Molecular Phylogeny and Taxonomy of the Coral Genus Cyphastrea (Cnidaria, Scleractinia, Merulinidae) in Japan, With the First Records of Two Species

Authors: Chukaew, Thanapat, Isomura, Naoko, Mezaki, Takuma, Matsumoto, Hisashi, Kitano, Yuko F., et al.

Molecular Phylogeny and Taxonomy of the Coral Genus *Cyphastrea* (Cnidaria, Scleractinia, Merulinidae) in Japan, With the First Records of Two Species

Thanapat Chukaew[1], Naoko Isomura[2], Takuma Mezaki[3], Hisashi Matsumoto[4†], Yuko F. Kitano[5], Yoko Nozawa[6], Hiroyuki Tachikawa[7], and Hironobu Fukami[8]*

[1]*Graduate School of Agriculture, University of Miyazaki, Miyazaki, Miyazaki 889-2155, Japan*
[2]*Bioresources Engineering, Institute of Technology, Okinawa College, 905 Henoko, Nago-city, Okinawa 905-2192, Japan*
[3]*Kuroshio Biological Research Foundation, 560 Nishidomari, Otsuki, Kochi 788-0333, Japan*
[4]*Miyakojima Sunagawa Elementary School, 605 Miyakojima, Okinawa 906-0108, Japan*
[5]*Japan Wildlife Research Center, 3-3-7 Kotobashi, Sumida-ku, Tokyo 130-8606, Japan*
[6]*Biodiversity Research Center, Academia Sinica, Taipei, 115, Taiwan*
[7]*Coastal Branch of Natural History Museum and Institute, Chiba, 123 Yoshio, Katsuura, Chiba 299-5242, Japan*
[8]*Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, Miyazaki University, Miyazaki, Miyazaki 889-2155, Japan*

The scleractinian coral genus *Cyphastrea* is widely distributed in the Indo-Pacific region and is common from the subtropical to the warm-temperate regions in Japan. Three new species in this genus have recently been reported from south-eastern Australia or the Red Sea. However, taxonomic and species diversity have been little studied so far in Japan. In this study, we analyzed 112 specimens of *Cyphastrea* collected from the subtropical to the warm-temperate regions in Japan to clarify the species diversity in the country. This analysis was based on skeletal morphological and molecular analyses using three genetic markers of the nuclear 28S rDNA, histone H3 gene, and the mitochondrial noncoding intergenic region between COI and tRNAmet. The molecular phylogenetic trees showed that our specimens are separated mainly into four clades. Considering the morphological data with the molecular phylogenetic relationships, we confirmed a total of nine species, including two species, *C. magna* and *C. salae*, recorded for the first time in Japan. Although eight out of nine species were genetically included within *Cyphastrea*, one species, *C. agassizi*, was genetically distant from all other species and was closely related to the genus *Leptastrea*, suggesting the return of this species to the genus to which it was originally ascribed. Two newly recorded species were reciprocally monophyletic, while the other six species (excluding *C. agassizi*) clustered in two clades without forming species-specific lineages, including three polyphyletic species. Thus, the species boundary between species in *Cyphastrea* remains unclear in most species using these three sequenced loci.

Key words: scleractinia, histone, mitochondria, 28S, species diversity

INTRODUCTION

Cyphastrea Milne Edwards and Haime, 1848 is a zooxanthellate scleractinian coral genus in the family Merulinidae Milne Edwards & Haime, 1857 and is widely distributed in the Indo-Pacific Ocean (Veron, 2000). *Cyphastrea* represents a morphologically well-defined and monophyletic lineage at the genus level (Huang et al., 2011, 2014a; Arrigoni et al., 2012, 2017). Morphological characteristics of *Cyphastrea* are defined as follows: colonial with only extra-calicular budding, about 1–2 mm diameter of corallites with monomorphic and plocoid-type, spinose coenosteum, 24 or less septal number (three or fewer orders of septa) with poorly developed or no paliform lobes, and trabecular and compact columellae (Veron et al., 1977; Veron, 2000). Recently, taxonomic studies of *Cyphatrea* have advanced, and three new species have been described (Bouwmeester et al., 2015; Arrigoni et al., 2017; Baird et al., 2017): *Cyphastrea kausti* Bouwmeester and Benzoni, 2015 and *Cyphastrea magna*

Benzoni and Arrigoni, 2017 from the Red Sea, and *Cyphastrea salae* Baird, Hoogenboom and Huang, 2017 from Lowd How Island in Australia. These three species have not yet been reported in any other regions. *Cyphastrea* contains 12 valid extant species (Huang et al., 2014a; Hoeksema and Cairns, 2021). All species are massive or encrusting in colony shape, except for *Cyphastrea decadia* Moll and Borel-Best, 1984 with a branching colony, and *Cyphastrea zhongjianensis* Zou, 1980 with a free-living glomerate-branched colony.

Integrated analysis using molecular phylogenetic and morphological data is now a nearly required method to evaluate the species status of corals (e.g., Benzoni et al., 2012; Budd et al., 2012; Huang et al., 2014a, 2014b, 2016; Arrigoni et al., 2019). For *Cyphastrea*, this approach has been applied to specimens in the Red Sea using the nuclear 28S rDNA gene (28S), histone H3 gene (H3), and the mitochondrial noncoding intergenic region (mtIGR) between COI and tRNAmet (Arrigoni et al., 2017). In Australia, the existence of a new species, *C. salae*, was revealed using 28S and mtIGR (Baird et al., 2017). On the other hand, *Cyphastrea chalcidicum* (Forskål, 1775), *C. kausti*, *Cyphastrea microphthalma* (Lamarck, 1816), *Cyphastrea hexasepta* Veron, Turak and DeVantier, 2000, and *Cyphastrea serailia* (Forskål, 1775) were not genetically distinguishable (Arrigoni et al., 2017; Baird et al., 2017).

In Japan, *Cyphastrea* species are distributed from the subtropical coral reef region (the Ryukyu Islands and the Ogasawara Islands) to the warm-temperate non-reef region (mainland Japan). At present, a total of nine species of *Cyphastrea* have been reported: *Cyphastrea agassizi* (Vaughan, 1907), *C. chalcidicum*, *C. decadia*, *Cyphastrea japonica* Yabe and Sugiyama in Yabe, Sugiyama and Eguchi, 1936, *C. microphthalma*, *Cyphastrea ocellina* (Dana, 1846), *C. serailia* by Nishihira and Veron (1995), *Cyphastrea conferta* Nemenzo, 1959 by Sugihara (2014) and Sugihara et al. (2015), and *C. zhongjianensis* by Nishihira and Sugihara (2015) and Yokochi et al. (2019). Additionally, *Cyphastrea chalcidicum tanabensis* Yabe and Sugiyama in Yabe, Sugiyama and Eguchi, 1936 was described as a new subspecies of *C. chalcidicum* in Yabe et al. (1936). However, this subspecies is currently treated as a junior subjective synonym of *C. chalcidicum* or *C. japonica* (see Hoeksema and Cairns, 2022a, c). The authorship and the date of publication of *C. japonica* and *C. chalcidicum tanabensis* are frequently referred to as "Yabe and Sugiyama, 1932" (e.g., Veron, 2000; Hoeksema and Cairns, 2022a, c). However, as Yabe and Sugiyama (1932) only proposed the name of these taxa and did not give any description, Yabe et al. (1936), in which formal descriptions were made, should be considered as the original description of these taxa; consequently, the authorship and date of publication of these taxa should be written as "Yabe and Sugiyama in Yabe, Sugiyama and Eguchi, 1936". For *C. conferta*, Sugihara (2014) and Sugihara et al. (2015) resurrected this previously synonymized species as a valid species without any reason. Still, this species has generally been treated as a synonym of *C. serailia* since Veron et al. (1977) (Hoeksema and Cairns, 2022b).

Nishihira and Veron (1995) reported seven Japanese *Cyphastrea* species, but detailed surveys of the species composition and molecular phylogenetic analyses have not been conducted in Japan yet. Therefore, we investigate the species diversity of *Cyphastrea* in Japan in this study based on the morphological and molecular phylogenetic data of specimens collected from the subtropical to the warm-temperate region in Japan and also from Taiwan as a comparison. Also, we provide a brief diagnosis for a few species of *Cyphastrea* which are the ones recorded for the first time in Japan or with uncertain identification/taxonomy.

MATERIALS AND METHODS

Sampling

We collected samples from 11 sites in Japan in addition to the southern (Kenting) and northern (Bitou) parts of Taiwan (Fig. 1; see Supplementary Table S1). Samples (about 50–100 cm^3) were collected using a hammer and a chisel by SCUBA divers during 2017–2020. We took photographs of living specimens in the field. A small piece (about 25 mm^2) of each collected sample was put into a guanidine-based solution (4M guanidine thiocyanate, 0.1% N-lauroyl sarcosine sodium, 10 mM Tris-HCl pH 8, 0.1 M 2-mercaptoethanol; Fukami et al., 2004) to dissolve the tissue for DNA analysis. The rest of each sample was placed in sodium hypochlorite solution for up to 48 h to remove all tissue, rinsed in freshwater, and dried for skeletal morphological analysis. All Japanese skeletal specimens were deposited at the Department of Marine Biology and Environmental Sciences, University of Miyazaki (MUFS), Miyazaki, Japan, except the ones collected from Kikaijima Island that were deposited at KIKAI Institute for Coral Reef Sciences (KICRS), Kikaijima Island, Kagoshima, Japan. The Taiwanese specimens were deposited at Zoological Collection, Biodiversity Research Museum, Academia Sinica (ASIZC), Taiwan.

Museum abbreviations

AM, Australian Museum, Sydney, Australia; MNHN, Muséum national d'Histoire naturelle de Paris, France; NHMUK, Natural History Museum, London, UK (formerly British Museum of Natural History; BMNH); TU, Tohoku University, Sendai, Japan; UP, Marine Science Institute, University of the Philippines, Manila, the Philippines; YPM, Yale Peabody Museum of Natural History, New Haven, Connecticut, USA; ZMUC, Zoologisk Museum, University of Copenhagen, Denmark.

Molecular analyses

Total genomic DNA was extracted from the guanidine-based solution of each sample using the conventional phenol/chloroform extraction method. The nuclear 28S gene, H3 gene, and mtIGR region were amplified by polymerase chain reaction (PCR) using the following primers: 28SC1F (5′-ACC CGC TGA ATT TAA GCA T-3′) and 28sD2MAD (5′-TCG GAT GGA CCC ATA TGA-3′) (Cuif et al., 2003) for 28S, and H3F (5′-ATG GCT CGT ACC AAG CAG ACV GC-3′) and H3R (5′-ATA TCC TTR GGC ATR GTG AC-3′) (Colgan et al., 1998) for H3. Newly designed primers in this study were also considered, such as mtCRnewF1 (5′-AAT GGA CAT CGA AGT ACA CCA T-3′) and mtCRnewR1 (5′-AAT TGT CAA TCT GGC TAA GAC AAA C-3′) for mtIGR (first, we tried the previously published primers for this region, such as COIF3 and tRNAmetR [Fukami et al., 2004] but failed in amplifying it). The following conditions were considered: 94°C for 1 min followed by 5 cycles at 94°C for 30 s, 48°C for 45 s, 72°C for 90 s, and by 25 cycles at 94°C for 30 s, 55°C for 45 s, 72°C for 90 s, and 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, followed by treatment at 80°C for 20 min.

