Re-Examination of the Phylogenetic Relationship among Merulinidae Subclades in Non-Reefal Coral Communities of Northeastern Taiwan

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Abstract: Species identification for spawning corals relies heavily on morphology. Recent molecular phylogenetic approaches have demonstrated the limits of traditional coral taxonomy based solely on skeletal morphology. Merulinidae is considered a complex taxonomic group, containing 24 genera and 149 species. This family is one of the most taxonomically challenging and its taxonomy has largely improved in recent studies. However, studies of the phylogeny of Merulinidae are constrained by limited geographic scales. In Taiwan, merulinid corals are dominant in non-reefal communities on northeast coasts and they consistently spawn between summer and fall. This study is a first attempt to establish a molecular database of merulinid corals in this new area, including a volcanic island (Kueishan Island), and provide information about sexual reproduction. We analyzed 65 specimens, including 9 genera and 28 species collected from Taiwan using one mitochondrial marker (COI: cytochrome c oxidase subunit 1 gene) and three nuclear markers (ITS: nuclear ribosomal internal transcribed spacer, 28S rDNA D1 and D2, and histone H3) to re-examine phylogenetic relationships and search for new species. Overall, 58 COI sequences, 59 for ITS, 63 for 28S, and 62 histone sequences were newly obtained from the collected specimens. The reconstructed molecular tree demonstrates that all the specimens and reference sequences we examined are clustered within Merulinidae. Subclades A, B, C, D/E, F, G, H, and I are congruent with previous studies. However, Astrea curta is separated from the other congeneric species, Astrea annuligera (XVII-B), which is a sister to Favites and defined as a new subclade K. In addition, two new species (Paragoniastrea deformis and Paragoniastrea australensis) were discovered for the first time in Taiwan, and we defined them as a new subclade J. In addition, A. curta, P. australensis, and P. deformis are all hermaphroditic spawners and released bundles in July. This study greatly improves the accuracy of biodiversity estimates, systematic taxonomy, and reproduction for Taiwan’s coral ecosystem.

Keywords: taxonomy; Taiwanese corals; molecular phylogeny; scleractinian corals; reproduction

1. Introduction

1.1. Uncovering Taxonomic Progress

The identification of scleractinian corals based solely on morphology is challenging because some scleractinian species can exhibit environment-correlated variations in morphology, i.e., Ecomorphs [1]. In addition, species display phenotypic plasticity across their distribution, making it difficult to rely on shared morphological features to identify them [2,3]. Therefore, it is important to combine morphological and molecular characteristics to improve the accuracy of the determination of evolutionary relationships.

The traditional classification of scleractinia into seven suborders was out of date [4–7]. Given the comprehensive study of the entire taxon with morphological and molecular approaches, the scleractinian corals can be generally divided into three major groups: basal, robust and complex [8]. Furthermore, they are separated into 21 clades (I-XXI) [8,9]. Many
scleractinian corals at family and genus were revised or remained unclear taxonomic position (Scleractinia incertae sedis). For example, Diploastrea heliopora and Montastraea cavernosa were separated into Diploastreidae Chevalier & Beauvais, 1987, and Montastreaeidae Yabe & Sugiyama, 1941, respectively [10]. Euphylliidae Milne Edward & Haime, 1857, contains six genera: Ctenella Matthai, 1982; Euphyllia Dana, 1846; Galaxea Oken, 1815; Gyromithia Milne Edwards & Haime, 1851; Montigya Matthai, 1928; Simplantea Umbgrove, 1939; and Frimbraphyllia Veron & Pichon, 1980, the last of which was redefined from the conventional Euphyllia ancora, E. yaeyamaensis, and E. divisa [11]. In addition, the genera Nemenzophyllia, Physogyra, and Plerogyra were removed from Euphylliidae because they formed a separate clade with Blastomussa (clade XIV) [9,12].

1.2. Revision of Merulinidae (Clade XVII)

The species identification of Faviidae, Gregory, 1900 and Wells, 1956 was based on their budding patterns and macromorphological characteristics. They were traditionally subdivided into two subfamilies based on whether their budding was primarily intracalicular (Caulastraea, Favia, Diploria, Favites, Oulophyllia, Goniatrea, Platygura, Letoria, Hydnophora, Manicina, and Colopophyllia) or extracalicular (Montastraea, Diploastrea, Cyphastrea, and Echinopora). A third, smaller, subfamily displays intracalicular budding and very well-developed septal lobes (trabecular versus lamellar, continuous versus discontinuous). Genera within the Faviinae are distinguished by having a colony form (ceroid versus plocoid, mendroid versus phaceloid) and the columella structure (trabecular versus lamellar versus continuous versus discontinuous).

Based on the molecular results, the genera of Faviidae not only displayed a polyphyletic pattern, but were also clustered together with species from four conventional coral families: Faviidae Milne Edwards & Haime, 1857; Merulinidae Verrill, 1995; Pectinidae Rafinesque, 1815; and Trachyphylliidae Well, 1956, which had previously been recovered as Merulinidae (XVII) [10,13–17]. The faviid corals outside of clade XVII were assigned to other families. For example: Plesiastrea versipora, Diploastrea heliopora, and Montastraea cavernosa were reclassified as Plesiastreidae Dai & Horng, 2009, Diploastraeidae Chevalier & Beauvais, 1987, and Montastreaeidae Yabe & Sugiyama, 1941, respectively. Faviidae is limited to Atlantic corals such as Favia, Diploria, and Manicina because they are evolutionarily divergent to the Pacific corals [18,19]. Furthermore, phylogenies based on multiple genetic markers and morphological characteristics demonstrated that the species/genera Merulinidae are divided into nine subclades (A, B, C, D/E, F, G, H, and I) [10,14,16]. Paramontastraea, Orbicella, and Astrea are new genera in the Merulinidae, revised from Montastraea. Given these results, Merulinidae contains the most genera (with 24) and the second-most species (with 149) among the scleractinians [20] (Supplementary Table S1). Its species are commonly distributed in the Indo-Pacific [2,21].

