

## Diversity, Distribution, and Molecular Systematics of Octocorals (Coelenterata: Anthozoa) of the Penghu Archipelago, Taiwan

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**Yehuda Benayahu, Leendert Pieter van Ofwegen, Chang-feng Dai, Ming-Shiou Jeng, Keryea Soong, Alex Shlagman, Henryi J. Hsieh, and Catherine S. McFadden (2012)** Diversity, distribution, and molecular systematics of octocorals (Coelenterata: Anthozoa) of the Penghu Archipelago, Taiwan. *Zoological Studies* 51(8): 1529-1548. The 1st ever surveys of octocorals in the Penghu Archipelago, Taiwan were conducted in 2006 and 2009. Scuba collections were carried out at 17 sites in northern, eastern, south-central, and southern parts of the archipelago. The collection, comprising about 250 specimens, yielded 34 species of the family Alcyoniidae belonging to *Aldersladum*, *Cladiella*, *Klyxum*, *Lobophytum*, *Sarcophyton*, and *Sinularia*. These include 6 new species that were recently described and another 15 records new to Taiwanese reefs. The northern collection sites featured a lower number of species compared to most of the central/southern or southern ones. To facilitate identification of octocoral species in future surveys, DNA sequences were obtained for the mitochondrial barcoding markers, *COI* and *mtMutS*, and for *Cladiella* and *Klyxum* only, nuclear *28S* rDNA. These molecular markers reliably identified specimens to genus and clade, but could not discriminate among species within some clades. Phylogenetic reconstructions based on the barcode markers revealed paraphyly among *Cladiella*, *Klyxum*, and *Aldersladum*, thus emphasizing the need for taxonomic revisions of these genera. These results highlight the importance of octocoral surveys to elucidate patterns of biodiversity and zoogeography in the East and South China Seas, including their marginal reef systems. <http://zoolstud.sinica.edu.tw/Journals/51.8/1529.pdf>

**Key words:** Alcyonacea, Penghu, Taiwan, Species diversity.

Taiwan, a continental island with several offshore islets, is located at the junction of the Philippine-Japan island arc (Chen 1999), and features several well-developed coral communities at the northern edge of the South China Sea, particularly along its southern part and around offshore islands (Spalding et al. 2001). The occurrence of scleractinian corals in Taiwan is

influenced by sea surface currents and seawater temperatures (Chen 1999). Some of the best-known and most-developed coral reefs of southern Taiwan are those of the Hengchun Peninsula, mainly at Nanwan Bay, Kenting National Park (Dai 1997). Scleractinian corals are well developed around the east-coast offshore islands of Green Is. (*Lutao* in Chinese) and Orchid Is. (*Lanyu* in

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Chinese), the northeast coast, and the Penghu Archipelago (Chen et al. 2005; see below). In recent years, the Dongsha Atoll, located at the northern edge of the South China Sea, has also garnered attention due to its unique reefs (Dai 2004).

The octocorals of Taiwan have been a subject of several studies, including early taxonomic studies of the northeastern coast of Taiwan (Utinomi 1950a) and of the southernmost part of the island (Utinomi 1950b 1951 1959). Dai (1988 1990 1991a b) demonstrated the high abundance and ecological importance of octocorals of southern Taiwanese reefs. Benayahu and Perkol-Finkel (2004) and Benayahu et al. (2004) reviewed octocoral studies in Taiwanese waters and provided a species list resulting from surveys conducted in southern Taiwan and around Green Is. Their studies further emphasized the high abundance and live coverage of octocorals in Taiwanese coral reefs.

The Penghu Archipelago (Penghu, or the Pescadores), located off the western coast of Taiwan in the Taiwan Strait, is composed of 64 islands and islets assigned to 4 regions: northern, southern, eastern, and inner (Chen et al. 2005). As opposed to reefs of the southern tip of Taiwan and Green Is., which are categorized as “tropical reefs”, those of the Penghu Is. are clustered into high-latitude, subtropical “non-reefal communities”. Because the biogeographic setting of the Penghu Archipelago, between the western Pacific Ocean and East China Sea was intriguing, we conducted an octocoral survey there. So far, it has yielded 6 new species: Benayahu and Ofwegen (2011) described *Lobophytum hsiehi*, *Sinularia daii*, and *Sin. soongi*; Benayahu and McFadden (2011) assigned *Aldersladum jengi*, a new species from there, to that newly established genus; and Ofwegen and Benayahu (2012) described *Sin. penghuensis* and *Sin. wanannensis*. The current study provides a systematic list of octocoral species, aspects of their phylogeny, and information on their abundances and distributional patterns in the archipelago, and also compares them to reefs of southern Taiwan. In addition, to facilitate identification of octocoral material collected from Taiwan in the future and increase our understanding of phylogenetic relationships among Penghu octocorals, representative specimens of most species were sequenced for 2 molecular barcode markers: cytochrome oxidase I (COI), and the octocoral-specific mitochondrial MutS homolog, *mtMutS* (*msh1*) (McFadden et al.

2011).

## MATERIALS AND METHODS

### Collection and morphological characterization

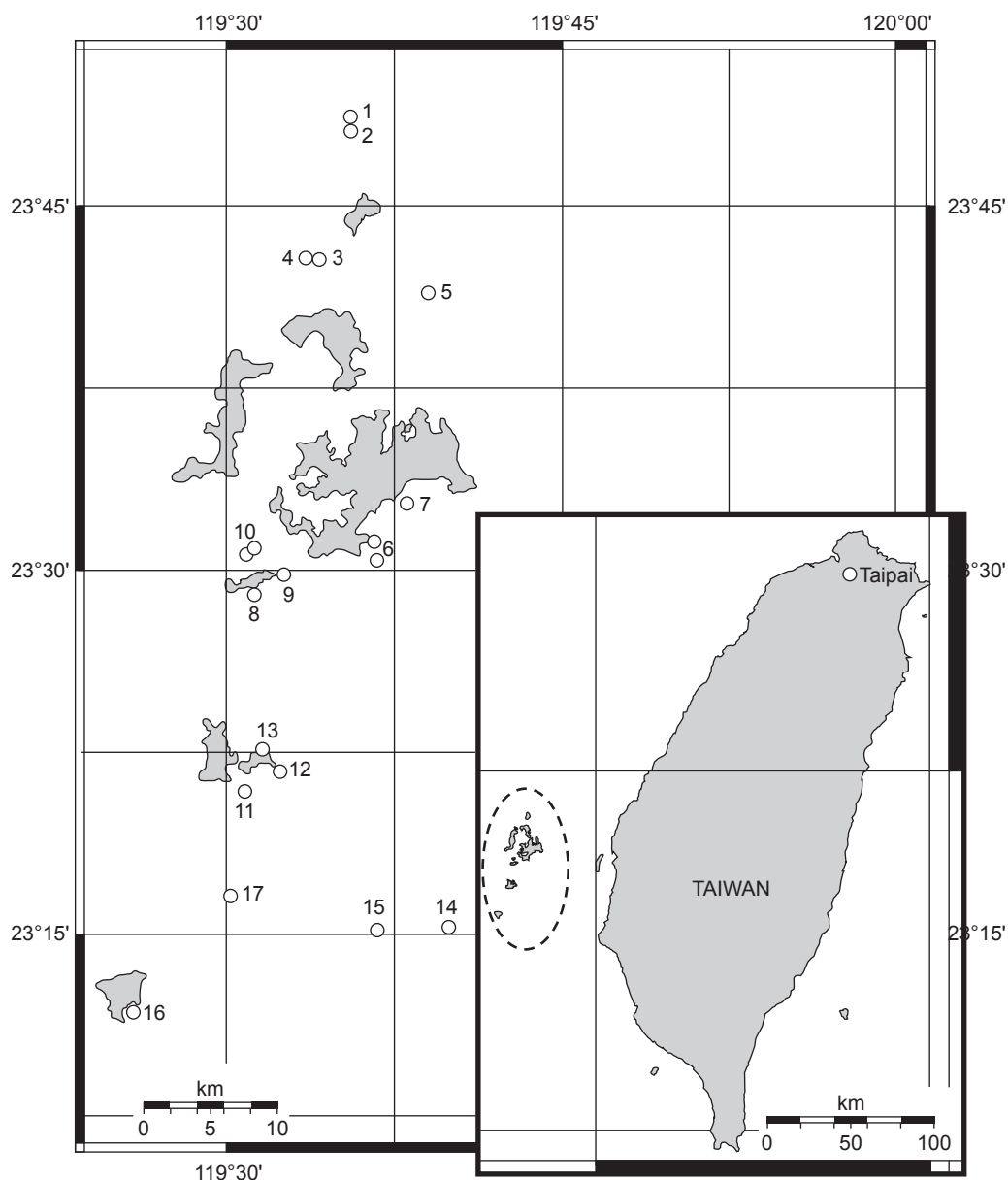
Material was collected, using scuba diving, on 2 field trips conducted in July 2006 (7 d) and July 2009 (5 d), which yielded about 250 specimens of octocorals, encompassing the variety of taxa occurring at the explored sites. The study covered the northern, eastern, central/southern, and southern parts of the Penghu Archipelago, totaling 17 sites (Fig. 1). The collection sites are listed below, with their respective number that appears in figure 1, along with their coordinates and regional affiliation in the archipelago:

1. Da-Chiaw, 23°48.651'N, 119°35.458'E (northern part);
2. Mu-Do, 23°48.079'N, 119°35.474'E (northern part);
3. Tieh-Jen, 23°42.824'N, 119°34.049'E (northern part);
4. Gu-Po, 23°42.859'N, 119°33.488'E (northern part);
5. Chu-Dra, 24°41.437'N, 119°38.941'E (eastern part);
6. San-Sui, 23°30.573'N, 119°36.569'E (south-central part);
7. Ai-Men, 23°32.685'N, 119°38.158'E (south-central part);
8. Hu-Gin 1, 23°28.994'N, 119°31.163'E (southern part);
9. Hu-Gin 2, 23°29.808'N, 119°32.641'E (southern part);
10. Ton-Pan, 23°30.674'N, 119°30.822'E (southern part);
11. Wan-An, 23°20.913'N, 119°30.753'E (southern part);
12. Hou-Dai-Dzai, 23°21.683'N, 119°32.366'E (southern part);
13. Chiang-Jun, 23°22.604'N, 119°31.533'E (southern part);
14. Tong-Gee, 23°15.273'N, 119°39.891'E (southern part);
15. Si-Gee, 23°15.223'N, 119°36.680'E (southern part);
16. Chi-Mei, 23°11.762'N, 119°25.989'E (southern part); and
17. Yu-Pin, 23°16.536'N, 119°30.154'E (southern part).

