Age and intraspecific diversity of resilient *Acropora* communities in Belize

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abstract

The corals *Acropora palmata* and *Acropora cervicornis* are important Caribbean reef-builders that have faced significant mortality in recent decades. While many studies have focused on the recent demise of these species, data from areas where *Acropora* spp. have continued to thrive are limited. Understanding the genetic diversity, recruitment, and temporal continuity of healthy populations of these threatened *Acropora* spp. and the hybrid they form *("Acropora prolifera")* may provide insights into the demographic processes governing them. We studied three reef sites with abundant *Acropora cervicornis*, *Acropora palmata*, and hybrid *Acropora* populations offshore of Ambergris Caye, Belize at Coral Gardens, Manatee Channel, and Rocky Point. Samples were collected from all three *Acropora* taxa. We used microsatellite markers to determine: 1) genotypic diversity; 2) dominant reproductive mode supporting local recruitment; 3) minimum and maximum genet age estimates of all three acroporids; and 4) the history of hybrid colonization at these sites. We found *Acropora* populations to be highly clonal with local recruitment primarily occurring through asexual fragmentation. We also estimated the ages of 10 *Acropora* genets using recent methodology based on somatic mutation rates from genetic data. Results indicate minimum ages between 62 – 409 years old for *A. cervicornis,* between 187-561 years for *A. palmata*, and between 156-281 years old for the *Acropora* hybrids at these sites. Our data indicate that existing *A. cervicornis, A. palmata,* and *Acropora* hybrid genets persisted during the 1980s Caribbean-wide *Acropora* spp. collapse, suggesting that these sites have been a refuge for Caribbean *Acropora* corals. Additionally, our data suggest that formation of extant hybrid *Acropora* genets predates the widespread collapse of its parent taxa.

introduction

101 The global decline of shallow coral populations in response to increasing sea-surface
102 temperatures, overfishing, rising sea level, disease, eutrophication, sedimentation, and ocean
103 acidification has been well documented (Hughes 1994; Hughes et al. 2003; Bellwood et al. 2004;
104 Hoegh-Guldberg et al. 2007; Kuffner et al. 2015). Caribbean populations of *Acropora*
105 *cervicornis* and *Acropora palmata* (Fig. 1), two of the most important Caribbean reef
106 framework-builders, have experienced up to 98% mortality over just a few decades in response
107 to increasing environmental stress and disease prevalence (Gladfelter 1982; Aronson and Precht
108 2001; Pandolfi 2002; Gardner et al. 2003; Eakin et al. 2010; Randall and van Woesik 2015).
109 Consequently, these were the first two coral species listed as threatened under the US
110 Endangered Species Act (NOAA 2005).

111 Despite Caribbean-wide declines, relatively abundant *A. cervicornis* and *A. palmata*
112 populations have been reported in Honduras (Keck et al. 2005; Purkis et al., 2006), the
113 Dominican Republic (Lirman et al. 2010), Mexico (Larson et al. 2014), Florida (Fort Lauderdale
114 area; Vargas-Angel et al. 2003), and Belize (Peckol et al. 2003; Brown-Saracino et al. 2007;
115 Macintyre and Toscano 2007; Busch et al. 2016). These studies have variably included general
116 habitat surveys, quantification of percent of algal and live coral coverage, *Acropora* colony sizes,
117 number of *Acropora* colonies, fish and invertebrate population densities, image-based spatial
118 characterization, and disease prevalence, but none included genetic data.

