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THE GENUS *Chrysaora* (SEMAEOSTOMEAE: PELAGIIDAE) IN COASTAL WATERS OF THE ANDAMAN SEA AND THE GULF OF THAILAND

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ABSTRACT: The scyphozoan jellyfish in the genus *Chrysaora* were collected from the coastal waters of the Andaman Sea from 2008 to 2018 and the Gulf of Thailand from 2014 to 2019. The total of 959 specimens were examined, of which 834 specimens were from the coastal waters of the Andaman Sea and 125 specimens from the Gulf of Thailand. The following characters were used to differentiate species of the genus *Chrysaora* from each other: shape and number of marginal lappets, number of marginal tentacles, number of tentacles per octant, the presence of quadralinga, shape of radial septa, shape of gonads, and size of nematocysts. The morphological identification indicated that all of the specimens belong to a single species, *Chrysaora chinensis*, which was supported by the results from the molecular DNA sequence analysis. The 16S and 18S sequences were deposited in NCBI genebank as KT982700–KT982711 and KT982732–KT982743, respectively.

Key words: *Chrysaora*, Scyphozoa, Andaman Sea, Gulf of Thailand

INTRODUCTION

The genus *Chrysaora* is a scyphozoan jellyfish, belonging to the Order Semaeostomeae and Family Pelagiidae, first described by Péron and Lesueur in 1810. Jellyfish of this genus are characterized by the following features: 32–48 (or more) simple marginal lappets, eight marginal sense organs, three or more (up to nine) tentacles between each successive pair of marginal sense organs, 16 radial stomach pouches with eight rhopalar pouches narrower than eight tentacular pouches in the marginal zone.

In 2010, Morandini and Marques reported 15 species of *Chrysaora* in their publication. Though the genus widely inhabits most of the world's oceans, many species have been recorded only from restricted geographic areas (Morandini and Marques 2010) *e.g*., *C. kynthia*, *C. pentastoma*, and *C. wurlerra* in South Australia waters (Gershwin and Zeidler 2008), *C. chinensis* and *C. pacifica* in the western Pacific Ocean (Morandini and Marques 2010; Lee *et al.* 2016; Rizman-Idid *et al*. 2016; Low *et al*. 2019). The main characters used to identify each member of the genus include bell diameter, number of all marginal tentacles, number of marginal tentacles per octants, number and shape of all marginal lappets, number of marginal lappets per octant, shape of radial septa, shape and size of gonads, presence or absence of quadralinga and type and size of tentacle nematocysts (Morandini and Marques 2010).

In the Andaman Sea, *Chrysaora* sp. A, *Chrysaora* sp. B, and *Chrysaora* sp. C were reported by Aungtonya and Chanachon in 2012. The only difference between these putative species was the shape of their rhopalia (Aungtonya and Chanachon 2012; fig. 18 A–C). The only DNA sequence of *C. chinensis* from the Andaman Sea was published by Gómez Daglio and Dawson (2017). *Chrysaora*

chinensis was also reported from Malaysian waters by Rizman-Idid *et al*. in 2016.

The aims of this study are to morphologically describe and assess the genetic diversity of *Chrysaora* from the Andaman Sea and the Gulf of Thailand as part of the continuing effort to understand the genetic diversity and also to DNA barcode the jellyfish in Thai waters.

MATERIALS AND METHODS

Specimen collection and morphological study

A total of nine hundred and fifty-nine specimens were investigated in this study and were collected as part of jellyfish distribution surveys in the Andaman Sea and the Gulf of Thailand. The monitoring project was carried out during the period 2008–2018 in the Andaman Sea and 2014–2019 in the Gulf of Thailand. The sampling sites covered 14 provinces from both water bodies of Thailand: the Andaman Sea (Ranong, Phang-nga, Phuket, Krabi, Trang, Satun) and the Gulf of Thailand (Trat, Chanthaburi, Rayong, Chonburi, Phetchaburi, Chumphon, Suratthani and Songkhla) (Fig. 1). Most of the samples were collected with shrimp trammel nets and hand nets, while some were obtained from local fishermen caught by typical fishing gears such as fishing nets, crab nets, etc. After tissue samples were taken for DNA analysis, the jellyfish were then fixed and preserved in 3% formalin solution. The sampling was done during Southwest and Northeast monsoon seasons (May to October and November to April, respectively) in both the Andaman Sea and the Gulf of Thailand.

The coloration pattern on the majority of the specimens were observed on some of the specimens. They were photographed before specimen's fixation. Furthermore, underwater images were taken on some specimens. The specimens were fixed and preserved in 3% formalin.

Investigated characteristics included body diameter size, number and shape of all marginal tentacles, number of marginal tentacles per octant, number and shape of all marginal lappets, number of marginal lappets per octant, shape of radial septa, shape and size of gonads, presence or absence of quadralinga (Morandini and Marques 2010) and type and size of tentacle nematocysts (Östman and Hydman 1997; Östman 2000). Bell diameter size was measured with a digital caliper in millimeters and statistically described. The nematocysts were observed and measured using a light microscope at up to 400x magnification.

DNA extraction, PCR amplification and sequencing

The tissues were prepared from 12 randomly selected *Chrysaora* specimens before they were fixed in 3% formalin. A small piece (0.5 cm x 0.5 cm) of tentacle or mesoglea was cut off from the selected specimens, and preserved in 99% alcohol at a ratio of 10-acl: 1 tissue (V:V). The fixed tissues were stored at 4 degree Celsius for DNA sequence analysis.