Prior to collection, many of the colonies were photographed underwater. Abundance estimates

were visually made at each site and divided into 4 categories: rare, sporadic, abundant, and dominant (see Benayahu et al. 2004). Samples were preserved in 70% alcohol, and subsamples were removed and preserved in absolute alcohol and dimethyl sulfoxide (DMSO) for molecular studies (see below). In order to identify the material, sclerites from different parts of colonies (polyp, polyparium surface and interior, and base surface and interior) were obtained by dissolving

the tissues in 10% sodium hypochlorite, followed by careful rinsing in fresh water. Identification of species was facilitated, when available, by comparisons to permanent sclerite preparations of type materials kept in the Zoological Museum, Department of Zoology, Tel Aviv Univ., Israel (ZMTAU) and at the Naturalis Biodiversity Center (NBC), formerly Rijksmuseum van Natuurlijke Historie (RMNH), Leiden, the Netherlands. The identified specimens are deposited at ZMTAU



**Fig. 1.** Map of the Penghu Archipelago showing locations of study sites with regional affiliations of the archipelago: northern part: 1. Da-Chiaw; 2. Mu-Do; 3. Tieh-Jen; 4. Gu-Po; eastern part: 5. Chu-Dra; south-central part: 6. San-Sui; 7. Ai-Men; southern part: 8. Hu-Gin 1; 9. Hu-Gin 2; 10. Ton-Pan; 11. Wan-An; 12. Hou-Dai-Dzai; 13. Chiang-Jun; 14. Tong-Gee, 15. Si-Gee, 16. Chi-Mei, and 17. Yu-Pin.

and RMNH as indicated below. Members of the family Clavulariidae Hickson, 1894; Nephtheidae Gray, 1862; and Nidaliidae Gray, 1869; and some material of the genera *Klyxum* Alderslade, 2000 and *Cladiella* Gray, 1869 (Alcyoniidae) could not be identified to species, since taxonomic revisions of the respective taxa are required.

### Molecular phylogenetic analyses

Extraction of DNA from ethanol-preserved tissue samples, polymerase chain reaction (PCR) amplification, and sequencing of the *mtMutS* and *COI* genes followed protocols published in McFadden et al. (2011). A fragment of the nuclear ribosomal 28S gene was also sequenced for *Aldersladum*, *Cladiella* and *Klyxum* using primers and protocols specified in McFadden and Ofwegen (2013). The L-INS-i method in MAFFT (Kato et al. 2005) was used to align sequences to reference datasets consisting of previously published sequences for the genera *Sinularia*, *Sarcophyton*, and *Lobophytum* (McFadden et al. 2006 2009). Sequences from *Cladiella* and *Klyxum* were aligned to reference sequences for members of those taxa included in McFadden et al. (2011), and to newly sequenced voucher material from other geographic regions (Table 1). MEGA vers. 5

(Tamura et al. 2011) was used to compute pairwise measures of genetic distances (uncorrected *p* values) among sequences. *COI* and *mtMutS* sequences were concatenated, and the combined dataset was analyzed using maximum-likelihood implemented in Garli-2.0 (Zwickl 2006) with the TVM+I+G model of evolution selected using ModelTest 3.0 (Posada and Crandall 1998). In addition, for *Cladiella* and *Klyxum*, all 3 gene regions (*COI*, *mtMutS*, and 28S ribosomal (r)DNA) were concatenated, and a partitioned likelihood model was run with a TVM+I+G model applied to the mitochondrial gene partition and GTR+I+G applied to the 28S rDNA partition.

## RESULTS

### Species diversity and distribution

In total, the study of collections from the Penghu Archipelago (2006 and 2009) yielded 34 species of the family Alcyoniidae belonging to the following 6 genera: *Aldersladum*, *Cladiella*, *Klyxum*, *Lobophytum*, *Sarcophyton*, and *Sinularia* (Table 2); these include 5 new species that were recently described (Benayahu and McFadden 2011, Benayahu and Ofwegen 2011, Ofwegen

**Table 1.** Specimens from the Penghu Archipelago included in molecular analyses. Vouchers of all specimens are deposited at the Zoological Museum, Tel Aviv University (ZMTAU). NA, no accession

Genus and Species	Voucher ZMTAU #	GenBank Acc. No.		
		<i>COI</i>	<i>mtMutS</i>	28S
<i>Aldersladum</i>				
<i>A. jengi</i>	Co 33607	JX991220	JX991144	JX991201
<i>Cladiella</i>				
<i>C. australis</i>	Co 33613	JX991221	NA	JX991202
<i>C. australis</i>	Co 33622	JX991222	NA	JX991203
<i>C. bottae</i>	Co 34648	JX991223	JX991145	JX991204
<i>C. krempfi</i>	Co 33592	JX991224	NA	JX991205
<i>C. pachyclados</i>	Co 33604	JX991225	JX991146	JX991206
<i>C. tuberculoides</i>	Co 34642	JX991226	JX991147	JX991207
<i>C. tuberculoides</i>	Co 34686	JX991227	JX991148	JX991208
<i>C. tuberosa</i>	Co 34669	JX991228	JX991149	JX991209
<i>Klyxum</i>				
<i>K. utinomii</i>	Co 33581	JX991229	NA	JX991210
<i>K. utinomii</i>	Co 33612	JX991230	NA	JX991211
<i>K. utinomii</i>	Co 34636	JX991231	JX991150	NA
<i>K. utinomii</i>	Co 34639	JX991232	JX991151	JX991212
<i>K. utinomii</i>	Co 34672	JX991233	JX991152	NA
<i>K. utinomii</i>	Co 34674	JX991234	JX991153	NA
<i>K. utinomii</i>	Co 34692	JX991235	JX991154	NA

Table 1. (continued)

Genus and Species	Voucher ZMTAU #	GenBank Acc. No.		
		<i>COI</i>	<i>mtMutS</i>	<i>28S</i>
<i>Lobophytum</i>				
<i>L. crassum</i>	Co 34657	JX991244	NA	NA
<i>L. hsiehi</i>	Co 33593	JX991245	JX991155	NA
<i>L. hsiehi</i>	Co 34694	NA	JX991156	NA
<i>L. hsiehi</i>	Co 34717	NA	JX991157	NA
<i>Sarcophyton</i>				
<i>S. ehrenbergi</i>	Co 34705	JX991246	JX991158	NA
<i>S. trocheliophorum</i>	Co 33621	JX991247	JX991159	NA
<i>S. trocheliophorum</i>	Co 33634	JX991248	JX991160	NA
<i>S. trocheliophorum</i>	Co 34641	JX991249	JX991161	NA
<i>S. trocheliophorum</i>	Co 34644	JX991250	JX991162	NA
<i>S. trocheliophorum</i>	Co 34660	JX991251	JX991163	NA
<i>S. trocheliophorum</i>	Co 34662	JX991252	JX991164	NA
<i>S. tortuosum</i>	Co 34684	JX991253	JX991165	NA
<i>S. tumulosum</i>	Co 34698	JX991254	JX991166	NA
<i>S. tumulosum</i>	Co 34710	JX991255	JX991167	NA
<i>Sinularia</i>				
<i>S. abrupta</i>	Co 33623	JX991256	JX991168	NA
<i>S. acuta</i>	Co 33617	JX991257	JX991169	NA
<i>S. daii</i>	Co 34665	JX991258	JX991170	NA
<i>S. erecta</i>	Co 33585	JX991259	NA	NA
<i>S. gibberosa</i>	Co 33611	JX991260	JX991171	NA
<i>S. lochmodes</i>	Co 33648	JX991261	NA	NA
<i>S. molesta</i>	Co 33615	JX991262	JX991172	NA
<i>S. ornata</i>	Co 34646	JX991263	JX991173	NA
<i>S. ornata</i>	Co 34647	JX991264	JX991174	NA
<i>S. ornata</i>	Co 34650	JX991265	JX991175	NA
<i>S. ornata</i>	Co 34651	JX991266	JX991176	NA
<i>S. ornata</i>	Co 34683	JX991267	JX991177	NA
<i>S. ornata</i>	Co 34712	JX991268	JX991178	NA
<i>S. pavidata</i>	Co 33630	JX991269	JX991179	NA
<i>S. peculiaris</i>	Co 34707	JX991270	JX991180	NA
<i>S. penghuensis</i>	Co 34630	JX991271	JX991181	NA
<i>S. penghuensis</i>	Co 34643	JX991272	JX991182	NA
<i>S. penghuensis</i>	Co 34659	JX991273	JX991183	NA
<i>S. penghuensis</i>	Co 34681	JX991274	JX991184	NA
<i>S. penghuensis</i>	Co 34706	JX991275	JX991185	NA
<i>S. penghuensis</i>	Co 34739	JX991276	JX991186	NA
<i>S. slieringsi</i>	Co 34654	JX991277	JX991187	NA
<i>S. soongi</i>	Co 33632	JX991278	NA	NA
<i>S. wanannensis</i>	Co 34695	JX991279	JX991188	NA
<i>S. wanannensis</i>	Co 34700	JX991280	JX991189	NA
<i>S. wanannensis</i>	Co 34704	JX991281	JX991190	NA
<i>S. sp.</i>	Co 33658	JX991282	JX991191	NA
<i>S. sp.</i>	Co 34691	JX991283	JX991192	NA
Additional reference material sequenced for this study:				
<i>A. sodwanum</i>	Co 31520	JX991236	JX991193	JX991213
<i>C. kashmani</i>	Co 32246	JX991237	JX991194	JX991214
<i>C. kashmani</i>	Co 32334	JX991238	JX991195	JX991215
<i>C. kashmani</i>	Co 32482	JX991239	JX991196	NA
<i>C. pachyclados</i>	Co 35507	JX991240	JX991197	JX991216
<i>C. pachyclados</i>	Co 35508	JX991241	JX991198	NA
<i>K. adii</i>	Co 32636	JX991242	JX991199	JX991217
<i>K. flaccidum</i>	Co 32221	JX991243	JX991200	JX991218
<i>Metalcyonium verseveldti</i>	Co 33097	GU355978	GU356012	JX991219



**Table 2.** List of species of Octocorallia of the order Alcyonacea (Lamouroux, 1812), their occurrence in the Penghu Archipelago with inventory numbers of the Zoological Museum, Tel Aviv University (ZMTAU Co) and Center for Biodiversity (NCB), Naturalis, Leiden (RMNH). Field notes include abundance estimates with numbers of sites where species were collected (in brackets) and an indication of respective underwater photographs when available

