Supplementary :

Targeted and non-targeted mass spectrometry to explore the chemical diversity of the genus *Gambierdiscus* in the Atlantic Ocean

Authors

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Table S 1 Summary of compounds identified in the Gambierdiscus *species reported in the Atlantic Ocean.*

	PIGMENTS	MAITOTOXINS	OTHER POLYETHERS	OTHER COMPOUNDS
G. australes		MTX1 (Rhodes *et al.* 2014) *MTX 5 (Estevez *et al.* 2021)	44-methylgambierone (Rhodes *et al.* 2014, Estevez *et al.* 2020a, Murray *et al.* 2021) *Gambieric acids C and D (Estevez *et al.* 2020a)	
G. belizeanus	Chlorophyll a (Gwinn *et al.* 2019)	Desulfo-MTX1 (Mazzola *et al.* 2019)	44-methylgambierone (Pisapia *et al.* 2017, Boente-Juncal *et al.* 2019) Gambierone (Rodriguez *et al.* 2015)	Dimethylsulfoniopropionate (Gwinn *et al.* 2019)
G. caribaeus			44-methylgambierone (Murray *et al.* 2021) *C-CTX1/2 (Mudge *et al.* 2023) *C-CTX5 (Mudge *et al.* 2023)	
G. carolinianus			44-methylgambierone (Pisapia *et al.* 2017)	
G. carpenteri			44-methylgambierone (Murray *et al.* 2021, Malto *et al.* 2022) Gambierone (Murray *et al.* 2021)	Carbohydrates Fatty acids (Malto *et al.* 2022)
G. excentricus	Peridinin Chlorophyll C2 Chlorophyll a Chlorophyll C1 Diadinoxanthin Dinoxanthin (Zapata *et al.* 2012)	MTX4 (Pisapia *et al.* 2017, Estevez *et al.* 2020a, Yon *et al.* 2021)	Sulfo-gambierone (Yon *et al.* 2021) Dihydro-sulfo-gambierone (Yon *et al.* 2021)	
G. silvae			44-methylgambierone (Pisapia *et al.* 2017) *29-methylgambierone (Mudge *et al.* 2022) Gambierone (Mudge *et al.* 2022) *C-CTX1/2 (Mudge *et al.* 2023) *C-CTX5 (Mudge *et al.* 2023)	

* Putatively reported

Table S 2 Quantification of Gambierones (Sulfo-gambierones, gambierone, 44-methylgambierone) and Maitotoxins (MTX1 and 4) in the different pool per strain per laboratory expressed in pg equivalent cell-1 measured in this study and reported in the literature.

Species	Strains	Laboratory	Sulfo-gambierones (pg eq. gambierone cell^{-1})	Gambierone (pg cell^{-1})	MTX4 (pg eq. MTX1 cell^{-1})	44-methylgambierone (pg cell^{-1})	MTX1 (pg cell^{-1})	References
G. excentricus	Pulley Ridge Gam 2	IFR	0.5		**16.4**			This study
		/			22.9			(Pisapia *et al.* 2017)
	Bahamas Gam 5	NOAA	**1.0**		**35.5**			This study
		IFR	**0.6**		**19.8**			This study
	UNR-08	IFR	**2.3**		**29.0**			This study
		UNIRIO	**4.9**		**38.4**			This study
		/			19.8			(Pisapia *et al.* 2017)
	UNR-07	UNIRIO	**2.8**		**19.2**			This study
		/			16.0			(Pisapia *et al.* 2017)
	VGO1035	/			13.0			(Pisapia *et al.* 2017)
	VGO790	/			23.2			(Pisapia *et al.* 2017)
	VGO792	/			20.0			(Pisapia *et al.* 2017)
	VGO 791	IEO	**0.6**		**~6 <LOQ)**			This study
		/			72.8			(Pisapia *et al.* 2017)
	IRTA-SMM-17-407	/			37.1			(Estevez *et al.* 2020a)
G. australes	AUS S08	IFR				**21.4**	**15.5**	This study
		IEO				**16.7**	**9.3**	This study
		UNIRIO				**34.7**	**15.8**	This study
	CAWD149			<0.01		259		(Murray *et al.* 2021)
	IRTA-SMM17-189					344 (9.4)*		(Estevez *et al.* 2020a)
	IRTA-SMM17-162					720 (19.7)*		(Estevez *et al.* 2020a)
	IRTA-SMM17-164					1661 (45.5)*		(Estevez *et al.* 2020a)
	IRTA-SMM17-271					1322 (36.2)*		(Estevez *et al.* 2020a)
	IRTA-SMM17-253					1081 (29.6)*		(Estevez *et al.* 2020a)
	IRTA-SMM17-244					479 (13.1)*		(Estevez *et al.* 2020a)
G. belizeanus	ST1 F4	NOAA		**17.9**		**1.7**		This study
	UNR-58	UNIRIO		**6.4**		**8.35**		This study
	UNR-51	UNIRIO		**10.0**		**13.1**		This study
G. caribaeus	ST1C5	NOAA		**0.7**		**19.3**		This study
	Dive 1F4	NOAA		**0.9**		**33.3**		This study
	CAWD301			<0.01		44		(Murray *et al.* 2021)
G. carolinianus	RROV5	NOAA		**0.6**		**0.1**		This study
	VGO 1197	VIGO		**15.8**		**0.6**		This study
	Kenny 6	NOAA		**1.3**		**0.1**		This study
G. silvae	UNR-30	UNIRIO		**29.8**		**2.0**		This study
	VGO 1358	IEO		**6.5**		**8.4**		This study

*Results reported in MTX1 equivalent (corrected with the response factor between MTX1 and 44-methylgambierone calculated in this study- Table S9).

