

Genetic and epigenetic insight into morphospecies in a reef coral

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1 **Abstract**

2 Incongruence between conventional and molecular systematics has left the delineation of many species
3 unresolved. Reef-building corals are no exception, with phenotypic plasticity among the most plausible
4 explanations for alternative morphospecies. As potential molecular signatures of phenotypic plasticity,
5 epigenetic processes may contribute to our understanding of morphospecies. We compared genetic and
6 epigenetic variation in Caribbean branching *Porites* spp., testing the hypothesis that epigenetics—
7 specifically, differential patterns of DNA methylation—play a role in alternative morphotypes of a group
8 whose taxonomic status has been questioned. We used reduced representation genome sequencing to
9 analyze over 1,000 single nucleotide polymorphisms and CpG sites in 27 *Porites* spp. exhibiting a range
10 of morphotypes from a variety of habitats in Belize. We found stronger evidence for genetic rather than
11 epigenetic structuring, identifying three well-defined genetic groups. One of these groups exhibited
12 significantly thicker branches, and branch thickness was a better predictor of genetic groups than depth,
13 habitat, or symbiont type. Epigenetic patterns were more subtle, with no clear groups. The more thickly
14 branched individuals in one of the genetic groups exhibited some epigenetic similarity, suggesting
15 potential covariation of genetics and epigenetics. This covariation was further supported by a positive
16 association between pairwise genetic and epigenetic distance. We speculate that epigenetic patterns
17 are a complex mosaic reflecting inheritance and diverse environmental histories. Given the role of
18 genetics in branching *Porites* spp. morphospecies we were able to detect with genome-wide
19 sequencing, use of such techniques throughout the geographic range may help settle their phylogeny.

20

21 **Introduction**

22

23 Inconsistencies between conventional and genetics-based taxonomy are common across taxa
24 (Patterson *et al.* 1993). Morphological characters have long been the basis of systematics, yet they can
25 be misleading in cases such as convergent evolution or extensive phenotypic plasticity (Potter *et al.*
26 1997; Fukami *et al.* 2004; Fritz *et al.* 2007). While molecular phylogenetics has illuminated species
27 relationships by identifying and resolving some of these issues, uncertainties often persist, and
28 disagreement among molecular studies is not uncommon. The inferences made by molecular studies are
29 influenced by numerous factors such as the number and type of markers and the models used to
30 analyze them (Brocchieri 2001; Yang & Rannala 2012). Moreover, the field of epigenetics has uncovered
31 novel mechanisms of phenotypic variation and inheritance that has led to reappraisal of the traditional
32 theory of molecular evolution (Noble 2015; Skinner 2015).

33 Reef-building corals exhibit substantial inter- and intraspecific variation in growth forms that has
34 proved particularly problematic for the taxonomic delineation of species (Fukami *et al.* 2004; Forsman *et*
35 *al.* 2009; Flot *et al.* 2011). Conventional taxonomy based on morphological features has obscured coral
36 phylogenies and in some cases overestimated species diversity revealed by way of genetic analyses
37 (Fukami *et al.* 2004; Forsman *et al.* 2010; Prada *et al.* 2014). In other cases, supposed ecomorphs of the
38 same species have turned out to be sibling species (Knowlton *et al.* 1992), and genetic data have also
39 uncovered cryptic species among similar growth forms of what was thought to be a single species
40 (Keshavmurthy *et al.* 2013; Schmidt-Roach *et al.* 2013). The confusion surrounding coral taxonomy has
41 consequences for coral conservation, because a species cannot be conserved and managed if it cannot
42 be defined. For example, 25 coral species are currently listed under the U.S. Endangered Species Act
43 (NOAA 2015), and the ambiguous taxonomic status of some of these species has necessitated revision
44 using molecular data (Forsman *et al.* 2010).

45 Several studies have concluded that morphological plasticity is among the most plausible
46 hypotheses for instances of both over- and underestimation of species diversity based on conventional

47 taxonomy (Forsman *et al.* 2010; Flot *et al.* 2011; Keshavmurthy *et al.* 2013; Prada *et al.* 2014). Indeed, a
48 recent reciprocal transplant study confirmed the role of phenotypic plasticity in alternative
49 morphotypes of *Pocillopora* spp. (Paz-García *et al.* 2015). Morphological plasticity is likely to be
50 particularly prevalent and adaptive in corals due to their sessility, longevity, modularity, and
51 indeterminate growth (Jackson & Coates 1986; Sebens 1987; Todd 2008). In a review of 26 studies that
52 tested for morphological plasticity in approximately 20 different species of reef corals, 92% of studies
53 documented evidence for plasticity (Todd 2008).

54 The expanding field of epigenetics may hold promise for understanding phenotypic plasticity in
55 corals. Epigenetic processes are increasingly recognized as molecular signatures of phenotypic variation
56 (Duncan *et al.* 2014). DNA methylation, the most widely studied epigenetic mark, involves the addition
57 of a methyl group to a cytosine, most commonly in the context of a CpG dinucleotide pair. Along with
58 other epigenetic features such as histone modifications and small RNA molecules, methylation patterns
59 can influence gene expression, though the mechanisms appear to be diverse, complex, and context-
60 dependent (Gavery & Roberts 2014; Duncan *et al.* 2014). Unlike the DNA sequences of the genome
61 itself, which change relatively little during an individual's lifetime, genome DNA methylation patterns
62 are not fixed, and can be influenced by environmental stimuli (Duncan *et al.* 2014). An expanding
63 number of studies have documented differential patterns of DNA methylation associated with
64 alternative phenotypes in a range of taxa (Kucharski *et al.* 2008; Fonseca Lira-Medeiros *et al.* 2010;
65 Smith *et al.* 2015, 2016; Schield *et al.* 2016). Among different morphotypes of mangroves living in
66 distinct habitats, for example, (Fonseca Lira-Medeiros *et al.* 2010) found little genetic variation but high
67 levels of epigenetic variation. In the genomes of two alternative morphotypes of threespine
68 sticklebacks, (Smith *et al.* 2015) identified 77 differentially methylated regions whose functions were
69 associated with known adaptive phenotypes. Interestingly, even among different species of Darwin's
70 finches, epigenetic differences were better correlated with traditional phylogenetic relationships than

71 were genetic differences (Skinner *et al.* 2014). Thus, an increased understanding of epigenetics may
72 revise definitions of biological diversity and evolution (Skinner 2015), with implications for future
73 conservation efforts.

74 The taxonomic status of branching corals of the genus *Porites* in the tropical Western Atlantic
75 has been uncertain. There are currently three recognized species: *P. porites* (Pallas, 1766), *P. furcata*
76 (Lamarck, 1816), and *P. divaricata* (Lesueur, 1820). Branch diameter and corallite features have
77 traditionally been used to define these species, and while one study found morphological variation to be
78 nearly continuous (Brakel 1977), other studies have identified morphological breaks, with some
79 supporting traditional taxonomic delineations (Weil 1992; Budd *et al.* 1994; Jameson 1997; Jameson &
80 Cairns 2012). Molecular studies have also failed to reach consensus (Weil 1992; Budd *et al.* 1994;
81 Forsman *et al.* 2009; Prada *et al.* 2014), but earlier studies used allozymes, which could be influenced by
82 epigenetic effects. The most recent and thorough population genetic analysis found no support for
83 upholding the three named species, finding no significant variation between supposed species across 11
84 genetic markers and multiple geographic sub-regions (Prada *et al.* 2014). The authors acknowledged
85 that it is possible that their study overlooked diversity somewhere in the genome, but identified
86 phenotypic plasticity as a plausible explanation for their results. To further explore the potential source
87 of phenotypic variation in branching *Porites* spp., we compared genetic and epigenetic diversity in these
88 corals using restriction site associated DNA (RAD) sequencing.

89

90 **Methods**

91

92 *Specimen collection and DNA extraction*

93 Corals were collected in May 2016 within approximately 4 km of the Smithsonian Institution's
94 Carrie Bow Cay Field Station in Belize (16° 48' 9.39" N, 88° 4' 54.99" W). In the field, the three Caribbean

95 branching *Porites* spp. are distinguished primarily by branch diameter and habitat type, so collections
96 targeted a broad range of branch diameters (6 - 26 mm), depths (0.5 - 17 m) and habitats (mangrove to
97 forereef) to sample as much variation as possible. A total of thirty specimens were collected, but we
98 obtained sufficient sequence data from only 27 of these (Table 1). Branch tips were cut with shears and
99 placed in a conical tube. Within one hour of collection, specimens were transferred to tubes containing
100 salt-saturated DMSO (SS-DMSO) for DNA preservation (Gaither *et al.* 2011). Coral tissue preserved in SS-
101 DMSO began to slough off the skeleton after several days and was easily removed using forceps. Small
102 pieces of tissue (~0.5 μ l volume) were washed three times via centrifugation with phosphate buffered
103 saline prior to DNA extraction using Qiagen DNeasy Blood and Tissue kits according to the
104 manufacturer's protocol, with an overnight lysis with proteinase K. Samples were further purified via
105 overnight ethanol precipitation, followed by resuspension in Qiagen AE buffer. Genomic DNA was
106 checked for yield and quality via fluorescence (Qubit BR assay) and gel electrophoresis, respectively.

107 The remaining coral skeletons were soaked in a 10% bleach solution for 24 hrs, then dried at 40
108 °C for 48 hrs. The diameter of branch tips at their widest cross-section was measured to the nearest 0.5
109 mm with vernier calipers.

