MOLECULAR ECOLOGY

Supplemental Information for:

Millennia-old coral holobiont DNA provides insight into future adaptive trajectories

Carly B. Scott, Anny Cárdenas, Matthew Mah, Vagheesh M. Narasimhan, Nadin Rohland, Lauren T. Toth, Christian R. Voolstra, David Reich, Mikhail V. Matz

Table of Contents:

Table S1. Age, species, and geography assignment by coral core sample	Page 2
Table S2. Reads mapped by scaffold and proportion identified as putatively ancient.	Page 3
Table S3. f_4 statistics for modern day acroporids reaffirm present day relationships.	Page 9
Table S4. Details on comparison data sets used for microbial analysis.	Page 10
Figure S1. Misincorporation rates at the terminal ends of reads do not show characteristic aDNA patterns in Symbiodineaceae.	Page 11
Figure S2. Modern acroporid data do not show the same misincorporation rate and length distribution patterns as ancient coral.	Page 12
Figure S3. PCA of ancient samples placed with respect to modern acrporids shows separation by site on PC2.	Page 13
Figure S4. Admixture plot with three clusters solidifies assignment of ancient samples to *Acropora palmata.*	Page 14
Figure S5. Taxonomic validation of reads mapped to bacterial genomes	Page 15
Figure S6. Constructing a PCA from just ancient samples and *Acropora palmata* highlights separation between present-day and past populations.	Page 16
Supplemental References	Page 17

MOLECULAR ECOLOGY

Table S1. **Age, species, and geography assignment by coral core sample**
Information on sample IDs, ages, species, and location (Toth et al., 2018). See also **Figure 1**. Age uncertainty given as two standard deviations.

Core	Sample	Age	Age Uncertainty	Species	Location
1	S17463	1099 CE	954-1267 CE	*Acropora palmata*	Looe Key, Florida Keys
	S17464	4215 BCE	4312-4096 BCE	*Acropora palmata*	Looe Key, Florida Keys
2	S17465	951 CE	849-1036 CE	*Acropora palmata*	Sombrero Reef, Florida Keys
	S17466	3737 BCE	3860-3640 BCE	*Acropora palmata*	Sombrero Reef, Florida Keys

MOLECULAR ECOLOGY

Table S2. Reads mapped by scaffold and proportion identified as putatively ancient. Table giving reads mapped to each part of concatenated reference by sample, after filtering for mapping quality (Q>30). Bolded scaffolds marked with a (*) indicate a reference to which at least one sample showed characteristic ancient DNA patterns. Bolded and italicized table entries indicate which samples were identified as putatively ancient for a given reference

Scaffold	Number Read Mapped by Sample			
	S17463	S17464	S17465	S17466
Acropora millepora *	***6566***	***1686***	***3278***	***741***
Symbiodinium	189	79	277	1013
Breviolum	55	43	68	1457
Cladicopium	95	60	70	308
Durisdinium	158	26	132	215
A19_0	1171	1652	557	645
A3_1	74032	3016	47032	26590
A6_0	7804	36191	250	826
A7_0	107605	4639523	35161	858351
Endolith_101	201	34	60	38
Endolith_106	26	2	99	102
Endolith_120	94	55	337	651
Endolith_124	17	14	35	48
Endolith_130	1719	1316	1472	870
Endolith_131 *	***1613***	25	125	37
Endolith_138	82	66	74	103
Endolith_141	169	22	35	49

Endolith_144	433	235	1103	1803
Endolith_147	9	8	169	57
Endolith_149 *	***12605***	***8153***	***43730***	***12736***
Endolith_159	858	622	580	730
Endolith_160	67	39	102	48
Endolith_161	30	9	120	34
Endolith_172	1440	1694	1460	2205
Endolith_175	40	15	466	128
Endolith_179 *	***137231***	***1998***	***38076***	***20791***
Endolith_183	38	34	24	16
Endolith_184	79	19	63	44
Endolith_188 *	719	218	***1313***	187
Endolith_189	44	7	127	57
Endolith_191	186	33	415	25
Endolith_200	228	29	53	38
Endolith_202	52	27	1677	37
Endolith_217	19762	51335	264391	2330836
Endolith_218	615	191	256	452
Endolith_221	43	32	89	7
Endolith_222	173	13	85	31
Endolith_224	9717	4650	94143	94792
Endolith_232	1002	199	261	299
Endolith_237	914	778	393	351

