

Pilot Survey of Corallimorpharian (Cnidaria) Diversity in the Gulf of Thailand

Bancha Nilkerd^{1,2*}, Akarapong Swatdipong³, Jintana Salaenoi¹
and Suriyan Tunkijjanukij¹

ABSTRACT

Corallimorpharians, or coral-like sea anemones, are globally distributed throughout the coral reef ecosystems. Their occurrence within Thai waters, however, has been less studied and information relating to their taxonomy is highly required. The present study aims to conduct a taxonomical survey of corallimorpharians along the Gulf of Thailand. Both morphology and nucleotide sequence information were used for identification. Extensive surveys were conducted from November 2010 to December 2011, during which corallimorpharian samples were collected through a series of scuba dives. Three genera of the family Discosomatidae were found. *Rhodactis* sp. was the most commonly encountered at nearly all sites, occupying both coral reefs and rocky habitats. *Rhodactis* sp. samples were notably dominant in all the sampling locations within Chuang Island (Chonburi). *Metarhodactis* sp. samples were found at Kut Island (Trat), Samet Island (Rayong) and Ngam Noi Island (Chumphon), typically on shallow coral reefs at depths of 3-10 meters. The single species of *Amplexidiscus*, *A. fenestrafer* Dunn and Hamner, 1980, was found at a small number of sites, including Chuang Island (Chonburi), Rang Island, and Kut Island (both in Trat). Nucleotide sequence of cytochrome *c* oxidase subunit I (COI) and small subunit ribosomal RNA (12S rRNA) genes, however, permits ready genus discrimination and identification which is feasibly informative in the taxonomical classification of other corallimorpharians.

Keywords: Corallimorpharian, mitochondrial sequence, mushroom anemone, diversity

INTRODUCTION

Corallimorpharians are marine invertebrates belonging to the phylum Cnidaria. They are morphologically similar to stony corals (Order Scleractinia) (Fautin *et al.*,

2002; Daly *et al.*, 2003; Medina *et al.*, 2006). Due to the lack of calcified skeleton, they are thus mostly akin to sea anemones (Order Actiniaria). Corallimorpharians are globally distributed throughout the marine ecosystems, from tropical to polar regions in both shallow

¹ Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok, Bangkok 10900 Thailand

² Faculty of Marine Technology, Burapa University, Chantaburi, Thailand

³ Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Bangkok 10900 Thailand

* Corresponding author, email: cnilkerd@hotmail.com

and deep waters (Fautin *et al.*, 2002). The corallimorpharian *Nectactis singularis* Gravier, 1918, for example, has been found at a depth of 5,005 meters (Gravier, 1922). Corallimorpharians may dominate benthic communities in both temperate (Chadwick, 1991) and notably tropical regions (den Hartog, 1980; Chen *et al.*, 1996) where they are found in abundance on coral reefs in shallow waters of depths up to 65 meters (den Hartog, 1980; Chadwick-Furman and Spiegel, 2000; Muhando *et al.*, 2002).

Asexual reproduction can be found in corallimorpharians, and its populations can increase in relatively short periods of time (Chadwick and Adams, 1991). They are arguably better adapted than stony corals in that they can endure a range of physical stresses, including exposure to air during low tides (Chadwick, 1991; Muhando *et al.*, 2002). Some species of corallimorpharian are aggressive by extruding mesenterial filaments through the tips of their discal tentacles and mouth. These filaments can be also used for self-defense when disturbed (Elliot and Cook, 1989; Fautin and Mariscal, 1991).

Whilst the morphological features characterizing each genus are distinct, these are likely insufficient to separate species within each genus to any degree of certainty. Corallimorpharians have been, however, surveyed and their morphological taxonomy has been attempted. *Rhodactis indosinensis* Carlgren, 1943, and *Rhodactis inchoata* Carlgren, 1943, were reported in Cambodia and Vietnam, respectively (Carlgren, 1943). *Pseudocorynactis* sp. den Hartog, 1980 and *Discosoma nummiformis* Rüppell and

Leuckart, 1828 were reported in the Gulf of Thailand (Carlgren, 1943; Putchakarn *et al.*, 1997; Dokkaew and Pichitkul, 2006). Also *Corynactis globulifera* Hemprich and Ehrenberg, 1834 was reported at Kut Island, Gulf of Thailand (Carlgren, 1943). The indigenous corallimorpharian fauna in Thailand, however, remain largely unknown.

The present study, therefore, aimed to conduct a taxonomical survey of corallimorpharians in the Gulf of Thailand, using both morphological and nucleotide sequence investigations.