*Table S 3 MRM transitions used for targeted LC-MS/MS analysis in negative ionization mode (ESI-, reproduced from (*Yon *et al.* 2021*). In bold the transitions used for quantification.*

Instrumental parameters	The mobile phases were 100% water (eluent A) and acetonitrile/water (95:5, v/v) (eluent B), both added with 2 mM ammonium formate and 50 mM formic acid and the elution gradient was as follows: 10 to 95% of B from 0 to 10 min, held at 95% B for 2 min and then back to the initial condition (10% B) at 12.1 min and held for 3 min.	
Compound name	Transitions (precursor ion/product ion)	Ion species
MTX1 (Murata *et al.* 1993)	**1689.8/1689.8**	**$[M-2H]^{2-}/[M-2H]^{2-}$**
	1689.8/96.9	$[M-2H]^{2-}/[HOSO_3]^-$
	1126.2/1126.2	$[M-3H]^{3-}/[M-3H]^{3-}$
	1126.2/96.9	$[M-3H]^{3-}/[HOSO_3]^-$
MTX2 (Lewis *et al.* 1994)	1637.8/1637.8	$[M-2H]^{2-}/[M-2H]^{2-}$
	1637.8/96.9	$[M-2H]^{2-}/[HOSO_3]^-$
	1091.8/96.9	$[M-3H]^{3-}/[HOSO_3]^-$
MTX4 (Pisapia *et al.* 2017, Pisapia *et al.* 2020)	**1646.2/1646.2**	**$[M-2H]^{2-}/[M-2H]^{2-}$**
	1646.2/96.9	$[M-2H]^{2-}/[HOSO_3]^-$
	1097.8/1097.8	$[M-3H]^{3-}/[M-3H]^{3-}$
	1097.8/96.9	$[M-3H]^{3-}/[HOSO_3]^-$
MTX Unknown1 (Mazzola *et al.* 2019)	1649.8/1649.8	$[M-2H]^{2-}/[M-2H]^{2-}$
	1649.8/96.9	$[M-2H]^{2-}/[HOSO_3]^-$
MTX Unknown2 (Mazzola *et al.* 2019)	1641.8/1641.8	$[M-2H]^{2-}/[M-2H]^{2-}$
	1641.8/96.9	$[M-2H]^{2-}/[HOSO_3]^-$
Gambierone (Rodriguez *et al.* 2015)	**1023.5/1023.5**	**$[M-H]^-/[M-H]^-$** **(SG) $[M\text{-}SO_3\text{-}H]^-$/ $[M\text{-}SO_3\text{-}H]^-$**
Sulfo-Gambierones (SG) (Yon *et al.* 2021)	1023.5/97.0	$[M-H]^-/[HOSO_3]^-$ (SG) $[M\text{-}SO_3\text{-}H]^-/[HOSO_3]^-$
	1023.52/899.4	$[M-H]^-/[C_{43}H_{63}O_{18}S]^-$
	1023.52/963.5	$[M-H]^-/[C_{49}H_{71}O_{17}S]^-$
44-methylgambierone (Murray *et al.* 2019)	**1037.5/1037.5**	**$[M-H]^-/[M-H]^-$**
	1037.5/97.0	$[M-H]^-/[HOSO_3]^-$
	1037.5/899.5	$[M-H]^-/[C_{43}H_{63}O_{18}S]^-$
	1037.5/977.6	$[M-H]^-/[C_{50}H_{73}O_{17}S]^-$
Gambieroxide (Watanabe *et al.* 2013)	1193.6/96.9	$[M-H]^-/[HOSO_3]^-$
	1193.6/987.6	
	1193.6/1193.6	$[M-H]^-/[M-H]^-$
Gambieric Acid A (Nagai *et al.* 1992)	1055.1/1037.1	$[M-H]^-/[M-H_2O-H]^-$
	1055.1/1055.1	$[M-H]^-/[M-H]^-$
Gambieric Acid B (Nagai *et al.* 1992)	1069.1/1069.1	$[M-H]^-/[M-H]^-$
	1069.1/1051.1	$[M-H]^-/[M-H_2O-H]^-$
Gambieric Acid C (Nagai *et al.* 1992)	1183.7/1183.7	$[M-H]^-/[M-H]^-$
	1183.7/1165.7	$[M-H]^-/[M-H_2O-H]^-$
Gambieric Acid D (Nagai *et al.* 1992)	1197.7/1197.7	$[M-H]^-/[M-H]^-$
	1197.7/1179.7	$[M-H]^-/[M-H_2O-H]^-$

Table S 4 MRM transitions used for LC-MS/MS targeted analysis in positive ionization mode (ESI+).

Instrumental parameters	LC mobile phase was 5mM ammonium formate and 0.1% formic acid in H_2O (A) and MeOH (B). The gradient used was: 78% to 88% B in 10 min and holding for 5 min. Return to initial conditions for 2 min	
Compound name	Transitions (precursor ion/product ion)	Ion species
C-CTX1/2 (Vernoux *et al.* 1997, Estevez *et al.* 2019, Estevez *et al.* 2020b)	1123.6/1105.6	$[M-H_2O+H]^+/[M-2H_2O+H]^+$
	1123.6/191.1	$[M-H_2O+H]^+$/fragment
	1141.4/1123.4	$[M+H]^+/[M-H_2O+H]^+$
C-CTX3/4 (Kryuchkov *et al.* 2020)	1143.6/1125.6	$[M+H]^+/[M-H_2O+H]^+$
	1143.6/1089.5	$[M+H]^+/[M-3H_2O+H]^+$
	1143.6/547.3	$[M+H]^+$/fragment
	1143.6/141.0	$[M+H]^+$/fragment
C-CTX-1159 (Pottier *et al.* 2002)	1159.4/1141.4	$[M+H]^+/[M-H_2O+H]^+$
	1159.4/1123.4	$[M+H]^+/[M-2H_2O+H]^+$
C-CTX-1127 (Pottier *et al.* 2002)	1127.4/1109.4	$[M+H]^+/[M-H_2O+H]^+$
	1127.4/1091.4	$[M+H]^+/[M-2H_2O+H]^+$
I-CTX3/4 (Hamilton *et al.* 2002)	1157.4/1139.4	$[M+H]^+/[M-H_2O+H]^+$
	1139.6/1121.6	$[M-H_2O+H]^+/[M-2H_2O+H]^+$
	1139.6/1103.4	$[M-H_2O+H]^+/[M-3H_2O+H]^+$
	1174.6/1139.6	$[M+NH4]^+/[M-H_2O+H]^+$
I-CTX5 (Diogène *et al.* 2017)	1121.6/1103.6	$[M-H_2O+H]^+/ [M-2H_2O+H]^+$
	1121.6/1085.6	$[M-H_2O+H]^+/ [M-3H_2O+H]^+$
	1156.6/1121.6	$[M+NH4]^+/[M-H_2O+H]^+$
I-CTX6 (Diogène *et al.* 2017)	1155.6/1137.6	$[M+H]^+/[M-H_2O+H]^+$
	1137.6/1119.6	$[M-H_2O+H]^+/ [M-2H_2O+H]^+$
	1137.6/1101.6	$[M-H_2O+H]^+/ [M-3H_2O+H]^+$
	1177.6/1137.6	$[M+Na]^+/[M-H_2O+H]^+$

Table S 5 Strains provided by each laboratory, culture conditions and harvesting methods.

