

Characterization of a *Karenia papilionacea*-like dinoflagellate from the South China Sea

P.K.K. Yeung, V.K.L. Hung, F.K.C. Chan and J.T.Y. Wong*

Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong Special Administrative Region, People's Republic of China.

*Corresponding author, e-mail: botin@ust.hk

The morphological characteristics of a new isolate of a dorso-ventrally flattened athecate dinoflagellate obtained from Hong Kong (HK) waters is described. Partial sequences of the large subunit ribosomal RNA gene were obtained and a high degree of similarity was observed with various species within the genus *Karenia*. Phylogenetic comparison suggested the *Karenia* sp. HK forms a clade with the newly described species *K. papilionacea* from New Zealand but not with authentic *K. brevis* from North America. *Karenia* sp. HK is the first *K. brevis*-like dinoflagellate characterized by molecular cladistics from the South China Sea and the results call for re-examination of the status of other *K. brevis*-like morphotypes described from the region.

INTRODUCTION

Karenia brevis (formerly *Gymnodinium breve*) Davis is a toxic dinoflagellate that is regularly found in warm temperate to tropical waters, in the Gulf of Mexico along the Texas and Louisiana coasts to the east coast of Florida. Mortality of marine fish and other animals have been reported in the coastal areas with *K. brevis* outbreaks. Athebate dinoflagellate species, including *K. brevis*, are easily distorted under stresses during the examination and preservation of specimens, reducing the accuracy of species identification. Daugbjerg et al. (2000) re-described the gymnodinioids into four genera based on the morphological, ultrastructural and molecular data. A new genus, *Takayama*, was recently introduced (Salas et al., 2003) to include those *Gymnodinium* species that have not been categorized by the Daugbjerg's classification.

A few records of *K. brevis* were previously reported in the western Pacific, including the Japan Sea (Fukuyo et al., 1990) and the South China Sea (Lin et al., 1997). However, there are doubts whether the Japanese *K. brevis* is identical to the West Atlantic species (Steidinger et al., 1989). The re-description of *K. brevis* 'look-alikes' from New Zealand waters (Haywood et al., 2004) encouraged us to examine the characters of South China Sea 'look-alikes'.

MATERIALS AND METHODS

In our routine water sampling at the pier of the Hong Kong University of Science and Technology, we observed a low density (10/l) of *K. brevis* 'look-alikes' during the winter months. We isolated one strain and for the purpose of discussion in this paper we name it *Karenia* sp. Hong Kong (HK). *Karenia* sp. HK was maintained in ES medium at 23°C under a photon flux of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ from fluorescent tubes (Phillips daylight) in 12L:12D h cycles. For the purpose of comparison, we also obtained

Karenia brevis (= *Gymnodinium breve* CCMP718) from the Provasoli-Guillard Center for Culture of Marine Phytoplankton (CCMP).

In order to obtain comparable molecular data for *Karenia* sp. HK, we isolated and sequenced the D1 and D2 domains of the large subunit ribosomal RNA gene, using conserved polymerase chain reaction (PCR) primers for dinoflagellates. The DNA extraction, PCR conditions and sequencing protocols were described previously (Yeung et al., 2002). The sequence data of *Karenia* sp. HK were compared with the data for other species/strains of gymnodinioids, which were available in the US National Center for Biotechnology Information database.

RESULTS AND DISCUSSION

Karenia sp. HK cells are dorso-ventrally flattened (similar to *K. brevis*), 20–25 μm (N=50) long, 20–30 μm (N=50) width. The width to length ratio is about 1:1. The epicone is in a delta triangular shape, broadly truncated and with a pointed apex (Figure 1A,G). An indentation or short linear apical groove at the apex leads to the right (Figure 1A). The hypocone is notched and bilobed with a concavity or deep indentation at the antapex, creating two posterior lobes. The whole cell forms a heart shape and does not form a chain or a pair with others. The cingulum is incised, and equatorial with girdle displacement <1/5 of the total cell length. This value falls within the range of the *Karenia* group. The cingulum is ~10–15% of total cell length. The sulcus extends from the antapex to immediately above the cingulum. Similar to *K. brevis* (CCMP 718), the nucleus of *Karenia* sp. HK (stained with the DNA dye DAPI) is spherical and is located in the left of the hypocone (Figure 1C,F). This contrasts with *K. brevisulcata*, where, the nucleus is elongated and extends from the left to the right hypocone and the chloroplasts are elongated (Chang, 1999). The cell contains elongated yellow-green chloroplasts varying in size and numbers. The chloroplasts

