Plankton Biol. Ecol. 50 (2): 61–64, 2003

plankton
biology & ecology

Note

Alexandrium acatenella (Gonyaulacales: Dinophyceae): Morphological characteristics of vegetative cell and resting cyst

MAKOTO YOSHIDA*, KOICHIRO MIZUSHIMA & KAZUMI MATSUOKA

Laboratory of Coastal Environmental Sciences, Faculty of Fisheries, Nagasaki University, 1–14, Bunkyo-machi, Nagasaki 852–8521, Japan

Received 3 October 2002; accepted 24 January 2003

The ecology and dynamics of dinoflagellate cysts are actively studied especially on harmful species such as some species of *Alexandrium*, a group that includes PSP (Paralytic Shellfish Poisoning) causatives, because it is very important for understanding the mechanism of bloom recurrence and geographic expansion (e.g. Anderson & Wall 1978; Hallegraeff 1998). Although the identification of *Alexandrium* cysts is necessary for these studies, cysts have been described only for half of the *Alexandrium* species (Table 1). This is due to the difficulty of performing excystments. Moreover, cysts do not have distinctive morphological characteristics, namely, almost all of them have a thin and smooth cyst-wall and lack of a distinct archeopyle shape. The cyst shape is rather simple and can be categorized into a few basic morphotypes (Table 1). *Alexandrium tamarense* and *A. catenella* are the major PSP causatives in Japan, and share exactly the same cyst morphology. Their cysts are thus counted as "tamarense-complex" cysts (Fukuyo 1985). In the present study, another *Alexandrium* species was obtained from the germination of ellipsoidal cysts collected from surface sediment of Kure Bay, Seto Inland Sea, West Japan. Based on the characteristics of germinated cells the species was identified as *Alexandrium acatenella*. The results of a morphological study on germinated cells and resting cysts of *Alexandrium acatenella* are presented. A sediment core sample of 81.5 cm in length was collected with a small piston core sampler (Nanba et al. 1998) in the northern part of Kure Bay (Fig. 1) in November, 2000. The first, 13 cm of the core was sliced into 1 cm subsamples, which were used for excystment experiments. The ellipsoidal cysts with smooth-wall morphology were picked up from sieved subsamples using a capillary under on inverted fluorescence light microscope (Olympus IX 70) equipped with DIC optics. Each cyst was placed in a well of a multiwell plate and incubated at 15°C, at a photon fluence rate of 10 μmol/m^2/s provided by cool white fluorescent tubes under continuous light. The shape of all cysts was recorded with a digital camera (Sony DKC-CM30) before the germination experiment. For testing germination, the presence of motile cells was checked in the incubation chamber every day for 10 days. After excystment, motile cells were preserved immediately in formalin and observed under the microscope. The cells were compared with natural plankton specimens of *Alexandrium acatenella*, collected in Kushimoto Bay, and *A. tamarense*, collected in Hiroshima Bay (Fig. 1).

In this study, 126 of the 312 incubated cysts germinated. Among them, 102 motile cells were identified as *Alexandrium tamarense*. *Alexandrium acatenella* was obtained from the germination of three cysts. The other 21 cells could not be identified owing to insufficient thecal plate development or failure of procedure.

Motile cells of *Alexandrium acatenella* (Figs. 2A–D, H–O) were longer than wide in the ventral view. Its epitheca was taller than the hypotheca. The ventral pore was located on the suture between the first and fourth apical plates. The shape of thecal plates was very similar to *Alexandrium tamarense*, but some small differences were observed. The first apical plate seems slightly longer than that of *Alexandrium tamarense*. The side connected to the anterior sulcal plate was rather shorter especially in well-developed thecae (compare Fig. 2P with Figs. 2C and 2N). The shape of the third apical plate (Figs. 2D and 2M) was different from *Alexandrium tamarense* (Fig. 2S). In the case of *Alexandrium tamarense*, the side connected to the second apical plate was much longer than the side connected to the fourth apical plate (Fig. 2S), but the ratio of the length of the two sides was almost the same in *A. acatenella*. The other precingular plates tended to be elongate. The posterior sulcal plate (Figs. 2B and 2O) was slightly wider than that of *Alexandrium tamarense* (Fig. 2T), but narrower than that of *A. catenella*. Thecal plate ornamentation was not observed. Even though there were no obvious differences in the shape of thecal plates between the two species, *Alexandrium acatenella* could be distinguished by the unique cell outline in the ventral