1.3. Taiwan Taxonomy and Species Diversity

Taiwan is located at the center of the Philippine–Japan Island arc at a latitude of 21.90° N to 25.3° N, crossing from the Tropic of Cancer close to the northern tip of the Coral Triangle [22]. To date, 317 scleractinian coral species have been reported in Taiwan and display a latitudinal gradient of decreasing species diversity from south to north [23,24]. In addition, coral assemblages contain 21 genera covering 87% of the total number of genera of merulinid corals and 89 species covering 60% of the total number of merulinid species [25]. Taxonomic phylogenetic studies of scleractinian corals collected from Taiwan are very limited [9,11,26–29]. In addition, biogeographical integration is needed on a larger scale. For example, Polycyathus chaishanensis (Caryophyllidae) was proposed to be endemic to Taiwan [27]. Later, this species was also found to inhabit Indonesia, based on molecular evidence [30]. Euphyllia ancora has been a model species for studies on sexual reproduction [31] and its genus was recently revised to Frimbraphyllia [11]. This revision created an important foundation on the convergent and divergent functionalities...
of genes and compared functional genes among the cnidarians underlaying precisely the phylogenetic position of the studied species.

1.4. Purpose of This Research

The phylogeny of Merulinidae reconstructed in Huang et al. [16] was based on samples/taxa from Australia, Singapore, Japan, and the Philippines in the Pacific Ocean and the Atlantic Ocean. Taiwan is located in the Pacific Ocean; it is an important stepping stone between the Philippines and Japan. Merulinid corals are major spawning members and consistently spawn between summer and fall in non-reefal coral communities in northern Taiwan [32]. These spawning corals are important for maintaining local recruitment, providing heterogenetic materials to the local population and connecting across different populations. However, some convergent macro-morphological characteristics make it challenging to identify some genera in the field, such as Goniastrea (ceroid form), Favites (ceroid and plocoid forms), and Diploastrea (plocoid form) [24,33]. In addition, species identification for spawning corals based on morphological criteria in the fields and underwater photographs is difficult because the polyps are deformed when “the mature sperm and eggs move to the mouths of polys” (i.e., bundle setting).

As mentioned above, these challenges can be resolved by molecular approaches, as was demonstrated by Huang et al. [10]. For example, ceroid forms of Goniastrea, Diploastrea, and Favites are clearly separated in subclades A, B, and F based on phylogenetic reconstruction using multiple loci [10]. Therefore, Chen et al. [32] identified the species to the genus level of each specimen using molecular approaches and the BLAST tool [34]. Subsequently, specimens were identified to species level using the morphology of their skeletons. The established molecular database of merulinid corals can provide further insight into the phylogenetic relationships among the subclades of Merulinidae. The objectives in this present study were to: (1) establish a molecular database of spawning corals of Merulinidae from Taiwan, which have not been studied before; (2) re-examine the phylogenetic relationship between the specimens collected from northern Taiwan and the merulinid corals in previous studies, using phylogeny reconstructions based on multiple loci; and (3) record any new species or subclades we might find in this region.

2. Materials and Methods

2.1. Sample Collection

Chen et al. [32] demonstrated that the spawning season for merulinid corals is July to August, from 2014 to 2016, in northeast Taiwan. Merulinid corals with bundle-setting behavior and released bundles still attached outside of the mouths of polyps were collected at night by scuba diving. Some corals were collected at two offshore islands and their sexual reproductive behavior was observed using histological approaches [32]. A total of 65 specimens from four sites were chosen for this study: 26 specimens from Pitoujiiao (25°07′34″ N, 121°54′55″ E), 21 from Longdong (25°05′02″ N, 121°55′09″ E), 3 from Keelung Island (25°07′34″ N, 121°54′55″ E), and 12 from Kueishan Island (24°84′19″ N, 121°57′06″ E) (Figure 1). Coral fragments were collected by using chisels and hammers and separated into two parts. One was fixed in 90% ethanol for molecular analysis. The other was bleached in sodium hypochlorite until the tissue was entirely removed, rinsed in freshwater, and air-dried for the morphological analysis.

