Species composition and mitochondrial molecular phylogeny of Acropora corals in Funakoshi, Amami-Oshima Island, Japan: A proposal for its new taxonomic grouping

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Communicated by Frederic Sinniger (Associate Editor-in-Chief)
Received: 8 October 2020, Accepted: 4 June 2020
Published online: 28 October 2021

Abstract About 80 species of Acropora corals have been recorded in Japan to date. However, there are few information on its diversity in Amami-Oshima Island, which is located in the northern part of the central Ryukyu Islands. Multiple studies, including molecular work, have looked at the species diversity of Acropora in Japan, but many of them have not been effective in successful differentiation at the species. This is the first study reporting the species diversity and molecular phylogeny of the Acropora species in Amami-Oshima Island. We collected 89 Acropora specimens, including 26 species within reef lagoon in the southwestern area (Funakoshi) of the island. We recorded A. spathulata for the first time in Japan, and A. acuminata and A. papillare for the first time in the Amami Islands. For eight species that were uncommon or difficult to identify, we described their morphological characteristics. To infer the phylogenetic relationships between the Acropora species in Amami-Oshima Island and Japan, we also reconstructed the phyllycene of the specimens using a mitochondrial putative control region including published DNA data from other Japanese specimens. The results showed that Acropora species were genetically separated into seven clades. As previously reported, A. hyacinthus and A. cytherea were highly polyphyletic; nonetheless, most species were included in specific clades. In combination with previously published ecological data, the present data allowed us to propose a new species grouping (eight groups) for 36 Japanese Acropora species, which have morphological, molecular phylogenetic, and ecological similarities. This grouping will help identify the species and understand the species diversity of Acropora until a formal taxonomic revision of the genus is conducted.

Keywords Reef-building corals, Scleractinia, Mitochondrial DNA, Molecular phylogeny, Taxonomy

Introduction

The species identification of zooxanthellate scleractinian corals (hereafter, corals) in the field provides fundamental biological data that are useful for the conservation of coral reefs. However, this can be challenging, which may prevent coral scientists from accurately recording coral species diversity in the field. The difficulty in identifying coral species is due to the apparent lack of key morphological characteristics that distinguish species in many cases. Indeed, the morphological characteristics (e.g., size, septa, costae, and columnellae) of a corallite (the cup-shaped structure of each polyp) are unstable and variable even between the corallites within a single colony, although the fossil record (e.g., Santodomingo et al. 2015) showed that the morphological characteristics of Acropora can be relatively stable.

Molecular phylogenetic analyses have therefore been introduced into coral taxonomy in the last decades. In many cases, results have revealed that the molecular phylogenetic relationships of corals are not consistent with
morphology-based taxonomy at family and genus levels (e.g., Fukami et al. 2004b, 2008; Kitahara et al. 2010; Benzoni et al. 2012; Arrigoni et al. 2014ab, 2016). Subsequently, the identification of new morphological characteristics associated with molecular phylogenetic data has become important for the formal taxonomic revision of corals. Indeed, the taxonomy of several coral families, especially Merulinidae Verrill, 1865 (Huang et al. 2014ab) and Lobophylliidae Dai & Horng, 2009 (Huang et al. 2016; Arrigoni et al. 2014ab, 2018), and genera has been revised using the integrated data of morphology and molecular phylogeny. Hence, integrated morphological and molecular phylogenetic analysis has become a powerful tool for these taxonomic revisions. Nevertheless, species relationships among corals remain largely unclear in most genera due to the difficulty of species identification.

Species identification is particularly difficult in the genus Acropora Oken, 1815, which contains more than 150 species—the highest number among coral genera (Wallace 1999). Several molecular phylogenetic studies using Acropora corals have been reported (e.g., Hatta et al. 1999; van Oppen et al. 2001; Wolstenholme 2004; Richards et al. 2008; Rosser et al. 2017), but further studies are needed to clarify the species relationships in Acropora. In Australia, the phylogenetic relationships of 36 species in total have been reported, with 28 species from the Great Barrier Reefs (GBR) (van Oppen et al. 2001; Richards et al. 2013) and 20 species from Western Australia (Rosser et al. 2017). In addition, nine species from Papua New Guinea and seven species from the Republic of Marshall Islands were analyzed together with Australian species (Richards et al. 2008, 2013). Chen et al. (2009) also showed the species relationships using 28 species from GBR and Indonesia. On the other hand, only 20 species in Japan (Hatta et al. 1999; Fukami et al. 2003; Suzuki and Fukami 2012; Suzuki et al. 2008; Fukami et al. 2019) have been analyzed, even though a total of 80 species have been reported (Nishihira and Veron 1995).

Several genetic markers, including mini-collagen, ribosomal RNA gene including ITSs, PaxC, and mitochondrial putative control region (mtCR), have been used to date to build molecular phylogenetic trees for Acropora (van Oppen et al. 2001; Wei et al. 2006; Richards et al. 2008, 2013; Rosser et al. 2017). The total phylogenetic relationships are similar among trees inferred from nuclear and mitochondrial markers; although the differences among these trees occur at some points. They are largely due to misidentification, existence of cryptic species, or introgression by hybridization (van Oppen et al. 2001; Willis et al. 1997; Wolstenholme et al. 2004; Richards et al. 2008, 2013, 2016; Rosser et al. 2017; Mao et al. 2018, 2020). Therefore, given the cost, usability, and large amount of available published data, a mtCR marker would be useful as a convenient molecular maker to determine the phylogenetic relationships among Acropora species to a certain extent.

The reproductive information of Acropora is also useful to consider species boundary or species grouping. Most Acropora species spawn synchronously (Harrison et al. 1984; Babcock et al. 1986; Hayashibara et al. 1993), but three species, A. tenuis (Dana, 1846), A. donei Veron & Wallace, 1984, and A. yongei Veron & Wallace, 1984, spawned two hours earlier than the others (Babcock et al. 1986; Hayashibara et al. 1993; Fukami et al. 2003). These three species were also genetically distant from most other species (van Oppen et al. 2001; Fukami et al. 2003), while they are able to hybridize with each other (Fukami et al. 2003; Morita et al. 2019). In addition, the different timing of spawning is also strongly related to phylogenetic differences. For examples, van Oppen et al. (2001) showed that A. latistella (Brook, 1892), which typically spawned two weeks out of phase from most Acropora species (Willis et al. 1985; Babcock et al. 1986), formed an independent clade in the phylogenetic trees based on mtCR and PaxC. Furukawa et al. (2020) revealed that two morphotypes of A. divaricata were clearly separated by both genetic differences and a month differences in spawning time. Using PaxC, Rosser et al. (2017) also showed that different spawning seasons were associated with highly diverged lineages in Acropora species. On the other hand, hybridizing species tend to be genetically grouped together or can be inseparable genetically in Acropora (Hatta et al. 1999; Wolstenholme 2004).

In Japan, Amami-Oshima Island (28° 19’ N, 129° 22’ E) is one of the Amami Islands in Kagoshima prefecture; it is located in the middle of the Ryukyu Islands. Data on the species and genetic diversity of Acropora
in Amami-Oshima would be useful for understanding the regional differences in the relationships of *Acropora* species in Japan. Although the species diversity of corals in Amami-Oshima is yet to be reported in detail, 39 *Acropora* species have been recorded in one of the Amami Islands, Tokunoshima Island, which is located ~50 km southwest of Amami-Oshima (Nishihira and Veron 1995). Additionally, 25 *Acropora* species have been reported in Kikaijima Island located ~25 km east of Amami-Oshima (Fukami et al. 2016; Fujii et al. 2020). The corals in Amami-Oshima were severely damaged by a bleaching event in 1998 and two outbreaks of crown-of-thorns starfish, *Acanthaster* sp., from 1974 to 1983 (Hirata 1980; Yamaguchi 1986) and 2000 to 2007 (Council for Coral Reef Conservation in Amami Island 2013). Even in a protected area, known as Derikyonma, only 12 *Acropora* species remained, as shown by species surveys in 2009–2010 (Hata et al. 2013). However, in a preliminary survey in Funakoshi, the southwestern area of Amami-Oshima, we found abundant *Acropora* corals.