110

111 *Symbiont genotyping*

112 Reef corals commonly engage in species-specific associations with symbiotic dinoflagellates
113 (*Symbiodinium* spp.), and these associations can also be related to genetic structure within a given host
114 (Bongaerts *et al.* 2010; Finney *et al.* 2010). To identify the dominant *Symbiodinium* type associated with
115 each coral, an approximately 700 base pair region of domain V of the cp23S-rDNA region was PCR-
116 amplified using primer pair 23S1 (5'-CACGACGTTGTAACACGACGGC TGTAACATAACGGTCC-3') and 23S2
117 (5'-GGATAACAATTCACACAGGCCATCGTATTGAACCCAGC-3') (Santos *et al.* 2002). PCR was performed in
118 25 μ l volumes containing 1X green buffer (Promega), 2.5 mM MgCl₂, 240 μ M dNTP, 5 pmol of each

119 primer, 1U *Taq* (GoTaq, Promega), and 1-20 ng of template DNA. Reactions were carried out in an
120 Applied Biosystems Veriti thermocycler under the following conditions: initial denaturing period of 1 min
121 at 95 °C, 35 cycles of 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension period of 7
122 min. PCR products were cleaned (NEB Monarch kit) and checked on a 1% agarose gel. Cleaned PCR
123 products (10 ng/μL) were sent to Sequetech Corporation (Mountain View, CA) for Sanger sequencing
124 using the forward primer. Chromatograms were edited with Geneious v9.1.5, converting bases with a
125 Phred score < 30 to Ns. Sequences were then aligned using ClustalW and queried against the GenBank
126 nucleotide database in Geneious.

127

128 *RAD library preparation and sequencing*

129 Double digest RADseq (ddRADseq) libraries were prepared following the methods of (Peterson
130 *et al.* 2012). Only samples with high molecular weight DNA were used for library preparation. For each
131 sample, a minor variation of ddRADseq called EpiRADseq was also employed to evaluate methylated loci
132 (Schield *et al.* 2016). Both methods use two restriction enzymes—a rare cutter and a common cutter—
133 to perform a double digest of the DNA at specific restriction sites throughout the genome, with the only
134 difference that EpiRADseq uses a methylation-sensitive common cutter that will not cut methylated loci.
135 Both methods used the rare cutter *PstI* (5'-CTGCAG-3' recognition site), while the common cutter *MspI*
136 (5'-CCGG-3' recognition site) was used for ddRADseq and the methylation-sensitive isoschizomer *HpaII*
137 (also 5'-CCGG-3' recognition site) was used for EpiRADseq preparations. Double digests of 300-500 ng
138 gDNA per sample were carried out using 20 units of each enzyme in the manufacturer's supplied buffer
139 (New England Biolabs, NEB) for 5 hours at 37 °C. Samples were cleaned using magnetic beads (Sera-Mag
140 SpeedBeads) prior to ligation of barcoded Illumina adapters onto the fragments (Peterson *et al.* 2012).
141 After two rounds of bead cleanup, samples were pooled into 12 libraries (along with 18 other samples
142 not analyzed in this study), which were then subjected to automated size-selection of fragments

143 between 415 and 515 bp using a Pippin Prep (Sage Science). Libraries were then PCR amplified using
144 Phusion *Taq* (NEB) and Illumina-indexed primers (Peterson *et al.* 2012). Final library fragment sizes and
145 concentrations were evaluated with D1000 ScreenTape on an Agilent 2200 TapeStation. Libraries were
146 sent to the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley,
147 where their concentrations were verified via qPCR prior to 100 bp, paired-end sequencing in equimolar
148 ratios on the Illumina HiSeq 4000.

149

150 *RAD sequence assembly*

151 Sequences were assembled using *ipyrad* v0.3.41 (Eaton 2014). We used the ‘denovo - reference’
152 assembly method with the *Symbiodinium minutum* (clade B; GenBank accession GCA_000507305.1
153 (Shoguchi *et al.* 2013)) and *Symbiodinium kawagutii* (clade F;
154 http://web.malab.cn/symka_new/data/Symbiodinium_kawagutii.assembly.935Mb.fa.gzb.fa.gz (Lin *et al.*
155 2015)) genomes used as reference to subtract symbiont reads from the *de novo* assembly. We
156 concatenated these genomes into a single reference file. Step one of the *ipyrad* workflow demultiplexed
157 the data in each pool by identifying restriction overhangs and barcode sequences associated with each
158 sample; zero barcode mismatches were tolerated. Demultiplexed samples were then combined in a
159 single directory for further steps. In step two, reads were trimmed of barcodes and adapters and quality
160 filtered using a q-score threshold of 20, with bases below this score converted to Ns and any reads with
161 more than 5 Ns removed. Step three mapped reads to the concatenated symbiont reference genomes
162 with *BWA* using the default *bwa mem* setting and removed any mapped reads. With the remaining
163 reads, similar clusters of reads were identified using a threshold of 85% similarity and aligning them. We
164 chose 85% as a moderately conservative clustering threshold to avoid over-splitting of loci (Harvey *et al.*
165 2015). Next, step four performed joint estimation of heterozygosity and error rate (Lynch 2008) based
166 on a diploid model assuming a maximum of 2 consensus alleles per individual. Step five used the

167 parameters from step four to determine consensus bases calls for each allele, and removed consensus
168 sequences with greater than 5 Ns per end of paired-end reads. With consensus sequences identified,
169 step six clustered and aligned reads for each sample to consensus sequences. Finally, step seven filtered
170 the dataset according to maximum number of indels allowed per read end (8), maximum number of
171 SNPs per locus (20), maximum proportion of shared heterozygous sites per locus (0.5), and minimum
172 number of samples per locus (15).

173 Henceforth, we will use the term *locus* to refer to a consensus paired-end read. The term *SNP*
174 refers specifically to a single nucleotide polymorphism on a locus, while the term *CpG* refers to a
175 cytosine-guanine dinucleotide pair that can be either methylated or non-methylated at the 5'-CCGG-3'
176 restriction site of each locus.

177

178 *SNP analysis*

179 We analyzed unlinked SNPs that were sampled by *ipyrad* at 1 SNP per locus with the least
180 amount of missing data; SNPs were sampled randomly if they had equal amounts of missing data. We
181 were able to estimate the SNP error rate, defined as the proportion of SNP mismatches between pairs of
182 the same individuals (Mastretta-Yanes *et al.* 2015), by treating ddRADseq and EpiRADseq samples as
183 technical replicates and computing pairwise differences between individuals using the *dist.gene* function
184 in the R package *ape* (Paradis *et al.* 2004). We then analyzed a SNP dataset from ddRADseq libraries that
185 had no missing data across samples. While this reduced the dataset considerably, it allowed us to test
186 variation across a similar set of loci for both the genetic and epigenetic analysis, as explained further
187 below.

188 Genetic differentiation among samples was examined using multidimensional scaling ($k = 2$)
189 using the *cmdscale* R function. Discriminant analysis of principal components (DAPC) in the R package
190 *adegenet* (Jombart 2008) was used to further examine these patterns. Rather than applying prior

191 assumptions about the identity of each coral specimen to assign population groups, we used the
192 *find.clusters* function in *adeget* to identify groups. This k-means clustering function reduces the data
193 with principal components analysis (PCA) before estimating the number of clusters with the lowest
194 Bayesian information criterion (BIC). All PCs were retained for the analysis and a maximum of 10 clusters
195 was specified. Once groups were identified, cross-validation (*xvalDapc*) was used to estimate the
196 number of PCs to retain for the subsequent DAPC analysis. In the groups identified by the DAPC analysis,
197 genetic differentiation between groups was evaluated by computing pairwise Weir and Cockerham's F_{ST}
198 using the R package *hierfstat* (Goudet 2005).

199

200 *DNA methylation analysis*

201 Methylation detection with EpiRADseq data involves analysis of read counts (Schield *et al.*
202 2016). Presence of a given read in the EpiRADseq dataset indicates that the locus is not methylated at
203 the restriction cut site. Conversely, if a locus is methylated at the 5'-CCGG-3' cut site, *HpaII* is blocked
204 from cutting, and the locus will be absent from the dataset. Hence, read counts of zero are informative,
205 but they could also be the result of missing data through processes such as allele dropout or variation in
206 library size or fragment size selection. To control for this, we used ddRADseq data to normalize the
207 EpiRADseq data. Any loci with zeros in the ddRADseq library were treated as absent and removed,
208 thereby leaving zeros in the EpiRADseq library only where the locus was counted in the ddRADseq
209 library. This had the added benefit that it resulted in analysis of a similar set of loci as the SNP analysis
210 described above.

211 EpiRADseq and ddRADseq read counts per locus were highly correlated, with the exception of
212 methylated loci, which, as expected, had low or zero abundances in EpiRADseq libraries (Fig. 1A). The
213 dataset comprising read counts for both EpiRADseq and ddRADseq was subject to normalization of read
214 counts using TMM normalization in the R package *edgeR* (Robinson *et al.* 2010). Residuals of linear

215 regressions of EpiRADseq and ddRADseq libraries provided optimal differentiation of methylated loci
216 from non-methylated loci (Fig. 1B&C). Finally, a binary dataset was created from these data by setting a
217 residual threshold of -1 for methylated loci such that 1 = methylated and 0 = non-methylated (Fig. 1C).
218 These data were evaluated using multidimensional scaling as described above for the SNP analysis.

219 EpiRADseq read counts could theoretically represent variable levels of methylation within a
220 sample, such as through variation in methylation across pooled replicates, or among different tissue or
221 cell types (Schield *et al.* 2016). However, our analysis of ddRADseq and EpiRADseq read counts in
222 tandem indicated that much of the variability in read counts occurs in both libraries and is thus likely
223 related to library preparation effects such as fragment size-selection or PCR bias (Davey *et al.* 2013). In
224 the original EpiRADseq method developed by Schield *et al.* (2016), PCR effects were minimized by using
225 unique molecular identifier sequences attached to fragments. We suspect that fragment size-selection
226 effects could be equally important in driving read count variability. For example, abundant reads could
227 simply represent the mean or mode fragment size within the library after size-selection, while less
228 abundant reads could represent the tails of the distribution. In either case, our analysis suggests that
229 much of the read count variation above zero does not reflect methylation levels, and by normalizing
230 EpiRADseq data to the ddRADseq data and creating a binary dataset, we removed much of the potential
231 bias described above.

232 A repository with the complete bioinformatic workflow described above can be accessed at
233 <https://github.com/jldimond/Branching-Porites>.