Endolith_240	207	24	224	72
Endolith_245	24615	133801	7553579	7321526
Endolith_246	701	182	248	97
Endolith_254	58	16	64	29
Endolith_259	57	54	63	64
Endolith_264 *	***43175***	376	***13875***	225
Endolith_271	164	25	116	0
Endolith_272	97	561	189	37
Endolith_277	140	74	197	44
Endolith_280	92	66	127	27
Endolith_282	668	142	276	178
Endolith_283	543	643	357	88
Endolith_284 *	405	***2294***	***2603***	208
Endolith_290	45114	3608	5188	14492
Endolith_291	550	529	393	823
Endolith_294	29	6	217	22
Endolith_296	498	130	167	235
Endolith_298	114956	14712	4913	12538
Endolith_299	201	33	113	85
Endolith_303	582	461	207	160
Endolith_311	31	17	27	85
Endolith_313	532	280	149	50
Endolith_318	621	24	89	119

Endolith_339	131	11	98	26
Endolith_343	16	37	74	19
Endolith_352	130	10	88	29
Endolith_353	1271	367	437	275
Endolith_374	28	17	3	4
Endolith_37	191	182	182	77
Endolith_395	14	7	12	0
Endolith_422	552	151	184	660
Endolith_430	117	3	177	7
Endolith_55	335	114	183	44
Endolith_56	58	50	31	189
Endolith_60 *	***6563***	515	***14969***	253
Endolith_64	28	0	41	19
Endolith_66	183	81	155	19
Endolith_67	67	26	141	13
Endolith_86	162	9	33	22
Endolith_87	54	13	108	137
Endolith_89	1458	1414	2388	760
Endolith_91	150	33	235	61
Endolith_98	1414	198	606	1794
Endolith_99 *	***83779***	***16484***	***47652***	***6026***
G20_0	779	1237	5419	4995
G21_0	6065	180440	4115	1669

G2_1	204	54	69	265
G2_2	316	34	41	137
R10_1	0	0	0	0
R10_2	0	0	0	0
R11_0	99	516	660	1249
R12_1	0	0	0	0
R12_2	1	0	0	0
R13_0 *	***2452***	1511	***2396***	2494
R14_0	329	9199	9461	16702
R15_0	535	36617	15830	21483
R16_0	539	2902	3449	5156
R17_0	383	4118	7217	10953
R18_1	348	210926	50262	39309
R18_2	417	202013	49272	67092
R4_1	137	92	131	56
R4_2	110	1276	501	644
R5_0	922	1390	988	1122
R8_1	0	2	43	25
R8_2	0	0	0	0
R9_1	79	234	278	415
R9_2	65	292	235	555
R9_3	61	295	265	509
Total Mapped:	732459	5585076	8339968	10894216

Reads Sequenced:	57903514	56354049	72336825	60753563
Percent Mapped:	0.01265	0.099107	0.115294	0.179318
Putatively Ancient:	293984	30615	167892	42499
Proportion Ancient/ Total Mapped	0.4013658	0.00548157	0.02013101	0.00390106

MOLECULAR ECOLOGY

Table S3. f_4 statistics for modern day acroporids reaffirm present day relationships. Both *Acropora palmata* and *A. cervicornis* are more related to known hybrid *A. prolifera* than each other. Significance is indicated by |Z| > 3.

Outgroup	B	C	D	f_4	Z
Acropora millepora	*A. palmata*	*A. cervicornis*	*A. prolifera*	0.0119	27.05
Acropora millepora	*A. cervicornis*	*A. palmata*	*A. prolifera*	0.0077	23.36
Acropora millepora	*A. prolifera*	*A. cervicornis*	*A. palmata*	0.0046	19.52

MOLECULAR ECOLOGY

Table S4. Details on comparison data sets used for microbial analysis. Citations, details, and BioProject Accession numbers for all modern comparison data sourced for this study. Note there is more data associated with Westrich *et al.* than initially published, not all of which was used in comparison for this study.