2.2. Species Identification

Chen et al. [32] identified 54 coral species in 23 genera and 8 families (Acroporidae, Agariciidae, Fungiidae, Lobophylliidae, Merulinidae, Poritidae, Pocilloporidae, and Psammocoridae), which were sexually reproductive between July and October. For Merulinidae, nine genera and 26 species collected from northeast Taiwan were chosen for the molecular phylogenetic study: Astrea curta (n = 5), Astrea annuligera (n = 1), Coelastrea aspera (n = 2), Coelastrea palauensis (n = 1), Cyphastrea chalcidicum (n = 2), Diploastrea favus (n = 4), Dipsastraea lizardensis (n = 1), Dipsastraea matthaii (n = 1), Dipsastraea rotumana (n = 1),
**Favites flexuosa** \((n = 1)\), **Favites pentagona** \((n = 7)\), **Favites styllfera** \((n = 2)\), **Favites magnstellata** \((n = 2)\), **Favites valenciennesi** \((n = 2)\), **Mycedium elephantotus** \((n = 1)\), **Mycedium robokaki** \((n = 1)\), **Mycedium mancaoi** \((n = 1)\), **Paragoniastrea australensis** \((n = 5)\), **Paragoniastrea deformis** \((n = 6)\), **Pectinia paonia** \((n = 1)\), **Pectinia lactuca** \((n = 1)\), **Platygyra daedalea** \((n = 1)\), **Platygyra lamellina** \((n = 2)\), **Platygyra ryukyuensis** \((n = 5)\), **Platygyra pini** \((n = 2)\), **Platygyra sinensis** \((n = 1)\), and **Platygyra verweyi** \((n = 3)\). Those specimens were identified to the genus level using molecular sequences and BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/, accessed on 2 April 2020) [34]. Subsequently, individuals were identified to the species level using morphological keys, notably Dai and Cheng [25]. Specimens that could not be identified morphologically (cerioid corals: *Goniastrea* and *Favites*, plocoid corals: *Favites* and *Dipsastraea*, unknown species, etc.) were preliminarily identified to the genus level and then re-evaluated after molecular analyses. DNA extraction, PCR amplification, and sequencing methods

Figure 1. Map showing sampling sites at Pitoujiiao, Longdong, Keelung Island, and Kueishian Island in northeastern Taiwan.

Genomic DNA was extracted from 90% ethanol-preserved tissue specimens using the automated LabTurbo Nucleic Acid Mini Kit LGD480-220 (Taigen Bioscience Corporation), following the manufacturer’s protocols. A total of four genes were amplified from the collected specimens, including one mitochondrial marker and three nuclear markers, following Huang et al. [16]: (1) cytochrome c oxidase subunit I segment (MCOIF: 5′-TCTACAAATCATAAGACATAGG-3′, MCOIR: 5′-GAGAAATTATACCAAAACCCAGG-3′); (2) nuclear ribosomal internal transcribed spacer segment (ITS, A18S: 5′-GATCGAACGGTTTAGTGAGG-3′); (3) nuclear ribosomal internal transcribed spacer segment (ITS, A18S: 5′-GATCGAACGGTTTAGTGAGG-3′); (3) two variable domain (D1 and D2) at 5′end of 28S ribosomal RNA segment (C1′: 5′-ACCCGCTGAATTTAAGCAT-3′, D2MAD: 5′-GACGATCGATTGCACGTCA-3′); and (4) histone H3 segment (H3F: 5′-ATGGCTCGTACCAAGCAGACVC-3′, H3R: 5′-ATATCCTTTR GGCATRATRGTGAC-3′). PCR was carried out using 12.5 µL of Fast-Run™ Advanced Taq Master Mix (Protech, Taipei, Taiwan), 10 mM each of forward and reverse ITS primer, 10–100 ng/µL DNA template, and deionized water to a final volume of 25 µL. The PCR profiles were as follows: an initial denaturation stage (95 °C, 5 min); 35 cycles of a denaturation step (95 °C, 30 s), an annealing step (54 °C, 40 s); an elongation step (72 °C, 7 min); and a final extension at 72 °C, for 5 min. The PCR products were confirmed by electrophoresis and subcloned into a pGEM-T easy vector (Promega, Madison, WI, USA). Three inserted cDNA fragments were sequenced...
with the pUC/M13 forward and reverse primers using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Forster City, CA, USA).

2.3. Sequence Management, Alignment, and Matrix

The raw forward and reverse sequences were edited and assembled into consensus sequences by the CodonCode Aligner V6.0.2 program (CodonCode Corporation Dedham, MA, USA). To exclude sequences amplified from zooxanthellae, the consensus sequences obtained were used to perform the BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/, accessed on 2 April 2020) [34]. The sequences obtained from the collected specimens of spawning corals in northern Taiwan were deposited into the NCBI GenBank (accession numbers in Supplementary Table S1). Newly obtained sequences for COI \((n = 58)\), ITS \((n = 59)\), 28S \((n = 63)\), and histone \((n = 62)\) were combined with sequences retrieved from public sources (Table S1).

All sequences for each gene were automatically aligned with the accurate alignment option (E-INS-i) in MAFFT v.7 ([35]; http://mafft.cbrc.jp/alighment/server/, accessed on 10 January 2021) under default parameters. The resulting multiple sequence alignments were translated into inferred amino acid sequences as a guide for inferred gap placement between coding regions using Se-Al v.2.0a11 [36]. The amino acid residue and nucleotide were manually adjusted to minimize the gaps. PAUPRat software v.3.1 [37] on the CIPRES Science Gateway (http://www.phylo.org, accessed on 10 January 2021) [38] was used to calculate descriptive statistics (sequence variations and informative sites) for the compared sequences of each gene.

2.4. MolecularDatasets

Some sequences were not obtained because the gene failed to amplify during PCR. Operational taxonomic units (OTUs) were created for each gene for the phylogeny reconstruction. The phylogeny reconstructions were conducted based on the combined gene matrix (COI, 28S, ITS, and histone); IGR (noncoding intergenic region between COI and the formylmethionine transfer RNA gene) was ignored because the overall alignment was not similar enough.

