

**Morphology and taxonomy of chain forming species of the genus *Cochlodinium*  
(Dinophyceae)**

Kazumi Matsuoka<sup>a, \*</sup>, Mitsunori Iwataki<sup>a</sup>, Hisae Kawami<sup>b</sup>

<sup>a</sup>*Institute for East China Sea Research, Nagasaki University; 1551-7 Taira-machi, Nagasaki  
851-2213, Japan (Laboratory; 1-14, Bunkyo-machi, Nagasaki, 852-8521, Japan)*

<sup>b</sup>*Graduate School of Science and Technology, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki  
852-8521, Japan*

\* Corresponding author. Tel. & fax: +81 95 819 2812

*E-mail address:* kazu-mtk@nagasaki-u.ac.jp (K. Matsuoka)

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## 1. Introduction

An unarmored chain-forming dinoflagellate *Cochlodinium polykrikoides* Margalef is one of the harmful species for fish aquaculture in western Japan and southern Korea in the last two decades. In Japan, this species was first recognized in the Yatsushiro Sound in 1978 with a serious economic damage to yellowtail aquaculture industries, and thereafter this species has been continuously expanding its habitat and giving damages to fish aquaculture in both Japan and Korea (Matsuoka and Iwataki, 2004). Recently, the geographical distribution of *C. polykrikoides* seems to expand apparently in tropical to subtropical coastal waters of the Philippines and Saba of Malaysia, Southeast Asia (Relox and Bajarias, 2003; Azanza and Baula, 2005; Anton, pers. com.). However, its biological nature such as taxonomy, the optimum environmental conditions for reproduction, life history including cyst formation and ichthyotoxicity is still unclear for the moment. In particular, reliable identification of *C. polykrikoides* is important among these unsolved subjects, because morphologically similar chain-forming species of the genus *Cochlodinium* have been reported around Japanese and Korean coastal waters (e.g. Yuki and Yoshimatsu, 1989). For investigating on occurrence of *C. polykrikoides*, the precise morphology of these similar species and prominent morphological features, which can differentiate *C. polykrikoides* from other similar species, should be clarified.

In this paper, we describe the morphology of *C. polykrikoides* and its similar species such as *C. catenatum* Okamura, *C. convolutum* Kofoid and Swezy, and *C. fulvescens* ., which is probably a new species, in focusing a single cell stage and discuss on taxonomic problems in the relation to *C. polykrikoides*. In addition, the occurrence locations of *C. polykrikoides* and its related species are shown based on the previous reports.

## 2. Materials

For observation on the morphology of a single cell and chain-forming cells of *C. polykrikoides*, the cultured cells collected from the following sites were provided; Imari Bay, Usuka Bay, Nama Bay, off Shikamachi, Tachibana Bay and Isahaya Bay of Nagasaki Prefecture, Inokushi Bay of Oita Prefecture, off Mishima of Yamaguchi Prefecture, west Japan, Manila Bay of the Philippines and Kota Kinabalu of Sabah, Malaysia. For morphological comparison to *C. polykrikoides*, another chain-forming species, *C. fulvescens* . similar to *C. polykrikoides* collected from Tachibana Bay, west Japan was also observed. To understand the morphological feature of *C. catenatum*, the original description and line drawings given by Okamura (1916) were also employed, because after the last occurrence of this species reported by Hirasaka (1922), *C. catenatum* has never been observed from Japanese coastal waters. For the description of *C. fulvescens* . the photomicrographs and description of this species given by Yuki and Yoshimatsu (1989) was also provided for the description of *Cochlodinium* sp. with our original data. Cells of another *Cochlodinium* species forming short cell-chains, *C. convolutum*, collected from Inokushi Bay, Oita Prefecture in November 2002 and Imari Bay, Nagasaki in September 2005, were also provided for morphological comparison. Information of specimens photographed in this paper is shown in Table 1.

### 3. Results

#### 3.1. *Cochlodinium polykrikoides* Margalef (Fig. 1)

*Cochlodinium polykrikoides* Margalef 1961, p. 76, 78, fig. 27m

##### **Synonyms:**

*Cochlodinium heterolobatum* Silva 1967

*Cochlodinium* sp. Type 78 of Kumada et al. 1980

*Cochlodinium catenatum* Okamura; Hargraves and Viquez 1981

##### **Description based on the Japanese-Korean populations:**

Single cell: Small (30-40  $\mu\text{m}$  in length and 25-30  $\mu\text{m}$  in width) and ovoidal. Anterior part of the epicone is widely conical, and the hypocone is subspherical (Fig. 1-A, B, C). The cingulum encircles twice of the cell and well concave. The sulcus closely runs down just below the cingulum (Fig. 1-A, B). A reddish orange pigmented body is located at the dorsal side of the epicone (Fig. 1-B). A spherical nucleus is large and positioned at the anterior of the epicone (Fig. 1-C, F). Chloroplasts are many, and rod-like shape and extending longitudinally (Fig. 1-B, D, E).

Chain forming cell: At the blooming time, *C. polykrikoides* easily makes chains consisting two, four, eight and sometimes sixteen cells. However, these chains usually contain morphologically different cells; the anterior cell having the flattened hypocone, the posterior cell possessing the truncated epicone and lobed antapical end, and the intermediate cells being more or less compressed longitudinally (Fig. 1-F). However, other features such as the position of the sulcus, nucleus, and a single reddish orange pigmented body and are the same of the single cell stage.