119 Genotypic diversity (the number of distinct genotypes or clones per species per site) is a
120 critical factor when assessing the long-term population trends and evolutionary potential of these
121 sites because Caribbean acroporids do not self-fertilize and thus sexual reproduction requires the
122 presence of several genotypes (Szmant 1986; Baums et al. 2005a; Baums et al. 2006; Fogarty et
123 al. 2012). Clonal reproduction is also an important process in many populations. Caribbean

124 acroporids experience frequent breakage due to physical impacts such as storms and anchor
125 damage. Branches are genetically identical to their donor colonies (ie. ramets of the same genet)
126 and can re-attach to the benthos. Once re-attached they grow into new colonies, usually within
127 10s of meters of the donor colony (Highsmith 1982; Lirman 2000, Baums et al. 2006; Williams
128 et al. 2008). This sometimes results in large areas of a reef dominated by a single genotype
129 (Baums et al. 2006). Genotypic diversity of foundation fauna has been associated with overall
130 population health and has been predicted to have a positive relationship with persistence during
131 adverse conditions by providing a diverse set of alleles to the population (Altizer et al. 2003;
132 Reed and Frankham 2003; Reusch et al. 2005, Downing et al. 2012; Williams et al. 2014).
133 However, recent findings show that genotypic diversity in *Acropora* populations does not
134 necessarily increase their sustainability over short time scales as predicted (Williams et al. 2014).
135 Thus, the role of genotypic diversity in persistence remains unclear.

136 *A. cervicornis* and *A. palmata* hybridize to form "*Acropora prolifera"*, the only known
137 scleractinian hybrid in the Caribbean (Fig. 1; van Oppen et al. 2000; Vollmer and Palumbi 2002;
138 Willis et al. 2006*).* The hybrid has not been found in the fossil record (Budd et al. 1994), and has
139 been reported as rare compared to its parents (Lang et al. 1998; Willis et al. 2006; Fogarty, 2007;
140 Fogarty 2012). However, recently it has been observed more frequently and in higher abundance
141 in Florida (Wheaton et al. 2006), Curaçao (Fogarty 2010), Honduras (Keck et al. 2005), the
142 Lesser Antilles (Japaud et al. 2014), and Belize (Fogarty 2010; Fogarty et al. 2012; Richards and
143 Hobbs 2015). The apparent recent increase in the hybrid may suggest either that hybridization is
144 now occurring more frequently, or that hybridization has always occurred, but environmental
145 conditions now favor maturation of hybrid larvae over those of the diminishing parent species
146 (Willis et al. 2006).

147 In this study, we analyzed genetic data of *A. cervicornis, A. palmata,* and the hybrid from
148 Coral Gardens, Manatee Channel, and Rocky Point, sites offshore of Ambergris Caye, Belize,
149 where acroporids are highly abundant (Table 1, Fig. 2). At these sites our specific objectives
150 were to: 1) determine genotypic diversity of *Acropora* taxa; 2) calculate the degree to which
151 sexual vs. asexual recruitment has occurred; 3) estimate the age of *Acropora* genets; and 4)
152 determine the history and nature of hybrid colonization. To do this, we analyzed data from
153 microsatellite loci to identify genets and ramets and subsequently draw inference about the
154 genotypic diversity, as well as the degree of asexual versus sexual reproduction in recruitment to
155 these sites (Baums et al. 2005b). We will refer to a "genet" as an assemblage of genetically
156 identical colonies (clones) that are descendants of a single zygote (Harper 1977, Hughes 1989,
157 Carvalho 1994), and a genet's component colonies as "ramets" (Kays and Harper 1974) or clone
158 mates. Amethod based on accumulation of somatic mutations in clonal organisms (Weiher et al.
159 1999; Eriksson 2000; Ally et al. 2008; de Witte and Stocklin 2010), recently adapted to study
160 *Acropora* genets (Devlin-Durante et al. 2016), was applied to estimate clonal age and assess the
161 history of *Acropora* spp. and *A. prolifera* hybrid persistence at these sites.). We analyzed clonal
162 age data for all *Acropora* taxa to determine persistence and, for the hybrid at our study sites, to
163 elucidate the history of its emergence and spread.

