

# Analytical studies on ciguateric fish in Okinawa, Japan (II): the grouper *Variola albimarginata*

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## Supplementary Materials

**Table S1.** The specimens of *Variola albimarginata* analyzed in this study and their CTXs levels in the flesh.

ID	Collection		Age <sup>3</sup> (years)	SL <sup>4</sup> (cm)	BW <sup>5</sup> (g)	CF <sup>6</sup>	Sex <sup>7</sup>	CTX <sup>8</sup> level (μg/kg)			
	Date <sup>1</sup>	Area <sup>2</sup>						1B	epi-deoxy1B	deoxy1B	total
141640	04/Jun/2014	West-south, Okinawa	4	25.6	571	3.40	F	0	0	0	0
141641	06/Jun/2014	West-south, Okinawa	7	29.1	773	3.14	M	0	0	0	0
141643	13/Jun/2014	West-north, Okinawa	5	19.5	224	3.02	F	0	0	0	0
141645	21/Jun/2014	Ie	5	24.8	466	3.06	F	0	0	0	0
141651	30/Jun/2014	West-north, Okinawa	4	21.8	332	3.20	F	0	0	0	0
141652	NR	East, Okinawa	4	21.8	341	3.29	M	0	0	0	0
141653	NR	East, Okinawa	9	24.4	439	3.02	M	0	0	0	0.000
141654	01/Jul/2014	East, Okinawa	2	16.5	127	2.83	F	0	0	0	0
141655	01/Jul/2014	East, Okinawa	2	23.0	377	3.10	F	0.001	0.001	0.001	0.003
141658	22/Jul/2014	West-south, Okinawa	4	28.1	733	3.30	F	0	0	0	0
141659	23/Jul/2014	West-north, Okinawa	6	24.1	422	3.01	F	0	0	0	0
141660	23/Jul/2014	West-north, Okinawa	5	26.9	604	3.10	M	0.012	0.009	0.011	0.032
141661	23/Jul/2014	West-north, Okinawa	5	26.5	553	2.97	M	0	0	0	0
141662	23/Jul/2014	West-north, Okinawa	4	20.4	281	3.31	F	0	0	0	0
141664	28/Jul/2014	West-south, Okinawa	4	25.4	519	3.17	M	0	0	0	0
141666	30/Jul/2014	West-north, Okinawa	15	33.7	1251	3.27	M	0	0	0	0
141677	30/Jul/2014	West-north, Okinawa	7	29.6	745	2.87	M	0.006	0.001	0.001	0.008
153024	01/Sep/2014	East, Okinawa	4	23.8	440	3.26	M	0	0	0	0
153025	04/Sep/2014	Ie	5	25.1	523	3.31	F	0.007	0.001	0.001	0.009
153026	04/Sep/2014	Ie	5	25.7	503	2.96	M	0.009	0.001	0.001	0.011
153027	04/Sep/2014	Ie	4	24.5	396	2.69	F	0	0	0	0
153028	04/Sep/2014	Ie	8	32.0	915	2.79	M	0.009	0.001	0.005	0.015
153029	04/Sep/2014	Ie	6	23.7	396	2.97	F	0.005	0.001	0.001	0.007
153030	04/Sep/2014	Ie	3	21.8	304	2.93	F	0	0	0	0
153031	04/Sep/2014	Ie	5	22.9	360	3.00	F	0	0	0	0
153032	04/Sep/2014	Ie	3	19.5	203	2.74	F	0	0	0	0
153033	04/Sep/2014	Ie	4	20.3	247	2.95	F	0	0	0	0
153034	04/Sep/2014	Ie	4	22.3	346	3.12	M	0	0	0	0
153035	13/Sep/2014	Yaeyama	4	24.0	384	2.78	M	0	0	0	0
153036	19/Sep/2014	East, Okinawa	4	25.8	591	3.44	M	0	0	0	0
153037	19/Sep/2014	East, Okinawa	4	22.7	363	3.10	M	0	0	0	0
153038	19/Sep/2014	East, Okinawa	4	19.9	242	3.07	F	0	0	0	0
153039	19/Sep/2014	East, Okinawa	4	22.5	334	2.93	F	0	0	0	0
153040	28/Oct/2014	East, Okinawa	3	20.0	234	2.93	F	0	0	0	0
153041	28/Oct/2014	East, Okinawa	5	26.0	457	2.60	F	0	0	0	0
153042	30/Oct/2014	Yaeyama	6	27.0	568	2.89	F	0.005	0.001	0.001	0.007
153043	30/Oct/2014	Yaeyama	5	26.3	518	2.85	M	0.001	0.001	0.001	0.003
153044	30/Oct/2014	Yaeyama	4	26.2	529	2.94	F	0.005	0.001	0.001	0.007
153045	13/Sep/2014	Yaeyama	1	14.3	95	3.25	U	0	0	0	0
153046	22/Oct/2014	West-south, Okinawa	7	26.6	594	3.16	M	0	0	0	0
153047	04/Nov/2014	West-south, Okinawa	8	28.8	725	3.04	M	0	0	0	0
153048	04/Nov/2014	West-south, Okinawa	3	17.7	162	2.92	F	0	0	0	0
153049	13/Nov/2014	West-north, Okinawa	6	22.1	318	2.95	F	0	0	0	0
153050	13/Nov/2014	West-north, Okinawa	4	18.6	184	2.86	F	0	0	0	0
153051	13/Nov/2014	West-north, Okinawa	3	17.8	169	3.00	F	0	0	0	0
153052	13/Nov/2014	West-north, Okinawa	4	18.0	170	2.91	F	0	0	0	0
153053	13/Nov/2014	West-north, Okinawa	2	15.4	113	3.09	F	0	0	0	0