Therefore, in this study, we surveyed the species diversity of *Acropora* corals in Funakoshi, Amami-Oshima. We investigated the molecular phylogenetic relationships of identified *Acropora* species using mtCR. We also summarized the key characteristics to identify of *Acropora* species. In addition, we temporarily proposed a new grouping of *Acropora* species for future studies, considering their morphology, molecular phylogenetic relationships, and reproductive traits.

**Materials and methods**

**Sampling and species identification**

The survey area, Funakoshi, is on the coast of Uken village in Amami-Oshima (Fig. 1). We divided this area into five sites (I: the small bay next to the main reef, II: connection between the main reef area and the small bay, III: outer area in the lagoon of the main reef with large branching coral area, IV: inner area in the lagoon of the main reef with large branching coral area, IV: inner area in the lagoon of the main reef with large branching coral area) (Fig. 1). We investigated the molecular phylogenetic relationships of identified *Acropora* species using mtCR. We also summarized the key characteristics to identify of *Acropora* species. In addition, we temporarily proposed a new grouping of *Acropora* species for future studies, considering their morphology, molecular phylogenetic relationships, and reproductive traits.

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main reef, V: front of the beach with low coral density, next to a shrimp aquaculture area) considering the information obtained in our preliminary survey. During snorkeling on July 18–21 in 2014, we thoroughly searched to determine the extent of *Acropora* diversity present in this area, collecting 1–3 colonies (each 5 to 10 cm long) for every *Acropora* species we found during our surveys. Sample photos were taken in the field. Each site was surveyed 2–3 times by three researchers. A survey period in a single day lasted 5–6 h. We sampled down to a maximum depth of ~10 m; nevertheless, we covered all areas of the inner reef (lagoon) and reef crest (but not the outer reef). In the laboratory, a small sample piece (< 5 mm) was placed in a guanidine solution (4 M guanidine thiocyanate; 0.1% N-lauroyl sarcosine sodium; 10 mM Tris-HCl, pH 8; 0.1 M 2-mercaptoethanol) (Fukami et al. 2004a) for DNA analysis. The remaining sample pieces were bleached in preparation for observing the skeleton for species identification. Species identification was performed based on the monograph of Wallace (1999) using a stereomicroscope or digital stereomicroscope (VHX-5000, Keyence Co., Osaka, Japan). During species identification, we selected three major branches from each skeletal sample; we measured branch thickness and length, and the outer diameter of the axial corallite, using a vernier caliper. All skeletal and DNA samples are stored at the University of Miyazaki. The photos of living specimens are available on request.

**Molecular phylogenetic analyses**

Total DNA was extracted from coral tissues dissolved in a guanidine solution using a phenol/chloroform extraction method (Fukami et al. 2004a). The mtCR was amplified using polymerase chain reaction (PCR) with the following primers: rns_2F (5′-CAG AGT AAG TCG TAA CAT AG-3′) and block G_R (5′-AAT TCC GGT GTG TGT TCT CT-3′) (Suzuki and Fukami 2012) for the mtCR (700–900 bases). PCR conditions for the mtCR were 94°C for 30 s, followed by 30 or 35 cycles at 94°C for 30 s, 55°C or 60°C for 45 s, and 72°C for 90 s, with a final phase of 72°C for 5 min. PCR products were treated with shrimp alkaline phosphatase and exonuclease I at 37°C for 40 min, and then 80°C for 20 min. DNA sequences were then determined by direct sequencing using the ABI3730 sequencers of a research contract service (FASMAC Co., Ltd., Kanagawa, Japan). All DNA sequences obtained in the current study were submitted to the DDBJ (accession numbers: LC616156-LC616234; Supplementary Table 1).

All DNA sequences were aligned manually using Sequencher software (Gene Codes, MI, USA). Molecular phylogenetic relationships were inferred using neighbor-joining (NJ) and maximum likelihood (ML) analyses in MEGA X (Kumar et al. 2018) based on mtCR datasets. The published DNA sequences of the mtCR of Japanese *Acropora* species (Suzuki et al. 2008; Suzuki and Fukami 2012; Suzuki et al. 2016; Fukami et al. 2019) were used for comparison with our data. Although many mtCR DNA sequences of *Acropora* species from outside of Japan, e.g., Australia, have been reported (van Oppen et al. 2001; Richards et al. 2008, 2013, 2016; Rosser et al. 2017), we did not use these sequences because our focus was on Japanese *Acropora* species. For NJ analysis, the Kimura two-parameter model was used for both datasets. For ML analysis, the Kimura two-parameter model with Gamma distribution was used for both datasets, and this model was evaluated by MEGA X. All positions containing gaps were treated as pairwise deletion in the analysis because long deletion existed in several species. Missing data were eliminated from the analysis. Bootstrap analysis was performed with 500 replicates for ML and 1,000 replicates for NJ. Among the *Acropora* species, *A. tenuis* and closely related species have previously been categorized in the clades most distant from all others (van Oppen et al. 2001). Thus, in our molecular trees, we treated *A. tenuis* and the closely related species like an outgroup species.

**Results**

In total, 26 nominal species were identified from 81 of 89 specimens collected in Funakoshi; of the remaining eight specimens, one specimen was identified as *Acropora* sp. 1, previously reported by Hayashibara and Shimoyike (2002). The other seven specimens were not identified at species level, so they were treated as unidentified species (i.e., *Acropora* spp.) because of their different morphology, such as branch thickness and colony form. Of the 26 species, *A. spathulata* (Brook, 1891) (Fig. 2A,
B, C) was recorded for the first time in Japan (to our knowledge) and two species, *A. acuminata* (Verrill, 1864) (Fig. 3A, D) and *A. papillare* Latypov, 1992 (Fig. 2J, K, L), were recorded for the first time in the Amami Islands. The full list of species identified in this study is shown in Supplementary Table 1.

In Funakoshi, different species compositions were observed in each of the five study sites. Although we did not count the colony number nor measure the density of living corals, for future studies, we showed here the status
Fig. 3 Acropora species at Funakoshi in Amami-Oshima. A, D. Acropora acuminata. B, E. A. abrotanoides. C, F. A. robusta. G, J. A. cytherea. H, K. A. anthocercis. I, L. A. hyacinthus. M, P. A. microphthalmalma. N, Q. A. elseyi. O, R. A. verweyi. For each species, living specimen is shown on the upper and the close-up view of skeleton on the lower. Scale bar: 1 mm.
of coral distribution observed at that time. At site I, a large patch (approximately 10 × 10 m) of *A. intermedia* (Brook, 1891) was found (Fig. 1D), and 19 out of the total 26 *Acropora* species were recorded. At site II, a shallow area exposed to wave action connecting sites I and III, *Isopora cuneata* (Dana, 1846) and *I. palifera* (Lamarck, 1816) were commonly observed, and five *Acropora* species (*A. cytherea* (Dana, 1846), *A. robusta* (Dana, 1846), *A. samoensis* (Brook, 1891), *A. tenuis*, and *Acropora* sp.) were recorded. At site III, a large patch (approximately 20 × 20 m) of *A. spathulata* was found (Fig. 1E). At site IV, *A. aspera* (Dana, 1846) (Fig. 2D, E, F) and *A. pulchra* (Brook, 1891) (Fig. 2G, H, I) were abundant in the back-reef moat. At site V, which was also a lagoon, *Montipora* spp. and *Porites* spp. were dominant and four *Acropora* species (*A. digitifera* (Dana, 1846), *A. nasuta* (Dana, 1846), *A. gemmifera* (Brook, 1892), and *A. intermedia*) were recorded.