164

165 materials and methods

166 Study Site

167 The three reef sites sampled lie within roughly a 50-km distance between Caye Caulker
168 to the south and the northern end of Ambergris Caye in Belize (Fig. 2). Coral Gardens

(17°50'00.36"N, 87°59'32.45"W, Table 1, Fig. 1) is a shallow-water back reef setting with a maximum depth of ~7m. It is comprised of lagoonal patch reefs dominated by interconnected thickets of *A. cervicornis*, large peripheral colonies of *A. palmata*, and small hybrid colonies. The *Acropora* thickets are interspersed with mixed coral stands dominated by *Orbicella*, *Agaricia*, *Porites*, and *Millepora* species, and areas of sandy bottom. Peckol et al. (2003) and Brown-Saracino et al. (2007) reported live coral cover (all species) in this area exceeding 43%. More recently, Busch et al. (2016) reported an average value of ~30% live *A. cervicornis* coral cover (species specific) in the *Acropora* thickets sampled in this study. The extent of live *Acropora* thickets in the greater Coral Gardens area exceeded 7.5 hectares in 2015, making it one of the largest documented sites of its kind in the Caribbean (Busch et al. 2016).

Manatee Channel (17°47'58.08"N, 87°59'45.57"W; Table 1, Fig. 2) is approximately 1-2 m deep and located just inside the reef crest. It is comprised of patch reefs dominated by thickets of the hybrid and scattered stands of *A. cervicornis* and *A. palmata*. Acroporid thickets and colonies are surrounded by mixed coral stands dominated by *Orbicella*, *Agaricia*, *Porites*, and *Millepora* species and sandy bottom. Manatee Channel has been previously described in the literature by Fogarty (2012) as "north Caye Caulker."

Rocky Point, (18°12'37.95"N, 87°82'60.64"W; Table 1, Fig. 2) approximately 2-3 m depth, is dominated by individual colonies and small patches of the hybrid, both parent species, and some colonies of *Orbicella*, *Porites,* and *Siderastrea* species (Figures 2 and 3). Rocky Point lies within the Bacalar Chico National Park and Marine Reserve and is the only site among our study locations with marine protected area (MPA) status.

Sampling

Live coral samples approximately 1 cm^3 were collected from *Acropora* branch tips using surgical bone cutters. Samples were preserved in 95% ethanol and refrigerated prior to DNA extractions. Coral tissue samples were collected from Coral Gardens in June of 2013 and 2014, and from Manatee Channel and Rocky Point in June 2014. In 2013, coral tissue was collected at Coral Gardens from haphazardly selected *A. cervicornis* colonies (n=60) within the reef boundary, and each observed colony of *A. palmata* (n=78) and the hybrid (n=48) was sampled. Additional sampling in 2014 was designed to increase the likelihood of sampling ramets of the same genet: At Coral Gardens, *A. cervicornis* samples (n=158) were collected from 5-m radius plots in which a center point was designated and samples were collected along 4 randomly generated headings (at least 5° apart) every 1 m for a total of 20 samples per plot. The likelihood that the same colonies were sampled in 2013 and 2014 is small. At Manatee Channel, hybrid samples (n=80) were collected using the same circle plot method, with 4 plots (20 samples per plot) placed at areas of largest abundance and never overlapping. All observed colonies of *A. cervicornis* (n=20) and *A. palmata* (n=15) across the entire site were also sampled. We defined a colony as a continuous, upright entity of living coral attached to a base or seafloor. At Rocky Point the hybrid was sampled haphazardly (n=32) due to irregular patch shape. *A. cervicornis* and *A. palmata* were not sampled at this site.

Genotypic Analysis

Nuclear DNA was extracted from tissue of 3-5 polyps using the Qiagen DNEasy kit (Qiagen, Germany) and protocol with the following modifications: 1) we performed proteinase K digestion overnight at 56°C in a stationary water bath; and, 2) precipitates were dissolved in Buffer AL for 10 minutes 56°C in a stationary water bath. We amplified five microsatellite loci (166, 181, 182, 192, 207) via polymerase chain reaction in two multiplexes following methods

214 described by Baums et al (2005a). Locus 192 does not amplify in *A. cervicornis* and amplifies
215 only the *A. palmata* allele in the hybrid (making the marker appear homozygous). Fragment
216 lengths were determined using an ABI 3730 (Gene Scan 500-Liz, Applied Biosystems) and
217 electropherograms were scored using GeneMapper Software 3.0 (Applied Biosystems).

