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High abundance of the potentially maitotoxic dinoflagellate *Gambierdiscus carpenteri* in temperate waters of New South Wales, Australia

Gurjeet S. Kohli [a,b,*], Shauna A. Murray [b,c], Brett A. Neilan [a,d], Lesley L. Rhodes [e], D. Tim Harwood [e], Kirsty F. Smith [e], Lauren Meyer [f], Angela Capper [f], Steve Brett [g], Gustaaf M. Hallegraeff [h]

[a] School of Biotechnology and Biomolecular Sciences, University of New South Wales, New South Wales 2052, Australia
[b] Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, PO Box 123, Broadway, New South Wales 2007, Australia
[c] Sydney Institute of Marine Sciences, New South Wales 2088, Australia
[d] Australian Centre for Astrobiology, University of New South Wales, New South Wales 2052, Australia
[e] Cawthron Institute, 98 Halifax Street East, Private Bag 2, Nelson 7010, New Zealand
[f] Centre for Sustainable Tropical Fisheries and Aquaculture, School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia
[g] Microalgal Services, 308 Tucker Road, Ormond, VIC 3204, Australia
[h] Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, Tasmania, Australia

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ABSTRACT

Species of the genus *Gambierdiscus* are epiphytic dinoflagellates well known from tropical coral reef areas at water temperatures from 24 to 29 °C. *Gambierdiscus* spp. are able to produce ciguatoxins (CTXs) known to bioaccumulate in fish, and the ingestion of tropical fish that accumulated CTXs and possibly also maitotoxins (MTXs) can cause ciguatera fish poisoning (CFP) in humans. In Australia, ciguatera poisonings have been reported in tropical parts of Queensland and the Northern Territory. Here, we report for the first time the seasonal abundance (April–May 2012/13) of *Gambierdiscus* spp. (up to 6565–8255 cells g^{-1} wet weight algae) from Merimbula and Wagonga Inlets in temperate southern New South Wales, Australia (37° S) at water temperatures of 16.5–17 °C. These are popular shellfish aquaculture and recreational fisheries areas with no reports of ciguatera poisoning. Sequencing of a region of the 28S rRNA gene led to the conclusive identification of *Gambierdiscus carpenteri*. The cells differed however from the Belize type description, including the absence of a thecal groove, dorsal rostrum and variable hatchet- to rectangular-shaped 2′ plate, and were morphologically more similar to *Gambierdiscus toxicus*. To study the dinoflagellate community structure in detail, a pyrosequencing approach based on the 18S rRNA gene was applied, which confirmed the presence of a single *Gambierdiscus* species only. Neither CTXs nor MTXs were detected in natural bloom material by LC–MS/MS; however, the extracts were found to be toxic via mouse-bioassay, with symptoms suggestive of poisoning by MTX-like compounds. Understanding the abundance of *Gambierdiscus* populations in areas with no apparent human health impacts is important towards defining the alternate conditions where sparse populations can create ciguatera problems.

* Corresponding author at: Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, PO Box 123, Broadway, New South Wales 2007, Australia. Tel.: +61 2 9514 8404.
E-mail addresses: gurjeet.kohli@uts.edu.au, gurukohli@gmail.com (G.S. Kohli), shauna.murray@uts.edu.au (S.A. Murray), b.neilan@unsw.edu.au (B.A. Neilan), Lesley.Rhodes@cawthron.org.nz (L.L. Rhodes), tim.harwood@cawthron.org.nz (D.T. Harwood), kirsty.smith@cawthron.org.nz (K.F. Smith), lauren.meyer@my.jcu.edu.au (L. Meyer), angela.capper@jcu.edu.au (A. Capper), algae@bigpond.net.au (S. Brett), gustaaf.hallegraeff@utas.edu.au (G.M. Hallegraeff).

