Azaspiracids producing dinoflagellates

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Azadinium spp.

Found in 2009 as a producer of lipophilic polyether toxin Azaspiracids (AZAs), responsible for diarrhetic shellfish poisoning, which has been reported from northern Europe including Ireland, United Kingdom, Norway, Netherlands, France, Spain, Portugal and Italy. (Krock et al. 2009, Tillmann et al. 2011)

New genus and new species, *Azadinium spinosum* were described for the organism in 2011, showing characteristic features including thecal plate arrangements and rDNA phylogeny. (Tillmann et al. 2011)

10 species with 2 varieties were so far described in the genus. (Tillmann et al. 2014)

Cells are generally small (7–18 µm), autotrophic, and occur in planktonic form. (Tillmann et al. 2014)
Cell shape of *Azadinium* and *Amphidoma*

*Azadinium spinosum*\(^a\) (type species)

*Azadinium obesum*\(^b\)

*Azadinium poporum*\(^c\)

*Azadinium caudatum* var. *margeleffii*\(^d\)

*Azadinium caudatum* var. *caudatum*\(^d\)

*Azadinium polongum*\(^e\)

*Azadinium dexteroporum*\(^f\)

*Azadinium dalianense*\(^g\)

*Azadinium trinitatum*\(^h\)

*Azadinium cuneatum*\(^h\)

*Azadinium concinnum*\(^i\)

*Amphidoma languida*\(^i\)

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### Species discrimination (Tillmann et al. 2014, Protist)

<table>
<thead>
<tr>
<th>Species</th>
<th>Length range (mean) (mm)</th>
<th>Width range (mean) (mm)</th>
<th>L/W ratio</th>
<th>Number apical / intercalary plates</th>
<th>Antepical spine</th>
<th>Stalked pyrooid</th>
<th>1” adjacent to ta</th>
<th>Vp position</th>
<th>Pore plate symmetry</th>
<th>Shape of 1” plate</th>
<th>Rel. size first and last intercalary</th>
<th>Relative size apical plates</th>
<th>AZAs</th>
<th>Records</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. spinosum</td>
<td>12.3-15.7</td>
<td>7.4-10.3</td>
<td>1.6</td>
<td>4 / 3</td>
<td>spine</td>
<td>yes</td>
<td>left side of 1”</td>
<td>left side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>large</td>
<td>medium</td>
<td>A2A-1, 2, 716</td>
<td>North Sea, Atlantic, Pacific</td>
<td>a, b, c</td>
</tr>
<tr>
<td>A. obscurum</td>
<td>13.3-17.7</td>
<td>10.0-14.3</td>
<td>1.8</td>
<td>4 / 3</td>
<td>no</td>
<td>yes</td>
<td>left side of 1”</td>
<td>left side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>A2A-2, 848, trz, none</td>
<td>North Sea, Mediterranean, North Sea, Asian Pacific</td>
<td>d, e, f, g, h</td>
</tr>
<tr>
<td>A. poporum</td>
<td>11.3-16.3</td>
<td>7.4-10.3</td>
<td>1.3</td>
<td>4 / 3</td>
<td>no</td>
<td>yes</td>
<td>right side of 1”</td>
<td>right side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>North Sea, Mediterranean, North Sea, Atlantic</td>
<td>i, j, k, l, m</td>
</tr>
<tr>
<td>A. caudatum ver. margalese</td>
<td>21.0-42.1</td>
<td>25.0-36.7</td>
<td>1.2</td>
<td>4 / 3</td>
<td>short horn, long spine</td>
<td>not shown</td>
<td>up to four</td>
<td>yes</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>not tested</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td>n, o</td>
</tr>
<tr>
<td>A. caudatum ver. caudatum</td>
<td>35.0-32.5</td>
<td>25.0-36.7</td>
<td>1.2</td>
<td>4 / 3</td>
<td>long horn, short spine</td>
<td>not shown</td>
<td>up to four</td>
<td>yes</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>not tested</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td>p, q, r</td>
</tr>
<tr>
<td>A. prologum</td>
<td>10.1-17.4</td>
<td>7.4-13.6</td>
<td>1.3</td>
<td>4 / 3</td>
<td>spine</td>
<td>yes</td>
<td>left side of 1”</td>
<td>left side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td>s, t, u</td>
</tr>
<tr>
<td>A. diatropoporum</td>
<td>11.0-10.7</td>
<td>8.3-12.7</td>
<td>1.2</td>
<td>4 / 3</td>
<td>spine</td>
<td>yes</td>
<td>left side of 1”</td>
<td>left side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td>v, w, x</td>
</tr>
<tr>
<td>A. dalinaufana</td>
<td>11.5-10.7</td>
<td>8.3-12.7</td>
<td>1.4</td>
<td>4 / 3</td>
<td>rare, short spine</td>
<td>up to two</td>
<td>(unstable)</td>
<td>yes</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td>y, z</td>
</tr>
<tr>
<td>A. trinitatum</td>
<td>11.5-10.7</td>
<td>8.3-12.7</td>
<td>1.5</td>
<td>4 / 3</td>
<td>spine</td>
<td>no</td>
<td>middle of pore plate</td>
<td>middle of pore plate</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td></td>
</tr>
<tr>
<td>A. cuneatum</td>
<td>12.0-13.9</td>
<td>8.5-12.7</td>
<td>1.3</td>
<td>4 / 3</td>
<td>no</td>
<td>no</td>
<td>middle of pore plate</td>
<td>middle of pore plate</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td></td>
</tr>
<tr>
<td>A. concinum</td>
<td>12.5-12.5</td>
<td>9.7-14.1</td>
<td>1.3</td>
<td>4 / 3</td>
<td>no</td>
<td>no</td>
<td>right side of 1”</td>
<td>right side of 1”</td>
<td>suture to 1” symmetric</td>
<td>narrow posteriorly</td>
<td>small</td>
<td>medium</td>
<td>none</td>
<td>Mediterranean, North Sea, Atlantic</td>
<td></td>
</tr>
</tbody>
</table>