The sequences of merulinid corals published in Huang et al. [16] were retrieved from GenBank. They included 19 genera in Merulinidae: *Merulina* (2 species), *Caulastrea* (3 species), *Cyphastrea* (3 species), *Dipsastrea* (13 species), *Echinopora* (5 species), *Favites* (13 species), *Goniastrea* (5 species), *Hydnophora* (2 species), *Leptoria* (2 species), *Mycedium* (2 species), *Orbicella* (1 species), *Oulophyllia* (2 species), *Pectinia* (3 species), *Platygyra* (8 species), *Scapophyllia* (1 species), and *Trachyphyllia* (1 species); two resurrected genera (*Astrea* (2 species), *Coelastrea* (2 species)); and one new genus (*Paramontastraea* (1 species)). In total, we retrieved 124 sequences for 28S rDNA, 121 sequences for histone H3, 91 for ITS rDNA, and 112 for COI from the GenBank.

The clades distant from the merulinid corals (XVIII-XXI) were included for the phylogenetic inference following Huang et al. [10]. These sequences were comprised of three species of Lobophylliidae (*Moseleya latistellata, Acanthastrea echinata*, and *Lobophyllia corymbosa*), three species of Faviidae (*Montastraea multipunctata, Favia fragum*, and *Mussa angulosa*), and one species of Plesiastreidae (*Plesiastrea versipora*). Phylogeny reconstructions were created for each gene, along with four combined datasets based on maximum likelihood and Bayesian analyses.

2.5. Molecular Phylogenetic Analysis

The maximum likelihood (ML) trees of each partition were reconstructed with raxml-GUI v.2.0 [39] using the best model (GTR+I+G). The five datasets, including three nuclear genes (ITS, 28S, and histone H3), one mitochondrial gene (COI), and a combined gene dataset, were partitioned based on coding position. The combined gene datasets were conducted with five independent runs, and the tree with the best ML scores was selected.
Bayesian inference (BI) was carried out in MrBayes v.3.2.6 [41]. PartitionFinder was used to select the best partition scheme and accompanying substitution model, according to the Bayesian information criterion [42]. The best-fit substitution model was determined by ProtTest3. Two Monte Carlo Markov chains (MCMCs) were run for $4 \times 10^6$ million generations in two simultaneous runs, each with four different chains. The convergence of the estimates was checked by the standard deviation of split frequencies and by monitoring the likelihood score over time using Tracer v.1.6 [43]. Trees were sampled every 1000 generations, with the first 2500 (25%) discarded as “burn-in.” The remaining sampled trees were collected to construct a 50% majority-rule BI consensus tree. Nodal support from BI was assessed, and only nodes with $\geq 0.90$ posterior probabilities (PPs) were shown.

The output trees were further edited by FigTree v1.3.1 [44].

**3. Results**

**3.1. Characteristics of the Gene Data**

In the 65 specimens collected from Taiwan, 58 newly obtained COI sequences, 59 newly ITS sequences, 63 for 28S, and 62 for histone H3 sequences were obtained for the first time (Table S1). Examining the individual gene dataset, the aligned COI sequence was 744 base pairs (bp) long, with 180 variable and 81 parsimony informative sites. That of 28S was 865 bp, with 316 variable and 147 parsimony informative sites. That of ITS was 1249 bp, with 662 variable and 459 parsimony informative sites. That of histone H3 was 344 bp, with 109 variable and 82 parsimony informative sites.

**3.2. Results of the Analysis Matrix**

The dataset comprised a total of 3202 bp and 186 OTUs (123 OTUs from references and 65 OTUs from the present study). ML and BI methods (using raxmlGUI and MrBayes, respectively) were used to reconstruct the phylogenies for the combined dataset. The results from the partitioned ML analysis and BI conducted with the combined dataset were congruent (Figure 2). The ML analysis yielded a log-likelihood value of $-28,909.928401$ and the BI analysis yielded $(-3.069135 \times 10^4, -3.083239 \times 10^4)$.

**3.3. Phylogenetic Relationship**

The clades XV (Diploastreidae), XVI (Montasraeidae), and XVII (Merulinidae) were monophyletic, with high ML and BI support (100/1 and 100/0.99), whereas clades XVIII-XX (Lobophylliidae) and XXI (Mussidae) formed clusters but with weak support (63/0.8 and 58/0.84) (Figure 2). Within Merulinidae, eight major subclades (A, C, D/E, F, H, and I) formed with high ML (83–100) and BI (100) support. Subclades B and G, on the other hand, did not have high support (84/– and –/0.99). Subclade A is composed of *Paragoniastrea australensis*, *Scapophyllia cylindrica*, as well as species of *Goniastrea* and *Merulina*. Subclade B is composed of *Astrea annuligera*, *Favites valenciennesi*, and *Trachyphyllia geoffroyi*, as well as species of *Coelastrea* and *Dipsastrea*. Subclade C is composed of *Oulophyllia annulalis* and species of *Cuphastrea*. Subclade D/E is composed of species of *Caulastrea*, *Oulophyllia*, *Mycedium*, and *Pectinia*. Subclade F is composed of species of *Favites*. Subclade G is composed of *Favites stylifera* and species of *Platgyra* and *Leptoria*. Subclade H is composed of species of *Hydnophora*. Finally, subclade I is composed of species of *Echinopora* and *Paramontastra e salebrosa*. 
Figure 2. Phylogenetic tree of merulinid corals and their allies based on the combined gene dataset inferred with the maximum likelihood method using the GTR+G model. Molecular subclades within Merulinidae (XVII) are defined as being A to I following Huang et al. [10]. The other two novel clades (J and K) are defined in this study. Branch lengths are proportional to inferred nucleotide substitutions. Numbers at the nodes represent bootstrap values (only ≥ 70 shown) from the maximum likelihood method and posterior probability (only ≥ 0.9 shown) from the Bayesian inference. Bold branches on the tree indicate statistically robust nodes. The spawning month of specimens in Chen et al. [32] are in brackets.
Favites russilli and Astrea curta formed a distinct cluster (BP:100, PP:1), defined as a new subclade K, separated from Astrea annuligera, Paragoniastrea australensis, and P. deformis were monophyletic (BP:96, PP:0.97), so we defined the genus as a new subclade J. Paragoniastrea australensis, not monophyletic, was placed in subclade A and new subclade J. F. valenciennesi, not monophyletic, was placed in subclades B and F.