<sup>1</sup> NR: not recorded, <sup>2</sup> the locations of collection areas are shown in Figure 2, <sup>3</sup> U: unidentified, <sup>4</sup> SL: standard length, <sup>5</sup> BW: body weight, <sup>6</sup> CF: condition factor =  $100 \times \text{BW}/\text{SL}^3$ , <sup>7</sup> B: bisexual, F: female, M: male, U: unidentified, <sup>8</sup> 1B: CTX1B, epi-deoxy1B: 52-epi-54-deoxyCTX1B, deoxy1B: 54-deoxyCTX1B, 0: less than LOD (0.001 μg/kg), 0.001: detected but less than LOQ (0.005 μg/kg).

Table S1. Continued.

ID	Collection		Age <sup>3</sup> (years)	SL <sup>4</sup> (cm)	BW <sup>5</sup> (g)	CF <sup>6</sup>	Sex <sup>7</sup>	CTX <sup>8</sup> level (µg/kg)			
	Date <sup>1</sup>	Area <sup>2</sup>						1B	<i>epi</i> - deoxy1B	deoxy1B	total
153065	18/May/2015	West-south, Okinawa	5	26.1	560	3.15	U	0.088	0.039	0.098	0.225
153066	18/May/2015	West-south, Okinawa	6	19.8	226	2.91	U	0	0	0	0
153067	18/May/2015	West-south, Okinawa	4	21.1	281	2.99	U	0	0	0	0
153068	18/May/2015	West-south, Okinawa	4	18.9	202	2.99	F	0	0	0	0
153069	18/May/2015	West-south, Okinawa	3	19.4	226	3.10	U	0	0	0	0
153070	18/May/2015	West-south, Okinawa	2	16.3	132	3.05	U	0	0	0	0
163001	NR	Yaeyama	2	15.4	94	2.57	F	0	0	0	0
163002	29/Jan/2016	West-south, Okinawa	3	22.0	310	2.91	F	0	0	0	0
163003	30/Jan/2016	West-south, Okinawa	3	25.2	401	2.51	F	0	0	0	0
163004	30/Jan/2016	West-north, Okinawa	3	23.2	348	2.79	F	0	0	0	0
163005	30/Jan/2016	West-north, Okinawa	3	20.0	219	2.74	F	0	0	0	0
163006	30/Jan/2016	West-north, Okinawa	3	18.8	173	2.60	F	0	0	0	0
163007	30/Jan/2016	West-north, Okinawa	2	17.3	133	2.57	F	0	0	0	0
163008	12/Feb/2016	West-south, Okinawa	4	18.3	178	2.90	F	0	0	0	0
163009	12/Feb/2016	West-south, Okinawa	2	17.3	170	3.28	F	0	0	0	0
163010	12/Feb/2016	West-south, Okinawa	4	23.2	404	3.24	F	0	0	0	0
163011	12/Feb/2016	West-south, Okinawa	2	18.8	236	3.55	F	0	0	0	0
163012	12/Feb/2016	West-south, Okinawa	5	22.2	333	3.04	M	0	0	0	0
163013	12/Feb/2016	West-south, Okinawa	4	21.8	299	2.89	B	0	0	0	0
163014	12/Feb/2016	Unknown	3	19.3	252	3.51	F	0.011	0.001	0.001	0.013
163033	14/Dec/2015	West-south, Okinawa	6	24.5	384	2.61	F	0	0	0	0