Laboratory	Species	Strain	Reference	Origin	Culture Collection	Container	-Sea water -Salinity -Medium	--Temperature -Dark/ Light cycle -Intensity -Light source	-Cell counting - inoculate- harvest -Harvesting procedure -Quenching
Laboratório de Microalgas Marinhas, Departamento de Ecologia e Recursos Marinhos (UNIRIO)	*G. australes*	S080911_1	Nishimura *et al.* (2013)	Kochi, Susaki City, Kutsu, Japan		-1 L glass Erlenmeyer flask with vented cap	- filtered and autoclaved natural seawater (Arraial do Cabo, Rio de Janeiro state) -Salinity 32 (adjusted) -500 mL L2 modified medium (by omitting silicate, nickel, vanadium and chromium).	-24 °C. -12 h light/ 12 h dark -90 µmol m^{-2} s^{-1} -Led (yellow and white, alternated)	- Sedgewick rafter chamber or 'drop on slide' (100 µL, 3-5 replicates). - Harvested 2300 - 3400 cells mL^{-1}. -Centrifugation for 15 min (5000*g*) in 50 mL centrifuge tubes. -cells were stored at -80°C, then freeze-dried and stored at -20°C until shipment.
	G. belizeanus	UNR51	Nascimento et al. in prep	Fernando de Noronha Archipelago, Brazil	UNIRIO				
	G. belizeanus	UNR58	Nascimento et al. in prep	Fernando de Noronha Archipelago, Brazil	UNIRIO				
	G. excentricus	UNR-08	Nascimento *et al.* (2015)	Armação dos Búzios, Rio de Janeiro, Brazil	UNIRIO				
	G. excentricus	UNR-07	Nascimento *et al.* (2015)	Armação dos Búzios, Rio de Janeiro, Brazil	UNIRIO				
	G. silvae	UNR-30	Nascimento et al. in prep	Trindade Island, Brazil	UNIRIO				
National Oceanic and Atmospheric Administration, National Ocean Service, National Centers for Coastal Ocean Science (NOAA)	*G. australes*	S080911_1	(Nishimura *et al.* 2013)	Kochi, Susaki City, Kutsu, Japan		- 75 cm^2 plastic vented cap culture flask	-0.2µm filtered seawater (Gulf stream offshore North Carolina) -Salinity 33 -modified K medium, sterilized in microwave and 0.2µm filtered	- 27 °C -12 h light/ 12 h dark -90–100 µmol m^{-2} s^{-1} -Fluorescent tubes (Full Spectrum)	- Beckman Coulter Multisizer™ 3 particle counter - 500 cell mL^{-1} – 3000 cell mL^{-1} - 20 µm sieve to collect cells, then centrifugation at 3200g for 10 minutes -cells were stored at -80°C until shipment and freeze-dried upon arrival
	G. belizeanus	ST1F4	(Vandersea *et al.* 2012)	St. Thomas, U.S. Virgin Islands	CCFHR				
	G. caribaeus	ST1C5	(Vandersea *et al.* 2012)	St. Thomas, U.S. Virgin Islands	CCFHR				
	G. caribaeus	DIVE1Fa	(Vandersea *et al.* 2012)	Marathon key, Florida, United States of America	CCFHR				
	G. carolinianus	Kenny 6 (KEN6)	(Vandersea *et al.* 2012)	60 km offshore, Cape Fear North Carolina, United States of America	CCFHR				
	G. carolinianus	RR0V5		Puerto Rico, United States of America	CCFHR				
	G. excentricus	Bahamas Gam 5 (BG5)		Freeport, Grand Bahama Island	CCFHR				
Ifremer, Laboratoire METALG (IFR)	*G. australes*	S080911_1	(Nishimura *et al.* 2013)	Kochi, Susaki City, Kutsu, Japan		- 225 cm^2 plastic vented cap culture flask	-Sterilized natural seawater (English Channel) -Salinity 34.5 -L1-Si	-25 °C -12 h light/ 12 h dark -70-90 µmol m^{-2} s^{-1} -Fluorescent tubes (cool-white and pink)	- Beckman Coulter Multisizer™ 3 particle counter -500 cell mL^{-1} – 2000 cell mL^{-1} -Harvesting using a 20 µm sieve to reduce volume then centrifugation 3500 g for 4 min -Quenching with liquid nitrogen then freeze-drying and stored at -20°C
	G. excentricus	Pulley Ridge Gam 2 (PRG2)	(Litaker *et al.* 2017)	Pulley Ridge, Florida, United States of America	CCFHR				
	G. excentricus	Bahamas Gam 5 (BG5)		Freeport, Grand Bahama Island	CCFHR				
	G. excentricus	UNR-08	(Nascimento *et al.* 2015)	Armação dos Búzios, Rio de Janeiro, Brazil	UNIRIO				
Instituto Español de Oceanografía, Centro Ocenográfico de Vigo (IEO)	*G. australes*	S080911_1	(Nishimura *et al.* 2013)	Kochi, Susaki City, Kutsu, Japan		- 1000 mL Erlenmeyer flask	- 0.2µm filtered seawater (Gulf stream) -Salinity 32 - modified K/2-medium-Si	-25 °C -12 h light/ 12 h dark -90–100 µmol m^{-2} s^{-1} -Fluorescent tubes (Full Spectrum)	- Beckman Coulter Multisizer™ 3 particle counter -500 cell mL^{-1} – 2000 cell mL^{-1} -Harvesting using a 20 µm sieve to reduce volume then centrifugation 1000 g for 5 min -Quenching with liquid nitrogen then freeze at -80ºC then freeze-dried and maintain at -20ºC before shipment.
	G. carolinianus	VGO 1197	(Rodríguez *et al.* 2017)	Alcalá, Tenerife, Spain	CCVIEO				
	G. excentricus	VGO791	(Fraga *et al.* 2011)	Punta Hidalgo, Tenerife, Canary Islands, Spain	CCVIEO				
	G. silvae	VGO1358	(Rodríguez *et al.* 2017)	La Gomera, Valle Rey harbor, Canary Islands	CCVIEO				

UNIRIO: Rio de Janeiro State, Federal University, Brazil
CCFHR: National Oceanographic and Atmospheric Administration (NOAA), National Ocean Service, National Centers for Coastal Ocean Science, Center for Coastal Fisheries Habit Research, Beaufort, NC, USA
CCVIEO: Culture Collection of Harmful Microalgae of IEO, Centro de Vigo, Vigo, Spain

Table S 6 Number of cells, dry mass and final concentration in for each replicate.