234

235 **Results**

236

237 *Data yield*

238 An average of 3.24 million paired-end reads per sample were obtained based on restriction
239 overhangs and barcodes, with an average of 2.63 million reads per sample remaining after q-score and
240 adapter filtering. After filtering for symbionts and minimum read depth, an average of 26,330 consensus
241 loci per sample were obtained, with a total of 135,980 unique consensus loci across samples. Obtaining
242 datasets with no missing data across samples substantially reduced the number of loci; the final SNP
243 dataset consisted of 1,113 unlinked SNPs (1,113 consensus paired-end loci with 1 SNP sampled per
244 locus) across 27 samples, while the methylation dataset consisted of 1,712 CpGs (1,712 consensus
245 paired-end loci with one CpG cut site per locus) across 25 samples. Differences in the number of
246 SNPs/CpGs between datasets were due to the different numbers of samples; two samples, 101 and 112,
247 were removed from the methylation dataset due to low coverage. This reduced the number of samples
248 in the methylation dataset but resulted in a greater number of shared CpG sites.

249

250 *Symbiont identity*

251 BLAST searches of *Symbiodinium* cp23S sequences indicated that the dominant symbiont in the
252 majority of corals was a clade C *Symbiodinium* with high similarity to *Symbiodinium* subclade C3 (Table
253 1). Four corals occurring in shallow water habitats (\approx 2.7 m) hosted clade A *Symbiodinium* with high
254 similarity to subclade A3. We were unable to amplify cp23S in three of the specimens despite repeated
255 attempts.

256

257 *Genetic patterns*

258 Based on SNP mismatches between technical replicates, the SNP error rate was estimated to be
259 3.6% (standard deviation, SD, 3.1%), which is on the lower end of the range that has been reported
260 previously (Mastretta-Yanes *et al.* 2015) (Fig. 2). In other words, multilocus genotypes of technical
261 replicates were 96.4% similar on average. In contrast, nearly all non-replicate pairwise comparisons

262 exhibited greater differences, with the exception of two pairs of individuals (109 & 114, 127 & 115) that
263 exhibited similarity within the range of the SNP error (Fig. 2). The high similarity of these individuals
264 suggests they are possible clones. Specimens 109 and 114 were separated by approximately 45 m, while
265 specimens 127 and 115 were separated by approximately 450 m. If they are clones, these distances
266 make it unlikely that they resulted from fragmentation.

267 Multidimensional scaling of samples based on SNPs suggested genetic structure of *Porites* spp.,
268 with samples clustering into three generally well-separated groups (Fig. 3). K-means clustering of SNPs
269 showed the strongest support for three clusters according to BIC values (Fig. 4A). Based on results of
270 cross-validation, nine PCs (the maximum suggested given the sample size) were retained for DAPC
271 analysis of the three groups, which resulted in two discriminant axes that were both retained (Fig. 4B
272 inset). As with the MDS analysis, DAPC indicated clear separation of the three SNP groups (Fig. 4B). The
273 two pairs of potential clones were in two separate groups (109 & 114 in group 2, 127 & 115 in group 1).
274 Similar values of F_{ST} were observed between groups (F_{ST} for each pairwise comparison: 1&2 = 0.194; 1&3
275 = 0.207; 2&3 = 0.191).

276 We evaluated potential factors associated with genetic structure using multiple regression. For
277 the genetic variable, we used the first discriminant axis from the DAPC analysis of SNPs. This was
278 regressed against collection depth, symbiont type, habitat, and branch diameter. The model explained
279 61% of the variation in the SNP variable. The function *calc.relimp* in the R package *relaimpo* was used to
280 estimate the relative importance of each model component. The relative importance of depth, symbiont
281 type, habitat, and branch diameter in the model was 0.5%, 2%, 31%, and 66.5%, respectively. We further
282 evaluated branch diameter with a one-way ANOVA, followed by pairwise t-tests with Bonferroni
283 adjustment. Group 1 branch diameter was significantly different from both groups 2 and 3 ($p < 0.001$),
284 while groups 2 and 3 were not significantly different from each other ($p = 0.724$) (Fig. 5).

285

286 *Epigenetic patterns*

287 Among the 1,712 CpGs, a range of 314-360 were methylated per sample, yielding a mean
288 methylation level of 19.5% (SD 0.008%). However, there were two outliers with high levels of
289 methylation. This was due largely to low read count loci being categorized as methylated. A more
290 stringent minimum read count threshold of 10 reads per locus was therefore applied to the ddRADseq
291 dataset and this resulted in 1,368 CpGs with a range of 238-263 methylated CpGs per sample,
292 corresponding to a methylation level of 18.3% (SD 0.005%). Of the 1,368 CpGs, 208 were differentially
293 methylated, 131 were constitutively methylated across all samples, and 1029 were constitutively non-
294 methylated across all samples.

295 Methylation patterns in the MDS analysis were less clear than SNP patterns, with no clear
296 grouping and most of the variation spread across much of the horizontal axis (Fig. 6). K-means clustering
297 of CpG methylation indicated the strongest support for a single group (not shown), so DAPC was not
298 appropriate. Among the 208 differentially methylated CpGs, most were either methylated or
299 unmethylated across a majority of samples (Fig. 7). However, when visualized within the context of the
300 groups identified in the SNP analysis, individuals in group 1 appeared to cluster adjacent to each other
301 (far left color swatch, Fig. 7). These also tended to be samples with thicker branch diameter (bar plot,
302 Fig. 7).

303

304 *Linkages between genetic and epigenetic variation*

305 To evaluate potential associations between genetic and epigenetic variation, we asked whether
306 SNPs with high contributions to the DAPC analysis were linked to differentially methylated CpGs (i.e.,
307 from the same RAD loci). Unique locus IDs generated by *ipyrad* were used to merge differentially
308 methylated CpG data with the SNP contribution scores from the DAPC. There was no significant

309 difference between contribution scores associated with differentially methylated loci and scores from a
310 random sample of the data (Kolmogorov-Smirnov test; $p = 0.365$; Fig. 8).

311 We also compared genetic and epigenetic variation by calculating pairwise genetic and
312 epigenetic distances, again using the *dist.gene* function in the R package *ape*. This was the same statistic
313 generated in Fig. 2 for the SNP data, measuring the proportion of pairwise mismatches in multilocus
314 SNPs and CpG methylation status. The majority of pairwise comparisons did not exhibit a clear
315 association between genetic and epigenetic distance (Fig. 9). However, the two pairs of genetic outliers
316 identified earlier with very low genetic distances (109 & 114, 127 & 115) also had the lowest epigenetic
317 distances. A linear regression of these data was significant ($p < 0.001$).

318

319 Discussion

320

321 Our sample of branching *Porites* spp. from Belize exhibited clear genetic differentiation,
322 supporting the idea that these corals comprise three separate and fairly well-defined groups. While this
323 result is in general agreement with older studies based on allozymes (Weil 1992; Budd *et al.* 1994), it
324 contrasts with the most recent study based on 11 genetic markers (Prada *et al.* 2014). The inferences we
325 were able to make likely reflect the large number of markers we sampled. Reduced representation
326 genome sampling methods like RADseq have shown great promise in resolving phylogenetic
327 relationships, especially in recalcitrant taxa like cnidarians for which traditional mitochondrial and
328 nuclear markers have shown limited success (Pante *et al.* 2015; Combosch & Vollmer 2015; Herrera &
329 Shank 2016; Rosser *et al.* 2017). Despite the number of markers we sampled, however, our study lacks
330 sufficient geographic breadth to draw strong phylogenetic conclusions. Indeed, all evaluations of
331 branching *Porites* spp. to date, including ours, have been limited by geographic scope, insufficient colony
332 sample size, or low genome coverage. A thorough reexamination of branching *Porites* spp. phylogeny

333 across the Caribbean / Western Atlantic region may be warranted, assessing both genome-wide
334 variation and multivariate morphological traits. Ideally, such a study would incorporate randomized
335 collections in different habitats to permit assessment of potential habitat selection.

336 Differences in branch diameter, particularly between group 1 and the other two groups, suggest
337 that the genetic differentiation we observed is associated with colony-level morphological variation.
338 Variation in branch thickness is the primary diagnostic feature used for *Porites* spp. identification in the
339 field, and is likely what historically prompted delimitation of species groups. Corallite-level variation in
340 morphology is also well documented, and most studies using these methods have identified distinct
341 groups based on multivariate corallite characters (Weil 1992; Budd *et al.* 1994; Jameson 1997). Two of
342 these studies found evidence supporting the three recognized species (Weil 1992; Jameson 1997), while
343 one found up to five morphospecies among specimens collected from three regions (Budd *et al.* 1994),
344 and another identified continuous variation without any clear breaks (Brakel 1977). However, despite
345 the continuous variation observed by Brakel (1977), he judged the variation to be largely genetic (fixed)
346 based on the observation that 1) corals from very different environments can have very similar
347 morphologies, and 2) corals from very similar environments can have very different morphologies. This
348 concurs with our analysis suggesting that morphotype variation in *Porites* spp. has a genetic basis. On
349 the other hand, we also observed evidence for phenotypic plasticity, such as in the case of three
350 specimens collected from nearby mangrove prop roots exhibiting similar branch morphologies; one of
351 these colonies (122) was assigned to group 2 while the others (118, 121) were assigned to group 3.

352 Based on the lack of consensus among previous studies concerning the taxonomic status of
353 branching *Porites* spp., and hypotheses that phenotypic plasticity might underlie this incongruence, we
354 did not anticipate finding strong genetic differentiation and instead we hypothesized that epigenetic
355 patterns might prove more informative. Instead, patterns of DNA methylation were less conclusive than
356 genetic patterns. Levels of methylation did not vary greatly among samples, and even among

357 differentially methylated CpGs, most were either methylated or unmethylated in a majority of samples.
358 Similarly, little variation in methylation was observed in a recent study of threespine sticklebacks, with
359 only 737 differentially methylated CpG sites identified out of 1,445,567 sites examined across eight
360 individuals (Smith *et al.* 2015). However, these differentially methylated loci were associated with
361 alternative phenotypes (Smith *et al.* 2015). The only phenotypic pattern to emerge from our data was a
362 suggestion that individuals in group 1, which also tended to be more thickly branched, were generally
363 grouped close together. Thus, there is an intriguing possibility that epigenetic variation is coupled with
364 genetic variation in these individuals.