163034	30/Jan/2016	West-north, Okinawa	6	28.0	540	2.46	M	0	0	0	0
163035	30/Jan/2016	West-north, Okinawa	7	28.8	870	3.64	M	0	0	0	0
163036	12/Feb/2016	West-south, Okinawa	10	29.1	792	3.21	M	0.008	0.001	0.001	0.010
163037	12/Feb/2016	West-south, Okinawa	5	22.5	364	3.20	F	0	0	0	0
163038	12/Feb/2016	West-south, Okinawa	8	27.1	632	3.18	U	0	0	0	0
163042	18/Mar/2016	West-north, Okinawa	8	29.1	879	3.57	M	0.005	0	0.001	0.006
163043	18/Mar/2016	West-north, Okinawa	11	28.8	798	3.34	M	0.001	0.001	0.001	0.003
163044	18/Mar/2016	West-north, Okinawa	3	16.8	150	3.16	F	0	0	0	0
163054	14/Dec/2015	West-south, Okinawa	U	30.2	934	3.39	M	0	0	0	0
163055	14/Dec/2015	West-south, Okinawa	5	23.3	326	2.58	F	0.005	0.001	0.001	0.007
163056	30/Jan/2016	West-north, Okinawa	6	30.0	710	2.63	M	0	0	0	0
163057	30/Jan/2016	West-north, Okinawa	10	26.2	440	2.45	U	0	0	0	0
163058	30/Jan/2016	West-north, Okinawa	4	23.7	334	2.51	M	0	0	0	0
163059	12/Feb/2016	West-south, Okinawa	9	24.3	421	2.93	F	0	0	0	0
163060	12/Feb/2016	West-south, Okinawa	7	23.0	422	3.47	M	0	0	0	0
163079	26/Apr/2016	Unknown	12	30.0	860	3.19	M	0.005	0.001	0.001	0.007
163080	05/Sep/2016	Miyako	5	20.6	286	3.27	F	0	0	0	0
163081	05/Sep/2016	Miyako	6	20.7	277	3.12	F	0	0	0	0
163082	05/Sep/2016	Miyako	6	22.6	330	2.86	F	0	0	0	0
163083	05/Sep/2016	Miyako	3	17.6	154	2.82	F	0	0	0	0
163084	05/Sep/2016	Miyako	U	18.1	184	3.10	F	0	0	0	0
163085	05/Sep/2016	Miyako	12	26.2	589	3.28	M	0.001	0.001	0.001	0.003
163086	05/Sep/2016	Miyako	8	25.2	466	2.91	M	0	0	0	0
163087	05/Sep/2016	Miyako	6	22.8	388	3.27	M	0	0	0	0
163088	05/Sep/2016	Miyako	11	24.1	418	2.99	M	0	0	0	0
163089	05/Sep/2016	Miyako	5	21.3	287	2.97	M	0	0	0	0

<sup>1</sup> NR: not recorded, <sup>2</sup> the locations of collection areas are shown in Figure 2, <sup>3</sup> U: unidentified, <sup>4</sup> SL: standard length, <sup>5</sup> BW: body weight, <sup>6</sup> CF: condition factor =  $100 \times \text{BW}/\text{SL}^3$ , <sup>7</sup> B: bisexual, F: female, M: male, U: unidentified, <sup>8</sup> 1B: CTX1B, *epi*-deoxy1B: 52-*epi*-54-deoxyCTX1B, deoxy1B: 54-deoxyCTX1B, 0: less than LOD (0.001 µg/kg), 0.001: detected but less than LOQ (0.005 µg/kg).

