# Comparative genomics of two closely related coral species with different spawning seasons reveals genomic regions possibly associated with gametogenesis

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## Abstract

Marine invertebrates release their gametes at an optimal time to produce the next generation. In reef-building scleractinian corals, synchronous spawning is essential for reproductive success. Molecular mechanisms of scleractinian gametogenesis have been studied; however, the mechanism by which coral gametes mature at specific times has yet to be discovered. The present study focused on two *Acropora* species with different spawning seasons. In Okinawa, Japan, *Acropora digitifera* spawns from May to June, whereas *Acropora* sp. 1 spawns in August. Comparative genomic analyses revealed that 39 candidate genes are differentiated between the two species, suggesting a possible association with timing of gametogenesis. Among candidate genes, we identified an *Acropora* sp. 1-specific amino acid change in gene *WDR59*, one of the components of a mTORC1 activator, GATOR2. Since regulation of gametogenesis by mTORC1 is widely conserved among eukaryotes, the difference in timing of gamete maturation observed in the two *Acropora* species may be caused by a substitution in WDR59 that slightly affects timing of mTORC1 activation via GATOR2. In addition, this substitution may lead to reproductive isolation between the two species, due to different spawning periods. Thus,

we propose that A. digitifera and Acropora sp. 1 species pair is an effective model for studying coral speciation and understanding the molecular mechanisms that control coral spawning timing.

***Significance statement*** *(required)*:

For successful coral reproduction, conspecific corals must spawn synchronously. Gamete production initiates coral spawning. Regulation of gamete maturation by a protein complex, mTORC1, is widely conserved among organisms, but little is known about it in cnidarians. In this study, we analyzed genomes of two closely related *Acropora* species with different spawning months, May/June and August. Our analyses revealed that 39 genes are genetically differentiated between the two species. One of these is a component of mTORC1 activator, suggesting that this gene may be associated with the difference in spawning times of these two species.

*Key words: mTORC1, oogenesis, cnidaria*

## Introduction

In marine invertebrate reproduction, gametes are released into the water to be fertilized externally (spawning) (Mercier and Hamel 2010). Spawning occurs at an optimal time to produce the next generation (Forrest and Miller-Rushing 2010). Since fertilization in seawater can easily fail due to sperm dilution and other factors, marine organisms have evolved mechanisms such as synchronized spawning (Fukami, et al. 2003; Levitan, et al. 2004).

Spawning in reef-building, scleractinian corals is one of the most massive reproductive events on earth. In the Great Barrier Reef, most corals release their gametes once a year for a few nights (Harrison, et al. 1984). For example, over 100 coral species spawn in the Great Barrier Reef between the full and last quarter moon in late spring (Babcock, et al. 1986). Synchronous spawning within species is essential for fertilization because dilution and aging of sperm reduce fertilization success (Fukami, et al. 2003; Levitan, et al. 2004). In synchronous spawning, gametes spawned by different species are present in the water and may encounter each other. However, many *Acropora* species exhibit species specificity in gamete compatibility (Hatta, et al. 1999; Willis, et al. 1997),

and interspecific hybridization rarely occurs in the Indo-Pacific (Hatta and Matsushima 2008; Isomura, et al. 2013).

Environmental cues act on corals to regulate spawning months, days, and times (Babcock, et al. 1986; Baird, et al. 2009). Temperature strongly influences gamete maturation (Baird, et al. 2009), and in several coral species, spawning has become asynchronous, due to effects of recent climate change (Shlesinger and Loya 2019). Therefore, understanding mechanisms of synchronous gamete maturation will help us estimate the impact of climate change on coral reproduction and restoration using coral seedlings produced from gametes (Suzuki, et al. 2020). Gametogenesis in corals has been studied in the field (Harrison 2011) and by molecular biological approaches (Chiu, et al. 2020; Shikina and Chang 2016). However, the mechanism by which coral gametes mature at specific times has yet to be identified.

In the Indo-Pacific region, including Okinawa, Japan, the genus *Acropora* comprises the largest number of coral species (Veron 2000). In Okinawa, most *Acropora* species spawn around the full moon in May or June, with a few species spawning several months later (Hayashibara, et al. 1993). One species that spawns later is *Acropora* sp. 1.

This species was initially classified as *Acropora digitifera* (Wallace 1999); however, the two are now recognized as separate species, due to differences in morphology and spawning time (Hayashibara and Shimoike 2002; Nakajima, et al. 2012; Ohki, et al. 2015). *Acropora* sp. 1 has a flatter colony shape and shorter branches than *A. digitifera* (Hayashibara and Shimoike 2002; Ohki, et al. 2015). *Acropora* sp. 1 tends to inhabit reef edges with faster (offshore) currents than *A. digitifera*. In addition, in Okinawa, *A. digitifera* spawns from May to June, whereas *Acropora* sp. 1 spawns in August (Hayashibara and Shimoike 2002). Gametes of both species can cross-fertilize as indicated by artificial fertilization experiments (Ohki, et al. 2015). Under natural conditions, however, the two species do not interbreed because of the different spawning months (Ohki, et al. 2015).

Advances in analysis of genomic data with next-generation sequencers have revealed the genetic basis of specific traits (Ellegren and Sheldon 2008). In particular, comparative genomic analyses between genetically close species have identified genomic regions associated with their phenotypic differences (Poelstra, et al. 2014; Turner, et al. 2005). So far, genomes of various corals have been sequenced (Fuller, et al. 2020;

98 Shinzato, et al. 2021; Shinzato, et al. 2011; Voolstra, et al. 2015), and population genomic
99 approaches have identified loci associated with heat tolerance (Smith, et al. 2022).
100 Comparative genomic analysis has yet to be conducted to identify genomic regions
101 associated with differences in coral spawning timing due to the lack of closely related
102 species pairs to compare.