##### **Geographical distribution:**

**Type locality;** Phosphorescence Bay, Puerto Rico, Atlantic Ocean (Margalef, 1961).

**Western Pacific Ocean:** Sea of Japan in Eastern Russia?? (Orlova et al., 2004; questionable because of only record of cyst in sediment), several places in West Japan (Matsuoka and Iwataki, 2004), Southern Coast of Korean Peninsula (Kim, 1998), Manila Bay, Iligan Bay, Balayan Bay and Puerto Princesa of the Philippines (Relox and Bajarias, 2003; Azanza and Baula, 2005), Sabah of Malaysia (Azanza et al., 2006; Anton, pers. com.), Hong Kong (Lu and Hodgkiss, 1999).

**Eastern Pacific Ocean:** Pacific coast of Guatemala (Rosales-Loessener et al., 1996), Southern California, U.S.A. (Kudela, 2006), Central California U.S.A. (M. Silver in Kudela, 2006), the Gulf of Nicoya, Costa Rica (Vargas-Montero et al., 2004), La Ense-nada de La Paz of Baja California (Gárate-Lizárraga et al., 2000, 2004), Manzanillo Bay, Colima, Mexico (Morales-Blake et al., 2001), Pacific coast of Guatemala (Rosales-Loessener et al., 1996).

**Atlantic Ocean:** Coast of New Jersey, USA (as *C. heterolobatum* by Silva 1967), York River, Virginia, (as *C. heterolobatum* by Ho and Zubkoff, 1979), Peconic Estuary, Long Island, New York in U.S.A (Nuzzi, 2004; Kudela, 2006).

**Mediterranean Sea:** Coast of Naples, Italy (Zingone et al., 2006).

**Indian Ocean:** Goa, Southwest India (Bhat and Matondkar, 2004).

**Remarks:**

The holotype of *C. polykrikopides* was ca. 50 µm in cell length, collected from Puerto Rico and specimens of Malaysian Sabah however, specimens of *C. polykrikoides* occurring in Japanese and Korean coastal waters is significantly smaller (30-40 µm) than the type specimens. The smaller *C. polykrikoides* has been also reported from Gulf of California (Gárate-Lizárraga et al., 2004) and Gulf of Nicoya (Hargraves and Viquez 1981).

**3.2. *Cochlodinium catenatum* Okamura (Fig. 2)**

*Cochlodinium catenatum* Okamura 1916, p. 41, figs 1-3; Hargraves and Viquez 1981, p. 33, fig. 1.

**Synonym:** *Pouchetia* sp. of Nakazawa 1911, p. 304, fig. 4.

Non: *Cochlodinium catenatum* Okamura sensu Kofoid and Swezy (1921) from off La Jolla, California

**Description:**

This species was first described from plankton samples collected from Yokohama Harbor in Tokyo Bay by Okamura (1916). The following morphological features of *C. catenatum* are based on the drawings of the holotype using camera lucida and description given by Okamura (1916; Figs 1-3, and p. 41)

Organism is small (21-26 µm in length and 21-27 µm in width in chain-forming cells), catenate and unarmored species usually composed of four eight, or sixteen cells, which are weakly combined. Cell is sub-spherical to ovoidal with almost equal size of the epicone and the hypocone (Fig. 2-A, C). The cingulum located at the equator is approximately one-sixth of the cell length in width, and is descending levorotary with more than 1.5 turns. The sulcus is not extending into the epicone and making almost a half turn (Fig. 2-B). The large nucleus is located at the apex and occupies almost half of the cell (Fig. 2-A, C). A small reddish orange pigmented body, which was called as a stigma by Okamura (1916) located in the epicone (Fig. 2-B). Many yellowish-brown chloroplasts are rod-like in shape and extend antero-posteriorly (Fig. 2-A). No information on the apical groove for the moment.

The cell shape of this species is mostly subspherical, however, some cells shown in the original drawings such as the anterior and posterior cells of fig. 2 and the posterior cell of fig. 3 of Okamura (1916) seem to be rather ovoidal to shortly ellipsoidal in shape (Fig. 2-B and A, respectively). However, no single cell was illustrated in the original drawings.

**Geographical distribution:**

Type locality; Yokohama Harbor in Tokyo Bay, Pacific Ocean (Okamura, 1916; Nakazawa, 1911 only illustration)

Western pacific: Mikawa Bay and Gokasho Bay of Central Japan (Hirasaka, 1922)

East Pacific Ocean; Mazatlan Bay, Mexico (Altamirano et al., 2004), Nicoya Bay of Costa Rica (Hargraves and Viquez, 1981), Nicoya Bay of Costa Rica and Panama (Guzman et al., 1990)

**3.3. *Cochlodinium fulvescens* Iwataki, Kawami and Matsuoka (Fig. 3)**

*Cochlodinium fulvescens* Iwataki, Kawami and Matsuoka 2007, p.235, Figs. 1-9.

**Synonym:** *Cochlodinium* sp. of Yuki and Yoshimatsu 1989

**Description:**

*C. fulvescens* . collected from Tachibana Bay, west Japan, and Hurun Bay in south Sumatra of Indonesia is also making a chain consisting of two or four cells. Due to the formation of cell chains, this species is apparently similar to *C. polykrikoides*. Its morphological features are followings.

A single cell: Relatively large, 45-50  $\mu\text{m}$  in length and 30-35  $\mu\text{m}$  in width. Cell is more roundly and ellipsoidal in shape (Fig. 3-C). The cingulum also encircles twice of the cell as well as that of *C. polykrikoides*. Sulcus runs down apart from the cingulum and occupies at the intermediate position between the semicircular cingulum at the dorsal side (Fig. 3-A, B). A reddish orange pigmented body is located at the dorsal side of the epicone (Fig. 3-B, E). A nucleus is spherical and located at the anterior part of the epicone (Fig. 3-C, F). Chloroplasts are granular and probably distributed along the cingulum and the periphery of the cell (Fig. 3-D). No information on the apical groove.