MATERIALS AND METHODS

Field methods

For the current study, a number of locations along the eastern and western coasts of the Gulf of Thailand were surveyed over the period of November 2010 to December 2011 (Figure 1). The sampling locations along the eastern coasts were the Chang Islands, Kut Island, Nomsao Island, Man Islands, Talu Island, Samet Islands, Chuang Island, Chan Island, Samaesan Island, and Lan Islands. The sampling locations along the western coasts were Ngam Noi Island, Ang Thong Islands and Pha Chalarm Island (Table 1). At each site, corallimorpharian samples were collected during scuba dives. The distribution of corallimorpharians was recorded by visual estimates during each survey, typically covering an approximately 100 meters stretch along each site. Findings were supported by photographs taken with a Canon G11 camera and the measurement of the on-site environmental parameters

Table 1. Locations and distinguishable corallimorpharians surveyed in the study

Location	Station / Province	Code	Survey station	Morphotype
Eastern coasts of the Gulf of Thailand	Kut Island, Trat	Kut	11°38'4.86"N 102°32'33.39"E	2, 4 & 16
			11°40'20.66"N 102°36'11.15"E	2, 3, 4, 17, 18, 19, 20, 21, 22, 23, 24, 26, 27 & 28
			11°45'33.52"N 102°33'25.94"E	2 & 25
	Rang Islands, Trat	Rang	11°48'35.69"N 102°22'39.34"E	16
			11°47'19.96"N 102°23'42.70"E	2 & 3
			11°47'39.78"N 102°23'32.39"E	16
	Chang Islands, Trat	Chang	11°54'21.46"N 102°23'33.50"E	2 & 3
			11°55'32.15"N 102°24'47.74"E	2 & 4
			12° 9'32.06"N 102°14'48.83"E	2 & 4
	Nomsao Island, Chanthaburi	Nom	12°27'36.02"N 102°1'30.07"E	2, 3, 4 & 31
	Man Islands, Rayong	Man	12°36'35.30"N 101°41'10.34"E	3
			12°34'19.88"N 101°42'0.79"E	2, 4 & 31
	Talu Island, Rayong	Talu	12°33'6.31"N 101°34'5.33"E	2, 4, 6, 10, 11, 13, 14 & 31
			12°34'18.73"N 101°34'15.66"E	3, 11, 12, 13, 29 & 31
	Samet Islands, Rayong	Same	12°36'15.20"N 101°30'57.12"E	30 & 31
			12°31'33.86"N 101°26'47.49"E	2, 21, 29 & 31
	Samaesan Island, Chonburi	Sama	12°35'20.90"N 100°56'51.43"E	1 & 7
			12°34'24.94"N 100°56'42.53"E	2 & 31
	Chuang Island, Chonburi	Chu	12°30'30.20"N 100°57'25.70"E	2, 3, 4, 6, 7, 8, 11, 13, 15 & 16
	Chan Island, Chonburi	Chan	12°30'56.52"N 100°58'29.59"E	2, 3, 4 & 8
	Lan Islands, Chonburi	Lan	12°53'52.63"N 100°46'31.39"E	2, 4, 8, 15 & 31
			12°55'40.38"N 100°48'16.93"E	2
			12°56'43.92"N 100°47'27.32"E	2 & 31
Western coasts of the Gulf of Thailand	Ngam Noi Island, Chumphon	Ngam	10°29'11.89"N 99°25'6.23"E	2, 4, 5, 6, 32 & 33
			10°29'4.89"N 99°25'5.07"E	6, 7, 9 & 31
	Wao Island, Suratthani	Wao	9°46'55.80"N 99°40'44.37"E	2, 6, 7, 8, 9 & 31
	Ang Thong Islands, Suratthani	Ang	9°39'56.38"N 99°40'50.50"E	6, 7, 8, 9 & 30
			9°39'54.27"N 99°40'57.68"E	2 & 6
	Pha Chalarm Island, Suratthani	Pha	9°18'49.78"N 99°57'27.67"E	10 & 31