Laboratory	Species	Strains	Counting Method	Replicate	Number of cells (x10^6)	Dry mass of extracts* (mg)	Final mass concentration of extracts (mg mL^{-1})
UNIRIO	*G. australes*	S080911_1	- Sedgewick rafter chamber or 'drop on slide' (100 µL, 3-5 replicates).	1	1.1	57.0	13.0
				2	1.4	58.8	10.5
				3	1.2	99.3	20.7
				4	1.3	127.3	24.5
				5	1.8	94.8	13.2
	G. excentricus	UNR 08		1	0.8	26.8	8.0
				2	0.4	12.9	8.7
		UNR 07		1	0.1	/	/
	G. silvae	UNR 30		1	0.2	/	/
	G. belizeanus	UNR 51		1	0.9	12.0	3.3
				2	0.5	16.4	8.9
				3	0.1	6.2	14.1
		UNR 58		1	0.3	11.8	9.2
				2	0.3	10.6	10.2
IFR	*G. australes*	S080911_1	- Beckman Coulter Multisizer™ 3 particle counter	1	2.8	83.4	7.4
				2	2.8	59.1	5.3
				3	3.1	67.8	5.5
				4	2.5	68.0	6.8
				5	2.5	62.6	6.3
	G. excentricus	Bahamas Gam 5		1	1.1	37.4	8.5
				2	1.2	46.3	9.6
				3	1.4	47.5	8.5
				4	1.4	44.7	8.0
				5	1.5	46.6	7.8
		Pulley Ridge Gam 2		1	1.1	44.5	10.1
				2	1.4	42.3	7.5
				3	1.1	39.2	8.9
				4	1.2	47.9	10.0
				5	1.5	45.6	7.6
		UNR 08		1	1.9	52.9	7.0
				2	1.5	41.1	6.9
				3	1.5	40.7	6.8
				4	1.5	43.4	7.2
				5	1.3	49.0	9.4
IEO	*G. australes*	S080911_1	- Beckman Coulter Multisizer™ 3 particle counter	1	2.1	103.7	12.3
				2	2.3	121.3	13.2
				3	2.1	168.5	20.1
				4	2.4	97.5	10.2
				5	2.7	133.4	12.4
	G. carolinianus	VGO 1197		1	2.1	42.8	5.1
				2	2.2	45.0	5.1
				3	2.5	49.2	4.9
				4	2.7	101.6	9.4
				5	2.2	55.6	6.3
	G. excentricus	VGO 791		1	1.1	35.0	8.0
				2	1.0	37.5	9.4
				3	1.0	63.7	15.9
				4	1.0	40.7	10.2
				5	1.2	39.2	8.2
	G. silvae	VGO 1358		1	2.0	38.9	4.9
				2	2.3	36.9	4.0
				3	2.4	35.4	3.7
				4	2.6	41.0	3.9
				5	2.9	36.8	3.2
NOAA	*G. carolinianus*	Kenny 6	- Beckman Coulter Multisizer™ 3 particle counter	1	1.1	32.4	7.4
				2	1.0	33.5	8.4
				3	1.1	30.8	7.0
				4	1.4	33.4	6.0
		RROV5		1	1.3	32.5	6.3
				2	#	25.6	5.1
				3	1.2	35.4	7.4
				4	1.4	39.4	7.0
	G. excentricus	Bahamas Gam 5		1	1.1	74.1	17.3
				2	1.1	38.0	9.0
				3	1.1	65.0	14.4
				4	#	35.0	8.3
				5	#	68.5	16.3
	G. belizeanus	ST1F4		1	1.1	30.1	7.1
				2	1.1	35.4	8.1
				3	1.0	38.6	9.7
				4	1.1	28.4	6.5
				5	1.7	50.5	7.4
	G. caribaeus	ST1C5		1	1.0	38.2	9.5
				2	1.2	35.5	7.4
				3	1.0	38.0	9.5
				4	1.0	35.5	8.9
				5	1.0	43.7	10.9
		Dive1 F4		1	1.0	56.0	14.0
				2	1.1	69.8	15.9
				3	1.2	39.9	8.3
				4	1.4	77.0	13.8
				5	1.2	57.6	12.0

* Dry mass was estimated by evaporating 1/3 of each sample. # When cell number was missing, it was estimated at 1x10^6

*Table S 7 Limits of detection and quantification of gambierone, 44-methylgambierone (determined in this study) and MTX1 (from (*Pisapia *et al.* 2017*)) in ng mL-1 and pg cell-1.*

Toxin	transition	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	LOD* (pg $cell^{-1}$)	LOQ* (pg $cell^{-1}$)
Gambierone	1023.5/1023.5	1	2	0.004	0.008
44-methylgambierone	1037.5/1037.5	1	2	0.004	0.008
MTX1	1689.8/1689.8	640	2120	2.56	8.48

* in a methanolic 90% extract corresponding to 250 000 cells mL^{-1}

Table S 8 Steps and parameters for peak picking and grouping procedure used in MZmine 2.53.

Steps	Parameters	Value for ESI+	Value for ESI-
1) Raw data methods > Feature detection > Mass detection	Retention time range (min)	0.46-12.0	0.46-12.0
	MS level	1	1
	Mass detector	Centroid	Centroid
	Noise (counts)	5000	1000
2) Raw data method > Peak detection > ADAP Chromatogram builder	Min groupe size (number of scans)	5	5
	Group intensity threshold (counts)	10000	2000
	Min highest intensity (counts)	5000	1000
	m/z tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
3) Feature list methods > Peak detection > Chromatogram deconvolution> Wavelets (ADAP)	S/N threshold	10	10
	S/N estimator	Intensity window SN	Intensity window SN
	Min feature height (counts)	3000	1000
	Coefficient/ area threshold	100	100
	Peak duration range (min)	0.02-2.0	0.02-2.0
	RT wavelet range	0.02-0.2	0.02-0.2
4) Feature list methods >Isotopes > Isotope peak grouper	*m/z* tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	Retention time tolerance (min)	0.05	0.05
	Monotonic shape	false	false
	Representative isotope	Lowest *m/z*	Lowest *m/z*
5) Feature list methods >Filtering > Duplicate peak filter	Filter mode	OLD AVERAGE	OLD AVERAGE
	m/z tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	RT tolerance (min)	0.05	0.05
6) Feature list methods > Normalization >Retention time calibration	*m/z* tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	RT tolerance (min in absolute)	0.1	0.1
	Minimum standard intensity (counts)	2500	1000
7) Feature list methods > Alignment >Join aligner	*m/z* tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	Weight for *m/z*	1	1
	RT tolerance (min in absolute)	0.1	0.1
	Weight for RT	1	1
8) Peak list methods >Gap filling > Peak finder	Intensity tolerance	30.0%	30.0%
	m/z tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	RT tolerance	0.08	0.08
	RT correction	True	True
9) Peak list methods >Filtering > Duplicate peak filter	Filter mode	OLD AVERAGE	OLD AVERAGE
	m/z tolerance	0.001 *m/z* or 5.0 ppm	0.001 *m/z* or 5.0 ppm
	RT tolerance (min)	0.05	0.05