Classification
<p>Family Alcyoniidae Lamouroux, 1812  <i>Aldersladum</i> Benayahu &amp; McFadden, 2011  <i>Aldersladum jengi</i> Benayahu &amp; McFadden, 2011            Occurrence: ZMTAU Co 33607, holotype, Penghu, Gu-Po Reef (23°42.859'N, 119°33.488'E); 2-8 m, 29 July 2006.            Field notes: Rare (1 site) (Benayahu and McFadden 2010: fig. 6b).            Genus <i>Cladiella</i> Gray, 1869  <i>Cladiella australis</i> (Macfadyen, 1936)            Occurrence: ZMTAU Co 34640, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009; ZMTAU Co 33613, Co 33622, Co 33633, Co 33635, Co 33636, Co 33637, Co 33639, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 33576, Co 33596, Co 33580, Co 33591, Co 33603, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006; ZMTAU Co 33656, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34666, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July 2009; ZMTAU Co 34740, Penghu, Ton-Pan (23°30.674'N, 119°30.822'E), 5-7 m, 31 July 2009; ZMTAU Co 34629, Penghu, San-Sui (23°30.573'N, 119°36.569'E), 7-11 m, 27 July, 2009; ZMTAU Co 34656, Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009.            Field notes: Common (8 sites) (Fig. 2A), mostly as aggregations (Fig. 2B).  <i>Cladiella bottai</i> (Tixier-Durivault, 1948)            Occurrence: ZMTAU Co 34648, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009.            Field notes: Rare (1 site).  <i>Cladiella krempfi</i> Hickson, 1919            Occurrence: ZMTAU Co 33592, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006.            Field notes: Rare (1 site) (Fig. 2C).  <i>Cladiella latissima</i> (Tixier-Durivault, 1948)            Occurrence: ZMTAU Co 34638, Penghu, San-Sui (23°30.573'N, 119°36.569'E), 7-11 m, 27 July 2009; ZMTAU Co 33606, Penghu, Gu-Po (23°42.859'N, 119°33.488'E), 2-8 m, 29 July 2006; ZMTAU Co 33578, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006.            Field notes: Sporadic (3 sites).  <i>Cladiella pachyclados</i> (Klunzinger, 1877)            Occurrence: ZMTAU Co 33604, Penghu, Gu-Po (23°42.859'N, 119°33.488'E), 2-8 m, 29 July 2006.            Field notes: Rare (1 site).  <i>Cladiella papillosa</i> (Tixier-Durivault, 1948)            Occurrence: ZMTAU Co 34734, Penghu, Hu-Gin 2 (23°29.808'N, 119°32.641'E), 15-21 m, 31 July 2009.            Field notes: Rare (1 site).  <i>Cladiella tuberculoides</i> (Tixier-Durivault, 1948)            Occurrence: ZMTAU Co 34642, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009; ZMTAU Co 34685, Co 34686, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009.            Field notes: Rare (2 sites).  <i>Cladiella tuberosa</i> (Tixier-Durivault, 1948)            Occurrence: ZMTAU Co 34669, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July, 2009.            Field notes: Rare (1 site).            Genus <i>Klyxum</i> Alderslade, 2000  <i>Klyxum utinomii</i> (Verseveldt, 1971)            Occurrence: ZMTAU Co 34672, Co 34673, Co 34674, Penghu, Da-Chiaw (23°48.651'N, 119°35.458'E), 8-10 m, 29 July, 2009; ZMTAU Co 34708, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009; ZMTAU Co 33581, Co 33583, Co 33587, Co 33588, Co 33600, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006; ZMTAU Co 34690, Co 34692 Penghu, Tieh-Jen (23°42.824'N, 119°34.049'E), 7-9 m, 29 July 2009; ZMTAU Co 33612, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 34680, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009; ZMTAU Co 34636, Penghu, San-Sui (23°30.573'N, 119°36.569'E), 7-11 m, 27 July 2009; ZMTAU Co 34722, Penghu, Hu-Gin 1 (23°28.994'N, 119°31.163'E), 12-16 m, 31 July 2009; ZMTAU Co 34737, Penghu, Hu-Gin 2 (23°29.808'N, 119°32.641'E), 15-21 m, 31 July 2009; ZMTAU Co 34639, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009.            Field notes: Abundant (10 sites), occasionally as patches comprised of numerous colonies (Fig. 2D, E).            Genus <i>Lobophytum</i> von Marenzeller, 1886</p>

**Table 2.** (continued)

## Classification

*Lobophytum batarum* Moser, 1919

Occurrence: ZMTAU Co 34645, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009.

Field notes: Rare (1 site).

*Lobophytum crassum* von Marenzeller, 1886

Occurrence: ZMTAU Co 33646, Co 33652, Co 33667, Co 33672, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34657, Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009; ZMTAU Co 34716, Penghu, Chiang-Jun (23°22.604'N, 119°31.533'E), 6-8 m, 30 July 2009.

Field notes: Sporadic (3 sites), growing as solitary colonies (Fig. 2F) or aggregations.

*Lobophytum hsiehi* Benayahu & Ofwegen, 2011

Occurrence: ZMTAU Co 33629 holotype and 4 microscope slides, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; paratypes: ZMTAU Co 33579, Co 33582, Co 33593, Co 33594, Co 33599, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006; ZMTAU Co 33605, Penghu, Gu-Po (23°42.859'N, 119°33.488'E), 2-8 m, 29 July 2006; ZMTAU Co 33609, Co 33620, Co 33628, Co 33638, Co 33641, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 33671, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34628, Co 34634, Penghu, San-Sui (23°30.573'N, 119°36.569'E), 7-11 m, 27 July 2009; ZMTAU Co 34687, Co 34676, Co 34679, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009; ZMTAU Co 34688, Penghu, Tieh-Jen (23°42.824'N, 119°34.049'E), 7-9 m, 29 July 2009; ZMTAU Co 34694, Penghu, Wan-An (23°20.913'N, 119°30.753'E), 8-10 m, 30 July 2009; ZMTAU Co 34663, Co 34670, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July 2009; ZMTAU Co 34709, Co 34711, Co 34715, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009; ZMTAU Co 34717, RMNH Coel 39920, Co 34726, Penghu, Hu-Gin 1 (23°28.994'N, 119°31.163'E), 12-16 m, 31 July 2009; ZMTAU Co 34687, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009; ZMTAU Co 34688, Penghu, Tieh-Jen (23°42.824'N, 119°34.049'E), 7-9 m, 29 July 2009.

Field notes: Dominant (11 sites) mostly forming either large colonies (Fig. 3A) or aggregations (Benayahu and Ofwegen 2011: fig. 4).

*Lobophytum sarcophytoides* Moser, 1919

Occurrence: ZMTAU Co 33610, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006.

Field notes: Rare (1 site).

Genus *Sarcophyton* Lesson, 1834*Sarcophyton ehrenbergi* von Marenzeller, 1886

Occurrence: ZMTAU Co 34702, 34705, Penghu, Wan-An (23°20.913'N, 119°30.753'E), 8-10 m, 30 July 2009; ZMTAU Co 34664, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July 2009.

Field notes: Rare (2 sites) (Fig. 3B).

*Sarcophyton tortuosum* Tixier-Durivault, 1946

Occurrence: ZMTAU Co 34684 Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009.

Field notes: Rare (1 site) (Fig. 3C).

*Sarcophyton trocheliophorum* von Marenzeller, 1886

Occurrence: ZMTAU Co 33616, Co 33621, Co 33625, Co 33634, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 33659, Co 33669, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34660, Co 34662 Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009; ZMTAU Co 34641, Co 34644, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009.

Field notes: Sporadic (4 sites) (Fig. 3D).

*Sarcophyton tumulosum* Benayahu & Ofwegen, 2009

Occurrence: ZMTAU Co 34710, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009; ZMTAU Co 34735, Penghu, Hu Gin 2 (23°29.808'N, 119°32.641'E), 15-21 m, 31 July 2009; ZMTAU Co 34698, Co 34703, Wan-An (23°20.913'N, 119°30.753'E), 8-10 m, 30 July 2009; ZMTAU Co 33644, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.

Field notes: Sporadic (4 sites), growing in patches (Fig. 3E).

Genus *Sinularia* May, 1898*Sinularia abrupta* Tixier-Durivault, 1970

Occurrence: ZMTAU Co 33623, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 33666, Co 33670, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.

Field notes: Rare (2 sites).

*Sinularia acuta* Manuputty & Ofwegen, 2007

Occurrence: ZMTAU Co 33617, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006.

Field notes: Rare (1 site).

*Sinularia daii* Benayahu & Ofwegen, 2011

Occurrence: ZMTAU Co 34665, holotype and 4 microscope slides, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July 2009.

Field notes: Rare (1 site) (Benayahu and Ofwegen 2011: fig. 8).