**Antapical spine** (Tillmann et al. 2014, *Protist*)

<table>
<thead>
<tr>
<th>Antapical spine</th>
<th>A. spinosum</th>
<th>A. obesum</th>
<th>A. poporum</th>
<th>A. caudatum var. margaritiferum</th>
<th>A. caudatum long horn</th>
<th>A. polongum</th>
<th>A. dactyloponum</th>
<th>A. dalianense</th>
<th>A. trinatum</th>
<th>A. consertum</th>
<th>A. concinnum</th>
<th>Amphidoma longuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>a, b, c</td>
<td>d</td>
<td>e, f, g, h</td>
<td>i, j</td>
<td>i</td>
<td>k</td>
<td>l, m</td>
<td>n</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>p, h, m</td>
</tr>
</tbody>
</table>

- **A. spinosum** (Tillmann et al. 2009)
- **A. poporum** (Tillmann et al. 2011)
- **A. cuneatum** (Tillmann et al. 2014)
- **A. concinnum** (Tillmann et al. 2014)

Ventral pore position (Tillmann et al. 2014, Protist)

<table>
<thead>
<tr>
<th>Species</th>
<th>vp position</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. spinosum</td>
<td>left side of 1'</td>
<td>a, b, c</td>
</tr>
<tr>
<td>A. obesum</td>
<td>left side of 1'</td>
<td>d</td>
</tr>
<tr>
<td>A. poporum var. margaritae</td>
<td>pore plate, left side</td>
<td>e, f, g, h</td>
</tr>
<tr>
<td>A. caudatum var. caudatum</td>
<td>pore plate, right side</td>
<td>i</td>
</tr>
<tr>
<td>A. poligonum</td>
<td>left side of 1'</td>
<td>k</td>
</tr>
<tr>
<td>A. dexteroorum</td>
<td>end of pore plate, right side</td>
<td>l</td>
</tr>
<tr>
<td>A. dalianense</td>
<td>pore plate, left side</td>
<td>m</td>
</tr>
<tr>
<td>A. trinitatum</td>
<td>end of pore plate, left side</td>
<td>n</td>
</tr>
<tr>
<td>A. consetum</td>
<td>middle of pore plate, left side</td>
<td>o</td>
</tr>
<tr>
<td>A. cuneatum</td>
<td>pore plate, right side</td>
<td>p</td>
</tr>
<tr>
<td>Amphidinium longica</td>
<td>right side of 1' (anterior position)</td>
<td>p, h, m</td>
</tr>
</tbody>
</table>
Resting cyst like cells were observed only in *A. polongum*. But germination from sediments was reported also in *A. poporum* and *A. dalianense* (Gu et al. 2013, Luo et al. 2013).
Toxicity of *Azadinium* and *Amphidoma* (Tillmann et al. 2014, Protist)

AZAs were detected in three *Azadinium* and one *Amphidoma* species.