Chain forming cells: This species usually appear as two, rarely four cells chain (Fig. 3-E, F, G). However, these chain-forming cells easily separate. In the two-cells form, a boundary of these cells becomes mostly linear (Fig. 3-F), and in the four-cells form, the two cells of the anterior and posterior parts of the intermediate two cells are truncated at the both anterior and posterior ends as the same as *C. polykrikoides* (Fig. 3-G).

*Cochlodinium* sp. described by Yuki and Yoshimatsu (1989, p. 452, fig. 2) is identical to *C. fulvescens* . of the present article judging from the larger cell size, the position of the sulcus and granular chloroplasts. According to Yuki and Yoshimatsu (1989), *Cochlodinium* sp. occurred in Harima-Nada, Seto Inland Sea in west Japan with a small-scaled red tide and showed ichthyotoxicity to juveniles of *Leiognotus nuchalis*. For the moment, the physiological condition such as the optimum growth condition has not examined yet, however, the geographical distribution suggests that this species is probably a warm temperate to tropical species as well as *C. polykrikoides*.

**Geographical distribution:**

Tachibana Bay of West Kyushu, Seto Inland Sea (Yuki and Yoshimatsu, 1989) of west Japan  
Hurun Bay, South Sumatra of Indonesia (Fig. 5).

**3.4. *Cochlodinium convolutum* Kofoid and Swezy 1921 (Fig. 4)**

*Cochlodinium convolutum* Kofoid and Swezy 1921, p. 363, pl. 10, fig. 115.

**Description:**

Cell is elongated ovoidal to roundly fusiform in shape and ca. 60-70  $\mu\text{m}$  in length and 30-45  $\mu\text{m}$  in width (Fig. 4-A). The epicone becomes slender toward the apex, and the hypocone is rounder with two broad lobes; the left being always larger than the right (Fig. 4-B). An orange pigmented body developed in *C. polykrikoides* is absent. Torsion of the cingulum is approximately 1.5 times (Fig. 4-A). The sulcus is deep and extends into the hypocone, and also immediately apart at the crossing part of the cingulum. A nucleus is roundly rectangular in shape and located in the center

and at the left ventral side of the cell (Fig. 4-B). The chloroplast is reticulate, but sometimes fairly (Fig. 4-C). Many small grains are evenly scattered in the periphery (Fig. 4-D). A hyaline membrane is often formed around the cell (hyaline cyst) (Fig. 4-E). Two-cell chains are rarely observed in natural condition (Fig. 4-F), but mainly appeared as a single cell.

*Cochlodinium convolutum* once made a red tide in the most inner part of Kagoshima Bay, west Japan in September 1997 (Wada pers. com.), and thereafter appeared in Yatsushiro Sound in September 2001. Recently this organism was recorded from Inokushi Bay, Oita Prefecture of west Japan in November 2002, Omura Bay and Imari Bay in Nagasaki Prefecture of west Japan. When this species occurred in Japan, the temperature of surface water was 26-27°C, and salinity was 32-33 psu. Kofoid and Swezy (1921) reported the occurrence of *C. convolutum* on July and August 1914 from off La Jolla of the west coast of U.S.A. and the surface temperature was 20.7-21.0°C. These data show that *C. convolutum* is the warm temperate to tropical species. This species caused neither fish nor shellfish mass mortality in the field where the red tide occurred and in the laboratory experiments that exposed *C. convolutum* of 2.8-2.0x10<sup>3</sup> cells/ml to yellow tails (Wada, pers. com.). According to these observations, *C. convolutum* is a red-tide forming species, but less harmful for fish and shellfish for the moment.

#### **Geographical distribution:**

Off La Jolla of the west coast of U.S.A. (Kofoid and Swezy, 1921).

Kagoshima Bay, Omura Bay, Yatsushiro Sound, Imari Bay and Ariake Sound of West Japan.

## **4. Discussions**

### **4.1. *Cochlodinium polykrikoides* Margalef vs. *Cochlodinium catenatum* Okamura**

*Cochlodinium polykrikoides* was established in 1961 by Margalef based on the plankton material collected from Phosphorescent Bay in Puerto Rico, Caribbean Sea. In Japan, *C. catenatum* was first reported as a new species by Okamura in 1916 from Tokyo Bay before *C. polykrikoides* being described. Unfortunately, Margalef (1961) did not make any morphological comparison between *C. catenatum* and *C. polykrikoides*, nevertheless these two species share several important morphological and ecological features such as naked, chain-forming, causative for red-tides and ichthyotoxic characteristics. The cell shape of *C. polykrikoides* is slightly different from that of *C. catenatum* in being rather ellipsoidal. However, both species have a small reddish orange pigmented body and many rod-like and yellowish-brown chloroplasts longitudinally distributed near the cell surface. In addition, the position of the nucleus located at the anterior part of the epicone is also common in both species. (Table 2)

According to Okamura (1916), the ichnotype of *C. catenatum* was illustrated using a camera lucida. If Okamura (1916) started to sketch the type specimens of *C. catenatum*, these specimens might stop swimming. Unfortunately, when unarmored dinoflagellates like *C. polykrikoides* stop moving, outlines of these motile cells are easily deformed time by time. The similar cell deformation in *C. heterolobatum* has been shown by Silva (1967, pl. 1, figs 5-7). Actually during our observation on vegetative cells of *C. polykrikoides*, we also encountered these morphological changes (Fig. 6).

The type locality of *C. catenatum* is Tokyo Bay in, Japan, but after the original report given by Okamura (1916), this species has never been observed in this bay. Subsequently in 1922 Hirasaka

reported the occurrence of this species from Gokasho Bay facing to the Pacific in west Japan, and this is the last record of *C. catenatum* in Japan. More than fifty years later, *C. polykrikoides* was found in Gokasho Bay in 1983 (Yuki, pers. com.). Thus these two species can share several important morphological and ecological features.

