

## **Peak calls of *A. cervicornis*, *A. palmata* and *A. prolifera* in the U.S. Virgin Islands used to determine genotypic richness.**

Genotypic richness, the total number of unique genotypes divided by the total number of samples, can be a useful metric for identifying population distributions particularly with an uneven sample size. Here, we use microsatellites developed by Baums et al. (2009) and with protocols slightly modified by Fogarty et al. (2012) to understand population variation and admixture within and between *A. cervicornis*, *A. palmata* and *A. prolifera* populations (Nylander-Asplin et al. 2021). After DNA extraction protocols (Baums et al. 2009), samples were quantified via microplate spectrophotometer (Nanodrop, ThermoFisher Scientific) and amplified using PCR. Microsatellite primers (loci 166, 181, 187, 182, 207; Table 1-3) were then multiplexed using two combinations of primers 166, 181, 187 in one multiplex and primers 182 and 207 in a separated multiplex. These combinations prevented duplication of the fluorescent colors assigned to each primer. After genotypic analysis was conducted at Florida State University Sequencing Facility, samples were binned and analyzed using Genemapper 5 software. In addition, Micro-checker 2.3.3 was used to isolate stutter peaks, allele dropout and null alleles, if present.

The product lengths ranged between 15-59 base pairs with 6-12 alleles being detected for all taxa. Each primer resulted in diploid or in some cases, triploid, bases being observed. These bases can appear homozygous (same calls) or heterozygous (different calls) depending on the peaks identified at each loci. The tables below display how base calls were completed and unique genotypes assigned. Each color represents a unique genotype with samples of the same color indicating a clone rather than sexually reproduced offspring. In some cases, the genotypic richness was similar to that of a nearby colony (indicated by percentages listed on the right side of each graph) with anything less than 60% similarity resulting in a distinct genotype. Comparisons of genotypic richness are influenced by both the number of unique genotypes observed in a sample and the evenness of the distribution (sample size) (Stoddart and Taylor 1988; Baums et al. 2006).

Baums IB, Devlin-Durante MK, Brown L, Pinzón JH (2009) Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean acroporid corals. *Mol Ecol Resour* 9(4):1155-8

Fogarty ND, Vollmer SV, Levitan DR (2012) Weak prezygotic isolating mechanisms in threatened Caribbean Acropora corals. *PLoS One* 7(2):e30486

Nylander-Asplin HF, Hill RL, Doerr JC, Greer L, Fogarty ND (2021) Population dynamics and genotypic richness of threatened Acropora species and their hybrid in the US Virgin Islands. *Coral Reefs* 40(3):965-71.

Table 1: Peak calls for *A. cervicornis* using five microsatellite primers (166, 187, 181, 182, 207). Peak calls were performed using Genemapper 5 software and any primers that did not amplify were re-run. If calls were unable to be confidently identified, an asterisk (\*) was used. Each color represents a unique genotype based on allelic variations at each locus. Each section represents independent sampling locations at No Name Bay, Thach Cay, and Lovango Cay, respectively.