The genomic DNA was extracted using the commercial animal tissue extraction kit (OMEGA bio-tek, Inc., USA) or a CTAB/chloroform method described by Dawson *et al*. (1998). The nuclear 18S and mitochondrial 16S gene fragments were amplified using the primer pairs: L18S: 5'-CGGAAGGGCACCACCAGGAG-3',18Sb: 5'-GATCCTTCTGCAGGTTCACCTAC-3' and BRDGP-1:5'-TCGACTGTTTACCAAAAACATAGC-3',BRDGP-2: 5'-ACGGAATGAACTCAAATCATGTAAG-3', respectively (Bayha 2005; Bayha *et al*. 2010). PCR reactions were performed on the thermal cycler (Bio-Rad MyCycler (USA)) following the optimized protocol in Liu *et al*. (2016). The amplicons were directly sequenced bi-directionally on the ABI3130XL genetic analyzer (Applied Biosystems, Inc., USA). In cases where direct sequencing was not applicable, amplicons were cloned using the TA cloning kits (Takara Biomedical Technology Co., Ltd., China) and then sequenced.

DNA Sequence analysis

The resulting DNA sequences were cleaned for vector, primers and ambiguous nucleotides and subsequently aligned with the reference sequences downloaded from GenBank using ClustalW algorithm (Thompson *et al*. 1994). The phylogenetic relationship between unknown specimen and references was analyzed using maximum likelihood (ML) and neighbor joining (NJ) methods. The nucleotide substitution model of Tamura 3-parameter based on the BIC criterion was applied for the phylogenetic analyses. Robustness of resulting phylogenetic trees were assessed by bootstrapping for 1000 replicates. The best assignment of the unknown specimens were hypothesized, and the results from different genes were compared to evaluate the consistency of the conclusions.

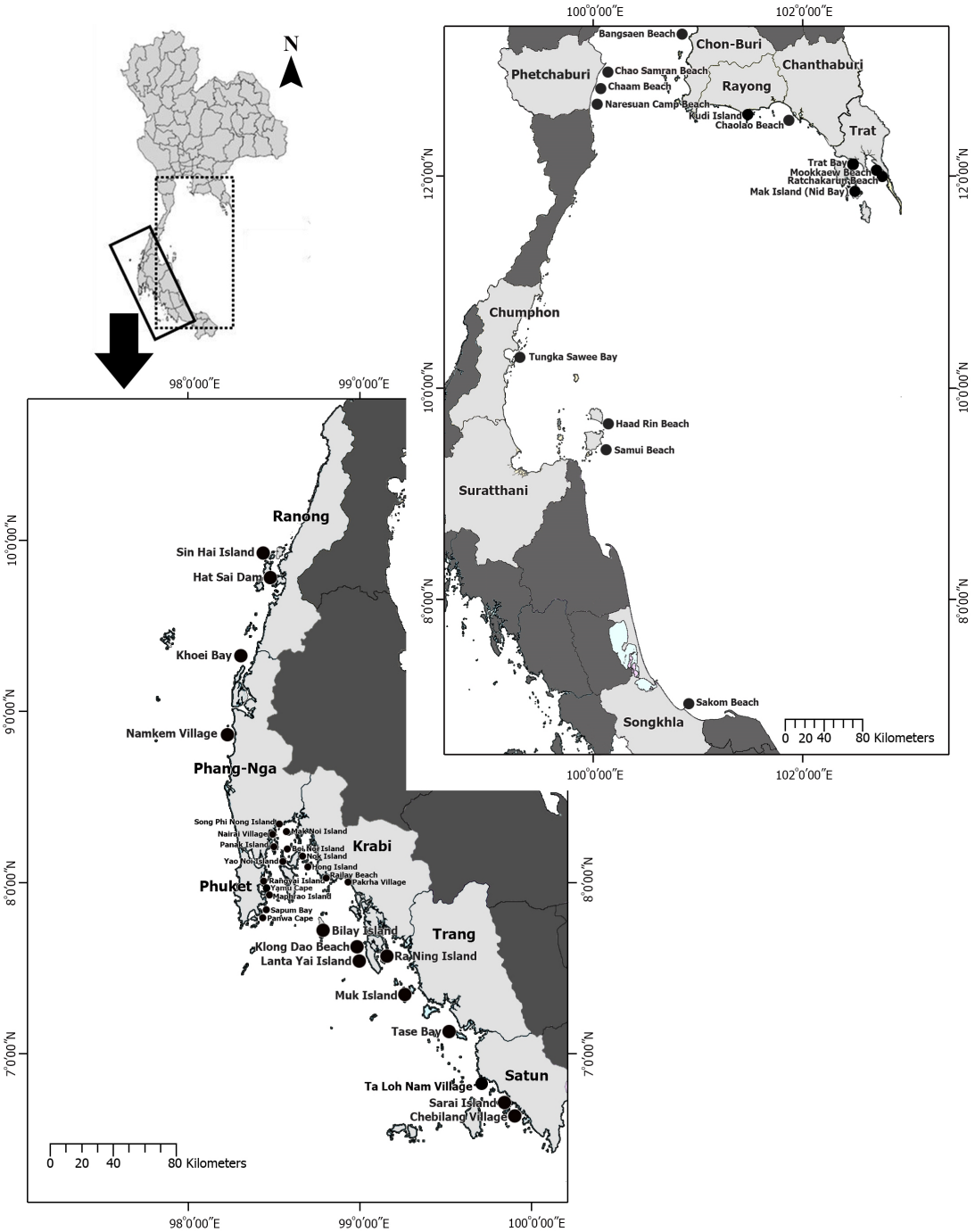

Figure 1. Localities of the Andaman Sea and the Gulf of Thailand where *Chrysaora* samples were collected.

RESULTS

A total of 959 specimens (834 specimens from the Andaman Sea and 125 specimens from the Gulf of Thailand) were examined. They have umbrella flatter than a hemisphere with 24 tentacles (Fig. 2). Shape and number of marginal lappets, number of marginal tentacles, number of tentacles

per octant, the presence of quadralinga, shape of radial septa, shape of gonads, and size of nematocysts in all specimens matched with *C. chinensis*, which was described by Morandini and Marques (2010), Östman and Hydman (1997), Östman (2000). The 16S and 18S sequences supported the morphological study and suggested a single *C. chinensis* species existing in Thai waters.