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1. Introduction

Epiphytic marine dinoflagellates of the genus *Gambierdiscus* are unique in their ability to produce the polyether ladder toxins ciguatoxins (CTXs) and maitotoxins (MTXs) (Lewis and Holmes, 1993; Murata et al., 1993; Chinain et al., 2010; Rhodes et al., 2010; Fraga et al., 2011; Holland et al., 2013). The ingestion of tropical fish that have orally accumulated effective levels of CTXs and possibly MTXs can cause ciguatera fish poisoning (CFP) in humans. It is the most common non-bacterial illness associated with seafood consumption (Friedman et al., 2008), globally affecting between 50,000 and 500,000 people per year (Fleming et al., 1998). Despite a 60% increase in CFP in Pacific Island nations over the last decade, significant under-reporting is suspected (Skinner et al., 2011). Recently, it has been determined using molecular tools that the causative organisms do not belong to the single species "*Gambierdiscus toxicus*", but rather a species complex of at least 12 unique lineages with differing morphologies and toxic potencies (Litaker et al., 2009; Fraga et al., 2011). This has significantly altered our approach to studying CFP and its causes. At least four *Gambierdiscus* species (*Gambierdiscus belizeanus*, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus* and *Gambierdiscus carpenteri)*, are distributed globally, with the other seven found only in the Pacific (*Gambierdiscus australes*, *Gambierdiscus pacificus*, *Gambierdiscus polynesiensis*, *G. toxicus*, *Gambierdiscus yasumotoi*), Indian (*G. toxicus)* or the Atlantic Oceans (*Gambierdiscus ruetzleri*, *Gambierdiscus excentricus*; Faust, 1995; Holmes, 1998; Chinain et al., 1999a; Litaker et al., 2009; Fraga et al., 2011). Recently, five new genotypes of *Gambieridiscus*, known as *Gambierdiscus* ribotype 1, *Gambierdiscus* ribotype 2, *Gambierdiscus* sp. type 1, *Gambierdiscus* sp. type 2 and *Gambierdiscus* sp. type 3, have also been described (Kuno et al., 2010; Litaker et al., 2010; Nishimura et al., 2013). Each species has a distinct toxin profile. Typically, *G. polynesiensis* and *G. excentricus* are efficient producers of CTXs and MTXs (Chinain et al., 2010; Fraga et al., 2011), whereas *G. australes* is only known to produce MTXs (Rhodes et al., 2010). These findings have shed new light on the previously recognised >100-fold variation in toxicity, but also suggests a much broader range of environmental tolerances for potentially CFP-causing dinoflagellates.

In Australia, *Gambierdiscus* is well known from the tropical waters of Queensland (QLD), with more than 1400 cases of CFP reported in the past 15 years, including two fatalities (Gillespie et al., 1986; Stewart et al., 2010). *Gambierdiscus* (reported as *Gambierdiscus toxicus*) has been documented from the tropical Great Barrier Reef (Flinders Reef, Arlington Reef, Hastings Reef, Heron Island (23° S) to Platypus Bay (27° S) (Gillespie et al., 1985; Hallegraeff, 1993; Holmes and Lewis, 1994). However, previous Australian toxicological studies (Holmes et al., 1990; Holmes and Lewis, 1994) do not allow for an unambiguous identification of the precise *Gambierdiscus* species involved, since no archived cultures or samples are available. Along the coast of New South Wales (NSW), 31 estuaries are monitored fortnightly for occurrences of harmful planktonic microalgae, as part of the state's shellfish safety program. *Gambierdiscus* species were found to be present at five sites along the NSW coast in the period 2005–2009 (Camden Haven River, Wallis Lake, Tuross Lake, Wapengo Lagoon, Merimbula Lake and Womboyn River) (Ajani et al., 2013). Murray (2010; Fig. 5.15) and Hallegraeff (2010) first drew attention to this unusual warm-temperate *Gambierdiscus* population, stretching as far as 37° S.