Sample Number	Primers										Percent Clones	
	166		187		181		182		207			
15	138	138	105	111	159	159	*	*	*	*	60%	
27	138	141	105	108	159	162	153	165	153	153	100%	
28	138	141	105	108	159	162	153	165	153	153	100%	
29	138	141	108	111	159	162	153	165	153	153	88%	
30	138	141	111	111	159	162	153	168	171	153	153	75%
31	138	141	135	108	111	159	162	153	165	153	153	100%
32	138	141	135	108	111	159	162	153	165	153	153	100%
33	138	135	108	111	159	159	*	*	*	*	60%	
34	138	135	114	114	162	162	153	141	153	153	0%	
35	138	138	108	108	159	159	153	153	153	153	100%	
37	138	138	*	*	159	159	153	153	153	153	100%	
38	138	138	108	111	159	165	153	153	153	153	100%	
39	138	138	108	111	159	165	153	153	153	153	100%	
40	135	141	108	111	159	162	153	165	153	153	80%	
95	138	141	108	111	177	177	*	*	153	153	60%	
96	138	141	105	108	159	162	153	153	*	*	40%	
45	138	141	105	108	174	174	141	153	153	153	100%	
46	138	141	108	108	174	174	*	*	*	*	50%	
47	138	141	105	108	174	174	153	153	153	153	100%	
48	138	141	105	108	174	174	153	153	153	153	100%	
49	138	141	105	108	174	174	141	153	153	172	90%	
50	138	141	105	108	174	174	*	*	*	*	60%	
51	138	141	105	108	174	174	141	153	153	153	100%	
52	138	138	105	108	111	165	165	153	165	153	165	100%
53	138	138	105	108	111	165	165	153	165	153	165	100%
54	138	141	105	108	174	174	141	153	153	153	100%	
55	138	141	105	108	*	*	141	153	153	153	80%	
56	138	141	105	108	174	174	141	153	153	153	100%	
57	138	141	105	108	154	174	141	153	153	172	175	90%
58	138	141	105	108	174	174	141	153	153	153	100%	
59	138	141	105	108	174	174	141	153	153	153	100%	
60	138	138	108	114	159	180	*	*	153	153	100%	
61	138	138	105	114	159	180	153	158	153	153	80%	
62	138	138	108	114	159	159	*	*	153	153	100%	
64	138	141	105	108	174	174	141	153	153	153	100%	
66	141	*	105	108	174	174	141	153	153	153	100%	
69	141	138	105	108	174	174	141	153	153	153	100%	
73	138	141	108	111	162	162	153	165	153	153	0%	
95	138	141	108	111	177	177	*	*	153	153	0%	
96	138	141	105	108	159	162	153	*	153	153	50-100%	
101	138	141	111	111	159	159	156	156	153	168	80%	
102	138	141	108	111	159	159	156	165	153	153	75%	
103	138	138	111	111	159	159	153	156	165	153	153	80%
105	138	138	111	111	159	159	156	165	153	153	100%	
106	138	138	111	111	*	*	156	165	*	*	60%	
107	138	138	111	111	159	159	156	165	153	153	100%	
108	138	138	111	111	159	159	156	165	153	153	100%	
109	138	138	111	111	159	159	156	165	153	153	100%	
110	138	141	108	111	159	159	158	158	153	168	100%	
115	138	141	108	111	159	159	158	158	153	168	100%	
117	138	141	108	111	159	159	158	158	153	168	100%	

Table 2: Peak calls for *A. palmata* using five microsatellite primers (166, 187, 181, 182, 207). Peak calls were performed using Genemapper 5 software and any primers that did not amplify were re-run. If calls were unable to be confidentially identified, an asterisk (\*) was used. Each color represents a unique genotype based on allelic variations at each locus. Each section represents independent sampling locations at No Name Bay, Thach Cay, and Lovango Cay respectively.

Sample Number	166		187		181		182		207		Percent Clones		
21	138	138	105	111	153	153	141	143	*	*	90%		
22	138	138	105	108	153	159	141	162	171	175	60%		
23	138	138	105	111	153	159	141	162	165	171	60%		
24	138	138	105	111	153	159	141	143	166	165	171	80%	
25	138	138	105	111	153	159	141	143	165	171	100%		
26	131	138	105	111	153	159	141	143	165	171	100%		
41	144	144	105	108	114	153	153	*	*	148	175	178	0%
42	138	141	108	111	153	153	141	162	148	175	0%		
44	138	141	105	108	153	162	141	141	*	*	40%		
63	138	144	105	108	111	153	162	141	146	168	171	100%	
65	138	144	111	111	153	153	141	171	168	171	100%		
72	138	141	111	111	153	153	141	171	168	171	100%		
74	138	144	105	108	111	153	162	141	146	168	171	100%	
75	138	129	105	111	153	153	141	154	172	171	193	100%	
76	138	129	105	105	153	153	165	175	171	181	80%		
83	138	138	105	105	153	154	165	175	175	181	100%		
84	138	138	105	111	153	153	165	175	175	181	100%		
85	138	131	108	111	153	159	141	175	175	184	50%		
86	138	148	105	108	153	162	141	175	168	171	100%		
87	138	148	105	108	153	162	141	175	168	171	100%		
88	135	148	105	108	153	163	*	*	*	*	80%		
89	138	128	105	111	153	153	141	154	172	171	193	100%	
90	138	128	105	111	153	153	141	154	172	171	193	100%	
98	138	144	108	111	153	153	171	173	168	171	90%		
99	138	138	108	111	153	153	171	171	168	171	90%		
111	141	144	105	105	153	153	162	172	181	168	171	100%	
112	138	138	108	111	153	153	141	172	175	181	100%		
113	138	138	108	114	153	153	141	175	181	184	70%		
114	138	138	108	111	153	153	141	175	175	181	100%		
116	*	*	*	*	*	*	151	175	175	178	30%		
120	138	141	105	105	153	153	141	172	175	175	181	50%	
125	141	144	105	111	153	153	141	161	171	175	100%		
126	141	144	105	111	153	153	141	161	171	175	100%		
127	138	144	108	108	153	162	*		178	184	0%		
128	138	138	105	111	153	153	141	141	171	175	0%		
129	141	147	111	111	153	153	141	161	171	175	80%		
130	141	141	105	111	153	153	141	141	171	175	178	60%	