Table S 9 Average peak area (n=2) of standards injected at three concentrations and comparison of response areas between Gambierones and MTX1

Concentration of standards (ng mL^{-1})	Average peak area (n=2) 1023.5/1023.5	Average peak area (n=2) 1037.5/1037.5	Average peak area (n=2) 1689.8/1689.8	Ratio Gamierone/ MTX1	Ratio 44-Methyl-gambierone/MTX1
200	977 895	952 935	24 731	40	39
400	1 403 000	1 388 200	49 257	28	28
800	4 065 100	4 256 150	98 381	41	43
				Average ratio	Average ratio
				36.5	36.5

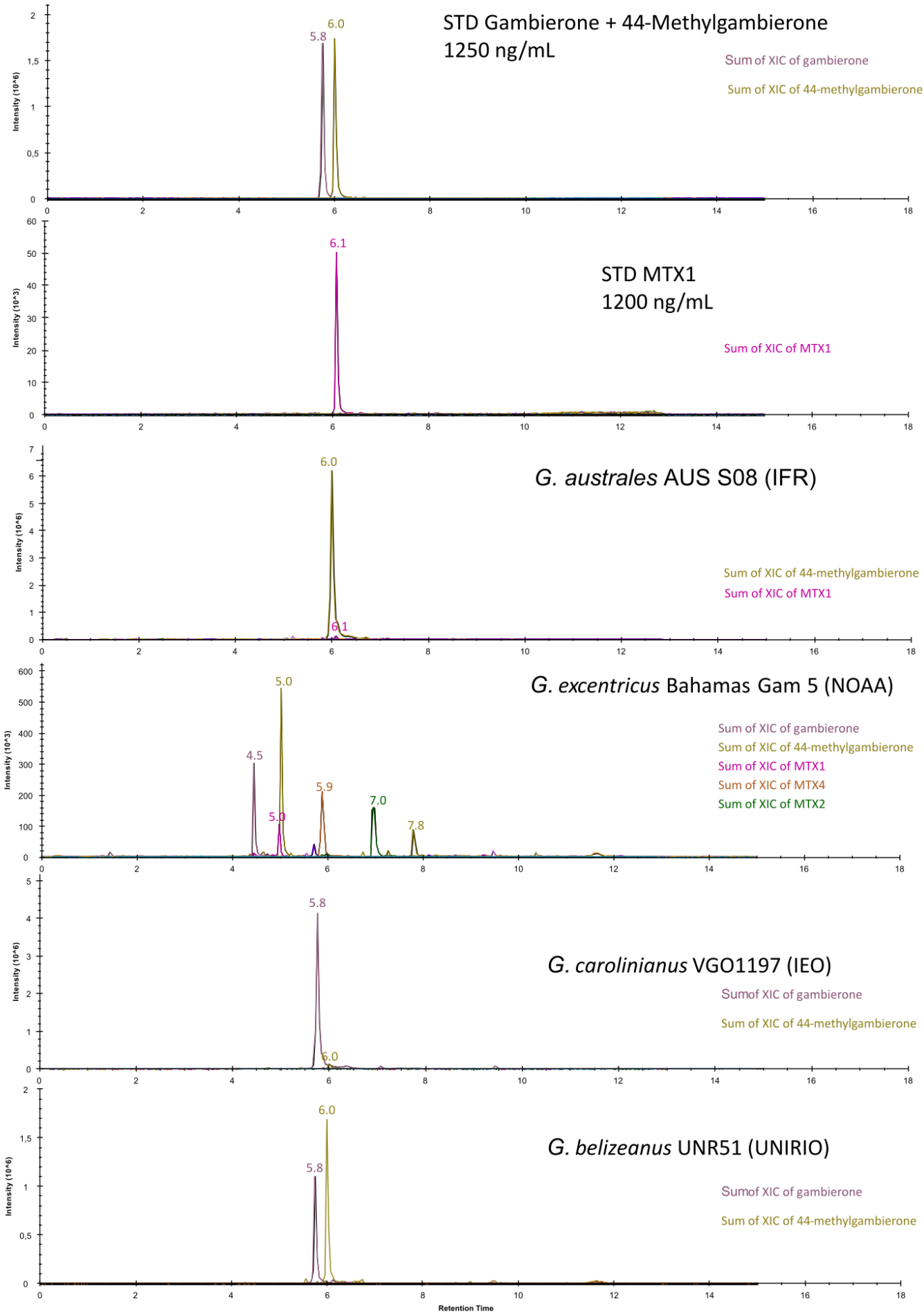

Figure S 1 Overlay of the EICs resulting from the sum of MRM transitions for each molecule as described in Table S3 acquired in negative ionization mode for standards of gambierone, 44-methylgambierone, MTX1 and algal extracts provided by each laboratory: (IFR) METALG Laboratory, Ifremer; (NOAA) Beaufort Laboratory, National Oceanic and Atmospheric Administration; (UNIRIO) Marine Microalgae Laboratory, Federal University of Rio de Janeiro and (IEO) Spanish Institute of Oceanography, Vigo Oceanographic.