Study	Accession	Data Type	# Samples	Sample Source	Used?
Kitchen *et al.* (2019)	PRJNA473816	Whole-genome sequencing	21	*Acropora cervicornis*	Yes
			26	*Acropora palmata*	Yes
			13	*Acropora prolifera*	Yes
Rosales *et al.* (2019)	PRJNA546259	16sRNA sequencing	96	*Acropora cervicornis* and *Acrpora palmata* tissue	Yes
Westrich *et al.* (2016)	PRJNA299413	16sRNA sequencing	81	*Acrpora palmata* mucus	Yes
			44	Water sample	No

MOLECULAR ECOLOGY

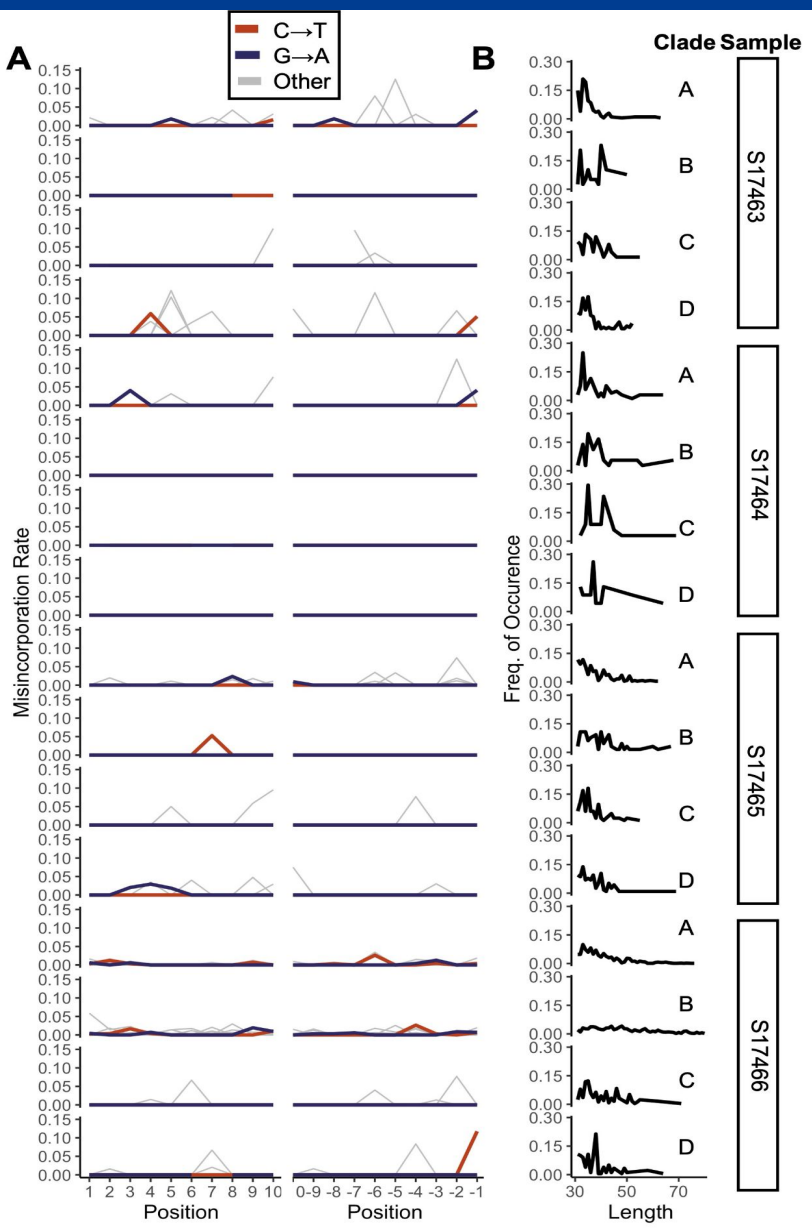

Figure S1. Misincorporation rates at the terminal ends of reads do not show characteristic aDNA patterns in Symbiodineaceae. Clade refers to species of symbiont (formerly clades A-D), and sample refers to the source. (A) Misincorporation plots for each sample/symbiont combination. Note that we do not observe an elevated C→T and G→A misincorporation pattern at the terminal bases in any sample. This is especially clear when compared to **Figure 2.** (B) Read length distribution for each sample and symbiont combination. The sample with the most symbiont reads (S17466) does not show the same length distribution as expected from aDNA.