*Sinularia erecta* Tixier-Durivault, 1945

**Table 2.** (continued)

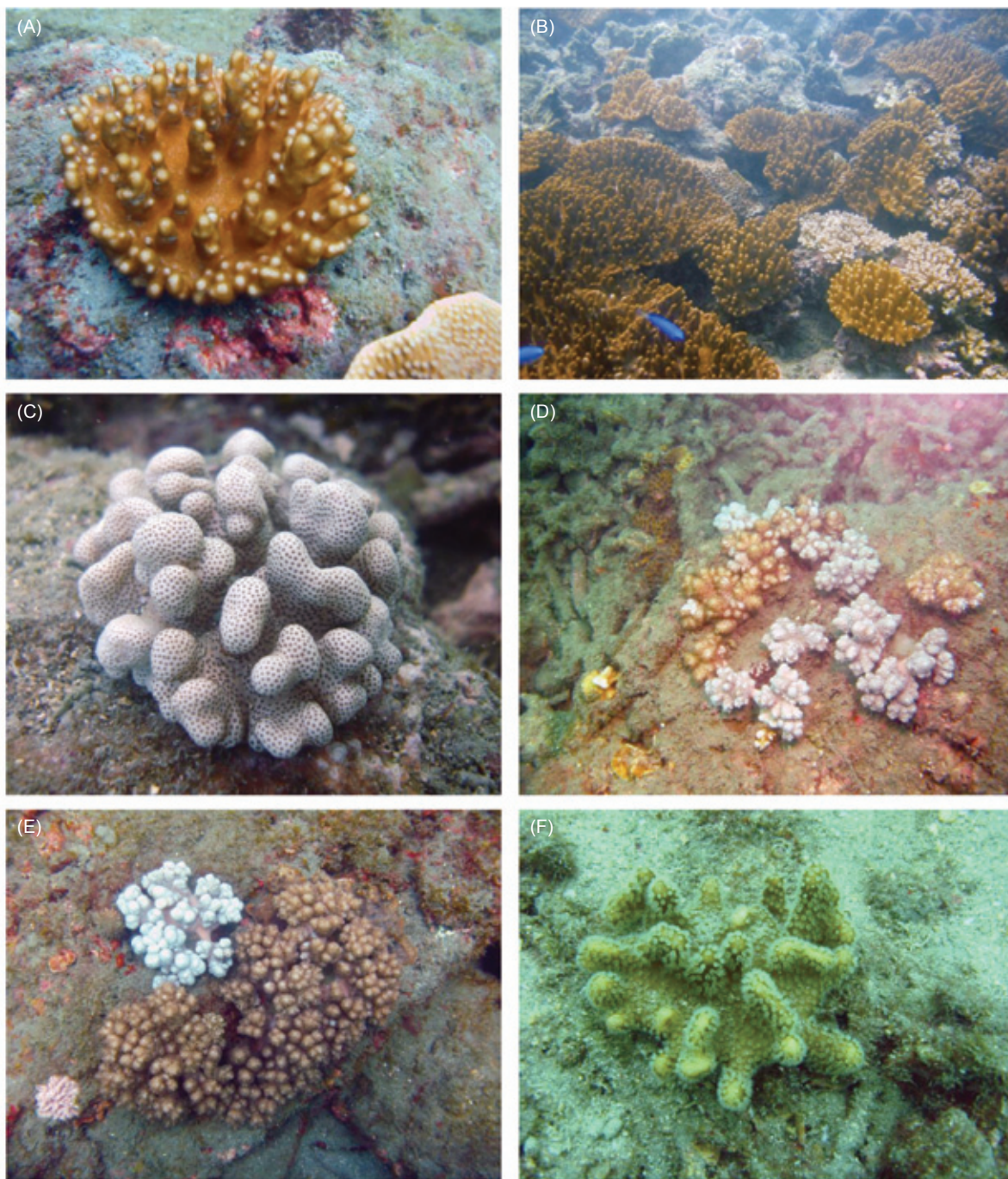
Classification
<p>Occurrence: ZMTAU Co 33585, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006; ZMTAU Co 33655, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (2 sites) (Fig. 3F).</p> <p><i>Sinularia gibberosa</i> Tixier-Durivault, 1970</p> <p>Occurrence: ZMTAU Co 33611, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (1 site).</p> <p><i>Sinularia hirta</i> (Pratt, 1903)</p> <p>Occurrence: ZMTAU Co 34511, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (1 site).</p> <p><i>Sinularia lochmodes</i> Kolonko, 1926</p> <p>Occurrence: ZMTAU Co 33648, Co 33651, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (1 site) (Fig. 4A).</p> <p><i>Sinularia maxima</i> Verseveldt, 1971</p> <p>Occurrence: ZMTAU Co 34510, Co 34514, Co 33653, Co 34654, Co 33660, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (1 site).</p> <p><i>Sinularia molesta</i> Tixier-Durivault, 1970</p> <p>Occurrence: ZMTAU Co 33657, Co 33665, Co 33661, Co 33663, Co 33664, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU 34671, Penghu, Si-Gee (23°15.223'N, 119°36.680'E), 6-8 m, 28 July 2009; ZMTAU Co 33615, Co 33624, Co 33626, Co 33640, Co 33642, Co 33631, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 34649, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009; ZMTAU Co 34697, Co 34699, Penghu, Wan-An (23°20.913'N, 119°30.753'E), 8-10 m, 30 July 2009; ZMTAU Co 34655, Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009.</p> <p>Field notes: Dominant (8 sites), forming aggregations (Fig. 4B).</p> <p><i>Sinularia ornata</i> Tixier-Durivault, 1970</p> <p>Occurrence: ZMTAU Co 34651, Co 34646, Co 34647, Co 34650 Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009; ZMTAU Co 34712, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009; ZMTAU Co 33668, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34683, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009.</p> <p>Field notes: Sporadic (4 sites) (Fig. 4C).</p> <p><i>Sinularia pavida</i> Tixier-Durivault, 1970</p> <p>Occurrence: Co 33630, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; ZMTAU Co 33645, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (2 sites).</p> <p><i>Sinularia peculiaris</i> Tixier-Durivault, 1970</p> <p>Occurrence: ZMTAU Co 33614, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006; Co 34509, Penghu, Chi-Mei (23°11.762'N, 119°25.989'E), 2-8 m, 30 July 2006; ZMTAU Co 34513, Co 33584, Co 33601, Penghu, Chu-Dra (24°41.437'N, 119°38.941'E), 2-8 m, 29 July 2006; ZMTAU Co 34707, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009.</p> <p>Field notes: Sporadic (4 sites) (Fig. 4D).</p> <p><i>Sinularia penghuensis</i> Ofwegen &amp; Benayahu, 2012</p> <p>Occurrence: ZMTAU Co 34706, holotype, Penghu, Hou-Dai-Dzai (23°21.683'N, 119°32.366'E), 6-7 m, 30 July 2009; paratypes: ZMTAU Co 34630, 4 colonies, RMNH Coel. 40123, 2 colonies Penghu, San-Sui (23°30.573'N, 119°36.569'E), 7-11 m, 27 July 2009; ZMTAU Co 34643, 1 colony, Penghu, Ai-Men (23°32.685'N, 119°38.158'E), 7-11 m, 27 July 2009; ZMTAU Co 34659, Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009; ZMTAU Co 34739, Penghu, Ton-Pan (23°30.674'N, 119°30.822'E), 5-7 m, 31 July 2009; ZMTAU Co 34681, Penghu, Mu-Do (23°48.079'N, 119°35.474'E), 7-9 m, 29 July 2009.</p> <p>Field notes: Abundant (6 sites), occasionally growing as small aggregates (Ofwegen and Benayahu 2012: fig. 5).</p> <p><i>Sinularia slieringsi</i> Ofwegen &amp; Vennam, 1994</p> <p>Occurrence: ZMTAU Co 34654, 1 specimen broken into 2 pieces, Penghu, Tong-Gee (23°15.273'N, 119°39.891'E), 6-8 m, 28 July 2009.</p> <p>Field notes: Rare (1 site) (Ofwegen and Benayahu 2012: fig. 16).</p> <p><i>Sinularia soongi</i> Benayahu &amp; Ofwegen, 2011</p> <p>Occurrence: ZMTAU Co 33632, holotype and 4 microscope slides, Penghu, Yu-Pin (23°16.536'N, 119°30.154'E), 2-8 m, 30 July 2006.</p> <p>Field notes: Rare (1 site).</p> <p><i>Sinularia wanannensis</i> Ofwegen &amp; Benayahu, 2012</p> <p>Occurrence: ZMTAU Co 34695, holotype, Penghu, Wan-An (23°20.913'N, 119°30.753'E), 8-10 m, 30 July 2009; paratypes: ZMTAU Co 35311, 2 colonies; RMNH Coel. 40124, 2 colonies; ZMTAU Co 34700, 7 colonies; ZMTAU Co 34704, 3 colonies, same data as for holotype.</p> <p>Field notes: Sporadic (1 site) (Ofwegen and Benayahu 2012: fig. 9).</p>



and Benayahu 2012). Table 2 includes collection details of specimens and their abundances, and it refers to underwater photographs of the respective species, when available.

The collection also included ~35 colonies that were tentatively assigned to the genera

*Carijoa* (Fig. 4E), *Dendronephthya* (Fig. 4F), *Nephthea* (Fig. 5A), *Scleronephthya* (Fig. 5B), and *Siphonogorgia* with a likely addition of 6-8 species to the list. Similarly, it is estimated that the ~30 *Cladiella* and *Klyxum* (e.g., Fig. 5C, D) colonies not yet identified may yield 1 or 2 additional species.



**Fig. 2.** Underwater photographs of Penghu Archipelago octocorals. (A) *Cladiella australis* (Macfadyen, 1936) with polyps retracted; (B) colony aggregation of *C. australis* on the reef; (C) *C. krempfi* Hickson, 1919; (D, E) Aggregation of *Klyxum utinomii* (Verseveldt, 1971), some with expanded polyps and others with retracted polyps (gray colonies); (F) *Lobophytum crassum* von Marenzeller, 1886.



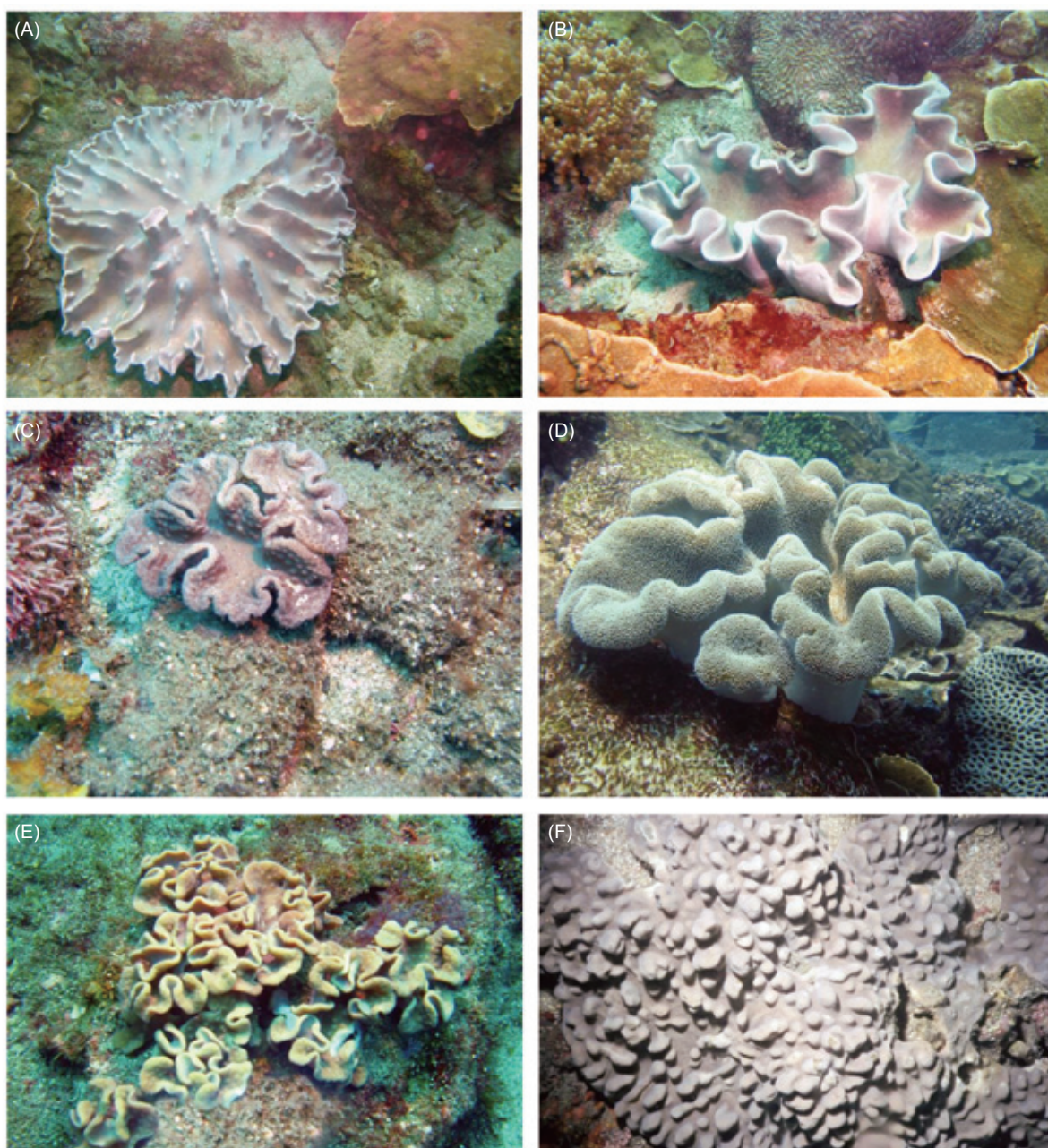
Table 3 presents the numbers of colonies, genera, and species obtained from the different collection sites in the Penghu Archipelago (Fig. 1). Although the current study does not provide quantitative community structure data, it does indicate that the northern collection sites featured a lower number of genera and species compared to most of the south-central and southern ones. It should be noted that among the latter, the

southernmost sites, Chi-Mei and Yu-Pin, were the most diverse (14 and 12 species, respectively).