In this study, we summarized the specific morphological traits of local ecomorphs (see below) for eight of the 26 *Acropora* species. Some species are not common in Japan, and it was somewhat difficult to distinguish them morphologically from closely related species. The morphological characteristics of each species described below were measured from our Funakoshi samples (Supplementary Table 1). For species information, we mainly referred to Wallace (1999), and the following species information fall within the range of variation described for each species in Wallace (1999), except *A. elseyi* (Brook, 1892) (see “remarks” for *A. verweyi* Veron & Wallace, 1984). For more details on the morphological characteristics of these species, see Wallace (1999) or Wallace (2012).

**Family Acroporidae**  
**Genus Acropora**

*A. spathulata* (Brook, 1891) (Fig. 2A, B, C)  
Sample numbers: AOU025, AOU047, AOU084, AOU097, AOU101  
Clade: VIb  
Specific characteristics: Colony form is corymbose. Branches are 10–15 mm thick and 50–80 mm long. Axial corallites are 3.3–4.2 mm in diameter, while radial corallites are open scale-like shaped and evenly sized. This species is sometimes confused with *A. aspera* with short branches.  
Similar species:  
*A. millepora* (Ehrenberg, 1834): Tabular-corymbose colony form with much shorter and thinner branches. Radial corallites look a pinecone-like shape. Not found in this study, but recorded at a different area (Keten Bay) in Amami-Oshima (Hata et al. 2013).  
*A. aspera* (Fig. 2D, E, F): Arborescent colony form with much longer branches, but similar length in the lagoon. Radial corallites are a pinecone-like, rather than scale-like, shape with primary septa presenting 1/3 of radius. Sample numbers: AOU057, AOU100.  
*A. papillare* Latypov, 1992 (Fig. 2J, K, L)  
Sample numbers: AOU012, AOU034  
Clade: VII  
Specific characteristics: Colony form is digitate, encrusted with thick branches (14–18 mm thick). Branches are 37–63 mm long with little branching. Axial corallites are 2.9–3.4 mm in diameter, while radial corallites are open scale-like shaped and evenly sized.  
Similar species:  
*A. spathulata* (Fig. 2A, B, C): Corymbose colony form. For more details, see the description of *A. spathulata*. Sample numbers: AOU025, AOU047, AOU084, AOU097, AOU101.  
*A. robusta* (Fig. 3C, F): Arborescent to digitate colony form. Usually branches are much longer than those of *A. papillare*. For more details, see the description
of the similar species of *A. abrotanoides* (Lamarck 1816). Sample numbers: AOU001, AOU014, AOU045, AOU054, AOU074.

*Acropora acuminata* (Verrill, 1864) (Fig. 3A, D)
Sample numbers: AOU031, AOU033, AOU053
Clade: VII
Specific characteristics: Colony form is arborescent, tabular, with slender, upwardly curving and tapering branches (9–12 mm thick and 55–81 mm long). Axial corallites are 2.0–2.6 mm in diameter, while radial corallites are long, tubular, with an outward opening resembling a needle hole.

Note: Three specimens of this species were collected, but DNA could not be extracted from two (AOU033, AOU053). For an unknown reason, the guanidine solution turned black with these specimens only, suggesting that DNA preservation failed. Therefore, it may be advisable to avoid using a guanidine solution to extract DNA from *A. acuminata*.

Similar species:
*Acropora valenciennesi* (Edwards & Haime, 1860): Colony form very similar but with much longer branches. Radial corallites are tubular but shorter and with a more oval-shaped opening. This species has not been recorded in Amami-Oshima.

*Acropora abrotanoides* (Lamarck, 1816) (Fig. 3B, E)
Sample number: AOU065
Clade: III
Specific characteristics: Colony form is arborescent, tabular, with broad thick branches. Branches are 19–21 mm thick and >100 mm long. Axial corallites are small (1.8–2.0 mm in diameter), while radial corallites have two types: very long upward tubular and submerged.

Similar species:
*Acropora robusta* (Fig. 3C, F): Colony form is arborescent to digitate and sometimes similar to that of *A. abrotanoides*. Shape of the radial corallites differs between the species. *Acropora robusta* has two different radial corallite types: one is the submerged type, similar to *A. abrotanoides*, but the other is a much shorter tubular type than that of *A. abrotanoides*. Lower lips of the tubular corallites of *A. robusta* elongate horizontally to resemble a grater ("Oroshi-gene" in Japanese). Sample numbers: AOU013, AOU014, AOU045, AOU054, AOU074.

*Acropora anthocercis* (Brook, 1893) (Fig. 3H, K)
Sample number: AOU064
Clade: VIa
Specific characteristics: Colony form is a thick plate with very short branches (16–22 mm in length and 8–12 mm thick). Axial corallites are 2.3–2.7 mm in diameter and sometimes elongate. Radial corallites are dish-like, as in *A. hyacinthus* (Dana, 1846), and the lip extends upward.

Similar species:
*Acropora hyacinthus* (Fig. 3I, L): Tabular colony form. Branches are much thinner, radial corallites are much smaller, and the lip does not extend upward. Sample numbers: AOU005, AOU037, AOU052, AOU072.

*Acropora cytherea* (Fig. 3G, J): Tabular colony form. Branches are much thinner, radial corallites are much smaller, but the lip does extend upward. Sample numbers: AOU015, AOU79.

*Acropora microphthalma* (Verrill, 1869) (Fig. 3M, P)
Sample numbers: AOU020, AOU022, AOU088, AOU090, AOU092
Clade: V
Specific characteristics: Colony form is arborescent with slender (9–13 mm thick) tapering branches (≤79 mm long). Axial corallites are 2.0–2.8 mm in diameter. Wallace (1999) described a branching pattern at 45° to 90° as one of the specific characteristics of this species, but other branching *Acropora* species also show this feature. The most specific characteristic is well-developed spinules (spines) on the radial corallites and coenosteum. When observed under a microscope or loupe, the radial corallites or coenosteum look sponge-like or sometimes meandering. According to this characteristic, the species is easily distinguishable from other similar species such as *A. muricata* (Linnaeus, 1758). In addition, *A. microphthalma* usually looks whitish or blue-whitish, as described by Wallace (1999).

Similar species:
*Acropora muricata*: Colony form is similar but branches are usually a little thicker. A lack of well-developed spinules on the radial corallites and coenosteum. This
species has not been recorded in Amami-Oshima.

*Acropora solitaryensis* Veron & Wallace, 1984  
(supplementary Fig. 1A, B)  
Sample number: AOU098  
Clade: IV  
Specific characteristics: Colony form is tabular with fusion of branches extending upwardly and horizontally. Upper branches are 39–47 mm in length and 9–12 mm thick. Axial corallites are 2.5–2.8 mm in diameter. Primary septa of radial corallites extend one-third of the radius.

Note: In the Ryukyu Islands, this species may be confused with *A. divaricata* (Dana, 1846). These two species have only one morphological difference, which is the length of their upper branches: branches of *A. solitaryensis* are ≤45 mm while those of *A. divaricata* are ≤70 mm [see Wallace (1999) for details]. However, this difference is sometimes unclear because several colonies have intermediate-sized branches (50–60 mm length). Thus, additional taxonomic studies are required for these two species.