MOLECULAR ECOLOGY

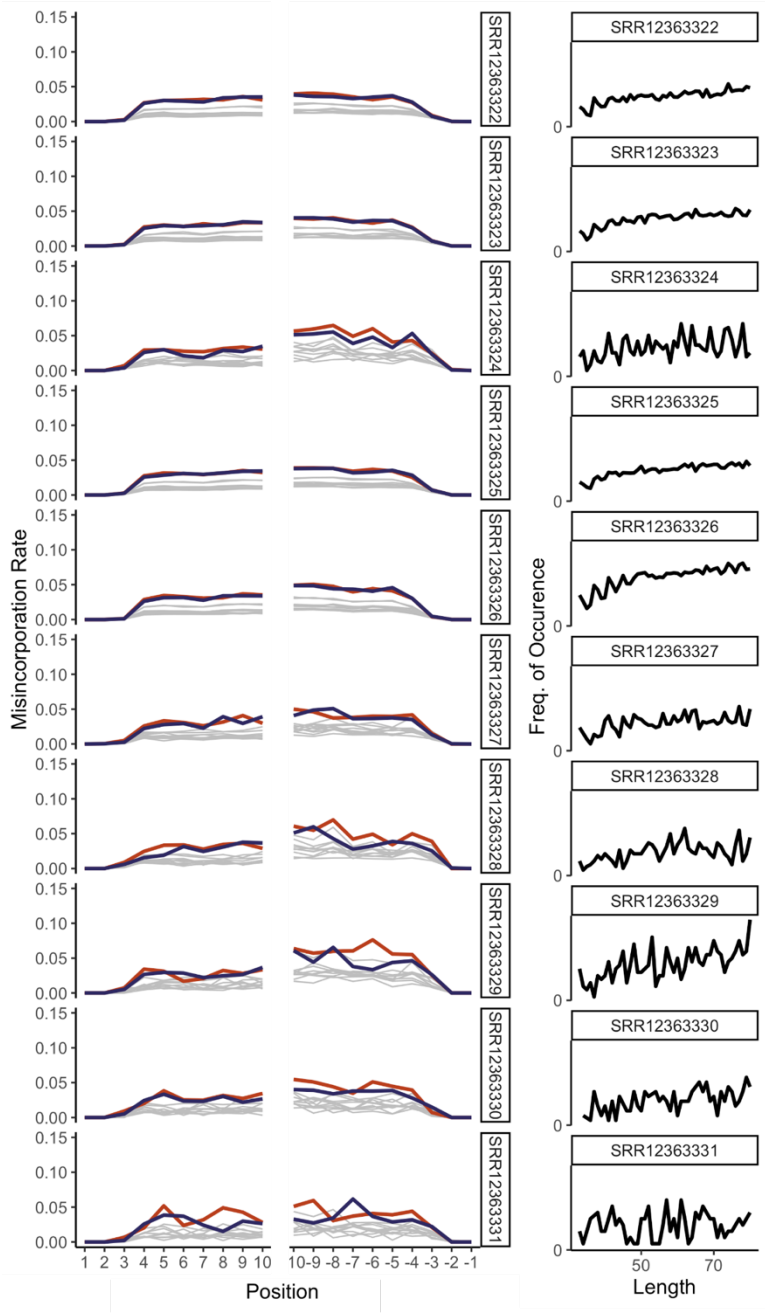

Figure S2. Modern acroporid data do not show the same misincorporation rate and length distribution patterns as ancient coral. (A) Misincorporation plots broken down by sample for ten modern files. This pattern holds for all modern acroporid data. (B) Read length distributions for the corresponding sample. Low misincorporation rate at terminal ends is an artifact of read mapping. However, these bases *were not* soft clipped from the modern files.