218 **Data Analyses**

219 *Genotypic diversity*:

220 We used data from highly polymorphic microsatellite loci to identify unique genotypes
221 (genets). Samples were considered ramets of the same genet, i.e. genotypically identical colonies
222 arisen from fragmentation, if they have identical genotypes across all five or four loci, or if they
223 have identical genotypes across all five or four (*A. cervicornis*) loci with an additional allele(s).
224 Because of the smaller number of loci retrieved for *A. cervicornis* the power to distinguish
225 clonemates from closely related individuals is lower for *A.cervicornis* (average *Pgen* = 1.11^{-04})
226 than the other taxa (*A palmata* average *Pgen* = 2.99^{-07}, hybrid average *Pgen* = 2.74^{-05}).
227 Nevertheless, using MLGSim 2.0 (Stenberg et al. 2003) we determined that each of the identified
228 genets, regardless of taxon, was highly likely to be the product of random mating within the
229 population (*Psex,* $p<0.05$ for each genet).

230 In addition, samples with matching genotypes across four or three (*A. cervicornis*) loci
231 were also considered to be ramets of a single genet if the fifth or fourth (*A. cervicornis*) locus
232 only differed by single- or double-step mutations. Such genotypic differences among ramets
233 were assumed to arise via mutations in somatic cell lines that can accumulate with age (Devlin-
234 Durante et al. 2016; see below). We calculated diversity metrics following methods described in

235 Baums et al (2006) as adapted from Stoddart and Taylor (1988). Observed genotypic diversity
236 (G_o) within and among sampling sites for each species was calculated as:

237 $$\frac{1}{\sum_i^k g_i^2}$$

238 Where g_i is the relative frequency of an observed genotype among all (k) genotypes. Expected
239 genotypic diversity under the condition of exclusively sexual reproduction (G_e) is assumed equal
240 to the number of colonies sampled (i.e. sample size, n), since only one sample was collected
241 from each separate colony. Thus, the ratio G_o/G_e provides an index of genotypic diversity within
242 the population that captures the relative degree to which sexual reproduction contributes to
243 recruitment. Genotypic evenness is represented as the ratio of observed genotypic diversity
244 versus genotypic richness (G_o/N_g), where N_g is the number of unique genets in the sample.
245 Finally, we calculated standardized genotypic richness among and within sites relative to sample
246 size (N_g/n). Baums et al. (2006) use these metrics to draw conclusions about the demography of
247 local recruitment, classifying sites with high clonality as "asexual" (most colony recruitment
248 arising from fragmentation) and those with the low clonality as "mostly sexual" (recruitment
249 arising from sexual reproduction and settlement of planulae). We used values for these metrics
250 from this study and those from Baums et al. (2006) to make relative inferences about clonal
251 structure and recruitment of *Acropora* at our sites compared to others in the Caribbean.

Somatic mutations:

Genotypes accumulate somatic mutations over time. Assuming a constant mutation rate, the number of somatic mutations can be used to estimate the age of the genome by applying coalescent methods (Ally et al. 2008). Microsatellites are ideal for estimating genetic divergence because they have high mutation rates and low technical error rates. Hence, they can provide adequate resolution of somatic mutations. A ramet was identified as having a somatic mutation if there was an amplification of an additional allele/s, but alleles were otherwise identical at all 5 or 4 (*A. cervicornis*) loci (Devlin-Durante et al. 2016). While the appearance of an additional allele at a locus (rendering an individual polyploid instead of diploid at this locus) may seem odd, it has been deemed common in *A. palmata*, and predicted to occur due to genomes accumulating somatic duplications over time.