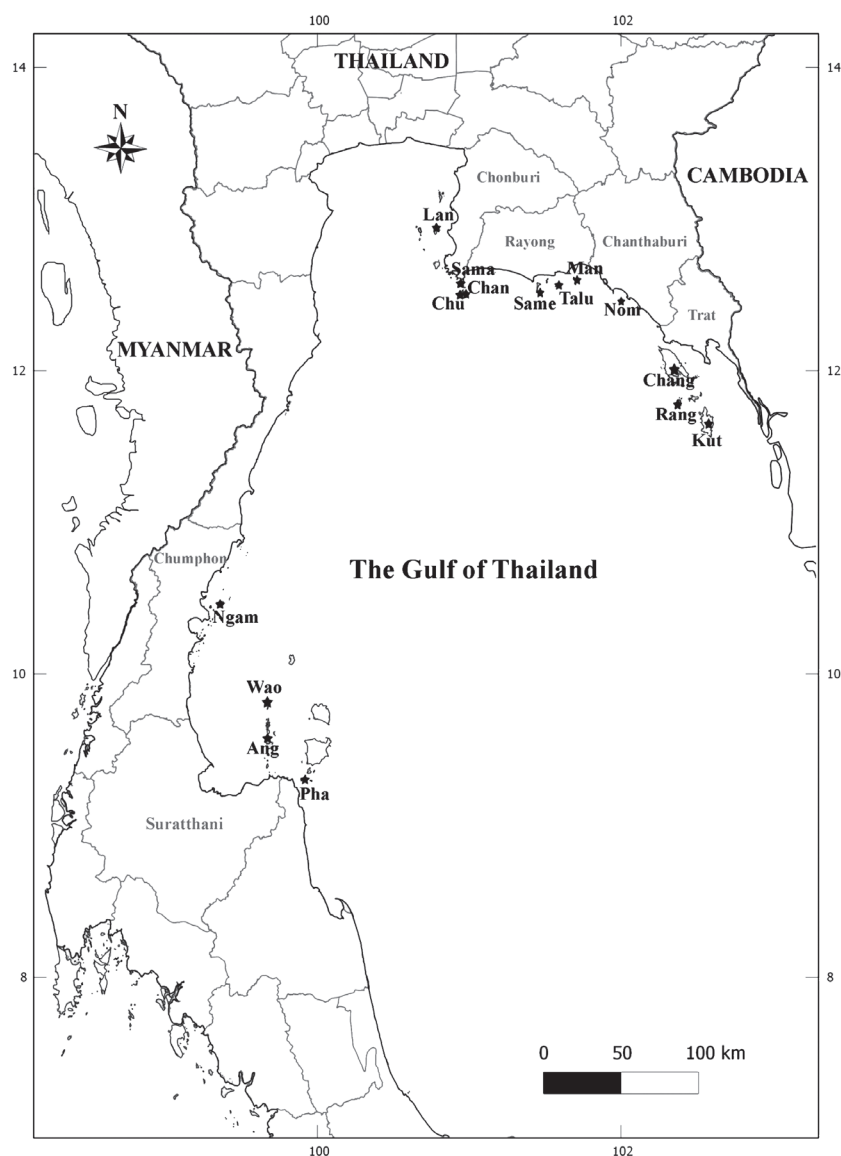


Figure 1. Map of the Gulf of Thailand indicating the locations where corallimorpharians were surveyed

which included substrate type, depth at the sampling point including the depth range. The morphology-based taxonomical identification of samples was based on the shape of discal and marginal tentacles, tentacle-free zones and other related characters (Carlgren, 1949; Cha, 2001).

DNA sequencing

Morphotypes of corallimorpharians were used for sequence analysis upon their preservation in absolute ethanol. Total genomic DNA was achieved using a DNeasy kit (Qiagen, Inc. Valencia, CA) following the

manufacturer's protocol. Molecular analysis was based on the derived mitochondrial DNA sequences for the cytochrome *c* oxidase subunit I (COI) and the small subunit ribosomal RNA (12S rRNA). The two genes were chosen following Barbeitos *et al.* (2010), Fukami *et al.* (2008) and Medina *et al.* (2006). The amplification of the COI gene was performed using the primer set AcMCOIF and AcMCOIR (Fukami *et al.*, 2008) and through the use of new internal forward primer AcMCOintF (5'-AGGGCC AAAACCCAAATWAC-3') designed for this study to get better sequence quality. The amplification profile began with a denaturation step at 95°C for 3 min, followed by 45 cycles of 95°C for 30 sec, 45°C for 30 sec and 72°C for 1 min, ending with an extension step at 72°C for 5 min. The amplification of the 12S rRNA gene was performed using the newly designed primer 12sF (5'-AATTTMTGRTTACAATTGGY TCA-3') and 12sR (5'-TGTTACGACTTA CTYCRYCTCAAA-3'). The amplification profile began with a denaturation step at 95°C for 3 min, followed by 45 cycles of 95 °C for 30 sec, 52 °C for 30 sec and 72 °C for 1 min, ending with an extension step at 72°C for 5 min. The PCR products were purified using a PCR purification kit (Favorgen Biotech Corp.) and then sent to a commercial sequencing service. The derived DNA sequences were checked by comparing to the ABI chromatogram files using BioEdit (Hall, 2007).

Molecular analysis

The partial COI (1583 bp) and 12S rRNA (987-992 bp) sequences obtained from each sample were subsequently used

for molecular analysis and phylogenetic reconstruction. For comparison, DNA sequences of closely related species (*Discosoma* and *Rhodactis*; NC_008072.1 and DQ640647.1, respectively) and the outgroup *Ricordea florida* Duchassaing and Michelotti, 1860 (NC_008159.1) were used when reconstructing phylogenetic trees. It is noted that the COI and 12S rRNA sequences of the genus *Metarhodactis* are unavailable in public database and thus are not used here. Distance estimations (D; Nei and Gojobori, 1986) were obtained for each region separately and in a combination; then analyzed and compared using the neighbor joining (Saitou and Nei, 1987) and maximum likelihood methods, conducted using MEGA 5 (Tamura *et al.*, 2011). The model of nucleotide evolution was chosen based on the Akaike information criterion determined using ModelTest (Posada and Crandall, 1998). The phylogenetic trees were reconstructed based on 1,000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Field observation

Corallimorpharians were found at almost all the surveyed locations along both the eastern and western coasts of the Gulf of Thailand. The collected samples were morphologically identified based on distinguishable characters according to the dichotomous key to the genera (Carlgren, 1949; Cha, 2001). The results were that all the collected samples belonging to the family Discosomatidae Duchassaing and Michelotti, 1864 with representatives from three genera,