* Corresponding author: Makoto Yoshida
E-mail: f1349@cc.nagasaki-u.ac.jp
Fax: +81-95-819-2812

Table 1. Shape of resting cysts of *Alexandrium* spp. Genera are unified to *Alexandrium* and some species are renamed based on the characteristics of vegetative cells illustrated in each study.

Species	Reference
Spheroid	
A. affine	Fukuyo et al. 1985
A. andersonii	Montresor et al. 1998
A. leei	Fukuyo and Pholpunthin 1990a
A. margalefii	Hallegraeff et al. 1991
A. ostenfeldii	Mackenzie et al. 1996
A. taylorii	Garces et al. 1998
Spheroid or ovoid	
A. monilatum	Walker and Steidinger 1979
Spheroid with paratabulation	
A. pseudogonyaulax	Montresor et al. 1993
Ellipsoid or elongated ellipsoid	
A. catenella	Fukuyo 1985
A. fundyense (as *Gonyaulax excavata*)	Anderson and Wall 1978
A. tamarense (as *Gonyaulax excavata*)	Fukuyo 1979
A. tamiyavanichii (as *A. cohorticula*)	Fukuyo and Pholpunthin 1990b
Hemispheroid or discoid	
A. hiranoi	Kita and Fukuyo 1988
A. lusitanicum	Blanco 1989
A. minutum	Bolch et al. 1991

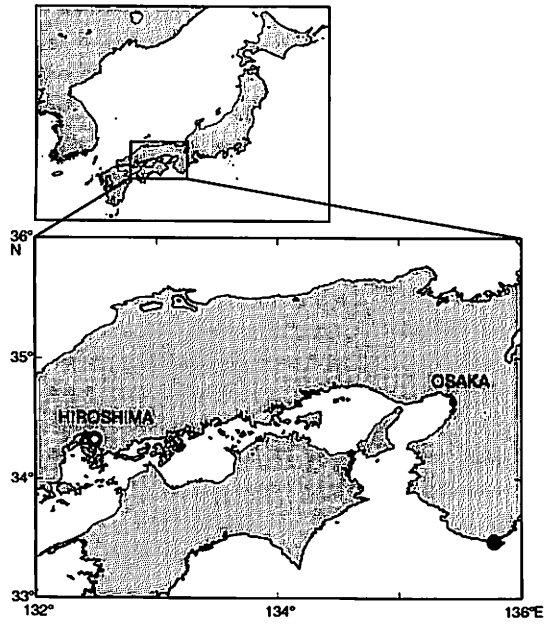

Fig. 1. Sampling location of *Alexandrium acatenella* (●: plankton sample, ○: cyst) and *A. tamarense* (△: plankton sample).

view.

The cyst of *Alexandrium acatenella* was ellipsoidal in shape, colorless and of the smooth-wall type, resembling cysts of *A. tamarense* and *A. catenella*. The size of the cysts (length: 43–60 μm; width: 22–25 μm) was also very similar. The cyst contained granular material and a red body like in the related species. Other distinctive features were not observed. There has never been a report on the cyst of *Alexandrium acatenella* before, and the present study demonstrates that the cyst of this species is very difficult to distinguish morphologically from those of *A. tamarense* and *A. catenella*.

