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LSU rDNA based RFLP assays for the routine identification of *Gambierdiscus* species

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ABSTRACT

The *Gambierdiscus* genus is a group of benthic dinoflagellates commonly associated with ciguatera fish poisoning (CFP), which is generally found in tropical or sub-tropical regions around the world. Morphologically similar species within the genus can vary in toxicity; however, species identifications are difficult or sometimes impossible using light microscopy. DNA sequencing of ribosomal RNA genes (rDNA) is thus often used to identify and describe *Gambierdiscus* species and ribotypes, but the expense and time can be prohibitive for routine culture screening and/or large-scale monitoring programs. This study describes a restriction fragment length polymorphism (RFLP) typing method based on analysis of the large subunit rDNA that can successfully identify at least nine of the described *Gambierdiscus* species and two *Fukuyoa* species. The software programs DNAMAN 6.0 and Restriction Enzyme Picker were used to identify a set of restriction enzymes (*Spe*I, *Hpy*CH4IV, and *Taq*α1) capable of distinguishing most of the known *Gambierdiscus* species for which DNA sequences were available. This assay was tested using *in silico* analysis and cultured isolates, and species identifications of isolates assigned by RFLP typing were confirmed by DNA sequencing. To verify the assay and assess intra-specific heterogeneity in RFLP patterns, identifications of 63 *Gambierdiscus* isolates comprising ten *Gambierdiscus* species, one ribotype, and two *Fukuyoa* species were confirmed using RFLP typing, and this method was subsequently employed in the routine identification of isolates collected from the Caribbean Sea. The RFLP assay presented here reduces the time and cost associated with morphological identification via scanning electron microscopy and/or DNA sequencing, and provides a phylogenetically sensitive method for routine *Gambierdiscus* species assignment.

1. Introduction

Ciguatera fish poisoning (CFP) is a human poisoning syndrome caused by the consumption of seafood contaminated with ciguatoxins. The genus *Gambierdiscus* represents a group of benthic dinoflagellates known to produce ciguatoxins (CTX); however, toxin production is variable among species and strains (Holmes et al., 1991; Chinain et al., 2010). Incidences of CFP are more common in tropical and subtropical latitudes, which correspond to the endemic range of *Gambierdiscus* spp., and the prevalence of poisonings and abundances of *Gambierdiscus* spp. are often site-specific (Dickey and Plakas, 2010). This dinoflagellate genus has been reported in tropical or sub-tropical regions around the world, including Okinawa in Japan (Nishimura et al., 2013), the South China Sea (Zhang et al., 2016), Hong Kong (Wong et al.,

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2005), Malaysia (Leaw et al., 2011), Thailand (Tawong et al., 2015), Texas, South Carolina, Hawaii, and Florida in U.S. (e.g., Babinchak et al., 1986; Centers for Disease Control and Prevention (CDC), 2006; Villareal et al., 2007; Rains and Parsons, 2015), French

Table 1
Isolate name and geographic origin of *Gambierdiscus* and *Fukuyoa* spp. used for RFLP assay testing, and comparison between RFLP typing results and identification based on DNA sequencing or alternative method. Percent identity levels based on BLAST sequence similarity searches in GenBank are shown in parentheses. In the interest of simplifying the assay description and results, the first four letters of each species name (shown in alphabetical order) is used to represent each *Gambierdiscus* and *Fukuyoa* species, except for *Gambierdiscus* ribotype 2 (Ribo2).