Similar species:  
*Acropora divaricata*: Corymbose or tabular colony form with fusion of branches extending upwardly and horizontally. Upper branches are about two times as long as those of *A. solitaryensis*. This species has not been recorded in Amami-Oshima.  
*Acropora glauca* (Brook, 1893): Corymbose to corymbose plate colony form with short cylindrical branches. Primary septa of radial corallites extend two-thirds of the radius, which is a main difference from *A. solitaryensis*. This species has not been recorded in Amami-Oshima.  
*Acropora cf. glauca* sensu Sugihara et al. (2015): Tabular colony form with short cylindrical branches. This taxon has almost the same morphological characteristics as *A. glauca* except for its colony form. This species has not been recorded in Amami-Oshima.

*Acropora verweyi* Veron & Wallace, 1984 (Fig. 3N, Q)  
Sample number: AOU061  
Clade: II  
Specific characteristics: Caespito-corymbose with terete branches (56–59 mm in length and 6–8 mm thick). Axial corallites are 2.7–3.2 mm in diameter. Radial corallites are tubular with a clear round opening. In the field, axial polyps look yellow.

Similar species:  
*Acropora elseyi* (Fig. 3O, R): Caespitose colony form. Branch length is about two times that of *A. verweyi*. Radial corallites are rounded, tubular, with an oblique opening and a thickened wall. Axial corallites have a similar diameter but are longer. In the field, axial polyps look yellow, as in *A. verweyi*. Sample number: AOU055.  
Remarks: We identified the specimen (AOU055) as *A. elseyi* (Fig. 3O, R) in this study, although the axial coral size (2.0–3.1 mm) was a little larger than that in the description of this species by Wallace (1999) (i.e., 0.9–2.3 mm). This species is common in the shallow water of the Ryukyu Islands; therefore, additional taxonomic study will be necessary to understand its species diversity in Japan.

**Phylogenetic analysis**

All 26 identified species and eight unidentified species (79 out of 89 specimens) were genetically analyzed using the mtCR marker (Supplementary Table 1). Figure 4 shows the mtCR molecular phylogenetic tree using the specimens. There were 801 positions with one long indel (698 positions without indel) in the final dataset. In this tree, two major groups were formed. The first major group contained two clades (I and II); clade I contains *A. donei* and *A. tenuis*, while clade II contains *A. austera*, *A. elseyi*, *A. verweyi*, and *Acropora sp.* (AOU060). All specimens in these two clades had one specific long gap (103 positions) in their DNA sequences, while all others except one specimen (AOU036 of *A. nana* (Studer, 1878)) had no gap. In the second major group, all other species were included and separated into five clades (III to VII). Clade III contains *A. abrotanoides* and *A. robusta*. Clade IV contains *A. digitifera*, *A. nasuta*, *A. pulchra*, *A. solitaryensis*, and *Acropora sp.* 1 (AOU096). Clade V contains *A. microphthalmal*, *A. nana*, *A. valida* (Dana, 1846), and *Acropora spp.* (AOU021, AOU029, AOU073, and AOU083). Although the bootstrap value to support this clade V was less than 50%, this group was genetically distant from all other clades, and all specimens in this clade were genetically closely related. There-
fore, despite low bootstrap value in this clade, we treat this group as an independent clade in this study. Clade VI has two subclades (a and b); VIa contains *A. anthocercis*, *A. cytherea*, and *A. hyacinthus*, while VIb contains *A. gemmifera*, *A. humilis* (Dana, 1846), *A. intermedia*, *A. monticulosa* (Brüggemann, 1879), *A. samoensis* (Brook, 1891), *A. spathulata*, and *Acropora* sp. (AOU043). Clade VII includes *A. acuminata*, *A. aspera*, *A. hyacinthus*, and *A. papillare*. Thus, each species used in this study was included within a single clade except *A. hyacinthus*, which was polyphyletic (clades VI and VII).

Notably, four morphologically similar species, namely *A. aspera*, *A. pulchra*, *A. spathulata*, and *A. papillare*, which belong to the morphologically similar group

**Fig. 4** Molecular phylogenetic tree of mitochondrial putative control region of *Acropora* species at Funakoshi in Amami-Oshima. Sample number is shown in parenthesis. Bootstrap values (ML) with more than 50% are shown on the main branches. Polyphyletic species are shown by asterisk.
Fig. 5  Molecular phylogenetic tree of mitochondrial putative control region of Acropora species in Japan. Amami-Oshima’s species are shown in bold, together with sample numbers. Species referred from DDBJ are shown with accession numbers. On the right side of tree, number of colony for each species is shown in parenthesis (left: this study, right: DDBJ data).
“Acropora aspera” group” proposed by Wallace (1999), were not included together in a single clade. Instead, *A. aspera* and *A. papillare* is in clade VII, *A. spathulata* is in clade VI (with *A. humilis*, *A. gemmifera*, and others) and *A. pulchra* is in clade IV (with various other species).

We also used the DNA sequences (Suzuki et al. 2008; Suzuki and Fukami 2012; Suzuki et al. 2016; Fukami et al. 2019) of the mtCR obtained from DDBJ (DNA Data Bank of Japan) to investigate the local genetic differences within Japan. There were 455 positions without indels in the final dataset. The total length differed from our original dataset because many mtCR DNA sequences from DDBJ were shorter. Figure 5 shows the mtCR molecular phylogenetic tree of Japanese *Acropora* species using the referenced data from DDBJ. The topology of this tree was similar to that from our data, but *A. horrida* (Dana, 1846) and *A. longicyathus* (Milne Edwards & Haime, 1860), which were not analyzed in our study, formed new sister clades of clades I and II. The local differences within species were not identified, although *A. hyacinthus* and *A. cytherea* were polyphyletic because they were additionally included in clade III. In addition, each of *A. japonica* Veron, 2000 and *A. nasuta* contained one genetically distant haplotype with a major haplotype. *Acropora muricata*, *A. loripes* (Brook, 1892), and *A. valida* were also separated into two different clades (clades IV and V).

**Discussion**

**Phylogeny of Acropora**

In this study, *Acropora* species were separated genetically into seven clades with two subclades. Although each clade/subclade contains 3–6 species, species classification based on the mtCR is useful because the species in each clade/subclade are relatively different in terms of morphology, e.g., in their colony form and skeletal morphology. The topology revealed here was similar to that of a molecular phylogeny in which 818 single-copy orthologous genes of 15 *Acropora* species were used (Shinzato et al. 2020), except that the phylogenetic position of *A. acuminata* considerably differed (also see Table 1). In future studies, more *A. acuminata* specimens should be analyzed by mtCR; we were only able to analyze one specimen in this study due to DNA extraction problems (see “Note” in this species’ description).

Although Wallace (1999) classified *Acropora* species into 20 groups based on their skeletal morphological characteristics, those groupings differed from our molecular data in several cases (Table 1), as indicated by van Oppen et al. (2001) and Richards et al. (2013) for non-Japanese *Acropora* species. For example, Wallace (1999) reported that *A. intermedia* and *A. robusta* belong to “Acropora robusta group,” but they were separated into different clades (clades III and VI, respectively) in our study. Veron (2000) also classified *Acropora* species into 38 groups based on their colony form and corallite’s morphology, but those grouping also differed from our molecular data (Table 1). Thus, new *Acropora* species groupings associated with molecular phylogenetic relationships are necessary to understand the species relationships among *Acropora*. Here we classified *Acropora* species into eight groups based on molecular phylogenetic relationships, morphological characteristics, and reproductive data (described in detail below). Despite some low support at higher nodes, the groups, except of group V, are all well supported in the molecular phylogeny and in the context of identification of Japanese *Acropora*. These groupings may be precious to facilitate reliable identifications.