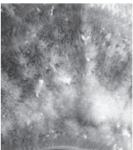
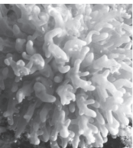
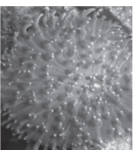
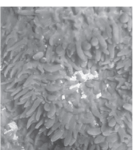
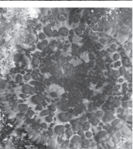
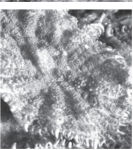
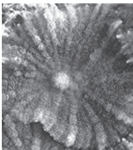
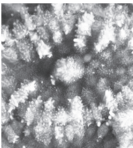
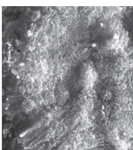
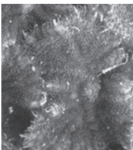
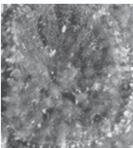
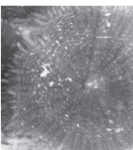
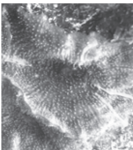
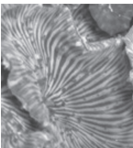
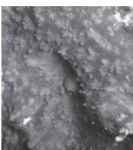

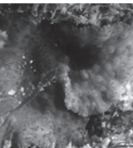
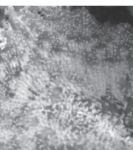
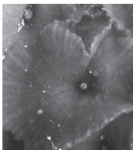
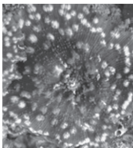
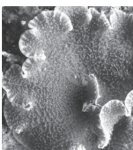
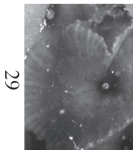
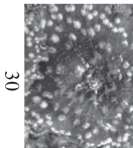
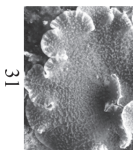
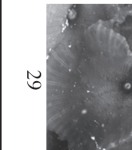
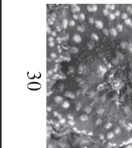
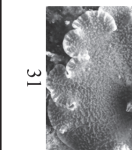
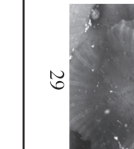
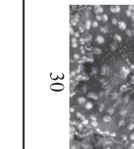
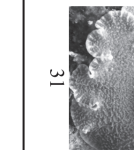
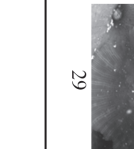
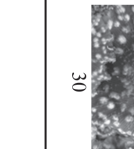
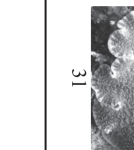
Rhodactis Milne Edwards and Haime, 1851, *Amplexidiscus* Dunn and Hamner, 1980 and *Metarhodactis* Carlgren, 1943.

Morphotypes 1 to 15, as labeled in Table 2, belong to the genus *Rhodactis*. This genus was found at all surveyed sites and widely distributed. Morphotypes 1 to 4 showed obviously long branched discal tentacles (Table 2). Morphotype 1 was found at only a single site, Samaesan Island (Chonburi), at a depth of 12 meters in a channel of rapid water where it used polyps to attach rocky substrate. Morphotype 2 to 4 showed variation in their tentacle tips and disc color. Morphotypes 2 to 4 were commonly encountered throughout the Gulf of Thailand at water depths ranging from 2–20 meters and on a broad range of habitats including live and dead coral reefs, rocky shores and on submerged rocks. These morphotypes were notably abundant at the surveyed site of Chuang Island (Chonburi), where they covered more than 70 % of the habitable area. Morphotype 2 (Table 1) was the most dominant and found at all survey sites. Morphotypes 1 to 15 had obviously long digitiform or marginal tentacles. However, divergent from morphotypes 1 to 4, morphotypes 5 to 15 possessed discal tentacles that were either papilliform or had a reduced degree of branching. These morphotypes were found on a broad range of habitats, similar to those occupied by representative morphotype 2 to 4, and were frequently abundant in the shallow waters of the intertidal to sub-tidal zones at depths of 1–22 meters. Morphotype 6 was found along both the eastern and western coasts of the Gulf of Thailand.

Corallimorpharian morphotype 16 was identified as *Amplexidiscus fenestrafer* based on the tentacle-free zone between the inner and outermost discal tentacles and digitiform marginal and discal tentacles. This was the single species of *Amplexidiscus* found throughout this survey and appeared to be restricted to a number of areas, such as, Chuang Island (Chonburi), Rang Island and Kut Island (Trat). The samples were found attached to hard substrates, i.e. rocks and coral reefs, at depths of 5–25 meters.

