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Diversity and Distribution of Jellyfish Polyps Along Coastal Areas of Chonburi and Rayong Provinces, Thailand

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ABSTRACT. – Jellyfish polyps can be difficult to identify based on their morphology due to a lack of precise references in Thailand, yet species identification is an important step for management of this marine resource. Here, we pursued a dual approach, morphology and DNA barcoding, to describe the diversity and distribution of jellyfish polyps in two coastal provinces that have various anthropogenic activities e.g., tourism, marine transportations, industrial estate, local fisheries, and aquacultures, that effect on water qualities or provide substrates for polyp settlement. Jellyfish polyps were collected in January, May, and July 2019 to represent the Northeast Monsoon, pre-Southwest Monsoon, and Southwest Monsoon, respectively, from eight stations along coastal areas from Chonburi and Rayong Provinces, eastern Thailand. The jellyfish polyps were sampled from substrates e.g., rocks, ropes, and shells, at sampling sites by scuba diving. Three genera of polyps were identified according to their morphology, while nine genera of jellyfish polyps were identified by their COI gene sequences from 29 individuals. Polyps of the genus *Clytia* were recorded during the sampling periods at most sampling sites, while the genus *Obelia* was found mainly at Rayong Province, when identified by both morphological and molecular approaches. These results can be used as part of a suitable management plan about jellyfish issues in Thailand.

KEYWORDS: COI, DNA, morphology, polyp, jellyfish

INTRODUCTION

The diversity and distribution of jellyfish in The Gulf of Thailand and The Andaman Sea off the coast of Thailand have received sparse coverage in the literature, however, important work has been done. For instance, the Department of Marine and Coastal Resources has two important summaries including a report on the diversity of jellyfish and occurrences of tourists stung by poisonous jellyfish in Thailand during 2010-2015 (Marine and Coastal Resources Research and Development Institute, 2015a), and a guideline handbook to identify jellyfish in Thailand (Marine and Coastal Resources Research and Development Institute, 2015b). Other studies have been conducted on the diversity and distribution of small jellyfish or hydromedusae in the Upper Gulf of Thailand and the Inner Gulf of Thailand with a total of 63, 31, and 45 species of hydrozoa recorded in 2000, 2012, and 2017, respectively (Wuttichareonmongkol, 2004; Phongphattarawat, 2013; Jitrapat, 2018). A major goal of our work was to extend and enhance these earlier reports.

Recently, jellyfish bloom events, mainly from *Catostylus* sp., have occurred frequently along the eastern coasts of the Gulf of Thailand including Chonburi, Rayong, Chanthaburi, and Trat Provinces (Patithanarak et al., 2014). Similarly, jellyfish bloom events are increasing in various coastal locations and oceans globally during the last decade (Brotz et al., 2012; Condon et al., 2013), examples include Japan (Uye, 2008), China (Dong et al., 2010), Mediterranean and Black Sea (Boero, 2013), and the northern Great Barrier Reef (Gershwin et al., 2014). It is likely that climate change, eutrophication, and anthropogenic activities including overfishing, marine transportation, and habitat modification are triggering mechanisms that induce jellyfish blooms in many regions (Purcell et al., 2007; Richardson et al., 2009; Dong et al., 2010). In particular, about 68% of published papers from 1995 to 2015 claimed that jellyfish bloom is caused by anthropogenic stressors in terms of eutrophication, climate change, overfishing, artificial substrates, and introduction species with the contribution of 25, 25, 23, 13, and 10% of citations, respectively (Pitt et al., 2018).

The jellyfish life cycle includes multiple stages: the medusa stage is the most easily recognized form and is the free-floating form associated with bloom events; medusa reproduce sexually to produce gametes that mature into the planula larvae, which settle on suitable substrates and morph into the sessile polyp stage; after maturation, the sessile polyp produces buds that are released (i.e., a process known as budding or strobilation) that will grow in the water column and become the mature medusa stage. Work reported in this paper focuses on polyp stage larvae for two major reasons. First, the sessile polyp stage of jellyfish plays an important role for ensuring the long-term survival of jellyfish as well as the formation of jellyfish blooms (Lucas et al., 2012). Appropriate conditions are

required for polyp settlement including temperature and substrate availability, whether natural or artificial (Holst and Jarms, 2007; Song et al., 2017; Green et al., 2018; Pinto, 2021). Hence, marine construction activities in coastal areas such as harbors, water-fronts, docks, aquaculture facilities, or even aquatic animal surfaces e.g., bivalve shells, etc., can provide increased substrate areas for jellyfish polyp settlement and formation of colonies (Lo et al., 2008; Hoover and Purcell, 2009; Uye, 2010; van Walraven et al, 2020). The second reason we focused on jellyfish in the polyp stage is, as mentioned above, the polyp form of jellyfish is difficult to identify based on morphological criteria, especially in Thailand where deficiency in a reference key.

Jellyfish polyps have been studied in Thailand, though only sparsely. For instance, there have been reports of jellyfish polyps including studies on the life cycle of *Catostylus* sp. (Choosri et al., 2016), the upside-down jellyfish *Cassiopea andromeda* **(**Phuangsanthia et al., 2018), box jellyfishes, *Chironex* sp. and *Chiropsella* sp. (Toshino et al., 2019), and the edible jellyfish *Rhopilema hispidum* **(**Phuangsanthia et al., 2020). However, these previous studies of jellyfish polyps were performed with cultures of jellyfish in the laboratory, and no studies were conducted in natural habitats.

Given the strong, but limited, background research conducted to date with jellyfish polyps in Thailand, this paper focused on the diversity and distribution of jellyfish polyps in natural habitats using both traditional and molecular-based techniques for species identification; we focused efforts along coastal areas of Chonburi to Rayong Provinces as these areas have extensive human activities including tourism, marine transportation, aquaculture, and local fisheries, hence these are areas requiring science-based management plans.

MATERIALS AND METHODS

Sample collection

Field samplings were conducted in January, May, and July 2019 to represent Northeast Monsoon, pre-Southwest Monsoon, and Southwest Monsoon, respectively. Jellyfish polyps were collected from eight stations, five were along the western coast of Chonburi Province and three were on the southern coast of Rayong Province, Thailand. Sampling sites were given a two or three-letter identification name based on their nearest land-based community and ecosystems such as coastal, beach, river mouth, and mangrove forests; human activities in each community and ecosystems are described and include tourism, marine transportation, industrial estate, aquaculture, and local fisheries (Fig. 1). Polyp samples were collected from hard substrates e.g., piers and rocks, in coastal areas by scuba diving. All samples were preserved in absolute ethanol and kept at -20 °C for further analysis including morphological identification, DNA extraction, PCR, and sequencing.

Jellyfish medusae were collected vertically from 1-2 meters above sea-based using conical plankton net meshed 300 microns during the same trips with polyp collections. Jellyfish medusae were preserved with ethanol as same as polyp preservation protocols for morphological identification and DNA extraction, PCR, and sequence analysis. This will be used for verification and/or reference points for jellyfish polyp data analysis.

Identification of jellyfish's polyps using morphological criteria

Jellyfish polyps were identified according to their morphology following description and illustration by several references (Cornelius, 1975; Cornelius, 1982; Schuchert, 2003; Calder, 2012; Zhen-zu et al., 2014; Gravili et al., 2015; Mendoza-Becerril et al., 2020) using a stereo microscope (model SZ30; Olympus Corp.; Tokyo, Japan) before sorted for DNA extraction, PCR and sequencing.

Identification of jellyfish polyps and medusa using COI gene amplification and DNA sequencing

The polyps (42 samples) and 14 medusae were sorted under a stereo microscope according to their morphology as described above. Next, each polyp and medusa were transferred to an Eppendorf tube (1.5 ml) and DNA extraction was performed using a DNA extraction kit (PureLink® Genomic DNA Mini Kit, Invitrogen, USA). Extracted DNA was amplified by PCR (Biometra TOne96, Analytik Jena, Germany) with the specific primers of the mitochondrial Cytochrome Oxidase subunit I gene (mtCOI). The primers and PCR conditions were modified from Ortman et al. (2010). Briefly, the universal primers (Folmer et al., 1994) were used in this study: forward primer, [LCO1490]: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and reverse primer, [HCO2198]: 5′-TAAAC TTCAGGGTGACCAAAAAATCA-3′. Each 50 µl PCR reaction (in 0.2 ml PCR tubes) contained 25 µl of pre-mixed buffer solution (OnePCR^{TM} Ultra; GeneDirex, the Bio-helix, Co., LTD., Taiwan), 1 µl each of forward and reverse primers (5~10 µM), 1 µl of DNA template, and added ddH_2O or nuclease-free water to a final volume of 50 µl. PCR conditions were: initial heat at 95 °C for 3 min, followed by 35 cycles (denaturation at 94 °C for 1 min, annealing at 45 °C for 2 min, and extension at 72 °C for 3 min) and final elongation (72 °C for 10 min). Of the 56 samples, 35