We showed that the mtCR marker was useful to a certain extent for separating Japanese *Acropora* species. However, this marker had some issues as six species (*A. japonica*, *A. loripes*, *A. muricata*, *A. nasuta*, *A. valida*, and *A. cytherea*) had two genetically distant haplotypes, while *A. hyacinthus* had three (Fig. 5). Each of *A. cytherea*, *A. nasuta*, and *A. japonica* had a genetically distant haplotype in addition to a dominant haplotype. Several species might rarely contain at least one genetically distant haplotype within a single species, suggesting the occurrence of ancestral polymorphisms or recent hybridization. Such unusual haplotypes may be found to be exceptions when many more specimens from each species will be analyzed.

*Acropora loripes*, *A. muricata*, and *A. valida*, which were separated into two independent clades (IV and V in Fig. 5), might contain cryptic species because they exhibited high morphological variation and were therefore difficult to identify at species level. A similar genetic pattern was reported in *A. solitaryensis* (Suzuki and Fukami...
Table 1  Summary of the proposed species grouping of *Acropora* in Japan and the comparison with other studies. Species which has only one DNA data in this study are shown with question mark in the group number. Polyphyletic species are shown with asterisks, and also with parenthesis in the group number as non-specific member. For van Oppen et al. (2001) and Rosser et al. (2017), we checked the phylogenetic relationship of species they used by adding their DNA sequences of mtCR into our data. For Shinzato et al. (2020), we just compared the topologies of the phylogenetic trees with our data and them. In species from other studies, ones included in same groups proposed in this study are shown in bold. N/A means not applicable. See Fig. 5 for clade number in this study. See discussion “Proposal of future grouping of *Acropora*” for group number.

| This study          | Wallace (1999) | Veron (2000) | van Oppen et al. (2001) | Rosser et al. (2018) | Shinzato et al. (2020) |
|---------------------|----------------|--------------|-------------------------|----------------------|------------------------|
| Acropora            | group | clade | group | species | clade | species | clade | species | clade |
| donei               | 1     | I     | selago | tenuis | IA    | donei   | I     | N/A     | N/A   |
| tenuis              | 1     | I     | selago | tenuis | 8     | tenuis  | I     | N/A     | N/A   |
| yongei              | 1     | I     | selago | yongei | 29    | yongei  | I     | N/A     | N/A   |
| austere             | 2     | II    | echinata | audrey | 8     | N/A     | N/A   | N/A     | N/A   |
| elseyi              | 2     | II    | echinata | verweyi | 32    | N/A     | N/A   | N/A     | N/A   |
| verweyi             | 2     | II    | verweyi | verweyi | 32    | N/A     | N/A   | N/A     | N/A   |
| longicyathus        | extra | extra | echinata | latistella | 14   | longicyathus | IB | N/A     | N/A   |
| horrida             | extra | extra | horrida | horrida | 14   | N/A     | N/A   | N/A     | N/A   |
| robusta             | 3     | III   | robusta | robusta | 7     | N/A     | N/A   | N/A     | N/A   |
| abrotanoides        | 3?    | III   | abrotanoides | abrotanoides | 7     | N/A     | N/A   | N/A     | N/A   |
| cytherea*           | (3)   | III   | cytherea* | cytherea* | 7     | N/A     | N/A   | N/A     | N/A   |
| hyacinthus*         | (3)   | III   | hyacinthus* | hyacinthus* | 7     | N/A     | N/A   | N/A     | N/A   |
| digitifera           | 4     | IV    | humilis | divaricata | 22   | divaricata | III | N/A     | N/A   |
| divaricata           | 4     | IV    | divaricata | divaricata | 9     | N/A     | N/A   | N/A     | N/A   |
| sp.1                | 4     | IV    | nasuta | nasuta | 34   | N/A     | N/A   | N/A     | N/A   |
| nasuta              | 4     | IV    | aspera | aspera | 26   | N/A     | N/A   | N/A     | N/A   |
| pulchra             | 4     | IV    | N/A    | pulchra* | 15   | N/A     | N/A   | N/A     | N/A   |
| pruinosa            | 4     | IV    | selago | selago | 29   | N/A     | N/A   | N/A     | N/A   |
| selago              | 4     | IV    | divaricata | divaricata | 9     | N/A     | N/A   | N/A     | N/A   |
| solitarensis        | 4     | IV    | loripec | loripec | 32   | N/A     | N/A   | N/A     | N/A   |
| loripec*            | (4)   | IV    | muricata | muricata | 9     | N/A     | N/A   | N/A     | N/A   |
| muricata*           | (4)   | IV    | nasuta | nasuta | 35   | N/A     | N/A   | N/A     | N/A   |
| varida*             | (4)   | IV    | varida* | varida* | 35   | N/A     | N/A   | N/A     | N/A   |
| microphthalmal       | 5     | V     | horrida | latistella | 12   | N/A     | N/A   | N/A     | N/A   |
| nana                | 5     | V     | cytherea* | cytherea* | 19   | muricata | IV | N/A     | N/A   |
| cf. glauca           | 5     | V     | cytherea* | cytherea* | 19   | cytherea* | IV | N/A     | N/A   |
| loripec*            | (5)   | V     | loripec | loripec | 19   | cytherea* | IV | N/A     | N/A   |
| loripec*            | (5)   | V     | loripec | nasuta | 35   | cytherea* | IV | N/A     | N/A   |
| cytherea*           | 6     | VIa   | hyacinthus | hyacinthus | 19   | loripec | IV | N/A     | N/A   |
| hyacinthus*         | 6     | VIa   | hyacinthus | hyacinthus | 19   | N/A     | N/A   | N/A     | N/A   |
| anthocercis         | 6     | VIa   | hyacinthus | hyacinthus | 19   | N/A     | N/A   | N/A     | N/A   |
| cytherea*           | 6     | VIa   | hyacinthus | hyacinthus | 30   | loripec | IV | N/A     | N/A   |
| cytherea*           | 6     | VIa   | hyacinthus | hyacinthus | 19   | N/A     | N/A   | N/A     | N/A   |
| cytherea*           | 6     | VIa   | hyacinthus | hyacinthus | 19   | N/A     | N/A   | N/A     | N/A   |
| florida             | 7     | VIb   | florida | aspera | 11   | florida | IV | N/A     | N/A   |
| gemmifera           | 7     | VIb   | gemmifera | aspera | 11   | gemmifera | IV | N/A     | N/A   |
| humilis             | 7     | VIb   | humilis | humilis | 21   | humilis | IV | N/A     | N/A   |
| japonica            | 7     | VIb   | humilis | humilis | 21   | humilis | IV | N/A     | N/A   |
| intermedia          | 7     | VIb   | robusta | robusta | 7    | N/A     | N/A   | N/A     | N/A   |
| monticulosa         | 7     | VIb   | humilis | humilis | 21   | N/A     | N/A   | N/A     | N/A   |
| samoensis           | 7     | VIb   | aspera | aspera | 21   | N/A     | N/A   | N/A     | N/A   |
| spathulata          | 7     | VIb   | aspera | aspera | 21   | N/A     | N/A   | N/A     | N/A   |
| aspera              | 8     | VII   | aspera | aspera | 26   | N/A     | N/A   | N/A     | N/A   |
| papillare           | 8     | VII   | aspera | aspera | 26   | N/A     | N/A   | N/A     | N/A   |
| acuminata           | 8?    | VII   | muricata | muricata | 8    | N/A     | N/A   | N/A     | N/A   |
| hyacinthus*         | (8)   | VII   | hyacinthus | hyacinthus | 19   | N/A     | N/A   | N/A     | N/A   |
2012); this species was clearly divided into two clades. Although this situation has not yet received taxonomic attention, one of these *A. solitaryensis* could be a different species, and it was tentatively named *A. cf. glauca* (Sugihara et al. 2015). Therefore, it will be necessary to conduct further taxonomic study to delimit these three species.