103 In this study, we performed a comparative genomic analysis between *A.*
104 *digitifera* and *Acropora* sp. 1 to identify genomic regions likely involved in trait
105 differences between them. We expected that *A. digitifera* and *Acropora* sp. 1 were
106 genetically closely related based on analysis of short sequences (Nakajima, et al. 2012)
107 and their fertilization ability (Ohki, et al. 2015). Therefore, we determined the genome
108 sequences of both species. This comparative genomic analysis identified genomic regions
109 likely associated with differences in their spawning times. Since differences in spawning
110 time can lead to reproductive isolation, these species will be a useful model to study coral
111 speciation and to understand molecular mechanisms that regulate spawning time in corals.
112

113 **Results**

114 ***The spawning month of Acropora sp. 1***

115 We collected 16 *Acropora* sp. 1 colonies during 2018-2020 at Sesoko and Bise, Okinawa,

116 Japan (Fig.1), and observed mature oocytes or spawning in August (Table S1). This later-

117 spawning month of *Acropora* sp. 1 is consistent with previous observations (Hayashibara

118 and Shimoike 2002; Nakajima, et al. 2012; Ohki, et al. 2015).

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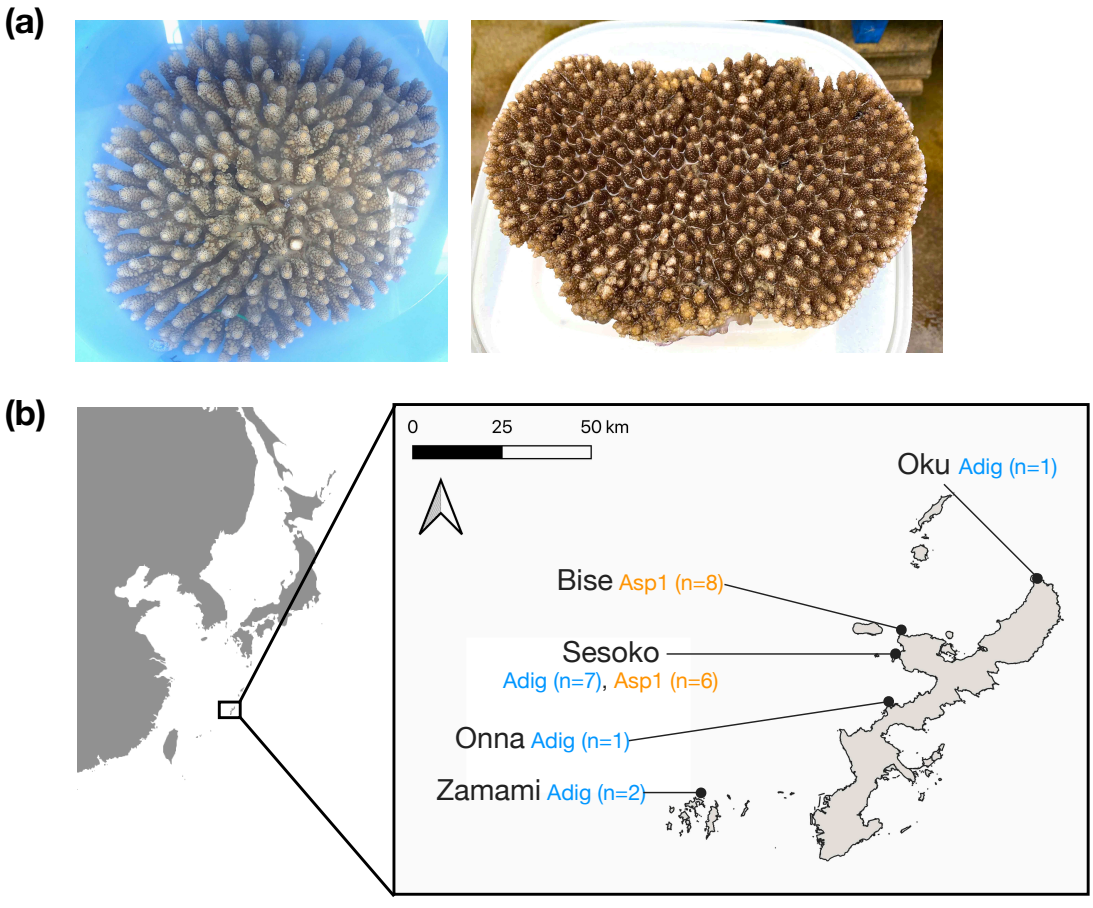



120

121 Figure 1

122 ***Phylogenetic tree and principal component analysis***

123 We sequenced genomes from 16 colonies of *Acropora* sp. 1 (Table S1). Then, we mapped
124 these reads to the *A. digitifera* whole-genome assembly ver. 2.0 (Shinzato, et al. 2021)
125 and selected 14 colonies with coverage >10x for analyses. In addition, we downloaded
126 genomic sequence data for 11 colonies of *A. digitifera* and one colony each of 15 other
127 *Acropora* species from the DNA Data Bank of Japan (DDBJ) and mapped them as well.