Bell diameters of the specimens from the Andaman Sea ranged from 24 mm to 240 mm, while those from the Gulf of Thailand lie between 52 mm and 185 mm. The smallest and largest specimens were both collected from the Andaman Sea, in Ranong and Krabi Province, respectively. The average bell size was 88.30 ± 24.35 mm in the Andaman Sea and 102.8 ± 22.88 mm in the Gulf of Thailand (Appendix 1).

The specimens from the upper part of the Andaman Sea (Ranong, Phang-nga, and Phuket Provinces) exhibited a trend for larger umbrella size during the northeast monsoon, whereas those from the lower Andaman Sea (Krabi, Trang, and Satun Provinces) were apparently larger during the southwest monsoon season (Fig. 3). The differences, however, were statistically not significant. As for the Gulf of Thailand, no size trends were detected due to the small numbers of specimens from the area.

About 50 lots of *C. chinensis* materials were selected for deposit in the PMBC Reference Collection (Appendix 2).

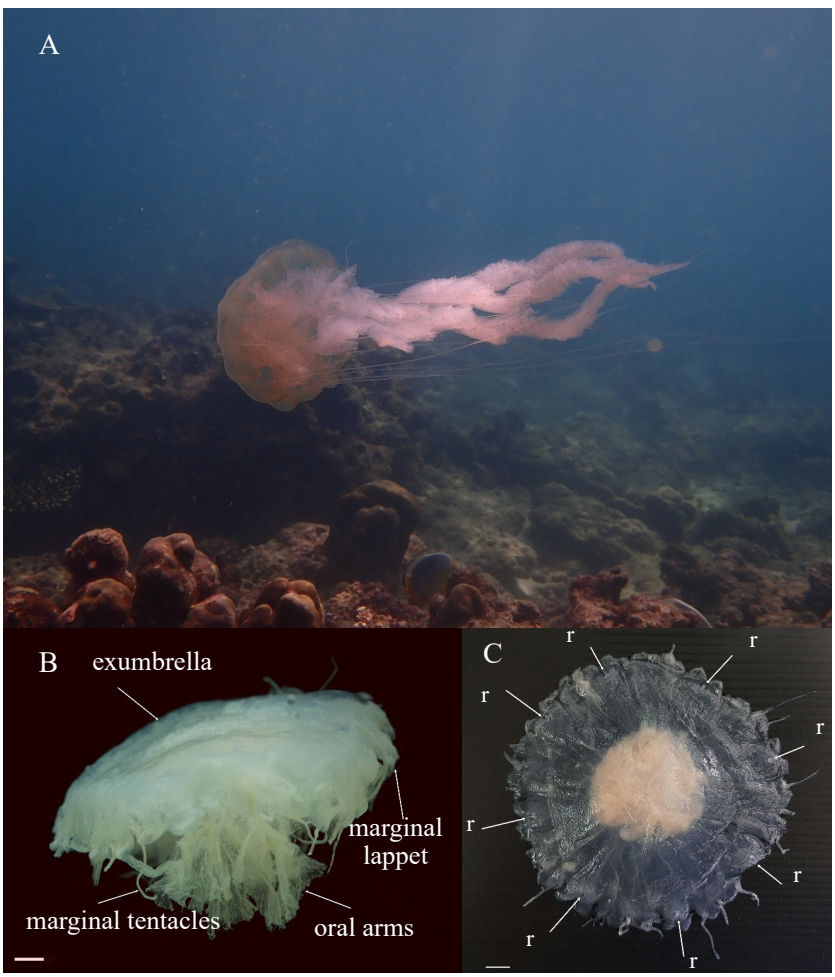

Figure 2. *Chrysaora chinensis*. A. In coral reef at Bang Tao Bay, Phuket; B. General morphological characters; C. Exumbrella (finely granulated surface with eight rhopalia (r)). A: photographed by Mr. Chaimongkol Yaemarunpattana; B–C: PMBC 20820. Scale bar = 1 cm.

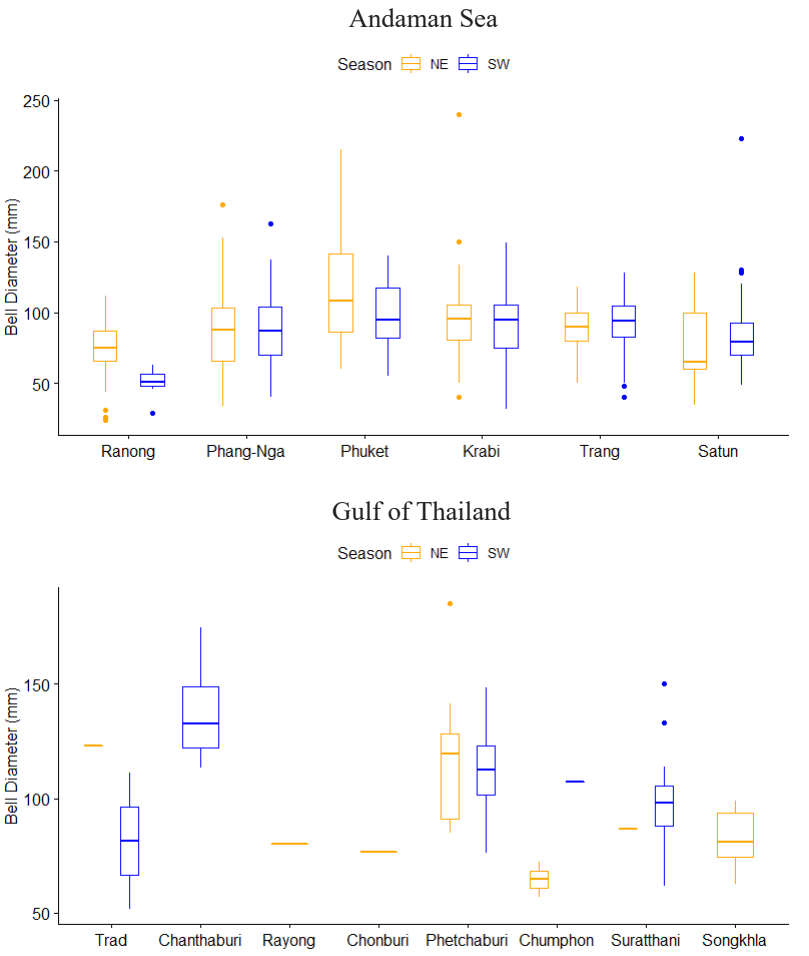

Figure 3. Average bell diameter of *Chrysaora chinensis* collected from the Andaman Sea from 2008 to 2018 and the Gulf of Thailand from 2014 to 2019.