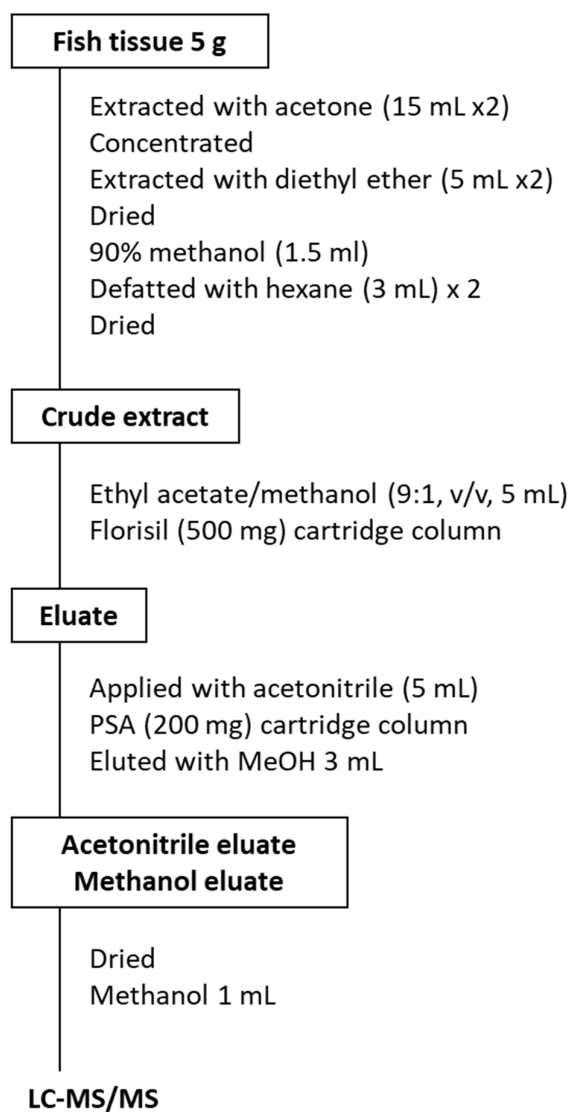
Table S1. Continued.

ID	Collection		Age <sup>3</sup> (years)	SL <sup>4</sup> (cm)	BW <sup>5</sup> (g)	CF <sup>6</sup>	Sex <sup>7</sup>	CTX <sup>8</sup> level (µg/kg)			
	Date <sup>1</sup>	Area <sup>2</sup>						1B	<i>epi</i> - deoxy1B	deoxy1B	total
163090	05/Sep/2016	Miyako	4	21.4	303	3.09	B	0	0	0	0
163091	05/Sep/2016	Miyako	5	21.5	304	3.06	F	0	0	0	0
163092	05/Sep/2016	Miyako	5	18.8	216	3.25	F	0	0	0	0
163093	05/Sep/2016	Miyako	4	19.3	206	2.87	F	0	0	0	0
163094	05/Sep/2016	Miyako	5	17.1	151	3.02	F	0	0	0	0
163133	18/Mar/2016	West-north, Okinawa	5	27.1	642	3.23	M	0	0	0	0
163134	18/Mar/2016	West-north, Okinawa	6	26.5	614	3.30	M	0	0	0	0
163135	18/Mar/2016	West-north, Okinawa	5	25.3	522	3.22	M	0	0	0	0
163136	18/Mar/2016	West-north, Okinawa	7	23.2	412	3.30	F	0	0	0	0
163137	18/Mar/2016	West-north, Okinawa	6	22.2	341	3.12	F	0	0	0	0
163138	06/May/2016	Unknown	3	20.5	300	3.48	F	0	0	0	0
163139	04/Jul/2016	Unknown	12	26.6	575	3.06	M	0.023	0.031	0.032	0.086
163140	25/Jul/2016	Yaeyama	7	26.1	566	3.18	M	0.008	0.010	0.012	0.030
163141	25/Jul/2016	Yaeyama	10	28.0	640	2.92	M	0.001	0.001	0.001	0.003
163142	25/Jul/2016	Yaeyama	7	28.6	658	2.81	M	0.001	0.001	0.001	0.003
163143	25/Jul/2016	Yaeyama	9	28.8	760	3.18	M	0.010	0.010	0.009	0.029
163144	25/Jul/2016	Yaeyama	10	25.0	500	3.20	F	0.001	0.001	0.001	0.003
163145	05/Sep/2016	Miyako	10	26.8	538	2.79	M	0	0	0	0
163146	05/Sep/2016	Miyako	8	24.6	454	3.05	F	0.006	0.006	0.008	0.020
163147	05/Sep/2016	Miyako	14	28.4	720	3.14	M	0.013	0.018	0.014	0.045
163148	05/Sep/2016	Miyako	6	22.3	366	3.30	F	0	0	0	0
163149	05/Sep/2016	Miyako	7	23.9	419	3.07	F	0.001	0.001	0.006	0.008
163150	05/Sep/2016	Miyako	10	23.2	359	2.87	F	0	0	0	0
163151	05/Sep/2016	Miyako	3	19.0	210	3.06	F	0	0	0	0
163152	05/Sep/2016	Miyako	3	18.4	176	2.83	F	0	0	0	0
163153	05/Sep/2016	Miyako	4	15.1	100	2.90	U	0	0	0	0
163154	05/Sep/2016	Miyako	U	13.5	68	2.76	F	0	0	0	0
163156	26/Oct/2015	Miyako	2	16.9	146	3.02	F	0	0	0	0
163157	26/Oct/2015	Miyako	2	16.7	141	3.03	F	0	0	0	0
163158	26/Oct/2015	Miyako	1	13.0	61	2.78	U	0	0	0	0
163159	26/Oct/2015	Miyako	3	22.6	404	3.50	F	0	0	0	0
163160	26/Oct/2015	Miyako	5	24.3	446	3.11	F	0	0	0	0
163161	26/Oct/2015	Miyako	4	22.9	341	2.84	F	0	0	0	0
163162	26/Oct/2015	Miyako	6	20.8	263	2.92	F	0	0	0	0
163163	26/Oct/2015	Miyako	8	21.7	317	3.10	F	0	0	0	0
163164	26/Oct/2015	Miyako	3	18.7	187	2.86	F	0	0	0	0
163165	26/Oct/2015	Miyako	3	17.8	173	3.07	F	0	0	0	0
163166	26/Oct/2015	Miyako	3	17.2	143	2.81	F	0	0	0	0
		Maximum	15	33.7	1251	3.64		0.088	0.039	0.098	0.225
		Minimum	1	13.0	61	2.45					
		Average	5.3	22.8	398	3.03					
		Median	5	22.9	360	3.04					
		SD	2.7	4.2	218	0.24					
		RSD	51%	18%	55%	8%					