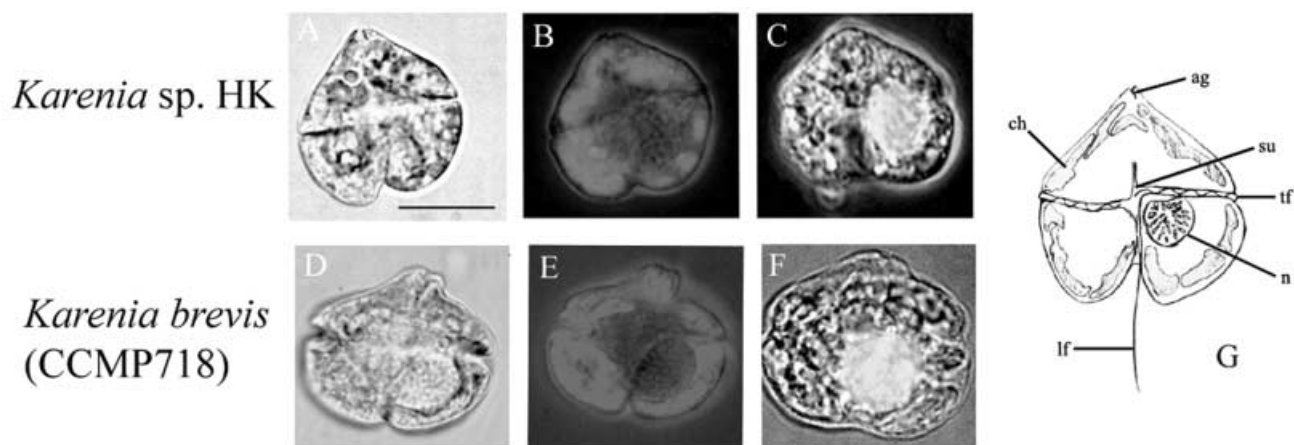


Figure 1. Morphological comparisons between *Karenia* sp. HK cells (A,B,C) and *K. brevis* cells (D,E,F). (A & D) phase contrast photomicrographs showing the ventral view of the cells; (B & E) fluorescence photomicrographs, showing autofluorescence (filter 490 nm) of chloroplasts located at the periphery of the cells; (C & F) overlay of phase contrast with fluorescent images (DAPI-stained). For nuclei staining, fixed cells (2% paraformaldehyde) were stained in $0.2 \mu\text{g ml}^{-1}$ DAPI for 10 min before being washed in phosphate buffered saline; (G) schematic representation of *Karenia* sp. HK in ventral view. ag, apical groove; ch, chloroplasts; lf, longitudinal flagellum; tf, transverse flagellum; n, nucleus; su, short anterior extension of the sulcus.

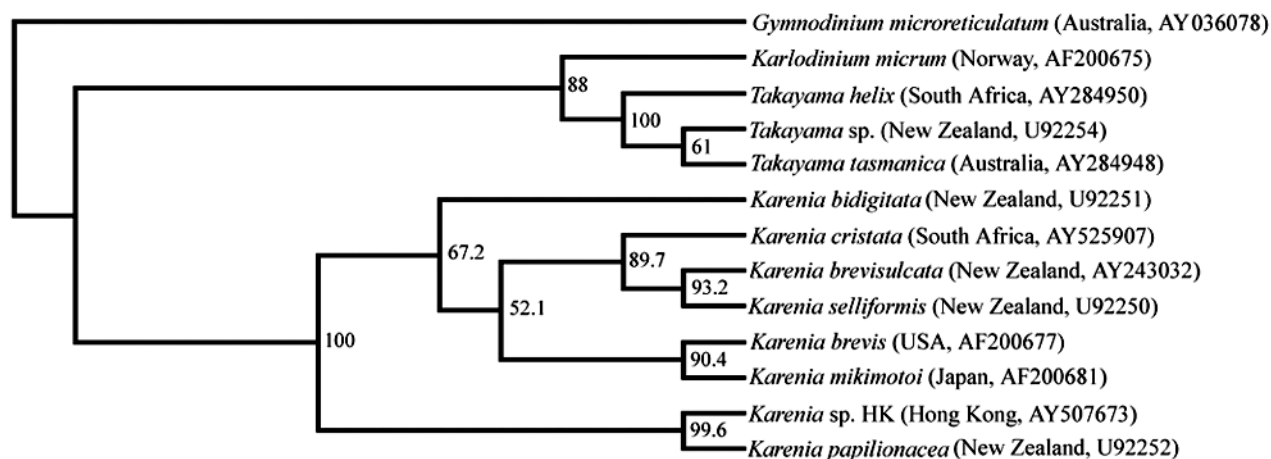


Figure 2. Phylogenetic tree of the *Gymnodinium* (*sensu lato*) species constructed by using the neighbour joining (NJ) method of sequence differences in base pairs. The percentage of 1000 cumulative bootstrap re-samplings of the aligned data set that support the topological elements is indicated at the left of each grouping. Outgroup species was the Australia isolate (*Gymnodinium microreticulatum*). Japanese *Karenia mikimotoi* was chosen as the representative species of the 'mikimotoi complex'. Horizontal branch-lengths reflect the relatedness of the sequences. The brackets show the places of isolation and the accession numbers. Numbers in the branching points indicate bootstrap values (in percentages) for groups (PHYLIP 3.6).

are located along the periphery of the cell, a situation similar to *K. brevis* (Figure 1B,E). Live cells are yellow-green in colour and have two flagellae. During swimming, the cells rotate along the longitudinal axis in a clockwise direction and they make abrupt stops and change direction frequently, a pattern very similar to *K. brevisulcata*.