128 First, we investigated the genetic relationship between *A. digitifera* and
129 *Acropora* sp. 1. We extracted 885,405 biallelic single-nucleotide polymorphisms (SNPs)
130 from mapping data of 17 species using our criteria (Materials and Methods). With these
131 SNPs, we constructed a phylogenetic tree (Figure 2a). *Acropora digitifera* and *Acropora*
132 sp. 1 colonies formed a monophyletic clade. In this clade, *A. digitifera* and *Acropora* sp.
133 1 colonies each formed monophyletic clades. A monophyletic *A. digitifera*/*Acropora* sp.
134 1 clade formed a monophyletic clade with *A. acuminata, A. microphthalma,* and *A. nasuta*
135 (Fig. 2a). Using five species in this monophyletic clade, we performed principal
136 component analysis (PCA). We used 80,490 SNPs extracted from *A. digitifera*, *Acropora*
137 sp. 1, and three out-group species (*A. acuminata, A. microphthalma*, and *A. nasuta*). The

three out-group species were separated along the PC1 axis from *A. digitifera* and *Acropora*. sp. 1 colonies, forming distinct genetic clusters. *Acropora digitifera* colonies were separated from *Acropora* sp. 1 along the PC2 axis (Fig. 2b). Among *Acropora* sp. 1 colonies, two (Colony IDs: Asp1B1906 and Asp1B1904) were separated from other *Acropora* sp. 1 colonies by PCA. In addition, these two colonies (Colony IDs: Asp1B1906 and Asp1B1904) formed a single clade with high bootstrap support in the phylogenetic tree (Fig. 2a). These two colonies were sampled from the same sites as other colonies sampled in the same year, indicating no geographic isolation.

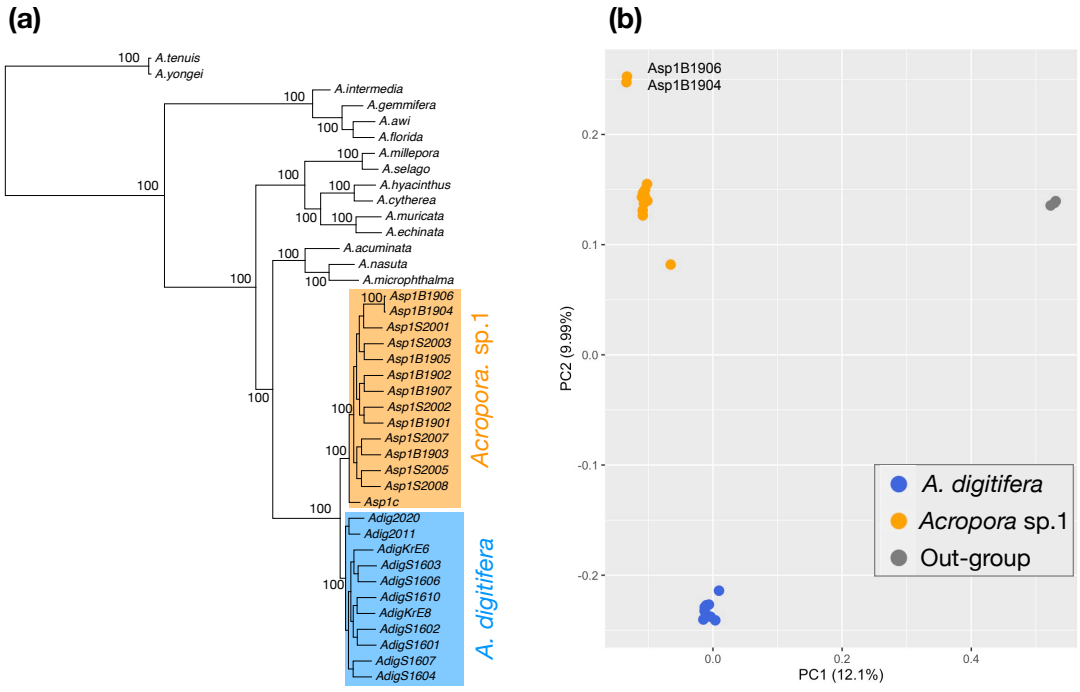



Figure 2

148 ***Highly differentiated regions between A. digitifera and Acropora sp. 1***

149 Since phylogenetic analysis indicated that *A. digitifera* and *Acropora* sp. 1 are closely
150 related, the degree of differentiation between the two species was calculated ($F_{ST}$) (Weir
151 and Cockerham 1984) using 1,459,328 SNPs. The $F_{ST}$ (Weir and Cockerham 1984) value
152 across the genomes of these two species was 0.10225. This is comparable to the genetic
153 divergence of species pairs used in comparative genome analysis in previous studies
154 (Ellegren, et al. 2012; Geraldes, et al. 2011; Nadeau, et al. 2013). Despite low
155 differentiation throughout their genomes, genomic regions responsible for differences in
156 traits between *A. digitifera* and *Acropora* sp. 1 are expected to differ in the two species.
157 To extract differentiated regions, we performed a sliding window analysis of 10 kb in
158 1 kb increments between *A. digitifera* and *Acropora* sp. 1. Genomic regions with the top
159 0.1% $F_{ST}$ (Hudson, et al. 1992) values ($F_{ST}$ >0.6157) in each 10 kb window were then
160 selected. We further selected windows containing differentiated SNPs (Materials and
161 Methods) from the top 0.1% $F_{ST}$ (Hudson, et al. 1992) windows. When these windows
162 overlapped, they were combined. As a result, 34 genomic regions, called highly
163 differentiated regions (HDRs) (Table S2), were extracted from the whole genome.