In this study, *A. hyacinthus* was highly polyphyletic, consistent with previous studies (Suzuki et al. 2016; Nakabayashi et al. 2019); therefore, *Acropora hyacinthus* might be an ancestral species with highly variable haplotypes or may contain several cryptic species.

**Proposal of future grouping of Acropora**

The following new grouping of *Acropora* species is proposed here until a formal taxonomic revision of the genus is conducted. This grouping was based mainly on identified molecular phylogenetic relationships and previous studies (Hatta et al. 1999; Fukami et al. 2003; Suzuki et al. 2008; Suzuki and Fukami 2012; Fukami et al. 2019). We also considered the morphological characteristics identified here and by Wallace (1999) and reproductive traits (Hatta et al. 1999; Fukami et al. 2003; Suzuki and Fukami 2012; Isomura et al. 2013, 2016) because closely related species by genetics tend to hybridize (Hatta et al. 1999; Fukami et al. 2000; Suzuki and Fukami 2012; Isomura et al. 2013, 2016). Each of the eight groups (1 to 8) was tentatively named below. When more species in Japan are added and more molecular and biological (reproduction) data are obtained, proper grouping names should be determined for each group or these grouping might be revised. Note that each of the polyphyletic species is shown within parentheses, and the morphological and other features of these species were not considered for this grouping. Unidentified species recorded in our study were also excluded from this grouping. For some species, DNA data have been obtained from only one sample; the phylogenetic position of these species might change because the haplotype obtained from one sample could be rare in the species, as discussed above (such species are indicated by asterisks). Table 1 shows the summaries of this grouping.

We chose to only investigate the species in Japan because of the inherent difficulty in evaluating the skeletal morphology of specimens that was phylogenetically analyzed outside of Japan (e.g., in Australia). The phylogenetic relationships of *Acropora* from Australia were reported (van Oppen et al. 2001; Richards et al. 2008, 2013), and these differed from Japanese analysis in several ways, such as in the phylogenetic position of *A. intermedia* (Fukami et al. 2019). In future studies, it would be interesting to perform morphological and phylogenetic comparisons between Japanese and Australian or other sites’ samples to clarify the geological, genetic, and morphological differences in *Acropora*.

For reference, we compared the species groupings of *Acropora* by Wallace (1999) and Veron (2000), and the phylogenetic relationships on *Acropora* by van Oppen et al. (2001), Richards et al. (2013), and Shinzato et al. (2020) with this grouping (Table 1).

**Species grouping of Acropora**

**Group 1: “Early hour spawner group”**

Members: *Acropora tenuis, A. donei*, and *A. yongei*

These three species spawn just after sunset (around 19:00 to 19:30 local time) in May to June in Okinawa, Japan (Hayashiraba et al. 1993; Fukami et al. 2003). They are also genetically closely related in terms of nuclear markers (intron region of the mini-collagen gene; see Fukami et al. 2003) and can hybridize (Fukami et al. 2003; Morita et al. 2019). One specific shared morphological characteristic is the species’ large dish-like radial corallites.

**Group 2: “Second early hour spawner group”**

Members: *Acropora austera, A. elseyi*, and *A. verweyi*

These three species spawn 1.0–1.5 h after sunset (around 20:00 to 20:30 local time) in May to June in Okinawa, Japan (Fukami et al. 2003). We reidentified specimens named “*A. vaughani*-like” by Fukami et al. (2003) as *A. elseyi*. A molecular phylogeny based on the intron region of the mini-collagen gene has shown that these three species form a single clade (Fukami et al. 2003). A specific shared morphological characteristic is a conspicuous, yellow-colored axial corallite. This feature is similar to that of group 7, but axial corallites in the present group are smaller. In addition, these species are all roundish in shape.
Note that Groups 3 to 8 (below) take part in multi-specific synchronous spawning (21:00 to 23:00 local time) (Hayashibara et al. 1993).

**Group 3: “Robust species group”**
Members: *Acropora robusta*, *A. abrotanoides*, *(A. hyacinthus and A. cytherea)*

A specific shared characteristic of this group is the shape of the radial corallites, which is a mixture of two types: long tubular and submerged. Branches are very thick. Published DNA data are not available for *A. abrotanoides* and only one sample was analyzed in this study, so it will be necessary to add more *A. abrotanoides* samples to confirm the phylogenetic position of this species.

**Group 4: “Complex species group”**
Members: *Acropora digitifera*, *A. divaricata*, *Acropora sp.1 sensu* Hayashibara and Shimoike (2002), *A. nasuta*, *A. pulchra*, *A. pruinosa* (Brook, 1892), *A. selago* (Studer, 1879), *A. solitaryensis*, *(A. lorisipes, A. muricata, and A. valida)*

There are no specific shared morphological characteristics in this group. The shape of the radial corallites is nariform or pinecone-like. Colony form is arborescent, corymbose, or arborescent and tabular. Members of this group are common in the Ryukyu Islands except for *A. pruinosa* and *A. solitaryensis*, which are dominant in Kyushu, Shikoku, and Honshu; *A. pruinosa* and *A. solitaryensis* can hybridize (Suzuki and Fukami 2012). For *Acropora sp. 1*, we analyzed only one sample; however, Ohki et al. (2015) used the nuclear Pax-C marker to show that this species was genetically closely related to *A. digitifera*.

**Group 5: “Slender branch species group”**
Members: *Acropora microphthalma*, *A. nana*, *(A. cf. glauca, A. lorisipes, A. muricata, and A. valida)*

This group exhibits various colony forms but has tapering slender branches regardless of length. In this study, we renamed “*A. solitaryensis PL*” (plate type of *A. solitaryensis*), as previously named by Suzuki and Fukami (2012), as *(A. cf. glauca)* following Sugihara et al. (2015) because a proper scientific name for this species is not available at present. This species clearly differs from the true *A. solitaryensis* in Group 4 according to both morphological and molecular data. Data on hybridization are not currently available for this group.

**Group 6: “Tabular species group”**
Members: *Acropora cytherea*, *A. hyacinthus*, and *A. anthocercis*

*Acropora hyacinthus* and *A. cytherea* can hybridize (Willis et al. 1997; Suzuki et al. 2016). All species have dish-like radial corallites and a more-or-less tabular colony form. However, *A. hyacinthus* is highly polyphyletic and could contain several cryptic species (Ladner and Palumbi 2012; Suzuki et al. 2016). Thus, additional molecular and morphological analyses are required for *A. hyacinthus*. Published DNA data are not available for *A. anthocercis* and only one sample was analyzed in this study; therefore, it will be necessary to analyze more *A. anthocercis* samples to confirm the phylogenetic position of this species.

**Group 7: “Conspicuous axial corallite species group”**
Members: *Acropora florida* (Dana, 1846), *A. gemmifera*, *A. humilis*, *A. intermedia*, *A. monticulosa*, *A. samoensis*, and *A. spathulata*

These species have one specific shared morphological characteristic, which is a large round axial corallite (3–5 mm). In the field, the axial corallite is conspicuous and usually orange-colored. The colony form varies from arborescent to corymbose. *Acropora florida*, *A. gemmifera*, *A. humilis*, and *A. intermedia* can hybridize (Hatta et al. 1999; Wolstenholme 2004; Isomura et al. 2013, 2016); however, there is no record of hybridization in *A. samoensis* and *A. spathulata*. We analyzed only one sample of *A. monticulosa*, but Wolstenholme (2004) showed that this species was genetically closely related to *A. humilis*, *A. gemmifera*, and *A. samoensis*.