365 Genetic-epigenetic coupling is further suggested by the positive relationship between pairwise
366 genetic and epigenetic distance. This relationship implies a potential heritable component to the
367 methylation patterns, although it was strongest when including two pairs of outliers. The physical
368 distances separating these pairs suggests that they are unlikely to be clones resulting from
369 fragmentation, but possibly clones from asexually produced larvae (Harrison 2011). The interpretation
370 that these pairs are clones is more likely, because even though some branching *Porites* spp. colonies are
371 hermaphrodites capable of self-fertilization (Schlöder & Guzman 2008), Mendelian segregation leads to
372 an extremely low probability of identical parental and offspring genotypes (Stoddart 1983). If they are
373 indeed clones, the similar methylation patterns in these individuals would reflect inheritance via mitosis
374 rather than sexual recombination through the germline. However, regardless of whether methylation
375 profiles were transmitted sexually or asexually in the two pairs of outliers, it is notable that while
376 genetic distance was near zero, epigenetic distance was relatively high. This could reflect divergence of
377 the methylome due to environmental effects. Methylation levels in corals have recently been shown to
378 be at least partially under environmental influence over short time scales (Putnam *et al.* 2016). Clearly,
379 the methylation patterns we observed in *Porites* spp. exhibit a high degree of noise, and this noise may
380 reflect the diverse habitat conditions and environmental histories experienced by the corals analyzed

381 here. Environmental influence is also suggested by the case of the three corals collected from adjacent
382 mangrove prop roots (118, 121, 122); while these corals came from two different genetic groups, they
383 were clustered closely to each other epigenetically.

384 Beyond simply covarying with genetic variation, some studies have reported epigenetic variation
385 potentially driving genetic variation (Skinner *et al.* 2015; Smith *et al.* 2016). In our analysis, differentially
386 methylated CpGs were not more likely to be linked to SNPs with strong contributions to genetic
387 structuring. However, it is important to keep in mind that SNPs and differentially methylated CpG sites
388 could be separated by up to ~500 bp on the RAD loci we generated. Moreover, given the read lengths
389 and profiling techniques, the number of CpG sites we were able to sample per locus with EpiRADseq was
390 a fraction of the number of nucleotides we were able to assess for SNPs. For example, for a given paired-
391 end locus, nearly 200 nucleotides were assayed for potential SNPs whereas only a single CpG site was
392 assayed for methylation. Nonetheless, if we consider the EpiRADseq method a random sample of CpGs,
393 the 18% methylation we observed is within the range expected for invertebrates (Zemach *et al.* 2010;
394 Sarda *et al.* 2012; Dimond & Roberts 2016).

395 Reduced representation genome sequencing techniques are currently very popular and are
396 enhancing our ability to probe molecular processes, but they are not without error. Our analysis of
397 ddRADseq and EpiRADseq libraries in tandem provided robust control for error in both the SNP and
398 methylation datasets. Technical replicates have been advocated as a means to assess genotyping error,
399 and by assessing genotypes of the same individuals from the two libraries we confirmed that this error
400 was within the range documented elsewhere (Mastretta-Yanes *et al.* 2015; Recknagel *et al.* 2015). For
401 the methylation analysis, comparing EpiRADseq libraries to ddRADseq libraries was a key factor in
402 controlling for library composition effects, reducing the likelihood of false positives. This paired library
403 approach appears to be a strong alternative to the unique molecular identifier approach used by Schield
404 *et al.* (2016).

405 In addition to genetic data, prevailing symbiont populations could provide an additional means
406 to evaluate whether branching *Porites* spp. exhibit species-level differentiation. Species-specific
407 associations between host corals and *Symbiodinium* are common in the Caribbean, particularly among
408 brooding species such as *Porites* (Finney et al. 2010; Bongaerts et al. 2015). Furthermore, branching
409 *Porites* spp. appear to associate with host-specialist symbionts that are not common in other hosts
410 (Finney et al. 2010; Bongaerts et al. 2015). Branching *Porites* spp. also tend to host distinct symbionts
411 from their congener *P. astreoides* (Finney et al. 2010; Bongaerts et al. 2015). Although cp23S is not a
412 fine-scale genetic marker, the majority of corals we analyzed hosted the same *Symbiodinium* clade C
413 phylotype, while three individuals in group 3 and one in group 2 hosted the clade A phylotype (Table 1).
414 While host-symbiont pairings can be regionally specific (LaJeunesse 2002; Finney et al. 2010), if
415 branching *Porites* spp. have the same symbiont profile on a given reef regardless of potential differences
416 in host genetics or morphology, this could be an argument against considering them separate species. A
417 comprehensive reexamination of branching *Porites* spp. would be wise to include an assessment of
418 *Symbiodinium* communities.

419

420 *Conclusion*

421 Contrary to our expectations, branching *Porites* spp. morphotype variation was better explained
422 by genetic patterns than epigenetic patterns. This analysis benefited from the resolution afforded by
423 genome-wide sequencing, and may justify a more thorough analysis of branching *Porites* spp. phylogeny
424 throughout the tropical Western Atlantic. Although patterns of DNA methylation were not as conclusive
425 as genetic patterns, there was some evidence of covariation between genetic and epigenetic variation.
426 This possibility, as well as potential environmental influence on methylation in corals, will require
427 further study. Given the increasingly powerful molecular biology tools available for work in

428 environmental epigenomics, stronger inferences about the extent, variability, and potential functions of
429 epigenetic processes in corals are only a matter of time.

430

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432

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440

441 **Data Accessibility**

442

443 -DNA sequences: GenBank accession numbers KY649212-KY649238; SRA accession numbers
444 SAMN06566335-SAMN06566364.

445 -Repository detailing analysis methods: <https://github.com/jldiamond/Branching-Porites>

446

447 **Author Contributions**

448

449 J.D. conceived and designed the study, collected the specimens, prepared RAD libraries,
450 analyzed the data, and drafted the manuscript. S.G. processed the specimens, extracted DNA, and
451 performed the symbiont genotyping. S.R. contributed reagents, equipment and analysis tools.

452 **Literature Cited**

- 453
- 454 Bongaerts P, Carmichael M, Hay KB *et al.* (2015) Prevalent endosymbiont zonation shapes the depth
455 distributions of scleractinian coral species. *Royal Society open science*, **2**, 140297.
- 456 Bongaerts P, Riginos C, Ridgway T *et al.* (2010) Genetic divergence across habitats in the widespread
457 coral *Seriatopora hystrix* and its associated Symbiodinium. *Plos One*, **5**, e10871.
- 458 Brakel W (1977) Corallite variation in *Porites* and the species problem in corals. *Proceedings of the Third*
459 *Internatioeedinal Coral Reef Symposium*, **1**, 457–462.
- 460 Brocchieri L (2001) Phylogenetic inferences from molecular sequences: review and critique. *Theoretical*
461 *Population Biology*, **59**, 27–40.
- 462 Budd AF, Johnson KG, Potts DC (1994) Recognizing morphospecies in colonial reef corals: I. Landmark-
463 based methods. *Paleobiology*, **20**, 484–505.
- 464 Combosch DJ, Vollmer SV (2015) Trans-Pacific RAD-Seq population genomics confirms introgressive
465 hybridization in Eastern Pacific Pocillopora corals. *Molecular Phylogenetics and Evolution*, **88**, 154–
466 162.
- 467 Davey JW, Cezard T, Fuentes-Utrilla P *et al.* (2013) Special features of RAD Sequencing data: implications
468 for genotyping. *Molecular Ecology*, **22**, 3151–3164.
- 469 Dimond JL, Roberts SB (2016) Germline DNA methylation in reef corals: patterns and potential roles in
470 response to environmental change. *Molecular Ecology*, **25**, 1895–1904.
- 471 Duncan EJ, Gluckman PD, Dearden PK (2014) Epigenetics, plasticity, and evolution: How do we link
472 epigenetic change to phenotype? *Journal of Experimental Zoology. Part B, Molecular and*
473 *Developmental Evolution*, **322**, 208–220.
- 474 Eaton DAR (2014) PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*,
475 **30**, 1844–1849.
- 476 Finney JC, Pettay DT, Sampayo EM *et al.* (2010) The relative significance of host-habitat, depth, and
477 geography on the ecology, endemism, and speciation of coral endosymbionts in the genus
478 Symbiodinium. *Microbial Ecology*, **60**, 250–263.
- 479 Flot J, Blanchot J, Charpy L *et al.* (2011) Incongruence between morphotypes and genetically delimited
480 species in the coral genus *Stylophora*: phenotypic plasticity, morphological
481 convergence, morphological stasis or interspecific hybridization? *BMC Ecology*, **11**, 22.
- 482 Fonseca Lira-Medeiros C, Parisod C, Fernandes R *et al.* (2010) Epigenetic variation in mangrove plants
483 occurring in contrasting natural environment. *Plos One*, **5**, e10326.
- 484 Forsman ZH, Barshis DJ, Hunter CL, Toonen RJ (2009) Shape-shifting corals: molecular markers show
485 morphology is evolutionarily plastic in *Porites*. *BMC Evolutionary Biology*, **9**, 45.
- 486 Forsman ZH, Concepcion GT, Haverkort RD *et al.* (2010) Ecomorph or endangered coral? DNA and
487 microstructure reveal hawaiian species complexes: *Montipora dilatata/flabellata/turgescens* & *M.*
488 *patula/verrilli*. *Plos One*, **5**, e15021.
- 489 Fritz U, Hundsdoerfer A, Široký P *et al.* (2007) Phenotypic plasticity leads to incongruence between
490 morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo*
491 *graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia*, **28**, 97–121.
- 492 Fukami H, Budd AF, Paulay G *et al.* (2004) Conventional taxonomy obscures deep divergence between
493 Pacific and Atlantic corals. *Nature*, **427**, 832–835.
- 494 Gaither M, Szabó Z, Crepeau M, Bird C, *et al.* (2011) Preservation of corals in salt-saturated DMSO buffer
495 is superior to ethanol for PCR experiments. *Coral Reefs*, **30**, 329–333.
- 496 Gavery MR, Roberts SB (2014) A context dependent role for DNA methylation in bivalves. *Briefings in*
497 *functional genomics*, **13**, 217–222.
- 498 Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular*
499 *ecology resources*, **5**, 184–186.