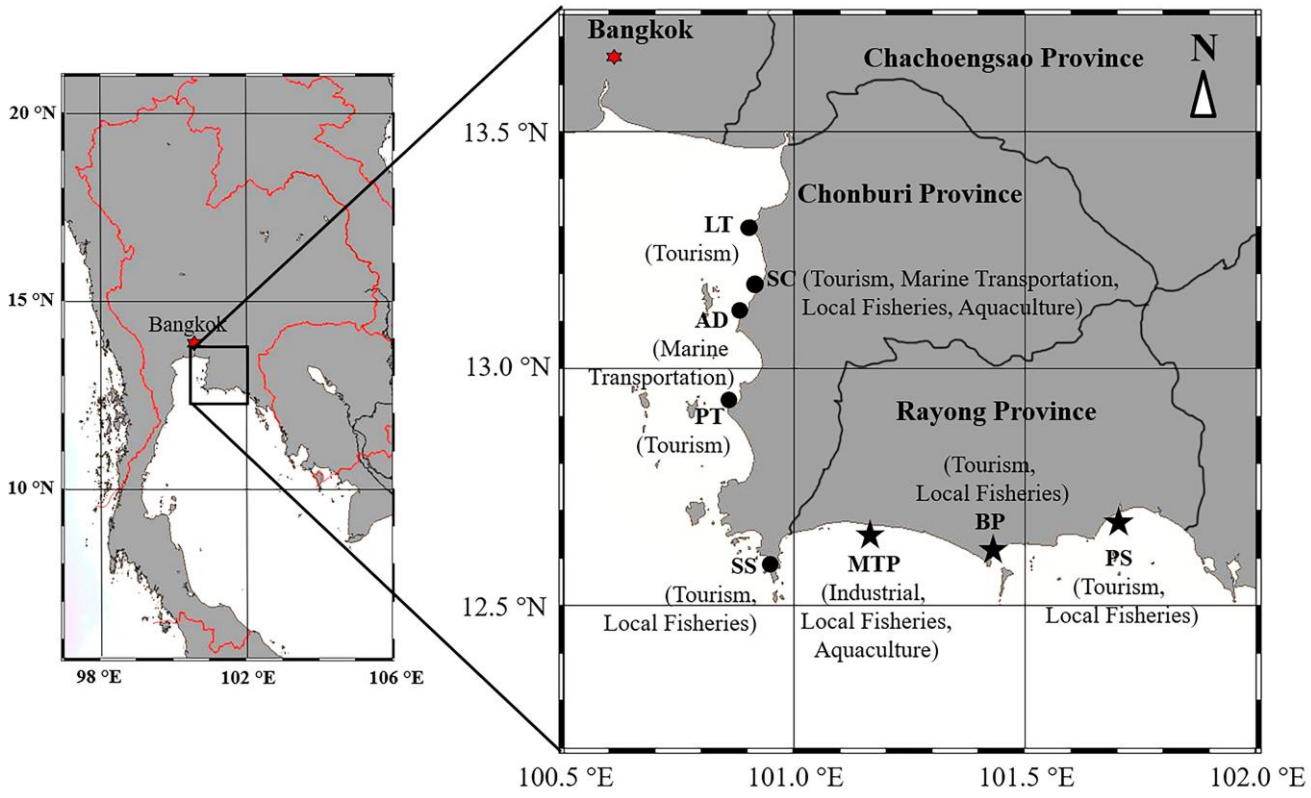

FIGURE 1. Eight sampling stations of jellyfish located along coastal areas of Chonburi Province (circles) and Rayong Province (stars), Thailand, and including a summary of the different anthropogenic activities carried out at study sites.

polyps from 42 polyp samples and 13 medusae from 14 medusa samples gave a recognizable PCR product of 657 to 698 bp, the expected size of the COI sequence. All the PCR products were subsequently purified and sequenced by a private company (Macrogen, Korea). Chromatogram evaluation, editing, and assemblage were performed using BioEdit version 7.2.5 (Hall, 1999). The edited sequences were blasted against the GenBank nucleotide database (http://www.ncbi.nlm.nih.gov/). The corresponding genus or species of the edited sequences were defined by the highest scores and/or identical percentages matching with the GenBank nucleotide database. Unfortunately, of the 35 polyp sequences, six (unclassified polyps) were subsequently found to be non-jellyfish polyps (e.g., bryozoan or entoprocta). This might due to the limitation of the investigator's experience and lack of reference keys for jellyfish polyps in Thailand especially in the natural habitats not from laboratory culture of the known species. However, the presence of six misidentified polyps in this study reinforces a central problem that jellyfish polyps can be very difficult to identify based on their morphology and, further, provides more support for the use of DNA barcoding, which is somewhat technically difficult, but in reality, is far easier to master than the fine art of jellyfish polyp morphological analysis.

To generate a phylogenetic tree, all of the DNA sequences were trimmed to the most highly conserved region of all COI genes, and these concatenated sequences were then aligned together with selected reference sequences from the GenBank database (the highest matching score with our query sequences) and some of the non-jellyfish sequences e.g., non-jellyfish polyps and copepods (samples from this study), as references (outgroup), using ClustalW, an optional menu in MEGA X program (version 10.2.6). Genetic distance within species, genera, families, and order of all sequences were also calculated in MEGA X (Kumar et al, 2018) using Kimura Two-parameter (K2P) models. Unrooted Neighbor-Joining (NJ) phylogenetic trees were established using MEGA under the K2P evolutionary model with 1,000 bootstrapping replicates.

RESULTS

Diversity and distribution of jellyfish polyps according to morphology

In the present study, 42 polyps were identified according to their morphological characteristics. Of the 42 polyps, 36 polyps can be classified into 6 families comprised of the family Campanulariidae, Bougainvilliidae, Thyroscyphidae, Halopteridiidae, Aequoreidae, and Geryoniidae, and including 6

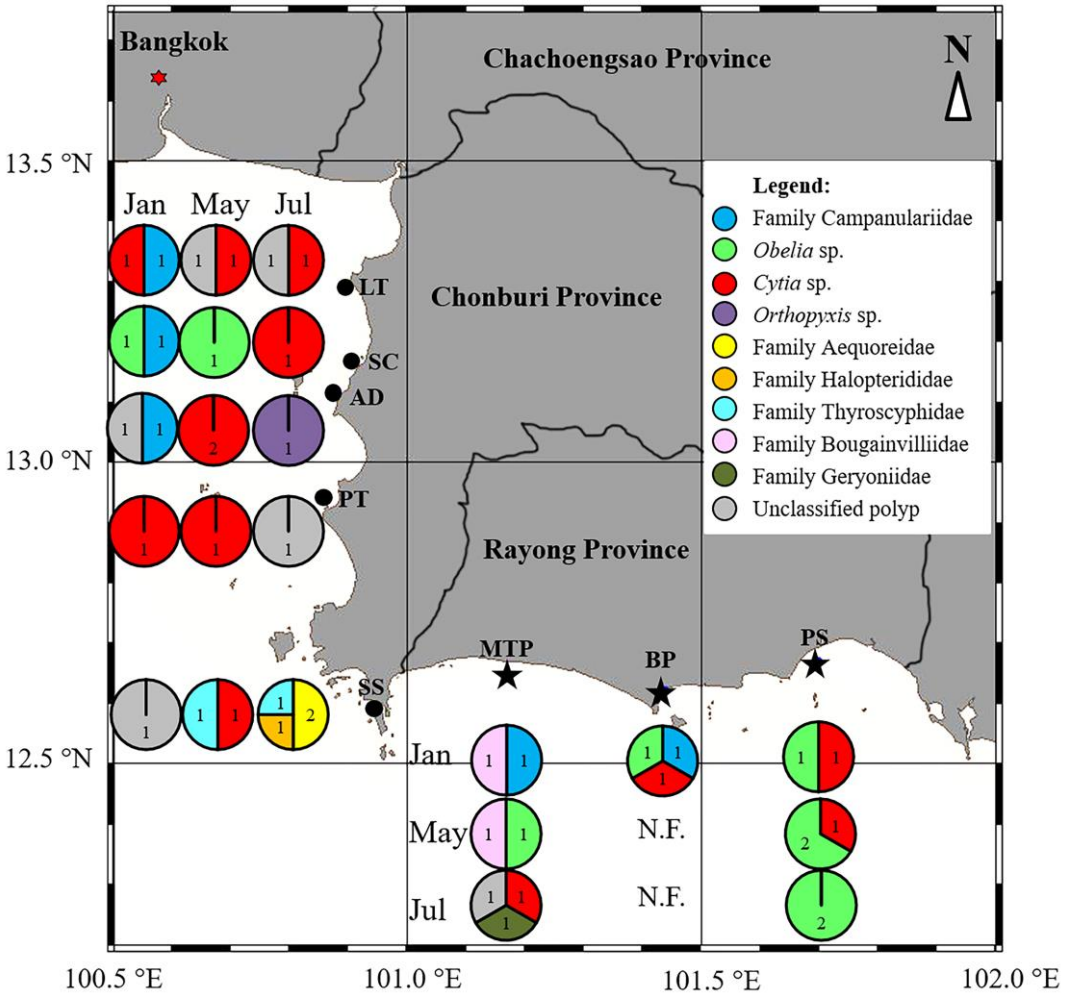

FIGURE 2. Distribution of jellyfish polyps (according to morphological identification) along coastal areas of Chonburi Province and Rayong Province, during sampling periods in January, May, and July 2019. N.F. = Not found.

unclassified polyps (Table 1). Twenty-three individuals of jelly polyps belonging to the family Campanulariidae were composed of the genus *Clytia* (13 individuals), *Obelia* (9 individuals), *Orthopysis* (1 individual), and unidentified Campanulariidae (5 individuals). In particular, the polyp of *Clytia* sp. might be a common polyp found along the coastal areas of Chonburi and Rayong Provinces since they can be occurred in every station during study periods, while the polyp of *Obelia* sp. can be detected mainly in Rayong Province and only at Sriracha (SC), Chonburi Province. Differ from the previous 2 species, *Orthopyxis* sp. can be found only at Ao Udom (AD), Chonburi Province. On the other hand, the rest polyps can be classified into the family level. In detail, the family Aequoreidae, Halopteridiidae, and Thyroscyphidae, can be found only at Samae San (SS), Chonburi Province, while the family Bougainvilliidae and Geryoniidae have appeared at Mabtaput (MTP), Rayong Province (Fig. 2).