As somatic gene duplications accumulate, multiple copies of the microsatellite locus become available for replication slippage (Devlin-Durante et al. 2016). For example, consider the case where an unmutated ramet had the ancestral genotype of allele 1 = 160 bp, allele 2 = 172 bp (in short: 160/172) at locus 1, whereas a mutated ramet had alleles of 160/172/175 at locus 1. In this instance, the ramet mutated when its second allele duplicated, and the new third allele underwent a single-step mutation, adding a tri-nucleotide repeat. Alternatively, one of the alleles may have mutated in some of the cells or polyps without a prior gene duplication event (Devlin-Durante et al. 2016). Because DNA was extracted from multiple polyps this could also lead to the appearance of three alleles in the electropherograms. A ramet was also identified as having a somatic mutation if it matched a genotype across four or three (*A. cervicornis*) loci, but at the fifth or fouth (*A. cervicornis*) locus only differed by single- or double-step mutations.

Next we required that a genet have at least five ramets, a minimum requirement set by Devlin-Durante et al. (2016) to improve accuracy of aging. The mutation rates established by Devlin-Durante et al. (2016) (lowest=1.542-05 per locus per year, highest=1.195-04 per locus per year) were then applied to the equation described in Ally et al. (2008) to obtain minimum and maximum ages of each genet. The methods for calculating clonal age utilizing genetic divergence are described in Ally et al. (2008). In brief, there are two statistics, π_k and S_k, that describe genetic divergence within a clone (Slatkin 1996). We calculated the average number of pairwise differences per locus for the *kth* clone

$$\pi_k = \frac{1}{\binom{n}{2}} \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} s_{ij}$$

where *n* is the number of sampled ramets and sij is the number of genetic differences between ramet *i* and *j* averaged across loci (Ally et al. 2008). We chose π_k to measure the level of genetic divergence because it has been shown to be more robust to deviations from a starlike phylogeny than S_k (the observed proportion of polymorphic loci) (Ally et al. 2008).

results

289 **Genotypic diversity**

290 Collectively we identified 18 genets among n = 227 individuals of *A. cervicornis*
291 sampled, 31 genets among n = 92 *A. palmata*, and 14 genets among n = 147 hybrid samples
292 (Table 2). Acroporids at our study sites showed a range in genotypic diversity suggesting that
293 local recruitment in some locations was predominantly asexual, i.e. supported by fragmentation,
294 while at other sites recruitment was supported to a greater degree by sexual reproduction (Table
295 2; Fig.3). At Manatee Channel, all hybrid colonies (n=70) shared the same genotype and were
296 therefore ramets of a single genet (Table 2, Fig. 3). Thus, recruitment of existing hybrid colonies
297 at Manatee Channel had occurred only through fragmentation from existing colonies. By
298 contrast, *A. cervicornis* and *A. palmata* at Manatee Channel showed the highest genotypic
299 diversity (G_o/G_e = 0.26 and 0.37 respectively) and evenness (G_o/N_g = 0.62 and 0.69 respectively)
300 among locations[1], indicating that a substantial proportion of local recruitment was via sexual
301 reproduction (following Baums et al. 2006; Table 2, Fig. 3). Coral Gardens was characterized by
302 low genotypic diversity across all three taxa, indicating a high degree of asexual recruitment
303 (Table 2, Fig. 3). It is possible but unlikely that a few individual colonies were re-sampled from
304 2013 to 2014. If so, this could partially contribute to the low genotypic diversity seen at Coral
305 Gardens. Rocky Point, at which only hybrids were sampled, was characterized by low genotypic
306 diversity and predominantly asexual recruitment as well (Table 2, Fig. 3).

307 When samples were pooled across all three sites, both diversity and evenness metrics
308 were relatively low and indicated predominantly asexual recruitment (Table 2, Fig. 3). Overall,
309 *A. palmata* showed the highest genotypic diversity (G_o/G_e=0.0.43), evenness (G_o/N_g=0.15), and

[1] Note that although the calculated value for evenness is highest for *A. prolifera* hybrid at Manatee Channel (Go/Ng = 1), this value is not meaningful as all individuals shared the same genotype.

richness (N_g/N=0.34) across all three species (Table 2, Fig. 3). *A. cervicornis* and the hybrid were comparatively much lower in genotypic diversity and richness (Table 2, Fig. 3).

