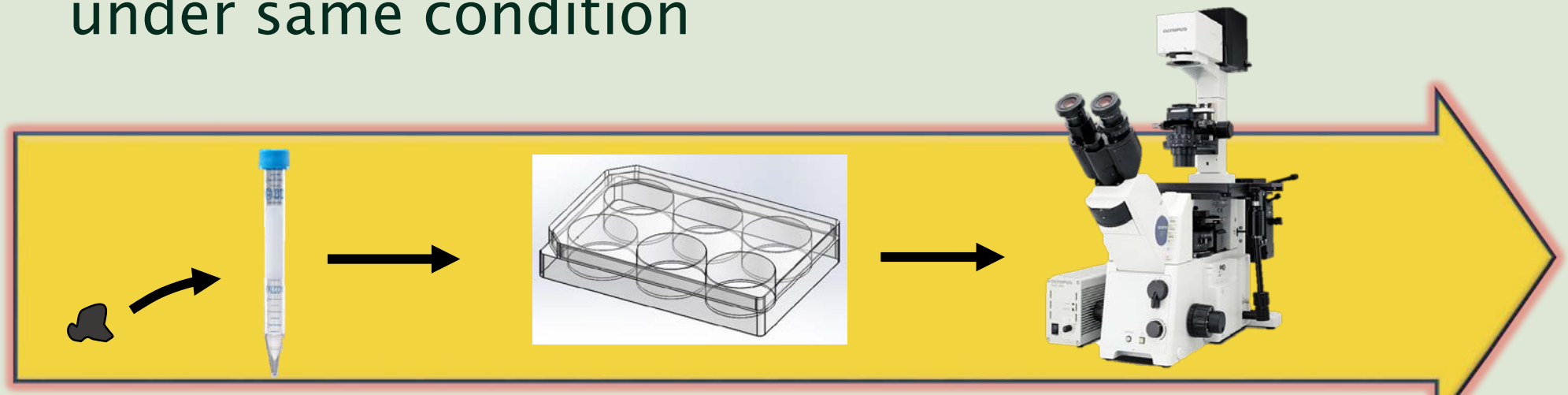
DNA extraction

qPCR assay

Detection & Quantification

Isolation & culture

- Sediment suspension was diluted with ESNW-Si medium in 6-well plate under 18 ºC, 12:12 L:D cycle condition
- After 5 days, *Azadinium* like cells were isolated by capillary pipette
- Isolated cells were transferred into 96-well plate and incubated under same condition



Sediment suspension (0.1g / mL)