### Genetic diversity and molecular identifications

#### Genera *Aldersladum*, *Cladiella*, and *Klyxum*

Species belonging to the genera *Aldersladum*, *Cladiella*, and *Klyxum* from Penghu were divided



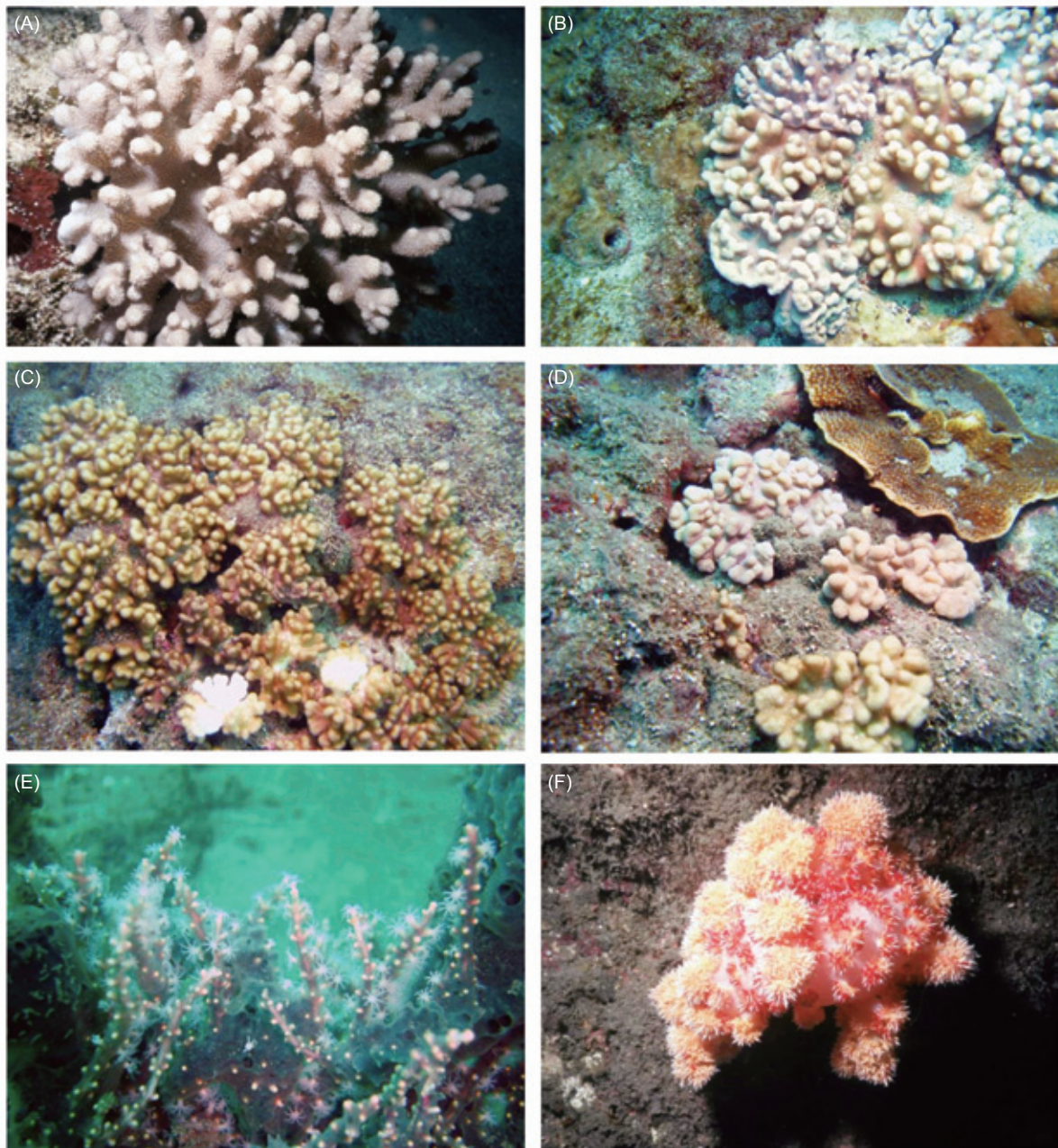
**Fig. 3.** Underwater photographs of Penghu Archipelago octocorals. (A) *Lobophytum hsiehi* Benayahu & Ofwegen, 2011; (B) *Sarcophyton ehrenbergi* von Marenzeller, 1886; (C) *Sar. tortuosum* Tixier-Durivault, 1946; (D) *Sar. trocheliophorum* von Marenzeller, 1886; (E) *Sar. tumulosum* Benayahu & Ofwegen, 2009; (F) *Sinularia erecta* Tixier-Durivault, 1945.



between 2 well-defined, genetically divergent clades that differed by a mean genetic distance (uncorrected  $p$ ) of 3.5% ( $COI + mtMutS$ ) (Fig. 6). The *Klyxum* clade included all species of that genus and 2 species of *Cladiella*: *C. kashmani* and *C. australis*. The *Cladiella* clade included all other species of *Cladiella* and both species of the recently described genus *Aldersladum*; there was no significant phylogenetic separation of those 2 morphologically distinct genera. *Metalcyonium*

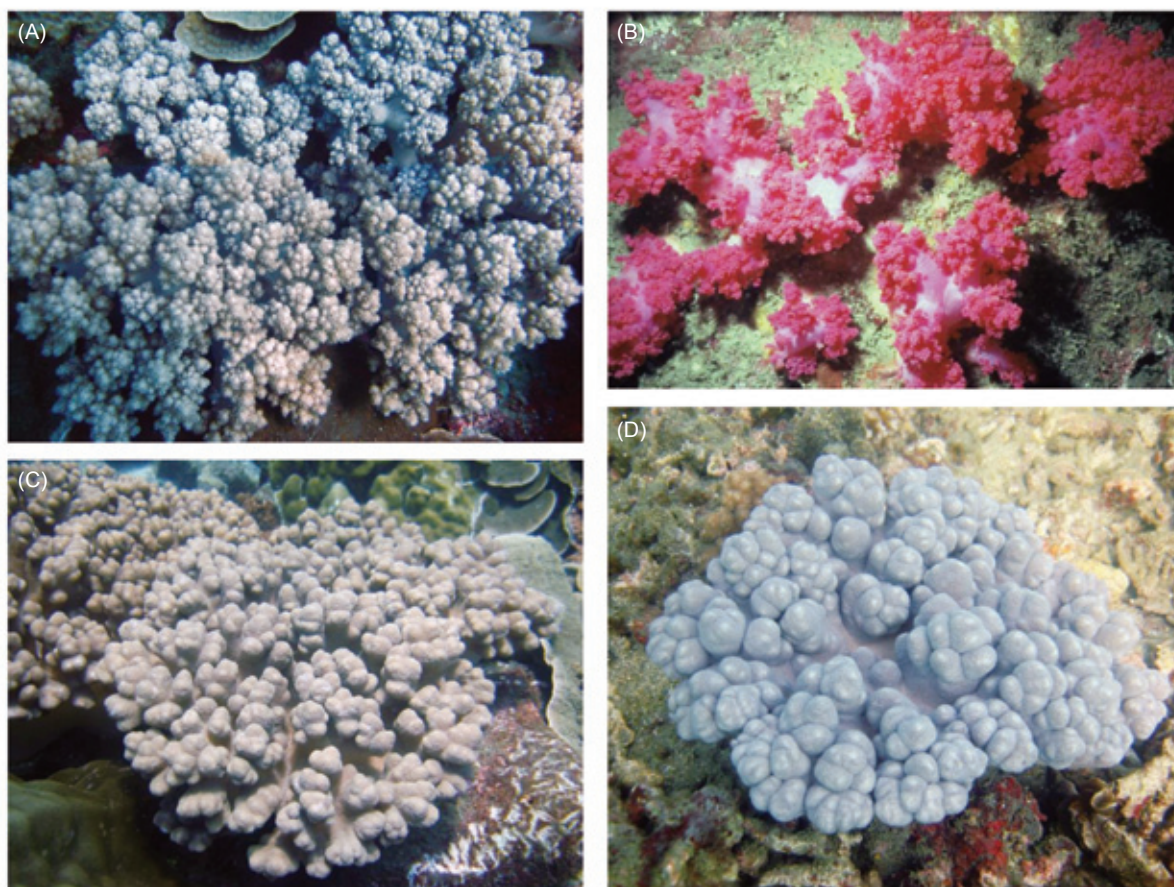
*verseveldti* Benayahu, 1982 is the sister taxon to the *Klyxum* clade, but was well separated from all other genera.

Within each of the 2 clades, mitochondrial genes provided little or no resolution among species. Species in the *Klyxum* clade all shared identical *mtMutS* haplotypes, and differed from one another by  $< 0.2\%$  (uncorrected  $p$ ) at  $COI$ ; the maximum genetic distance among species in the *Cladiella* clade was only 0.4%. The addition



**Fig. 4.** Underwater photographs of Penghu Archipelago octocorals. (A) *Sinularia lochmodes* Kolonko, 1926; (B) *Sin. molesta* Tixier-Durivault, 1970; (C) *Sin. ornata* Tixier-Durivault, 1970; (D) *Sin. peculiaris* Tixier-Durivault, 1970; (E) *Carijoa* sp.; (F) *Dendronephthya* sp.





**Fig. 5.** Underwater photographs of Penghu Archipelago octocorals. (A) *Nephthea* sp.; (B) *Scleronephthya* sp.; (C) *Klyxum* sp. with expanded polyyps; (D) *Klyxum* sp. with retracted polyyps.