DNA sequences were determined by direct sequencing using ABI3730 sequencers by a contracted research service (FASMAC Co. Ltd., Kanagawa, Japan). All DNA sequences were manually aligned using BioEdit version 7.2 (www.mbio.ncsu.edu/BioEdit/bioedit.html). Molecular phylogenetic trees were reconstructed
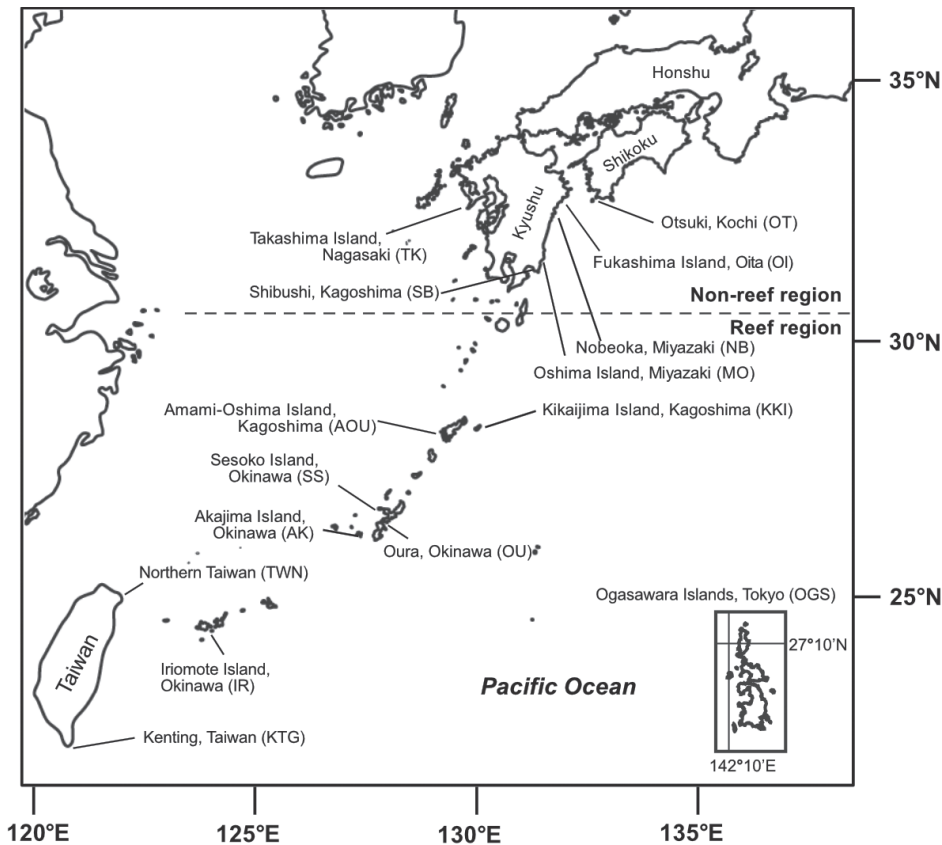
Fig. 1. Map of sampling sites. Taiwan: Northern Taiwan (TWN) and Kenting (KTG). Japan: Otsuki (OT) in Kochi; Fukashima Island (OI) in Oita; Nobeoka (NB) and Oshima Island (MO) in Miyazaki; Takashima Island (TK) in Nagasaki; Shibushi (SB), Amami Oshima Island (AOU), and Kikaijima Island (KII) in Kagoshima; Sesoko Island (SS), Akajima Island (AK), Oura (OU), and Iriomote Island (IR) in Okinawa; and Ogasawara Islands (OGS) in Tokyo.

using the neighbor-joining (NJ) and maximum-likelihood (ML) methods in MEGA X (Kumar et al., 2018). MEGA X was also used to estimate a model of nucleotide evolution for each marker (T92+G+I for 28S, JK+G for H3, and T92+I for mtIGR) and to conduct a bootstrap analysis (with 1000 replicates). All DNA sequences obtained in this study were submitted to the DNA Data Bank of Japan (DDBJ) (accession numbers LC750494–LC750685, see Supplementary Table S1). DNA sequences of *Cyphastrea* spp. from the Red Sea, Australia, Singapore, and the Philippines (Arrigoni et al., 2017; Baird et al., 2017; Huang et al., 2011) (see Supplementary Table S2) were used for comparison with our sequence data in this study. Additionally, DNA sequences (Cuif et al., 2003; Huang et al., 2011) of *Leptastrea* sp., *Paramontastraea salebrosa* (Nemenzo, 1959), and *Diploastrea heliopora* (Lamarck, 1816) were used as outgroups (see Supplementary Table S1).

Morphological examination

Skeletal specimens were observed and analyzed using a VHX-100 digital microscope (Keyence Co. Osaka, Japan) and Miniscope TM-1000 (Hitachi Ltd.) to examine the skeletal morphological characteristics. Five mature individuals were randomly selected for each specimen. We counted or measured the following characters (Fig. 2): the diameter of the corallite, calice, columella, septal number of the first order, and total septal and costal numbers. Additionally, we counted the number of corallites per 1 cm^2, the number of spines on the coenosteum per 1 mm^2, and the number of granules on the spine tip on the coenosteum (three replicates per specimen) to examine the morphological differences between *C. salae* and *C. serailia* (see Results). A generalized linear model was used to examine whether these morphological characters were conserved within and between species for *C. salae* and *C. searailia* (see Results). The responsive variables were the morphological characters, and the explanatory variable was the combination of species. The error distribution and link function of the model were gamma distribution and log, respectively, and the above analysis was performed using R ver. 4.2.1 (R Development Core Team, 2022) with the "lme4" package. The multivariate dataset was processed by principal components analysis (PCA) using the R with the "ggbiplot" and "devtools" packages to determine critical characteristics to separate the species.

In *Cyphastrea*, the total septal number is 24 in most species, but the counting is not concordant among the reports previously published. For the scleractinian corals, a septal number with 24 septa is generally counted as "six primary septa, six secondary septa, and 12 tertiary septa" or "six septa in the first cycle, six septa in the second cycle, and 12 septa in the third cycle" (e.g., Duerden, 1904; Veron, 2000). In the description of *Cyphastrea* species with 24 septa, the septal number has been described as "Septa are in two very unequal orders of 12 each" and "Septa of the first order can be divided into two hexameral cycles" by Veron et al. (1977).
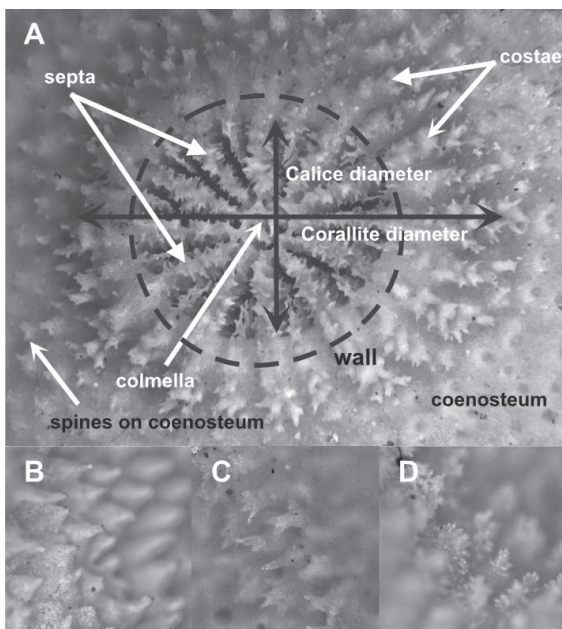

Fig. 2. Morphological characters of *Cyphastrea*. Main morphological characters of a corallite and coenosteum **(A)**, and different numbers of granules on the spine tip on coenosteum **(B–D)**. No granules **(B)**, a few granules **(C)**, and many granules **(D)**.
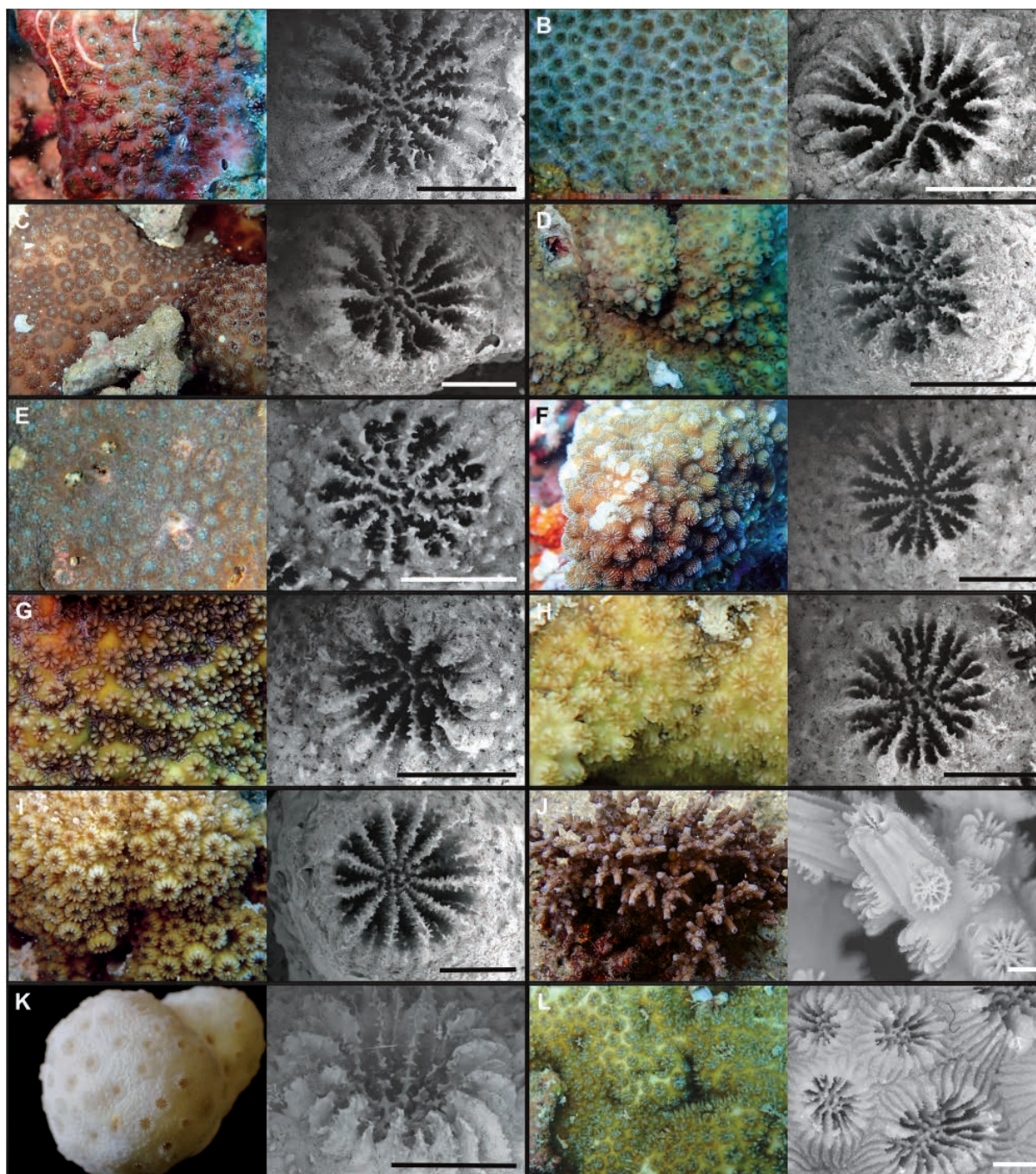
Fig. 3. In situ (left) and skeletal (right) images of *Cyphastrea* species identified in this study. *Cyphastrea serailia* (AOU373) in clade I **(A)**, *C. serailia* (MO247) in clade III **(B)**, *C. chalcidicum* (SS108) in clade I **(C)**, *C. chalcidicum* (IR496) in clade III **(D)**, *C. microphthalma* type I (MO338) **(E)**, *C. microphthalma* type I (IR377) **(F)**, *C. microphthalma* type II (IR413) **(G)**, *C. ocellina* (SS124) **(H)**, *C. magna* (SS121) **(I)**, *C. decadia* (AOU458) **(J)**, *C. zhongjianensis* (OU42) **(K)**, and *C. agassizi* (IR538) **(L)**. Scale bars: 1 mm.