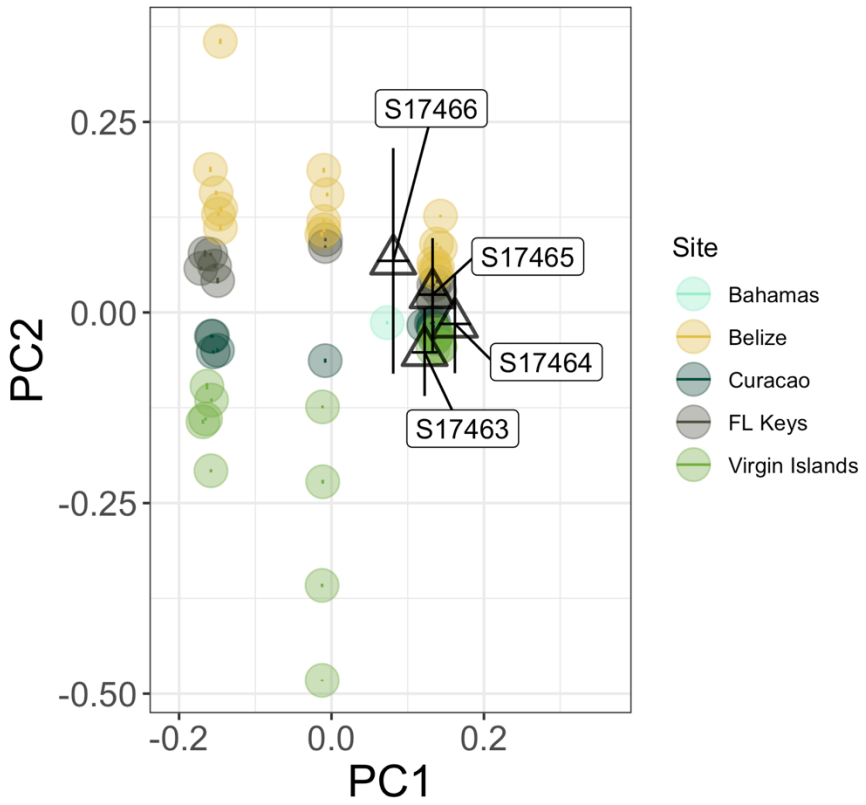

Figure S3. PCA of ancient samples placed with respect to modern acrporids shows separation by site on PC2.
Projection-based PCA colored by sample origin. This recapitulates the structure found between sites in Kitchen *et al*. (2019) Note that ancient samples could not be placed with confidence along PC2, represented by large vertical error bars.

MOLECULAR ECOLOGY

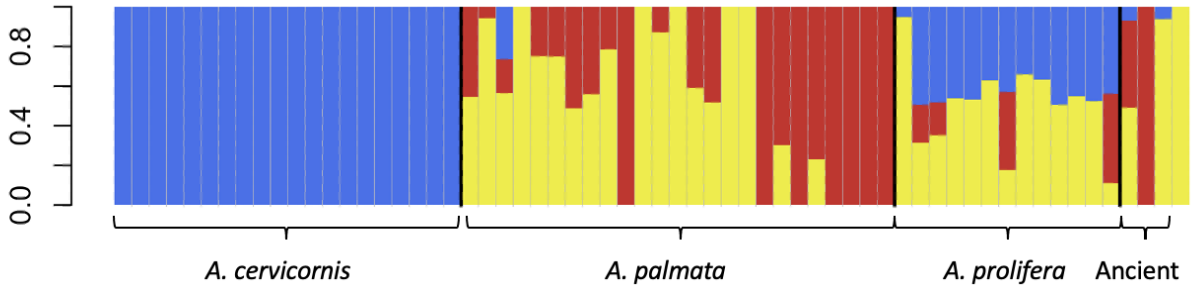

Figure S4. Admixture plot with three clusters solidifies assignment of ancient samples to *Acropora palmata.*