164

165 ***Genes in highly differentiated regions***

166 In the HDRs, 39 genes harbor differentiated SNPs. We performed a Blast search using
167 these 39 genes as queries (Figure S1) and found that 23 of them are similar to high-quality
168 manually annotated genes (Table 1). Ten genes are similar to genes with automated
169 annotations related to known genes (Table S3). Four genes are similar to uncharacterized
170 genes (Table S3), and two genes have no similarity to any others in the NCBI nucleotide
171 database.

172 Table 1

| Gene ID | Protein Name (Uniprot Reviewed) | Identity (%) | E-value | Entry |
|---|---|---|---|---|
| adig_s0002.g57.t1 | Growth/differentiation factor 11 | 29.6 | 2.6E-35 | Q9Z217 |
| adig_s0002.g67.t1 | Nipped-B-like protein A | 26.1 | 5.1E-15 | F5HSE3 |
| adig_s0002.g68.t1 | Kelch-like protein diablo | 41 | 5.9E-122 | B0WWP2 |
| adig_s0013.g139.t1 | Transcription factor Atoh1 | 27.9 | 9.6E-08 | P48985 |
| adig_s0013.g67.t1 | Stromelysin-2 | 28.6 | 6.3E-17 | O55123 |

| | | | | |
|---|---|---|---|---|
| adig_s0015.g100.t1 | RNA-binding motif protein, X | 35 | 3.8E-40 | Q6IRQ4 |
| adig_s0015.g101.t1 | Glycine-rich RNA-binding protein 2 | 58.1 | 9.5E-52 | Q99070 |
| adig_s0020.g143.t1 | - | - | - | - |
| adig_s0028.g24.t1 | - | - | - | - |
| adig_s0034.g150.t1 | Collectin-12 | 49.2 | 3.9E-07 | Q4V885 |
| adig_s0034.g151.t1 | Collagen alpha-1(XVI) chain | 46.2 | 1.3E-05 | Q07092 |
| adig_s0042.g174.t1 | Inactive tyrosine-protein kinase transmembrane receptor ROR1 | 31.5 | 2.3E-99 | Q9Z139 |
| adig_s0046.g74.t1 | - | - | - | - |
| adig_s0048.g28.t1 | GATOR complex protein WDR59 | 42.1 | 0 | Q6PJI9 |
| adig_s0048.g29.t1 | Guanine nucleotide-binding protein G(o) subunit alpha | 72.6 | 0 | P08239 |
| adig_s0058.g4.t1 | - | - | - | - |
| adig_s0064.g90.t1 | Putative nucleotidyltransferase MAB21L1 | 23.4 | 3.8E-06 | Q0V9X7 |
| adig_s0064.g91.t1 | Survival of motor neuron-related-splicing factor 30 | 50.5 | 7.8E-20 | Q4QQU6 |
| adig_s0064.g92.t1 | Probable N-acetyltransferase CML1 | 35.9 | 4.3E-23 | Q9JIZ0 |
| adig_s0087.g2.t1 | Zinc finger protein 862 | 26.6 | 2E-06 | O60290 |
| adig_s0087.g3.t1 | Pogo transposable element with KRAB domain | 38.7 | 1.3E-91 | Q9P215 |

| | | | | |
|---|---|---|---|---|
| adig_s0087.g8.t1 | - | - | - | - |
| adig_s0091.g43.t1 | - | - | - | - |
| adig_s0091.g44.t1 | - | - | - | - |
| adig_s0096.g12.t1 | Brevican core protein | 37.8 | 1.4E-15 | P55068 |
| adig_s0113.g15.t1 | - | - | - | - |
| adig_s0125.g55.t1 | - | - | - | - |
| adig_s0130.g47.t1 | - | - | - | - |
| adig_s0150.g20.t1 | Peroxidasin | 36.8 | 3.9E-92 | A4IGL7 |
| adig_s0150.g21.t1 | - | - | - | - |
| adig_s0164.g12.t1 | - | - | - | - |
| adig_s0164.g14.t1 | - | - | - | - |
| adig_s0164.g40.t1 | Isoform 5 of Microtubule-actin cross-linking factor 1 | 24.1 | 0 | Q9UPN3-4 |
| adig_s0164.g41.t1 | - | - | - | - |
| adig_s0171.g21.t1 | Collagen alpha chain | 47.6 | 0 | B8V7R6 |
| adig_s0181.g15.t1 | - | - | - | - |
| adig_s0181.g16.t1 | - | - | - | - |
| adig_s0184.g19.t1 | Microtubule-associated proteins 1A/1B light chain 3A | 62.1 | 9E-46 | Q2HJ23 |
| adig_s0184.g20.t1 | Isoform 2 of Rho GTPase-activating protein 39 | 58.1 | 1.3E-147 | P59281-2 |

We surveyed the literature related to the annotated genes. Amino acid

175 sequences of two genes (Gene IDs: adig_s0034.g151 and adig_s0171.g21) were similar
176 (Table 1) to collagen alpha chain, which is associated with skeletogenesis in *Acropora*
177 corals (Ramos-Silva, et al. 2013). The amino acid sequence of another gene (Gene ID:
178 adig_s0048.g28) showed similarity (see Table 1) to a gene encoding WD repeat-
179 containing protein 59 (WDR59).

180 To identify genes whose function is affected by differentiated SNPs, we
181 identified amino acid changes between the two species caused by differentiated SNPs.
182 Among 39 genes, 14 had at least one amino acid change between *A. digitifera* and
183 *Acropora* sp. 1 (Table S4). Compared with the *A. digitifera* reference genome, *Acropora*
184 sp. 1 had three amino acid changes in *WDR59* (Gene ID: adig_s0048.g28) (Table S4).
185 WDR59 is a component of the GTPase-activating protein toward Rags (GATOR)
186 complex, GATOR2 (Bar-Peled, et al. 2013). In *Drosophila*, GATOR2 controls meiotic
187 entry and oocyte development (Wei, et al. 2014). Therefore, we focused further on this
188 gene.