- 500 Harrison PL (2011) Sexual Reproduction of Scleractinian Corals. In: *Coral Reefs: An Ecosystem in*
501 *Transition* (eds Dubinsky Z, Stambler N), pp. 59–85. Springer Netherlands, Dordrecht.
- 502 Harvey MG, Judy CD, Seeholzer GF *et al.* (2015) Similarity thresholds used in DNA sequence assembly
503 from short reads can reduce the comparability of population histories across species. *PeerJ*, **3**,
504 e895.
- 505 Herrera S, Shank TM (2016) RAD sequencing enables unprecedented phylogenetic resolution and
506 objective species delimitation in recalcitrant divergent taxa. *Molecular Phylogenetics and Evolution*,
507 **100**, 70–79.
- 508 Jackson JBC, Coates AG (1986) Life cycles and evolution of clonal (modular) animals. *Philosophical*
509 *Transactions of the Royal Society B: Biological Sciences*, **313**, 7–22.
- 510 Jameson S (1997) Morphometric analysis of the Poritidae (Anthozoa: Scleractinia) off Belize. In:
511 *Proceedings of the 8th International Coral Reef Symposium* (eds Lessios H, McIntyre I), pp. 1591–
512 1596. International Society for Reef Studies, Smithsonian Tropical Research Institute, Panama.
- 513 Jameson S, Cairns S (2012) Neotypes for *Porites porites* (Pallas, 1766) and *Porites divaricata* Le Sueur,
514 1820 and remarks on other western Atlantic species of *Porites* (Anthozoa: Scleractinia). *Proceedings*
515 *of the Biological Society of Washington*, **125**, 189–207.
- 516 Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*,
517 **24**, 1403–1405.
- 518 Keshavmurthy S, Yang S-Y, Alamaru A *et al.* (2013) DNA barcoding reveals the coral “laboratory-rat”,
519 *Stylophora pistillata* encompasses multiple identities. *Scientific reports*, **3**, 1520.
- 520 Knowlton N, Weil E, Weigt LA, Guzmán HM (1992) Sibling Species in *Montastraea annularis*, Coral
521 Bleaching, and the Coral Climate Record. *Science*, **255**, 330–333.
- 522 Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in
523 honeybees via DNA methylation. *Science*, **319**, 1827–1830.
- 524 Lajeunesse T (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean
525 coral reefs. *Marine biology*, **141**, 387–400.
- 526 Lin S, Cheng S, Song B *et al.* (2015) The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene
527 expression and coral symbiosis. *Science*, **350**, 691–694.
- 528 Lynch M (2008) Estimation of nucleotide diversity, disequilibrium coefficients, and mutation rates from
529 high-coverage genome-sequencing projects. *Molecular Biology and Evolution*, **25**, 2409–2419.
- 530 Mastretta-Yanes A, Arrigo N, Alvarez N *et al.* (2015) Restriction site-associated DNA sequencing,
531 genotyping error estimation and de novo assembly optimization for population genetic inference.
532 *Molecular ecology resources*, **15**, 28–41.
- 533 NOAA (2015, October 15). Corals. Retrieved March 11, 2017, from
534 <http://www.nmfs.noaa.gov/pr/species/invertebrates/corals.htm>
- 535 Noble D (2015) Evolution beyond neo-Darwinism: a new conceptual framework. *The Journal of*
536 *Experimental Biology*, **218**, 7–13.
- 537 Pante E, Abdelkrim J, Viricel A *et al.* (2015) Use of RAD sequencing for delimiting species. *Heredity*, **114**,
538 450–459.
- 539 Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language.
540 *Bioinformatics*, **20**, 289–290.
- 541 Patterson C, Williams DM, Humphries CJ (1993) Congruence between molecular and morphological
542 phylogenies. *Annual review of ecology and systematics*, **24**, 153–188.
- 543 Paz-García DA, Hellberg ME, García-de-León FJ, Balart EF (2015) Switch between Morphospecies of
544 *Pocillopora* Corals. *The American Naturalist*, **186**, 434–440.
- 545 Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive
546 method for de novo SNP discovery and genotyping in model and non-model species. *Plos One*, **7**,
547 e37135.

- 548 Potter D, Lajeunesse T, Saunders G, et al. (1997) Convergent evolution masks extensive biodiversity
549 among marine coccoid picoplankton. *Biodiversity and conservation*, **6**, 99–107.
- 550 Prada C, DeBiase MB, Neigel JE *et al.* (2014) Genetic species delineation among branching Caribbean
551 Porites corals. *Coral reefs (Online)*, **33**, 1019–1030.
- 552 Putnam HM, Davidson JM, Gates RD (2016) Ocean acidification influences host DNA methylation and
553 phenotypic plasticity in environmentally susceptible corals. *Evolutionary applications*, **9**, 1165–
554 1178.
- 555 Recknagel H, Jacobs A, Herzyk P, Elmer KR (2015) Double-digest RAD sequencing using Ion Proton
556 semiconductor platform (ddRADseq-ion) with nonmodel organisms. *Molecular ecology resources*,
557 **15**, 1316–1329.
- 558 Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression
559 analysis of digital gene expression data. *Bioinformatics*, **26**, 139–140.
- 560 Rosser NL, Thomas L, Stankowski S *et al.* (2017) Phylogenomics provides new insight into evolutionary
561 relationships and genealogical discordance in the reef-building coral genus *Acropora*. *Proceedings.*
562 *Biological Sciences / the Royal Society*, **284**.
- 563 Rützler K, Macintyre I (1982) The habitat distribution and community structure of the barrier reef
564 complex at Carrie Bow Cay, Belize. In: *The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize,*
565 *I. Structure and communities.* , pp. 9–45.
- 566 Santos S, Taylor D, Kinzie R *et al.* (2002) Molecular phylogeny of symbiotic dinoflagellates inferred from
567 partial chloroplast large subunit (23S)-rDNA sequences. *Molecular Phylogenetics and Evolution*, **23**,
568 97–111.
- 569 Sarda S, Zeng J, Hunt BG, Yi SV (2012) The evolution of invertebrate gene body methylation. *Molecular*
570 *Biology and Evolution*, **29**, 1907–1916.
- 571 Schield DR, Walsh MR, Card DC *et al.* (2016) EpiRADseq: scalable analysis of genomewide patterns of
572 methylation using next-generation sequencing. *Methods in ecology and evolution / British*
573 *Ecological Society*, **7**, 60–69.
- 574 Schlöder C, Guzman H (2008) Reproductive patterns of the Caribbean coral *Porites furcata* (Anthozoa,
575 Scleractinia, Poritidae) in Panama. *Bulletin of marine science*, **82**, 107–117.
- 576 Schmidt-Roach S, Lundgren P, Miller KJ *et al.* (2013) Assessing hidden species diversity in the coral
577 *Pocillopora damicornis* from Eastern Australia. *Coral reefs (Online)*, **32**, 161–172.
- 578 Sebens KP (1987) The ecology of indeterminate growth in animals. *Annual review of ecology and*
579 *systematics*, **18**, 371–407.
- 580 Shoguchi E, Shinzato C, Kawashima T *et al.* (2013) Draft assembly of the *Symbiodinium minutum* nuclear
581 genome reveals dinoflagellate gene structure. *Current Biology*, **23**, 1399–1408.
- 582 Skinner MK (2015) Environmental Epigenetics and a Unified Theory of the Molecular Aspects of
583 Evolution: A Neo-Lamarckian Concept that Facilitates Neo-Darwinian Evolution. *Genome Biology*
584 *and Evolution*, **7**, 1296–1302.
- 585 Skinner MK, Guerrero-Bosagna C, Haque MM (2015) Environmentally induced epigenetic
586 transgenerational inheritance of sperm epimutations promote genetic mutations. *Epigenetics*, **10**,
587 762–771.
- 588 Skinner MK, Gurerrero-Bosagna C, Haque MM *et al.* (2014) Epigenetics and the evolution of Darwin's
589 Finches. *Genome Biology and Evolution*, **6**, 1972–1989.
- 590 Smith TA, Martin MD, Nguyen M, Mendelson TC (2016) Epigenetic divergence as a potential first step in
591 darter speciation. *Molecular Ecology*, **25**, 1883–1894.
- 592 Smith G, Smith C, Kenny JG, Chaudhuri RR, Ritchie MG (2015) Genome-wide DNA methylation patterns
593 in wild samples of two morphotypes of threespine stickleback (*Gasterosteus aculeatus*). *Molecular*
594 *Biology and Evolution*, **32**, 888–895.
- 595 Stoddart J (1983) Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine Biology*.

- 596 Todd PA (2008) Morphological plasticity in scleractinian corals. *Biological Reviews of the Cambridge*
597 *Philosophical Society*, **83**, 315–337.
- 598 Weil E (1992) Genetic and morphological variation in Caribbean and eastern Pacific Porites (Anthozoa,
599 Scleractinia). Preliminary results. *Proc 7th int coral Reef Symp.*
- 600 Yang Z, Rannala B (2012) Molecular phylogenetics: principles and practice. *Nature Reviews. Genetics*, **13**,
601 303–314.
- 602 Zemach A, McDaniel IE, Silva P, Zilberman D (2010) Genome-wide evolutionary analysis of eukaryotic
603 DNA methylation. *Science*, **328**, 916–919.

Table 1. *Porites* spp. specimen data. For descriptions of habitat types on the Belize Barrier Reef refer to Rützler & Macintyre (1982).