NGSAdmix plot for modern and ancient samples with K =3. Ancestry attributed to a third cluster in ancient samples is likely due to noise rather than actual ancestry from *Acropora cervicornis.*

MOLECULAR ECOLOGY

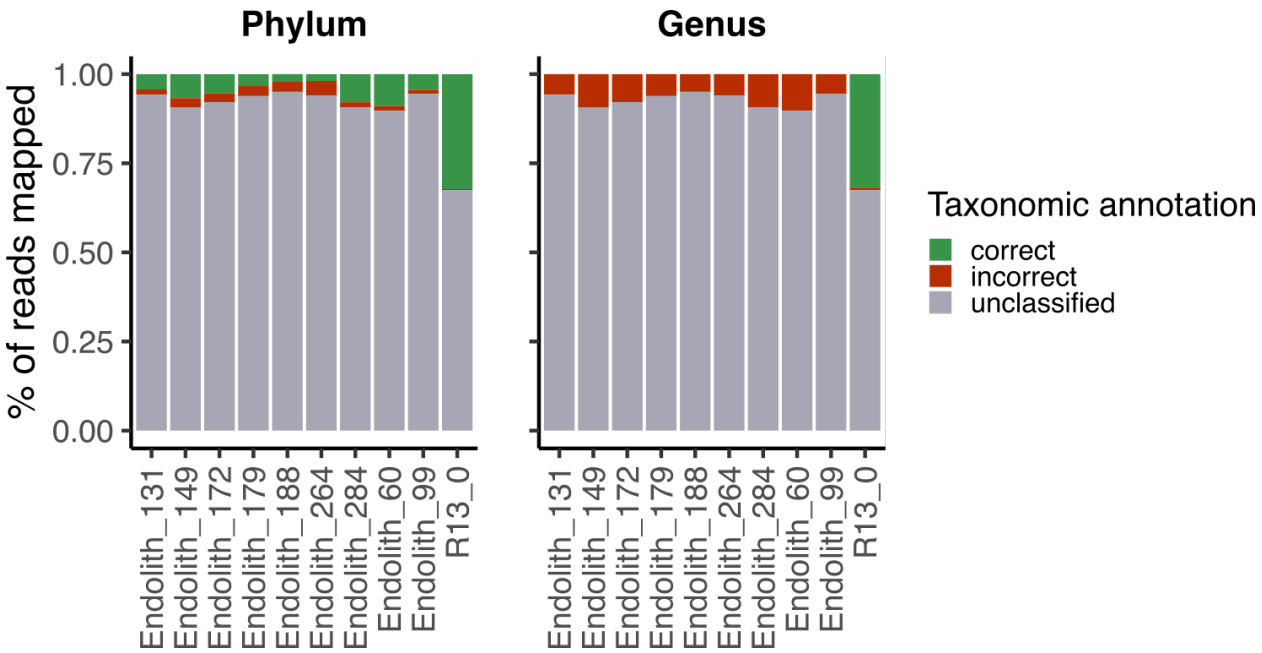

Figure S5. Taxonomic validation of reads mapped to bacterial genomes. Reads that mapped coral-associated bacterial genomes were taxonomically annotated using KAIJU. Taxonomic annotations were considered correct when the taxonomy assigned by KAIJU coincided with the genome taxonomy. Reads were unclassified if either did not map to any reference on the NCBI nr_euk database or ambiguously mapped to more than one genome of different taxa. The taxonomic level reported corresponded to the level at which the proportion of correct affiliations was higher than the incorrect ones.