Still, the number was also described as "12 equal primary septa" by Arrigoni et al. (2017) and "two septal cycles, and 12 primary septa and 12 secondary septa" by Baird et al. (2017). In this study, based on Veron et al. (1977), we used the following counting of septa for species with 24 septa (see Supplementary Table S2): six primary septa (first cycle), six secondary septa (second cycle), and 12 tertiary septa (third cycle) for septal number and cycle. In addition, 12 septa in first order and 12 septa in second order for septal order because of the primarily equivalent size of primary and secondary septa. The counting of costae for specimens with 24 costae also

follows that of septa, i.e., six primary costae (first cycle), six secondary costae (second cycle), and 12 tertiary costae (third cycle), and 12 costae in first order and 12 costae in second order. For several species with less than 24 septa, such as *C. microphthalma*, the counting of septa and costae is also shown in Supplementary Table S2, partially following that shown by Bouwmeester et al. (2015).

Species identification

We examined type materials (holotype or syntype series), the photographic images of type materials, or figures shown in the orig-

Fig. 4. Phylogenetic relationships of *Cyphastrea* spp. based on the DNA sequences of 28S. Numbers on main branches show percentages of bootstrap values (> 50) in ML. DNA sequences obtained from DDBJ are shown in bold (accession numbers or colony numbers). Species names for pre-identification are shown in square brackets after species names. In clades I and III, number of colonies are shown in parentheses. ● for *C. magna*, ★ for *C. salae*.

inal descriptions of all nine species we used in this study. We also summarized the identification of key morphological characters for the main eight species (*C. chalcidicum*, *C. conferta*, *C. japonica*, *C. magna*, *C. microphthalma*, *C. ocellina*, *C. salae*, and *C. serailia*) (see Supplementary Table S3) based on the type materials and the original descriptions. We did not summarize the morphological characteristics of *C. agassizi*, *C. decadia*, *C. hexasepta*, *C. kausti*, or *C. zhongjianensis* because they are morphologically easily distinguished from the other species. We preliminarily identified species based on the information described above. Later, we considered the molecular phylogenetic relationships to determine species identification, considering the morphological variation that is known for this genus (Veron et al., 1977). In particular, *C. salae* is morphologically nearly indistinguishable but genetically distinct from *C. serailia* (see Discussion). Therefore, we did not distinguish between them at first and treated *C. serailia*-like or *C. salae*-like specimens as *C. serailia* at the preliminary species identification.

The morphological difference between *C. serailia* and *C. chalcidicum* is generally recognized as the unequal costal length between the first order (primary and secondary) costae and the second order (tertiary) costae due to abortive tertiary costae in *C. chalcidicum* (Veron et al., 1977, 2016; Veron, 2000). However, in many cases, corallites with subequal costal length between the first and second orders were observed in our specimens. These specimens could not be confidently identified as *C. serailia* or *C. chalicidicum*. We treated such specimens with corallites with subequal costal length as *C. serailia* at identification in this study because the type specimen of *C. chalcidicum* (neotype shown in Veron et al., 1977) displays apparent unequal costal length between the first and second orders with reduced or absent tertiary costae.

Cyphastrea japonica is a species with uncertain taxonomic status in *Cyphastrea* (see Discussion). The holotype specimen of *C. japonica* (Yabe et al., 1936, pl. 17: figs 4–6, photographic images of holotype TU 40323) is superficially similar to *C. chalcidicum* (neotype shown in Veron et al., 1977, fig. 347). The distinct differences between *C. japonica* and *C. chalcidicum* are colony shape (rounded mass composed of humpy branches for *C. japonica* and massive for *C. chalcidicum*) and the size of corallites (smaller in *C. japonica*). In this study, we could not find any specimens with similar colony shapes and corallite sizes to the holotype of *C. japonica*. *Cyphastrea conferta* has
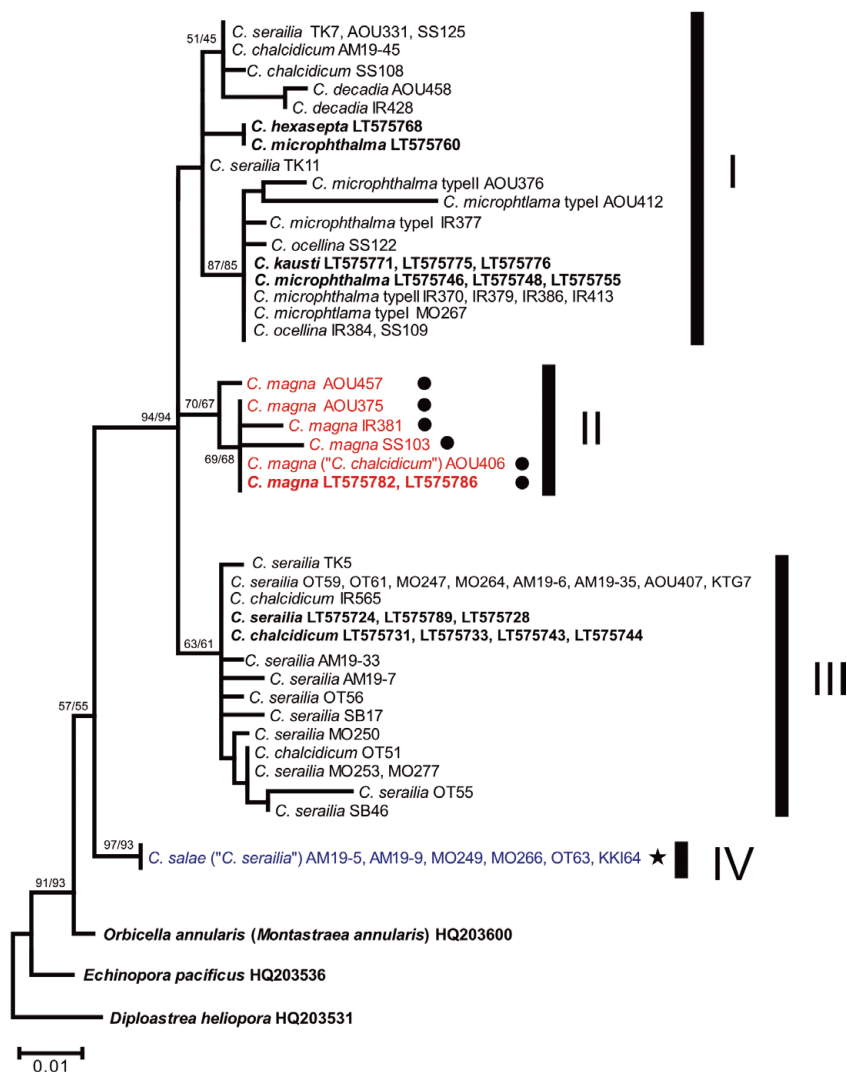
Fig. 5. Phylogenetic relationships of *Cyphastrea* spp. based on the DNA sequences of H3. Numbers on main branches show percentages of bootstrap values (> 50) in ML. DNA sequences obtained from DDBJ are shown in bold (accession numbers or colony numbers). Species names for pre-identification are shown in square brackets after species names. In clades I and III, number of colonies are shown in parentheses. ● for *C. magna*, ★ for *C. salae*.

C. ocellina, C. serailia, and *C. zhongjia-nensis* (Fig. 3). We also found two apparent intraspecific morphological variations for *C. microphthalma* and separated these variations into two morph-types: typical as type I (Fig. 3E, F) and ones with prominent exsert septa and costae as type II (Fig. 3G). These characters of type II are similar to those of *C. ocellina* (Fig. 3H).

Molecular phylogeny

Topologies of the NJ and ML trees inferred using each of the three markers (28S, H3, and mtIGR) were nearly concordant. Thus, only ML trees are shown in this report. Many samples could not be amplified by PCR using the mtIGR marker, even though we modified the primers several times. On the other hand, the other two markers could be amplified successfully for most samples.