Somatic Ages

From our 466 *Acropora* samples, we were able to calculate preliminary minimum and maximum age estimates for 10 of the 63 genets (Table 3). We were unable to estimate ages for the remaining 53 genets using this method either because we sampled fewer than five ramets of that genet (44 genets) or because there were no observed somatic mutations in the loci amplified (9 genets). In the latter scenario, we concluded these genets lack somatic mutations because they are of recent origin.

At Coral Gardens, *A. cervicornis* genets were estimated to be between 62-460 years old (two genets), 179-1337 years old (one genet), 393-2931 years old (one genet), and 409-3052 years old (one genet) (Table 3). *A. palmata* genets at this site were estimated to be between 187-1397 years old (one genet), 219- 1636 years old (one genet), and 561-4191 years old (one genet) (Table 3). A single hybrid genet at this site was estimated to be between 281-2096 years old (Table 3). At Manatee Channel, no somatic mutations were observed at these loci for any *A. cervicornis*, *A. palmata,* and hybrid genets. At Rocky Point, we were able to estimate the age for one hybrid genet to be between 156-1164 years old (Table 3).

discussion

In this study we had the rare opportunity to analyze large living stands of ecologically significant *Acropora* spp. and the hybrid they form, at a time when the existence of acroporids in the Caribbean is in peril. From our collection and analyses of 466 samples, we determined that acroporids offshore of Ambergris Caye, Belize were mostly clonal, with little sexual recruitment. Although genetic diversity was low, we found that both veteran and more recent genets were represented in all three acroporid taxa at these sites. Furthermore, our data suggested that hybrid colonization at some of these sites pre-dated the widespread Caribbean collapse of its parent acroporid species.

The genotypic diversity and evenness of acroporid populations at our study sites was low overall, with the exception of *A. palmata* and *A. cervicornis* at Manatee Channel, which exhibited a relatively high degree of genotypic diversity and evenness. From this information, we deduced that there is minimal input from local sexual reproduction or from 'upstream' source populations for all three Caribbean acroporids at Coral Gardens and the hybrid at Manatee Channel and Rocky Point. Only the Manatee Channel *A. palmata* and *A. cervicornis* samples in our study indicated substantive inputs from sexual reproduction. This may be due to localized variation in current patterns or subsequent differences in delivery or retention of planulae at these sites. A similar prevalence of asexual recruitment was observed by Baums et al. (2006) at a nearby *A. palmata* population in Chinchorro, Mexico (~49 km north of our northernmost site, Rocky Point), as well as in most other *A. palmata* populations at Western Caribbean sites (Baums et al. 2005b). Our results further validate their claim that Western Caribbean *Acropora* populations exhibit little sexual reproduction.

Previous studies from other abundant *Acropora* sites predicted that large populations were critical sources of propagules that might contribute to genetic diversity and coral coverage at neighboring sites (Keck et al. 2005; Zubillaga et al. 2008; Lirman et al. 2010, Vargas-Angel et al. 2003). However, because Caribbean acroporids usually do not self-fertilize, production of sexual offspring is a function of the number of genotypes present and not just a function of colony density or size (Levitan and McGovern 2005; Fogarty et al. 2012; Williams et al. 2014). Our study suggests that while Coral Gardens may be the largest extant acroporid site in the Caribbean (Busch et al., 2016), this may not translate into successful downstream sexual recruitment. Only observation of spawning and gamete-crossing experiments at these sites would provide additional insight into the production of gametes by these stands.