**Table 3.** Numbers of colonies, genera, and species obtained at Penghu collection sites also in relation to the region. In brackets, number of collected specimens in each site; \*, cases when the number of genera exceeded the number of species due to difficulties in identifying specimens to the specific level

Collection site	Region	# of genera	# of species
Da-Chiaw	Northern part (4)	2	1*
Mu-Do	" (12)	5	6
Tieh-Jen	" (5)	3	3
Gu-Po	" (5)	3	4
Chu-Dra	Eastern part (36)	4	8
San-Sui	Central/ southern part (19)	7	6*
Ai-Men	Central/ southern part (13)	5	9
Hu-Gin 1	Southern part (11)	5	2*
Hu-Gin 2	" (11)	5	3*
Ton-Pan	" (7)	5	2*
Wan-Ann	" (14)	5	5
Hou-Dai-Dzai	" (10)	5	6
Chiang-Junn	" (1)	1	1
Tong-Gee	" (10)	6	6
Si-Gee	" (9)	6	6
Chi-Mei	" (35)	6	14
Yu-Pin	" (35)	6	12

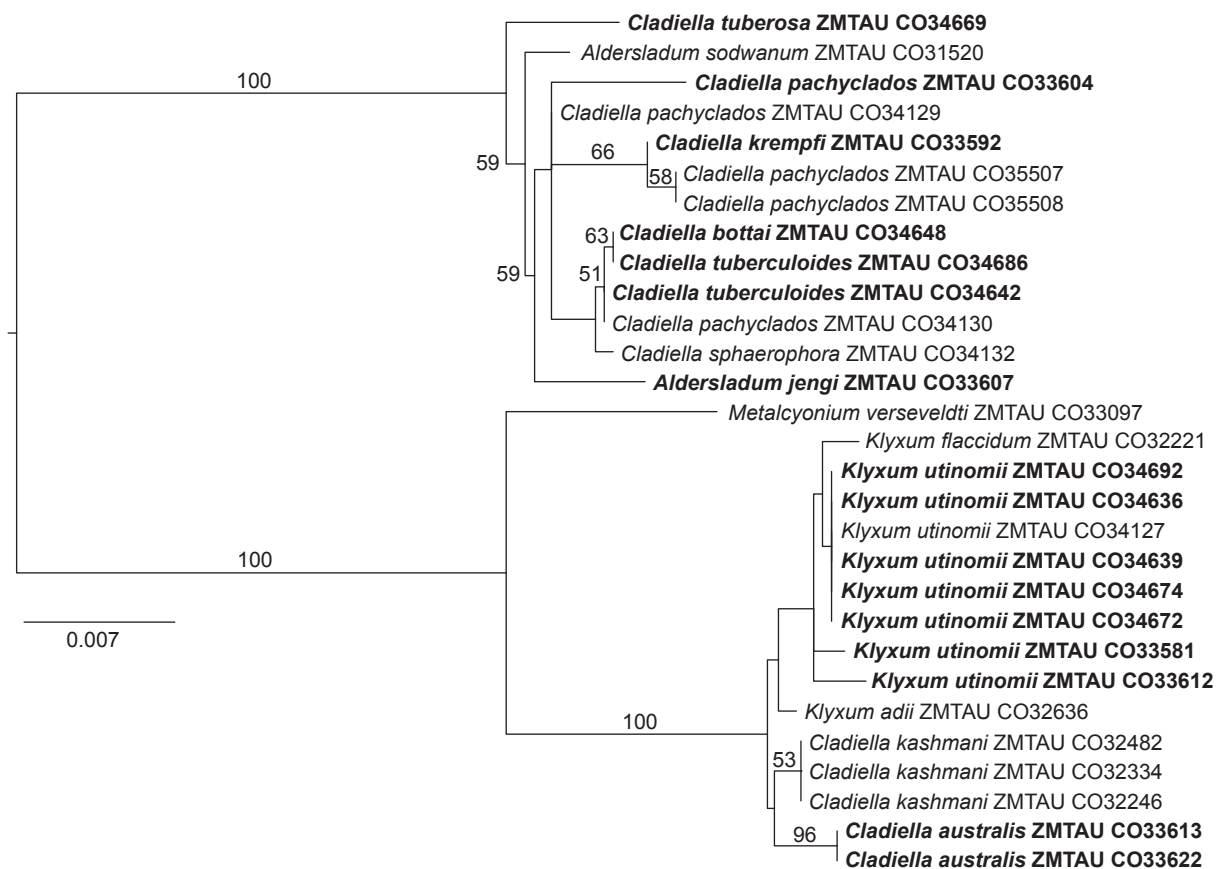
of 28S rDNA provided better resolution among species of *Cladiella* and *Klyxum* than did the mitochondrial genes alone (Fig. 6). Among the *Cladiella* species from Penghu, *C. tuberosa*, *C. krempfi*, and *A. jengi* were each genetically distinct from the other species (Fig. 6). *Cladiella bottai* and *C. tuberculoides*, however, shared haplotypes with one another and with a specimen of *C. pachyclados* from the Red Sea (Co 34130). Other specimens of *C. pachyclados*, including Co 33604 from Penghu, all differed from one another genetically, and were distributed throughout the clade (Fig. 6). We obtained no DNA sequences for *C. latissima* or *C. papillosa*.

Within the *Klyxum* clade, 2 specimens of *C. australis* from Penghu were genetically identical to one another. Although this species shared *mtMutS* and *COI* haplotypes with *C. kashmani* Benayahu & Schleyer, 1996, the 2 species were distinct at 28S (uncorrected  $p = 0.9\%$ ). The 7 specimens of *K. utinomii* had identical *mtMutS* haplotypes, but slightly differed at *COI* (uncorrected  $p = 0\%-0.1\%$ )

and 28S (uncorrected  $p = 0\%-0.9\%$ ). At least 1 specimen from Penghu (Co 34639) was identical at all 3 loci to a reference specimen of *K. utinomii* from Eilat, northern Red Sea (Co 34127).

### Genera *Sarcophyton* and *Lobophytum*

Species belonging to the genera *Sarcophyton* and *Lobophytum* were divided among 3 distinct clades, consisting of *Sarcophyton* only, *Lobophytum* only, and a “mixed” clade that included representatives of both genera (McFadden et al. 2006). Two species from Penghu, *Sar. tortuosum* and *Sar. trocheliophorum*, belonged to the *Sarcophyton* clade. The 6 specimens of *Sar. trocheliophorum* that were sequenced were genetically identical to one another and to representatives of that species from other regions of the Indo-Pacific; *Sar. tortuosum* Co 34684 was genetically close (uncorrected  $p = 0.1\%$ ) to a reference specimen of that species, NTM C14129 (McFadden et al. 2006) (Fig. 7). We only obtained

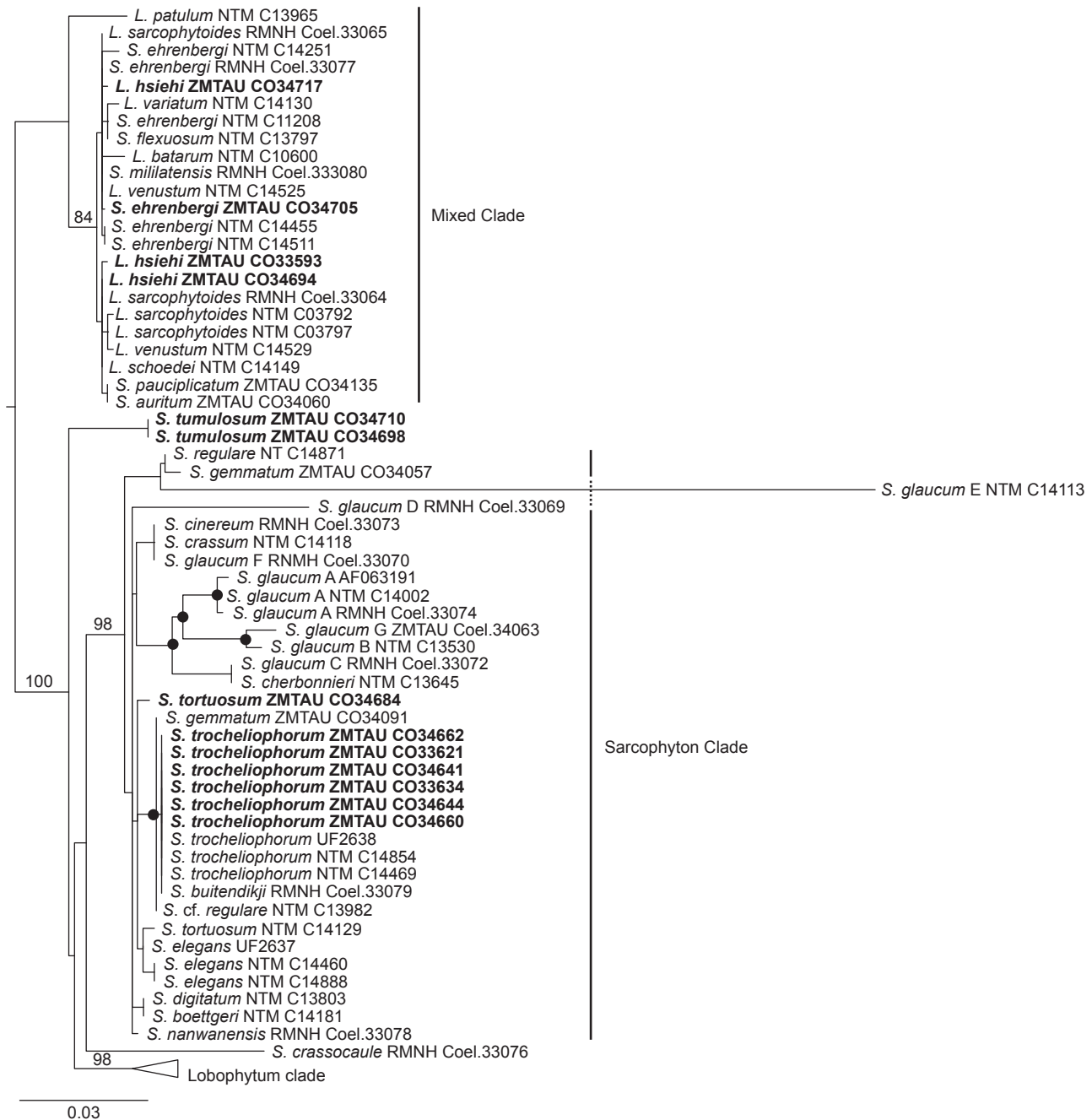


**Fig. 6.** Maximum-likelihood trees of *Cladiella* and *Klyxum* based on (A) combined analysis of *mtMutS* and *COI*, and (B) combined, partitioned analysis of *mtMutS*, *COI*, and 28S rDNA sequences. Boldface, specimens from the Penghu Archipelago. Numbers above the branches are bootstrap percentages. Trees are midpoint-rooted.



a *COI* sequence for *L. crassum* from Penghu; this sequence and results of previous work placed this species in the *Lobophytum* clade (McFadden et al. 2006). Because *COI* data were unavailable for the majority of reference species in that clade, we did not include *L. crassum* in our phylogenetic analysis (Fig. 7).

*Sarcophyton ehrenbergi* and *L. hsiehi* both belonged to the mixed clade. All of the species within this clade were very genetically similar to each other, and neither of the species from the current collection could safely be separated from one another or from other species in the clade based on *mtMutS* or *COI* sequences alone.