Morphotypes 17 to 33 belong to the genus *Metarhodactis*. Species belonging to this genus either lack discal tentacles or possess them in a reduced form, i.e. papilliform or short branches. Similarly, the marginal tentacles are either absent or in a reduced digitiform state. The species of *Metarhodactis* were typically found in association with shallow coral reefs at depths of 3–10 meters, such as, those surveyed at Kut Island (Trat), Samet Island (Rayong) and Ngam Noi Island (Chumphon). The morphotypes 17 to 28 were all encountered together at Kut Island displaying a variety of distinguishable characters in their tentacles, patterning (i.e. striping) and in the color of their oral disc. Representative, morphotypes 32 and 33 were found only at Ngam Noi Island (Chumphon), whilst representative morphotypes 21, 29 and 30 were found in many areas. Of the samples belonging to the genus *Metarhodactis*, representative morphotype 31 was the most dominant and was found distributed in the most of surveyed locations on both the eastern and western coasts of the Gulf of Thailand.

Table 2. Taxa and distinguishable characteristics of the corallimorpharians surveyed in the Gulf of Thailand.

Family/Genus/Species	Morphology /Habitat	Morphotypes with distinguishable characteristics				
Discosomatidae/ <i>Rhodactis</i> (Morphotype 1-15; n=73)	Shape of marginal tentacle: digitiform.					
	Shape of discal tentacles: branched, papilliform					
	Tentacle-free zone on oral disc: No.					
<i>Aplexidiscus fenestrafer</i> (Morphotype 16; n=4)	Coral reef and rocky habitats, shallow water of intertidal to sub-tidal zone at 1-22 metres depth.					
	Shape of marginal tentacle: digitiform.					
	Shape of discal tentacles: digitiform.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Tentacle-free zone on oral disc: Yes (within a field of discal tentacles).					
	Coral reef and rocky habitats, sub-tidal zone at 5-25 metres depth.					
	Shape of marginal tentacle: absent or reduced digitiform (very short).					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
	Coral reef habitats, shallow water of intertidal to sub-tidal zone at 3-10 metres depth.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					
<i>Metarhodactis</i> (Morphotype 17-33; n=31)	Shape of marginal tentacle: absent or reduced digitiform (very short).					
	Shape of discal tentacles: reduced papilliform or branched.					
	Tentacle-free zone on oral disc: No.					

Molecular observation

The neighbor joining and maximum likelihood clustering methods applied to the COI and 12S rRNA sequences, which were based on the best model Jukes-Cantor (JC) plus the proportion of invariable sites (I), reconstructed trees with similar configurations and only a small degree of differentiation in certain branches. The neighbor joining tree displayed a greater number of minor branches and slightly different bootstrap values when compared with the maximum likelihood tree. When the two genes were used together for phylogenetic tree reconstruction, based on the best model JC +I, it revealed that the clustering of genera corresponded with the morphological identification and also with the published sequence information of *Discosoma*, *Rhodactis* and *Ricordea florida* (Ricordeidae), wherein the last was used as the outgroup (Fig. 2). All of the corallimorpharian samples collected during the current Thai survey were clustered within either the genus *Metarhodactis* or *Rhodactis*. Additionally, the combined sequence information revealed that the genetic distance among the *Metarhodactis* samples ($D=0.001$) was smaller than that found among the *Rhodactis* samples ($D=0.004$) while the genetic distance between both genera was 0.021.

DISCUSSION

In the current study, the diversity of corallimorpharians was morphologically identified using a dichotomous key to the genera. *Amplexidiscus* was established based on digitiform marginal tentacles and tentacle-

free zone on oral disc, within morphotype 16. *Metarhodactis* was established based on reduced marginal tentacles or none at all and Hoplotelic *p*-mastigophores present in mesenterial filaments, within morphotype 17-33 and *Rhodactis* was established based on digitiform marginal tentacles and no tentacle-free zone on oral disc, branched discal tentacles, within morphotype 1-15 (Carlgren, 1949; Cha, 2001). The three genera were well identified using either COI, 12S rRNA or combined COI-12S rRNA sequence. However, among corallimorpharians, species composition and the number of valid genera have been unclear due to the inconsistency of name usage (Stephenson, 1922; Carlgren, 1949; den Hartog, 1980; den Hartog *et al.*, 1993; Fautin, 2006). The descriptions of some genera were also very short and difficult to be used for species identification (Cha, 2001). Therefore, samples were only identified to generic level, obstructing the gene sequence analysis in species level, even though some sequence variations and split branches of phylogenetic tree were observed among different corallimorpharian morphotypes. All of the samples were found belonging to three separate genera *Amplexidiscus*, *Metarhodactis* and *Rhodactis* within the family Discosomatidae. The occurrence of these three genera within the Gulf of Thailand supports the findings of an earlier survey conducted by Dokkaew and Pichitkul (2006). However, no representatives belonging to the genus *Pseudocorynactis* or samples of *C. globulifera*, as reported by Carlgren (1943), were found. Note that, the dichotomous key of Cha (2001) uses a morphological character, the presence and absence of hoplotelic *p*-mastigophores in mesenterial filaments, to identify the genus.