The 28S tree showed that our specimens were separated into four clades, I, II, III, and IV, except for *C. agassizi* (Fig. 4). *Cyphastrea agassizi*, originally described as a species of *Leptastrea* by Vaughan (1907), was genetically distant from all other *Cyphastrea* species and formed a clade with *Leptastrea* sp. Therefore, we excluded this species from the subsequent analyses using the other two markers because this species will need a taxonomic revision after further morphological and molecular analyses. Clade I was the largest group and contained *C. chalcidicam* (eight specimens), *C. decadia* (three specimens), *C. microphthalma* type I (12 specimens), *C. microphthalma* type II (seven specimens), *C. ocellina* (six specimens), *C. serailia* (26 specimens), and *C. zhongjianensis* (one specimen) in this study. Additionally, *C. kausti* and *C. hexasepta* from the Red Sea (Arrigoni et al., 2017), *C. microphthalma* from Australia

(Baird et al., 2017) and the Red Sea (Arrigoni et al., 2017), and *C. serailia* from Australia (Huang et al., 2011; Baird et al., 2017) and the Philippines (Huang et al., 2011) were included in clade I. Within clade I, one subclade with a high bootstrap value (98) was formed by some of the collected specimens of *C. ocellina* (four out of six specimens) and *C. microphthalma* type I (two out of 12 specimens). Clade II mainly contained *C. magna* (six specimens) in this study and also from the Red Sea (Arrigoni et al., 2017), in addition to one specimen (IR381) in this study and one from the Great Barrier Reef (GBR, Australia) referred from Huang et al. (2011), both of which were identified as *C. chalcidicum*. Clade III contained *C. serailia* (26 specimens), *C. chalcidicum* (four specimens), and *C. microphthalma* type I (three specimens), in addition to *C. serailia* from Australia and Singapore (Baired et al., 2017) and from the Red Sea (Arrigoni et al., 2017), and *C. chalcidicum* from the Red Sea (Arrigoni et al., 2017). Clade
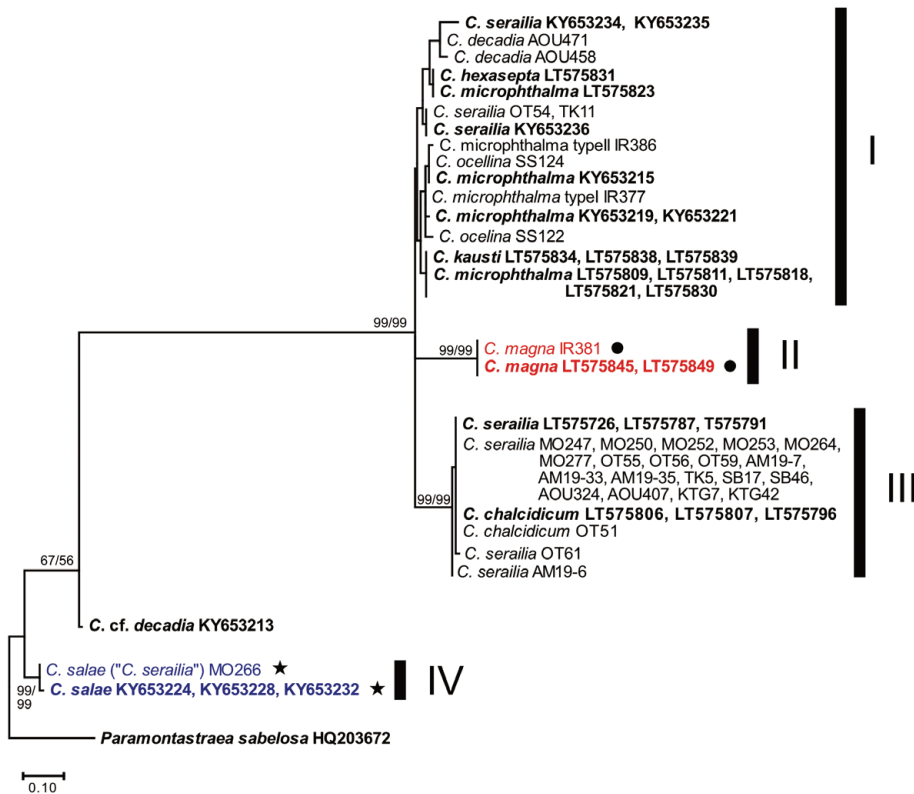
been treated for a long time as a junior synonym of *C. serailia* since Veron et al. (1977). Still, in this study, we first tried to distinguish this species from the other species because it has recently been used as a valid name in Japan (Sugihara, 2014; Sugihara et al., 2015). *Cyphastrea conferta* is morphologically similar to *C. ocellina* except for the height of exsert septa and costae (slightly less exsert in *C. conferta*), based on the photographic images of the type specimens [*C. conferta*: Nemanzo, 1959, pl XV; fig. 1, photographic images of holotype UP C-23 shown in Luzon et al. (2022), *C. ocellina*: photographic images of syntypes YPM IZ 474, 4330]. Therefore, we treated all *C. conferta*-like and *C. ocellina*-like specimens as *C. ocellina* in this study.

RESULTS

Preliminary species identification based on morphology

In total, eight species were preliminarily identified from the 112 samples collected in this study. They are *C. agassizi, C. chalcidicum, C. decadia, C. micropththalma, C. magna,*

Fig. 6. Phylogenetic relationships of *Cyphastrea* spp. based on the DNA sequences of mtIGR. Numbers on main branches show percentages of bootstrap values (> 50) in ML. DNA sequences obtained from DDBJ are shown in bold (accession numbers or colony numbers). Species names for pre-identification are shown in square brackets after species names. In clades I and III, numbers of colonies are shown in parentheses. ● for *C. magna*, ★ for *C. salae*.

IV contained *C. serailia* (eight specimens) in this study and *C. salae* from Australia (Baird et al., 2017). Thus, *C. chalcidicum* (clades I, II, III), *C. microphthalma* type I (clade I, III), and *C. serailia* (clades I, III, IV) were polyphyletic. *Cyphastrea* cf. *decadia* from Fiji (Baird et al., 2017) was genetically different from our collected *C. decadia* (clade I) and was not related to any of the four clades I–IV recovered in this study.

The topology of H3 was similar to that of 28S, and clade IV was distant from the other clades (Fig. 5). Clades II and III were supported with 60–70 bootstrap values, but clades I had low bootstrap values (49).

For mtIGR, many samples could not be amplified. Still, four clades were also formed by this marker, including a distant clade IV, with one specimen of *C. serailia* (MO266) in this study and *C. salae* in Australia (Fig. 6). Notably, in clade IV, the DNA sequences of mtIGR of *C. serailia* in this study and *C. salae* from Australia (Baired et al., 2017) were highly divergent and were challenging to align manually with those sequences from all other species. In addition, the mtIGR sequence of *C. cf. decadia* from Fiji (Baired et al., 2017) was highly divergent from those of others, including *C. decadia,* in this study. Like H3, clades II and III were formed with high bootstrap values (99), but clade I had a low bootstrap value (43).

Re-identification of species with morphology and molecular phylogeny

We re-identified several specimens, considering the molecular phylogenetic relationships and morphological data, because topologies inferred using three markers were concordant, as shown in previous papers (Arrigoni et al., 2017; Baird et al., 2017), in which all specimens of *C. magna* and *C. salae* were monophyletic.

One specimen (IR381, Fig. 7B) identified preliminarily as *C. chalcidicum*, due to the smaller corallite (1.8–2.3 mm) and calice (1.0–1.1 mm) size, was included in clade III. We re-identified this specimen as a morphological variation of *C. magna* (2.53–3.71 mm in corallite size, 1.6–1.9 mm in calice size for *C. magna*) (Fig. 3J, Fig. 7A, B), because it was not only phylogenetically included in the specific clade of *C. magna* but also shared the morphological characters of *C. magna*, except for the corallite and calice size. In addition, one *C. chalcidicum* specimen from GBR referred from Huang et al. (2011), which was also included in clade III, might be a morphological variation of *C. magna*. We could not make a further discussion on this specimen because no morphological data of the specimen is available.

Our specimens preliminarily identified as *C. serailia* in clade IV, in which all of the specimens used in the original description of *C. salae* (Baird et al., 2017) were included, were re-identified as *C. salae* (Fig. 7). Additionally, the DNA sequences of mtIGR of all specimens in clade IV were unique and distinct from those of all other specimens. As far as we examined the skeletal morphology of specimens used in this study, *C. salae* (clade IV) has denser columellae, and tends to have slightly larger and more numerous spines on the coenosteum than *C. serailia* in clades I and III (see Discussion).

Morphological analysis

Basic morphological data of species (after re-identification) are summarized in Table 1. We statistically compared the morphological differences between *C. serailia* (clades I and III) and *C. salae* (Table 2). The results showed that the two characters (total corallite number per 1 cm^2 and granule number on the spine tip of septa) of *C. salae* were significantly different from those of *C. serailia* (clades I and III) (Table 2). We also performed a PCA analysis using the morphological data with significant differences for *C. serailia* (clades I and III) and *C. salae* (Fig. 8). This PCA plot showed that *C. serailia* was roughly distinguishable from *C. salae*. As a result, the total corallite number per 1 cm^2 and the number of granules on the spine tip on the coenosteum seemed to be critical characters to separate *C. salae* from *C. serailia*. However, these data overlapped partially between the two species. Conversely, *C. serailia* was not distinguishable
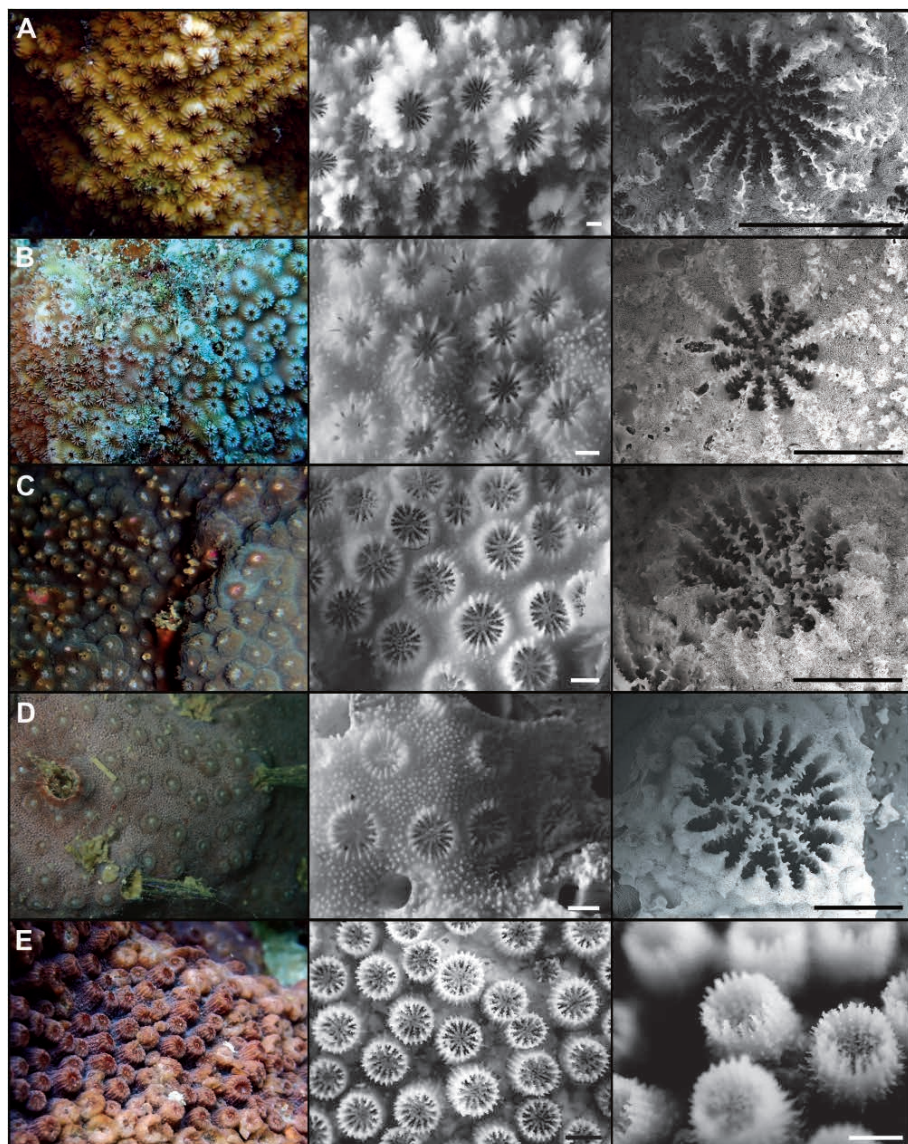
Fig. 7. In situ (left) and skeletal (right) images of *Cyphastrea magna* and *C. salae*. *Cyphastrea magna* (AOU457) **(A)**, *C. magna* (IR381) **(B)**, *C. salae* (MO249) **(C)**, *C. salae* (NB251) **(D)**, and *C. salae* (KKI64) **(E)**. Scale bars: 1 mm.

between clades I and III.

