

Short communication

Gymnodinoid genera *Karenia* and *Takayama*
(Dinophyceae) in New Zealand coastal waters

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INTRODUCTION

Micro-algae in the family Gymnodiniaceae Lankester include the genera *Gymnodinium* Stein, *Karenia* G. Hansen & Moestrup, *Karlodinium* J. Larsen, and *Takayama* de Salas, Bolch, Botes & Hallegraeff (Daugbjerg et al. 2000; de Salas et al. 2003). These three closely related genera have been responsible for fish kills and shellfish contamination events worldwide (Taylor et al. 2003), and several events have been ascribed to species of *Karenia* in New Zealand coastal waters over the last decade.

In 1993, a nationwide closure of shellfish harvesting was instigated in New Zealand after 180 cases of human illness occurred, following shellfish consumption, which fitted the Neurotoxic Shellfish Poisoning (NSP) illness definition (Jasperse 1993). The contamination of shellfish with dinoflagellate derived brevetoxins was confirmed (Ishida et al. 1995, 1996), but the causative organism was not definitively identified at the time. Subsequently there has been considerable debate as to the possible causative species of that event, and it is believed that *Karenia mikimotoi* (Miyake & Kominami ex Oda) G. Hansen & Moestrup, which produces both brevetoxins and gymnocin-C (Satake et al. 2002), was primarily responsible (Todd 2002). *Gymnodinium aureolum* Hulbert and *K. brevisulcata* (Chang) G. Hansen & Moestrup were also present in low numbers (Todd 2002); DNA sequence data confirming the morphological identification of *G. aureolum* was obtained for a strain (CAWD87) isolated in 1994 by Haywood (2001).

During 1994, blooms of *K. selliformis* Haywood et al. occurred around the southern South Island, gradually moving northwards up the eastern coastline and finally reaching the outer Marlborough Sounds (Mackenzie et al. 1996). This extensive bloom was associated with widespread fish and shellfish deaths, in particular tuatua (a surf clam);

Abstract New Zealand has been subject to massive blooms of gymnodinoid dinoflagellates over the last decade and in some instances marine biota mortalities. Respiratory problems for people impacted by aerosols from these blooms have occurred. However, because of the difficulty in definitively identifying gymnodinoid dinoflagellates, the identities of the causative organisms have not always been established with certainty. This paper documents the occurrence of several newly described species in the genera *Karenia* and *Takayama* in New Zealand's coastal waters as determined by analysis of DNA sequence data. The species include *Karenia umbella* de Salas, Bolch & Hallegraeff, *Takayama helix* de Salas, Bolch, Botes & Hallegraeff, and *T. tasmanica* de Salas, Bolch & Hallegraeff, which are known to have caused fish kills in Tasmanian (Australia) waters in the past. Liquid chromatography-mass spectrometry analyses of mass cultures of these dinoflagellates have been negative for neurotoxic brevetoxins. The species designation of several unidentified gymnodinoid species (designated *Gymnodinium* sp.) isolated from New Zealand waters and maintained in the Cawthron Collection of Microalgae were also identified on the basis of their DNA sequences.

Paphies subtriangulata). Gymnodimine, a fast acting toxin (Seki et al. 1995), accumulated in filter feeding shellfish and may have been responsible for the mortalities described. It has since been determined that this compound has a very low risk of toxicity to humans (Munday et al. 2004).

Karenia brevisulcata (first described by Chang (1999) as *Gymnodinium brevisulcatum*), was responsible for far greater devastation in January–February 1998, killing much of the marine biota in Wellington Harbour at that time (Chang et al. 2001). The toxic agent associated with this dinoflagellate is highly potent and is currently being chemically characterised.

No fish kills or toxin events have been ascribed to either *Karlodinium* or *Takayama* species in New Zealand to date, although both genera have been implicated in fish kills in Australia.

Many unnamed gymnodinoid isolates, as well as isolates of known species of *Karenia* and *Karlodinium* (Table 1), have been held for up to a decade in the Cawthron Collection of Micro-algae (Ponikla 2004: Cawthron Culture Collection of Micro-algae, catalogue of strains: www.cawthron.org.nz/

microalgae_culture_collection.htm). The focus of this study was to determine the correct designations for these isolates, based on morphology and DNA sequence data, and to determine whether they included any of the newly described *Karenia* and *Takayama* species found in Tasmanian waters. The possible presence of *K. cristata*, a newly described species from South Africa, was also investigated (Botes et al. 2003).