In the eastern side of the Pacific, these two chain-forming *Cochlodinium* species have been reported by different authors for the moment (Table 3). Hargraves and Viquez (1981) examined a photosynthetic, non-thecate dinoflagellates occurring with fish mass mortality in Nicoya Bay of Costa Rica and identified this species as *C. catenatum* after careful observation and comparison with *C. polykrikoides* and *C. heterolobatum*. In the examination of phytoplankton species of harmful algae forming blooms in Mazatlan Bay in Mexico. Altamirano et al. (2004) reported *C. catenatum* as one of HAB causative species, but neither morphological description nor photographs of this species were given. On the other hand, Vargas-Montero et al. (2004) reported extensive blooms caused by a chain-forming species identical to *C. polykrikoides*, which newly occurred in the same bay, Nicoya Bay. However, based on their LM and SEM photographs (Vargas-Montero et al., 2004, figs 1, 2), the organisms seem to have very similar morphological characters of the species shown by Hargraves and Viquez (1981, fig. 1). This case shows that from the same Nicoya Bay, two different *Cochlodinium* species, *C. catenatum* and *C. polykrikoides* have been identified by different authors. These observations and reports strongly suggest that *C. polykrikoides* is probably a synonym of *C. catenatum*.

For getting the clear taxonomic conclusion on these two species, molecular phylogenetic examination should be carried out on morphologically identical organisms to *C. catenatum* sensu Okamura should be collected from the type locality. Unfortunately, it is almost impossible to find out *C. catenatum* in present Tokyo Bay, because the water quality of this bay has been changed drastically to eutrophic condition since 1916 when *C. catenatum* occurred. However, instead of this situation, from Gokasho Bay facing to the Pacific in central Japan where is an upper stream region of Kuroshio Current, both *C. catenatum* sensu Okamura and *C. polykrikoides* were observed by different authors in different times, in 1922 by Hirasaka and in 1983 by Yuki (pers. com.), respectively. Therefore, for molecular phylogenetic examination, specimens collected from Gokasho Bay will be useful in future.

#### **4.2. *Cochlodinium catenatum* Okamura vs. *Cochlodinium catenatum* Okamura sensu Kofoid and Swezy 1921**

After the original description of *C. catenatum* given by Okamura (1916), Kofoid and Swezy (1921) also described this species from off La Jolla, California. However, Hirasaka (1922) pointed out that *C. catenatum* reported by Kofoid and Swezy (1921) was not identical to *C. catenatum* sensu Okamura in having no chloroplast, no reddish orange pigmented body, and the nucleus located at the center of the cell (Fig. 7). Thereafter, during the study on red-tides caused by a catenate dinoflagellate in Nicoya Bay of Costa Rica, as above mentioned Hargraves and Viquez (1981) identified this species as *C. catenatum* once again. Hargraves and Viquez (1981) also suggested this unarmored dinoflagellate not to be identical to *C. catenatum* sensu Kofoid and Swezy as well as Hirasaka (1922), and probably identical to *C. catenatum* sensu Okamura (1916). On the other hand, Rosales-Loessener et al. (1996) reported the dense bloom caused by *C. polykrikoides* from coastal waters of the Pacific side of Guatemala. These observations also support that *C. catenatum* may be

the senior synonym of *C. polykrikoides* (Table 3).

#### **4.3. *Cochlodinium polykrikoides* Margalef vs. *Cochlodinium heterolobatum* Silva**

*Cochlodinium heterolobatum* was first described from the coast of New Jersey, U.S.A. by Silva in 1967. Again unfortunately, Silva (1967) did not examine morphological comparisons between *C. polykrikoides* and *C. heterolobatum*, and did not discuss the differences between two species. According to the original description and the holotype of *C. heterolobatum*, this species is characterized by a cannonball shape in the single cell stage, with many chloroplasts rod-like shaped, an orange spot (= reddish orange pigmented body) positioned in the epicone, and a large nucleus located at the anterior part of the epicone. This species also makes chain usually consisting of four cells. Later, Hargraves and Viquez (1981) discussed the morphological differences among chain-forming species of *Cochlodinium* including the material collected from Nicoya Bay, and suggested that there is no remarkable morphological difference among *C. polykrikoides*, *C. heterolobatum* and Nicoya Bay specimens. Thereafter, Yuki and Yoshimatsu (1989) re-examined Japanese *Cochlodinium* sp. type 78-Yatsushiro, and concluded that *Cochlodinium* sp. type 78-Yatsushiro is attributable to *C. polykrikoides* and *C. heterolobatum* is a junior synonym of *C. polykrikoides* (Table 2, 3).

#### **Conclusions**

1. Two ichthyotoxic chain-forming *Cochlodinium* species, *C. polykrikoides* and *C. fulvescens* . are inhabiting in the coasts of west Japan and southern Korea, and the East China Sea, and their distribution is expanding toward the south.
2. Morphological features of three species, *C. polykrikoides*, *C. convolutum* and *C. fulvescens* . at the single cell stage are similar to each other, however, the cell size, the position of the nucleus and the sulcus, and shape of chloroplasts are clearly different among them.
3. *Cochlodinium catenatum* Okamura reported from off La Jolla, U.S.A. by Kofoid and Swezy in 1921 is a different species of *C. catenatum* sensu Okamura in lacking chloroplasts, and the nucleus located at the center of the cell.
4. The type specimen of *Cochlodinium polykrikoides* Margalef is significantly larger than the *C. polykrikoides* of Japanese-Korean populations in cell length.
5. *Cochlodinium polykrikoides* Margalef distributing along the Japanese and Korean coasts is possibly the same species of *C. catenatum* Okamura in having many yellowish-brown chloroplasts with rod-like shape, making a chain consisting of more than four cells, the nucleus located at the anterior part of the epicone, excepting only ellipsoidal body. However, the adequate taxonomic name for these species should be re-examined based on morphological and molecular phylogenetic data using the topotype of *C. catenatum*.