TAXONOMY

Family Pelagiidae Gegenbaur, 1856

Genus *Chrysaora* Péron and Lesueur, 1810

Type species. *Chrysaora chinensis* Vanhöffen, 1888

Diagnosis

Pelagiidae with an average body diameter of 10 cm; marginal lappets slightly elongated, up to 6 per octant; marginal tentacles 24–40 (3–5 per octant), clefts with similar depth; quadralinga absent. The only known distinction between *C. chinensis* and *C. pacifica* is the size of the holotrichous O-isorhiza nematocysts, which are two times smaller in *C. pacifica.* The average of holotrichous O-isorhiza nematocysts of *C. chinensis* is 19.17 x 16.65 µm (N=3); range 13.7–29.4 x 12.7–24.5 µm (Morandini and Marques, 2010).

Chrysaora chinensis Vanhöffen, 1888

Figs. 2A–B, 4A–B, 5–6, Appendix 3

Chrysaora sp. A - Aungtonya and Chanachon, 2012: 21–22, figs. 14–18, table 6.

Chrysaora sp. B - Aungtonya and Chanachon, 2012: 23, figs. 14–18, table 6.

Chrysaora sp. C - Aungtonya and Chanachon, 2012: 23, figs. 15–18, table 6.

Material examined

A total of 959 specimens examined were collected from the Andaman Sea and the Gulf of Thailand and preserved in good condition.

Description

Umbrella diameters between 2.4 and 24 cm, umbrella flatter than a hemisphere (Fig. 2A). Exumbrellar surface finely granulated (Fig. 2B), their colorations either pinkish or milky translucent, or translucent (in preserved specimens). Marginal lappets elongated, 4–6 per octant (Fig. 4A). Tentacles 24 (3 per octant). Rhopalia 8 (Figs. 2C, 4B), without ocelli. Quadralinga absent. Radial septa thin; rounded at base; straight up to 1/4 of margin, then making an "S"; ending near tentacular base at rhopalar lappet (Fig. 4B). Gonads greatly folded and yellowish (Fig. 5). Holotrichous O-isorhiza nematocysts measurement showed the average of 16.84 x 15.48 µm and range between 12.2–26.84 x 12.2–26.84 µm (N=30) (Fig. 6).

Remarks

Some specimens have smooth exumbrella surface, which is probably the result of long-time preservation. Each octant is of different sizes in most individuals. Approximately, 2.15% of the specimens from the Andaman Sea exhibit abnormal numbers of rhopalia (5–7, 9–12), which affect the shapes and numbers of lappets, tentacles and radial septa. Characteristic of tentacles varies; some are thick, while others are thin; translucent and transparent tentacles are also present.

Distribution

Western Pacific Ocean, China (South China Sea), Indonesia (Sumatra), Philippines, Malaysia, Thailand (Andaman Sea and Gulf of Thailand).

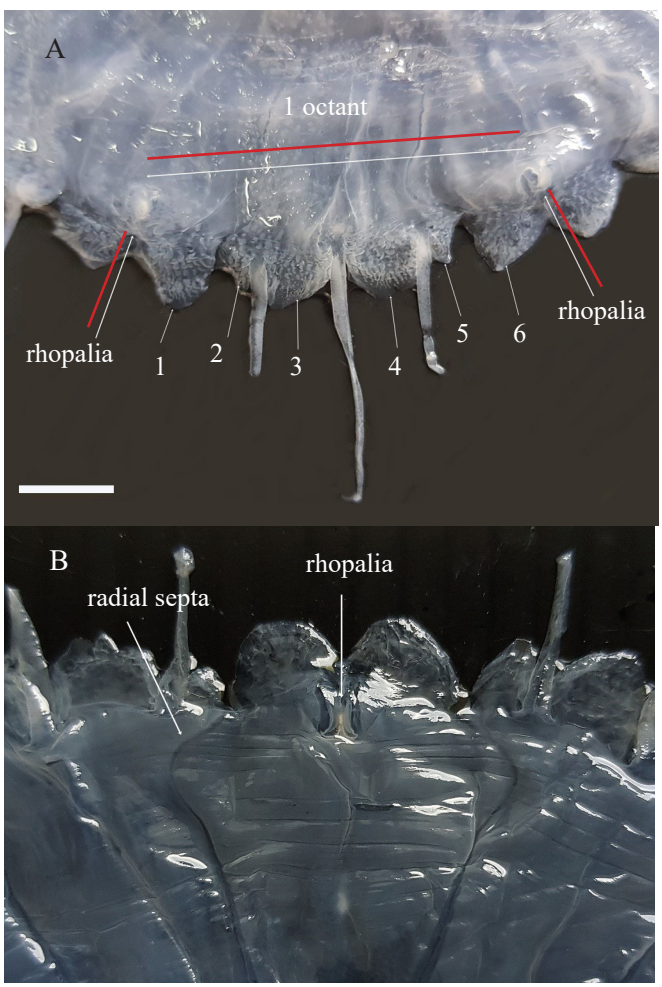

Figure 4. *Chrysaora chinensis*, PMBC 20820: A. Shape and number of marginal lappets and marginal tentacles per octant (scale bar = 1 cm); B. Radial septa.