Sample #	Coordinates	Habitat	Depth (m)	Symbiont clade	Branch diameter (mm)
101	N 16° 48.126', W 88° 4.729'	Lower spur & groove	7.9	C	19.5
103	N 16° 48.294', W 88° 4.666'	Inner reef slope	16.2	C	10.5
104	N 16° 48.294', W 88° 4.666'	Inner reef slope	16.5	C	9
105	N 16° 46.792', W 88° 4.599'	Lower spur & groove	9.8	C	11.5
106	N 16° 46.792', W 88° 4.599'	Lower spur & groove	7.0	C	17
107	N 16° 46.821', W 88° 4.521'	Inner reef slope	12.5	C	11.5
108	N 16° 48.120', W 88° 4.751'	Upper spur and groove	7.0	NA	10.5
109	N 16° 46.821', W 88° 4.521'	Inner reef slope	14.6	C	10
110	N 16° 48.146', W 88° 4.658'	Inner reef slope	14.0	C	14
111	N 16° 48.120', W 88° 4.751'	Upper spur and groove	7.9	C	14
112	N 16° 46.571', W 88° 4.524'	Lower spur & groove	8.5	C	13
114	N 16° 46.798', W 88° 4.511'	Outer ridge	15.5	C	9
115	N 16° 48.354', W 88° 4.998'	Backreef	1.2	C	20
116	N 16° 48.354', W 88° 4.998'	Backreef	1.2	NA	10
117	N 16° 48.354', W 88° 4.998'	Backreef	1.2	A	13
118	N 16° 49.645', W 88° 6.364'	Mangrove	0.6	C	7
121	N 16° 49.645', W 88° 6.364'	Mangrove	0.6	C	7.5
122	N 16° 49.645', W 88° 6.364'	Mangrove	0.6	C	6
123	N 16° 48.181', W 88° 4.885'	Backreef	0.8	C	21
124	N 16° 48.115', W 88° 4.983'	Lagoon/cut	4.6	C	16.5
125	N 16° 46.798', W 88° 4.511'	Outer ridge	16.5	C	10
126	N 16° 46.798', W 88° 4.511'	Outer ridge	17.4	C	26
127	N 16° 48.115', W 88° 4.983'	Lagoon/cut	2.7	A	15
128	N 16° 48.181', W 88° 4.885'	Backreef	0.6	NA	10
129	N 16° 48.115', W 88° 4.983'	Turtlegrass	0.9	A	10.5
130	N 16° 48.181', W 88° 4.885'	Backreef	0.8	C	17.5
131	N 16° 48.181', W 88° 4.885'	Turtlegrass	0.5	A	10

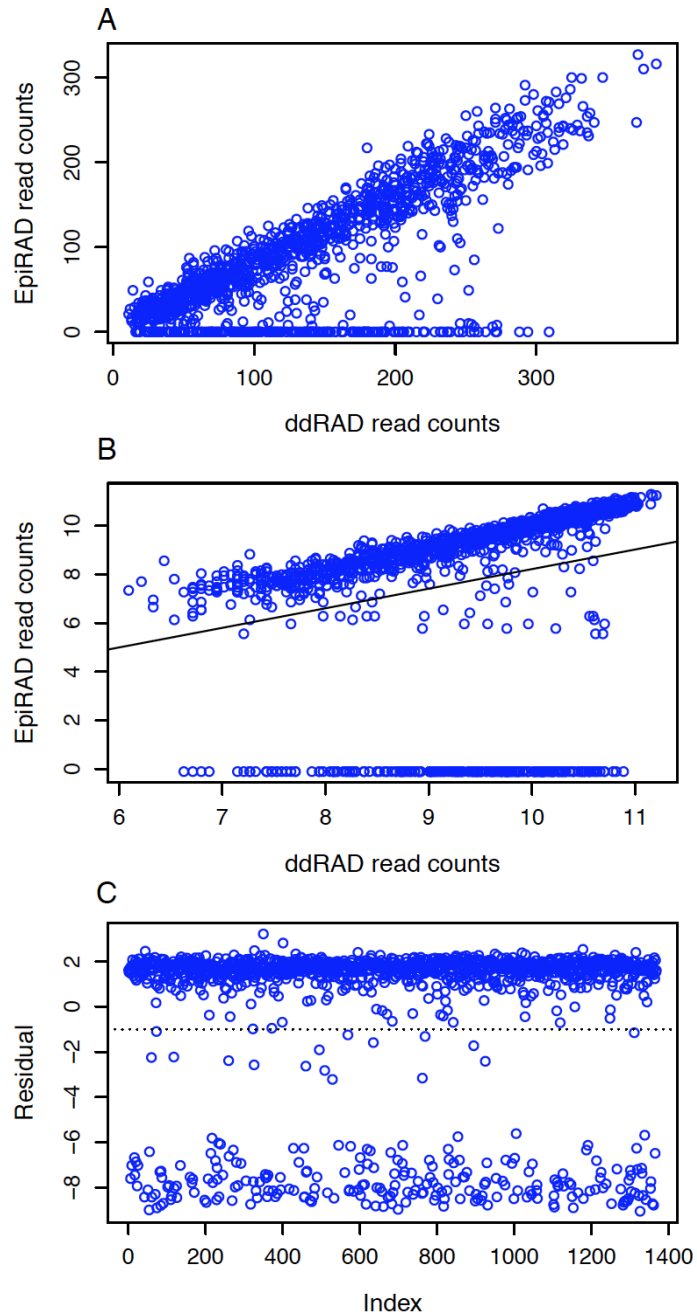


Fig. 1. Determining methylated loci in a representative sample. (A) ddRADseq and EpiRADseq raw read counts for each locus (each point is a locus) were highly correlated except for methylated reads, which were absent or at low abundance in the EpiRAD library and cluster at the bottom of the y-axis. (B) ddRADseq and EpiRADseq read counts after TMM normalization, showing regression line from linear model used for derivation of residuals. (C) Residuals from the linear regression of the normalized data. Non-methylated reads have positive residuals and cluster at the top while methylated reads have negative residuals and cluster at the bottom. The binary dataset derived from these data was based on a residual threshold of ≥ -1 (dotted line) for designating loci as methylated.

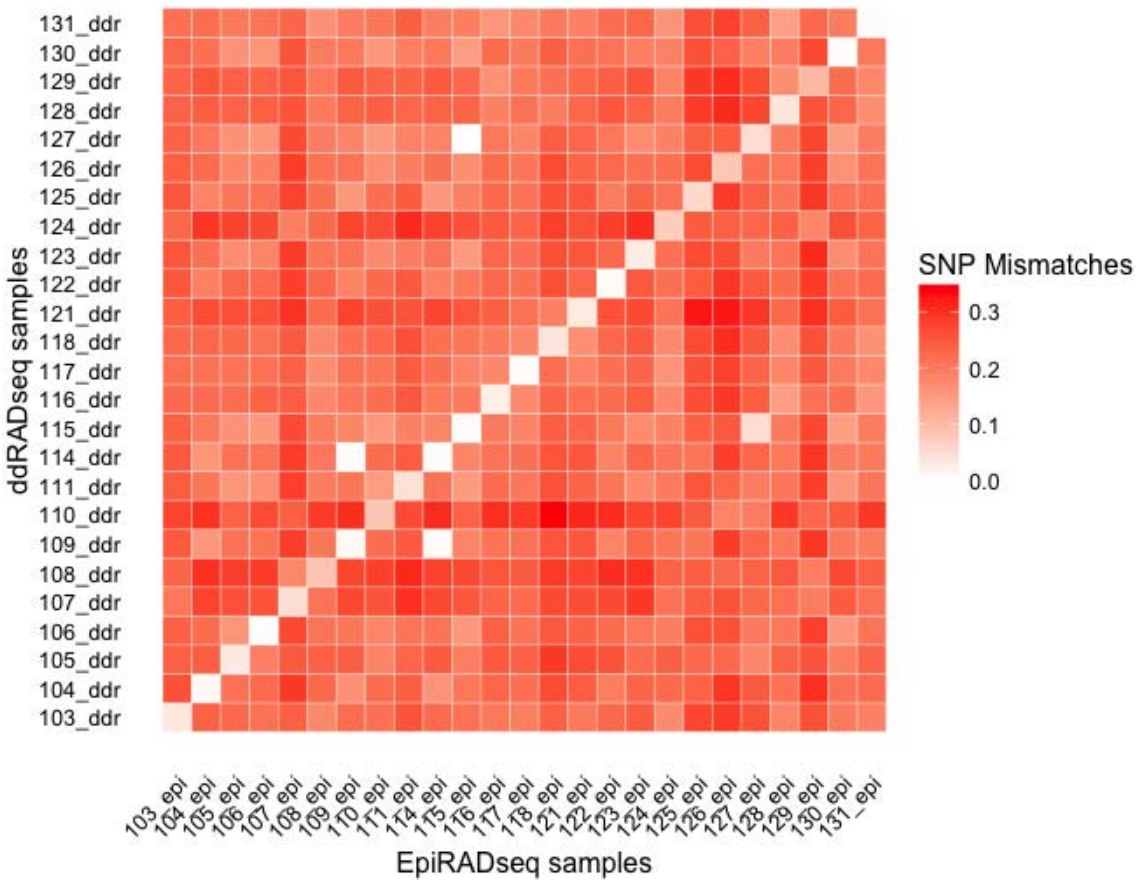


Fig. 2. Pairwise comparisons of SNP mismatches between all 25 individuals with both ddRADseq and EpiRADseq data. The color ramp represents the proportion of SNP mismatches, also known as genetic distance, between pairs. ddRADseq and EpiRADseq data for the same individual were used as technical replicates to estimate the SNP error rate and are expressed along the diagonal. Two pairs of outliers with a low proportion of mismatches (109/104, 115/127) are possible clones.