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## Abstract

Morphology of an unarmored chain-forming harmful dinoflagellate *Cochlodinium polykrikoides* and its similar species such as *C. catenatum*, *C. fulvescens*, and *C. convolutum* was carefully observed in focusing a single cell stage for clarifying taxonomically important morphological features. To differentiate *C. polykrikoides* from *C. convolutum*, the shape and the position of the nucleus are useful characters. *C. polykrikoides* is also different from *C. fulvescens* in being smaller in size, having many rod-like chloroplasts and the sulcus running just below the cingulum on the dorsal surface. Careful observation on the ichnotype of *C. catenatum* suggests that *C. catenatum* sensu Kofoid and Swezy collected from off La Jolla is not identical to *C. catenatum* sensu Okamura and probably a different species in having no chloroplast and a nucleus positioned at the center of the cell. In addition, *C. polykrikoides* has many common morphological features with *C. catenatum* sensu Okamura only except for slightly elongate cells and is probably a junior synonym of this species.

## Explanation of Figures

**Fig. 1.** Light and fluorescent micrographs of *Cochlodinium polykrikoides*. (A) Ventral view; (B) Dorsal view showing the position of the sulcus (s), chloroplasts (ch) and reddish orange pigmented body (arrowhead); (C) Optical cross section showing the nucleus (n) positioned in the anterior of the cell; (D) Fluorescent micrograph, showing rod-like chloroplasts; (E) A four cells chain showing different cell morphology in cell-chain; (F) Deeper focus of a cell chain indicates the positions of nuclei. Scale bars: 10  $\mu\text{m}$ .

**Fig. 2.** *Cochlodinium catenatum* Okamura. A-C are the same of figs. 3-1 of Okamura 1916, but abbreviations are changed from the original, cingulum (c), chloroplasts (ch), nucleus (n), sulcus (s), arrowheads; reddish orange pigmented body.

**Fig. 3.** Light and fluorescent micrographs of *C. fulvescens*. (A) Ventral view showing the start of the sulcus (s); (B) Dorsal view showing reddish orange pigmented body (arrowhead) and the position of the sulcus (s); (C) Optical cross section in dorso-ventral view showing the nucleus (n); (D) Fluorescent micrograph, showing granular chloroplasts peripherally located; (E, F) A chain-form consisting of two cells; (G) A chain-form consisting of four cells. Scale bars: 20  $\mu\text{m}$ .

**Fig. 4.** Light and fluorescent micrographs of *Cochlodinium convolutum*. (A) Ventral view showing the sulcus (s); (B) Optical cross section showing rectangular nucleus (n); (C) Fluorescent micrographs, showing reticulate chloroplasts; (D) Right lateral view, many small grains evenly scattered in the cell; (E) Optical cross section, showing the hyaline membrane (arrowhead); (F) A chain-form consisting of two cells. Scale bars: 20  $\mu\text{m}$ .

**Fig. 5.** Geographical distribution of *Cochlodinium polykrikoides*, *C. catenatum* and *C. fulvescens*. Temperatures in summer season.

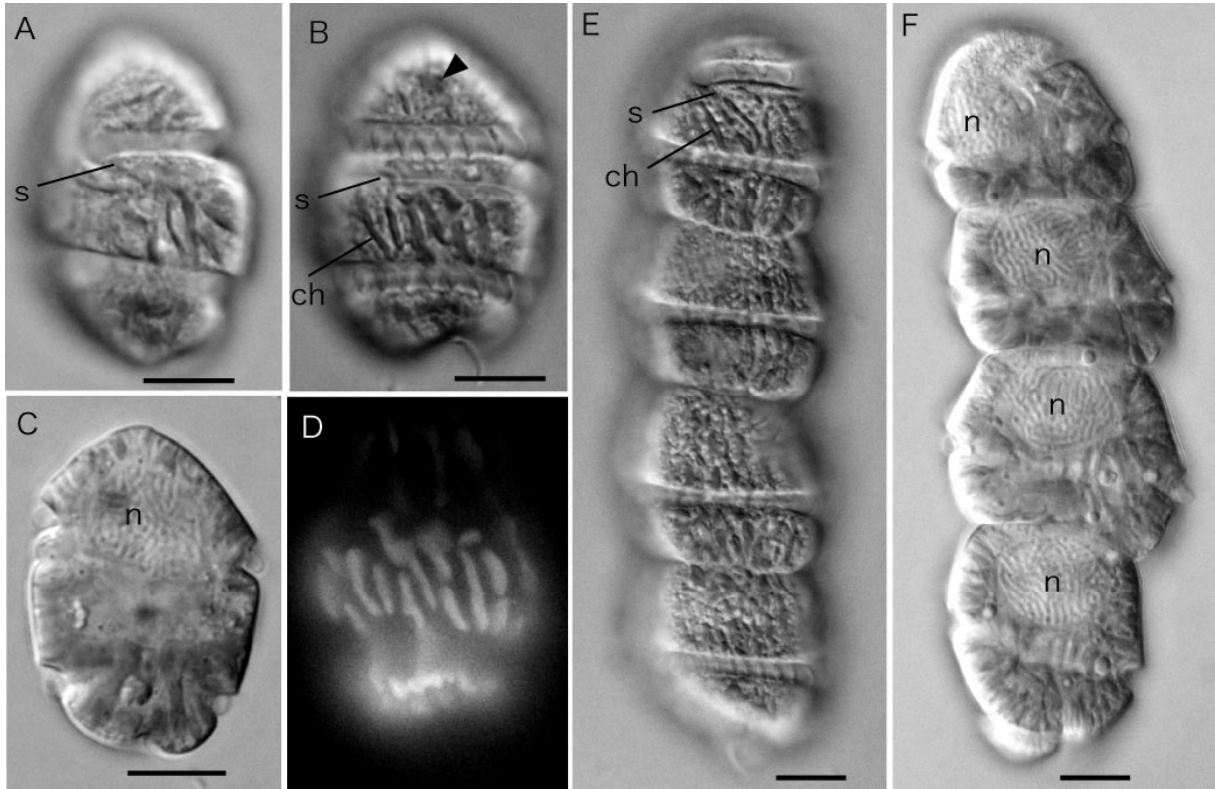
**Fig. 6.** Morphological change of *Cochlodinium polykrikoides* during the observation under a light microscope. Specimens collected at Busan, Korea in August 2002. (A) Cells stopping to swim and becoming round in outline of cells and chloroplasts; (B) Cells remarkably deformed and original rod-like chloroplasts decomposed after approximately two hours from microscopic observation. Scale bar: 20  $\mu\text{m}$