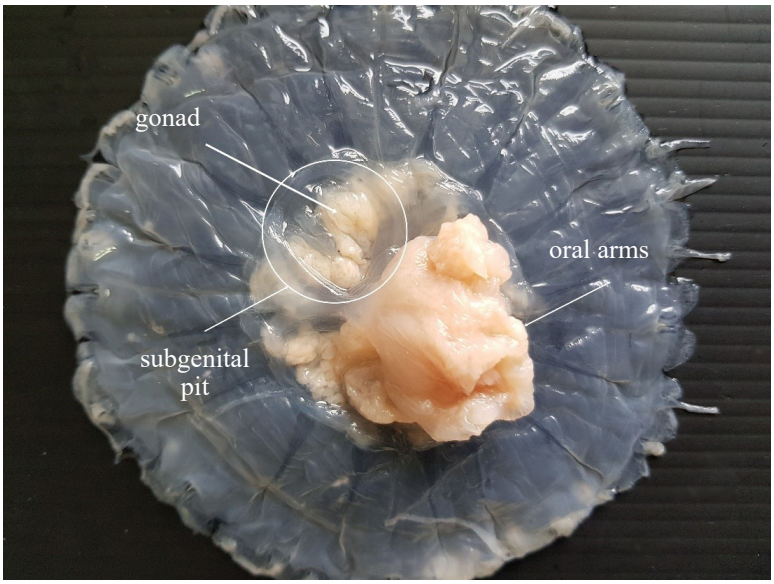

Figure 5. Gonads of *Chrysaora chinensis*, PMBC 20820.

Figure 6. Holotrichous O-isorhiza nematocysts of *Chrysaora chinensis*, PMBC 20820.

Sequence phylogeny

The partial sequences of 16S and 18S genes were generated from 12 samples of *Chrysaora* specimens from the Andaman Sea (PMBC 3246 (18S-KT982732 and 16S-KT982700), PMBC 3247 (18S-KT982732 and 16S-KT982700), PMBC 3248 (18S-KT982732 and 16S-KT982700), PMBC 30395 (18S-KT982732 and 16S-KT982700), PMBC 3249 (18S-KT982732 and 16S-KT982700), PMBC 3250 (18S-KT982732 and 16S-KT982700), PMBC 3251 (18S-KT982732 and 16S-KT982700), PMBC 3253 (18S-KT982732 and 16S-KT982700), PMBC 30393 (18S-KT982732 and 16S-KT982700), PMBC 30392 (18S-KT982732 and 16S-KT982700), PMBC 3258 (18S-KT982732 and 16S-KT982700), PMBC 3259 (18S-KT982732 and 16S-KT982700) (Appendix 2) and aligned with relevant sequences of *Chrysaora* species retrieved from the NCBI database. All 12 samples were grouped with the other *C. chinensis* in both phylogenetic trees of 16S and 18S genes with high bootstrap support (93%–98%, Figs. 7–8), suggesting a single *C. chinensis* species existing in Thai waters. Biography of the deposited *C. chinensis* sequences indicated that this species was widespread

off the coasts of tropical southeastern Asia, including Thailand and Malaysia (Rizman-Idid *et al*., 2016;). Whereas, the phylogenetic relationships among the *Chrysaora* spp. and the other genera (*Pelagia*, *Sanderia* and *Mawia etc*.) in Pelagiidae were still under investigation (Bayha *et al*., 2017; Daglio *et al*., 2017).

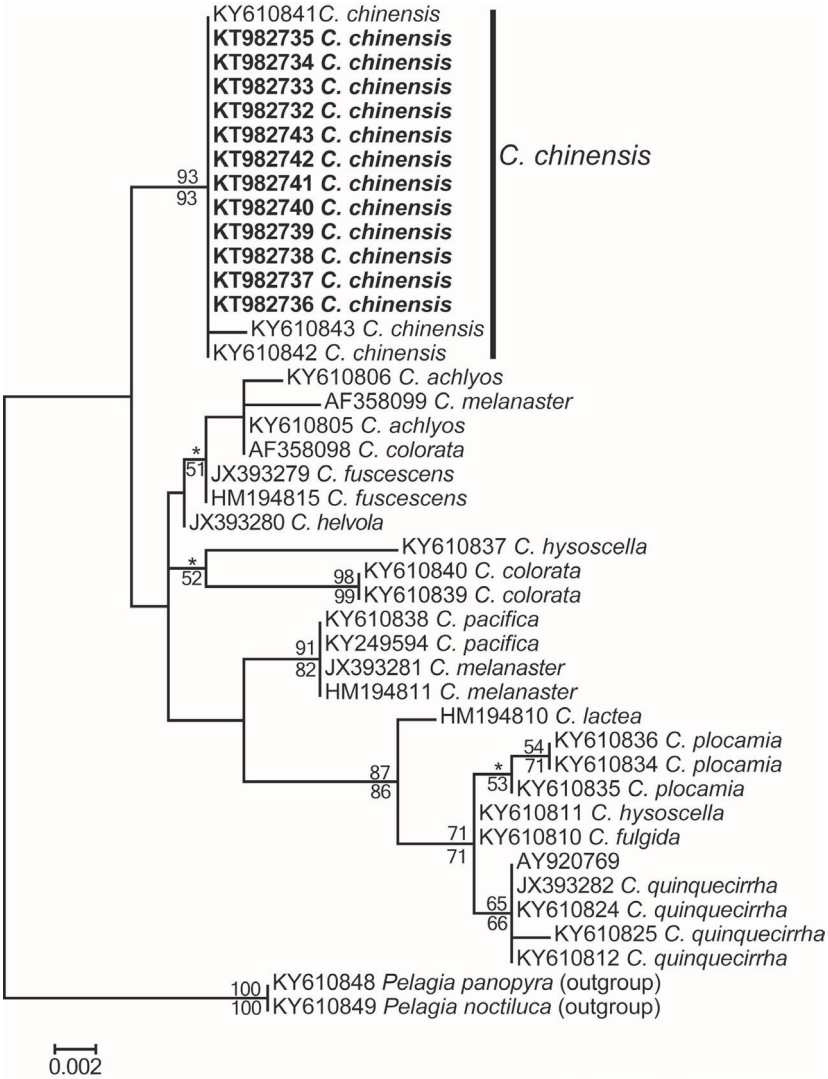

Figure 7. Phylogenetic tree based on the partial 18S gene sequences. Numbers on each node are bootstrapping support for NJ (above) and ML (below) analyses. Values larger than 50% were shown and * denotes those less than 50%. The sequences of *Pelagia panopyra* (KY610804) and *Pelagia noctiluca* (KY610849) are treated as the outgroup. The **bold** fonts are derived from this study.