Diversity and distribution of jellyfish's polyps using COI gene sequencing

In this work, 35 polyps (of the 42 polyp samples) gave a PCR product and were then sequenced. Of the 35 polyps isolated, six unclassified polyps were found to be non-jellyfish polyps as mentioned above. From the remaining 29 jellyfish polyp samples, 16 sequences (55%) were perfectly matched to *Clytia* sp. and *Obelia* cf. *bidentata*, which shared more than 95% identities with the database sequences from GenBank. In addition, another 8 isolates (28%) showed highly identical percentages (> 90%), and corresponded to jellyfish in the family Campanulariidae including *Clytia* sp., *Obelia* cf. *bidentata*, *Campanulariidae* sp. and *Orthopyxis* cf. *integra*, family Bougainvilliidae (*Bougainvillia* sp.), and family Thyroscyphidae (*Thyroscyphus* cf. *ramosus*). The rest of the 5 sequences (17%) were matched to the GenBank database with an identity of less than 90%, which were composed of *Clytia* cf. *elsaeoswaldae*, jellyfish belonging to the family Halopteridiidae, family Aequoreidae and family Geryoniidae (Table 1).

TABLE 1. Identification of DNA samples extracted from jellyfish polyps and medusa and aligned with the reference sequences. The data of PCR product sizes (bp), concatenated DNA sequence length (bp), query cover (%) and identities (%) compare to the reference sequences (GenBank) are indicated.

No.	Sample code (stage)	Identification methods		Query cover (%)	Identity (%)	Sizes of PCR Products (bp)	Lengths of concatenated DNA (bp)
		Morphology	COI Gene (Accession No. of the reference sequences in GenBank)				
1	LT-Jan (Polyp1)	*Clytia* sp.1	*Clytia* sp. JRH-2014 XMCL1 (KF962101.1)	94	98.94	672	585
2	LT-Jan (Polyp2)	Family Campanulariidae	No PCR product				
3	SC-Jan (Polyp1)	*Obelia* sp.1	No PCR product				
4	SC-Jan (Polyp2)	Family Campanulariidae	No PCR product				
5	AD-Jan (Polyp1)	Unknown polyp 1	*Calloporina angustipora* isolate CangGI02 (JF950411.1)	87	82.69	672	509
6	AD-Jan (Polyp2)	Family Campanulariidae	*Campanulariidae* sp. JG-2020 voucher 254805 (MT949549.1)	89	90.08	690	585
7	PT-Jan (Polyp1)	*Clytia* sp.1	*Clytia* sp. JRH-2014 XMCL1 (KF962101.1)	91	99.53	684	585
8	SS-Jan (Polyp1)	Unknown polyp 2	*Amathia vidovici* BRBRYO-A238 (KM373394.1)	68	86.97	684	414
9	MTP-Jan (Polyp1)	Family Bougainvilliidae	*Bougainvillia* sp. JRH-2014 isolate 1 (JQ716058.1)	54	90.93	664	417
10	MTP-Jan (Polyp2)	Family Campanulariidae	No PCR product				
11	BP-Jan (Polyp1)	*Clytia* sp.1	*Clytia* sp. GL JRH-2014 XMCG1 (KF962086.1)	94	99.25	682	585
12	BP-Jan (Polyp2)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2818 (KX665211.1)	94	98.64	686	585
13	PS-Jan (Polyp1)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2818 (KX665211.1)	95	97.87	690	584
14	PS-Jan (Polyp2)	*Clytia* sp.1	No PCR product				
15	LT-May (Polyp1)	*Clytia* sp.1	*Clytia* sp. GL JRH-2014 XMCG11 (KF962096.1)	92	99.07	673	583
16	LT-May (Polyp1)	Unknown polyp 3	*Amathia vidovici* BRBRYO-A57 (KM373363.1)	89	87.24	670	569
17	SC-May (Polyp1)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2818 (KX665211.1)	94	91.09	688	585
18	AD-May (Polyp1)	*Clytia* sp.1	*Clytia* sp. JRH-2014 XMCL1 (KF962101.1)	97	96.14	689	586
19	AD-May (Polyp2)	*Clytia* sp.2	*Clytia elsaeoswaldae* MZUSP:2762 (KX665227.1)	95	89.17	687	587
20	PT-May (Polyp1)	*Clytia* sp.1	*Clytia* sp. JRH-2014 XMCL1 (KF962101.1)	96	97.41	683	585
21	SS-May (Polyp1)	*Clytia* sp.1	No PCR product				
22	SS-May (Polyp1)	Family Thyroscyphidae	*Thyroscyphus ramosus* clone DMS-HA-Tr-Hap-01 (MH580282.1)	90	92.77	683	584
23	MTP-May (Polyp1)	Family Bougainvilliidae	*Bougainvillia* sp. PM JRH-2014 XMBP9 (KF962079.1)	95	94.39	691	585
24	MTP-May (Polyp2)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2818 (KX665211.1)	94	99.25	687	585
25	MTP-May (Polyp3)	Family Campanulariidae	No PCR product				
26	PS-May (Polyp1)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2817 (KX665213.1)	93	97.51	685	585
27	PS-May (Polyp2)	*Clytia* sp.1	*Clytia* sp. GL JRH-2014 XMCG1 (KF962086.1)	93	97.70	690	587
28	PS-May (Polyp3)	*Obelia* sp.1	*Obelia bidentata* MZUSP:2817 (KX665213.1)	94	99.85	685	585
29	LT-Jul (Polyp1)	*Clytia* sp.1	*Clytia* sp. GL JRH-2014 XMCG11 (KF962096.1)	97	97.44	680	585
30	LT-Jul (Polyp2)	Unknown polyp 4	*Barentsia gracilis* (FJ196079.1)	93	86.52	682	586
31	SC-Jul (Polyp1)	*Clytia* sp.1	*Clytia* sp. JRH-2014 XMCL1 (KF962101.1)	92	92.27	686	587
32	AD-Jul (Polyp1)	*Orthopyxis* sp.	*Orthopyxis integra* 823AS (AY789885.1)	85	90.86	675	580
33	PT-Jul (Polyp1)	Unknown polyp 5	*Calloporina angustipora* isolate CangGI02 (JF950411.1)	87	82.28	695	585
34	SS-Jul (Polyp1)	Family Thyroscyphidae	*Thyroscyphus ramosus* clone DMS-HA-Tr-Hap-01 (MH580282.1)	89	93.16	687	585
35	SS-Jul (Polyp2)	Family Halopterididae	*Antennella secundaria* STRI CJM75(MH282660.1)	88	82.54	673	555
36	SS-Jul (Polyp3)	Family Aequoreidae	*Aequorea australis* isolate 7 (JQ716196.1)	78	81.96	657	455
37	SS-Jul (Polyp4)	Family Aequoreidae	*Aequorea* sp. FRPRVSM (MF742056.1)	93	87.27	673	586
38	MTP-Jul (Polyp1)	Family Geryoniidae	*Liriope tetraphylla* isolate LEM: S05 (MG791813.1)	87	79.10	673	585
39	MTP-Jul (Polyp2)	Unknown polyp 6	*Barentsia discreta* (GU125772.1)	78	82.76	670	539
40	MTP-Jul (Polyp3)	*Clytia* sp.	*Clytia* sp. GL JRH-2014 XMCG1 (KF962086.1)	91	95.96	689	587
41	PS-Jul (Polyp1)	*Obelia* sp.	*Obelia bidentata* MZUSP:2818 (KX665211.1)	95	99.40	682	585
42	PS-Jul (Polyp2)	*Obelia* sp.	*Obelia bidentata* MZUSP:2818 (KX665211.1)	97	99.70	683	585
43	SC-Jan (Medusa1)	*Liriope* sp.	*Liriope tetraphylla* isolate KUFOS-LT1 (KU364622.1)	91	99.07	690	585
44	SS-Jan (Medusa1)	*Nemopsis* sp.	*Nemopsis bachei* clone M3491F02 (KX265108.1)	90	87.74	689	585
45	BP-Jan (Medusa1)	*Nemopsis* sp.	*Nemopsis bachei* clone M3491F02 (KX265108.1)	90	87.54	690	585
46	BP-Jan (Medusa2)	*Liriope* sp.	*Liriope tetraphylla* isolate KUFOS-LT6 (MH444768.1)	92	98.76	698	585
47	PS-Jan (Medusa1)	Family Malagazziidae	*Malagazzia carolinae* XMMC1 (KF962150.1)	92	99.54	687	585
48	PS-Jan (Medusa2)	*Eirene* sp.	*Eirene menoni* isolate 2 (JQ716133.1)	88	99.03	693	562
49	LT-May (Medusa1)	*Nemopsis* sp.	*Nemopsis bachei* clone M3491F02 (KX265108.1)	93	87.42	669	585
50	AD-May (Medusa1)	Family Campanulariidae	No PCR product				
51	SS-May (Medusa1)	Family Proboscidactylidae	*Proboscidactyla ornata* isolate 1 (JQ716077.1)	87	92.11	685	561
52	BP-May (Medusa1)	*Nemopsis* sp.	*Nemopsis bachei* clone M3491F02 (KX265108.1)	89	92.92	684	583
53	BP-May (Medusa2)	Obelia sp.	*Obelia dichotoma* voucher MZUSP:2820 (KX665209.1)	98	94.65	685	585
54	SS-Jul (Medusa1)	*Liriope* sp.	*Liriope tetraphylla* isolate KUFOS-LT6 (MH444768.1)	93	99.69	686	585
55	MTP-Jul (Medusa1)	*Cytaeis* sp.	*Cytaeis* sp. USNM IZ 1447971 (MW124761.1)	91	89.41	671	586
56	PS-Jul (Medusa1)	*Bougainvillia* sp.	*Bougainvillia* sp. JRH-2014 isolate 1 (JQ716058.1)	69	86.68	670	455