**Fig. 7.** The different type of *C. catenatum* given by Kofoid and Swezy (1921): (A, B) Re-drawings of Kofoid and Swezy in 1921. Showing the position of nucleus (n), note the cell of *C. catenatum* sensu Kofoid and Swezy having no chloroplast.

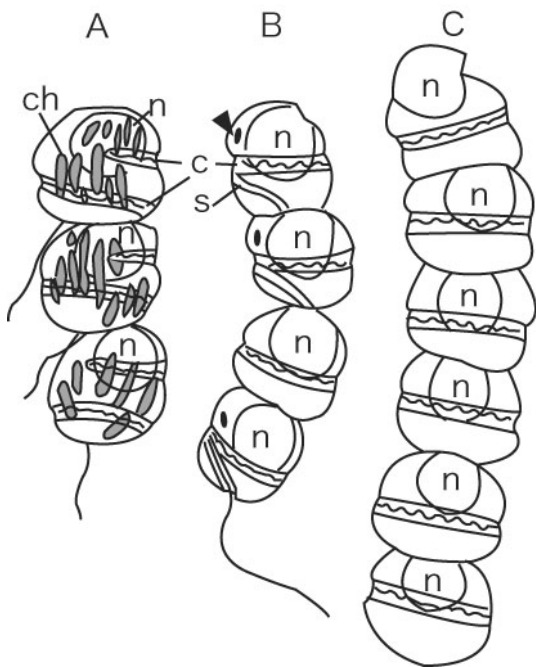
**Table 1.** Chain-forming *Cochlodinium* species examined in this study.

**Table 2.** Taxonomic history of chain-forming species of the genus *Cochlodinium*.

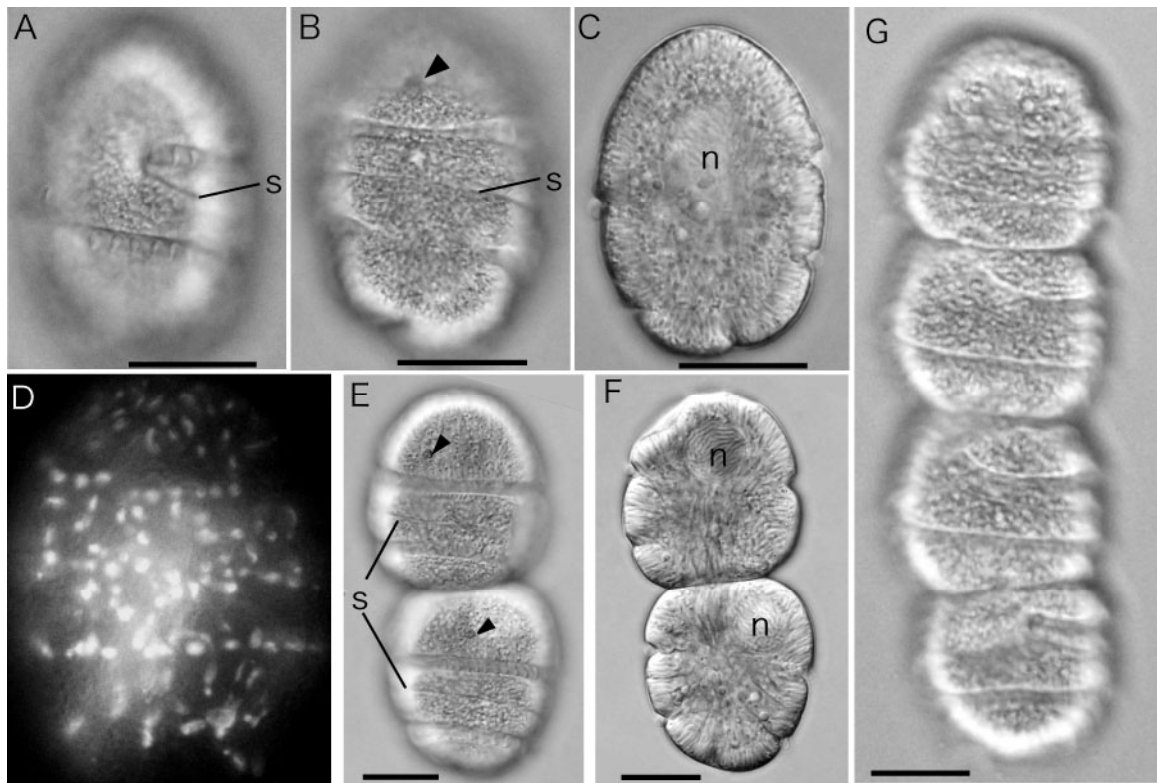
**Table 3.** Morphological comparison of a single cell stage of four *Cochlodinium* species occurring around Japanese coastal waters.