Incubation

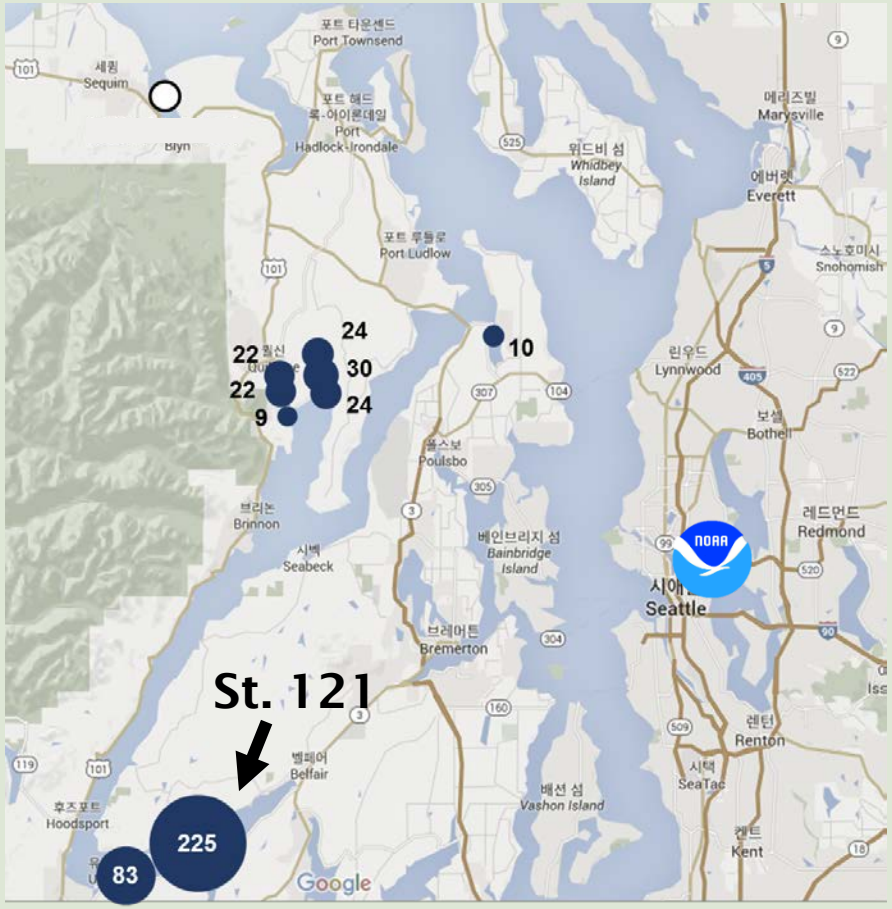
isolation

incubation

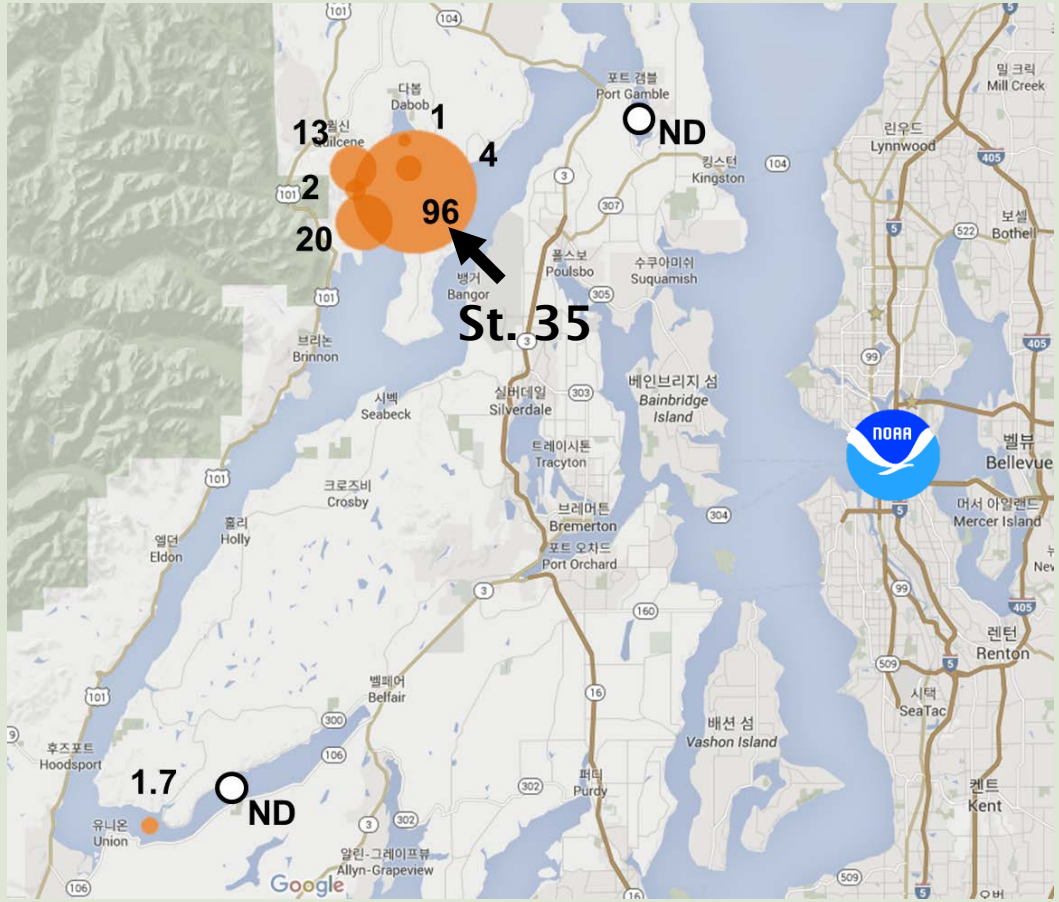
Results

- Detection of *Azadinium* using molecular tools

Amphidomataceae

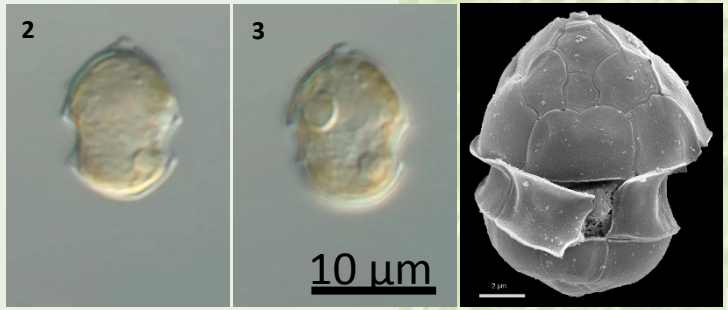


A. poporum

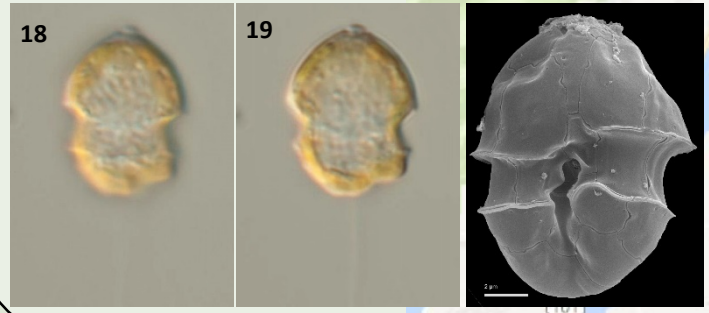


- Total 5 *Azadinium* species were isolated and identified from Puget Sound