A more detailed description of Table 1 reveals that jellyfish polyps analyzed here belonged to the genus *Clytia* (corresponded to strain JRH-2014 XMCL1, GL JRH-2014 XMCG1, and GL JRH-2014 XMCG11), and

the genus *Obelia*, especially *Obelia* cf. *bidentata* (corresponded to strain MZUSP:2817 and 2018) were recovered during the sampling periods with 10 and 8 individuals, respectively. Digging deeper into these results includes contextual information on the genus *Clytia* (corresponded to strain JRH-2014 XMCL1 and GL JRH-2014 XMCG11), which were recorded in coastal areas of Chonburi Province such as Laem Tan (LT), Sri Racha (SC), Au Udom (AD) and Pattaya (PT), while the other strain of *Clytia* (corresponded to GL JRH-2014 XMCG1) was noted in coastal areas of Rayong Province including Ban Phe (BP), Mabtaput (MTP) and Prasae (PS). Similarly, *Obelia* (corresponded to *Obelia* cf. *bidentata*) was also mainly found in Rayong Province, especially at Prasae (PS) where they were recorded in January, May, and July 2019. In contrast, jellyfish polyps found in Samae San (SS) included sequences agree with *Thyroscyphus* cf. *ramosus*, which was found in May and July 2019, as well as *Antennella* cf. *secundaria* and *Aequorea* spp., which were found in July; these jellyfish polyps were not observed from other stations (Table 1).

A phylogenetic analysis of COI genes was constructed using 77 concatenated DNA sequences including 29 jellyfish polyps, 13 jellyfish medusae, 8 sequences of non-jellyfish, and 27 reference sequences from GenBank, and a total of 5 clades were clustered (Fig. 3). The first clade in the phylogenetic analysis of the COI gene was the largest clade included 25 of the 29 polyps and 4 of the 13 medusae found in this study and reference sequences from GenBank database (16 sequences), which mainly belong to the order Leptothecata. In detail, it is likely that jellyfish in this clade can be divided into 3 sub-groups as follows; 1.1) the jellyfish in the family Campanulariidae comprised of polyps belonging to the genus *Clytia*, (including reference sequences of *Clytia* corresponded to strain JRH-2014, GL JRH-2014, *Clytia elsaeoswaldae*, *Clytia gracilis*, and *Clytia folleata*), polyps and medusa of the genus *Obelia* (corresponded to *Obelia bidentata* and *Obelia dichotoma*), polyp of the genus *Orthopyxis* (corresponded to *Orthopyxis integra*), unidentified polyp of the family Campanulariidae (corresponded to *Campanulariidae* sp.), and a medusa of *Eirene* sp. (corresponded to *Eirene menoni*), 1.2) the mixture of jellyfish belonging to the order Leptothecata including the family Malagazziidae (medusa) and family Aequoreidae (polyps), and the medusa form of jellyfish belonging to the order Anthoathecata (family Proboscidactylidae), and 1.3) the polyps belonging to the family Thyroscyphidae. The second clade composed of the jellyfish (both polyp and medusa forms) belongs to the order Anthoathecata including the family Bougainvilliidae (genus *Bougainvilla* and *Nemopsis*) and family Cytaeididae. The third clade was the jellyfish in the order Limnomedusae, which included the polyp and medusa forms of *Liriope* sp. (family Geryoniidae). The fourth clade was a remaining polyp in the family Halopteridídae which belonged to the order Leptothecata. Finally, the last clade was an outgroup reference of non-jellyfish including pelagic organisms (copepods) and sessile organisms (bryozoa and entoprocta), and this was clearly separated from the jellyfish COI sequences. In addition, as described above, there are two of the jellyfish polyp COI gene sequences were perfect matches to the jellyfish medusa DNA, family Bougainvilliidae (corresponded to *Bougainvillia* sp.) and family Geryoniidae (corresponded to *Liriope* cf. *tetraphylla*), hence there is no uncertainty about the identity of these polyps.

DISCUSSION

The present work aimed to study the diversity and distribution of jellyfish polyps collected from natural habitats along the coast of two provinces situated on the eastern shore of the Gulf of Thailand, areas with intense anthropogenic activity. To study the diversity of marine organisms including jellyfish, species identification is a significant step and there have fundamentally been based on their morphological and anatomical characteristics. Unfortunately, due to rarely references and taxonomic keys to identify jellyfish polyps in Thailand, the identification and classification of jellyfish polyps seem insufficient using traditional morphological criteria. In addition, such conventional ways may have some limitations in precisely identifying jellyfish species and necessitate special training or professional skills. Another difficulty may also be the existence of closely related taxa/characteristics that are barely distinguishable (Sathirapongsasuti et al., 2021). Therefore, the application of a rapid and promising tool for species identification is needed for analysis of jellyfish diversity. However, genetic analysis cannot replace morphologically based taxonomy in studies on species' population dynamics, physiology and ecology. Thus, most information is achieved by combining both methods in integrative studies using both morphological and molecular taxonomy (Laakmann and Holst, 2014). Consequently, the combination of morphology-based identification with DNA barcoding using field-collected samples represents the first attempt to conduct this type of work with jellyfish polyps in Thailand.

Detecting fluctuations in the species composition of jellyfish involves correct identification of each species

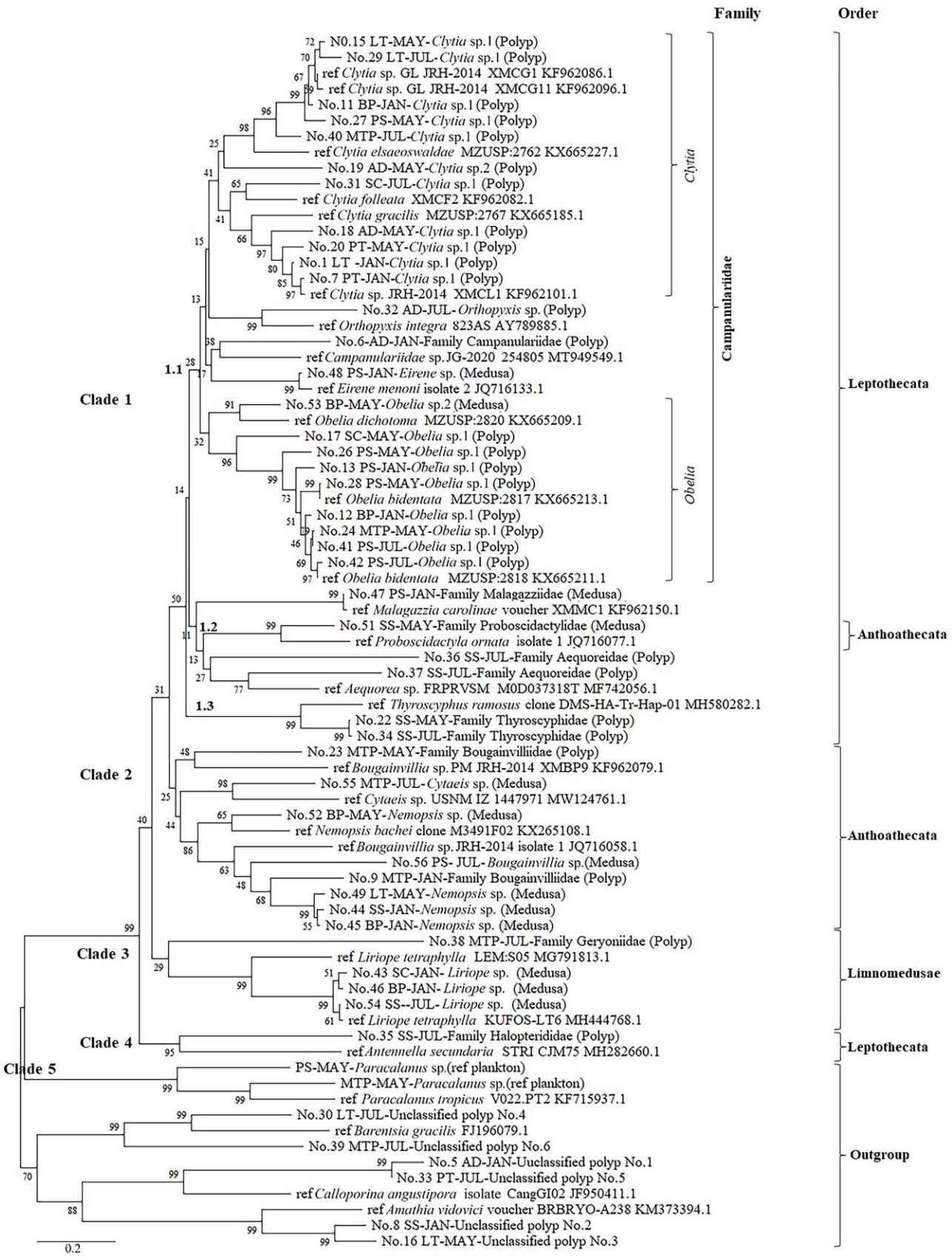

FIGURE 3. The unrooted Neighbor-Joining (NJ) tree was constructed with 77 nucleotide sequences of COI gene from jellyfish polyps and medusae collected in coastal areas of Chonburi and Rayong Provinces, and some reference samples from our study sites and GenBank database. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura-2-parameter (K2P) method and are in the units of the number of base substitutions per site.