Alexandrium acatenella and *A. catenella* were initially described by Whedon & Kofoid (1936) as members of the genus *Gonyaulax*, *G. acatenella* and *G. catenella* respectively. Since then, *Alexandrium catenella* has been recognized as one of the common PSP causatives, but occurrences of *A. acatenella* were not frequent. In the 1960s, *Alexandrium acatenella* caused red tides along the west coast of the United States (Prakash & Taylor 1966). Although PSP was detected during that bloom, a culture strain was never established and toxicity of this species was not examined. There are no reports of toxic blooms of the species afterwards, so the toxicity of the species is still doubtful. Balech (1995) described the species based on Japanese and Argentine materials. The specimens observed herein were similar to his Argentine specimens and they differed from his Japanese specimens, which possess an angular cell outline in the ventral view. Konovalova (1989, 1998) also described this species collected in Kamchatka. It closely resembled to our specimens, especially in the ventral view and its possession of a wider first apical plate.

Our observation demonstrated that five *Alexandrium* species, namely *A. acatenella*, *A. catenella*, *A. fundyense*, *A. tamarense* and *A. tamiyavanichii*, have an ellipsoidal cyst. Among them, the cyst of *A. tamiyavanichii* is slightly different in shape, rounder than in the other species (Fukuyo & Pholpunthin 1990b) and this may be a distinctive characteristic. However, it is very difficult to distinguish the other four species morphologically. Their morphology of the motile forms also is highly similar for some characteristics, namely the connection of the apical pore plate and the first apical plate, pentagonal posterior sulcal plate and the fact that they do not make long chains (usually less than 8 cells).

The genus *Alexandrium* is subdivided into two subgenera, *Alexandrium* and *Gessnerium*, and the former was further subdivided into six groups by Balech (1995). These species except *Alexandrium catenella*, belong to the "tamarense group", whereas *A. catenella*, together with *A. compressum* are placed in the "catenella group". This grouping seems to be inadequate or overclassified in view of the similarity of motile cells. In addition, cyst similarity found in them may reflect their phylogenetic closeness. However, as there have been very few studies dealing with the relationship between morphological characteristics of motile cell and cyst, we feel it is premature to discuss validity of Balech's groupings at this stage. In this respect, phylogenetic relationships of *Alexandrium acatenella* to related species based on gene sequences should also be clarified. These molecular data could be applied to development of new practical techniques, such as *in situ* hybridization using molecular probes, for cyst identification.