The major aim of this study was to conclusively identify the species of *Gambierdiscus* species present in above three estuaries in southern NSW. Light microscopy and 18S rRNA gene ribosomal Tag-Encoded FLX 454-Pyrosequencing (rTEFP) methods were applied to study the dinoflagellate community structure. Detailed morphological studies via electron microscopy were carried out to confirm the identity of the *Gambierdiscus* species in the samples, and samples were taken to estimate cell densities. Samples were tested for the presence of CTXs and MTXs using liquid chromatography–mass spectrometry (LC–MS) analysis. The toxicity of the sample extracts was also determined via mouse-bioassay.

2. Materials and methods

2.1. Sample collection

In May 2012 and 2013, samples were collected from various sites for rTEFP analysis, cell density estimation and toxin analysis (Table 1; Figs. 1–3). Several dinoflagellate species are known to live epibenthically on a variety of macroalgae (Bomber et al., 1989; Holmes, 1998). Hence, during sampling the most abundant types of macroalgae at each of the sites were collected (Table 1). Various amounts of macroalgae (750 g for rTEFP analysis, 5–10 g for cell density estimates, 1 kg for toxin analysis) were collected from approximately 1 m depth and placed in plastic bags containing ambient seawater (300–500 ml for rTEFP analysis, 5–30 ml for cell density estimates, 2 L for toxin analysis). The bag was shaken vigorously for 5 min. The liquid contents were then passed through a 104 μm mesh filter to remove larger fauna and debris. For rTEFP analysis, 50 ml of each sample was filtered using a 3 μm filter (Merck Millipore®, Billerica, MA) and cells were washed from the filters using 5 ml RNAlater (Ambion®, Austin, TX) for preservation and stored at 4 °C. For cell density estimates, the filtrate obtained after mesh filtration was preserved in Lugol's solution and stored at 4 °C for further analysis. For toxin analysis, a 1 ml sub-sample was

Table 1
Sampling sites, the macroalgae the samples were collected from, the water temperature at the time of sample collection and the type of analysis done with each sample.

Sampling site (latitude–longitude)	No. of samples collected (sample name)	Date collected (water temperature)	Macroalgae	Analysis conducted
Wagonga Inlet (36°12′43.04″ S–150°06′33.92″ E	1 (WG1)	May, 2012 (16.5 °C)	*Padina* sp.	rTEFP analysis
Wapengo Lagoon (36°35′51.21″ S–150°00′38.61″ E	1 (WP1)	May, 2012 (17 °C)	*Phyllospora* sp.	rTEFP analysis
Merimbula Inlet (36°53′29.80″ S–149°54′39.18″ E)	1 (MB1)	May, 2012 (17.5 °C)	*Phyllospora* sp.	rTEFP analysis
Merimbula Inlet (36°53′29.80″ S–149°54′39.18″ E)	1 (MB2)	May, 2012 (17.5 °C)	*Phyllospora* sp.	rTEFP analysis
S1-mid (Merimbula Inlet, 36°53′29.80″ S–149°54′39.18″ E)	4 (S1-mid-N1 to N4)	May, 2013 (17 °C)	*Phyllospora* sp.	Abundance counts
S1-low (Merimbula Inlet, 36°53′29.80″ S-149°54′39.18″ E)	4 (S1-low-N1 to N4)	May, 2013 (17 °C)	*Phyllospora* sp.	Abundance counts
S2 (Merimbula Inlet, 36°53′50.47″ S–149°55′0.45″ E)	4 (S2–N1 to N4)	May, 2013 (17 °C)	*Zostera*a sp.	Abundance counts
S3 (Merimbula Inlet, 36°53′56.33″ S–149°55′37.81″ E)	4 (S3–N1 to N4)	May, 2013 (17 °C)	*Phyllospora* sp.	Abundance counts
S4 (Merimbula Inlet, 36°53′46.28″ S–149°54′36.87″ E)	5 (S4–N1 to N5)	May, 2013 (17 °C)	S4-N1 and N2 – *Phyllospora* sp., S4-N3 and N4 – *Ecklonia* sp. S4-N5 – *Phyllospora* sp. and *Ecklonia* sp.	Abundance counts