Although the genotypic diversity is generally low, our age estimates of acroprid genets vary. Using a new genetic aging technique, we were able to estimate age ranges for 10 genets in this study. While the uncertainty in the age range estimates may be considerable (Devlin-Durante et al. 2016), we believe that the more conservative minimum age estimates may be valuable at least in distinguishing new from 'older' genets. Our data suggested that both new (minimal somatic mutations) and veteran (minimum ages from 62-561 years) genets co-exist at these sites and that veteran Belize acroporids pre-date the widespread Caribbean collapse of acroporids. Future determination of microsatellite mutation rates in *Acropora* coral would help to narrow the currently rather wide range for age estimates.

Two of the veteran genets we identified are from hybrid samples. We estimated that these hybrid genets have existed for at least 156 and 281 years, respectively. It is thought that the hybrid only recently expanded its presence in Caribbean reef areas (Willis et al. 2006, Fogarty 2010, Richards and Hobbs 2015); however, no data on the ages of extant hybrid genets exist in

372 the literature. Here we show that at least a few hybrid genets originated before the Caribbean
373 wide acroporid die-off event in the 1980s. It is unclear whether the persistence of *A. cervicornis*,
374 *A. palmata*, and hybrid veteran genets at our sites is a function of: 1) their innate ability to
375 rebound from disturbances (Eriksson 2000; Riegl et al. 2009); 2) whether certain colonies simply
376 got lucky and survived environmental changes at random; or 3) whether environmental or
377 oceanographic conditions at this site have been particularly conducive to acroporid persistence at
378 these sites.

379 Our study sites are home to acroporid populations observed in high abundances in a time
380 when they are facing Caribbean-wide decline. The existence of *Acropora* populations like these
381 provides hope that perhaps these species and their hybrid can continue to persist. However,
382 without analyzing the demographic processes of such populations, their potential resilience
383 cannot be known. Here, we studied genotypic diversity and age in unison. The genotypic
384 diversity of acroporids at our study sites was relatively low, which when considered alone does
385 not bode well with respect to the potential of these populations to combat future environmental
386 stresses (Schmid 1994; Steinger et al. 1996; Reusch et al. 2005; Garcia et al. 2008; Williams et
387 al. 2008). However, we also know that some of the genets in these populations are relatively old
388 and may have survived past environmental stresses, perhaps due to increased (and unknown)
389 fitness attributes. Such veteran populations have hypothesized potential to expand (Noss 2001;
390 Taberlet and Cheddadi 2002; Loarie et al. 2008; Keppel et al. 2012). Thus, we are left with two
391 contradicting conclusions: the populations have low genotypic diversity so they are vulnerable to
392 environmental change, and the populations have veteran genets whose past fitness may indicate
393 that they will continue to survive in the future. In either scenario, these sites have potential to be
394 valuable resources to *Acropora* spp. recovery initiatives, and should be investigated further. We

hope that the methods applied here can be useful to future studies that aim to identify sites of conservation priorities.

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572

573 figure captions

574 **Fig. 1** Caribbean *Acropora* species: A) *Acropora cervicornis*, or Staghorn coral; B) *A. palmata*,
575 or Elkhorn coral; C) "*A. prolifera",* the hybrid of *A. cervicornis* and *A. palmata*, also known as
576 "Fused Staghorn" coral; all from Coral Gardens, Belize.

577 **Fig. 2** Map of Belize, inlayed with map of study sites Coral Gardens, Manatee Channel, and
578 Rocky Point. Reef area is depicted in blue.

579 **Fig. 3** Comparison of genotypic diversity and evenness metrics calculated in our study with
580 those calculated by Baums et al (2006) for Caribbean populations of *A. palmata*, with inference
581 on demography of local recruitment. Values for our locations are labeled by site (MC, *Manatee*
582 *Channel*; CG, *Coral Gardens*; RP, *Rocky Point*; BZ, Belize i.e. all sites combined) and color
583 coded by species (blue, *A. cervicornis*; red, *A. palmata*; green, hybrid). Data from Baums et al.
584 (2006) are shown as unfilled circles. Ellipses grouping sites according to relative degree of
585 sexual versus asexual recruitment are redrawn from Figure 4 in Baums et al. (2006).