in each developmental stage. Jellyfish polyps have never before been extensively studied in Thailand from natural populations, even though jellyfish blooms are common in Thailand (e.g., Choosri et al., 2016; Phuangsanthia et al., 2018; Hwai et al., 2019; Toshino et al., 2019; Phuangsanthia et al., 2020). These earlier studies of jellyfish did identify the poly stages of the species involved, but the approach was different from the approach used in the current study. Earlier, researchers collected jellyfish medusa, cultured them in the laboratory, and observed the polyp stages. While these earlier reports represent important contributions to science, they are not the same as identifying jellyfish polyps in natural field collections and then, importantly, using these data to better inform management policies. Our long-term goal, which is still a work in progress, is to help make field identification of jellyfish polyps more applicable to management policy solutions.

Historically, the dearth of studies on jellyfish polyps in Thailand dates back to important contributions made by The Department of Marine and Coastal Resources (DMCR), which focused on the medusa form of jellyfish but did not refer to jellyfish polyps (Marine and Coastal Resources Research and Development Institute, 2015b). We are cautiously optimistic that DMCR will be able to extend their earlier work, including jellyfish polyps with their medusa data, and, equally important as revealed by our data, include DNA barcoding as an important step in species identification.

DNA barcoding is widely applied in species identification and biodiversity studies. The ease with which DNA barcoding can now be conducted has led to widespread adoption of this technique, including species-level analysis of marine diversity including cnidarians (Lindsay et al., 2015). Several genes have been used to identify metazoans such as the COI gene (Huang et al., 2008; Ortman et al., 2010), 16S RNA (Moura et al., 2008; Lianming et al., 2014; Klomthong et al., 2016), and 18S rDNA (Bendezu et al., 2005; Muhammad et al., 2021). Remarkably, the most frequently-used gene region for species-level identification of marine zooplankton is a ~570 bp region of COI (Hebert et al., 2003; Bucklin et al., 2011). The COI barcode region can provide valuable insights into evolutionary processes, demographic history, population genetic diversity, structure, and connectivity of a species (Bucklin et al., 2021). The COI gene has been studied and successfully utilized as a marker for DNA barcoding for various aquatic organisms including, medusozoan e.g., hydrozoa, scyphozoa, and cubozoa (Ortman et al., 2010), arthropods, mollusks, chaetognaths, and echinoderms (Bucklin et al., 2010; Radulovici et al., 2010), copepods (Baek et al., 2016), gastropods (Galan et al., 2018; Ran et al., 2020), and fish (Bingpeng et al., 2018; Zou et al., 2020), as well as the use of COI with benthic organisms including foraminifera (Ge et al., 2020; Macher et al., 2021). Nowadays, COI has been analyzed for more than 100 species and has proven to be useful for species delimitation in hydrozoa, including clades with multiple copies, and allows matching pelagic and benthic life history stages of one species (Bucklin et al., 2021). However, there is evidence that misidentifications, due to the lack of taxonomic expertise and/or the presence of morphologically cryptic species, are frequent in public DNA barcode sequence repositories, and these should be used with caution (Lindsay et al., 2015). Furthermore, the universal COI primers used with hydrozoan have yet to be designed (Moura et al., 2018). At present, the mt16S rRNA, 18S rDNA, or 28S rDNA have now frequently been used for hydrozoa (Zheng et al., 2014; Lindsay et al., 2015; Parracho and Morais, 2015; Chae et al., 2018; Muhammad et al., 2021), while using COI in paralleled with mt16S rRNA or 18S rDNA, is becoming more commonplace since its promotion as the universal barcode locus (van Walraven et al., 2016; Schuchert, 2018; van Walraven et al., 2020; Bucklin et al., 2021). Nevertheless, the COI gene is still developing as metabarcoding for the study of marine zooplankton community (Andújar et al., 2018; Mariac et al., 2018; Ershova et al., 2021; Zhao et al., 2021; Bucklin et al., 2022). Overall, we conclude that DNA barcoding is a useful technique, a technique that is evolving rapidly, and more work will be needed with this technique in Thailand to gain a deeper, more complete, understanding of jellyfish distributions and life cycles. Our work with jellyfish DNA barcoding especially for the COI gene is only the third such study in Thailand.

A total of 9 genera (estimated to be 11 species which corresponding to the GenBank database) of jellyfish polyps were recognized by COI sequencing in this study; this number was higher than the number of genera identified using morphological characters (i.e., only 3 genera were recognized based on morphology). Importantly, as mentioned above, identification based on DNA sequences permitted genus/species-level identification, while the more conservative methods of morphology only allowed us to identify to family or genus levels, and DNA barcoding allowed us to clearly distinguish polyps of jellyfish and polyps of bryozoan. There have been two previous studies of jellyfish in Thailand that utilized COI gene barcoding. In the first study, jellyfish were collected from coastal areas of Trat Province, Thailand. The results of this study were somewhat surprising because the morphology-based

results did not match the DNA barcoding results; morphological analysis indicated six different types of jellyfish while DNA barcoding revealed that all six were the same species, i.e., *Catostylus mosaicus* (Patithanarak et al., 2014). In a second, more recent, study both 16S rRNA and COI sequences were used to identify unknown samples of box jellyfish, *Chironex* species, in Thailand (Sathirapongsasuti et al., 2021). In addition to these two studies of jellyfish in Thailand that relied upon the COI gene, other studies have used nuclear 18S rDNA and mitochondrial 16S rDNA to identify box jellyfishes and scyphozoa in Thailand (Ruijuan et al., 2016; Toshino et al., 2019). Overall, we can conclude that identification of Thai jellyfish based on morphology may, or may not, be different from results obtained based on DNA barcoding. For instance, both methods in the present work gave similar results with the genus *Obelia* and *Clytia*, which were found frequently in most study areas; the agreement of results from the two methods does not extend to other polyps sampled in this study.

One of the surprising findings of our work is that we did not identify jellyfish polyps known to be associated with earlier bloom events. Four prior jellyfish bloom events stand out for their widespread, destructive nature in Thailand: 1) blooms along the eastern coast of Thailand associated with *Catostylus* sp. (Choosri et al., 2016); 2) blooms associated with an upside-down jellyfish, *Cassiopea andromeda* **(**Phuangsanthia et al., 2018); 3) blooms associated with the box jellyfishes, *Chironex* sp. and *Chiropsella* sp. (Toshino et al., 2019); and 4) blooms associated with the edible jellyfish, *Rhopilema hispidum* **(**Phuangsanthia et al., 2020). These four cases of jellyfish blooms were mentioned above in the context of identifying polyps that were reared in the laboratory, but here, we want to focus on a different question – why did we not observe polyps of these species in our studies? Our failure to find these well-known jellyfish may be due to the small area of sampling sites that were covered on selected coasts (Fig. 1). Furthermore, we suspect that water currents may have played a role in these earlier events. For example, the distribution of the giant jellyfish, *Nemopilema nomurai*, in East Asian Marginal Seas, was affected by several currents e.g., Tsushima current in Japan (Uye, 2008; Kitajima et al., 2015), Yellow Sea Coastal current, Subei Shoal Coastal current, Taiwan current, and Kuroshio Branch current in China (Sun et al., 2015). We further speculate that the blooms of *Catostylus* sp. in Rayong Province, Thailand (Choosri et al., 2016), were driven by currents. The currents in Rayong, on the west coast of Thailand in the Gulf of Thailand, are very different from the currents in the Inner Gulf of Thailand including Chonburi Province; in the Gulf of Thailand, the currents move in a clockwise direction during the Southwest monsoon and move in a counter clockwise direction in Northeast monsoon (Buranapratheprat, 2008). In contrast, in coastal areas of Rayong, Chantaburi and Trat Provinces currents run counter clockwise during the Southwest monsoon and move southward in a clockwise direction during the Northeast monsoon (Sojisuporn et al., 2010). We hope to extend our studies of jellyfish polyps to more sites throughout Thailand, following the same sampling strategy used here of examining different meteorological conditions, in hopes of gaining a better understanding of jellyfish blooms throughout the Gulf of Thailand.

