Phylogeny and geographical distribution of \textit{Cochlodinium polykrikoides} population (\textit{Gymnodiniales, Dinophyceae}) collected from Japanese and Korean coasts

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A harmful dinoflagellate *Cochlodinium polykrikoides*

An unarmored dinoflagellate *Cochlodinium polykrikoides* has formed red tides responsible for mass mortalities of caged and wild fishes in Japanese and Korean coasts.
Occurrences of *C. polykrikoides* in Japan and Korea

Red tides of *C. polykrikoides* have continuously occurred along coastal waters, does this imply that the Japanese and Korean population are identical?

Miyahara et al. 2005

Sep. 2003

Matsuoka & Iwataki 2004

Jun-Aug 2002

Matsuoka & Iwataki 2004

Jun-Aug 2002
The genus *Cochlodinium*

The genus *Cochlodinium* has been established by Schütte (1894), circumscribing unarmored dinoflagellates possessing the cingulum encircling the cell >1.5 times (Kofoid & Swezy 1921).

Unarmored dinoflagellates have been classified based mainly on the position and torsion of the cingulum, however, this classification was incongruent with phylogenetic relationship based on recent molecular analyses.

*Cochlodinium* is still classified based on characteristics of the cingulum, because their phylogenetic relationships have not been examined.

**Objectives**

To clarify the phylogenetic positions of *Cochlodinium* species among dinoflagellates.

To distinguish populations of a harmful red tide forming species *C. polykrikoides*, and investigate distributions of each *C. polykrikoides* population.
Materials and Methods

Morphology
- Light and fluorescence microscopy

Molecular phylogeny
- SSU rDNA
- LSU rDNA (D1-D3)
  Trees constructed by weighted NJ method

Map indicating locations:
- Manila Bay, Philippines
- Sabah, Malaysia
- Lampung Bay, Indonesia
- Various other locations in the region
Red tide occurred in Malaysian, Brunei and Philippine waters.
Three Cochlodinium species

Three morphotypes assigned to the genus Cochlodinium, with the cingulum encircling the cell more than 1.5 times, have so far been observed and analyzed rDNAs.

C. convolutum  C. polykrikoides  Cochlodinium sp.
Cochlodinium convolutum

Cingulum
Sulcus
Cingulum
Cochlodinium polykrikoides

August 1999, Imari Bay, Japan
January 2004, Sabah, Malaysia
October 2003, Usuka Bay, Japan
August 2003, Mishima Is, Japan
June 2003, Shikamachi, Japan
July 2003, Tachibana Bay, Japan
May 2004, Inokushi Bay, Japan
August 2003, Isahaya Bay, Japan

Single cell

August 1999, Imari Bay, Japan
January 2004, Sabah, Malaysia
October 2003, Usuka Bay, Japan
August 2003, Mishima Is, Japan

Chain-forming cell

Eye spot
Cingulum
Sulcus
Cingulum
Cochlodinium sp.

Resembling *C. polykrikoides*, but different in the position of the sulcus and chloroplasts.
### Morphological characters of *Cochlodinium* spp.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>C. convolutum</em></th>
<th><em>C. polykrikoides</em></th>
<th><em>Cochlodinium</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td>ca. 60-70 µm</td>
<td>ca. 30-40 µm</td>
<td>ca. 45-50 µm</td>
</tr>
<tr>
<td><strong>Eye spot</strong></td>
<td>Absent</td>
<td>Dorsal, epicone</td>
<td>Dorsal, epicone</td>
</tr>
<tr>
<td><strong>Cingulum</strong></td>
<td>ca. 1.5 times</td>
<td>ca. twice</td>
<td>ca. twice</td>
</tr>
<tr>
<td><strong>Sulcus</strong></td>
<td>Deeper</td>
<td>Shallow, immediately below the cingulum</td>
<td>Shallow, intermediate of the cingulum</td>
</tr>
<tr>
<td><strong>Nucleus</strong></td>
<td>Rectangular</td>
<td>Spherical, anterior</td>
<td>Spherical, anterior</td>
</tr>
<tr>
<td><strong>Chloroplast</strong></td>
<td>Reticulate (?)</td>
<td>Rod-like, aligned longitudinally</td>
<td>Granulate</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Forming hyaline cyst</td>
<td>Cell-chains (&lt;16 cells)</td>
<td>Cell-chains (&lt;4 cells)</td>
</tr>
</tbody>
</table>
Phylogenetic positions of Cochlodinium (SSU rDNA)