DISCUSSION

Cyphastrea magna and *C. salae* in Japan

Cyphastrea magna was first recorded in Japan, and was found in the subtropical regions of Japan (Akajima Island, Iriomote Island, and Sesoko Island in Okinawa, and Amami-Oshima Island in Kagoshima; Fig. 1). The morphological characters of *C. magna* in the Red Sea are concordant with those of Japanese ones, except for one specimen (IR381) with a 1.4–1.6 times smaller corallite size. On the other hand, in molecular phylogenetic trees (Figs. 4–6), the Japanese specimens differed slightly from the Red Sea specimens regarding their genetic features. We treated this genetic difference as an intraspecific variation in this study and identified Japanese specimens as *C. magna.* However, Japanese ones might be a distinct species from *C. magna* in

the Red Sea. This is because, in several coral species, morphologically identical but genetically different populations from the Pacific Ocean and the Red Sea or the Indian Ocean had been reported to be different species (Flot et al., 2011; Stefani et al., 2011; Arrigoni et al., 2012; Keshavmurthy et al., 2013). Considering that *C. magna* in Japan is relatively common in Okinawa, it is highly possible that this species could be found in other localities, such as Southeast Asia and Australia (e.g., a GBR specimen in clade II in Figs 4–5, identified as *C. chalcidicum* [accession No.: HQ2030404 for 28S, HQ203525 for H3] referred from Huang et al. [2011]). Further molecular and morphological analysis of more samples from other localities could be vital in determining whether Pacific specimens are a different species from *C. magna* in the Red Sea.

In this study, *C. salae* was also recorded for the first time in Japan, and was found mainly in the southern part of the temperate regions of Miyazaki, Kumamoto, and Kochi (31–32° N), where the latitude is similar to that of Lord Howe Island (31° S), the type locality of *C. salae* (Baird et al., 2017). When comparing the morphological characters of *C. salae* and *C. serailia* Japanese specimens, we found slight morphological differences between these species (e.g., the number of granules on the spine tip on the coenosteum and the total number of corallites per cm^2). In particular, *C. salae* has more space (wide coenosteum) between corallites than *C. serailia*. In the original description of *C. salae*, Baird et al. (2017) described that *C. salae* was distinguishable from *C. serailia* by the degree of variation in the size of corallites (mostly one size in *C. salae* vs. mixed sizes in *C. serailia*), but we could not find such a difference of this character between the two species from our specimens.

Additionally, based on mtIGR DNA, *C. salae* was highly divergent from all other *Cyphastrea* species except *C. agassizi*, which would belong to a different genus (see below). The other two nuclear markers (28S and H3) also had genetically distant clades of *C. salae* from those of the other *Cyphastrea* species. We do not know why the mtIGR DNA sequences of this species differed from the others, but the fact that *C. salae* is a distinct species from *C. serailia* is supported by this difference. However, we need continued efforts to search for additional key morphological characters

Table 1. Morphological characteristics of *Cyphastrea* spp. Upper row is average (standard deviation), lower row is minimum to maximum.

	N (Colony No.)	colony size (width × length) (mm)	Corallite diameter (mm)	Calice diameter (mm)	Columella diameter (mm)	Septal no. of first order	Costal No.
C. chalcidicum	6	30.2 (8.4) × 35.1 (9.0) / 19.8–38.3 × 25.7–46.8	2.85 (0.32) / 2.16–3.39	1.84 (0.40) / 1.15–2.36	0.57 (0.09) / 0.41–0.75	12.1 (0.4) / 11–13	23.9 (0.4) / 22–26
C. decadia	3	44.6 (18.2) × 67.3 (9.2) / 19.8–38.3 × 25.7–46.8	2.43 (0.05) / 1.98–3.04	1.53 (0.01) / 1.36–1.81	0.49 (0.00) / 0.35–0.67	10.1 (0.1) / 10–11	13.5 (6.0) / 10–22
C. magna	6	34.7 (10.2) × 50.0 (21.7) / 24.1–49.8 × 30.0–81.7	2.81 (0.51) / 1.88–4.80	1.65 (0.36) / 1.03–2.31	0.49 (006) / 0.36–0.69	12.0 (0.0) / 12	24.0 (0.0) / 24
C. microphthalma type I	9	31.3 (14.1) × 48.3 (24.5) / 12.6–54.3 × 18.6–99.1	2.43 (0.35) / 1.84–3.44	1.48 (0.14) / 1.17–1.80	0.43 (0.08) / 0.20–0.64	10.2 (02) / 10–12	20.3 (0.4) / 20–24
C. microphthalma type II	5	24.1 (4.7) × 38.7 (9.6) / 20.0–29.3 × 28.3–51.2	2.03 (0.14) / 1.62–2.38	1.23 (0.14) / 1.01–1.63	0.36 (0.03) / 0.27–0.51	9.9 (0.2) / 9–11	15.8 (5.3) / 10–22
C. ocellina	6	26.0 (8.0) × 34.9 (10.3) / 15.3–34.3 × 23.8–47.9	2.31 (0.12) / 1.94–2.64	1.49 (0.12) / 1.14–1.80	0.47 (0.04) / 0.37–0.58	11.7 (0.8) / 9–17	23.0 (1.0) / 19–24
C. salae	7	35.3 (6.5) × 46.3 (12.8) / 24.8–42.4 × 29.8–68.4	2.31 (0.33) / 1.94–2.64	1.64 (0.23) / 1.11–2.12	0.55 (0.09) / 0.33–0.84	11.7 (0.5) / 10–12	23.4 (0.8) / 20–24
C. serailia	38	32.1 (11.2) × 46.4 (20.4) / 13.6–63.1 × 23.2–90.6	2.36 (0.34) / 1.42–3.40	1.63 (0.24) / 1.02–2.38	0.51 (0.08) / 0.22–0.89	11.7 (0.5) / 8–18	23.4 (1.0) / 16–28
C. zhongjianensis	1	23.2 × 27.5 / –	2.62 (0.25) / 2.23–2.91	1.52 (0.14) / 1.34–1.62	0.46 (0.08) / 0.35–0.56	11.8 (0.4) / 11–12	23.6 (0.9) / 22–24

Table 2. Estimated coefficients for the generalized linear mixed effects model of morphological characters at *Cyphastrea salae* and *C. serailia* clade I and III. *Cyphastrea salae* was used as the basal group (Intercept).

morphological characters	Residual deviance	Degree of freedom (df)	Residual deviance/df	AIC	Chisq, df, Pr (>Chisq)	Tukey test df, t.ratio, p value *C. salae* vs *C. serailia* clade I / *C. salae* vs *C. serailia* clade III / *C. serailia* clade I vs *C. serailia* clade III
corallite diameter	3.87	172	0.02	147.12	1.399, 2, 0.497	172, −1.097, 0.5173 / 172, −0.345, 0.9366 / 172, −0.860, 0.6662
calice diameter	4.37	172	0.03	25.19	4.246, 2, 0.120	172, −0.212, 0.9756 / 172, −1.725, 0.1987 / 172, 1.769, 0.1831
columela diameter	7.84	172	0.05	−279.04	10.23, 2, 0.00602	172, 1.431, 0.3274 / 172, −1.328, 0.3815 / 172, 3.196, 0.0047**
septal teeth no.	3.68	172	0.02	242.08	4.425, 2, 0.109	172, −2.047, 0.1041 / 172, −0.905, 0.6381 / 172, −1.301, 0.3968
ceonostem spine no. per 1 mm^2	23.89	102	0.23	691.16	8.948, 2, 0.0114	102, 0.656, 0.7894 / 102, 2.707, 0.0215* / 102, −2.403, 0.0471*
total corallite no. per 1 cm^2	7.63	102	0.07	651.62	71.56, 2, 2.89e-16	102, −6.054, <.0001*** / 102, −8.700, <.0001*** / 102, 3.184, 0.0054**
granule no. on spine tip	44.04	102	0.43	431.32	68.19, 2, 1.56e-15	102, −9.259, <.0001*** / 102, −7.562, <.0001*** / 102, −1.832, 0.0699

to delimitate *C. salae* from *C. serailia* because the analyzed morphological data overlapped partially between the two species in PCA (Fig. 8) and *Cyphastrea* species have large intraspecific morphological variation and ecomorphs, as suggested by Veron et al. (1977). Investigation of the reproductive characters such as fertilization rate or spawning tim-
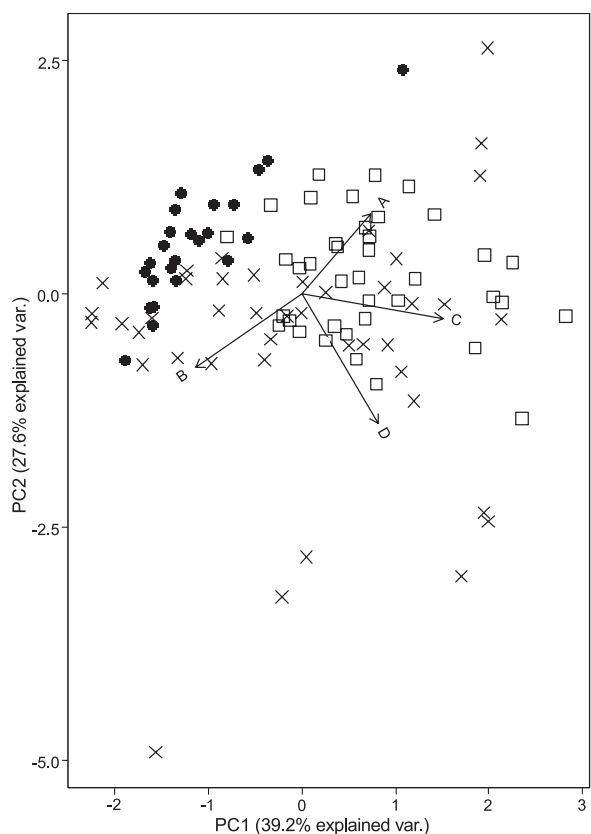
Fig. 8. Principal component analysis on morphological characters of specimens in *Cyphastrea salae* (●), *C. serailia* clade I (×) and clade III (□). Arrows indicate diameter of columellae **(A)**, number of spines on the coenosteum per 1 mm^2 **(B)**, number of corallites per 1 cm^2 **(C)**, number of granules on the spine tip on the coenosteum **(D)**.

ing might be a key to distinguishing these two species because they coexist in the temperate region of Japan. The difference in spawning timing and molecular phylogenetic relationships within a species in *Acropora* has been reported, suggesting that the difference would be due to a distinct species (Ohki et al., 2015; Rosser, 2015; Rosser et al., 2017; Furukawa et al., 2020).