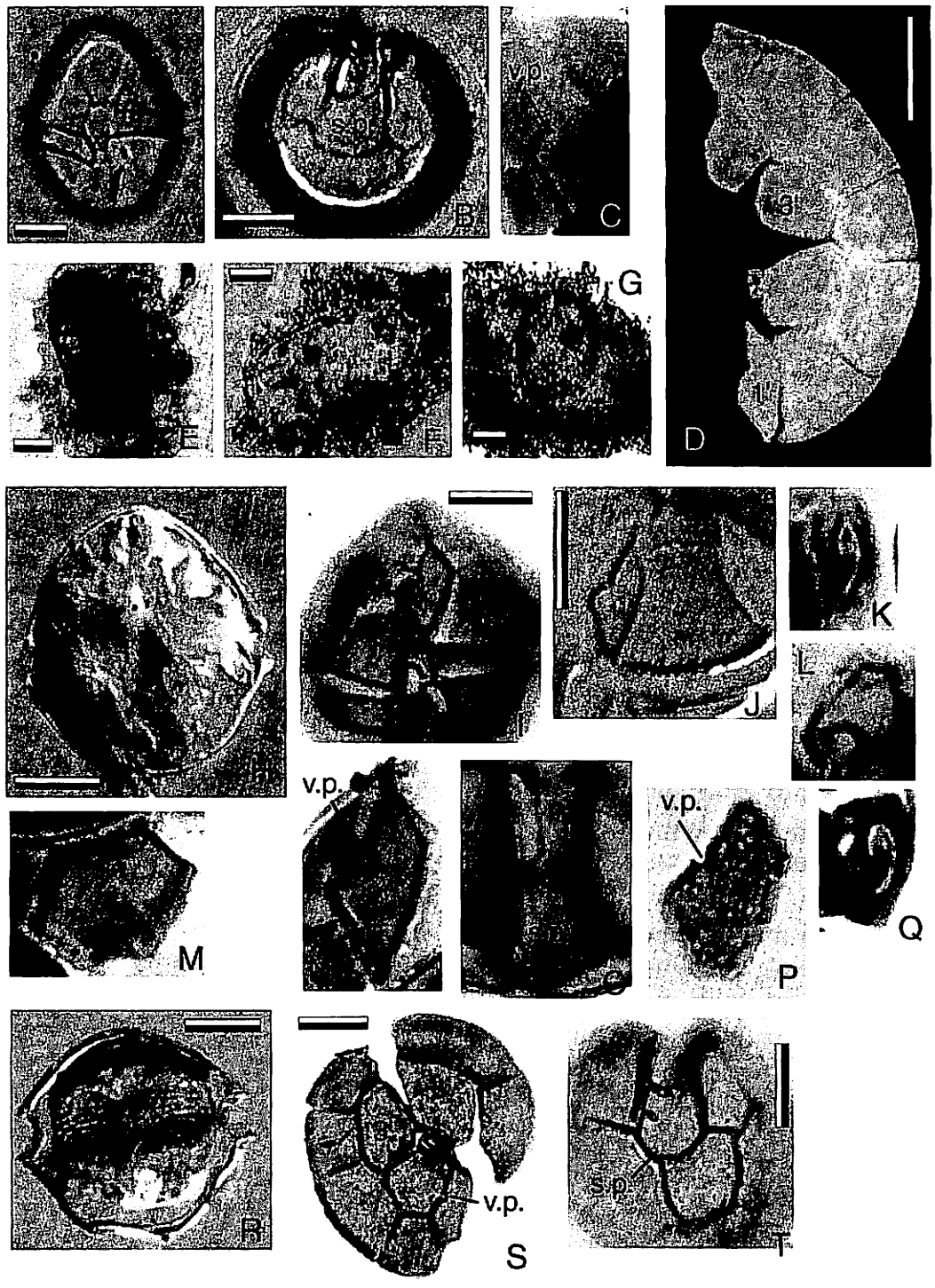

Fig. 2. *Alexandrium acatenella* (A–O) and *A. tamarense* (P–T). A–D: Specimens obtained from excystment; E–G: Resting cysts before excystment; H–O: motile cell from plankton sample collected in Kushimoto Bay; P–T: *A. tamarense* in plankton sample collected in Hiroshima Bay. A, H and R: Ventral view; B and T: Antapical view; C, N and P: The first apical plate with surrounding plates; D and S: Apical plates; I and J: Apical ventral view; K and Q: Apical pore plate; L: Anterior sulcal plate; M: The third apical plate; O: Sulcal plates. C and D are taken by fluorescent microscopy. s.p.: Posterior sulcal plate; v.p.: ventral pore; 1′: The first apical plate; 3′: The third apical plate. Scale bars represent 10 μm.

Acknowledgements

We thank Dr. Haruyoshi Takayama of Hiroshima Fisheries Experimental Station, Dr. Mitsunori Iwataki of Nagasaki University, and Dr. Shigeru Itakura of the National Research Institute of Fisheries and Environment of Inland Sea for plankton and sediment sample collection. This study was partially carried out during Research Fellowship sponsored by the JSPS Research Fellowships for Young Scientist program.

Literature cited

Anderson, D. M. & D. Wall 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in intiating toxic dinoflagellate blooms. *J. Phycol.* **14**: 224–234.

Balech, E. 1995. *The genus* Alexandrium *Halim*. Sherkin Island Marine Station, Sherkin Island, 151 pp.

Blanco, J. 1989. Quistes de Dinoflagelados de las costas de Galicia. I. Dinoflagelados gonyaulacoides. *Scient. Mar.* **53**: 785–796.

Bolch, C. J., S. I. Blackburn, J. A. Cannon & G. M. Hallegraeff 1991. The resting cyst of the red-tide dinoflagellate *Alexandrium minutum* (Dinophyceae). *Phycologia* **30**: 215–219.

Fukuyo, Y. 1979. Theca and cyst of *Gonyaulax excavata* (Braarud) Balech found at Ofunato Bay, Pacific coast of northern Japan. p. 61–64. In *Toxic Dinoflagellate Blooms* (eds. Taylor, D. L. & H. H. Seliger). Elsevier, New York.