Gamma weighted NJ tree, Substitution model: Tamura & Nei (1993), Proportion of invariable site: 0.3834, Shape parameter: 0.5580

- **Amphidinium**
- **Gyrodinium instriatum**
- **Akashiwo sanguinea**
- **Gymnodinium sensu stricto**
- **Gymnodinium**
- **Gyrodinium**
- **Cochlodinium convolutum**
- **Karenia + Karlodinium**
- **Gyrodinium sensu stricto**
- **Cochlodinium polykrikoides**
- **Cochlodinium sp.**
Phylogenetic relationship in *C. polykrikoides* (SSU rDNA)

- Cochlodinium polykrikoides Geoje, Korea
- Cochlodinium polykrikoides Saryang-do, Korea
- Cochlodinium polykrikoides Busan, Korea
- Cochlodinium polykrikoides Yoesu, Korea
- Cochlodinium polykrikoides Yoesu, Korea
- Cochlodinium polykrikoides Kamigoto, Japan
- Cochlodinium polykrikoides Hirado, Japan
- Cochlodinium polykrikoides Mishima, Japan
- Cochlodinium polykrikoides Inokushi Bay, Japan
- Cochlodinium polykrikoides Isahaya Bay, Japan
- Cochlodinium polykrikoides Tachibana Bay, Japan
- Cochlodinium polykrikoides Imari Bay, Japan
- Cochlodinium polykrikoides Omura Bay, Japan
- Cochlodinium polykrikoides Manila Bay, Philippines
- Cochlodinium polykrikoides Sabah, Malaysia
- Cochlodinium polykrikoides Sabah, Malaysia
- Cochlodinium sp. East China Sea, Japan
- Cochlodinium sp. Lampung Bay, Indonesia
- Cochlodinium sp. Lampung Bay, Indonesia
- Cochlodinium sp. Lampung Bay, Indonesia
Phylogeny of Cochlodinium (LSU rDNA, D1-D3)

Gamma weighted NJ tree, Substitution model: Tamura & Nei (1993),
Proportion of invariable site: 0.1580, Shape parameter: 0.7286

Phylogeny:
- Karenia + Karlodinium
- Gyrodinium sensu stricto
- Gymnodinium sensu stricto
- Togula
  - Cochlodinium polykrikoides
  - Cochlodinium sp.
  - Akashiwo sanguinea
  - Amphidinium
Phylogenetic relationship in *C. polykrikoides* (LSU rDNA)

- *Cochlodinium polykrikoides* Busan, Korea
- *C. polykrikoides* Inokushi Bay, Japan
- *C. polykrikoides* Tachibana Bay, Japan
- *C. polykrikoides* Hirado, Japan
- *C. polykrikoides* Kamigoto, Japan
- *C. polykrikoides* Mishima, Japan
- *C. polykrikoides* Isahaya Bay, Japan
- *C. polykrikoides* Manila Bay, Philippines
- *C. polykrikoides* Omura Bay, Japan
- *C. polykrikoides* Sabah, Malaysia
- *C. polykrikoides* Sabah, Malaysia
- *Cochlodinium* sp. East China Sea, Japan
Distribution of *C. polykrikoides* population

- **Manila Bay, Philippines**
- **Sabah, Malaysia**
- **Lampung Bay, Indonesia**

**Other sequences** include:
- Japan, Korea

**C. polykrikoides**

**Cochlodinium sp.**
Taxonomy and phylogeny of Cochlodinium

- *Cochlodinium convolutum* could be distinguished from other chain-forming *Cochlodinium* species based on, 1) shape of nucleus, 2) the deeper sulcus, and 3) formation of hyaline cyst; molecular data also indicates phylogenetic separation among them.

- *Cochlodinium* sp. is superficially similar to *C. polykrikoides*, but they could be distinguished by 1) cell size, 2) position of the sulcus, and 3) shape of chloroplast; their affinity was supported by molecular phylogeny.

Population and distribution of *C. polykrikoides* in southeastern Asia

- Majority of *C. polykrikoides* red tides occurred in Japanese and Korean coasts are derived from the same population; it could be distinguished from other populations distributed in SE Asia.

- SE Asian populations of *C. polykrikoides* scarcely exist in Japanese coastal waters.
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