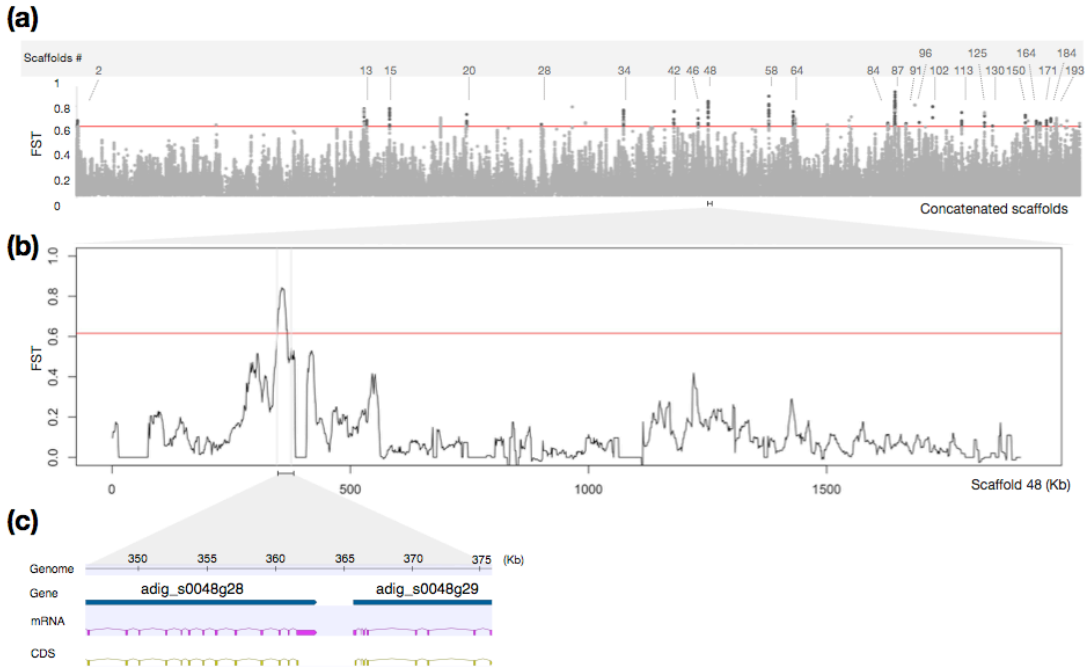



Figure 3

***Differences in WDR59 between A. digitifera and Acropora sp. 1***

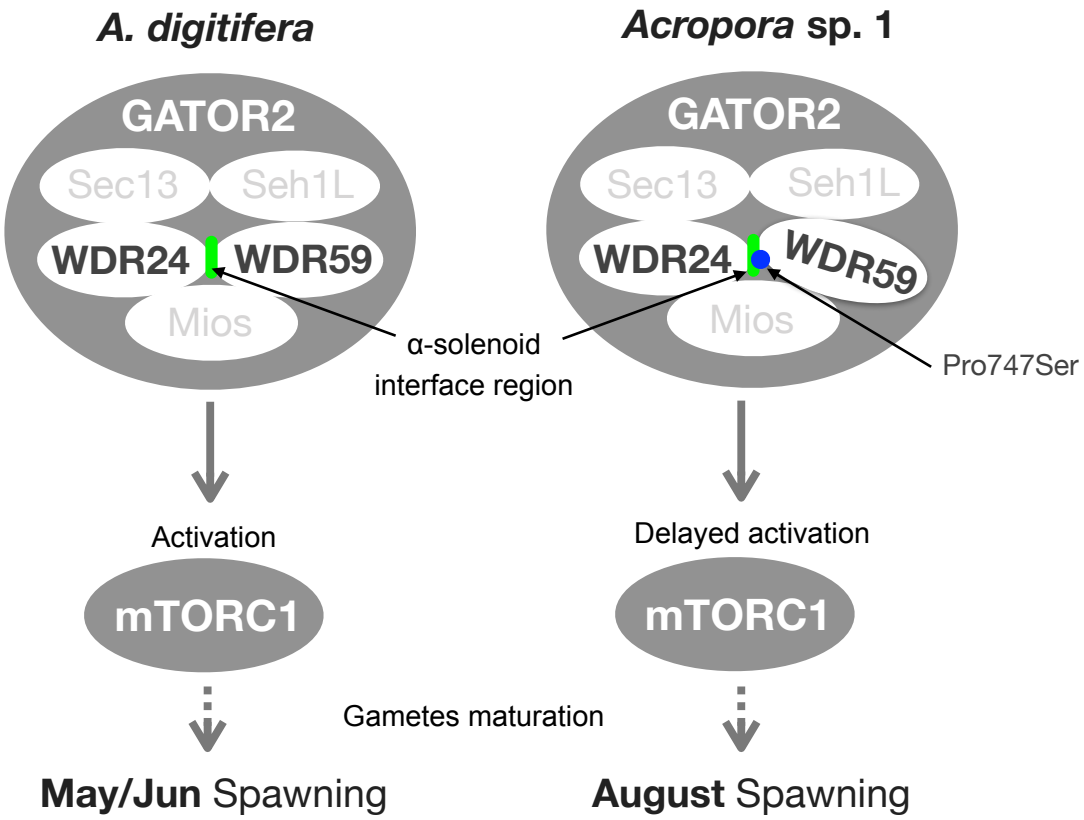
To determine whether three amino acid differences in *WDR59* between *A. digitifera* and *Acropora* sp. 1 are shared with other species or are specific to *Acropora* sp. 1, we analyzed *WDR59* in 15 *Acropora* species (Table S5). First, we aligned the WDR59 sequence of 14 *Acropora* species (excluding *A. cytherea* due to a possible partial sequence) with that of *A. digitifera* and *Acropora* sp. 1 (Figure S2) and found that one of the three amino acid changes (adig_s0048.g28.t1: CDS; 2239 C>T, amino acid sequence; Pro747Ser) is specific to *Acropora* sp. 1 (Figure S2).