**Fig. 1**



**Fig. 2**



**Fig. 3**



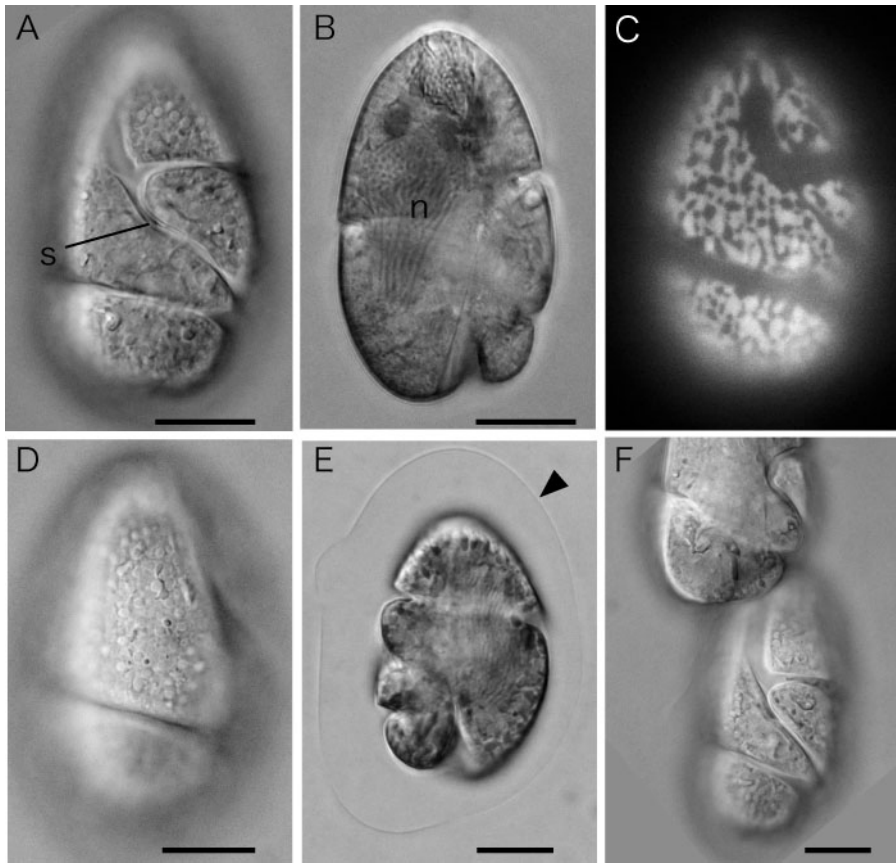


Fig. 4

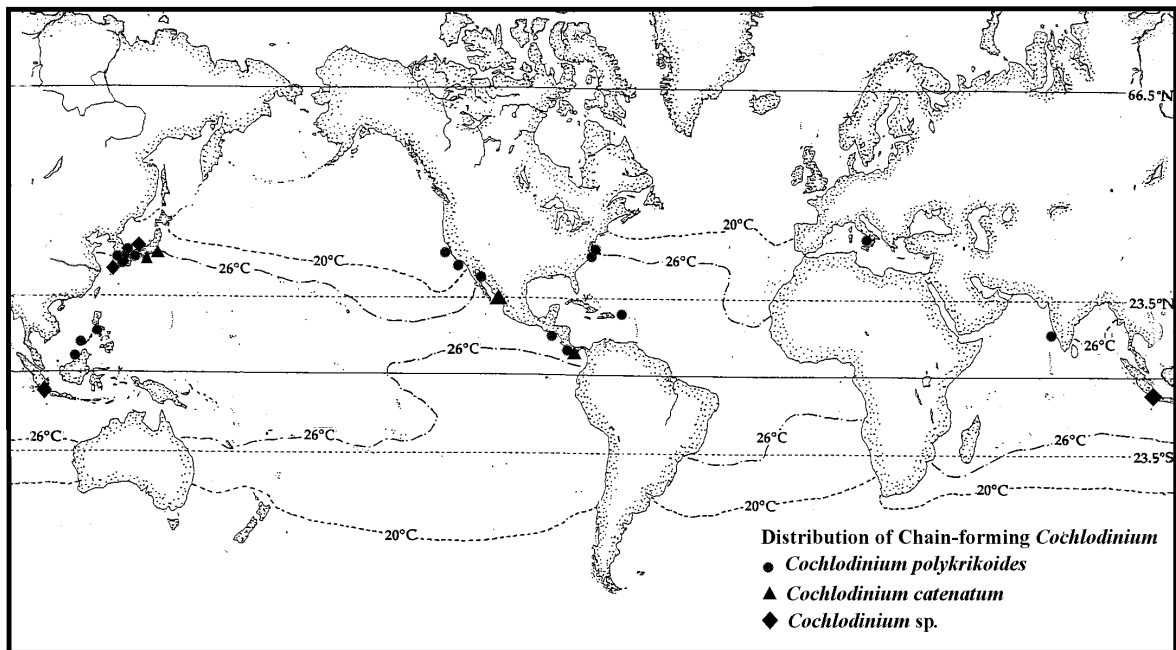


Fig. 5

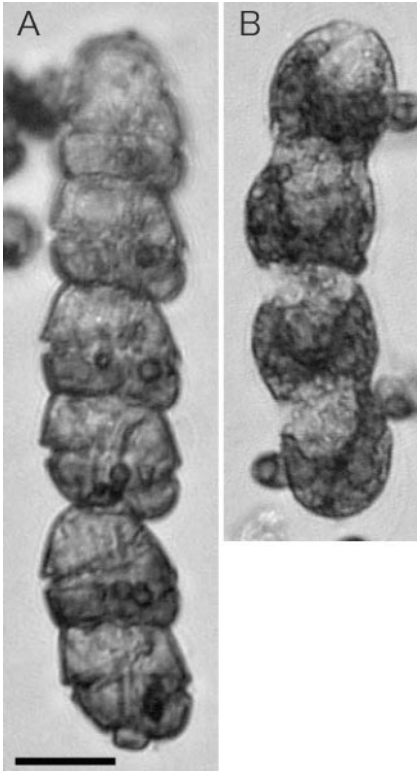


Fig. 6

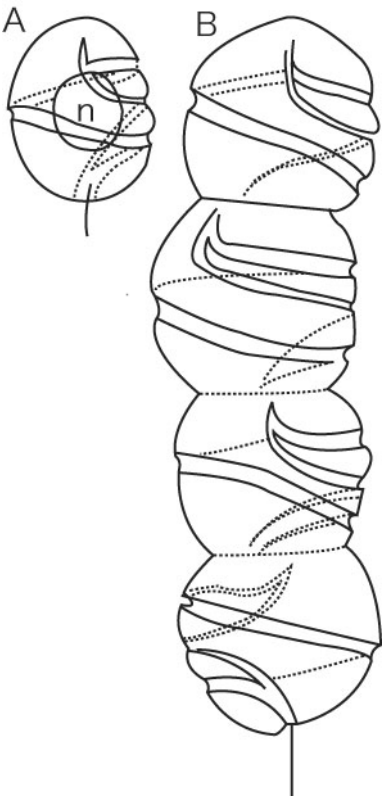


Fig. 7

Table 1. Chain-forming *Cochlodinium* species examined in this study.

Species	Strain	Location	Date	Photographs
<i>Cochlodinium polykrikoides</i>	KG8-ND14	Nama Bay, West Japan	27 Aug 2002	Fig. 1, A-F
<i>C. fulvescens</i>	TC10-ND102	Tachibana Bay, West Japan	17 Oct 2004	Fig. 2, A-D, G
<i>C. fulvescens</i>	non	Hurun Bay, Indonesia	23 Dec 2003	Fig. 2, E-F
<i>Cochlodinium convolutum</i>	non	Imari Bay, West Japan	18 Sep 2005	Fig. 4, A-C
<i>Cochlodinium convolutum</i>	non	Inokushi Bay, West Japan	5 Nov 2002	Fig. 4, D-F

Table 2. Taxonomic history of chain-forming species of the genus *Cochlodinium*.

	<i>C. catenatum</i>	<i>C. polykrikoides</i>	<i>C. heterolobatum</i>	<i>Cochlodinium</i> sp. type Yatsushiro '78
Nakazawa 1911	Illustrated as <i>Pouchetia</i> sp. in Tokyo Bay			
Okamura 1916	Described as a new species, <i>C.</i> <i>catenatum</i> in Tokyo Bay			
Hirasaka 1922	Last records in Gokasho Bay and Mikawa Bay			
Kofoed & Swezy 1921	Described as <i>C. catenatum</i> , but different species			
Margalef 1961		Described as a new species in Phosphorescence Bay		
Silva 1967			Described as a new species in New Jersey	
Kumada et al. 1980				Reported as <i>Cochlodinium</i> sp. type Yatsushiro '78
Hargraves & Viquez 1981	Recorded from Nicoya Bay, Costa Rica			
Yuki (pers. com.)		Observed in Gokasho Bay in 1983		