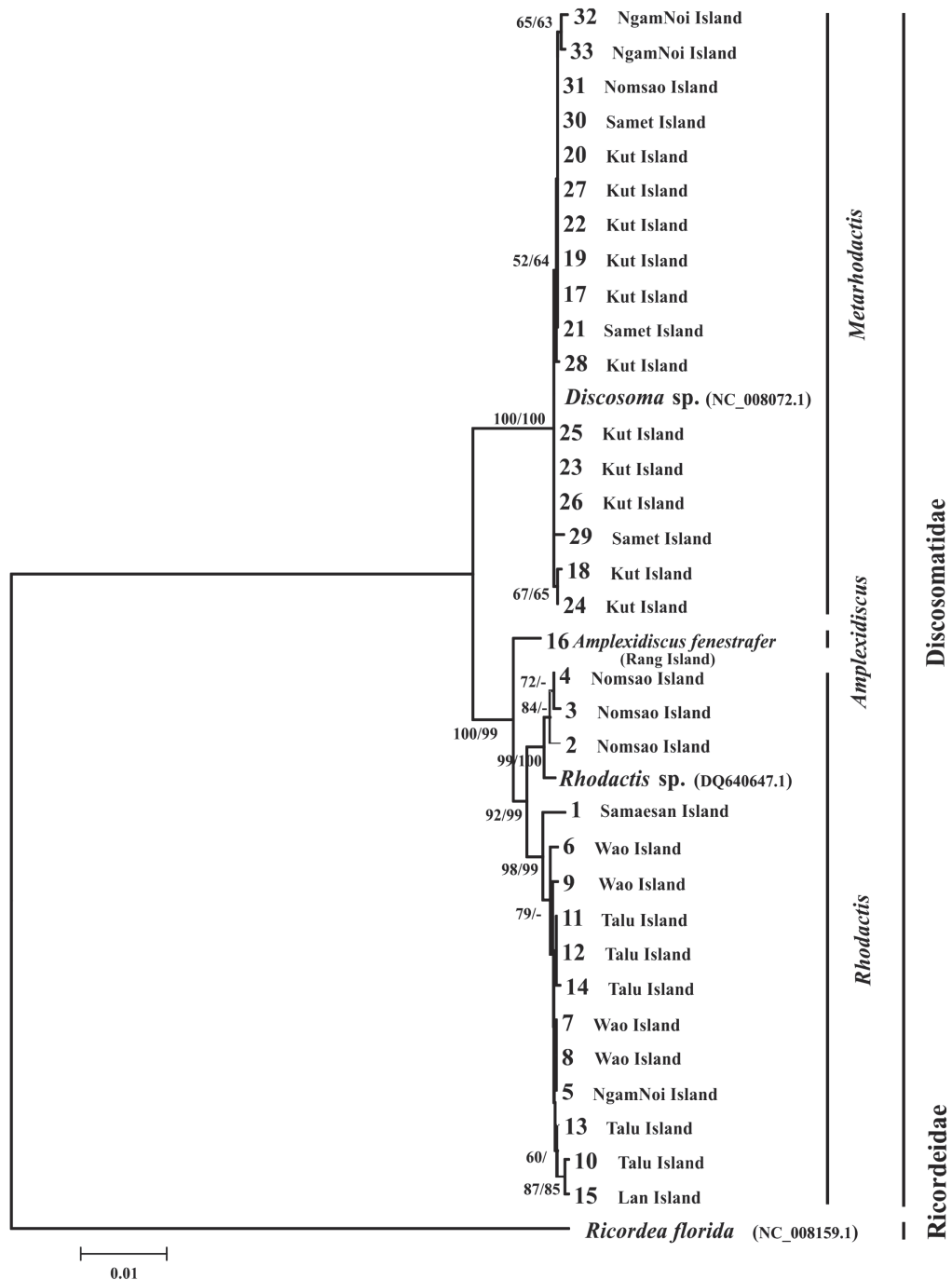


Figure 2. Neighbor joining tree based on the partial COI and 12S rRNA sequences derived from the Corallimorpharians collected within the Gulf of Thailand, as indicated using morphotype numbers and survey stations. The numbers on each branch represent the bootstrap support (values <50 not shown) while ‘-’ indicates incongruence between the neighbor joining and maximum likelihood clustering methods.

Samples displaying morphotype 17 to 33 show the hoplotelic *p*-mastigophores and thus they belong to the genus, *Metarhodactis*. On the other hand, no character information has been documented in the report of an earlier survey conducted by Dokkaew and Pichitkul (2006). Therefore the identification to genus of the earlier survey is hardly confirmable.

Representatives belonging to the genus *Rhodactis* were the most dominant corallimorpharians encountered at all the sites that were surveyed where they frequently had a greater coverage of residential areas than *Amplexidiscus* and *Metarhodactis*. The success of the *Rhodactis* species in covering large areas is due to the morphology that typifies this genus (Muhandó *et al.*, 2002), i.e. the possession of large marginal tentacles and penetrating nematocysts that can disturb and kill surrounding organisms (den Hartog, 1977; Chadwick and Adams, 1991). Marginal tentacles filled with penetrating nematocysts have been reported to have a major role in the competitive success to other cnidarians, including stony corals, for occupying residential areas (Langmead and Chadwick-Furman, 1999). These features may help explain the success and common occurrence of *Rhodactis* species throughout the Gulf of Thailand.