199 Next, we manually checked mapping reads around *WDR59* and found that
200 *Acropora* sp. 1 colonies have a 24 bp deletion 38 bp downstream of the *Acropora* sp. 1-
201 specific amino acid change. To verify this deletion, we amplified and sequenced the
202 region containing the deletion by PCR from genomic DNAs of *A. digitifera* (n=7) and
203 *Acropora* sp. 1 (n=14). We confirmed the deletion and found two additional amino acid
204 differences between *A. digitifera* and *Acropora* sp. 1, upstream (15 bp) and downstream
205 (14 bp) of the 24 bp deletion (Fig. S3). Among the differences between *A. digitifera* and
206 *Acropora* sp. 1, two amino acid changes and a deletion are shared with *A. nasuta,* and one
207 amino acid change is specific to *Acropora* sp. 1 (Figs. S4 and S5).
208 To estimate the position of the amino acid change specific to *Acropora.* sp. 1,
209 we used Phyre2 (Kelley, et al. 2015) to search for proteins highly similar to *A. digitifera*
210 *WDR59* in known structure databases. As a result, *S. cerevisiae* Sea3, the yeast
211 counterpart of mammalian WDR59, was highly similar to *A. digitifera WDR59* (E-
212 value=0, Identity=29%). *S. cerevisiae* Sea3 (WDR59) has an α-solenoid interface region
213 where Sea3 (WDR59) interacts with the other subunit to form a complex, Sea2 (WDR24)
214 (Tafur, et al. 2022). The α-solenoid interface region is located from amino acids 782 to

215 1,061 of *S. cerevisiae* Sea3 (WDR59) (Tafur, et al. 2022). An alignment of *A. digitifera*

216 WDR59 with *S. cerevisiae* Sea3 (*WDR59*) (Fig. S6) showed that the amino acid changes

217 specific to *Acropora* sp. 1 are located in the α-solenoid interface region.




218

219 Figure 4

220 **Discussion**

221 ***A. digitifera and Acropora sp. 1 are useful for understanding timing of gametogenesis***

222 ***in Acropora***

223 Studying the timing of gamete maturation in corals using a population genetic approach,
224 as in this study, provides insights into genetic mechanisms of coral gametogenesis and
225 speciation in corals. Therefore, we propose *A. digitifera* and *Acropora* sp. 1 as a model
226 species pair for studying mechanisms of spawning month determination and speciation
227 in corals.

228 One of the advantages of using these two species is their clear phenotypic
229 difference in timing of spawning. In Okinawa, *A. digitifera* spawns in May or June,
230 whereas *Acropora* sp. 1 spawns in August (Hayashibara and Shimoike 2002; Nakajima,
231 et al. 2012; Ohki, et al. 2015). Continuous observations of oocyte volume revealed that
232 gamete maturation is later in *Acropora* sp. 1 than in *A. digitifera* (Hayashibara and
233 Shimoike 2002). The difference in gamete maturation is expected to lead to reproductive
234 isolation. Indeed, phylogenetic analysis and PCA showed that the two species are
235 genetically differentiated, despite their low genetic differentiation. Therefore, gene flow
236 between *A. digitifera* and *Acropora* sp. 1 is limited, which is considered an initial stage
237 of speciation.

238 The low genetic differentiation between *A. digitifera* and *Acropora* sp. 1 is
239 another advantage in studying genes responsible for spawning timing mechanisms and
240 speciation. Genomic differentiation between these two species is low ($F_{ST}$ = 0.10225),
241 consistent with a previous microsatellite marker study (Nakajima, et al. 2012). Using this
242 low-genomic differentiated species pair, we identified 34 HDRs and selected 39 genes
243 located in HDRs. These genomic regions and candidate genes may be responsible for
244 morphological and ecological differences between the two species. Further analyses of
245 gene expression differences in different months, functional changes resulting from highly
246 differentiated substitutions are expected to advance research on the mechanism of
247 spawning month determination and speciation in corals.
248
249 ***Genes that may determine morphological differentiation between two species***
250 Morphological characteristics of *Acropora* sp. 1 include shorter branches and a flatter
251 colony shape than *A. digitifera* (Hayashibara and Shimoike 2002; Ohki, et al. 2015).
252 These morphological differences reflect differences in skeletal form (Todd 2008). The
253 alpha collagen-like proteins are skeletal organic matrix proteins involved in skeletal

254 formation in *Stylophora pistillat*a (Drake, et al. 2013; Mummadisetti, et al. 2021) and *A.*

255 *millepora* (Ramos-Silva, et al. 2013). In this study, we identified two alpha collagen-like

256 genes (Gene IDs: adig_s0034.g151 and adig_s0171.g21) in HDRs, and these genes are

257 likely responsible for species-specific differences in skeletal morphology. *ATOH1* (Gene

258 ID: adig_s0013.g139), encodes the transcription factor Atoh1, which regulates primary

259 cilia of calcifying cells in mice (Chang, et al. 2019). Since the possibility of cilia in coral

260 skeletogenesis has been discussed in *S. pistillata* (Tambutté, et al. 2021), *ATOH1* may

261 help to define skeletal morphology in the two species.