**Fig. 7.** Maximum-likelihood tree of *Sarcophyton* and *Lobophytum* based on a combined analysis of *mtMutS* and *COI* sequences. Boldface, specimens from the Penghu Archipelago. Clade labels correspond to clades discussed in McFadden et al. (2006); the *Lobophytum* clade was collapsed to improve readability. The tree is rooted to *Sinularia* (outgroup taxa not shown). Numbers above the branches are bootstrap percentages.

Within this clade, interspecific genetic distances (uncorrected  $p$  values) ranged 0%-1.0%, with a median of 0.3%, which is identical to the median intraspecific distance. Among specimens of *L. hsiehi*, intraspecific genetic distances were 0.1%-0.4%; between *Sar. ehrenbergi* from Penghu and other *Sar. ehrenbergi* specimens included in figure 7, they ranged 0.1%-0.5%. We did not obtain sequence data for *L. batarum* or *L. sarcophytoides*, but both of these species are known from previous work to also belong to the mixed clade (McFadden et al. 2006).

*Sarcophyton tumulosum* was genetically distinct from all other species of *Sarcophyton* or *Lobophytum*, and lay phylogenetically intermediate to the mixed clade and the 2 other clades (Fig. 7). Mean genetic distances between this species and members of the mixed and *Sarcophyton* clades were respectively 6.0% and 4.7%, comparable to the mean distances separating the 3 main clades (McFadden et al. 2006).

### Genus *Sinularia*

All of the *Sinularia* species from Penghu for which sequences were obtained belonged to just four of the 10 subclades previously defined for this speciose genus (McFadden et al. 2009). *Sinularia soongi* was the most genetically distinct of the Penghu Archipelago species, and belonged to clade 2 (Fig. 8). Nine species (*Sin. abrupta*, *Sin. acuta*, *Sin. daii*, *Sin. erecta*, *Sin. lochmodes*, *Sin. molesta*, *Sin. penghuensis*, *Sin. slieringsi*, and *Sin. wanannensis*) belonged to clade 5C, which was morphologically characterized by the presence of "leptocladus-type" sclerites. There was little to no genetic differentiation among many of the species in this clade; interspecific genetic distances ranged 0%-1.6% with a median value of 0.4%, significantly overlapping intraspecific values that ranged 0%-1.2% with a median of 0.1%. Within this genetically homogeneous clade, specimens of *Sin. acuta*, *Sin. abrupta*, and *Sin. erecta* from Penghu were identical or nearly identical (uncorrected  $p = 0.1%$ ) to reference specimens of those species. *Sinularia molesta* Co 33615 differed by 0.5% at *mtMutS* from another specimen identified as that same species (RMNH Coel. 38440; McFadden et al. 2009). No reference specimens were available for any of the other clade 5C species from Penghu, but all five of those species were genetically distinct from each other and from all other species in the clade (Fig. 8). Three specimens of *Sin. wanannensis* were genetically

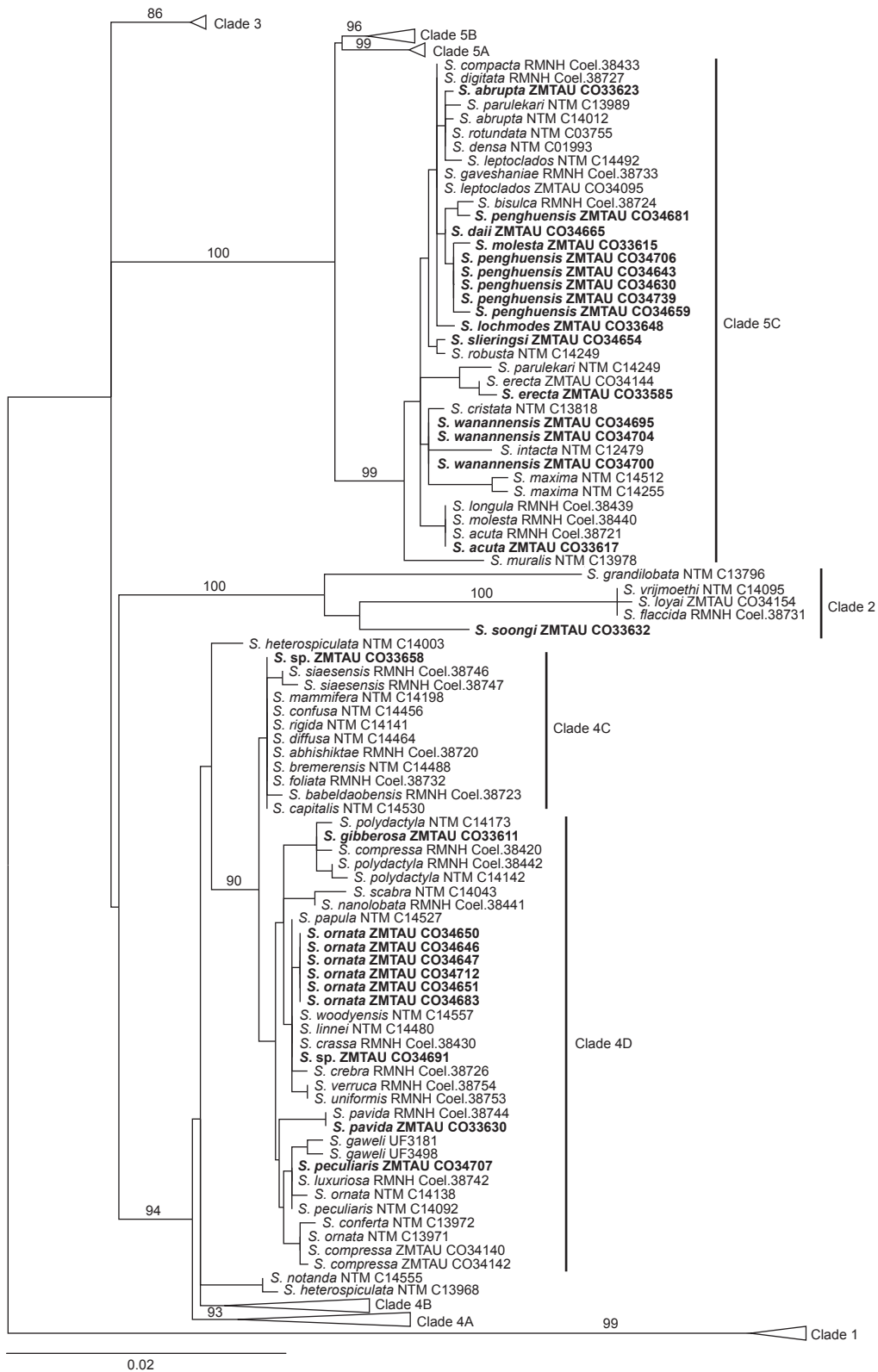
identical to each other; among the 6 specimens of *Sin. penghuensis* we sequenced, 4 were identical to each other, and the other 2 (Co 34659 and Co 34681) differed from them by genetic distances of 0.3% and 0.4%. We did not obtain sequences for *Sin. hirta* or *Sin. maxima* from Penghu. Based on previous work (McFadden et al. 2009), *Sin. maxima* also belonged to clade 5C, but was genetically quite distinct from all other species in that clade. *Sinularia hirta* belonged to clade 5B, to which no other species from the current collection belonged.

The remaining *Sinularia* species from the Penghu Archipelago (*Sin. gibberosa*, *Sin. ornata*, *Sin. pavidata*, *Sin. peculiaris*, and 2 unidentified species, Co 33658 and Co 34691), fell into clade 4C/D (McFadden et al. 2009). Similar to clade 5C, there was little genetic differentiation among species in this clade; the median interspecific genetic distance among species was 0.7%, while the median intraspecific distance was 0.4%, and many morphospecies shared identical *mtMutS* haplotypes (Fig. 8). The 6 specimens of *Sin. ornata* we sequenced were genetically identical to each other, but distinct (uncorrected  $p = 0.5\%-0.7%$ ) from 2 reference specimens of that species (NTM C14138 and NTM C13971; McFadden et al. 2009) that also differed from one another by 0.4%. *Sinularia gibberosa*, *Sin. pavidata*, and *Sin. peculiaris* differed from *Sin. ornata* and from one another by  $> 0.5%$  at *mtMutS* and 0.1% at *COI*. *Sinularia pavidata* Co 33630 and *Sin. peculiaris* Co 34707 each shared identical *mtMutS* haplotypes with reference specimens of those species (Fig. 8). The 2 unidentified *Sinularia* species (Co 33658 and Co 34691) differed from one another and from the other Penghu species, but each shared identical *mtMutS* haplotypes with as many as 8 other species in the clade (Fig. 8).

## DISCUSSION

### Discrimination and identification of Penghu octocorals using DNA barcoding

Identification of specimens to the species level poses a significant challenge in regard to all of the genera of the family Alcyoniidae recorded so far from the Penghu Archipelago. Considerable taxonomic training and expertise are required to separate species based on sclerite morphology, and the need for generic revisions often prevents names from being unequivocally assigned to



**Fig. 8.** Maximum-likelihood tree of the genus *Sinularia* based on a combined analysis of *mtMutS* and *COI* sequences. Boldface, specimens from the Penghu Archipelago. Clade numbers correspond to McFadden et al. (2009). Clades and subclades with no Taiwanese representatives were collapsed to improve readability. The tree is rooted to *Sarcophyton* and *Lobophytum* (outgroup taxa not shown). Numbers above the branches are bootstrap percentages.

specimens (Fabricius and Alderslade 2001). In addition, studies of these genera using molecular markers have now revealed a number of cases of cryptic species that cannot as yet be separated reliably using morphological characters (McFadden et al. 2006 2009 2011). Species-specific molecular markers, so-called DNA “barcodes”, are being used with considerable success to identify species in many such taxonomically difficult marine groups (Bucklin et al. 2010). Development of barcode markers sufficiently variable to reliably discriminate species has, however, been problematic in the anthozoan cnidarians, due to their unusually slow rates of mitochondrial gene evolution (Shearer et al. 2002, Hellberg 2006, Huang et al. 2008, Chen et al. 2009). Neither *mtMutS* nor *COI*, the 2 mitochondrial markers that have been most widely used so far as DNA barcodes for octocorals, are variable enough to discriminate all species pairs (McFadden et al. 2011). These genetic markers do, however, effectively discriminate genera and distinct clades within genera, and can narrow species identities to one of a few likely candidates (McFadden et al. 2011).

Among the octocoral genera we collected at Penghu, *mtMutS* and *COI* effectively identified specimens to distinct clades, and so facilitated initial steps of identifying species. The ability to unequivocally identify a specimen to species level is thus dependent on the genetic diversity of the clade to which it belongs. The 4 species of *Sarcophyton* found at the archipelago could each easily be identified using *mtMutS*: *Sar. tortuosum* and *Sar. trocheliophorum* were genetically distinct from one another within the *Sarcophyton* clade; *Sar. ehrenbergi* belonged to the mixed clade; and *Sar. tumulosum* was genetically well separated from any other species of *Sarcophyton* or *Lobophytum*. Among *Lobophytum* species; however, 3 species (*L. batarum*, *L. hsiehi*, and *L. sarcophytoides*) all belonged to the genetically homogeneous mixed clade, and could not reliably be separated from each other using the *mtMutS* or *COI* barcodes.