Fukuyo, Y. 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. *Bull. Mar. Sci.* **37**: 529–537.

Fukuyo, Y. & P. Pholpunthin 1990a. *Alexandrium leei* Balech. p. 92–93. In *Red Tide Organisms in Japan—An Illustrated Taxonomic Guide* (eds. Fukuyo, Y., H. Takano, M. Chihara & K. Matsuoka). Uchida Rokakuho, Tokyo.

Fukuyo, Y. & P. Pholpunthin 1990b. *Alexandrium cohorticula* (Balech) Balech. p. 88–89. In *Red Tide Organisms in Japan—An Illustrated Taxonomic Guide* (eds. Fukuyo, Y., H. Takano, M. Chihara & K. Matsuoka). Uchida Rokakuho, Tokyo.

Fukuyo, Y., K. Yoshida & H. Inoue 1985. p. 33–38. *Protogonyaulax* in Japanese coastal waters. In *Toxic Dinoflagellates* (eds. Anderson, D. A., A.W. White & D.G. Baden). Elsevier, New York.

Garces, E., M. Delgado, M. Maso & J. Camp 1998. Life history and *in situ* growth rates of *Alexandrium taylori* (Dinophyceae, Pyrrophyta). *J. Phycol.* 34: 880–887.

Hallegraeff, G. M. 1998. Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Mar. Ecol. Prog. Ser.* **168**: 297–309.

Hallegraeff, G. M., C. J. Bolch, S. I. Blackburn & Y. Oshima 1991. Species of the toxic dinoflagellate genus *Alexandrium* in southeastern Australian waters. *Bot. Mar.* **34**: 575–587.

Kita, T. & Y. Fukuyo 1988. Description of the Gonyaulacoid dinoflagellate *Alexandrium hiranoi* sp. nov. inhabiting tidepools on Japanese Pacific coast. *Bull. Plankton Soc. Jpn.* **35**: 1–7.

Konovalova, G. V. 1989. Morphology of three *Alexandrium* (Dinophyta) species from coastal waters of the Eastern Kamchatka. *Botanicheskii Zhurnal.* **74**: 1401–1409.

Konovalova, G. V. 1998. *Dinoflagellatae (Dinophyta) of the far eastern seas of Russia and adjacent waters of the Pacific ocean.* Dalnauka, Vladivostok, 300 pp.

Mackenzie, L., D. White, Y. Oshima & J. Kapa 1996. The resting cyst and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in New Zealand. *Phycologia* **35**: 148–155.

Montresor, M., A. Zingone & D. Marino 1993. The paratabulate resting cyst of *Alexandrium pseudogonyaulax* (Dinophyceae), p. 159–164. In *Toxic Phytoplankton Blooms in the Sea* (eds. Smayda, T. J. & Y. Shimizu). Elsevier, New York.

Montresor, M., A. Zingone & D. Sarno 1998. Dinoflagellate cyst production at a coastal Mediterranean site. *J. Plankton Res.* **20**: 2291–2312.

Nanba, K., T. Iida, Y. Fukuyo & K. Matsuoka 1998. A Handy Piston Core Sampler for Sediments in Shallow Water. *Fisheries Sci.* **64**: 985–986.

Prakash, A. & F. J. R. Taylor 1966. A "Red Water" Bloom of *Gonyaulax acatenella* in the strait of Geogia and its relation to paralytic shellfish toxicity. *J. Fish. Bd. Canada* **23**: 1265–1270.

Walker, L. M. & K. Steidinger 1979. Sexual reproduction in the toxic dinoflagellate *Gonyaulax monilata*. *J. Phycol.* **15**: 312–315.

Whedon, W. F. & C. A. Kofoid 1936. Dinoflagellates of the San Francisco region. I. On the skeletal morphology of two new species, *Gonyaulax catenella* and *G. acatenella*. *Univ. Calif. Publ. Zool.* **41**: 25–34.