262

263 **mTOR**C1 **may contribute to gametogenesis of *A. digitifera***

264 In this study, we identified an amino acid change specific to *Acropora* sp. 1 in *WDR59*.

265 WDR59 is one of the components of a mechanistic target-of-rapamycin complex 1

266 (mTORC1) activator, GATOR2 (Bar-Peled, et al. 2013; Wolfson, et al. 2016) (Fig. 4).

267 mTORC1 is involved in meiotic entry and gametogenesis. Regulation of meiotic entry by

268 mTORC1 is conserved from yeast to mammals. Downregulation of mTORC1 activity

269 promotes the transition from mitotic to meiotic cycles in *Saccharomyces cerevisiae*,

*Schizosaccharomyces pombe* (van Werven and Amon 2011; Zheng and Schreiber 1997), and *Drosophila* (Wei, et al. 2014). In mice, mTORC1 is required for spermatogonial differentiation (Busada, et al. 2015) and oogenesis (Guo, et al. 2018). Activated mTORC1 drives oocyte development and growth in *Drosophila* oogenesis (LaFever, et al. 2010). To the best of our knowledge, the function of mTORC1 in gametogenesis among Cnidarians has been little discussed. One exception is a study about the kinase, Mos, which regulates oocyte maturation in the jellyfish, *Clytia hemisphaerica* (Amiel, et al. 2009). Treatment of oocytes with rapamycin, a potent inhibitor of mTORC1, suggested that the mTORC1 signaling pathway controls one *Mos* paralog translation during oocyte growth (Amiel, et al. 2009). Moreover, in *Hydra oligactis*, continuous exposure to rapamycin results in fewer mature sperm cells than in untreated individuals (Tomczyk, et al. 2020). Hence, mTORC1 is likely associated with gametogenesis in cnidarians, including *Acropora* species.

The *Acropora* sp. 1-specific amino acid change in WDR59 is located in a region where WDR59 interacts with one of the other GATOR2 components to form the complex （GATOR2）. This amino acid change may cause slight differences in stability or structure

of GATOR2 through affinity of WDR59 with its counterpart. In *Drosophila* oogenesis, GATOR2 activates mTORC1, and active mTORC1 is required to start oocyte development (Wei, et al. 2014). Since regulation of gametogenesis by mTORC1 is reported in *Drosophila*, meiotic entry and oocyte development in *Acropora* species is also likely controlled by mTORC1 activity, regulated by GATOR2. In other words, the difference in timing of gamete maturation in *A. digitifera* and *Acropora* sp. 1 (Hayashibara and Shimoike 2002) may be caused by an amino acid substitution in WDR59 that slightly affects timing of mTORC1 activation via GATOR2. Note that even though we focused on WDR59 in this study, a combination of genetic factors, including genes in other HDRs, may be responsible for differences in spawning timing. Since the phylum Cnidaria, including corals, is located in the basal lineage of the animal kingdom, studies revealing the function of mTORC1 in gametogenesis in corals will provide insights into evolution of gametogenesis regulation. Future studies of the two coral species used in this study will shed light on mechanisms that determine the timing of coral spawning.

## Materials and Methods

### *Specimen collection and species identification*

Coral samples were collected from two reefs at Okinawa, Japan, between 2018 to 2020 (Table S1) with permission of the Aquaculture Agency of Okinawa Prefecture (permit numbers 30-29, 31-43, and 31-68). Sixteen colonies of *Acropora* sp. 1 with visible gametes, were collected in the field and subsequently maintained in an aquarium at the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus. In 2018, gametes of one *Acropora* sp. 1 colony were collected during spawning, and sperm were preserved at -80ºC until genome extraction. After we placed the coral colonies in the aquarium, we preserved branch fragments in RNAlater (Waltham, MA, USA) for genome extraction in 2019 and 2020.

### *DNA extraction and sequencing*

We extracted genomic DNAs from 15 branch fragments originating from 15 *Acropora* sp. 1 colonies using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). We used DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany) for DNA extraction from

318 sperm originating from one *Acropora* sp. 1 colony. Following the manufacturer's
319 instructions, we constructed DNA libraries from 16 samples using an NEBNext Ultra II
320 DNA Library Prep Kit (Illumina). The 15 libraries from branch tissues were sequenced
321 on an Illumina HiSeqX Ten, and one library from sperm was sequenced on an Illumina
322 HiSeq 2500.
323
324 ***Mapping and variant calling***
325 We downloaded genome sequence data from 11 colonies of *A. digitifera* and 15 *Acropora*
326 species (*A. tenuis, A.yongei, A. intermedia, A. gemmifera, A. awi, A. florida, A. millepora,*
327 *A. selago, A. hyacinthus, A. cytherea, A. muricate, A. echinate, A. acuminata, A. nasuta,*
328 *and A. microphthalma*). We trimmed raw sequences and removed low-quality reads
329 before mapping with fastp (Chen, et al. 2018). Trimmed reads were mapped to the *A.*
330 *digitifera* genome assembly ver. 2.0 (Shinzato, et al. 2021) using bowtie2 ver. 2.3.3.1
331 (Langmead and Salzberg 2012). Among 16 *Acropora* sp. 1 colonies, we used 14 colonies
332 with mapping bam coverage ≥10 for variant calling. Variants were called using Genome