Our approach of combining morphology and DNA barcoding of jellyfish polyps allowed us to conclusively identify two polyps to species; now, we want to revisit our original goal of 'describing jellyfish diversity and distribution', and focus on distribution of *Clytia* and *Obelia*, two species which were found frequently in most study areas and, as discussed above, yielded the same identification based on both morphology and COI gene sequencing. Firstly, we want to focus our attention on the literature on the genus *Clytia*, which is very diverse, with over 50 species currently recognized (Schuchert, 2020). *Clytia* is widely distributed throughout the world (Zhou et al., 2013) including tropical and subtropical zones e.g., Indonesia (Di Camillo et al., 2008), Brazil (Lindner and Migotto, 2002), and East China Sea (He et al., 2015), and temperate zones e.g., Japan (Kubota, 1978), England (Lucas et al., 1995), and the Mediterranean Sea (Boero et al., 2005; Brotz and Pauly, 2012). The abundance and distribution of *Clytia* is generally correlated with the density of other zooplankton, such as copepods, ciliated zooplankton, cirripedia larvae, and nauplius larvae; this correlation is likely a result of the important ecological role *Clytia* plays in shallow-water benthic environments, acting as both competitor and predator (Fulton and Wear, 1985; Costello and Colin, 2002; Hansson et al., 2005; Adamík et al., 2006; Sutherland et al., 2016). It is tempting to suggest that the distribution of *Clytia* polyps will be correlated to the abundance of food sources in the water column, but which food sources remains completely unknown at this time. Secondly, the genus *Obelia*, in particular *Obelia* cf. *bidentata*, was recorded generally in coastal of Rayong Province, while the genus *Clytia* was mainly recognized in coastal areas of Chonburi Province (Fig. 3). It is tempting to speculate that coastal areas of Rayong Province, especially at the mouth of the Prasae River (PS), offered hospitable attachment sites, or substrates, or both, for *Obelia*

bidentata polyps, whereas coastal areas of Chonburi and Rayong Provinces provided hospitable substrates/sites for *Clytia* sp. polyps. While these observations are suggestive of the 'what', they do not yet address the question of 'why'. Looking at the literature for answers to 'why', we see that the genus *Obelia* is a cosmopolitan species and has been found from coastal areas in the Tropical East Pacific (Miglietta et al., 2008) extending to the marginal sea of the Arctic Ocean, the Barents Sea, Russia (Zelickman, 1972). In particular, *O. bidentata* is distributed worldwide in tropical, subtropical and temperate waters (Calder, 1991), and the hydroid is found attached to hard substrata such as wood, shells and wrecks, as well as on sandy bottoms (Wilson, 2002). Moreover, *O. bidentata* is tolerant of brackish water, and is found from the sublittoral zone down to 200 m. (Wilson, 2002) and this might be one of the reasons *O. bidentata* was found in this study mostly at stations near the mouth of a river, i.e., Prasae River (PS). We have a small set of data on the distribution of *Obelia* medusa based solely on morphology (data not shown); we look forward to extending this work with *Obelia* medusa to determine how much, if any, the abundance of this genus is impacted by riverine inputs.

Phylogenetic analysis showed obviously clusters in which the COI sequences of jellyfish were grouped in the same order and clearly separated from an outgroup. However, some results should be noticed such as a jellyfish belonging to the order Anthoathecata which was grouped with jellyfish belonging to the order Leptothecata (clade 1.2), and the separation of jellyfish in the order Leptothecata between clade 4 and clade 1 (a major group in the tree). To achieve the study of biodiversity and distribution of marine organisms, there are some recommendations to be concerned including implementing multiple methods for a taxonomic assignment, using multiple genetic markers, clustering the data into different types of molecular operational units, and more importantly, collaborating with taxonomists to develop a regional database of the groups of interest (Pappalardo et al., 2021). In particular, for Hydrozoa, photographic vouchers that carefully document key structures of samples before fixation in ethanol may be the best way for both morphological identification and DNA barcoding (Bucklin et al., 2021).

According to our results, it can be noted that this paper provided the first report about the diversity of jellyfish polyps in the Gulf of Thailand in natural habitats. In addition, molecular methods provided a useful tool to identify jellyfish in both polyp and medusa forms; DNA barcoding is both rapid and accurate, and can be used with samples or tissues preserved in absolute ethanol and stored at -20 ℃ for up to one year (unpublished data). The ability to easily provide long-term storage of samples inexpensively should be advantageous for local fishermen or residents working as members of citizen-science working group focused on coastal issues in Thailand. Finally, results from this work will help lay a firm foundation that can be used by local or national authorities or local working groups to make better-informed policy decisions and/or management plans for Thailand.

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LITERATURE CITED

Adamík P., Gallager S.M., Horgan E., Madin L.P., McGillis W.R., Govindarajan A. and Alatalo P. 2006. Effects of turbulence on the feeding rate of a pelagic predator: the planktonic hydroid *Clytia gracilis*. Journal of Experimental Marine Biology Ecology, 333: 159–165.

Andújar, C., Arribas, P., Yu, D.W., Vogler, A.P., and Emerson, B.C. 2018. Why the COI barcode should be the community DNA metabarcode for the Metazoa. Molecular Ecology, 27(20): 3968-3975.

Baek, S.Y., Jang, K.H., Choi, E.H., Ryu, S.H., Kim, S.K., Lee, J.H., Lim, Y.J., Lee, J., Jun, J., Kwak, M., Lee, Y.S., Hwang, J.S., Venmathi Maran, B.A., Chang, C.Y., Kim, I.H., and Hwang, U.W. 2016. DNA Barcoding of Metazoan Zooplankton Copepods from South Korea. PloS one, 11(7): e0157307.Bendezu, I.F., Slater, J.W. and Carney, B.F. 2005. Identification of Mytilus spp. and Pecten maximus in Irish Waters by Standard PCR of the 18S rDNA Gene and Multiplex PCR of the 16S rDNA Gene. Marine Biotechnology, 7: 687–696.

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., Jianjun, W. 2018) DNA barcoding for identification of fish species in the Taiwan Strait. PLoS ONE 13(6): e0198109.

Boero, F. 2013. Review of jellyfish blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 92. FAO, Rome, 53 pp.

Boero F., Di Camillo C. and Gravili C. 2005. Aquatic invasions: phantom aliens in Mediterranean waters. MarBEF Newsletter, 3: 21–22.

Brotz, L., Cheung, W.W.L., Kleisner, K., Pakhomov, E. and Pauly, D. 2012. Increasing jellyfish populations: trends in Large Marine Ecosystems. Hydrobiologia, 690: 3–20.

Brotz, L. and Pauly, D. 2012. Jellyfish populations in the Mediterranean Sea. Acta Adriatica, 53(2): 211–230.

Bucklin, A., Batta-Lona, P.G., Questel, J.M., Wiebe, P.H., Richardson, D.E., Copley, N.J. and O’Brien, T.D. 2022. COI Metabarcoding of Zooplankton Species Diversity for Time-Series Monitoring of the NW Atlantic Continental Shelf. Frontiers in Marine Science, 9: 867893.

Bucklin, A., Ortman, B.D., Jennings, R.M., Nigro, L.M., Sweetman, C.J., Copley, N.J., Wiebe, P.H. 2010. A “Rosetta Stone” for zooplankton: DNA barcode analysis of

holozooplankton diversity of the Sargasso Sea (NW Atlantic Ocean). Deep-Sea Res II, 57: 2234–2247.

Bucklin, A., Peijnenburg, K.T.C.A., Kosobokova, K.N., O'Brien, T.D., Blanco-Bercial, L., Cornils, A., Falkenhaug, T., Hopcroft, R.R., Hosia, A., Laakmann, S., Li, C., Martell, L., Questel, J.M., Wall-Palmer, D., Wang, M., Wiebe, P.H. and Weydmann-Zwolicka, A. 2021. Toward a global reference database of COI barcodes for marine zooplankton. Marine Biology, 168: 78.

Bucklin, A., Steinke, D. and Blanco-Bercial, L. 2011. DNA Barcoding of Marine Metazoa. Annual Review of Marine Science, 3: 471–508.

Buranapratheprat, A. 2008. Circulation in the Upper Gulf of Thailand: A review. Burapha Science Journal. 13(1), 75–83.

Calder, D.R. 1991. Shallow-water hydroids of Bermuda: the Thecatae, exclusive of Plumularioidea. Royal Ontario Museum, Toronto, Life Science Contributions, 154: 1–140.

Calder, D.R. 2012. On a collection of hydroids (Cnidaria, Hydrozoa, Hydroidolina) from the west coast of Sweden, with a checklist of species from the region. Zootaxa, 3171: 1–77.

Chae, J., Seo, Y., Yu, W.B., Yoon, W.D., Lee, H.E., Chang, S.J. and Ki, J.S. 2018. Comprehensive analysis of the jellyfish *Chrysaora pacifica* (Goette, 1886) (Semaeostomeae: Pelagiidae) with description of the complete rDNA sequence. Zoological Studies, 57: 51.

Choosri, S., Luangoon, N., Charoendee, W., Taweedetch, S. and Phuangsanthia, W. 2016. Life cycle of the jellyfish *Catostylus* sp. Final report, The Institute of Marine Science, Burapha University, Chonburi, 42 pp.

Condon R.H., Duarte, C.M., Pitt, K.A., Robinson, K.L., Lucas, C.H., Sutherland, K.R., Mianzan, H.W., Bogeberg, M., Purcell, J.E., Decker, M.B., Uye, S., Madin, L.P., Brodeur, R.D., Haddock, S.H.D., Malej, A., Parry, G.D., Eriksen, E., Quiñones, J., Acha, M., Harvey, M., Arthur, J.M. and Graham, W.M. 2013. Recurrent jellyfish blooms are a consequence of global oscillations. Proceedings of the National Academy of Sciences of the United States of America, 110(3): 1000–1005.