<sup>1</sup> NR: not recorded, <sup>2</sup> the locations of collection areas are shown in Figure 2, <sup>3</sup> U: unidentified, <sup>4</sup> SL: standard length, <sup>5</sup> BW: body weight, <sup>6</sup> CF: condition factor =  $100 \times \text{BW}/\text{SL}^3$ , <sup>7</sup> B: bisexual, F: female, M: male, U: unidentified, <sup>8</sup> 1B: CTX1B, *epi*-deoxy1B: 52-*epi*-54-deoxyCTX1B, deoxy1B: 54-deoxyCTX1B, 0: less than LOD (0.001 µg/kg), 0.001: detected but less than LOQ (0.005 µg/kg).

**Table S2.** LC-MS/MS condition for CTX analysis.

LC	Equipment:	Agilent 1290 Infinity (Agilent Technologies)
	Column:	Zorbax Eclipse Plus C18 (1.8 $\mu$ m, 2.1 x 50 mm, Agilent Technologies)
	Column Temp.:	40 $^{\circ}$ C
	Injection volume:	5 $\mu$ L
	Eluent A:	5 mM ammonium formate in 0.1% formic acid
	Eluent B:	methanol
	Flow rate:	0.4 mL/min
	Gradient condition:	0 min (60%B)–0.25 min (60%B)–0.5 min (75%B)– 12 min (90%B)–14 min (90%B)–14.1 min (100%B)
MS	Equipment:	6460 Triple Quad LC/MS (Agilent Technologies)
	Ionization:	ESI with Agilent Jet Stream
	Nebulizer gas:	N <sub>2</sub> , 50 psi
	Sheath gas:	N <sub>2</sub> , 380 $^{\circ}$ C, 11 L/min
	Dry gas:	300 $^{\circ}$ C, 10 L/min
	Capillary voltage:	5000 V
	Fragmentor voltage:	300 V
	Collision energy:	40 eV
	Monitor ions:	[M+Na] <sup>+</sup> > [M+Na] <sup>+</sup>
		<i>m/z</i> 1045.6: CTX3C and 49- <i>epi</i> CTX3C
		<i>m/z</i> 1061.6: 2-hydroxyCTX3C and 51-hydroxyCTX3C
		<i>m/z</i> 1079.6: 2,3-dihydroxyCTX3C
		<i>m/z</i> 1083.6: CTX4A and CTX4B
		<i>m/z</i> 1095.6: 2,3,51-trihydroxyCTX3C
		<i>m/z</i> 1117.6: 52- <i>epi</i> -54-deoxyCTX1B and 54-deoxyCTX1B
		<i>m/z</i> 1133.5: CTX1B



**Figure S1.** Sample preparation from flesh for LS-MS/MS.