333 Analysis Toolkit (GATK) version 4.0 and filtered according to a GATK-suggested hard-
334 filtering with a minor modification.
335
336 ***PCA and molecular phylogenetic tree construction***
337 We constructed a molecular phylogenetic tree of these *Acropora* corals using phyML
338 (Guindon, et al. 2010) with the GTR option (Guindon, et al. 2010). We performed PCA
339 analysis of *A. digitifera* and *Acropora* sp. 1 with three species, *A. acuminata*, *A.*
340 *microphthalma,* and *A. nasuta,* as an out-group, using PLINK v1.90 (www.cog-
341 genomics.org/plink/1.9/) (Weeks 2010).
342
343 ***Genome scan of highly differentiated regions***
344 We calculated $F_{ST}$ (Hudson, et al. 1992) for 10-kb windows with 1 kb increments along
345 each scaffold (>10 kb) using a sliding window approach with PopGenome (Pfeifer, et al.
346 2014). First, we extracted 10 kb windows that included the top 0.1% of $F_{ST}$ values. Among
347 these top windows, we selected windows with SNPs for which the allele is fixed in one
348 population and for which there is no homozygote for the allele in the comparison

349 population. We considered these SNPs to be differentiated SNPs. We merged overlapping
350 regions among these selected windows and considered these connected regions highly
351 differentiated.
352
353 ***Identification of genes in highly differentiated regions (HDRs)***
354 We considered genes with differentiated SNPs in HDRs as candidate genes related to
355 phenotypic differences between the two species. To identify functional annotations of
356 these genes, we searched orthologous genes in the NCBI nucleotide database and UniProt
357 (Bateman, et al. 2022) by Blast search (Altschul, et al. 1990). We regarded the top hit
358 with an *e*-value $\geq 1e^{-30}$ and identity $\geq 90\%$ for NCBI and *e*-value $\geq 1e^{-4}$ and identity $\geq$
359 20% for UniProt as an orthologous gene.
360
361 ***Identification of a deletion in WDR59 among Acropora sp. 1***
362 The presence of one deletion in the WDR59 gene in *Acropora* sp. 1, discovered by visual
363 confirmation of the mapping results, was revealed by amplifying the genomic region
364 containing the deletion using PCR and sequencing it.

365

366 ***Alignment of WDR59 sequences among Acropora species***

367 To determine whether other *Acropora* species have genetic variants other than those that

368 differentiate *A. digitifera* and *Acropora*. sp. 1, orthologous genes of *WDR59* were

369 searched in the reference genomes of each of the 15 *Acropora* species using Blastn

370 (Altschul, et al. 1990). A *WDR59 sequence of A. millepora* was downloaded from the

371 Kyoto Encyclopedia of Genes and Genomes (KEGG).

372

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STK: research concept, all experiments, data analysis, and manuscript preparation.
AI: sample collection planning, species identification.
YT: research concept, research planning, data analysis, and manuscript preparation.

**Spell out all abbreviations**

CDS: Coding sequence

DDBJ: DNA Data Bank of Japan

GATK: Genome Analysis Toolkit

HDRs: Highly Differentiated Regions

KEGG: Kyoto Encyclopedia of Genes and Genomes

mTORC1 : mechanistic target of rapamycin complex 1

NCBI: National Center for Biotechnology Information

PCA: principal components analysis

SNP: single nucleotide polymorphism

SEA/GATOR: the Seh1 associated/GTPase-activating protein toward Rags

Sea2: SEA (*Se*h1-*a*ssociated) protein complex 2

Sea3: SEA (*Se*h1-*a*ssociated) protein complex 3

WDR24: WD repeat-containing protein 24

WDR59: WD repeat-containing protein 59

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557

558 **Figure Legends**

559 Figure 1. (a) Adult colonies of *Acropora digitifera* (left) and *Acropora* sp. 1 (right). (b)

560 Sampling locations are shown as dots on the map of Okinawa Island.

561 Figure 2. Phylogenetic relationship of *Acropora* sp. 1 (a) Phylogenetic relationships of 17

562 *Acropora* corals were analyzed based on 885,405 SNPs using the maximum likelihood method

563 with the GTR option. Bootstrap support, shown next to each node for each clade, was obtained

564 from 1,000 replicates. (b) PC1 and PC2 were derived from PCA based on SNPs for all individuals

565 of *A. digitifera*, *Acropora* sp. 1, and three *Acropora* species as an out-group.

566 Figure 3. The genome-wide pattern of genetic differences between the two species. (a)

567 Genome-wide FST values were calculated in overlapping windows of 10 kb. The red line

568 indicates the top 0.1% of values. (b) FST was estimated across a region of scaffold 48

569 (adig_s0048). The red line indicates the top 0.1% of values. (c) A close-up view of

570 predicted gene structures on an HDR in scaffold 48 (adig_s0048). The flanking gene

571 structure of WDR59 (Gene ID: adig_s0048.g28) and guanine nucleotide-binding protein

572 G(o) subunit alpha (Gene ID: adig_s0048.g29) are indicated.

573 Figure 4. Schematic representation of a hypothesis proposed in this study. Regulation of

574 mTORC1 by GATOR2 and components of GATOR2 is based on previous studies (Bar-

575 Peled, et al. 2013; Valenstein, et al. 2022; Wei, et al. 2014). An *Acropora* sp. 1-specific

576 mutation in the WDR59 / WDR24 interaction region is indicated with a blue circle.

577