Species delimitation of other Japanese *Cyphastrea* species

Except for *C. magna*, *C. salae,* and *C. agassizi*, six species (*C. chalcidicum*, *C. decadia*, *C. micropthalma*, *C. ocellina*, *C. serailia*, and *C. zhongjianensis*) analyzed in this study were not genetically monophyletic. Additionally in clade I, in addition to these six species, *C. hexasepta* and *C. kausti* from the Red Sea (Arrigoni et al., 2017) were also included without apparent genetic differences from other species. Thus, these *Cyphastrea* species are not genetically distinguishable from each other based on the three sequenced markers. At present, we do not have any idea why these species were not genetically separated like *C. magna* and *C. salae*. Similar phylogenetic relationships have been reported in the *Acropora* species. In *Acropora*, species formed five and more clades containing several species rather than forming species-specific clades (van Oppen et

al., 2001; Cowman et al., 2020; Fukami et al., 2021). Furthermore, the species members within each clade have no apparent species-specific genetic differences from each other. So far, the possible reasons for this lack of genetic differences have been considered to be repeated hybridization or incomplete lineage sorting (e.g., Hatta et al., 1999; van Oppen et al., 2001; Wolstenholme, 2004; Willis et al., 2006; Richards et al., 2008; Mao, 2020). At present, no data of hybridization of *Cyphastrea* species have been reported but it is highly possible that some species will be able to hybridize with other species, although the possibility of incomplete lineage sorting cannot be ruled out.

Cyphastrea decadia and *C. zhongjianensis* are morphologically easily distinguishable (branching for *C. decadia* and free living and glomerate-branched for *C. zhongjianensis*) from the other species in clade I. For the free-living species, *C. zhongjianensis*, the corallite characteristics are similar to those of *C. microphthalma* or *C. chalcidicum* because the septal number of the first order of this species ranges from 10–12 and the length of the first and second order costae is unequal (Zou, 1980; Nishihira and Sugihara, 2015; this study). Oku et al. (2020) reported that a fungiid coral, *Fungia fungites* (Linnaeus, 1758), contained two morphotypes as intraspecific morphological variations, namely, attached and unattached (free-living), in which the attached type was previously recognized as a distinct species *Fungia* sp. (Nishihira and Veron, 1995). Thus, in a future study, we will need to consider the possibility that a free-living with glomerate-branched form occurred accidentally from a non-free-living species, such as *C. microphthalma* or *C. chalcidicum*.

The branching species *C. decadia* is morphologically unique and will not be considered a morphological variation of other species. Nevertheless, this species was not genetically distinguishable from other species in clade I. In addition, two more morphologically unique species, *C. kausti* and *C. hexasepta*, were included in clade I. Thus, we need to search for more molecular markers or apply other methods (e.g., MIG-seq has recently been used in taxonomic studies for several coral species or coral populations [Pipithkul et al., 2021; Takata et al., 2021]) to distinguish the species included in clade I.

Cyphastrea chalcidicum and *C. serailia* were included in clades I and III. So far, we could not find any specific character that differs between clades I and III for these polyphyletic species. These two clades were genetically distinct, and this genetic difference is likely at the species level, considering that *C. magna* formed clade II between these clades. Considering that the specimens with typical morphology of both *C. chalcidicum* and *C. serailia* were included in clades I and III, it is strongly suggested that cryptic species would exist in each species. Therefore, we need to find new morphological characters to separate them. Otherwise, it will likely be necessary to investigate differences of reproductive traits between them to clarify the species differences.

In this study, we could not find *C. japonica* in Japan, although we surveyed many locations, including the site near the type locality (Kochi, in the temperate region of Japan) of *C. japonica*. Morphologically, *C. japonica* looks remarkably similar to *C. chalcidicum*. Yabe et al. (1936) described that *C. japonica* is distinguishable from other spe-

cies by its smaller corallite size and spe-
cific colony shape (mass composed of
humpy branches). Based on our examina-
tion of the holotype of *C. japonica*, we
consider it likely that it had been strongly
attacked and dramatically transformed
into a colony shape by coral-dwelling
worms (Fig. 9AB). Such a drastic deforma-
tion of coral has sometimes been
observed for several *Cyphastrea* species
in the temperate region of Japan (Fig.
9CD). Thus, the holotype of *C. japonica*
would not display a typical colony shape
as a species. It is highly possible that *C.
japonica* is just an intraspecific morpho-
logical variation of *C. chalcidicum*. Other-
wise, coral deformation by borer attack
might be one of the characteristics of *C.
japonica*, as described by Yabe et al.
(1936). Therefore, more specimens must
be morphologically and genetically ana-
lyzed to determine the taxonomic status of
C. japonica. Another taxon described by
the same authors, *C. chalcidicum tana-
bensis*, also bears taxonomical confusion.
Currently, *C. chalcidicum tanabensis* is
treated as a subjective junior synonym for
C. chalcidicum in Hoeksema and Cairns
(2022a). At the same time, "*C. tanabensis*"
(although there is no literature that ele-
vated *C. chalcidicum tanabensis* to spe-
cies rank) is also treated as a subjective
junior synonym for *C. japonica* in
Hoeksema and Cairns (2022b). As far as
we could see by examining the photo-
graphic images (Plate XVII, figs. 1–3 in
Yabe et al., 1936) of the holotype of *C.
chalcidicum tanabensis*, it looks quite
similar to *C. chalcidicum* and also *C.
japonica*. Therefore, *C. chalcidicum
tanabensis* would be also an intraspecific
morphological variation of *C. chalcidicum*.

Cyphastrea japonica has also fre-
quently been confused with *C. ocellina* in
some publications (see Veron, 2000;
Veron et al., 2016). However, by compar-
ing the holotypes of these species, we

Fig. 9. Holotype of *Cyphastrea japonica* and colony deformation by worm attack. Holotype of *C. japonica* (TU 40323) **(A)**, close-up view of holotype of *C. japonica* **(B)**, in situ images of colony deformation of *Cyphastrea* spp. by worm attack **(C, D)**: a colony in Shirahama, Wakayama **(C)**, a colony in Tsushima Island, Nagasaki **(D)**. Scale bars: 1 cm for **(A, C, D)**, 1 mm for **(B)**.

Fig. 10. Tubes and elongate tubes on the colony surface of *Cyphastrea*. *Cyphastrea microphthalma* type II (AOU376) **(A)** and *C. ocellina* (SS109) **(B)**. Black arrows: tubes, white arrows: elongate tubes. Scale bars: 1 mm.

concluded that *C. japonica* did not possess prominent exsert septa and costa (Fig. 10A, B) like *C. ocellina*. Also, as we surveyed the field for decades, we have never observed *C. ocellina*-like specimens in the warm-temperate region in Japan, including the type locality of *C. japonica* (Kochi). Thus, we consider that *C. ocellina* is a distinct species from *C. japonica*. On the other hand, *C. ocellina* is much more morphologically similar to *C. conferta* than to *C. japonica*. Sugihara (2014) and Sugihara et al. (2015) identified the specimens with prominent exsert septa and costa and with tubes and elongated tubes on the coenosteum as *C. conferta* (shown as *C. confesta* in these studies), but such specimens were identified as *C. ocellina* in Nishihira and Veron (1995). In this study, we re-identified *C. conferta* shown in
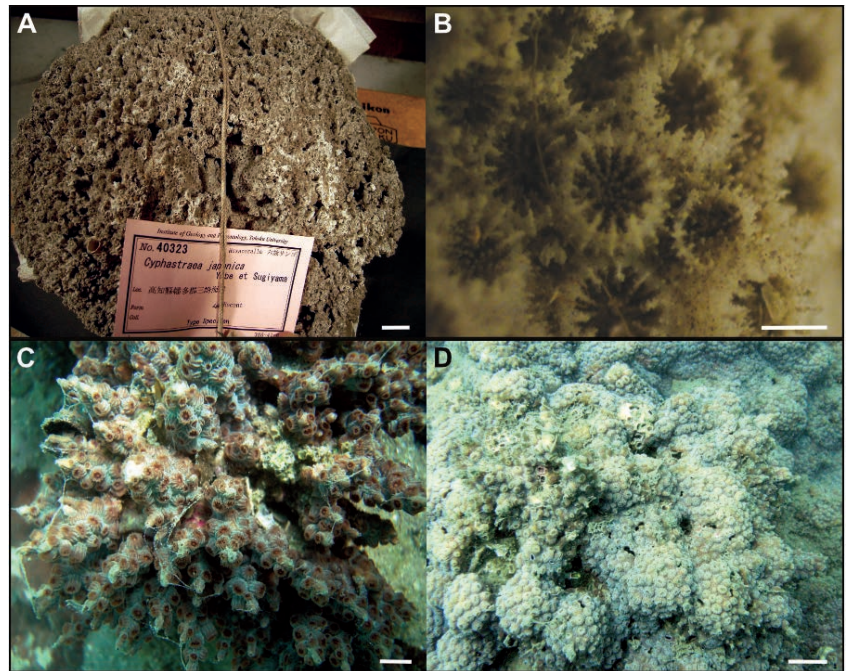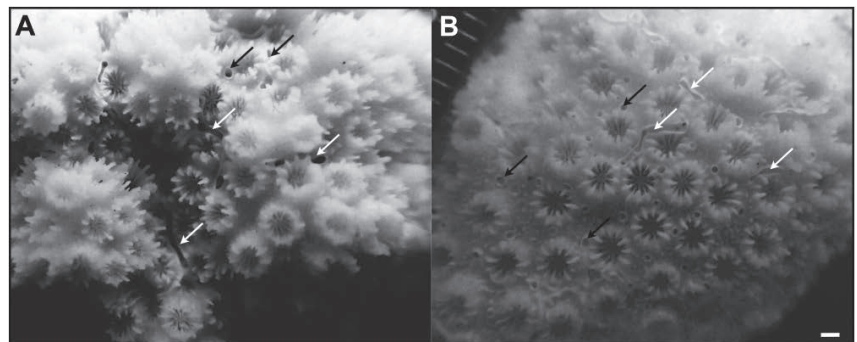
Sugihara (2014) and Sugihara et al. (2015) as *C. ocellina*. However, a possibility remains that Asian specimens might be *C. conferta* (type locality: Philippines), and *C. ocellina* might be a regional-specific species in Hawaii (type locality). Currently, we do not have any information on the molecular data of *C. ocellina* collected from the type locality. Therefore, we need to compare Hawaiian specimens with Asian specimens of *C. ocellina* genetically in a future study to clarify the species status of *C. conferta* and *C. ocellina*.