A. cuneatum



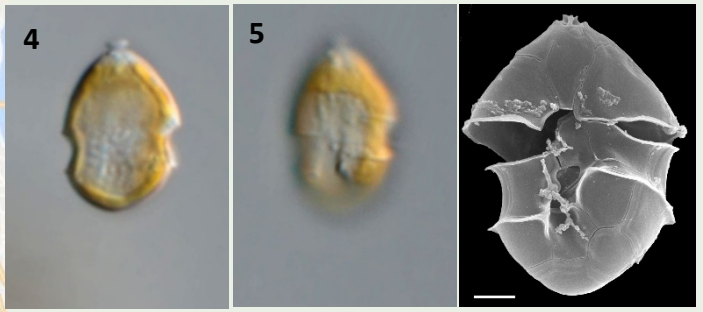
A. poporum



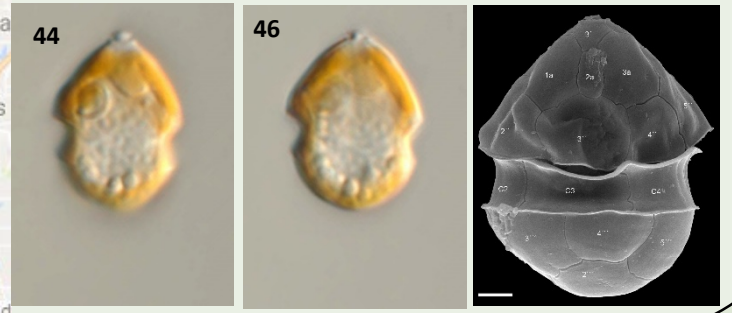
A. cf. dalianense

A. obesum

A. dalianense

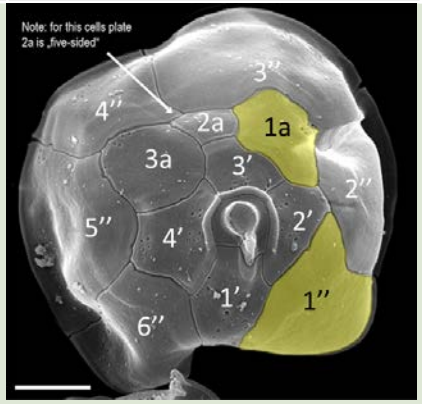


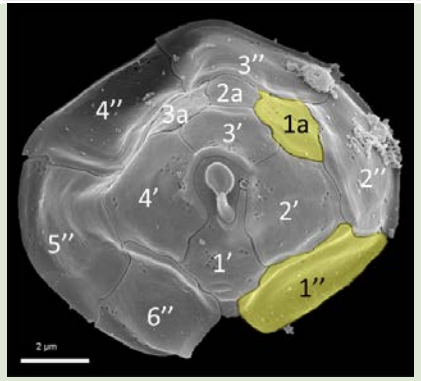
A. poporum

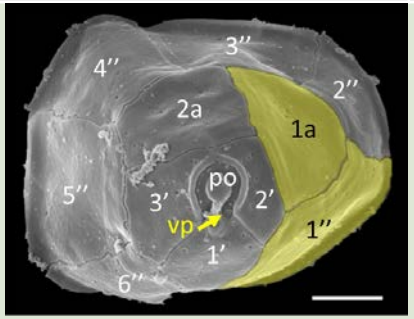


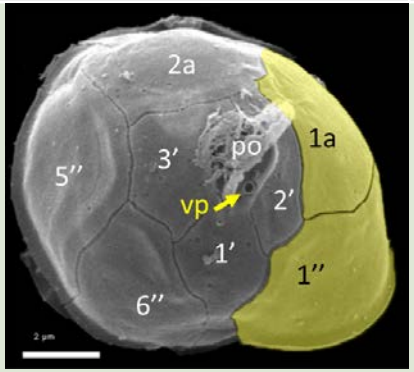
Morphological characteristics of the five species

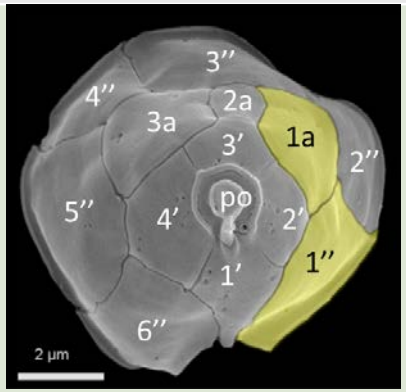
	A. obesum	*A. cuneatum*	*A. dalianense*	*A. cf. dalianense*	*A. poporum*
Apical plates	4	4	3	3	4
Intercalary plates	3	3	2	2	3
Vp position	left side of 1’	pore plate, left side	pore plate, left side	pore plate, left side	pore plate, left side
1’’ adjacent to 1a	no	no	yes	yes	yes