There are 54–62 nucleotide differences (out of 698 bps of PCR product) between *Karenia* sp. HK and seven other *Karenia* species. The highest similarity was observed with the newly described *K. papilionacea* from New Zealand, with only 25 nucleotide differences. A phylogenetic tree was constructed using the PHYLIP 3.6 with the neighbour joining (NJ) method and bootstrap of 1000 replicates (Figure 2). Thirteen gymnodinioids were compared and

G. microreticulatum was used as the outgroup. *Karenia* sp. HK forms a clade with *K. papilionacea* (New Zealand). However, the *Karenia* sp. HK has a position furthest from *K. brevis* (USA), which forms a clade with *K. mikimotoi* (Japan). *Gymnodinium microreticulatum* is closely related to the clade comprising *G. catenatum* and *G. nolleri*. These dinoflagellates are characterized in their production of a micro-reticulate, fossilizable cyst and have similar morphological features (Bolch et al., 1999).

Karenia papilionacea, in general, has a larger cell size (18–32 μm long and 18–48 μm wide) than *Karenia* sp. HK (20–25 μm long and 20–30 μm wide). *Karenia papilionacea* has an elliptical shape, wider than long, while *Karenia* sp. HK has similar width and length.

Although *Gymnodinium catenatum* and *Gymnodinium nolleri* share many morphological and ultrastructural similarities, they are classified as two separate species with 2.1% large subunit (LSU) ribosomal RNA sequence differences (Hansen et al., 2000). In this study, the LSU ribosomal RNA sequences of *Karenia* sp. HK and New Zealand *K. papilionacea* differed by 3.5% (including gaps) and 2.6% (without including gaps), a genetic divergence sufficient for the classification as two separate species. We are not suggesting that all previous records of *K. brevis* should be attributed to *Karenia* sp. HK, however, the results of the present study call for re-examination of the previous records of *K. brevis* in the South China Sea and the characterization of more 'look-alikes'. Our results also support the presence of a *K. papilionacea* group in the western Pacific.

[Gene Bank Accession Number: AY507673.]

Part of this study was supported by Grant ECWW97/98.SC06 from the Environment and Conservation Fund to J.W. We also thank Professor Y. Lin for discussion during an early stage of this study.

REFERENCES

- Bolch, C.J.S., Negri, A.P. & Hallegraeff, G.M., 1999. *Gymnodinium microreticulatum* sp. nov. (Dinophyceae): a naked, microreticulate cyst-producing dinoflagellate, distinct from *Gymnodinium catenatum* and *Gymnodinium nolleri*. *Phycologia*, **38**, 301–313.
- Chang, F.H., 1999. *Gymnodinium brevisulcatum* sp. nov. (Gymnodiniales, Dinophyceae), a new species isolated from the 1998 summer toxic bloom in Wellington Harbour, New Zealand. *Phycologia*, **38**, 377–384.
- Daughbjerg, N., Hansen, G., Larsen, J. & Moestrup, Ø., 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. *Phycologia*, **39**, 302–317.
- Fukuyo, Y., Takano, H., Chihara, M. & Matsuoka, K., ed., 1990. *Red tide organisms in Japan—an illustrated taxonomic guide*. Tokyo, Japan: Uchida Rokakuho.
- Hansen, G., Daughbjerg, N. & Henriksen, P., 2000. Comparative study of *Gymnodinium mikimotoi* and *Gymnodinium aureolum*, comb. nov. (= *Gyrodinium aureolum*) based on morphology, pigment composition, and molecular data. *Journal of Phycology*, **36**, 394–410.
- Haywood, A.J., Steidinger, K.A., Truby, E.W., Bergquist, P.R., Bergquist, P.L., Adamson, J. & MacKenzie, L., 2004. Comparative morphology and molecular phylogenetic analysis of three new species of the genus *Karenia* (Dinophyceae) from New Zealand. *Journal of Phycology*, **40**, 165–179.
- Lin, Y., Zhou, J. & Qiu, D., 1997. A study of red tide organisms and their ecological specificity along offshore South China. In *A study of eutrophication and red tide in the offshore area* (ed. Y. Lin), pp. 1–10. Beijing: Science Publisher. [In Chinese.]
- Salas, M.F., Bolch, C.J.S., Botes, L., Nash, G., Wright, S.W. & Hallegraeff, G.M., 2003. *Takayama* gen. nov. (Gymnodiniales, Dinophyceae), a new genus of unarmoured dinoflagellates with sigmoid apical grooves, including the description of two new species. *Journal of Phycology*, **39**, 1233–1246.
- Steidinger, K.A., Babcock, C. Mahmoudi, B., Tomas, C. & Truby, E., 1989. Conservative taxonomic characters in toxic dinoflagellate species identification. In *Red tides: biology, environmental sciences, and toxicology* (ed. T. Okaichi et al.), pp. 285–288. New York: Elsevier Science Publisher.
- Yeung, P.K.K., Wong, F.T.W. & Wong, J.T.Y., 2002. Large subunit rDNA sequences from *Alexandrium catenella* strains isolated during harmful algal blooms in Hong Kong. *Journal of Applied Phycology*, **14**, 147–150.

Submitted 16 November 2004. Accepted 12 May 2005.