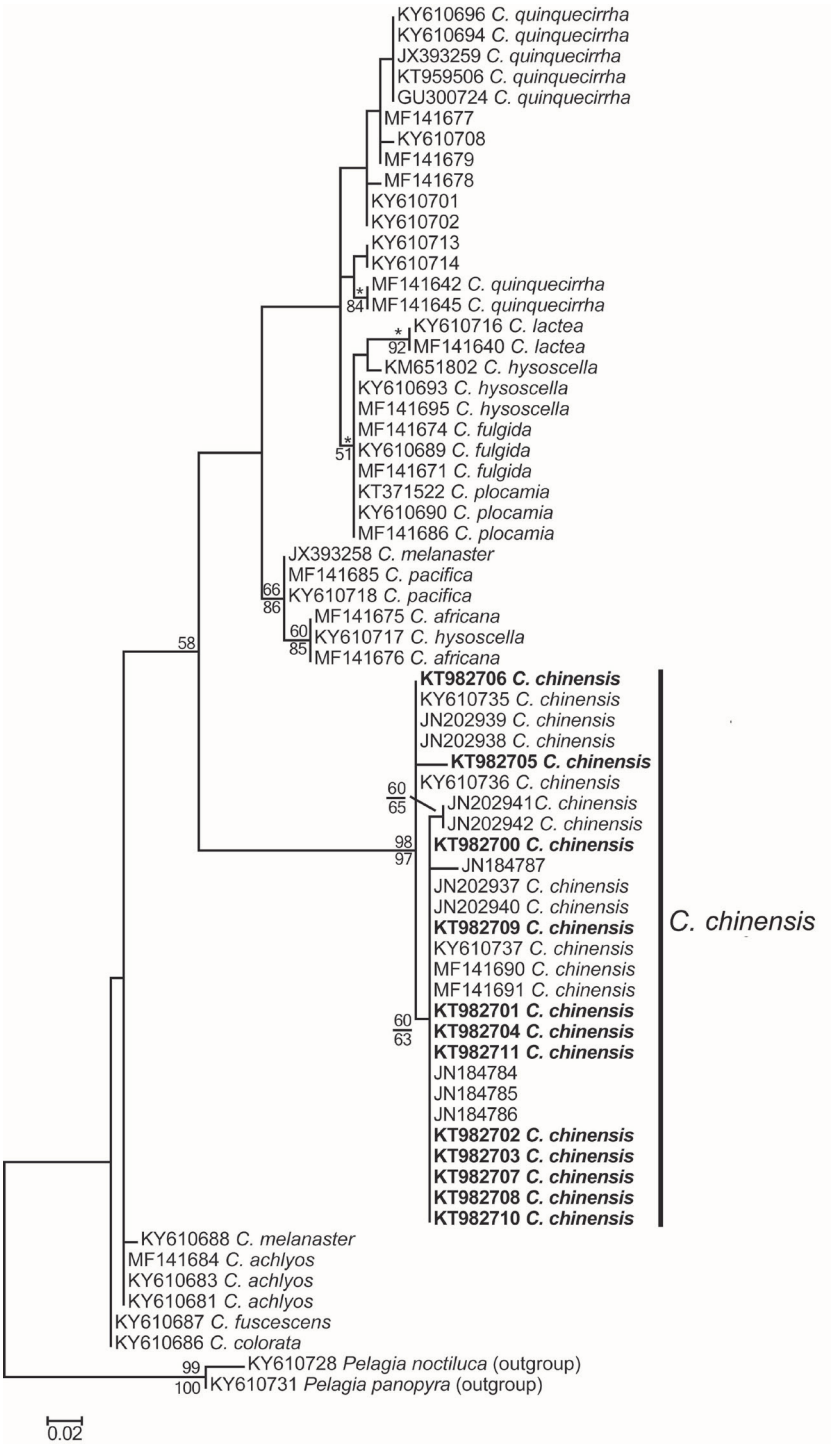

Figure 8. Phylogenetic tree based on the partial 16S gene sequences. Numbers on each node are bootstrapping support for NJ (above) and ML (below) analyses. The sequences of *Pelagia panopyra* (KY610731) and *Pelagia noctiluca* (KY610728) are treated as the outgroup. The bold fonts are derived from this study.

DISCUSSION AND CONCLUSION

Chrysaora chinensis was first described by Vanhöffen in 1888 based on a single specimen from Hong Kong, which could not be located. The genus was revised in 2010 by Morandini and Marques, in which a neotype specimen was designated, and additional specimens were observed and documented. The morphology of *C. chinensis* specimens inspected during this study agrees largely with the description of Morandini and Marques (2010). However, the exumbrella surfaces which were described as finely granulated in their report, were smooth in our specimen, which is probably the result of long-time preservation. Additionally, the size of the holotrichous O-isorhiza nematocysts which, according to Morandini and Marques (2010), is the only known distinction between *C. chinensis* and *C. pacifica* were also measured. The results clearly indicated that our specimens belong to the species *C. chinensis*. The identifying characteristics of *C. chinensis*, including other species in the genus were mentioned in Appendix 3.

This report will be the first official record of *C. chinensis* in the Andaman Sea, whose distribution was previously limited to the Western Pacific Ocean. Although there were some inconsistencies regarding the specimens from the Andaman Sea; such as the umbrella diameters which exceeded 16 cm than those given in Morandini and Marques, and the colouration and patterns of the exumbrella which were mostly undocumented; DNA sequence data suggested they belong to the same species as the specimens from the Gulf of Thailand, *C. chinensis.*

The results from both the morphological and meristic data indicate the specimens examined to be *C. chinensis.* However, 2.15% of the specimens from the Andaman Sea exhibited abnormal numbers of rhopalia (5–7, 9–12); some tentacles were thick, while others were thin; translucent and transparent tentacles were also present. Nevertheless, the results of the 16S and 18S sequence analyses confirmed them as *C. chinensis.*

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Appendix 1. Number of specimens and bell diameter of *Chrysaora chinensis* from 6 provinces along the Andaman Sea during 2008 – 2018 and from 8 provinces along the Gulf of Thailand during 2014 – 2019.