Isolates	Geographic Origin	Abbreviation	Species identification based on DNA sequencing or alternative method	RFLP Recognition
BB Apr 11–11	St. Thomas, USVI	Cari1	*G. caribaeus* (100%)	*G. caribaeus*
BB May 10–12	St. Thomas, USVI	Cari2	*G. caribaeus* (99%)	*G. caribaeus*
BP Aug 08	St. Thomas, USVI	Cari3	*G. caribaeus* (99%)	*G. caribaeus*
HGB7	Florida Keys, FL, USA	Cari4	*G. caribaeus* (100%)	*G. caribaeus*
LKH4	Florida Keys, FL, USA	Cari5	*G. caribaeus* (99%)	*G. caribaeus*
Tenn10	Florida Keys, FL, USA	Cari6	*G. caribaeus* (100%)	*G. caribaeus*
STT_Cari6	St. Thomas, USVI	Cari7	*G. caribaeus* (99%)	*G. caribaeus*
STT_Cari19	St. Thomas, USVI	Cari8	*G. caribaeus* (100%)	*G. caribaeus*
NH-1	Nuku-Hiva, Marquesas, French Polynesia	Cari9	*G. caribaeus* (100%)	*G. caribaeus*
Rik-1	Mangareva, Gambier, French Polynesia	Cari10	*G. caribaeus*[a]	*G. caribaeus*
BB May 10–11	St. Thomas, USVI	Caro1	*G. carolinianus* (99%)	*G. carolinianus*
FC Apr 11–2	St. Thomas, USVI	Caro2	*G. carolinianus* (99%)	*G. carolinianus*
BP May 10–5	St. Thomas, USVI	Caro3	*G. carolinianus* (99%)	*G. carolinianus*
LKH10	Florida Keys, FL, USA	Caro4	*G. carolinianus* (99%)	*G. carolinianus*
GHCG2-C6	San Salvador, Bahamas	Caro5	*G. carolinianus* (99%)	*G. carolinianus*
TRL26	Florida Keys, FL, USA	Caro6	*G. carolinianus* (99%)	*G. carolinianus*
GHCG2-A6	San Salvador, Bahamas	Caro7	*G. carolinianus* (99%)	*G. carolinianus*
GHCG2-B8	San Salvador, Bahamas	Caro8	*G. carolinianus* (99%)	*G. carolinianus*
CCMP399	St. Barthelemy Island	Beli1	*G. belizeanus*[b]	*G. belizeanus*
FC Dec 10–13	St. Thomas, USVI	Beli2	*G. belizeanus* (99%)	*G. belizeanus*
BP Apr 11–7	St. Thomas, USVI	Beli3	*G. belizeanus* (99%)	*G. belizeanus*
BP Mar 10–18	St. Thomas, USVI	Beli4	*G. belizeanus* (99%)	*G. belizeanus*
BP Mar 10–22	St. Thomas, USVI	Beli5	*G. belizeanus* (99%)	*G. belizeanus*
BP Mar 10–25	St. Thomas, USVI	Beli6	*G. belizeanus* (99%)	*G. belizeanus*
BP Mar 10–31	St. Thomas, USVI	Beli7	*G. belizeanus* (99%)	*G. belizeanus*
BP Mar 10–7	St. Thomas, USVI	Beli8	*G. belizeanus* (99%)	*G. belizeanus*
MUR-4	Moruroa, Gambier, French Polynesia	Paci1	*G. pacificus*[c]	*G. pacificus*
Hao1 (or HO-91)	Hao, Tuamotu, French Polynesia	Paci2	*G. pacificus*[c]	*G. pacificus*
Tub ET1	Tubuai, Australes, French Polynesia	Paci3	*G. pacificus*[a]	*G. pacificus*
BP Apr 11-6	St. Thomas, USVI	Ribo21	*G.* ribotype 2 (99%)	*G.* ribotype 2
SH Dec 10–10	St. Thomas, USVI	Ribo22	*G.* ribotype 2 (99%)	*G.* ribotype 2
SH Dec 10–12	St. Thomas, USVI	Ribo23	*G.* ribotype 2 (99%)	*G.* ribotype 2
TRL29	Florida Keys, FL, USA	Ribo24	*G.* ribotype 2 (100%)	*G.* ribotype 2