Amplexidiscus fenestrafer, commonly known as the giant cup mushroom, is the largest corallimorpharian. The largest sample found during this study is 40 cm in diameter; the record for this species is 45 cm (Dunn and Hamner, 1980). The genus *Amplexidiscus* has a natural distribution throughout the tropical Indo-Pacific Oceans and although

not commonly encountered, it inhabits the shallow waters of the subtidal zone where they attach to underwater rocks and corals (Dunn and Hamner, 1980). In the current study, this species was found only at Chuang Island, Rang Island and Kut Island, all on the eastern coast of the Gulf of Thailand. Samples of *A. fenestrafer* were also previously reported using underwater photos taken at Tao Island and Phangan Island (Chumphon), the western coasts of the Gulf of Thailand (www.the-diveinn.com; chaloklum-diving.com).

The genus *Metarhodactis* represents one of the largest groups within the Corallimorpharia. The species within the genus *Metarhodactis* possesses fantastic color tentacles and oral discs, arguably more so than any other genus. *Metarhodactis* shows natural behavior and habitat used similar to *Discosoma* which typically attaches to rocks, corals and sponges in shallow waters (den Hartog, 1980). Most of the *Metarhodactis* samples collected in the current study were on the coral reefs at depths of 3–7 meters around Kut Island (morphotype 17-28), Talu Island (morphotype 29), Samet Islands (morphotype 30), Ngam Noi Island (morphotype 32 and 33) and Ang Thong Islands (morphotype 30), except for the species of *Metarhodactis* represented by morphotype 31. The latter was commonly found along both the eastern and western coasts of the Gulf of Thailand. Carlgren (1943) reported finding *D. nummiformis* in the Gulf of Thailand, but they were not found in this study. All the samples collected in the current study were identified until genera and not to the species level, thus the finding of this species could not be confirmed.

The molecular and phylogenetic analyses corresponded with the morphological assignment to the generic level.

The phylogenetic tree reconstructions based on the neighbor joining and maximum likelihood clustering methods gave similar results. The neighbor joining method, however, produced a greater number of branches at the generic level. All three genera of corallimorpharians were separated from the species of *Ricordea* (Family Ricordeidae) that was used as the outgroup. From the trees, the samples within the genus *Rhodactis* clustered closer to those in the genus *Amplexidiscus* than to those of *Metarhodactis*, supporting the suggestions of Cha (2001) and Fukami *et al.* (2008). The samples within the genus *Rhodactis* can be divided into two clades. The first clade comprising morphotype 2–4 and *Rhodactis* sp. (DQ640647.1), all of which had long branched discal tentacles, whilst the second clade which consisted of morphotype 1 and 5–15 had short, reduced branched or papilliform discal tentacles, except morphotype 1 which had morphological characters akin to those in the first clade. The *Metarhodactis* clustering consisted of morphotype 17–33 and *Discosoma* sp. (NC 008072.1), *Metarhodactis* samples were morphologically varied, but DNA information is not very distinct. These observations were supported by the genetic distance indicating that the genetic diversity among *Rhodactis* species ($D=0.004$) was 4-times higher than that for *Metarhodactis* species ($D=0.001$). All the corallimorpharian morphotypes that were analyzed by sequencing, with the exception of morphotype 23 and 26, could be identified and separated based on the derived COI and 12S rRNA sequences. While the

approaches used here were able to speciate the samples readily to the generic level, this study does, however, highlight the need for additional sequences and taxonomy experts to assist in the taxonomical identification of the collected materials to achieve species identification. These ongoing studies may also require the description of additional taxonomical characters.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science and Technology, Thailand and the TRF/BIOTEC Special Program for Biodiversity Research and Training Grant BRT T154001.

LITERATURE CITED

- Barbeitos, M.S., S.L. Romano and H.R. Lasker. 2010. Repeated loss of coloniality and symbiosis in scleractinian corals. **Proc Natl Acad Sci USA** 107: 11877–11882.
- Cha, H. 2001. **Systematics of the order Corallimorpharia (Cnidaria: Anthozoa)**. Department of Ecology and Evolutionary Biology, The University of Kansas, 217 pp.
- Chadwick, N.E. and C. Adams. 1991. Locomotion, asexual reproduction and the killing of corals by the corallimorpharian *Corynactis californica*. In: **Coelenterate biology: recent research on Cnidaria and Ctenophora** (ed. R.B. Williams, P.F.S. Cornelius, G.R. Hughes and A.E. Robson), pp. 263–269. Kluwer Academic Publishers, Dordrech.