3.4. The Phylogenetic Tree

The spawning specimens we examined were all nested within Merulinidae (taxa in bold font in Figure 2). These specimens were placed in five subclades B, C, E, F, and G but not in the subclades A, D, H, or I. The following were nested in subclade B: one specimen each of Astrea annuligera, Coelastrea palauensis, Dipsastraea rotumana, Dipsastraea mathaii, and Favites valenciennesi and two specimens of Coelastrea aspera collected from Kueishan Island; one specimen of Dipsastraea favus collected from Pitoujiiao and two from Kueishan Island; one specimen of Dipsastraea lizardensis collected from Longdong. Two Cyphastrea chalcidicum specimens, collected from Longdong, were nested in subclade C. One specimen each of Mycedium mancaoi, Mycedium robokaki, and Pectinia paonia, collected from Longdong, and one Mycedium elephantotus, collected from Pitoujiiao, were nested in subclade E. Subclade F consisted of one specimen each of Favites valenciennesi and Favites flexuosa, collected from Pitoujiiao, and two Favites magnistellata, collected from Pitoujiiao and Kueishan Island. All the Favites pentagona specimens collected from Kueishan Island, Pitoujiiao, and Longdong were clustered with F. pentagona from Singapore and the Philippines. Subclade G consisted of two Platgyra pini, five Platgyra ryukyuensis, three Platgyra veraweyi, and one Platgyra sinensis, collected from Longdong; one species each of Platgyra lamellina and Platgyra daedalea, collected from Pitoujiiao; and two Favites stylifera from Pitoujiiao and Longdong. Six Paragoniastrea deformis, collected from Pitoujiiao, four Paragoniastrea australensis specimens from Pitoujiiao, and one from Longdong were clustered into subclade J. Subclade K was a monophyletic clade, consisting of three specimens of Astrea curta, collected from Keelung Island, and two from Pitoujiiao, which were clustered with those from the Great Barrier Reef and the Philippines (BP:98, PP:1).

4. Discussion

This is the first study to establish a molecular database for spawning corals, an important contribution to our understanding of genetic diversity in coral communities. We sequenced 1 species from Keelung Island, 9 from Kueishan Island, 13 from Longdong, and 12 from Pitoujiiao. In total, we sequenced 9 genera and 28 species, and most subclades were consistent with those of previous studies.

4.1. Phylogenetic Relationship of Merulinid Subclades

The Merulinidae are defined as monophyletic in this study, confirming previous findings [10,16]. Of four Atlantic species, Favia fragum, Mussa angulosa, Orbicella annularis, and Montastraea cavernosa, only O. annularis is nested within the subclade C and a sister to Cyphastrea spp. The genus Paramontastraea Huang et al. 2014 [10] examined in this study was also a sister to Echinopora Lamarck, 1816, and nested within subclade I.

Increasing the sequence lengths, taxon sampling, and sampling locations may improve the phylogenetic relationship among taxa. Adding new sequences of merulinid corals from Taiwan generated longer aligned sequences with which to examine the phylogenetic relationships among the subclades of Merulinidae; as a result, most subclades changed their phylogenetic positions (Figure S1). For example, the tree topology reconstructed in Huang et al. [10] showed that Hydnophora (subclade H) is closer to Favites (subclade F). However, our reconstructed phylogenetic tree showed that the Hydnophora lineages only closer to Favites pentagona and the rest of Favites spp. are close to novel subclade K, which comprises Astrea curta and Favites russelli. In addition, subclade B shifted its position from D/E clades to subclade H and Favites pentagona.
As mentioned in Huang et al. [10,15,16], *Favites pentagona* and *Paragoniastrea australensis* displayed polyphyletic patterns that require further investigation [10]. *Paragoniastrea australensis* was far from subclade A (*Goniastrea* spp.) and was clustered together with *Astrea curta*, *Astrea annuligera*, *Astrea devatieri*, and *Favites russelli* as a novel clade. *Favites pentagona* renders *Favites* polyphyletic in the molecular phylogeny and sister to the *Favites* spp. (subclade F) and subclade D/E [10]. Therefore, Huang et al. [10,16] suggested that these two species require further study with increasing sample collection from other locations. According to our new analysis, the molecular phylogenetic tree implied that *P. australensis* displayed a polyphyletic pattern, which is consistent with Huang et al. [10,16]. However, they were close to subclade A (*Goniastrea* spp.) and formed a novel subclade J (*Paragoniastrea* spp.). *Favites pentagona* formed a monophyletic pattern, which is different from Huang et al. [10,16]. They are separate from the major *Favites* spp. (subclade F) and are close to subclade H (*Hydnophora*).