Likewise, *mtMutS* and *COI* sequences clearly separated the 2 distinct clades that encompassed the genera *Aldersladum*, *Cladiella*, and *Klyxum*. Within each of these 2 clades, however, neither mitochondrial marker significantly varied; the slightly more-variable 28S rDNA discriminated species in these genera somewhat more reliably. *Klyxum utinomii* and *C. australis*, both of which belong to the *Klyxum* clade, were easily distinguished using 28S, despite some intraspecific

variation in *K. utinomii*. In the *Cladiella* clade, *C. krempfi*, *C. tuberosa*, and *A. jengi* were all genetically distinct from each other and from other species reported from Penghu, but *C. bottai* and *C. tuberculoides* were not separable from one another. Specimens identified as *C. pachyclados* were genetically diverse, and it is possible that this species represents yet another cryptic species complex. The specimen from Penghu (Co 33604) particularly differed from those from other geographic regions, but was also distinct from all other *Cladiella* species found in the current study.

*Sinularia* is the most speciose genus of alcyoniid octocorals found in the Penghu Archipelago and throughout the Indo-Pacific in general (Ofwegen 2002, McFadden et al. 2009). Although this large genus is divided among a number of genetically well-defined clades, within each clade, numerous species often share *mtMutS* haplotypes (McFadden et al. 2009). Among those species reported from the archipelago, *Sin. soongi* (clade 2) and *Sin. hirta* (clade 5B) could easily be genetically distinguished from all other species, and the 4 species belonging to clade 4D (*Sin. gibberosa*, *Sin. ornata*, *Sin. pavidata*, and *Sin. peculiaris*) were also clearly separable from one another and from species in clade 5C. Within clade 5C; however, the large number of species that shared the same or similar *mtMutS* haplotypes made species identity difficult to confirm. The 9 Penghu species belonging to clade 5C each had unique *mtMutS*+*COI* haplotypes; but in most cases, they differed by no more than a single substitution, which is within the range of the observed intraspecific variation. Although there is evidence for some intraspecific variation in *Sin. abrupta*, *Sin. erecta*, *Sin. molesta*, and *Sin. penghuensis*, the full range of such variation is known for no species. Additional sampling and sequencing of these species should, however, reveal the limits to intraspecific variations and lead to refinement of our abilities to accurately assign species identities based on mitochondrial haplotypes.

### Phylogenetic relationship between *Cladiella* and *Klyxum*

Previous molecular systematic studies examined inter- and intrageneric relationships among *Lobophytum*, *Sarcophyton*, and *Sinularia*, leading to recognition of distinct genetic clades that define those genera (McFadden et al. 2006 2009). The present study, however, represents the



1st such molecular systematic work on *Cladiella* and *Klyxum*, and highlights a clade structure that crosses the morphological boundaries between these 2 genera. Morphological distinctions between *Cladiella* and *Klyxum* are clear and well-defined: *Klyxum* has sclerites in the form of spindles in the colony interior and flat rods in the polyps, while *Cladiella* is characterized by dumbbell-shaped sclerites on the colony surface and interior and minute disks or figure-eights in the polyps (Fabricius and Alderslade 2001). The recently described genus, *Aldersladum*, lacks both spindles and dumbbells, and has only minute figure-eights throughout the colony (Benayahu and McFadden 2011).

Our molecular data (Fig. 6) suggested that morphological distinctions among these genera are not congruent with the phylogenetic history of the group. Two species of *Cladiella* belonged to the genetically distinct clade that otherwise included all species of *Klyxum* (although the number of species of *Klyxum* included here is small, many additional specimens identified to this genus also belong to this clade (unpubl. data)). The other distinct clade included both *Cladiella* and *Aldersladum*. Although preliminary molecular work on the relationship between those 2 genera suggested a phylogenetic distinction between them (Benayahu and McFadden 2011), the inclusion of additional *Cladiella* species in the present analysis blurred that distinction. Comprehensive revisions of *Cladiella* and *Klyxum* are long overdue, and will be necessary if we are to reconcile discrepancies between the observed morphological differences and molecular phylogenetic relationships among all 3 genera.

### Diversity and biogeography of octocorals in Taiwan

A final estimate of the total species inventory of octocorals in the Penghu Archipelago cannot yet be made, due to a lack of appropriate taxonomic revisions of certain genera of the families Alcyoniidae (*Cladiella* and *Klyxum*), Clavulariidae (*Carijoa*), and Nephtheidae (*Dendronephthya*, *Nephthea*, and *Scleronephthya*) (see “Results”). It is estimated; however, that the current collection will yield ~44 species, once the remaining material has been identified. Notably, no species of the octocoral families Briareidae, Helioporidae, Tubiporidae, or Xenidae were recorded at Penghu, despite being present in southern Taiwan and at Green Is. (Benayahu et al. 2004). These findings

further suggest that the Penghu Archipelago represents marginal non-reefal communities at the edge of the geographic distribution of tropical octocorals (see also Chen et al. 2005, Wei et al. 2012). The subtropical conditions prevailing in the archipelago (Chen et al. 2005) may limit the northernmost distribution of certain reef-dwelling octocorals in the East China Sea. Nonetheless, the current findings indicate that the major reef-dwelling genera of the family Alcyoniidae (Table 1) are able to withstand these conditions, and these genera contribute considerably to the live coverage on the reefs.

A comparison of the species diversity of alcyoniid octocorals between Nanwan Bay (southern Taiwan) (Benayahu et al. 2004) and the Penghu Archipelago (Table 4) reveals a higher number of species in the former region (48 vs. 34). To date, species of *Eleutherobia* and *Paraminabea* have not been recorded at Penghu, nor *Aldersladum* in Nanwan Bay. The difference between the 2 regions is further reflected in the relatively low number of *Sinularia* species at Penghu compared to southern-Taiwan reefs (16 vs. 30). *Sinularia* is considered the most speciose tropical alcyoniid octocoral genus (Ofwegen 2002, McFadden et al. 2009). The Penghu Archipelago collection yielded 4 new *Sinularia* species (Table 1; *Sin. daii*, *Sin. soongi*, *Sin. penghuensis*, and *Sin. wanannensis*) that comprise a substantial portion of the species inventory of that genus there; in addition, 2 as yet unidentified specimens (Co 33658 and Co 34691) may also represent new species. The 2 regions share in common 3 *Sinularia* species (*Sin. erecta*, *Sin. gibberosa*, and *Sin. lochmodes*), whereas another 7 (*Sin. abrupta*, *Sin. acuta*, *Sin. molesta*, *Sin. ornata*, *Sin.*

**Table 4.** Generic affiliation of species of the family Alcyoniidae from Penghu Archipelago and Nanwan Bay (southern Taiwan)

Genus	Penghu Archipelago	Nanwan Bay
<i>Aldersladum</i>	1	-
<i>Cladiella</i>	8	4
<i>Eleutherobia</i>	-	1
<i>Klyxum</i>	1	1
<i>Lobophytum</i>	4	4
<i>Paraminabea</i>	-	1
<i>Sarcophyton</i>	4	7
<i>Sinularia</i>	16	30
Total	34	48



*pavida*, *Sin. peculiaris*, and *Sin. slieringsi*) have not been recorded in southern Taiwan. A similar distributional pattern was revealed for *Cladiella*, with only *C. australis* and *C. pachyclados* found in both regions and another 6 *Cladiella* species only at Penghu. Among the 4 *Lobophytum* species, *L. crassum* and *L. sarcophytoides* were found in both regions, whereas *L. batarum* and *L. hsiehi* were only present in the Penghu Archipelago. Regarding *Sarcophyton*, with the recent finding of *Sar. tumulosum* in Nanwan Bay (ZMTAU Co 34596: Benayahu unpubl.), 3 species have now been recorded in both regions (Table 4, Benayahu et al. 2004) with *Sar. tortuosum* only present at Penghu. Therefore, it is evident that the Penghu Archipelago octocoral fauna includes 15 octocoral species not yet recorded on the more-diverse reefs of southern Taiwan that are considered to be new records for the region. The tropical Kuroshio Current originates in the northern Philippines and enters the East China Sea from the east side of Taiwan, although a branch of the Kuroshio goes through the Taiwan Strait in warmer seasons of the year (references in Chen 1999). This current, in combination with the effect of seawater temperatures, was shown to determine distributions of hermatypic corals in the region. Our findings suggest that these environmental conditions similarly affect octocoral distributions, which are characterized by a lower diversity in the Penghu Archipelago compared to southern-Taiwan reefs (Table 4).

The relatively low octocoral diversity of the Penghu Archipelago compared to reefs of southern Taiwan is likely a result of geography. Examination of the numbers of taxa found at the different collection sites in Penghu (Fig. 1) revealed a decrease in octocoral diversity from south to north (Table 3). This pattern corresponds to the attenuation of diversity noted between southern-Taiwan reefs and the more-northerly Penghu ones (Table 4). The Penghu Archipelago has a record of severe chilling events, the latest being during the winter of 2007-2008, with water temperatures dropped to 11.73°C (Hsieh 2008). The 1st survey of the current study was conducted prior to that event (2006) and the subsequent survey 1 yr after its occurrence (2009). Collections for both years were not carried out at the same reef-sites, and therefore, the effects of those extreme low temperatures on octocorals could not be quantitatively assessed. However, in both years, the northern collection sites (Da-Ciaw, Mu-Do, and Tieh-Jen) featured a high abundance of

*Klyxum* (Fig. 5C, D), which was the most prevalent octocoral there. Furthermore, sites where mass mortality of stony corals was evident in 2009 still retained quite-diverse octocoral assemblages. These findings imply that at least certain alcyoniid octocorals are able to withstand such unprecedented low-temperature conditions. Their detailed reactions to such severe chilling events nonetheless remain to be studied; and the Penghu Archipelago offers an appropriate system for such studies.

This 1st ever survey of octocorals of the Penghu Archipelago has already yielded 6 new species (Benayahu and McFadden 2011, Benayahu and Ofwegen 2011, Ofwegen and Benayahu 2012), and 15 new records of species not recorded on reefs of southern Taiwan. These results highlight the importance of such surveys in the region which undoubtedly will continue to elucidate patterns of biodiversity and zoogeography in the East and South China Seas, including their marginal reef systems. Application of molecular barcoding to such biodiversity studies will facilitate the identification of taxa in groups such as the Octocorallia, in which morphological traits are difficult to assess and taxonomic experts are few. In addition to flagging new and previously unrecorded species (Bucklin et al. 2010), molecular barcoding allows direct comparisons of biodiversity to be made between regions, even in the absence of confirmed species identifications. The combination of classical taxonomy with molecular approaches benefited this study, and is recommended for similar future studies.

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