On the other hand, *C. ocellina* is morphologically remarkably similar to *C. microphthalma* type II (Fig. 10), except for the differences of septal and costal numbers. As far as we observed, *C. ocellina* contains some corallites with 10 septa and costae in the first order in a colony, but the

other corallites in the same colony had 12 septa and costae in the first order. In contrast, *C. microphthalma* type II had 10 septa and costae in the first order in almost all corallites. Thus, *C. ocellina* and *C. microphthalma* type II are morphologically distinguishable. However, it was shown by the molecular phylogenetic tree of 28S (Fig. 4) that *C. microphthalma* type II was genetically closely related to two out of all six specimens of *C. ocellina*, forming a subclade-like group together with a meager bootstrap value (51). On the other hand, the other four specimens of *C. ocellina* formed another subclade with three specimens of *C. microphthalma* type I with a high bootstrap value (98). Thus, the septal number might not be an adequate key morphological character to delimitate the species for this genus. More molecular and morphological analyses are also needed to clarify the relationships between these species. Such complicated relationships between molecular data and morphology imply that most species of *Cyphastrea* contain a considerable intraspecific morphological variation overlapping with other species.

For *C. agassizi*, based on the holotype illustrations and the original description (Vaughan, 1907: pl. XXV, figs. 2–3), the present specimens were identified as *C. agassizi.* This species was genetically distant from all other species and closely related to *Leptastrea* sp. This species also differs from the other *Cyphastrea* species in the coenosteum and the costal morphology. Our specimens of *C. agassizi* had well developed and thickened costae over the compact coenosteum, and costae were covered with many granules, which characteristics are not observed in the other *Cyphastrea* species, but are common in *Leptastrea* species. Thus, it is highly possible that *C. agassizi* would be returned to *Leptastrea*, the genus in which the species was originally described by Vaughan (1907). Arrigoni et al. (2020) also discussed the same thing based on morphological examination. However, there are several species to be compared morphologically and phylogenetically with *C. agassizi* before the taxonomic revision. *Leptastrea inaequalis* Klunzinger, 1879 and *L. gibbosa* Benzoni and Arrigoni, 2020 are superficially quite similar to *C. agassizi* in in situ images (Fig. S1_4–5 in Arrigoni et al., 2020) because the calice outline is circular and corallites are sometimes exsert. *Leptastrea hawaiiensis* Vaughan, 1907 (Vaughan, 1907: pl. XXV, fig. 1) is also very similar to *C. agassizi.* Although *L. hawaiiensis* is presently treated as a junior synonym of *Leptastrea bottae* (Milne Edwards & Haime, 1849) (Hoecksema and Cairns, 2023), *C. agassizi* looks different from *L. bottae* because the holotype of *L. bottae* has no exsert corallites (a holotype photograph shown in Hoecksema and Cairns, 2023). Thus, further morphological and molecular analyses of these species would be necessary for the taxonomic revision of *C. agassizi*.

Species description of three *Cyphastrea* species in Japan

In this study, *C. magna* and *C. salae* were recorded in Japan for the first time. Here, we describe their morphological features for Japanese specimens. Also, we describe *C. ocellina* to solve the taxonomic confusion.

Cyphastrea magna Benzoni and Arrigoni in Arrigoni,

Berumen, Huang, Terraneo and Benzoni, 2017
New Japanese name: haguruma-togekikumeishi

Material studied. Iriomote Island, Okinawa (IR381: MUFS C497), Sesoko Island, Okinawa (SS103: MUFS C495, SS121: MUFS C496), Amami-Oshima Island, Kagoshima (AOU375: MUFS C492, AOU406: MUFS C493, AOU457: MUFS C494)

Holotype. MNHN-IK-2012–14235 (Figs. 5–6 in Arrigoni et al., 2017)

Type locality. Ras Al-Ubayd, Red Sea, Saudi Arabia

Specific characteristics of Japanese specimens. Colony is massive or submassive. Corallites are usually circular or oval, and rarely conical. Corallites with typical morphology look like "spur gears" due to the 12 conspicuous thickened and prominent costae of the first order. The diameter of the corallites ranged from 1.8 to 4.8 mm. The diameter of the calices ranged from 1.1 to 2.3 mm. The septal number is 24 or less. Primary and secondary septa are six each, equal or subequal in length, and reach the columellae. Tertiary septa are 12 or less, rarely absent, short (< 1/4R), and do not reach the columellae. The costal number ranged from 12 to 24. Primary and secondary costa are six each, conspicuous, with equal length, thickened on the wall, and have paddle-shaped ornamentation, as described by Arrigoni et al. (2017). Tertiary costae are 12 or less, or sometimes absent, much shorter than primary and secondary costae. Paliform lobes are absent. The coenosteum is covered with spines. The color of living colony is cream to light brown.

Similar species. None, but colonies with small corallites might be misidentified as *C. chalcidicum* due to the abortive tertiary costae.

Distribution in Japan. Ryukyu Islands

Remarks. This species is phylogenetically monophyletic (this study, Arrigoni et al., 2017). Corallite size is more variable in Japanese specimens (mean 2.74 ± 0.65 mm, ranging from 1.8 to 4.8 mm) than in those from the Red Sea (mean 2.94 ± 0.31 mm, ranging from 2.53 to 3.71 mm, referred from Arrigoni et al., 2017).

In this study, we proposed a new Japanese name, "Haguruma-togekikumeisi," because the appearance of the corallites of this species looks like a spur gear ("Haguruma" in Japanese). The specimen MUFS C494 (sample #: AOU457) is designated as a standard specimen for this newly proposed Japanese name.

Cyphastrea salae Baird, Hoogenboom and Huang, 2017
New Japanese name: Arata-fuka-togekikumeishi

Material studied. Oshima Island, Nichinan, Miyazaki (MO249: MUFS C521, MO266: MUFS C522, MO351: MUFS C523), Shimanoura-shima Island, Nobeoka, Miyazaki (NB251: MUFS C524), Fuka-shima Island, Saiki, Oita (OT63: MUFS C525), Ushibuka, Amakusa, Kumamoto (AM19-5: MUFS C519, AM19-9: MUFS C520), Kikaijima Island, Kagoshima (KKI64: KICRS-L-00132)

Holotype. AM 81_1530 (Fig. 1 in Baird et al., 2017)

Type locality. South Flat, Lord Howe Island, Australia

Specific characteristics of Japanese specimens. Colony is encrusting or submassive. Corallites are circular, and slightly conical. The diameter of the corallites ranged

from 1.1 to 2.8 mm. The diameter of the calices ranged from 1.0 to 2.3 mm. The septal number is 24, as usual. Primary and secondary septa are six each, equal in length, and reach columella. Tertiary septa are 12 and short (< 1/4R) and do not reach columella. The costal number is 24. Primary and secondary costa are six each, equal or subequal in length. Tertiary costae are 12 and equal to or subequal in length with primary and secondary costae. Paliform lobes are sometimes present. The coenosteum is spinous. The color of the living colony is blue, brown, or yellow.

Similar species. *C. serailia*. It is very difficult to distinguish *C. salae* from *C. serailia* at present without statistical analyses of skeletal morphology. In general, *C. salae* has denser columellae and is likely to have a bit larger and more numerous spines on the coenosteum than *C. serailia*.

Distribution in Japan. Miyazaki, Oita, Kumamoto, and Kikajima Island

Remarks. This species is phylogenetically monophyletic and genetically distant from other congeners (this study and Baird et al., 2017).

We propose a new Japanese name in this study, "Arata-fuka-togekikumeishi." We added "Arata," which means "new" in English, to "Fuka-togekikumeishi," which is the Japanese name of *C. serailia* because this species is morphologically quite similar to *C. serailia.* The specimen MUFS C524 (sample #: NB251) is designated as a standard specimen for this newly proposed Japanese name.

Cyphastrea ocellina (Dana, 1846)
Japanese name: Hime-toge-kikumeishi

Material studied. Iriomote Island, Okinawa (IR384: MUFS C513), Sesoko Island, Okinawa (SS101: MUFS C514, SS104: MUFS C515, SS109: MUFS C516, SS122: MUFS C517, SS124: MUFS C518)

Syntypes. YPM IZ 474, 4330
Type locality. Sandwich Islands (Hawaii Islands)
Synonymy.
Asterina (*Orbicella*) *ocellina* Dana, 1846: 218.
Cyphastrea ocellina (Dana, 1846): Nishihira and Veron, 1995, 378; Sugihara, 2014: 64; Veron, 2000: 244
Cyphastrea conferta. –Sugihara 2014: 64 (misspelled as *C. confesta*) [not *C. conferta* Nemenzo, 1959]; Sugihara et al., 2015: 118 (misspelled as *C. confesta*) [not *C. conferta* Nemenzo, 1959]
Cyphastrea japonica. –Veron, 2000: 240, figs. 1–4 [not *C. japonica* Yabe and Sugiyama in Yabe, Sugiyama and Eguchi, 1936]; Veron et al., 2021: *Cyphastrea japonica,* a part of images [not *C. japonica* Yabe and Sugiyama in Yabe, Sugiyama and Eguchi, 1936]

Specific characteristics of Japanese specimens. Colony is encrusting to massive with a hillocky surface. Corallites are circular. The diameter of the corallites ranged from 2.1 to 2.6 mm. The diameter of the calices ranged from 1.4 to 1.6 mm. The septal number is usually 24, but rarely mixed 20 and 24 in a colony. Primary and secondary septa are usually six each, but rarely five each, equal or subequal in length. Primary and secondary septa prominently exsert vertically up to 1 mm on the wall. Tertiary septa are 12 or fewer and noticeably short. The costal number is usually 24, but rarely mixed 20 and 24 in a colony. Primary and secondary costae

are six each, but rarely five each (costal numbers correspond to those of septa), equal or subequal length, thickened, and prominently exsert up to 1 mm on the wall. Tertiary costae are reduced to absent. Columella trabecular, well developed, and wide (> 1/4 of calice width). Paliform lobes are absent or sometimes developed. The coenosteum is densely spinose. Tubes or elongated tubes are conspicuous (Fig. 10; also see Remarks). The color of the living colony is brown or dark brown.

Similar species. Remarkably similar to *C. microphthalma* type II in this study, but distinguishable by 10 septa and costae of the first order in a whole colony in *C. microphthalma* type II.

Remarks. *Cyphastrea ocellina*, in this study and also in south-eastern Asia (shown by Veron, 2000), has a common feature, commonly known as a "groove-and-tubercle structure" formed by polychaete worm attacks (Randall and Eldredge, 1976). Randall and Eldredge (1976) investigated this structure (described as a "groove-and-tube structure") of several corals and distinguished the structure as "groove" and "tube or elongate tube" clearly. According to Randall and Eldredge (1976), *C. ocellina* in this study displayed only tubes or elongated tubes, but not grooves (Fig. 10). Tubes or elongated tubes were not observed in the holotype of *C. ocellina*. Still, other morphological characteristics are concordant between our specimens and the holotype.