Years	Ranong				Phang-nga				Phuket			
	No. of specimens	Bell diameter (mm)			No. of specimens	Bell diameter (mm)			No. of specimens	Bell diameter (mm)		
		min	max	mean±sd		min	max	mean±sd		min	max	mean±sd
2009									2	116	120	118.0± 2.83
2010					1	-	-	95.00	10	80	130	101.5±18.19
2011					41	60	130	90.24±16.74	2	90	95	92.50± 3.536
2012					107	50	163	96.22±21.18	4	60	215	134.0± 75.64
2013					36	73	176	109.1±22.35				
2014	37	26	112	78.00± 18.42	79	34	125	70.95±22.11	7	55	95	71.00± 17.50
2015	20	24	102	60.50± 20.31	76	40	120	76.33±19.21	1	-	-	140.0
2016									3	117	145	130.7±14.01

	Krabi				Trang				Satun			
2008	13	33	149	98.38± 30.91								
2009												
2010	7	60	120	93.29±23.49					31	50	223	81.26±31.39
2011	26	32	107	76.42±18.93					14	35	119	70.57±25.37
2012	11	61	240	115.4±47.91								
2013	69	43	134	96.65±19.12	151	50	128	95.70± 14.23				
2014	5	50	126	76.40± 31.03	54			81.91±18.68	4	103	128	112.0±11.34
2016	1	-	-	114.0								
2018									22	49	128	88.05±21.96

Appendix 1. (Continued).

Years	Trat				Chanthaburi				Rayong			
	No. of specimens	Bell diameter (mm)			No. of specimens	Bell diameter (mm)			No. of specimens	Bell diameter (mm)		
		min	max	mean± sd		min	max	mean±sd		min	max	mean± sd
2014	2	52.61	123.0	87.31±50.48	4	113.3	174.5	138.2±26.59				
2016									1	-	-	80.18
2017												
2018	1	-	-	113.3								
2019												
	Chonburi				**Phetchaburi**				**Chumphon**			
2016	1	-	-	76.44	6	92.00	148.4	118.3±19.53				
2017					1	-	-	185.3				
2018					54	76.25	148.1	110.0±16.28				
2019					3	85.60	140.3	116.5±28.05	3	56.85	107.3	78.82± 25.82
	Surat Thani				**Songkhla**							
2014					22	62.40	99.07	82.32±11.08				
2015	1	-	-	86.46								
2016	26	61.75	150.3	97.69±18.47								

Appendix 2. *Chrysaora chinensis*: selected materials for deposit in the PMBC Reference Collection (* indicates samples used for DNA sequencing).

PMBC no.	Locality	Province	CollectedDate	IndividualCount
18904	Mak Island (Nid Bay), Kut Island	Trat	14/8/2014	1
18914	Mookkaew Beach, Mueang Trat	Trat	13/9/2018	1
18900	Trat Bay, Mueang Trat	Trat	15/2/2014	1
3228	Ratchakarun Beach	Trat	26/06/2015	1
18913	Chaolao Beach, Tha Mai	Chanthaburi	29/10/2014	4
18903	Kudi Island, Mueang Rayong	Rayong	10/11/2016	1
18134	Bangsaen Beach, Mueang Chon Buri	Chonburi	10/11/2016	1
3217	Cha-Am Beach	Phetchaburi	14/2/2018	3
16021	Chao Samran Beach	Phetchaburi	6/11/2017	1
11649	Naresuan Camp Beach	Phetchaburi	28/6/2018	4
3238	Tungka-Sawee Bay	Chumphon	19/6/2016	1
3245	Haad Rin Beach	Suratthani	10/8/2016	25
3240	Samui Island	Suratthani	5/11/2015	1
26877	Sakom Beach, Thepha	Songkhla	6/11/2014	1
3246*	Hat Sai Dam, Mueang Ranong	Ranong	25/11/2014	1
3247*	Hat Sai Dam, Mueang Ranong	Ranong	25/11/2014	1
3248*	Hat Sai Dam, Mueang Ranong	Ranong	23/12/2014	1
30395*	Hat Sai Dam, Mueang Ranong	Ranong	21/4/2015	5
30396	Sin Hai Island, Mueang Ranong	Ranong	24/6/2014	20
30271	Boi Noi Island, Ko Yao	Phang-Nga	16/5/2012	2
3249*	Khoei Bay, Khura Buri	Phang-Nga	26/11/2014	1
3250*	Khoei Bay, Khura Buri	Phang-Nga	26/11/2014	1
3251*	Khoei Bay, Khura Buri	Phang-Nga	24/12/2014	1
30267	Mak Noi Island, Mueang Phang-nga	Phang-Nga	14/5/2013	5
3253*	Nai Rai Village, Takua Thung	Phang-Nga	13/03/2014	1
30393*	Nai Rai Village, Takua Thung	Phang-Nga	28/11/2014	1
3258*	Nam Kem Village, Takua Pa	Phang-Nga	12/3/2015	1
3259*	Nam Kem Village, Takua Pa	Phang-Nga	23/04/2015	1
30392*	Nam Kem Village, Takua Pa	Phang-Nga	12/3/2015	1
26868	Nok Island, Ko Yao	Phang-Nga	20/9/2011	7
26409	Panak Island, Takua Thung	Phang-Nga	4/2/2011	6
30264	Song Phi Nong Island, Mueang Phang-nga	Phang-Nga	18/6/2012	2