4.2. Application of Molecular Phylogenetic Approaches

Merulinidae corals with plocoid and ceroid forms are difficult to accurately identify to the genus level in the field because of their macro-morphological homoplasies [24,33]. In our phylogenetic analysis, all of the corals in the plocoid form were placed into subclades B, C, F, I, and/or K, including 18 *Dipsastraea* spp. (subclade B), 2 *Favites* spp. (B and F), 5 *Cyphastrea* spp. (C), and 2 *Astrea* spp. (B and K). Merulinidae corals in the ceroid form included 6 *Goniastrea* spp. (subclade A), 14 *Favites* spp. (F), 2 *Coelastrea* spp. (B), and 9 *Platygyra* spp. (G). Most of the samples we examined were either *Coelastrea* spp., *Favites pentagona*, *Platygyra* spp., *Paragoniastrea australensis*, or *Paragoniastrea deformis*. The genetic divergence between these four groups (subclades B, F, G, and J) may be driven by the differences in their sexually reproductive timing. *Favites pentagona* and *P. deformis* spawn in July, while *Platygyra* spp. and *P. australensis* spawn in August [32].

4.3. Sexual Reproduction in Merulinidae

Scleractinian corals have a complex sexual reproduction system, with the same species displaying different sexual reproduction patterns based on their geographic distribution [45]. The systematic pattern in the reproductive biology of Merulinidae, a hermaphroditic spawner, is highly conserved [45–47]. In this study, *Coelastrea aspera* collected from northern Taiwan was identified as a hermaphroditic spawner and placed in subclade B with the Singapore *Coelastrea aspera* [32,48]. *Coelastrea aspera*, from the Great Barrier Reef, is a spawner [49–52], whereas the nonspecific populations distributed in Palau are brooders [53,54]. The hermaphroditic *Coelastrea aspera*, from Okinawa, performs as a spawner [55] and a brooder [56–58]. A similar example, *Pocillopora damicornis*, is a brooder in most locations, but a spawner in western Australia [59–62].

5. Conclusions

This study integrates reproduction information, morphological characteristics, and molecular phylogenetic analysis to increase our understanding of the genetic diversity of Merulinidae. Ten major subclades (A, B, C, D/E, F, G, H, I, J, and K) were reconstructed. Our study identified *Paragoniastrea deformis* and *Paragoniastrea australensis* in Taiwan for the first time. Together, the two species form the new subclade J. *Astrea curta* were separated from another congeneric species, *Astrea annuligera* (XVII-B), clustered with *Favites russelli* into the new subclade K. Finally, we contributed information on the species diversity of coral communities in Taiwan and fill gaps involving merulinid corals between Japan and the Philippines in the Western Pacific.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14020144/s1, Table S1: Species and DNA sequences examined in this study. The species name in Huang et al. 2011 was updated according to World Register of Marine Species (WoRMs). GenBank accession numbers are displayed for each molecular marker (28S rDNA, histone H3, ITS rDNA and mt COI). N.D.: sequences were failed from PCR. Accession dates
of 28S rDNA sequences: 21-FEB-2011 [16], 26-OCT-2021 (this study); histone H3: 25-JUL-2016 [16], 23-NOV-2021 (this study); ITS rDNA: 21-FEB-2011 [16], 04-DEC-2021 (this study). Figure S1. Phylogeny of Merulinidae reconstructed from Huang et al. [10] and this study.

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References
1. Veron, J.E.N.; Pichon, M. Scleractinia of Eastern Australia; Australian Govt. Pub. Service: Townsville, Australia, 1976; Volume 1.
2. Veron, J.E.N. Corals of the World; Australian Institute of Marine Science: Townsville, Australia, 2000; Volume 3.
3. Todd, P.A. Morphological plasticity in scleractinian corals. Biol. Rev. Camb. Philos. Soc. 2008, 83, 315–337. [CrossRef] [PubMed]
4. Wells, J.W. Scleractinia. In Treatise on Invertebrate Paleontology, Coelenterata; Teichert, C., Ed.; Geological Society of America and University of Kansas Press: Lawrence, KS, USA, 1956; pp. 328–444.
5. Veron, J.E. Corals in Space and Time: The Biogeography & Evolution of the Scleractinia; Cornell University Press: Ithaca, NY, USA, 1995.
6. Romano, S.L.; Palumbi, S.R. Evolution of scleractinian corals inferred from molecular systematics. Science 1996, 271, 640–642. [CrossRef]
7. Romano, S.L.; Palumbi, S.R. Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. J. Mol. Evol. 1997, 45, 397–411. [CrossRef] [PubMed]
8. Kitahara, M.V.; Cairns, S.D.; Stolarski, J.; Blair, D.; Miller, D.J. A comprehensive phylogenetic analysis of the scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. PLoS ONE 2010, 5, 1490. [CrossRef]
9. Fukami, H.; Chen, C.A.; Budd, A.F.; Collins, A.; Wallace, C.; Chuang, Y.Y.; Chen, C.; Dai, C.F.; Iwao, K.; Sheppard, C.; et al. 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 2008, 3, e3222. [CrossRef]
10. Huang, D.; Benzoni, F.; Fukami, H.; Knowlton, N.; Smith, N.D.; Budd, A.F. Taxonomic classification of the reef coral families Merulinidae, Montastraeidae, and Diploastreaidae (Cnidaria: Anthozoa: Scleractinia). Zool. J. Linnean Soc. 2014, 171, 277–355. [CrossRef]
11. Kuzon, K.S.; Lin, M.F.; Lagman, M.C.A.A.; Licuanan, W.R.Y.; Chen, C.A. Resurrecting a subgenus to genus: Molecular phylogeny of Euphyllia and Fimbriaphyllia (order Scleractinia; family Euphylliidae; clade V). PeerJ 2017, 5, e4074. [CrossRef]
12. Benzoni, F.; Arrigoni, R.; Waheed, Z.; Stefani, F.; Hoeksema, B.W. Phylogenetic relationships and revision of the genus Blastomussa (Cnidaria: Anthozoa: Scleractinia) with description of a new species. Baffles Bull. Zool. 2014, 62, 358–378.
13. Budd, A.F. Systematics and evolution of scleractinian corals. In Encyclopedia of Life Synthesis Meeting Report; National Museum of Natural History: Washington, DC, USA, 2009.
14. Budd, A.F.; Stolarski, J. Coralite wall and septal microstructure in scleractinian reef corals: Comparison of molecular clades within the family Faviidae. J. Morphol. 2011, 272, 66–88. [CrossRef]
15. Huang, D.; Benzoni, F.; Arrigoni, R.; Baird, A.H.; Berumen, M.L.; Bouwmeester, J.; Chou, L.M.; Fukami, H.; Licuanan, W.Y.; Lovell, E.R.; et al. Towards a phylogenetic classification of reef corals: The Indo-Pacific genera Merulina, Goniastrea and Scaphophyllia (Scleractinia, Merulinidae). Zool. Scr. 2014, 43, 531–548. [CrossRef]
16. Huang, D.; Licuanan, W.Y.; Baird, A.H.; Fukami, H. Cleaning up the ‘Bigmessidae’: Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. BMC Evol. Biol. 2011, 11, 37. [CrossRef]
Diversity 2022, 14, 144