- Chadwick, N.E. 1991. Spatial distribution and the effects of competition on some temperate Scleractinia and Corallimorpharia. **Mar Ecol Prog Ser** 70: 39–48.
- Chadwick-Furman, N. E. and M. Spiegel. 2000. Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. **Invertebrate Biology** 119: 351–360.
- Carlgren, O. 1943. East-Asiatic Corallimorpharia and Actiniaria. **Kungliga Svenska Vetenskaps-Akademiens Handlingar** 20: 1–43.
- Carlgren, O. 1949. A survey of the Ptychodactaria, Corallimorpharia and Actiniaria. **Kungliga Svenska Vetenskaps-Akademiens Handlingar** 1: 1–121.
- Chen, C.A., B.L. Willis and D.J. Miller. 1996. Systematic relationships between tropical Corallimorpharians (Cnidaria: Anthozoa: Corallimorpharia): Utility of the 5.8S and internal transcribed spacer (ITS) regions of the rRNA transcription unit. **Bull Mar Scien** 59: 196–208.
- Daly, M., D.G. Fautin and A.V. Cappola. 2003. Systematics of the Hexacorallia (Cnidaria: Anthozoa). **Zool J Linn Soc** 139: 419–437.
- Dunn, D.F. and W.M. Hamner. 1980. *Amplexidiscus fenestrafer* n. gen., n. sp. (Coelenterata: Anthozoa), a tropical Indo-Pacific Corallimorpharian. **Micronesica** 16: 29–36.
- Dokkaew, S. and P. Pichitkul. 2006. A survey of Corallimorpharian (Class Anthozoa : Order Corallimorpharia) in Amphoe Ban Chang, Rayong Province. Faculty of Fisheries, Kasetsart University, 9 pp.
- Elliott, J. and C.B. Cook. 1989. Did variation in prey capture behavior by the Corallimorpharian *Discosoma sanctithomae*: Mechanical and chemical activation of feeding. **Biol. Bull.** 176: 218–228.
- Fautin, D.G. 2006. **Hexacorallians of the world: sea anemones, corals, and their allies**. Available at: <http://hercules.kgs.ku.edu/hexacoral/anemone2/index.cfm> (accessed on 2 January 2013)
- Fautin, D.G. and R.M. Mariscal. 1991. Cnidaria: Anthozoa. In: **Microscopic Anatomy of Invertebrates** (ed. F.W. Harrison and J.A. Westfall), pp. 267–358., Vol. 2. New York: Wiley-Liss.
- Fautin, D.G., T.R. White and K.E. Pearson. 2002. Two new species of deep-water Corallimorpharia (Cnidaria: Anthozoa) from the Northeast Pacific, *Corallimorphus denhartogi* and *C. Pilatus*. **Pac Sci** 56: 113–124.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. **Evolution** 39: 783–791.
- Fukami, H., C.A. Chen., A.F. Budd., A. Collins., C. Wallace., Y-Y. Chuang., C. Chen., C.F. Dai., K. Iwao., C. Sheppard and N. Knowlton. 2008. Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (order Scleractinia, class Anthozoa, phylum Cnidaria). **Plos One** 3: 1–9.
- Gravier, C. 1922. **Résultats des campagnes scientifiques accomplies sur son yacht par Albert Ier Prince Souverain de Monaco publiés sous sa direction avec le concours de M. Jules Richard**. Imprimerie de Monaco. Monaco. 104 pp.

- Hall, T. 2007. **BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP**. Carlsbad, CA: Ibis Biosciences. Available at: <http://www.mbio.ncsu.edu/> (accessed on 13 September 2011)
- den Hartog, J.C. 1977. The marginal tentacles of *Rhodactis sanctithomae* (Corallimorpharia) and the sweeper tentacles of *Montastrea cavernosa* (Scleractinia): Their cnidom and possible function, pp. 1:463-469. In: **Proceeding of the 3rd international coral reef symposium**. Miami. USA.
- den Hartog, J.C. 1980. Caribbean shallow water Corallimorpharia. **Zool Verh** 176: 1-82.
- den Hartog, J.C., O. Ocaña and A. Brito. 1993. Corallimorpharia collected during the CANCAP expeditions (1976-1986) in the south-eastern part of the North Atlantic. **Zool Verh** 282: 3-76.
- Langmead, O. and N.E. Chadwick-Furman. 1999. Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma* 1. Role in competition for space. **Mar Biol** 134: 479-489.
- Medina, M., A.G. Collins., T.L. Takaoka., J.V. Kuehl and J.L. Boore. 2006. Naked corals: Skeleton loss in Scleractinia. **Proc Natl Acad Sci USA** 103: 9096-9100.
- Muhandu, C.A., B.L. Kuguru., G.M. Wagner., N.E. Mbije and M.C. Öhman. 2002. Environmental effects on the distribution of corallimorpharians in Tanzania. **Ambio** 31: 558-561.
- Nei, M. and T. Gojobori. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. **Mol Biol Evol** 3:418-426.
- Posada, D. and K.A. Crandall. 1998. MODELTEST: Testing the model of DNAsubstitution. **Bioinformatics** 14: 817-818.
- Putchakarn, S., S. Munkongsomboon., T. Noiraksar and P. Sonchaeng 1997. **Biodiversity of marine animals in coral reefs along the eastern coast of Thailand (Chonburi Province)**. Institute of Marine Science, Burapha University, 131 pp.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. **Mol Biol Evol** 4: 406-425.
- Stephenson, T.A. 1922. On the classification of Actiniaria. Part III. **Q J Microsc Sci (New Series)** 66: 247-319.
- Tamura, K., D. Peterson., N. Peterson., G. Stecher., M. Nei and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. **Mol Biol Evol** 28: 2731-2739.