HGB	Florida Keys, FL, USA	Yasu	*F. yasumotoi* (94%)	*F. yasumotoi*
HGB6	Florida Keys, FL, USA	Carp1	*G. carpenteri* (99%)	*G. carpenteri*
KML1	Florida Keys, FL, USA	Carp2	*G. carpenteri* (99%)	*G. carpenteri*
TPH12	Florida Keys, FL, USA	Carp3	*G. carpenteri* (99%)	*G. carpenteri*
STT_Carp5	St. Thomas, USVI	Carp4	*G. carpenteri* (99%)	*G. carpenteri*
STT_Carp8	St. Thomas, USVI	Carp5	*G. carpenteri* (99%)	*G. carpenteri*
STT_Carp9	St. Thomas, USVI	Carp6	*G. carpenteri* (99%)	*G. carpenteri*
STT_Carp11	St. Thomas, USVI	Carp7	*G. carpenteri* (99%)	*G. carpenteri*
STT_Carp24	St. Thomas, USVI	Carp8	*G. carpenteri* (99%)	*G. carpenteri*
Rik-5	Mangareva, Gambier, French Polynesia	Carp9	*G. carpenteri* (98%)	Inconclusive
NH-2	Nuku-Hiva, Marquesas, French Polynesia	Carp10	*G. carpenteri* (99%)	Inconclusive
PO	Tahiti, Society, French Polynesia	Aust1	*G. australes*[a]	*G. australes*
RAV-1	Raivavae, Australes, French Polynesia	Aust2	*G. australes*[a]	*G. australes*
G3-93	Mangareva, Gambier, French Polynesia	Aust3	*G. australes*[a]	*G. australes*
S080911_1	Kutsu, Kochi, Japan	Aust4	*G. australes*[d]	*G. australes*
ISC5G	Touzato, Ishigaki Island, Okinawa, Japan	Aust5	*G. australes*[d]	*G. australes*
I080606_1	Sawada Beach, Irabu Island, Okinawa, Japan	Aust6	*G. australes*[d]	*G. australes*
Rai1	Raivavae, Australes, French Polynesia	Poly1	*G. polynesiensis*[c]	*G. polynesiensis*
Rik-8	Mangareva, Gambier, French Polynesia	Poly2	*G. polynesiensis*[a]	*G. polynesiensis*
RG-92	Rangiroa, Tuamotu, French Polynesia	Poly3	*G. polynesiensis*[c]	*G. polynesiensis*
TB-92	Tubuai, French Polynesia	Poly4	*G. polynesiensis*[e]	*G. polynesiensis*
GTT-1	Tahiti, Society, French Polynesia	Toxi1	*G. toxicus*[a]	*G. toxicus*
Rik-13	Mangareva, Gambier, French Polynesia	Toxi2	*G. toxicus*[a]	*G. toxicus*
HIT-0	Tahiti, French Polynesia	Toxi3	*G. toxicus*[c]	*G. toxicus*
CCMP 3143	Carrie Bow Cay, Belize	Ruez	*F. ruetzleri*[b]	*F. ruetzleri*
BP Mar 10–23	St. Thomas, USVI	Silv1	*G. silvae* (100%)	*G. silvae*
FC May 10–9	St. Thomas, USVI	Silv2	*G. silvae* (99%)	*G. silvae*
SH Apr 11–1	St. Thomas, USVI	Silv3	*G. silvae* (99%)	*G. silvae*
TRL23	Florida Keys, FL, USA	Silv4	*G. silvae* (99%)	*G. silvae*
M080828_3	Muroto Promontory, Kochi, Japan	Scab	*G. scabrosus*[d]	*G. scabrosus*

[a] Isolate from culture collection maintained by the Institut Louis Malardé, Tahiti, French Polynesia.
[b] Isolate from culture collection maintained by the National Center for Marine Algae and Microbiota at Bigelow Laboratory, East Boothbay, ME, USA.
[c] see Chinain et al. (2010).
[d] see Nishimura et al. (2013).
[e] see Chinain et al. (1999a).