17. Huang, D.W.; Meier, R.; Tood, P.A.; Chou, L.M. More evidence for pervasive paraphyly in scleractinian corals: Systematic study of Southeast Asian Faviidae (Cnidaria; Scleractinia) based on molecular and morphological data. *Mol. Phyloge net. Evol.* 2009, 50, 102–116. [CrossRef] [PubMed]

18. Fukami, H.; Budd, A.F.; Paulay, G.; Solé-Cava, A.; Chen, C.A.; Iwao, K.; Knowlton, N. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* 2004, 427, 832–834. [CrossRef] [PubMed]

19. Schwartz, S.A.; Budd, A.F.; Carlon, D.B. Molecules and fossils reveal punctuated diversification in Caribbean “faviid” corals. *BM C Evol. Biol.* 2012, 12, 123. [CrossRef] [PubMed]

20. World List of Scleractinia. Available online: http://www.marinespecies.org/scleractinia (accessed on 2 August 2021).

21. Khalil, H.M.; Fathy, M.S.; Sawy, S.M.A. Quaternary corals (Scleractinia: Merulinidae) from the Egyptian and Saudi Arabian Red Sea Coast. *Geol. J.* 2021, 56, 4150–4188. [CrossRef]

22. De Palmas, S.; Ho, M.J.; Denis, V.; Chen, C.A.; Iwao, K.; Knowlton, N. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* 2004, 427, 832–834. [CrossRef] [PubMed]

23. Dai, C.F.; Soong, K.; Chen, C.A.; Fan, T.Y.; Hsieh, H.J.; Jeng, M.S.; Chen, C.H.; Horng, S. *Status of Coral Reefs of Taiwan; Institute of Oceanography, National Taiwan University*; Taipei, Taiwan, 2009.

24. Ribas-Deulofeu, L.; Denis, V.; Palmass, S.; Kuo, C.Y.; Hsieh, H.; Chen, C. Structure of benthic communities along the Taiwan latitudinal gradient. *PloS ONE* 2016, 11, e0160601. [CrossRef] [PubMed]

25. Dai, C.F.; Cheng, Y.L. *Corals of Taiwan; Institute of Oceanography: Taipei, Taiwan*, 2020; Volume 1.

26. Chen, C.A.; Wallace, C.C.; Yu, J.K.; Wei, N.V. Strategies for amplification by polymerase chain reaction of the complete sequence of the gene encoding nuclear large subunit ribosomal RNA in corals. *Mar. Biotechnol.* 2000, 2, 558–570. [CrossRef] [PubMed]

27. Lin, M.F.; Kitahara, M.V.; Tachikawa, H.; Keshavmurthy, S.; Chen, C.A. A new shallow-water species, *Pocillopora chaishanensis* sp. nov. (Scleractinia: Caryophylliidae), from Chaishan, Kaohsiung, Taiwan. *Zool. Stud.* 2012, 51, 213–221.

28. De Palmas, S.; Soto, D.; Denis, V.; Ho, M.J.; Chen, C.A. Multispecies spawning of *Pocillopora verrucosa* (Scleractinia; Pocilloporidae) distribution along a depth gradient in Ludao, Taiwan. *PeerJ* 2018, 6, e5797. [CrossRef]

29. Soto, D.; De Palmas, S.; Ho, M.J.; Denis, V.; Chen, C.A. Spatial variation in the morphological traits of *Pocillopora verrucosa* along a depth gradient in Taiwan. *PloS ONE* 2018, 13, e020586. [CrossRef]

30. Hoeksema, B.W.; Arrigoni, R. DNA barcoding of a stowaway reef coral in the international aquarium trade results in a new distribution record. *Mar. Biodivers.* 2020, 50, 41. [CrossRef]

31. Shikina, S.; Chang, C.F. Sexual Reproduction in Stony Corals and Insight into the Evolution of Oogenesis in Cnidaria. In *The Cnidaria, Past, Present and Future*; Goffredo, S., Dubinsky, Z., Eds.; Springer: Cham, Switzerland, 2016; pp. 249–268.