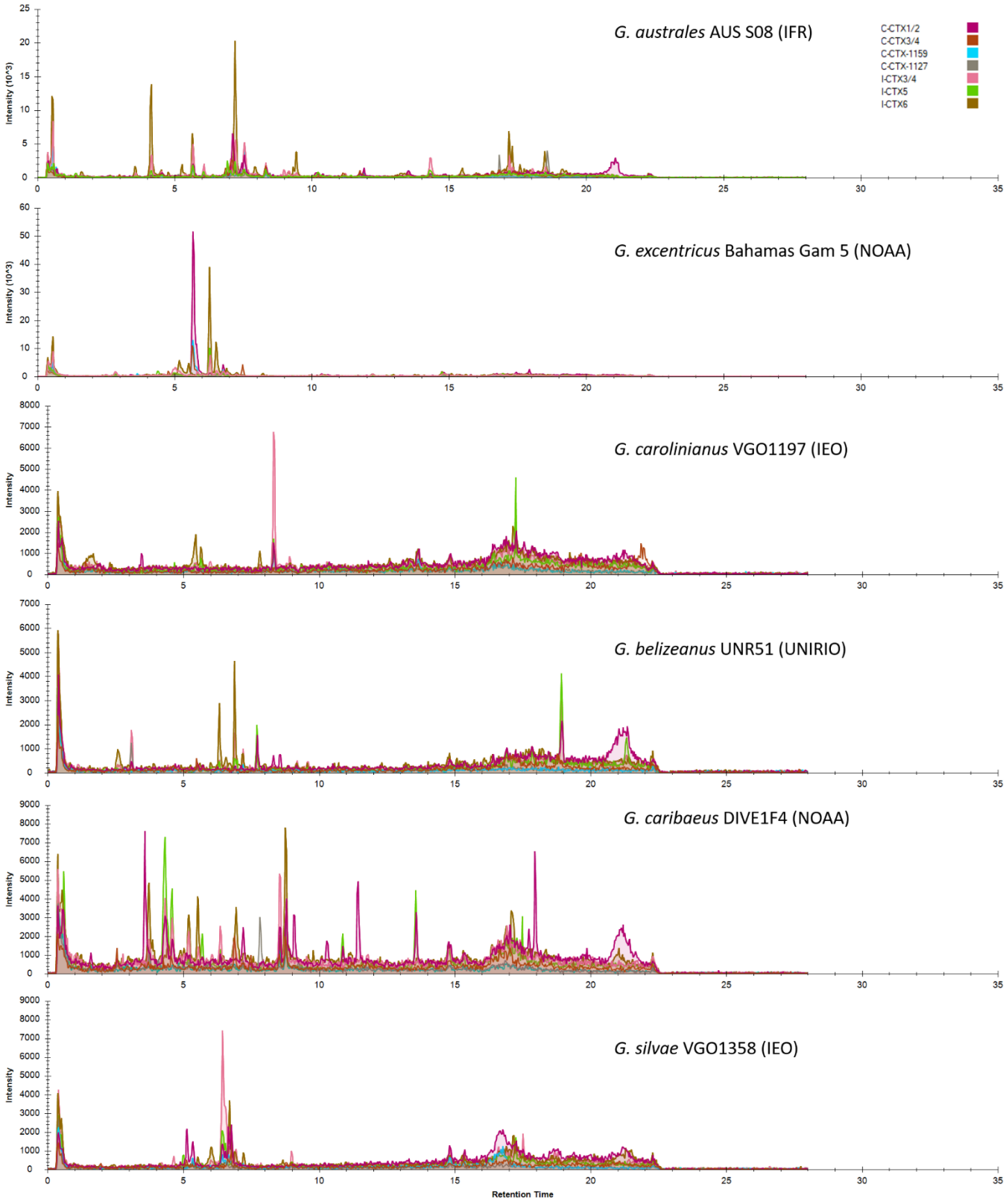

Figure S 2 Overlay of the EICs resulting from the sum of MRM transitions for each molecule as described in Table S4 acquired in positive ionization mode for algal extracts provided by each laboratory: (IFR) METALG Laboratory, Ifremer; (NOAA) Beaufort Laboratory, National Oceanic and Atmospheric Administration; (UNIRIO) Marine Microalgae Laboratory, Federal University of Rio de Janeiro and (IEO) Spanish Institute of Oceanography, Vigo Oceanographic.

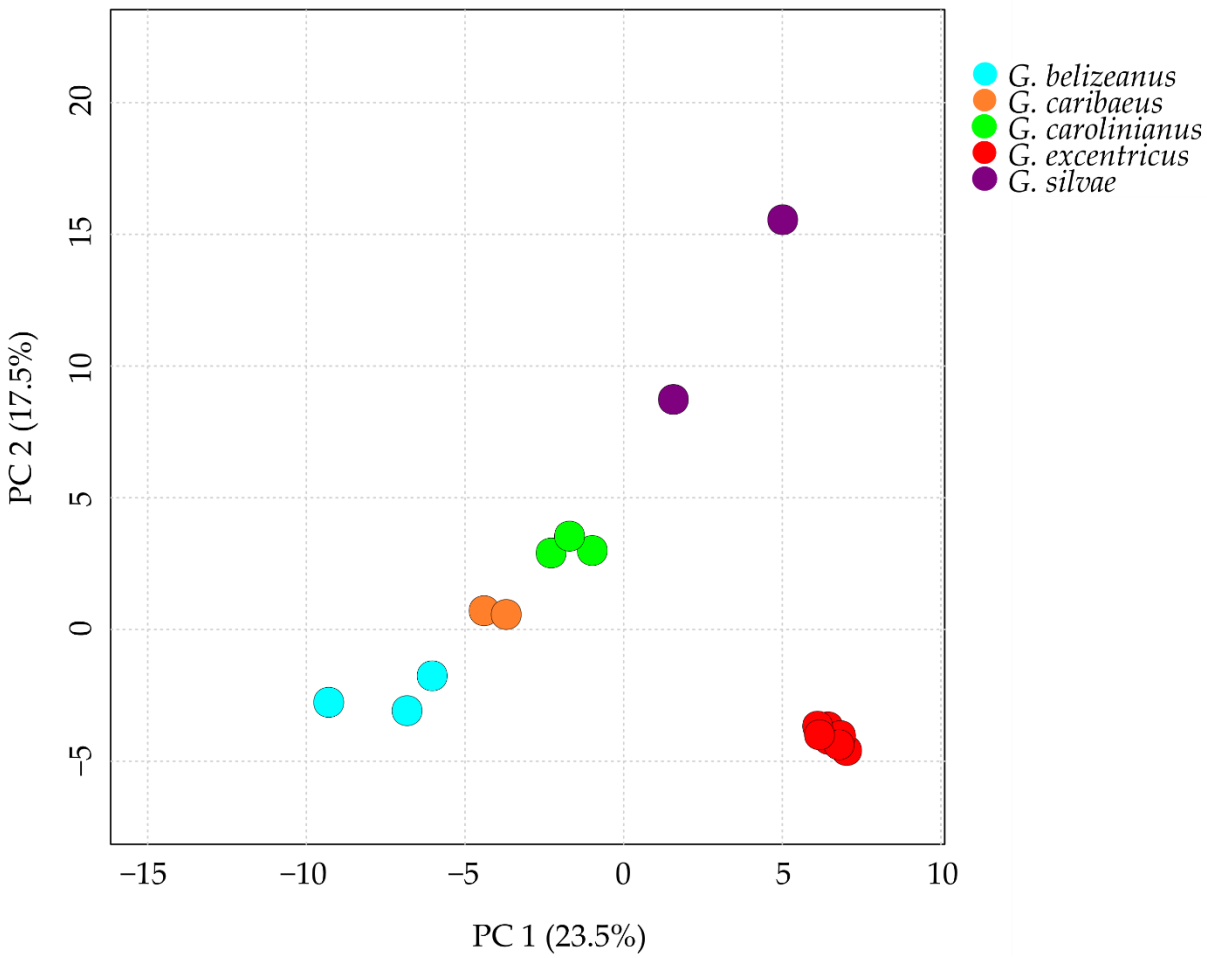

Figure S 3. Partial least square – discriminant analysis score plot of targeted analysis data acquired on the pool per strain per laboratory (based on 298 features manually integrated, R^2: 0.97; Q^2: 0.83; p-value 0.02 with 100 permutations). The color coding represents the species.