Distribution in the Western Pacific

Two species were observed from Korea, China, Vietnam and Japan.

A. poporum from Korea

A. dalianense from China
(Luo et al. 2013, Phycologia)

A. dalianense from China

A. poporum
ITS phylogeny of *A. poporum*

ML analysis based on 689 positions

Ribotype A: North Sea
Ribotype B: Asia
Ribotype C: Asia, Southwest Atlantic
Ribotype D: Gulf of Mexico

Takahashi, unpubl.
High variability of AZA profile in *A. poporum*

3 strains did not contain AZAs, and 13 strains contained different combinations of AZAs.
What factor is related to AZA productivity in *A. poporum*?

No clear relationship among ribotype, biogeography, and AZA combination was seen.
There are 10 described species in *Azadinium*, and closely related *Amphidoma languida* as potential producers of AZAs.

Resting form is most likely present in *A. polongum*, *A. poporum* and *A. dalianense*.

In Asia, two species *A. poporum* and *A. dalianense* were reported.

In *A. poporum*, some strains did not contain AZAs, and some strains contain different combinations of AZAs.
Are other *Azadinium* species distributed in Western Pacific?  
*Azadinium* species were mainly observed from Europe, but in Asia only two species were reported.

Do *Azadinium* species produce resting cyst?  
Presence/absence of productivity, morphology, distribution of resting cysts in each species will estimate AZA risk in each locality of Asia.

Do toxic species always produce AZAs?  
Do non-toxic species never produce AZAs?  
Analysis using different species and culture strains will help to clarify what factor (species, ribotype, biogeography) is related to AZA productivity.
Pinnatoxins producing dinoflagellates

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**Vulcanodinium rugosum**

Initially described as a new genus and new species in 2011 from Italy, and subsequently identified in the same year as a producer of cyclic imine toxin Pinnatoxin (PnTxs), using culture strains from Japan, New Zealand and South Australia. (Nezan et Chomerat 2011, Rhodes et al. 2011)

PnTxs were first characterized in pen shell *Pinna muricata* from Japan in 1995, but were detected widely including New Zealand, Australia, Japan, Norway, Canada and Cook Islands. (Rhodes et al. 2011)

Photosynthetic, armored and benthic species, and produces spherical immotile cells. (Nezan et Chomerat 2011)
Morphology and life history


Phylogeny

Nezan et Chomerat 2011, Cryptogamie, Algologie
Recent reports from China, Florida and Qatar

Florida

China

Qatar

Fig. 1. Scanning electron micrographs of motile and nonmotile cells of the *Vulcanodinium rugosum* strain CCFWCS16 isolated from the *M/V Southern Fighter*. (A) Comma-shaped apical pore plate (arrowhead) and side view of the invaginated 1\(^\prime\) plate and sa plate on motile cell. (B) Mucus exuding from apical pore complex (APC, arrowhead) and connectivity of the 3 pentagonal intercalary plates on motile cell. (C) Motile cell showing the overgrowth of the 2\(^\prime\) and 4\(^\prime\) plates obscuring the 1\(^\prime\) plate. The sa plate is visible. (D) Cluster of nonmotile cells. Arrowheads show division planes. Scale bar on (A) and (B) equals 10 \(\mu\)m, on (C), 5 \(\mu\)m, and on (D), 20-\(\mu\)m.

Garrett et al. 2014, Harmful Algae


Al Muftah et al. 2016, Toxicon
Fig. 5. Maximum likelihood tree of partial ITS region sequences of *Vulcanodinium rugosum* and closely related species. This tree was extracted from a larger ITS region sequence tree including other dinoflagellate species isolated in Qatar (Fig. S2) using Mega, version 5.2 (Tamura et al., 2011). Bootstrap values above 50% are shown next to the nodes. Branch lengths are proportional to the number of substitutions per site (see the scale bar).

Al Muftah et al. 2016, Toxicon
Summary

- *V. rugosum* was identified as a source organisms of PnTxs. In culture strains, coccoid stage was dominant in the life history.

- *V. rugosum* strains established at the same time were identical in ribotypes and combination of PnTxs.

- In Asia, *V. rugosum* was reported from Japan and China.
Future study

✓ How is the distribution of this species in other areas? If present, which genotypes are classified to them?

✓ How the combination of PnTxs is defined? (genotypes, locations).

PnTxs H was detected in the strain from Southern China, but more formerly other PnTxs were also reported in the environment near the place (Zeng et al. 2012).