The new records will underpin New Zealand's phytoplankton monitoring programmes, which are carried out to provide risk assessments of toxic events for the shellfish industry, finfish aquaculture (salmon farm managers), and public health regulators.

METHODS

Culture conditions

Dinoflagellates were isolated by micro-pipette from seawater samples and on-grown in plastic pottles (50 ml; Biolab, New Zealand) under standard conditions (100 $\mu\text{Ein m}^{-2} \text{s}^{-1}$; 14:10 h light:dark; 19°C \pm 1°C).

Table 1 *Karenia* and *Karlodinium* species isolated from New Zealand waters and maintained in the Cawthron Collection of Micro-algae.

| Species | CAW Code | Site of isolation |
|---------------------------|-----------------------|-------------------------------------|
| <i>Karenia bidigitata</i> | D92 | Hawke Bay |
| <i>K. brevisulcata</i> | D82 | Wellington Harbour |
| <i>K. mikimotoi</i> | D63, D117, D118, D119 | Hauraki Gulf and Marlborough Sounds |
| <i>K. papilionacea</i> | D91 | Hawke Bay |
| <i>K. selliformis</i> | D79 | Foveaux Strait |
| <i>Karlodinium micrum</i> | D66, D83 | Marlborough Sounds |

Table 2 DNA sequence-based identification of unidentified gymnodinoid species (designated *Gymnodinium* sp.) isolated from New Zealand waters and maintained in the Cawthron Collection of Micro-algae. Pairwise distances are expressed as percentage difference between partial 26S sequences, and absolute number of differing bases, when compared with a reference strain in GenBank.

| CAW code | Site of isolation | Isolator (date) | Identification based on DNA sequences | GenBank accession no. | Pairwise distance to reference strain |
|----------|--------------------|---------------------|---------------------------------------|-----------------------|---------------------------------------|
| D59 | Leigh | A. Haywood (1993) | <i>Gymnodinium aureolum</i> | AF200671 | 0.105 (1 base) |
| D65 | Tasman Bay | L. Mackenzie (1994) | <i>Karenia umbella</i> | AY263963 | 0 |
| D80 | Foveaux Strait | L. Mackenzie (1995) | <i>Karenia bidigitata</i> | U92251 | 0.141 (1 base) |
| D81 | Foveaux Strait | L. Mackenzie (1995) | <i>Karenia bidigitata</i> | U92251 | 0.141 (1 base) |
| D84 | Kawau Island | A. Haywood (1994) | <i>Karlodinium micrum</i> | AF200675 | 0.408 (4 bases) |
| D85 | Kawau Island | A. Haywood (1994) | <i>Gymnodinium aureolum</i> | AF200671 | 0.105 (1 base) |
| D90 | Wedge Point | A. Haywood (1995) | <i>Gymnodinium aureolum</i> | AF200671 | 0.105 (1 base) |
| D93 | Ponui Island | L. Mackenzie (1995) | <i>Karlodinium micrum</i> | AF200675 | 0.408 (4 bases) |
| D114 | East Bay | J. Adamson (2001) | <i>Takayama tasmanica</i> | AY284948 | 0 |
| D115 | Glenhaven hatchery | J. Adamson (2001) | <i>Takayama tasmanica</i> | AY284948 | 0 |
| D128 | Tasman Bay | L. Mackenzie (2000) | <i>Takayama helix</i> | AY284950 | 0.114 (1 base) |