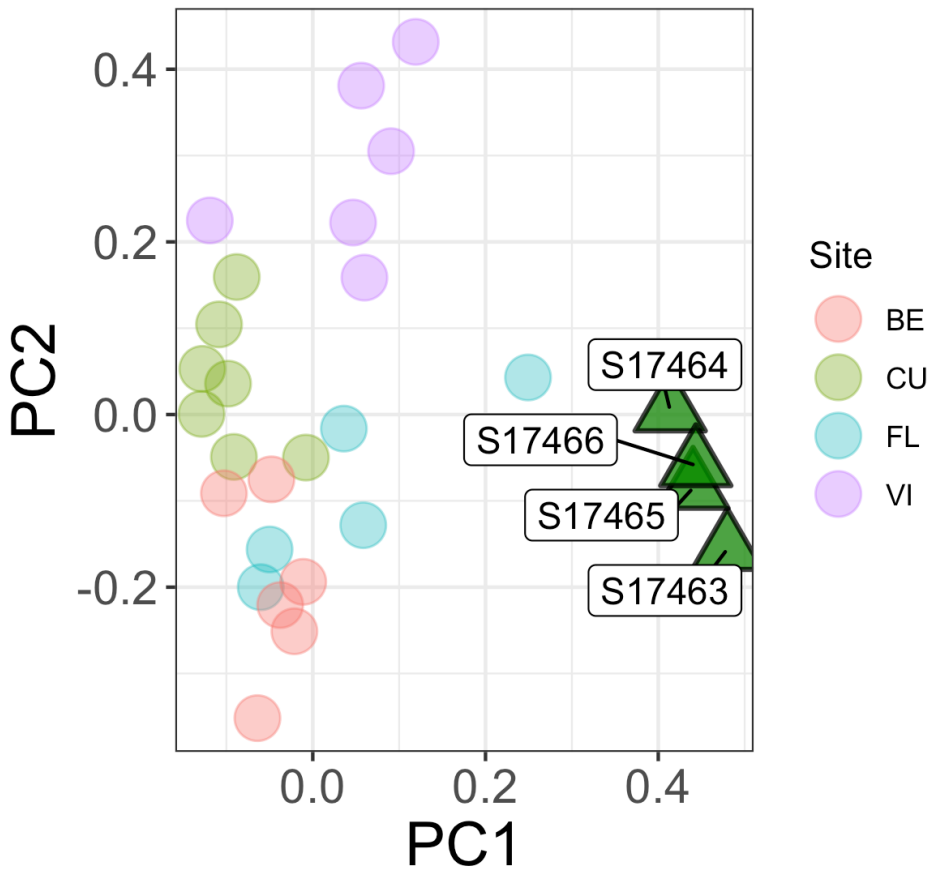

Figure S6. Constructing a PCA from just ancient samples and *Acropora palmata* highlights separation between present-day and past populations. ANGSD-based PCA constructed by using only ancient and *Acropora palmata* samples to calculate genotype likelihoods. Here, the PCA is colored by site of origin with green triangles showing ancient samples. BE= Belize, CU = Curacao, FL = FL Keys, VI = Virgin Islands. Note, most of the variation along PC1 is representative of the age of the samples. This segregation is likely due to the combination of actual accumulated genetic differences over the past 1,000-6,000 years and artifacts arising from ancient DNA.

Supplemental References

Toth, L., Stathakopoulos, A., & Kuffner, I. (2018). Descriptive core logs, core photographs, radiocarbon ages, and accretion data from Holocene reef cores collected throughout the Florida Keys reef tract [dataset]. In *U.S. Geological Survey data release*. USGS. doi: 10.5066/F7NV9HJX

Kitchen, S. A., Ratan, A., Bedoya-Reina, O. C., Burhans, R., Fogarty, N. D., Miller, W., & Baums, I. B. (2019). Genomic variants among threatened Acropora corals. *G3: Genes, Genomes, Genetics*, *9*(5), 1633–1646. doi: 10.1534/g3.119.400125

Rosales, S. M., Miller, M. W., Williams, D. E., Traylor-Knowles, N., Young, B., & Serrano, X. M. (2019). Microbiome differences in disease-resistant vs. susceptible Acropora corals subjected to disease challenge assays. *Scientific Reports*, *9*(1), 18279. doi: 10.1038/s41598-019-54855-y

Westrich, J. R., Ebling, A. M., Landing, W. M., Joyner, J. L., Kemp, K. M., Griffin, D. W., & Lipp, E. K. (2016). Saharan dust nutrients promote Vibrio bloom formation in marine surface waters. *Proceedings of the National Academy of Sciences*, *113*(21), 5964–5969. doi: 10.1073/pnas.1518080113