Cornelius, P.F.S. 1975. The hydroid species of *Obelia* (Coelenterata, Hydrozoa: Campanulariidae), with notes on the medusa stage. Bulletin of the British Museum, Zoology 28: 251–293.

Cornelius, P.F.S 1982. Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of genera. Bulletin of the British Museum, Zoology 42: 37–148.

Costello, J.H. and Colin, S.P. 2002. Prey resource use by coexistent hydromedusae from Friday Harbor, Washington. Limnology and Oceanography, 47(4): 934–942.

Di Camillo, C.G., Bavestrello, G., Valisano, L. and Puce, S. 2008. Spatial and temporal distribution in a tropical hydroid assemblage. Journal of the Marine Biological Association of the United Kingdom, 88(8): 1589–1599.

Dong, Z., Liu, D. and Keesing, J.K. 2010. Jellyfish blooms in China: Dominant species, causes and consequences. Marine Pollution Bulletin, 60: 954–963.

Ershova, E.A., Wangensteen, O.S., Descoteaux, R., Barth-Jensen, C. and Prabel, K. 2021. Metabarcoding as a quantitative tool for estimating biodiversity and relative biomass of marine zooplankton. ICES Journal of Marine Science, 78(9): 3342–3355.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5): 294–299.

Fulton, R.S. and Wear, R.G. 1985. Predatory feeding of the hydromedusae *Obelia geniculata* and *Phialella quadrata*. Marine Biology, 87: 47–54.

Galan, G.L., Mendez, N.P. and dela Cruz, R.Y. 2018. DNA barcoding of three selected gastropod species using cytochrome oxidase (COI) gene. Annales of West University of Timisoara, Series of Biology, 21(1): 93–102.

Ge, Y., Xia, C., Wang, J., Zhang, X., Ma, X., and Zhou, Q. 2021. The efficacy of DNA barcoding in the classification, genetic differentiation, and biodiversity assessment of benthic macroinvertebrates. Ecology and evolution, 11(10): 5669–5681.

Gershwin, L., Condie, S.A., Mansbridge, J.V. and Richardson, A.J. 2014 Dangerous jellyfish blooms are predictable. Journal of The Royal Society Interface, 11: 20131168.

Gravili, C, Vito, D.D, Camillo, C.G.D., Martell, L., Piraino, S., Boero, F., 2015. The non-Siphonophoran Hydrozoa (Cnidaria) of Salento, Italy with notes on their life-cycles: an illustrated guide. Zootaxa, 3908 (1): 001–187.

Green, T.J., Wolfenden, D.C.C. and Sneddon, L.U. 2018. An investigation on the impact of substrate type, temperature, and iodine on moon jellyfish production. Zoo Biology, 37: 434–439.

Hall, T.A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95-98.

Hansson, L.J., Moeslund, O., Kiørboe, T. and Riisgård, H.U. 2005. Clearance rates of jellyfish and their potential predation impact on zooplankton and fish larvae in a neritic ecosystem (Limfjorden, Denmark). Marine Ecology Progress Series, 304: 117–131.

He, J., Zheng, L., Zhang, W., Lin, Y. and Cao, W. 2015. Morphology and molecular analyses of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) from the East China Sea. Journal of the Marine Biological Association of the United Kingdom, 95(2): 289–300.

Hebert, P.D., Cywinska, A., Ball, S.L. and deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proceedings. Biological sciences, 270(1512): 313–321.

Holst, S. and Jarms, G. 2007. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. Marine Biology, 151: 863–871.

Hoover, R.A., and Purcell, J.E. 2009. Substrate preferences of scyphozoan *Aurelia labiata* polyps among common dock-building materials. Hydrobiologia, 616(1): 259–267.

Huang, D., Meier, R., Todd, P.A. and Chou, L.M. 2008. Slow Mitochondrial COI Sequence Evolution at the Base of the Metazoan Tree and Its Implications for DNA Barcoding. Journal of Molecular Evolution, 66: 167–174.

Hwai, A.T.S., Peng, C.T.C., Nilamani, N., and Yasin, Z. 2019. Harmful Jellyfish Country Report in Western Pacific. Centre for Marine and Coastal Studies, Universiti Sains Malaysia, Penang, Malaysia. 61 pp.

Jitrapat, H. 2018. Abundance and diversity of gelatinous zooplankton in the Inner Gulf of Thailand. Master thesis, Department of Marine Science, Faculty of Science, Chulalongkorn University, 145 pp.

Kitajima, S., Iguchi, N., Honda, N., Watanabe, T. and Katoh, O. 2015. Distribution of *Nemopilema nomurai* in the southwestern Sea of Japan related to meandering of the Tsushima Warm Current. Journal of Oceanography, 71: 287–296.

Klomtong, P., Phasuk, Y., and Duangjinda, M. 2016. Animal Species Identification through High Resolution Melting Real Time PCR (HRM) of the Mitochondrial 16S rRNA Gene. Annals of Animal Science, 16(2): 415–424.

Kubota, S. 1978. The Life-History of *Clytia edwardsi* (Hydrozoa; Campanulariidae) in Hokkaido, Japan. Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology, 21(3): 317–354.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across

computing platforms. Molecular Biology and Evolution, 35(6): 1547–1549.

Laakmann, S. and Holst, S. 2014. Emphasizing the diversity of North Sea hydromedusae by combined morphological and molecular methods. Journal of Plankton Research, 36(1): 64–76.

Lianming, Z., Jinru, H., Yuanshao, L., Wenqing, C. and Wenjing, Z. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. Acta Oceanologica Sinica, 33(4): 55–76.

Lindsay, D.J., Grossmann, M.M., Nishikawa, J., Bentlage, B., and Collins, A.G. 2015. DNA barcoding of pelagic cnidarians: current status and future prospects. Bulletin of the Plankton Society of Japan, 62(1): 39–43.

Lindner, A. and Migotto, A.E. 2002. The life cycle of *Clytia linearis* and *Clytia noliformis*: metagenic campanulariids (Cnidaria: Hydrozoa) with contrasting polyp and medusa stages. Journal of the Marine Biological Association of the United Kingdom, 82: 541–553.

Lo, W.T., Purcell, J.E., Hung, J.J., Su, H.M. and Hsu, P.K. 2008. Enhancement of jellyfish (*Aurelia aurita*) populations by extensive aquaculture rafts in a coastal lagoon in Taiwan. ICES Journal of Marine Science, 65: 453–461.

Lucas, C.H., Graham, W. and Widmer, C.L. 2012. Jellyfish life histories: role of polyps in forming and maintaining scyphomedusa populations. In: Lesser, M. (Ed.) Advances in Marine Biology, Vol.63, Academic Press, 133–196.

Lucas, C.H., Williams, D.W., Wiliams, J.A. and Sheader, M. 1995. Seasonal dynamics and production of the hydromedusan *Clytia hemisphaerica* (Hydromedusa: Leptomedusa) in Southampton water. Estuaries, 18: 362–372.

Macher, J.N., Wideman, J.G., Girard, E.B., Langerak, A., Duijm, E., Jompa, J., Sadekov, A., Vos, R., Wissels, R., and Renema, W. 2021. First report of mitochondrial COI in foraminifera and implications for DNA barcoding. Scientific reports, 11(1): 22165.

Mariac, C., Vigouroux, Y., Duponchelle, F., García-Dávila, C., Nunez, J., Desmarais, E., and Renno, J.F. 2018. Metabarcoding by capture using a single COI probe (MCSP) to identify and quantify fish species in ichthyoplankton swarms. PLOS ONE, 13(9): e0202976.

Marine and Coastal Resources Research and Development Institute. 2015a. Thai Marine and Coastal Resources Scriptures 2015. Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment, Bangkok, 246 pp.

Marine and Coastal Resources Research and Development Institute. 2015b. Guide to the diversity of jellyfish in the waters Thailand. Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment, Bangkok, 127 pp.

Mendoza-Becerril, M.A., Estrada-GonzÁlez, M.C., Mazariegos-Villarreal, A., Restrepo-AvendaÑo, L., Villar-BeltrÁn, R.D., AgÜero, J., and Cunha, A.F. 2020. Taxonomy and diversity of Hydrozoa (Cnidaria, Medusozoa) of La Paz Bay, Gulf of California. Zootaxa, 4808(1): 1–37.

Miglietta, M.P., Rossi, M. and Collin, R. 2008. Hydromedusa blooms and upwelling events in the Bay of Panama, Tropical East Pacific. Journal of Plankton Research, 30(7): 783–793.

Moura, C.J., Harris, D.J., Cunha, M.R. and Rogers, A.D. 2008. DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. Zoologica Scripta, 37: 93–108.

Moura, C.J., Lessios, H., Cortés, J., Nizinski, M.S., Reed, J., Santos, R.S., and Collins, A.G. 2018. Hundreds of genetic barcodes of the species-rich hydroid superfamily Plumularioidea (Cnidaria, Medusozoa) provide a guide toward more reliable taxonomy. Scientific reports, 8(1): 17986.