Yuki & Yoshimatsu 1989		Senior synonym for <i>C.</i> <i>heterolobatum</i> and <i>Cochlodinium</i> sp. type Yatsushiro '78		
Vargas-Montero et al. 2004		Recorded from Nicoya Bay, Costa Rica as a new occurrence		

Table 3. Morphological comparison of a single cell stage of four *Cochlodinium* species occurring around Japanese coastal waters.

	<i>C. catenatum</i>	<i>C. polykrikoides</i> *	<i>C. fulvescens</i>	<i>C. convolutum</i>
Length	Small (24-27 $\mu\text{m}$ )**	Small (30-40 $\mu\text{m}$ )***	Intermediate (45-50 $\mu\text{m}$ )***	Large (60-70 $\mu\text{m}$ )***
Cell shape	Subspherical to ovoidal	Ovoidal	Roundly ellipsoidal	Roundly subconical
Pigmented body	Present at the dorsal side of the epicone	Present at the dorsal side of the epicone	Present at the dorsal side of the epicone	Absent
Cingulum	Encircling 1.5 times of the cell	Encircling 1.8 to 2 times of the cell	Encircling ca. 2 times of the cell	Encircling 1.5 times of the cell
Sulcus		Running just below the cingulum	Running between the cingulum	
Nucleus	Spherical, at the anterior of the epicone	Spherical, at the anterior of the epicone	Spherical, at the anterior of the epicone	Rectangular
Chloroplasts	Rod-like	Rod-like	Granular	A few and light in color
Cell-chain	Chain forming (> 4 cells)	Chain forming (> 4 cells)	Chain forming (4 cells)	Chain forming (2 cells) Hyaline cyst

\* Cell length of the holotype given by Marglaef (1961) 50  $\mu\text{m}$

\*\*Measuring chain-forming cells only because of no illustration of a single cell stage (Okamura, 1916).

\*\*\*Measuring cells in a single stage.