32. Chen, C.J.; Chen, W.J.; Chang, C.F. Multispecies spawning of scleractinian corals in non-reefal coral communities of northern Taiwan in the northwestern Pacific Ocean. *Bull. Mar. Sci.* 2021, 97, 351–371. [CrossRef]

33. Lin, Y.V.; Denis, V. Acknowledging differences: Number, characteristics, and distribution of marine benthic communities along Taiwan coast. *Ecosphere* 2019, 10, e02803. [CrossRef]

34. Madden, T. *The NCBI Handbook; National Center for Biotechnology: Bethesda, MD, USA*, 2002.

35. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 2013, 30, 772–780. [CrossRef]

36. Rambaut, A. Se-Al Sequence Alignment Editor, Version 2.0a11. Available online: http://tree.bio.ed.ac.uk/software/seal/ (accessed on 8 August 2002).

37. Swoford, D.L. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1*; Illinois Natural History Survey: Champaign, IL, USA, 1991.

38. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In *Gateway Computing Environments Workshop (GCE)*; IEEE: Piscataway, NJ, USA, 2010.

39. Edler, D.; Klein, J.; Antonelli, A.; Silvestro, D. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods Ecol. Evol.* 2012, 12, 373–377. [CrossRef]

40. Felsenstein, J. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 1981, 17, 368–376. [CrossRef]

41. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 2012, 61, 539–542. [CrossRef]

42. Lanfear, R.; Calcott, B.; Ho, S.Y.; Guindon, S. Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 2012, 29, 1695–1701. [CrossRef]

43. Rambaut, A.; Suchard, M.A.; Xie, D.; Drummond, A.J. *Tracer* v1.6. Available online: http://beast.bio.ed.ac.uk/Tracer (accessed on 30 November 2009).

44. Rambaut, A. FigTree v1.4.2. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 10 March 2019).

45. Harrison, P.L. Sexual Reproduction of Scleractinian Corals. In *Coral Reefs: An Ecosystem in Transition*; Dubinsky, Z., Stambler, N., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 59–85.

46. Kerr, A.M.; Baird, A.H.; Hughes, T.P. Correlated evolution of sex and reproductive mode in corals (Anthozoa: Scleractinia). *Proc. R. Soc. B* 2010, 278, 75–81. [CrossRef]

47. Baird, A.H.; Guest, J.R.; Willis, B.L. Systematic and biogeographical patterns in the reproductive biology of scleractinian coral. *Annu. Rev. Ecol. Evol.* 2009, 40, 551–571. [CrossRef]
48. Dai, C.F.; Soong, K.; Fan, T.Y. Sexual reproduction of corals in northern and southern Taiwan. In Proceedings of the International Coral Reef Symposium (ICRS), Guam, Micronesia, 22–27 June 1992; Volume 1, pp. 448–455.

49. Babcock, R. Reproduction and distribution of two species of Goniastrea (Scleractinia) from the Great Barrier Reef Province. *Coral Reefs* **1984**, *2*, 187–195.

50. Babcock, R.C.; Bull, G.D.; Harrison, P.L.; Heyward, A.J.; Oliver, J.K.; Wallace, C.C.; Willis, B.L. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* **1986**, *90*, 379–394. [CrossRef]

51. Harrison, P.L.; Babcock, R.C.; Bull, G.D.; Oliver, J.K.; Wallace, C.C.; Willis, B.L. Mass spawning in tropical reef corals. *Science* **1984**, *223*, 1186–1189. [CrossRef] [PubMed]

52. Harrison, P.L.; Babcock, R.C.; Bull, G.D.; Oliver, J.K.; Wallace, C.C.; Willis, B.L. Mass spawning in tropical reef corals. *Science* **1984**, *223*, 1186–1189. [CrossRef] [PubMed]

53. Abe, N. Postlarval development of the coral Fungia actiniformis var. palawensis Doderlein. *Palao Trop. Biol. Stat. Stud.* **1937**, *1*, 73–93.

54. Motoda, S. Observation of period of extrusion of planula of Goniastrea aspera (Verrill.). *Kagaku Nanyo* **1939**, *1*, 5–7.

55. Sakai, K. Gametogenesis, spawning, and planula brooding by the reef coral Goniastrea aspera (Scleractinia) in Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **1997**, *151*, 67–72. [CrossRef]

56. Heyward, A.K. Sexual reproduction of corals in Okinawa. *Galaxea* **1987**, *6*, 331–343.

57. Hayashibara, T.; Shimoike, K.; Kimura, T.; Hosaka, S.; Heyward, A.; Harrison, P.L.; Kudo, K.; Omori, M. Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Mar. Ecol. Prog. Ser.* **1993**, *101*, 253–262. [CrossRef]

58. Nozawa, Y.; Harrison, P.L. Temporal settlement patterns of larvae of the broadcast spawning reef coral Favites chinensis and the broadcast spawning and brooding reef coral Goniastrea aspera from Okinawa, Japan. *Coral Reefs* **2005**, *24*, 274–282. [CrossRef]

59. Castrillón, A.; Muñoz, C.; Zapata, F. Reproductive patterns of the coral Pocillopora damicornis at Gorgona Island, Colombian Pacific Ocean. *Mar. Biol. Res.* **2015**, *15*, 1065–1075. [CrossRef]

60. Richmond, R.H.; Jokiel, P.L. Lunar periodicity in larva release in the reef coral Pocillopora damicornis at Enewetak and Hawaii. *Bull. Mar. Sci.* **1984**, *34*, 280–287.

61. Stoddart, J.A.; Black, R. Cycles of gametogenesis and planulation in the coral Pocillopora damicornis. *Mar. Ecol. Prog. Ser.* **1985**, *23*, 153–164. [CrossRef]

62. Ward, S. Evidence for broadcast spawning as well as brooding in the scleractinian coral Pocillopora damicornis. *Mar. Biol.* **1992**, *112*, 641–646. [CrossRef]