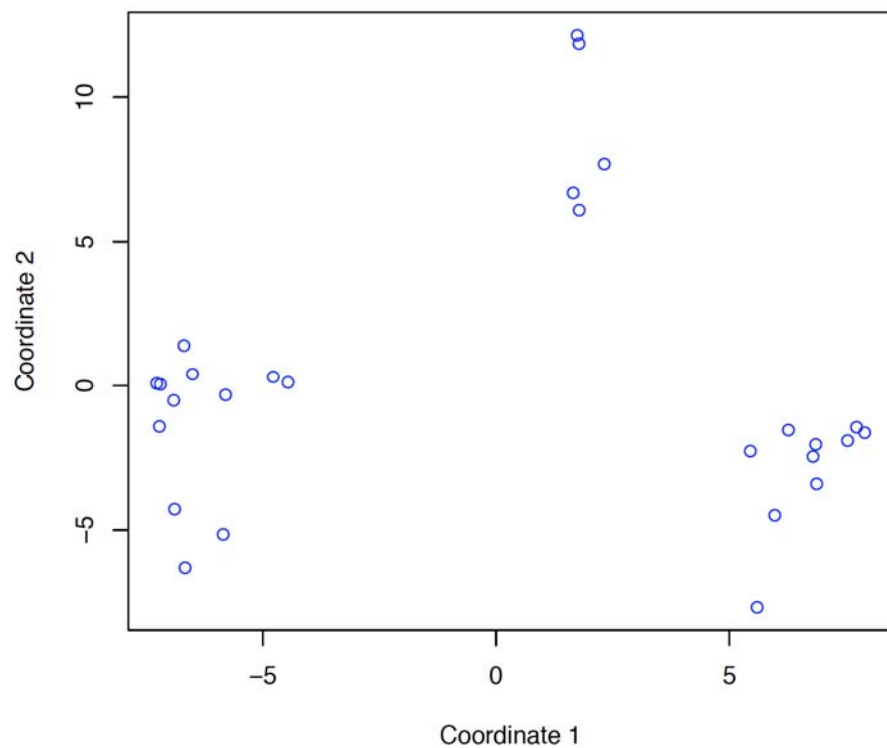


Fig 3. Multidimensional scaling plot of multilocus genotypes derived from SNPs in the *Porites* spp. specimens. Each point represents a specimen. Distances between points represent Euclidean distances projected in two dimensions.

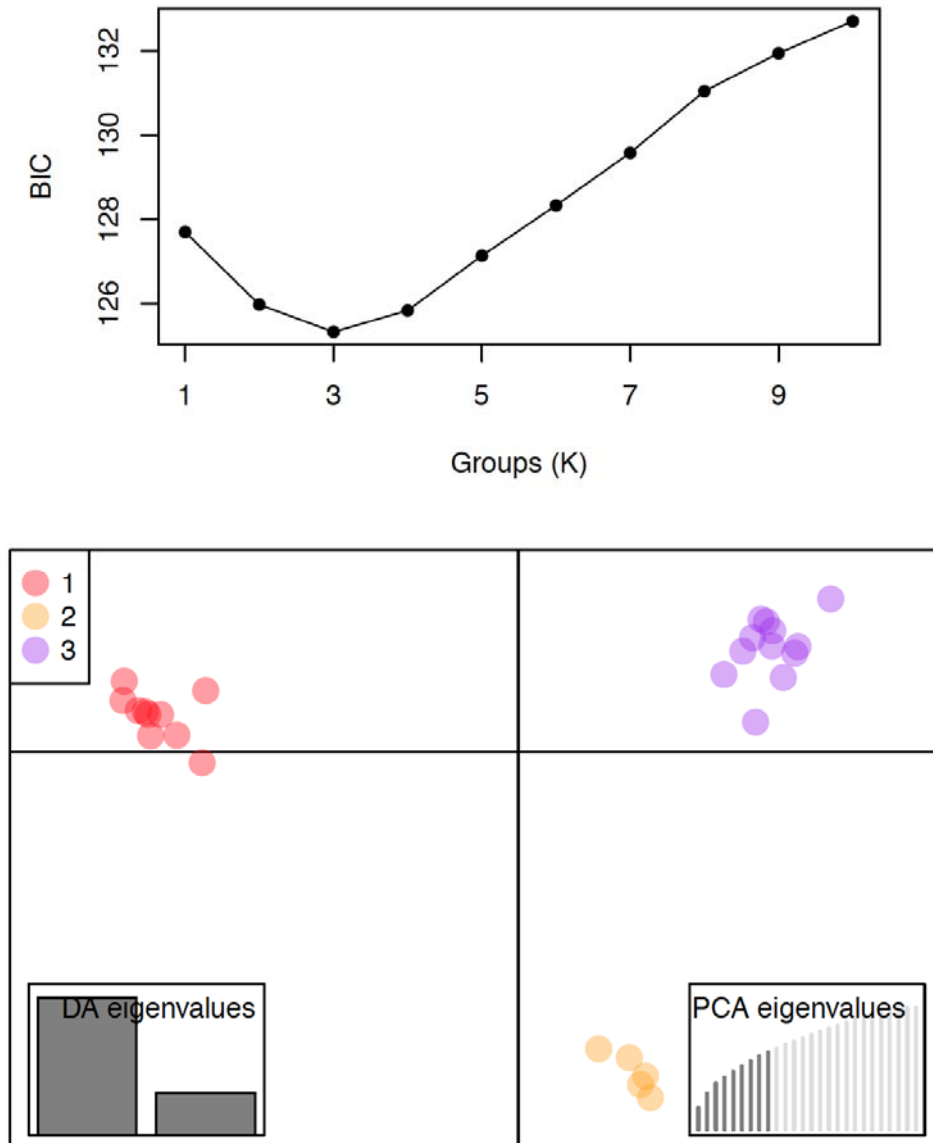


Fig. 4. DAPC analysis of SNP data. (Top) Results of *find.clusters* analysis on SNP data identifying optimal number of groups. $K = 3$ groups was determined to be optimal based on the lowest value of the Bayesian information criterion (BIC). (Bottom) Results of DAPC analysis on SNP data using the three optimal groups defined by *find.clusters*. Left inset shows DA eigenvalues illustrating the relative weight of the two DA axes ($x = \text{DA } 1$, $y = \text{DA } 2$). Right inset shows the relative amount of the variance explained by the 9 principal components used in the DAPC.

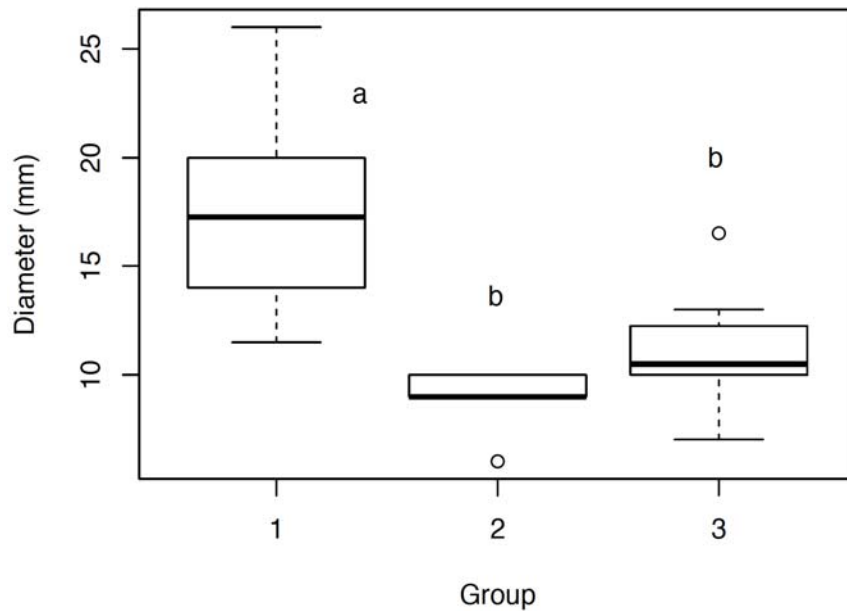


Fig. 5. Comparison of branch diameter in the three *Porites* groups identified by the DAPC analysis in Fig. 4. Boxes show the median (black horizontal bars) plus the 75th percentile and minus the 25th percentile; whiskers show these percentiles plus or minus 1.5 times the interquartile range. Extreme values are shown as points beyond these ranges. Different letters denote groups determined to be significantly different in pairwise t-tests.

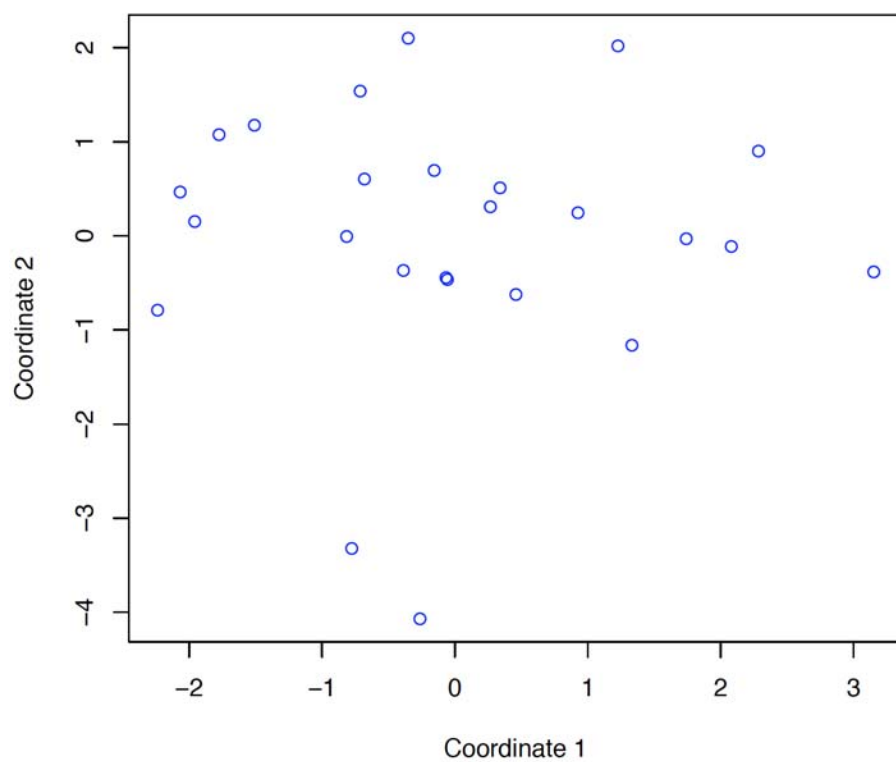


Fig. 6. Multidimensional scaling plot of multilocus epi-genotypes in the *Porites* spp. specimens. Each point represents a specimen. Distances between points represent Euclidean distances projected in two dimensions.