This species has been confused with *C. japonica* (see Veron, 2000; Veron et al., 2016), but the latter species does not possess prominent exsert septa and costae (see Discussion in detail). On the other hand, some specimens of *Cyphastrea* previously identified as *C. conferta* by Sugihara (2014) and Sugihara et al. (2015) were re-identified as *C. ocellina* in this study. Also, as far as we can consider from the photographic images of the holotypes, *C. conferta* is likely to be a synonym of *C. ocellina* despite slightly less exsert septa and costae in *C. conferta*. More morphological and molecular analyses are needed in future studies to clarify the status of these species.

This species is not genetically indistinguishable from other species, especially *C. microphthalama* type I and type II (see Fig. 4), but it is morphologically different from other species.

CONCLUSION

In this study, we recorded two *Cyphastrea* species, *C. magna* and *C. salae*, in Japanese waters for the first time. We also found that, in Japan, *C. salae* is mainly distributed in the warm-temperate region, whereas *C. magna* is specifically distibuted in the subtropical region in Japan. In addition, we also confirmed the occurrence of seven other species of *Cyphastrea* in Japan. Except for *C. agassizi*, they formed two genetically different clades (clades I and III). At present, these morphologically different species in each clade are genetically indistinguishable. It is presumed that some of these species have large intra-specific morphological variation overlapping each other, consequently making species distinction unclear. It will be necessary to combine novel approaches in morphological, ecological and molecular fields to solve this complex species problem of *Cyphastrea*.

ACKNOWLEDGMENTS

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

TC and HF designed the research. TC conducted morphological observations and molecular experiments. TC, NI, and HF performed the statistical analyses. HF and HT wrote the taxonomic descriptions. NI, TM, HM, YFK, YN, HT, and HF performed the sampling. All authors wrote and reviewed the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs230009)

Supplementary Table S1. List of specimens that we collected and used in this study.

Supplementary Table S2. List of species of *Cyphastrea* and other genera obtained from literature and used in molecular phylogenetic analyses.

Supplementary Table S3. Summary of morphological characteristics of type specimens of eight *Cyphastrea* species.

REFERENCES

Arrigoni R, Stefani F, Pichon M, Galli P, Benzoni F (2012) Molecular phylogeny of the Robust clade (Faviidae, Mussidae, Merulinidae, and Pectiniidae): an Indian Ocean perspective. Mol Pylogenet Evol 65: 183–193

Arrigoni R, Berumen ML, Huang D, Terraneo TI, Benzoni F (2017) *Cyphastrea* (Cnidaria: Scleractinia: Merulinidae) in the Red Sea: phylogeny and a new reef coral species. Invertebr Syst 31: 141–156

Arrigoni R, Berumen ML, Stolarski J, Terraneo TI, Benzoni F (2019) Uncovering hidden coral diversity: a new cryptic lobophylliid scleractinian from the Indian Ocean. Cladistics 35: 301–328

Baird AH, Hoogenboom MO, Huang D (2017) *Cyphastrea salae*, a new species of hard coral from Lord Howe Island, Australia (Scleractinia, Merulinidae). ZooKeys 662: 49–66

Benzoni F, Arrigoni R, Stefani F, Stolarski J (2012) Systematics of the coral genus *Craterastrea* (Cnidaria, Anthozoa, Scleractinia) and description of a new family through combined morphological and molecular analyses. Syst Biodivers 10: 417–433

Bouwmeester J, Benzoni F, Baird AH, Berumen ML (2015) *Cyphastrea kausti* sp. n. (Cnidaria, Anthozoa, Scleractinia), a new species of reef coral from the Red Sea. ZooKeys 496: 1–13

Budd AF, Fukami H, Smith ND, Knowlton N (2012). Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). Zool J Linn Soc 166: 465–529

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, et al. (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust J Zool 46: 419–437

Cowman PF, Quattrini AM, Bridge TCL, Watkins-Colwell GJ, Fadli N, Grinblat M, et al. (2020) An enhanced target-enrichment bait set for Hexacorallia provides phylogenomic resolution of the staghorn corals (Acroporidae) and close relatives. Mol Phylogenet Evol 153: 106944

Cuif JP, Lecointre G, Perrin C, Tillier A, Tillier S (2003) Patterns of septal biomineralization in Scleractinia compared with their 28S rRNA phylogeny: a dual approach for a new taxonomic framework. Zool Scr 32: 459–473

Duerden JE (1904) The morphology of the Madreporaria. V. Septal sequence. Biol Bull 7: 79–104

Flot JF, Blanchot J, Charpy L, Cruaud C, Licuanan WY, Nakano Y, et al. (2011) Incongruence between morphotypes and genetically delimited species in the coral genus *Stylophora*: phenotypic plasticity, morphological convergence, morphological stasis or interspecific hybridization? BMC Ecol 11: 22

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

Furukawa, M, Ohki S, Kitanobo S, Fukami H, Morita M (2020) Differences in spawning time drive cryptic speciation in the coral *Acropora divaricata*. Mar Biol 167: 163

Hoeksema BW, Cairns S (2021) World list of Scleractinia. *Cyphastrea* Milne Edwards and Haime, 1848. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetailsandid=206488 on 30 July 2021

Hoeksema BW, Cairns S (2022a). World List of Scleractinia. *Cyphastrea chalcidicum* (Forskål, 1775). Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=207415 on 14 Dec 2022

Hoeksema BW, Cairns S (2022b) World List of Scleractinia. *Cyphastrea conferta* Nemenzo, 1959. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=758404 on 14 Dec 2022

Hoeksema BW, Cairns S (2022c) World List of Scleractinia. *Cyphastrea japonica* Yabe & Sugiyama, 1932. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=288922 on 14 Dec 2022

Hoeksema BW, Cairns S (2023) World List of Scleractinia. *Leptastrea* Milne Edwards & Haime, 1849. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=204278 on 6 April 2023

Huang D, Licuanan WY, Baird AH, Fukami H (2011) Cleaning up the 'Bigmessidae': molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. BMC Evol Biol 11: 37

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

Huang D, Benzoni F, Arrigoni R, Baird AH, Berumen ML, Bouwmeester J, et al. (2014b) Towards a phylogenetic classification of reef corals: the Indo-Pacific genera *Merulina*, *Goniastrea* and *Scapophyllia* (Scleractinia, Merulinidae). Zool Scr 43: 531–548

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

Keshavmurthy S, Yang SY, Alamaru A, Chuang YY, Pichon M, Obura D, et al. (2013) DNA barcoding reveals the coral "laboratory-rat", *Stylophora pistillata* encompasses multiple identities. Sci Rep 3: 1520

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

Luzon KS, Alcantara DT, Licuanan WY (2022) Nemenzo Virtual Museum: *Cyphastrea conferta*. (Coenomap.) Available at

http://www.dlsu.edu.ph/research-1/centers/shore/coenomap/cyphastreaConferta.html (Verified 20 Dec 2022)

Nishihira M, Veron JEN (1995) Hermatypic Corals of Japan. Kaiyusha Publisher, Tokyo (in Japanese)

Nishihira M, Sugihara K (2015) A free-living coral *Cyphastrea zhongjianensis* found on the shingle bottom. Study Rev Iriomote Is 2014, ORRC, Tokai Univ 2014: 65–67 (in Japanese with English abstract)

Ohki S, Kowalski RH, 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

Oku Y, Iwao K, Hoeksema BW, Dewa N, Tachikawa H, Koido T, Fukami H (2020) *Fungia fungites* (Linnaeus, 1758) (Scleractinia, Fungiidae) is a species complex that conceals large phenotypic variation and a previously unrecognized genus. Contrib Zool 89: 188–209

Pipithkul S, Ishizu S, Shimura A, Yokochi H, Nagai S, Fukami H, Yasuda N (2021) High clonality and geographically separated cryptic lineages in the threatened temperate coral, *Acropora pruinosa*. Front Mar Sci 8: 668043

R Development Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Randall RH, Eldredge LG (1976) Skeletal modification by a polychaete annelid in some scleractinian corals. In "Coelenterate Ecology and Behavior" Ed by GO Mackie, Springer, Boston, pp 453–465

Rosser NL (2015) Asynchronous spawning in sympatric populations of a hard coral reveals cryptic species and ancient genetic lineages. Mol Ecol 24: 5006–5019

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

Stefani F, Benzoni F, Yang SY, Pichon M, Galli P, Chen CA (2011) Comparison of morphological and genetic analyses reveals cryptic divergence and morphological plasticity in *Stylophora* (Cnidaria, Scleractinia). Coral Reefs 30: 1033–1049

Sugihara K (2014) Catalogue of coral specimens from Nakagusuku bay Deposited in the University Museum (Fujukan), University of the Ryukyus. Catalogue of Materials deposited in the University Museum (Fujukan), University of the Ryukyus No. 9. Ryukyu University Museum (Fujukan), Nishihara (in Japanese)

Sugihara K, Nomura K, Yokochi H, Shimoike K, Kajiwara K, Suzuki G, et al. (2015) Zooxanthellate scleractinian corals of Tanegashima Island, Japan. Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Tsukuba (in Japanese)

Takata K, Iwase F, Iguchi A, Yuasa H, Taninaka H, Iwasaki N, et al. (2021) Genome-wide SNP data revealed notable spatial genetic structure in the deep-sea precious coral *Corallium japonicum*. Front Mar Sci 8: 667481

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

Vaughan TW (1907) Recent Madreporaria of the Hawaiian Islands and Laysan. US National Museum Bulletin 59: 1–427

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville

Veron JEN, Pichon M, Wijsman-Best M (1977) Scleractinia of eastern Australia. Part II. Families Faviidae, Trachyphylliidae. Australian Institute of Marine Science, Townsville

Veron JEN, Stafford-Smith MG, Turak E, DeVantier LM (2016) Corals of the World. http://www.coralsoftheworld.org/species_factsheets/species_factsheet_summary/cyphastrea-ocellina/ Accessed 08 Dec 2022

Yabe H, Sugiyama T (1932) Reef corals found in the Japanese Seas. Sci Rep Tohoku Imp Univ, 2nd Ser Geol 15: 143–168

Yabe H, Sugiyama T, Eguchi M (1936) Recent reef-building corals from Japan and the south sea islands under the Japanese mandate I. Sci Rep Tohoku Imp Univ, 2nd Ser Geol, Special volume 1: 1–66, 59 plates

Yokochi H, Shimoike K, Kajiwara K, Nomura K, Kitano Y, Matsumoto H, et al. (2019) A preliminary report on hermatypic corals of Amitori Bay, Iriomote-jima, Ryukyu Islands, Japan. Study Rev Iriomote Is 2018, ORRC, Tokai Univ 2018: 36–69

Zou RL (1980) Studies on the corals of the Xisha Islands IV. Two new hermatypic scleractinian corals. Nanhai Studia Marina Sinica 1: 113–118