Muhammad, B.L., Seo, Y. and Ki, J.S. 2021 Evaluation ofthe complete nuclear rDNA unit sequence of the jellyfish *Cyanea nozakii* Kishinouye (Scyphozoa: Semaeostomeae) for molecular discrimination, Animal Cells and Systems, 25:6, 424–433.

National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 202 0 Sep 13]. Available from: https://www.ncbi.nlm.nih.gov/

Ortman, B.D., Bucklin, A., Pages, F. and Youngbluth, M. 2010. DNA Barcoding the Medusozoa using mtCOI. Deep-Sea Research Part II, 57: 2148–2156.

Pappalardo, P., Collins, A.G., Pagenkopp Lohan, K.M., Hanson, K.M., Truskey, S.B., Jaeckle, W., Ames, C.L., Goodheart, J.A., Bush, S.L., Biancani, L.M., Strong, E.E., Vecchione, M., Harasewych, M.G., Reed, K., Lin, C., Hartil, E.C., Whelpley, J., Blumberg, J., Matterson, K., Redmond, N.E., Becker, A., Boyle, M.J., and Osborn, K.J. 2021. The role of taxonomic expertise in interpretation of metabarcoding studies. ICES Journal of Marine Science, doi:10.1093/icesjms/fsab082.

Parracho, T., and Morais, Z. 2015. *Catostylus tagi*: partial rDNA sequencing and characterisation of nematocyte structures using two improvements in jellyfish sample preparation. Journal of Venomous Animals and Toxins Including Tropical Diseases, 21: 40.

Patithanarak, T., Luang-on, N., Manhaphol, A., Taleb, S. and W. 2014. Molecular Identification of Colorful Jellyfish along the Coast of Trat Province. The 4th Marine Science Conference, Prince of Songkhla University, Songkhla, 287–296.

Phongphattarawat, S. 2013. Species diversity and abundance of zooplankton Class Hydrozoa (Cnidaria: Hydrozoa) in the Inner Gulf of Thailand. Senior project, Department of Biology, Faculty of Science, Chulalongkorn University, 79 pp.

Phuangsanthia, W., Choosri, S., Chunam, W. and Sripanomyom, J. 2018. Life Cycle and Development of Upside-Down Jellyfish, *Cassiopea andromeda* (Forsskal, 1775) in Laboratory Conditions. Burapha Science Journal, 23(2): 1178–1187.

Phuangsanthia, W., Choosri, S., Fakrajang, S. and Muthuwan, V. 2020. Life cycle of the edible jellyfish *Rhopilema hispidum* (Vanhöffen, 1888). Khon Kaen Agriculture Journal, 48(Suppl.1): 907–916.

Pinto, C.D.C., 2021. The effect of temperature and substrates on polyp settlement of the spotted jellyfish, *Phyllorhiza punctata* (Von Lendenfeld, 1884). Master thesis in Aquaculture, Politécnico de Leiria, Portugal. 30 pp.

Pitt, K.A., Lucas, C.H., Condon, R.H., Duarte, C.M., and Stewart-Koster, B. 2018. Claims That Anthropogenic Stressors Facilitate Jellyfish Blooms Have Been Amplified Beyond the Available Evidence: A Systematic Review. Frontiers in Marine Science, 5: 451.

Purcell, J.E., Uye, S. and Lo, W.T. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology Progress Series, 350: 153–174.

Radulovici, A.E., Archambault, P. and Dufresne, F. 2010. DNA Barcodes for Marine Biodiversity: Moving Fast Forward? Diversity, 2: 450–472.

Ran, K., Li, Q., Qi, L., Li, W., and Kong, L. 2020. DNA barcoding for identification of marine gastropod species from Hainan Island, China. Fisheries Research, 225: 105504.

Richardson, A.J., Bakun, A., Hays, G.C. and Gibbons, M.J. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. Trends in Ecology and Evolution, 24(6): 312–322.

Ruijuan, L., Jie, X., Xuelei, Z. and Aungtonya, C. 2016. Genetic analysis of common venomous Cubozoa and Scyphozoa in Thailand waters. Haiyang Xuebao. 38(6): 51-61. (in Chinese with English abstract).

Sathirapongsasuti, N., Khonchom, K., Poonsawat, T., Pransilpa,M., Ongsara, S., Detsri, U., Bungbai, S., Lawanangkoon, S.-a., Pattanaporkrattana,W. and Trakulsrichai, S. 2021. Rapid and Accurate Species-Specific PCR for the Identification of Lethal Chironex Box Jellyfish in Thailand. International Journal of Environmental Research and Public Health, 18, 219.

Schuchert, P. 2003. Hydroids (Cnidaria, Hydrozoa) of the Danish expedition to the Kei Islands. Steenstrupia, 27(2): 137–256.

Schuchert, P. 2018. DNA barcoding of some Pandeidae species (Cnidaria, Hydrozoa, Anthoathecata). Revue Suisse De Zoologie, 125(1): 101–127.

Schuchert, P. 2020. World Hydrozoa Database, Flanders Marine Institute. Available from: http://www.Marinespecies. org/hydrozoa/, (13 August 2020).

Sojisuporn, P., Morimoto, A. and Yanagi, T. 2010. Seasonal variation of sea surface current in the Gulf of Thailand. Coastal Marine Science, 34(1): 91–102.

Song, F., Jianing, L., Song, S. and Fang, Z. 2017. Artificial substrates preference for proliferation and immigration in *Aurelia aurita* (s. 1.) polyps. Journal of Oceanology and Limnology, 35(1): 153–162.

Sun, S., Zhang, F., Li, C.L., Wang, S.W., Wang, M.X., Tao, Z.C., Wang, Y.T., Zhang, G.T. and Sun, X.X. 2015. Breeding place, population development and distribution pattern of the giant jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in the Yellow Sea and East China Sea. Hydrobiologia, 754: 59–74.

Sutherland, K.R., Gemmell, B.J., Colin, S.P. and Costello, J.H. 2016. Prey capture by the cosmopolitan hydromedusae, *Obelia* spp., in the viscous regime. Limnology and Oceanography, 61: 2309–2317.

Toshino, S., Nishikawa, J., Srinui, K., Taleb, S. and Miyake, H. 2019. New records of two species of Cubozoa from Thailand. Plankton and Benthos Research, 14(3): 143–149.

Uye, S. 2008. Blooms of the giant jellyfish *Nemopilema nomurai*: a threat to the fisheries sustainability of the East Asian Marginal Seas. Plankton Benthos Research, 3 (Suppl.): 125–131.

Uye, S. 2010. Jellyfish Blooms in East Asian Coastal Seas: Loss of Productive Ecosystem. International Symposium on Integrated Coastal Management for Marine Biodiversity in Asia, Kyoto, Japan, 16–19.

van Walraven, L., Driessen, F., van Bleijswijk, J., Bol, A., Luttikhuizen, P.C., Coolen, J.W., Bos, O.G., Gittenberger, A., Schrieken, N., Langenberg, V.T., and van der Veer, H.W. 2016. Where are the polyps? Molecular identification, distribution and population differentiation of *Aurelia aurita* jellyfish polyps in the southern North Sea area. Marine biology, 163: 172.

van Walraven, L., van Bleijswijk, J., van der Veer, H.W. 2020. Here are the polyps: in situ observations of jellyfish polyps and podocysts on bivalve shells. PeerJ 8: e9260.

Wilson, E. 2002. *Obelia bidentata* A hydroid. In Tyler-Walters, H. and Hiscock, K. (eds.) Marine Life Information Network: Biology and Sensitivity Key Information Reviews, Plymouth: Marine Biological Association of the United Kingdom. Available from: http://www.marlin.ac.uk/species/detail/1270/, (10 August 2020)

Wutticharеonmongkol, N. 2004. Diversity and Abundance of Planktonic Hydrozoan, Class Hydrozoa, in the Upper Gulf of Thailand. Master Thesis, Graduate School, Kasetsart University, Bangkok, 265 pp.

Zelickman, E.A. 1972. Distribution and Ecology of the Pelagic Hydromedusae, Siphonophores and Ctenophores of the Barents Sea, Based on Perennial Plankton Collections. Marine Biology, 17: 256–264.

Zhao, L., Zhang, X., Xu, M., Mao, Y. and Huang, Y. 2021. DNA metabarcoding of zooplankton communities: species diversity and seasonal variation revealed by 18S rRNA and COI. PeerJ, 9: e11057.

Zheng, L., He, J., Lin, Y., Cao, W., and Zhang, W. 2014. 16S rRNA is a better choice than COI for DNA barcoding hydrozoans in the coastal waters of China. Acta Oceanologica Sinica, 33(4): 55–76.

Zhen-zu, X., Jia-qi, H., Mao, L., Donghui, G. and Chunguang, W. 2014. The superclass hydrozoa of the phylum Cnidaria in China, Vol.2, China Ocean Press, Beijing, 945 pp.

Zhou, K., Zheng, L., He, J., Lin, Y., Cao, W. and Zhang, W. 2013. Detection of a new *Clytia* species (Cnidaria: Hydrozoa: Campanulariidae) with DNA barcoding and life cycle analyses. Journal of the Marine Biological Association of the United Kingdom, 93(8): 2075–2088.

Zou, R., Liang, C., Dai, M., Wang, X., Zhang, X. and Song, Z. 2020. DNA barcoding and phylogenetic analysis of bagrid catfish in China based on mitochondrial COI gene, Mitochondrial DNA Part A, 31(2): 73-80.