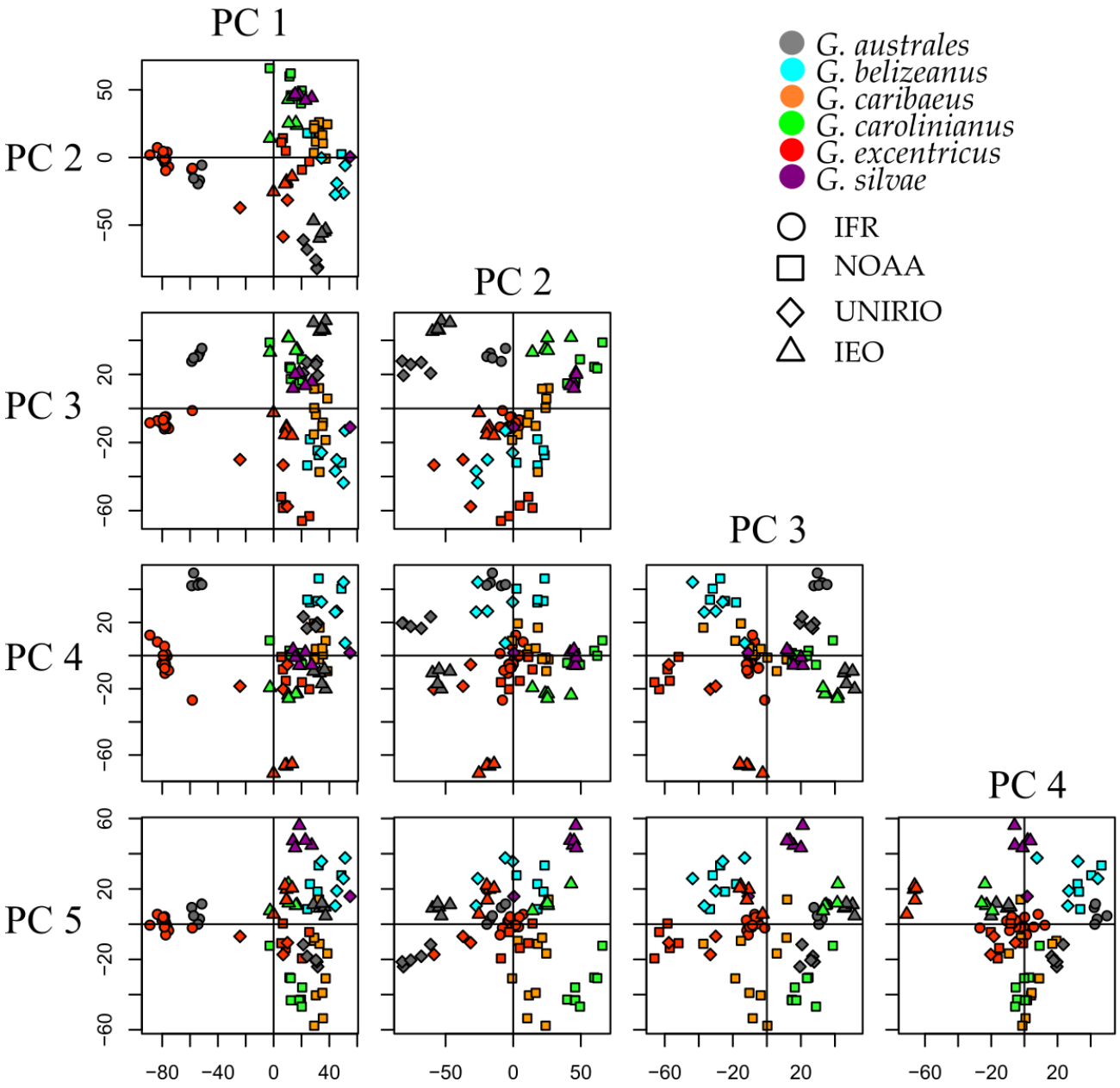

Figure S 4. ComDim score plot (PC1 to PC5) of untargeted analysis data acquired using full scan mode in both polarities (log-transformed and pareto-scaled data). The color coding represents the species while the symbol shapes represent the laboratory.

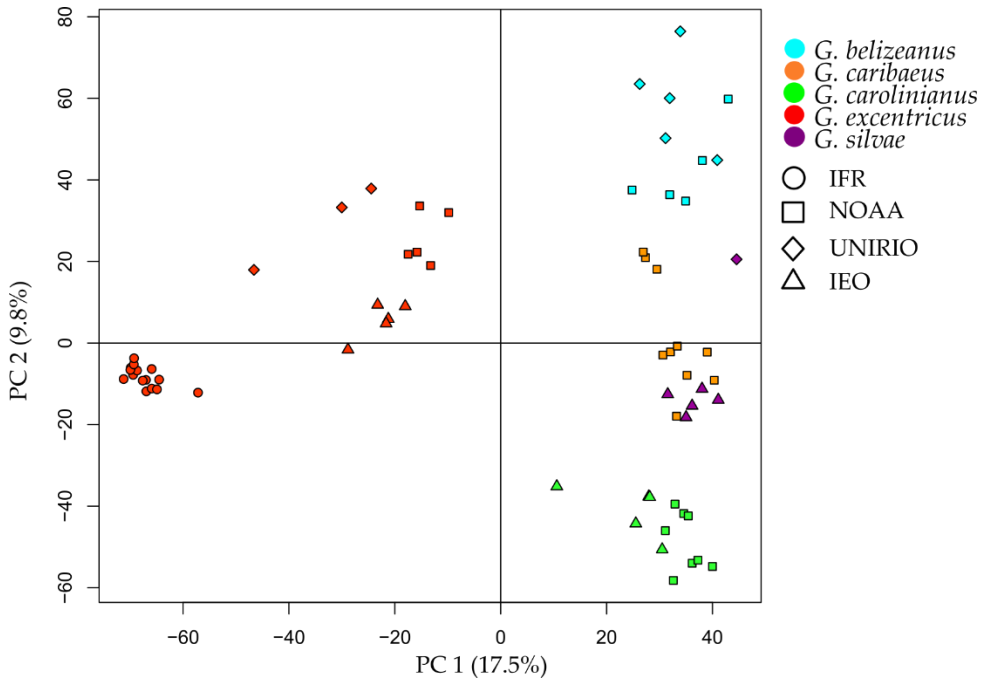

Figure S 5. Multi-blocks partial least square – discriminant analysis score plot of untargeted analysis data acquired using full scan mode in both polarities (log-transformed and pareto-scaled data). The color coding represents the species while the symbol shapes represent the laboratory.