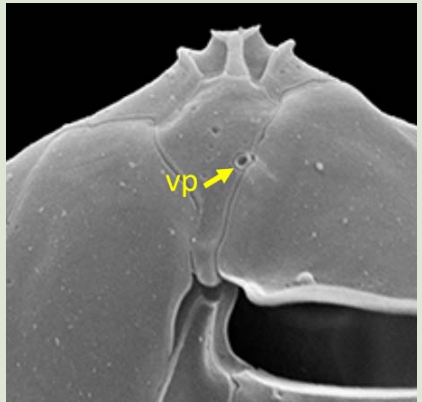


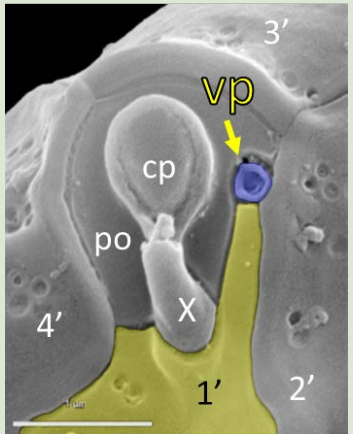


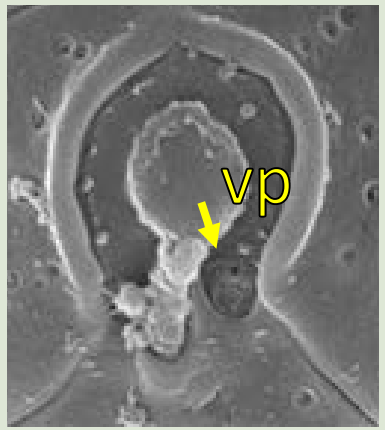


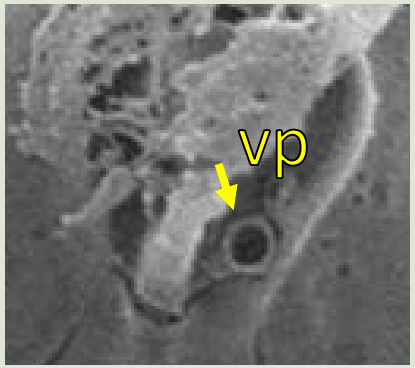


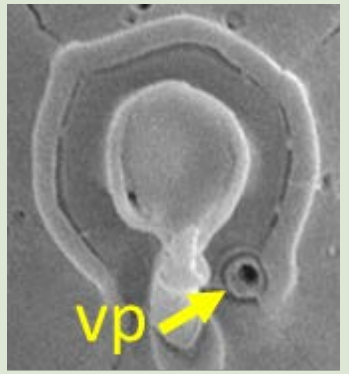








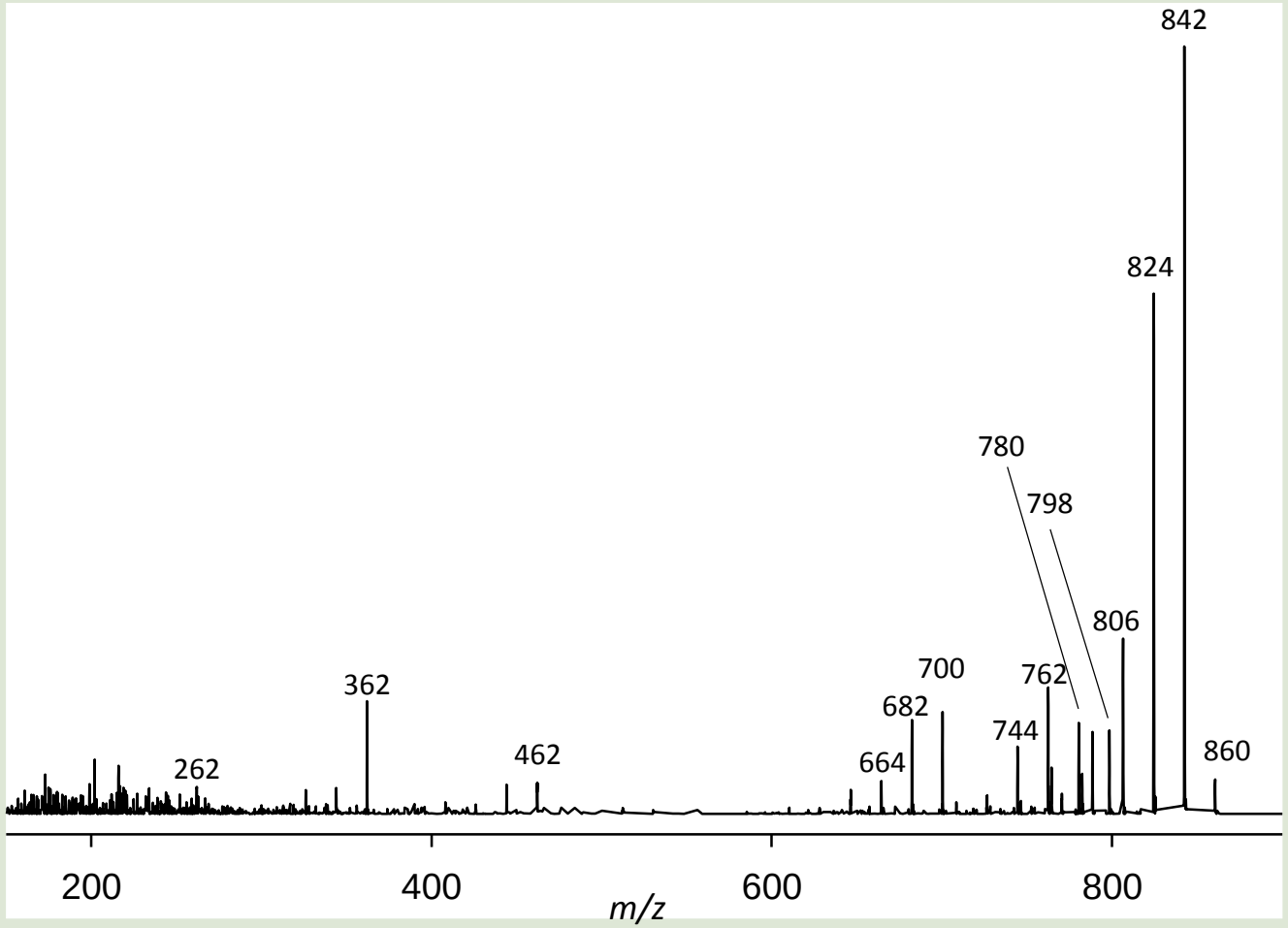




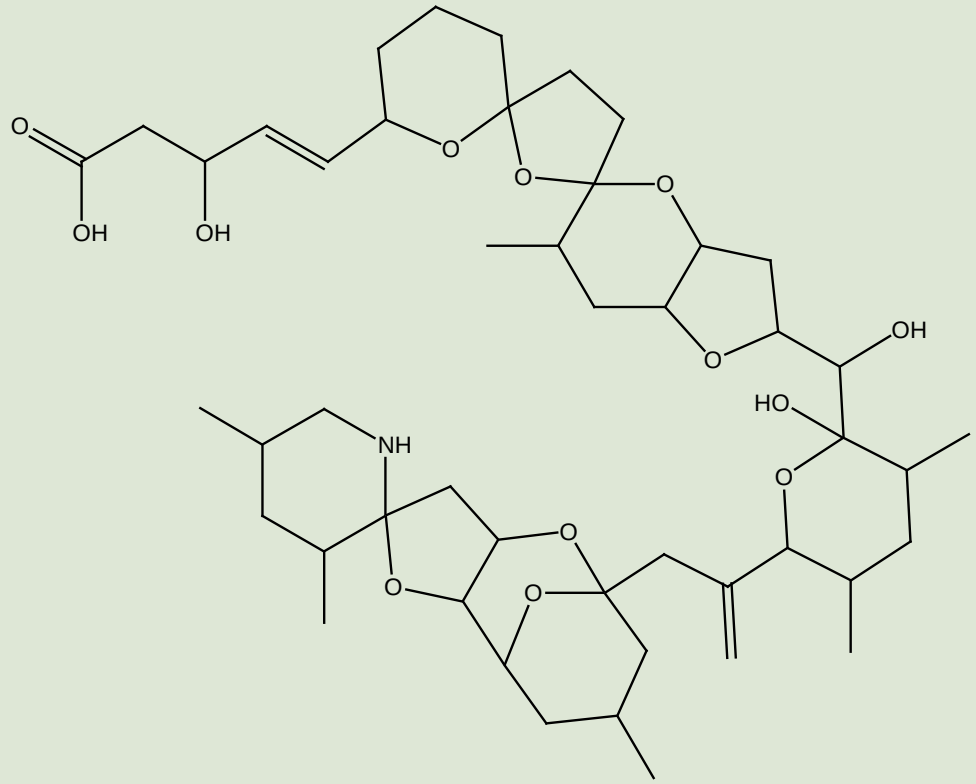
Toxin analysis result

Species	Strains	AZA
Azadinium cf. *dalianense*	481F8	Neg.
Azadinium cf. *dalianense*	962B3	Neg.
Azadinium cuneatum	965F5	Neg.
Azadinium cuneatum	966G8	Neg.
Azadinium cuneatum	968B10	Neg.
Azadinium cuneatum	S35A2	Neg.
Azadinium cuneatum	S35C4	Neg.
Azadinium dalianense	962B8	Neg.
Azadinium dalianense	S121F6	Neg.
Azadinium obesum	481F2	Neg.
Azadinium poporum	967B8	**Pos.**
Azadinium poporum	967G9	**Pos.**
Azadinium poporum	968B7	**Pos.**
Azadinium poporum	S121E10	**Pos.**

Structure of AZA-59 from Puget sound *A. poporum*



CID spectrum of AZA-59 = 7,8-hydro-3-hydroxy-AZA-1 (m/z 860)



AZA-59 = 7,8-hydro-3-hydroxy-AZA-1 (m/z 860)

Summary

- First report of *Azadinium* species at Northeast Pacific region
- *Azadinium* species were *A. cuneatum*, *A. obesum*, *A. dalianense*, *A.* cf. *dalianense*, *A. poporum*
- AZA-59 is a new toxin

Acknowledgements

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