**Group 8: “Aspera-Papillare species group”**
Members: *Acropora aspera*, *A. papillare*, *(A. acuminata)*, and *(A. hyacinthus)*

*Acropora aspera* and *A. papillare* are morphologically similar, in terms of axial and radial corallites, to Group 7 but genetically distinct. *Acropora acuminata* is morpho-
logically different from these two species and we analyzed only one sample in this study. More morphological and phylogenetic analyses are therefore required for this species. Data on hybridization are not currently available for this group.

Species diversity at Funakoshi in Amami-Oshima

In this study, *A. spathulata* was recorded in Japan for the first time. In particular, this species is abundant, forming a large community at site III (see Fig. 1C, E). This species is morphologically similar to *A. millepora* and *A. aspera*; indeed, was previously recognized as *A. millepora* (Willis et al. 2006). Given that *A. spathulata* is common in Australia and found in Amami-Oshima, but not in Tanegashima (Sugihara et al. 2015) nor the higher latitudinal area in Japan (personal observation by authors), the northern limit of this species is likely to be Amami-Oshima. *Acropora spathulata* probably inhabits the area around Okinawa, a more southern area than Amami-Oshima; however, as this species is rarely recognized in Japan, it might be identified as *A. millepora*, *A. aspera*, or a similar species at present.

To date, there have been few reports on the species diversity of corals in Amami-Oshima. Hata et al. (2013) reported 92 coral species, including 16 *Acropora* species, from three sites (Keten Bay, Kudadon, and Derikyonma) in Amami-Oshima. Additionally, in Tokunoshima Island and Kikaijima Island, both of which are close to Amami-Oshima, 220 (39 *Acropora* species) (Nishihira and Veron 1995) and 130 (25 *Acropora* species) (Fukami et al. 2016; Fujii et al. 2020) coral species, respectively, have been reported. In this study, we focused solely on *Acropora* species, and our survey was conducted by snorkeling in a limited area; in contrast, surveying by scuba diving would likely identify more species in deeper sites and other areas, especially outside the reefs. Therefore, additional surveys are required to determine species diversity and numbers in Amami-Oshima.

Acknowledgements

We would like to thank T. Koido, Y. Oku, and members of the Fukami laboratory, University of Miyazaki, for their assistance with molecular analysis, and reviewers and editors for their useful comments on the manuscript. We also thank to Enago (www. Enago.jp) for the English language review.

Compliance

Permissions relevant for Amami-Oshima to undertake the research were obtained from Kagoshima prefecture and fishermen’s cooperative of Uken village. This study was supported in part by JSPS KAKENHI Grant Number JP18K06423 to HF.

References

Arrigoni R, Terraneo TI, Galli P, Benzoni F (2014a) Lobophylliidae (Cnidaria, Scleractinia) reshuffled: pervasive non-monophyly at genus level. Mol Phylogenet Evol 73: 60–64

Arrigoni R, Richards ZT, Chen CA, Baird AH, Benzoni F (2014b) Phylogenetic relationships and taxonomy of the coral genera *Australomussa* and *Parascolymia* (Scleractinia, Lobophylliidae). Contrib Zool 83: 195–215

Arrigoni R, Benzioni F, Huang D, Fukami H, Chen CA, Berumen ML, Hoogenboom M, Thomson DP, Hoeksema BW, Budd AF, Zayasu Y, Terraneo T, Kitano Y, Baird AH (2016) When forms meet genes: revision of the scleractinian genera *Micromussa* and *Homophyllia* (Lobophylliidae) with a description of two new species and one new genus. Contrib Zool 85: 387–422

Arrigoni R, Berumen ML, Stolarski J, Terraneo TI, Benzioni F (2018) Uncovering hidden coral diversity: a new cryptic lobophylliid scleractinian from the Indian Ocean. Cladistics https://doi.org/10.1111/cla.12346

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, et al. (1986) Mass spawning of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90: 379–394

Benzoni F, Arrigoni R, Stefani F, Reijnen BT, Montano S, Hoeksema BW (2012) Phylogenetic position and taxonomy of *Cycloseris explanulata* and *C. welshi* (Scleractinia: Fungiidae): lost mushroom corals find their way home. Contrib Zool 81: 125–146

Chen IP, Tang CY, Chiou CY, Hsu JH, Wei NV, Wallace CC, Muir P, Wu H, Chen CA (2009) Comparative analyses of coding and noncoding DNA regions indicate that *Acropora* (Anthozoa: Scleractinia) possesses a similar evolutionary tempo of nuclear vs. mitochondrial genomes as in plants. Mar Biotechnol 11: 141–152

Council for coral reef conservation in Amami Islands (2013)
Records of extermination of the crown-of-thorns starfish. https://www.amami-sango.com/report

Fujii T, Kitano Y, Isomura N, Fukami H (2020) Zooxanthellate corals of Kikaijima Island. Kikai institute for coral reef sciences, Kagoshima. http://kikaireefs.org/download/4622/ (low resolution version) or http://kikaireefs.org/download/4646/ (high resolution version for the first half) and http://kikaireefs.org/download/4648/ (high resolution version for the second half) (in Japanese)

Fukami H, Omori M, Hatta M (2000) Phylogenetic relationships in the coral family Acroporidae, reassessed by inference from mitochondrial genes. Zool Sci 17: 689–696

Fukami H, Omori M, Shimoike K, Hayashibara T, Hatta M (2003) Ecological and genetic aspects of reproductive isolation by different spawning times in Acropora corals. Mar Biol 142: 679–684

Fukami H, Budd AF, Levitan DR, Jara J, Kernsanach R, Knowlton N (2004a) Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. Evolution 58: 324–337

Fukami H, Budd AF, Paulay G, Solé-Cava AM, Chen CA, Iwao K, Knowlton N (2004b) Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. Nature 427: 832–835

Fukami H, Chen CA, Budd AF, Collins AG, Wallace CC, Chuang YY, Chen C, Dai CF, Iwao K, Sheppard CRC, Knowlton N (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). PLoS ONE 3: e3222

Fukami H, Kitano FY, Tachikawa H (2016) Species list of zooxanthellate scleractinian corals in Kikaijima. Kaiyo Monthly/Special (56): 94–102 (in Japanese)

Fukami H, Iwao K, Kumagai NH, Morita M, Isomura N (2019) Maternal inheritance of F1 hybrid morphology and colony shape in the coral genus *Acropora*. PeerJ 7: e6429

Furukawa M, Ohki S, Kitanobo S, Fukami H, Morita M (2020) Differences in spawning time drive cryptic speciation in the coral *Acropora divaricata*. Marine Biology 167: 163 https://doi.org/10.1007/s00227-020-03781-z

Hatta M, Fukami H, Wang W, Omori M, Shimoike K, Hayashibara T, Ina Y, Sugiyama T (1999) Reproductive and genetic evidence for a reticulate evolutionary history of mass-spawning corals. Mol Biol Evol 16: 1607–1613

Hata H, Hirabayashi I, Hamaoka H, Mukai Y, Omori K, Fukami H (2013) Species-diverse coral communities on an artificial substrate at a tuna farm in Amami, Japan. Mar Environ Res 85: 45–53

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, et al. (1984) Mass spawning in tropical reef corals. Science 223: 1186–1189.