Supplementary Part 1. Strategies to remove the laboratory effect

As interlaboratory variations impair exploration of the data by ComDim, it was decided to tentatively remove such an effect from the data to confirm the species effect on the clustering. First, attempts to correct the data according only to *G. australes* interlaboratory variations were unsuccessful (data not shown) indicating that the impact of culture condition variation is at least species dependent. An attempt to remove variables related to the observed interlaboratory variation after selection by PLS-DA removed more than half of the features of the data matrices, and thus was not retained. The use of ANOVA decomposition of variance (Gémin *et al.* 2021) was not possible because it requires equal group size, which was not the case in the present study. Finally, as PLS-DA was able to accurately discriminate sample origin, orthogonal signal correction (OSC) was attempted. This chemometric approach was previously successfully applied in a few metabolomic studies (Bony *et al.* 2016, He *et al.* 2022). OSC allows for extracting a sub matrix that is highly related to a specific effect, and consequently provides a residual orthogonal matrix where this specific effect is removed. The OSC was thus performed to remove the interlaboratory effect from the original data matrix, providing two new data matrices: one related to the interlaboratory effect and one orthogonal (formerly the residue) where such effect is reduced. The latter residual matrix was finally explored to highlight species differences.

Supplementary Part 2. Chemical diversity of natural products

The untargeted approach performed in this study led us to build a large MN providing an informative overview of the different molecular families of compounds that can be produced by the 5 species of *Gambierdiscus* considered (Table S10).

In this study, 18 compounds were putatively identified by GNPS using MolNetEnhancer (Ernst *et al.* 2019) with a cosine score higher than 0.9 and an accurate mass error inferior to 10 ppm (Table 2).

Table S 10 . Putative annotation of the features with GNPS with the cluster number, Molecular families, node ID, putative compound name, precursor mass, retention time, library mass, delta ppm, cosine score and number of shared peaks (i.e. fragment ions).

Molecular families	Node ID	Putative compound name	Precursor mass	Rt (sec)	Library Mass	Delta ppm	Cosine score	Shared peaks
Betain lipids	P-507	Lyso DGCC (20:5)	536.358	484.0	536.36	1.48	0.99	7
Betain lipids	P-3610	DGCC (38 :7)	798.587	637.3	798.59	0.53	0.97	11
Betain lipids	P-671	Lyso DGCC (22:6)	562.374	526.9	562.37	0.54	0.97	7
Aliphatic compounds	P-374	monoelaidin	339.289	642.0	339.29	2.97	0.98	11
Aliphatic compounds	P-407	AEG(o-14:1/16:1)	523.471	782.3	523.47	1.28	0.96	10
Aliphatic compounds	P-1670	1-Hexadecanoyl-sn-glycerol	331.284	532.8	331.28	0.92	0.96	9
Aliphatic compounds	P-235	1-Palmitoyl-2-oleoyl-sn-glycerol	577.519	638.7	577.52	5.07	0.96	11
Aliphatic compounds	P-2800	Glycerol 1-myristate	285.242	770.2	285.24	0.32	0.94	10
Aliphatic compounds	P-181	AEG(o-16:2/16:0)	551.503	730.4	551.50	0.77	0.92	13
Aliphatic compounds	P-640	AEG(o-16:2/18:2)	575.503	704.4	575.50	0.53	0.92	12
Aliphatic compounds	P-1025	DAG (16:0/18:1)	612.556	773.3	612.56	0.10	0.90	15
Chlorophyll degradation product	P-4608	Pheophytin a	871.572	603.6	871.58	1.05	0.96	11
Polyphenols	P-18	Gossypol derivative	415.212	448.7	415.21	0.73	0.99	7
Single node	P-6228	Spermine	203.223	29.5	203.22	0.97	0.99	7
Single node	P-2581	Arginine	175.119	35.6	175.12	0.61	0.99	7
Single node	P-5188	4-Guanidinobutyric acid	146.092	48.9	146.09	3.45	0.98	7
Single node	P-961	Dimethylsulfoniopropionate	135.047	69.8	135.05	3.73	0.95	4
Single node	P-3899	Nicotinamide adenine dinucleotid	664.115	46.2	664.12	2.39	0.93	8

Among the putatively identified compounds, some of them were expected to be produced by photosynthetic micro-algae such as lipids, carbohydrates, amino acids or peptides and pigments but the putative identification of other compounds was more surprising. The lipids and lipid-like compounds were identified with different sub-structure types such as betain lipids (Figure 6, cluster 1): diacylglyceryl carboxy-hydroxy-methylcholines (DGCC) and Lyso-DGCC, mono-acylglycerols (Figure 6, cluster 10): monoelaidin, 1-Hexadecanoyl-sn-glycerol, 1-Palmitoyl-2-oleoyl-sn-glycerol and Glycerol 1-myristate or acyl/diacyl-glycerophopholipids (Figure 6, cluster 2) (AEG and DAG). The high presence of lipids in the MN is consistent with the extraction and analysis method well adapted for lipophilic compounds. In addition, micro-algae are known to be able to adapt to a variety of environmental conditions and one of the key factors explaining this adaptability is the production of a wide variety of lipids that are used for cell membrane synthesis or as storage products (Eichenberger *et al.* 1997, Guschina *et al.* 2006).

A large cluster of chlorophyll degradation products was observed in the MN (Figure 6 cluster 4) with the putative identification of pheophytin a (cosine score 0.96, 11 shared peaks) however, no other pigments were identified by GNPS in contrary to the pigments reported by Malto et al., (Malto *et al.* 2022).

A polyphenolic compound related to gossypol was also identified, this compound is a natural product extracted from cotton plant and was unlikely to be present in *Gambierdiscus* extracts (Zeng *et al.* 2019). In the single nodes, several compounds were identified with GNPS, e.g. spermine, a polyamine demonstrated as important in the synthesis of nucleic acids and proteins in marine algae (Hamana *et al.* 1982), 4-Guanidinobutyric acid, an amino acid derivative widely found in eukaryotes, dimethylsulfoniopropionate (DMSP), a compound produced by many species of marine phytoplankton that can act as an osmoregulator but this latter function was not confirmed in *Gambierdiscus* (Gwinn *et al.* 2019) and finally the Nicotinamide adenine dinucleotide (NAD) a co-enzyme essential in cell metabolism (Burlacot *et al.* 2019).

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