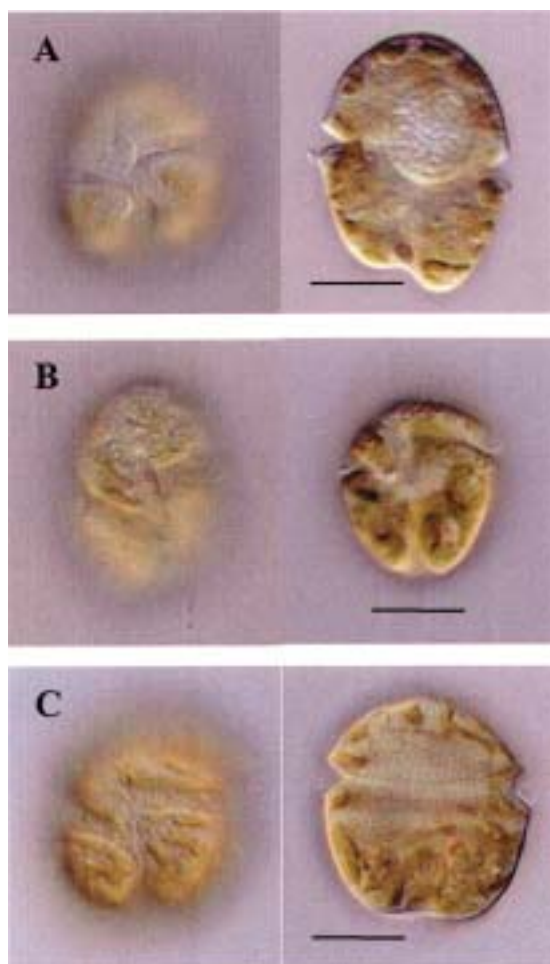


Fig. 1 Light micrographs of **A**, *Karenia umbella*, **B**, *Takayama tasmanica*, and **C**, *T. helix* (M. de Salas). Bar = 10 μ m.

Cultures were maintained in the Cawthron Culture Collection in modified GP medium (Loeblich & Smith 1968); GP was 50% strength, except for the soil extract (20%), and sea water was full strength Sigma Sea Salts (32 ppt; Sigma Aldrich, Germany).

Light microscopy

Live cells were photographed using a Zeiss Axioskop 2 Plus microscope (Carl Zeiss, Germany) with a Zeiss Axiocam Hr digital camera.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Cultures were grown to mid-exponential phase and c. 20 ml pelleted by gentle centrifugation. Genomic

DNA was extracted from the resultant pellet using DNeasy Plant Mini Kits (Qiagen, Hilden, Germany), following the manufacturers instructions. PCR conditions and cleanup, cycle sequencing, sequence alignment, and phylogenetic analysis parameters are detailed in de Salas et al. (2004b).

RESULTS AND DISCUSSION

The identifications of several unnamed gymnodinoid isolates held in the Cawthron Culture Collection of Micro-algae were determined by comparison of DNA sequence data with sequences held in GenBank (Table 2). The results confirmed the occurrence of *Karenia umbella* de Salas, Bolch & Hallegraeff, *Takayama helix* de Salas, Bolch, Botes & Hallegraeff, and *T. tasmanica* de Salas, Bolch & Hallegraeff in New Zealand coastal waters and morphological details concurred with the detailed descriptions by de Salas et al. (2003, 2004a; Fig. 1). Definitive identifications of isolates of *Gymnodinium aureolum*, *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen, and *Karenia bidigitata* Haywood et al. (Haywood et al. 2004) were also enabled by the DNA sequence data (Table 2).

All the gymnodinoid cultures referred to in this communication, including *K. umbella* and the two *Takayama* species, were mass cultured and analysed for brevetoxins by LC-MS and all proved non-toxic (McNabb et al. 2004).

Currently, oligonucleotide probes are available for the gymnodinoids *Karenia selliformis*, *K. papilionacea* Haywood et al., *K. brevisulcata*, *K. mikimotoi* (Rhodes et al. 2004), as well as for *Gymnodinium aureolum* and *Karlodinium micrum* (M. de Salas; Table 3). The trialing of oligonucleotide probes (targeted at ribosomal RNA) is underway for *Karenia umbella*, *Takayama tasmanica*, and for the genus *Takayama*.

The development of oligonucleotide probes for rapid and definitive identification in phytoplankton monitoring programmes, together with the use of chemical methods of analysis (e.g., LC-MS), will perhaps lead to the replacement of the mouse

Table 3 Oligonucleotide sequences for whole cell format DNA probes targeted at the rRNA of *Gymnodinium aureolum* (GaD2) and *Karlodinium micrum* (kdmmk).

| | |
|---------------|-------------------------------|
| GA 26S:562 | 5' gagcaatatgacagtgtgagttc 3' |
| kdmmk 26S:383 | 5' tagcgcacacaactctcacc 3' |

bioassay in the near future. Currently the mouse bioassay is a regulatory requirement in New Zealand, but there are ethical concerns about its use and questions as to its reliability (e.g., in consistently and accurately detecting brevetoxins in shellfish). Other toxins produced by the gymnodinoids, such as gymnodimine by *K. selliformis*, are already detected using LC-MS.

To ensure that phytoplankton monitoring can play an important part in a new monitoring system, all the species that might contribute to a toxicity risk need to be known. Methods of differentiation of non-toxic as well as toxic lookalikes should be developed. The identification of the species described in this study is therefore timely.

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