Hayashibara T, Shimoike K (2002) Cryptic species of *Acropora digittiera*. Coral Reefs 21: 224–225

Hayashibara T, Shimoike K, Kimura T, Hosaka S, Heyward A, Harrison P, Kudo K, Omori M (1993) Patterns of coral spawning at Akajima Islands, Okinawa, Japan. Mar Ecol Prog Ser 101: 253–262

Hirata K (1980) Outbreaks of crown-of-thorns starfish in Amami National Marine Park. Sizen-aigo 6: 2e4

Huang D, Benzioni F, Fukami H, Knowlton N, Smith ND, Budd AF (2014a) Taxonomic classification of the reef coral families Merulinidae, Montastreaeidae, and Diploastreidae (Cnidaria: Anthozoa: Scleractinia). Zool J Linn Soc 171: 277–355

Huang D, Benzioni F, Arrigoni R, Baird AH, Berumen ML, Bouwmeester J, Chou LM, Fukami H, Licuanaan WY, Lovell ER, Meier R, Todd PA, Budd AF (2014b) Towards a phylogenetic classification of reef corals: the Indo-Pacific genera *Merulina*, *Goniastrea* and *Scapophyllia* (Scleractinia, Merulinidae). Zool Sci 43: 531–548

Huang D, Arrigoni R, Benzioni F, Fukami H, Knowlton N, Smith ND, Stolarski J, Chou LM, Budd AF (2016) Taxonomic classification of the reef coral family Lobophylliidae (Cnidaria: Anthozoa: Scleractinia). Zool J Linn Soc 178: 436–481

Isomura N, Iwao K, Fukami H (2013) Possible natural hybridization of two morphologically distinct species of *Acropora* (Cnidaria, Scleractinia) in the Pacific: fertilization and larval survival rates. PLoS One 8: e56701

Isomura N, Iwao K, Morita M, Fukami H (2016) Spawning and fertility of F1 hybrids of the coral genus *Acropora* in the Indo-Pacific. Coral Reefs 35: 851–855

Kitahara MV, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. PLoS ONE 5: e11490.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35: 1547–1549

Ladner JT, Palumbi SR (2012) Extensive sympathy, cryptic diversity and introgression throughout the geographic distribution of two coral species complexes. Mol Ecol 21: 2224–2228
Mao Y, Economo EP, Satoh N (2018) The roles of introgression and climate change in the rise to dominance of *Acropora* corals. Curr Biol 28: 3373–3382. e3375

Mao Y (2020) Genomic insights into hybridization of reef corals. Coral Reefs 39: 61–67

Morita M, Kitanobo S, Nozu R, Iwao K, Fukami H, Isomura N (2019) Reproductive strategies in the intercrossing corals *Acropora donei* and *A. tenuis* to prevent hybridization. Coral Reefs 38: 1211–1223

Nakabayashi A, Yamakita T, Nakamura T, Aizawa H, Kitano YF, Iguchi A, Yamano H, Nagai S, Agostini S, Teshima KM, Yasuda N (2019) The potential role of temperate Japanese regions as refugia for the coral *Acropora hyacinthus* in the face of climate change. Scientific reports 9: 1892 https://doi.org/10.1038/s41598-018-38333-5

Nishihira M, Veron JEN (1995) Hermatypic corals of Japan. Kiyusha, Tokyo (in Japanese)

Ohki S, Kowalski RK, Kitanobo S, Morita M (2015) Changes in spawning time led to the speciation of the broadcast spawning corals *Acropora digitifera* and the cryptic species *Acropora* sp. 1 with similar gamete recognition systems. Coral Reefs 34: 1189–1198 https://doi.org/10.1007/s00338-015-1337-4

Richards ZT, van Oppen MJH, Wallace CC, Willis BL, Miller DJ (2008) Some rare Indo-Pacific coral species are probable hybrids. PLoS One 3: e3240

Richards ZT, Miller DJ, Wallace CC (2013) Molecular phylogenetics of geographically restricted *Acropora* species: implications for threatened species conservation. Mol Phylo Evol 69: 837–851

Richards ZT, Berry O, van Oppen MJH (2016) Cryptic genetic divergence within threatened species of *Acropora* coral from the Indian and Pacific Oceans. Conserv Genet 17: 577–591

Rosser NL, Thomas L, Stankowski S, Richards ZT, Kennington WJ, Johnson MS (2017) Phylogenomics provides new insight into evolutionary relationships and genealogical discordance in the reef-building coral genus *Acropora*. Proc R Soc B 284: 20162182 http://dx.doi.org/10.1098/rspb.2016.2182

Santodomingo N, Wallace CC, Johnson KG (2015) Fossils reveal a high diversity of the staghorn coral genera *Acropora* and *Isopora* (Scleractinia: Acroporidae) in the Neogene of Indonesia. Zool J Linn Soc 175: 677–763

Shinzato C, Khalturin K, Inoue J, Zayas Y, Kanda M, Kawamitsu M, Yoshioka Y, Yamashita H, Suzuki G, Satoh N (2020) Eighteen coral genomes reveal the evolutionary origin of *Acropora* strategies to accommodate environmental changes. Mol Biol Evol msaa216 https://doi.org/10.1093/molbev/msaa216

Sugihara K, Nomura K, Yokochi H, Shimoike K, Kajiwara K, Suzuki G, Zayas Y, Dewa N, Fukami H, Kitano Y, Matumoto H, Mezaki T, Nagata S, Tachikawa H, Kimura T (2015) Zoanthellate scleractinian corals of Tane-gashima Island, Japan. Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Tsukuba, Japan. 198pp (in Japanese)

Suzuki G, Hayashibara T, Shirayama Y, Fukami H (2008) Evidence of species-specific habitat selectivity of *Acropora* corals based on identification of new recruits by two molecular markers. Mar Ecol Prog Ser 355: 149–159

Suzuki G, Fukami H (2012) Evidence of genetic and reproductive isolation between two morphs of subtropical-dominant coral *Acropora solitaryensis* in the non-reef region of Japan. Zool Sci 29: 134–140

Suzuki G, Keshavmurthy S, Hayashibara T, Wallace CC, Shirayama Y, Chen CA, Fukami H (2016) Genetic evidence of peripheral isolation and low diversity in marginal populations of the *Acropora hyacinthus* complex. Coral Reefs 35: 1419–1432

van Oppen MJH, McDonald BJ, Willis B, Miller DJ (2001) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? Mol Biol Evol 18: 1315–1329

Veron JEN (2000) Corals of the World. Volumes 1–3. Australian Institute of Marine Science, Townsville, Australia

Wallace CC (1999) Staghorn corals of the world: a revision of the genus *Acropora*. CSIRO Publishing, Melbourne

Wallace CC, Done BJ, Muir PR (2012) Revision and catalogue of worldwide staghorn corals *Acropora* and *Isopora* (Scleractinia, Acroporidae) in the Museum of Tropical Queensland. Mem Qld Mus 57: 1–255

Wei NWV, Wallace CC, Dai CF, Pillay KRM, Chen CA (2006) Analyses of the ribosomal internal transcribed spacers (ITS) and the 5.8S gene indicate that extremely high rDNA heterogeneity is a unique feature in the scleractinian coral genus *Acropora* (Scleractinia; Acroporidae). Zool Stud 45: 404–418

Willis BL, Babcock RC, Harrison PL, Wallace CC (1997) Experimental hybridization and breeding incompatibilities within mating systems of mass spawning reef corals. Coral Reefs 16: 53–65

Willis BL, van Oppen MJH, Miller DJ, Vollmer SV, Ayre DJ
(2006) The role of hybridization in the evolution of reef corals. Annu Rev Ecol Evol Syst 37: 489–517

Wolstenholme JK (2004) Temporal reproductive isolation and gametic compatibility are evolutionary mechanisms in the Acropora humilis species group (Cnidaria; Scleractinia). Mar Biol 144: 567–582

Yamaguchi M (1986) Acanthaster planci infestations of reefs and coral assemblages in Japan: a retrospective analysis of control efforts. Coral Reefs 5: 23–30

Electronic supplementary material
ESM Fig. 1 and ESM Table 1 can be downloaded from the J-STAGE website: https://doi.org/10.3755/galaxea.G23–5

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