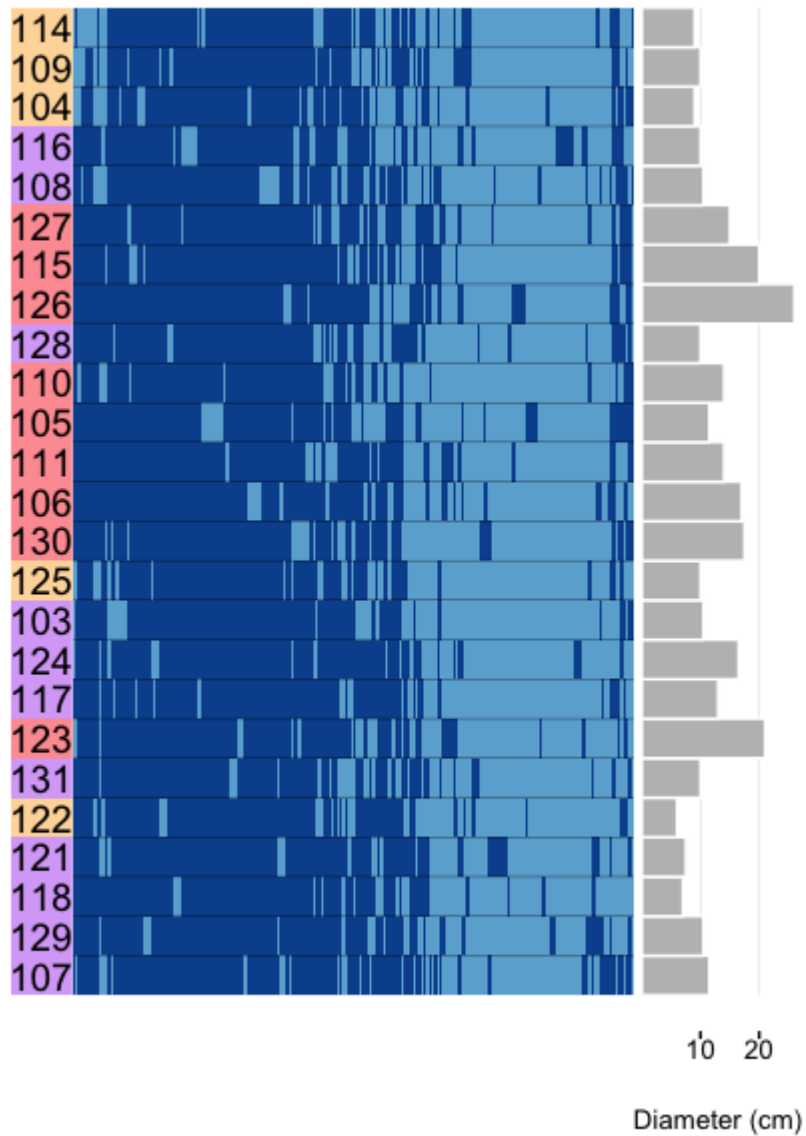


Fig. 7. Heatmap of differentially methylated CpGs. Methylated CpGs are shown in dark blue. Hierarchical clustering was used for ordering of both rows (samples) and columns (CpGs). On the left hand side of the plot, samples are colored by the three SNP groups from the DAPC in Fig. 4. On the right side, a bar plot of the branch diameter of each sample is shown.

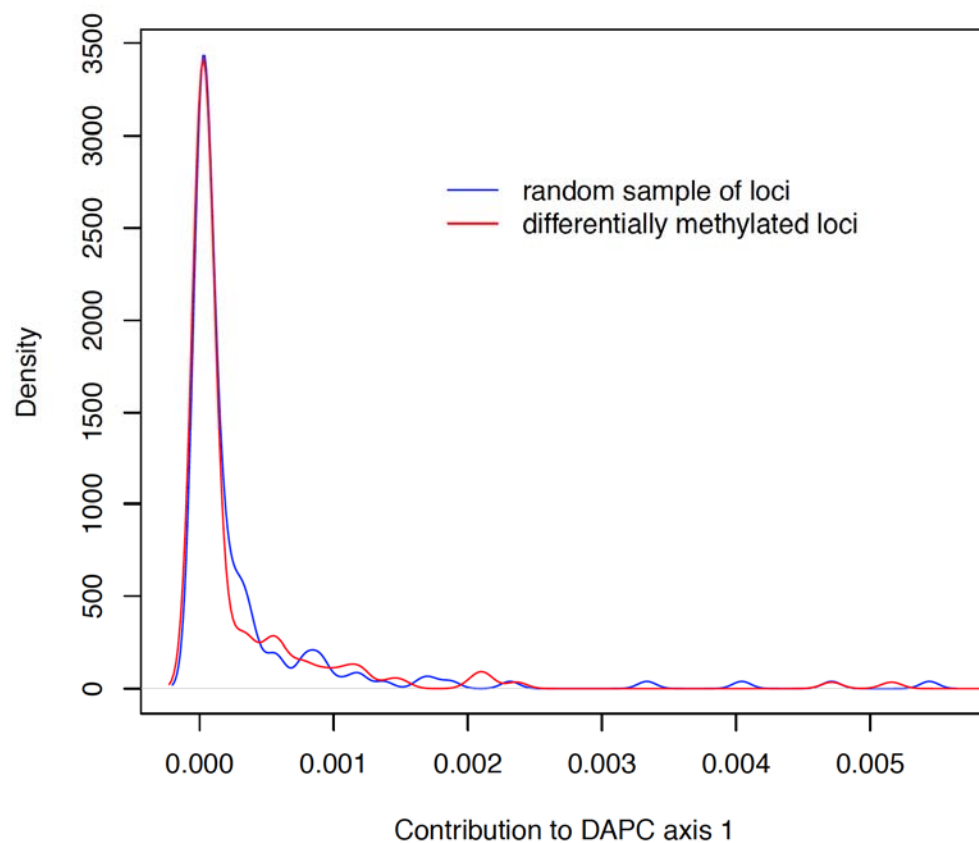


Fig. 8. Distribution of SNP contribution scores to discriminant axis one of the DAPC model in Fig. 3. The red line shows scores of SNPs linked to differentially methylated cut sites on the same loci, while the blue line shows scores from a random sample of the data.

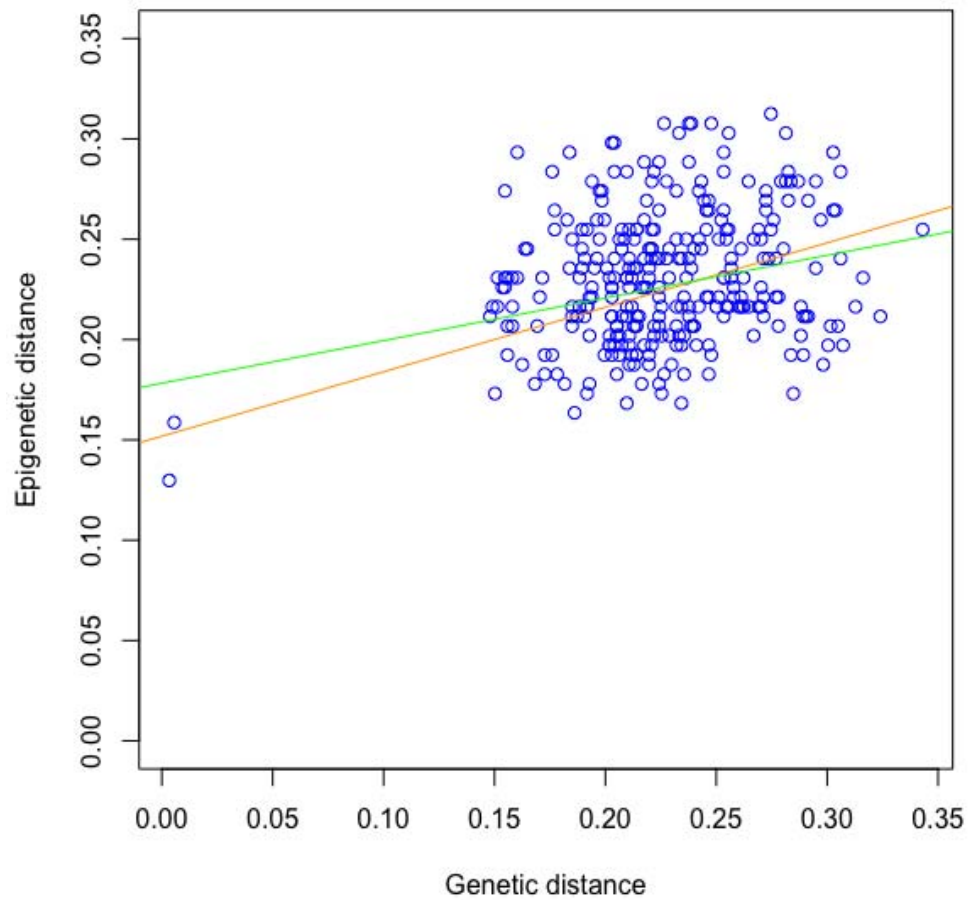


Fig. 9. Association between pairwise genetic distance and pairwise epigenetic distance. Each point represents a unique pairwise comparison between two samples among the 25 samples that had both genetic and epigenetic data (300 unique pairwise comparisons total). For a given pair, genetic and epigenetic distances represent the proportion of mismatches between multilocus genotypes (SNPs) and multilocus epi-genotypes (CpG methylation status), respectively. The two pairs of outliers are samples 109/114 and 127/115; these possible clones showed the lowest levels of both genetic and epigenetic differentiation. Linear regressions of all the data (orange line, $p < 0.001$) and exclusive of the outliers (green line, $p = 0.002$) are shown.