**Table 3** Peak calls for *A. proliferata* using five microsatellite primers (166, 187, 181, 182, 207). Peak calls were performed using Genemapper 5 software and any primers that did not amplify were re-run. If calls were unable to be confidentially identified, an asterisk (\*) was used. Each color (arbitrarily chosen for each site) represents a unique genotype based on allelic variations at each locus. Each section represents independent sampling locations at No Name Bay, Thach Cay, and Lovango Cay respectively.

Sample Number	166		187		181		182		207		Percent Clones	
1	135	141	108	111	159	162	141	153	153	153	0%	
2	138	141	105	111	159	159	141	183	153	168	40%	
3	138	141	108	111	159	159	141	183	153	168	70%	
4	135	141	105	111	159	159	141	153	153	168	100%	
5	135	141	105	111	159	159	141	153	153	168	100%	
6	138	141	105	111	159	159	141	153	153	168	100%	
7	138	141	105	111	159	159	141	153	*	*	90%	
8	138	141	105	117	153	153	*	*	168	168	30%	
9	138	135	108	111	153	153	141	154	*	*	100%	
10	138	135	108	111	153	153	141	154	*	*	100%	
11	138	138	108	111	153	153	141	168	153	183	100%	
12	138	138	108	111	153	153	141	168	153	183	100%	
13	135	135	108	111	153	153	141	168	153	183	100%	
14	135	135	108	111	153	153	141	168	153	183	100%	
16	135	135	108	111	153	153	141	168	153	183	100%	
17	135	135	108	111	153	153	141	168	153	183	100%	
18	138	141	105	111	159	159	141	183	153	168	100%	
19	138	141	105	111	159	159	141	183	153	168	100%	
20	138	141	105	111	159	159	141	183	153	168	100%	
43	138	141	108	111	159	165	141	153	153	162	60%	
92	135	141	108	111	153	153	141	168	*	*	90%	
93	135	135	108	111	153	153	141	168	184	153	183	70%
97	138	141	111	111	174	168	153	159	153	168	30%	
100	138	141	111	111	168	*	153	159	153	153	60%	
67	141	145	111	114	153	153	141	171	153	168	141	100%
68	141	145	111	114	153	153	141	171	153	168	141	100%
71	138	138	108	114	159	159	153	158	169	153	153	0%
77	138	141	105	108	159	162	153	168	184	156	168	100%
78	138	141	105	108	159	162	153	168	184	156	168	100%
79	138	141	105	108	159	162	153	168	184	156	168	100%
82	141	141	111	111	159	162	*	*	*	*		50%
91	141	138	105	108	159	162	153	184	156	168		100%
118	141	141	111	111	153	153	156	168	153	168		100%
119	141	141	111	111	153	153	156	168	153	168		100%
121	141	141	111	111	153	153	153	156	168	168		80%
122	141	141	111	111	153	165	156	159	168	153	168	80%
123	141	141	111	111	153	165	156	168	153	168		100%
124	141	141	111	111	153	165	156	168	153	168		100%