PMBC no.	Locality	Province	CollectedDate	IndividualCount
26403	Yao Noi Island, Ko Yao	Phang-Nga	20/2/2011	1
26414	Maphrao Island, Mueang Phuket	Phuket	23/5/2011	2
26287	Panwa Cape, Mueang Phuket	Phuket	18/9/2010	1
26299	Rang Yai Island, Mueang Phuket	Phuket	22/9/2010	1
26308	Sapum Bay, Mueang Phuket	Phuket	20/10/2010	1
26336	Yamu Cape, Thalang	Phuket	22/12/2010	1
26358	Bilae Island, Mueang Krabi	Krabi	26/10/2010	1
26881	Hong Island, Mueang Krabi	Krabi	20/4/2012	5
30262	Klong Dao Beach, Lanta Yai Island	Krabi	4/7/2017	1
30252	Lanta Yai Island, Ko Lanta	Krabi	24/9/2013	30
26384	Pakrha Village, Nuea Khlong	Krabi	25/10/2010	7
26394	Ra Ning Island, Ko Lanta	Krabi	23/4/2011	3
26851	Railay Beach, Nam Mao Bay, Mueang Krabi	Krabi	20/9/2011	12
20820	Tasae Bay	Trang	19/08/013	1
30398	Muk Island, Kantang	Trang	29/4/2014	2
26863	Chebilang Village, Mueang Satul	Satul	12/10/2011	14
26388	Sarai Island, Mueang Satul	Satul	21/4/2011	1
26313	Ta Loh Nam Village, Mueang Satul	Satul	22/10/2010	1

Appendix 3. Diagnosis characters of the species members in genus *Chrysaora* (Morandini and Marques, 2010 and present study).

	Distribution	**Shape of exumbrella**	**Bell diameter**	**Shape of marginal lappet**	**Marginal lappets per octant**
***C. achlyos* Martin, Gershwin, Burnett, Cargo and Bloom, 1997**	Eastern North Pacific Ocean	Hemisphere	up to 100 cm	Squared	up to 4 per octant
***C. caliparea* (Reynaud, 1830)**	Indian Ocean	Slightly hemisphere	about 40 cm	Rounded	up to 4 per octant
***C. chinensis* Vanhöffen, 1888**	Western Pacific Ocean and Indian Ocean (Bangladesh and Andaman Sea)	Flatter than a hemisphere	about 10 cm	Slightly elongate	up to 4 per octant
***C. colorata* (Russell, 1964)**	Northeastern Pacific	Hemisphere, flatter in younger specimen	up to 100 cm	Squared	up to 4 per octant
***C. fungida* (Reynaud, 1830)**	Coast of West Africa	Almost hemisphere	10–20 cm	Rounded	up to 8 per octant
***C. fuscescens* Brandt, 1835**	Northeast Pacific Ocean	Hemisphere, flatter in younger specimen	up to 30 cm	Rounded	4 per octant
***C. hysoscella* (Linnaeus, 1767)**	Eastern North Atlantic	Hemisphere, flatter in younger specimen	15–25 cm	Rounded	4 per octant
***C. kynthia* Gershwin and Zeidler, 2008**	Southwestern Australia	-	4.7–11.2 cm	Rounded	4 per octant
***C. lactea* Eschscholtz, 1829**	Western North and South Atlantic	Almost hemisphere, flatter in younger specimen	6–11 cm	Rounded	4–6 per octant
***C. melanaster* Brandt, 1835**	Northern Pacific Ocean	Hemisphere, flatter in younger specimen	up to 60 cm	Rounded	4 per octant
***C. pacifica* (Goette, 1886)**	North Pacific Ocean (Japan)	Almost hemisphere, flatter in younger specimen	10–15 cm	Rounded	4–10 per octant
***C. pentastoma* Péron and Lesueur, 1810**	South Australia	-	6.6 cm	Rounded and tongue-shaped	6 per octant
***C. plocamia* (Lesson, 1830)**	South Pacific Ocean, South Atlantic Ocean	Almost hemisphere	up to 100 cm	Squared	4 per octant
***C. quinquecirrha* (Desor, 1848)**	Western North Atlantic, East coast of USA	Almost hemisphere, flatter in younger specimen	up to 40 cm	Rounded	4–10 per octant
***C. wurlerra* Gershwin and Zeidler, 2008**	Southeastern Australia	-	12.59 cm	Bilobed and tongue-shaped, crescentic row of vacuoles at central tentacle base.	3 per octant

Appendix 3. (Continued).

	Tentacles	Tentacles per octant	Quadralinga	Rhopallium	Rhopalia	Canal in marginal lappet
C. achlyos **Martin, Gershwin, Burnett, Cargo and Bloom, 1997**	24	3 (2-1-2)	Present	closed	8, no ocelli	present, small canals
C. caliparea **(Reynaud, 1830)**	24–40	3 (2-1-2), 5	Not known	-	8	-
C. chinensis **Vanhöffen, 1888**	24–40	3-5 (3-2-1-2-3)	absent	closed	8, no ocelli	absent
C. colorata **(Russell, 1964)**	8	1	present	closed	8, no ocelli	present
C. fungida **(Reynaud, 1830)**	24, 56	3(2-1-2), 5(3-2-1-2-3), 7(4-3-2-1-2-3-4)	absent	opened	8, no ocelli	absent
C. fuscescens **Brandt, 1835**	24	3 (2-1-2)	absent	opened	8, no ocelli	absent
C. hysoscella **(Linnaeus, 1767)**	24	3 (2-1-2)	absent	opened	-	absent
C. kynthia **Gershwin and Zeidler, 2008**	24,32	3 (2-1-2)	not mentioned	-	-	-
C. lactea **Eschscholtz, 1829**	24–40	3-5 (2-3-1-3-2)	absent	-	yellowish	absent
C. melanaster **Brandt, 1835**	24	3 (2-1-2)	Absent	-	8, no ocelli	present
C. pacifica **(Goette, 1886)**	24–72	3-9 (5-4-3-2-1-2-3-4-5)	absent	opened	8, no ocelli	absent
C. pentastoma **Péron and Lesueur, 1810**	40	5	not mentioned	-	-	-
C. plocamia **(Lesson, 1830)**	24	3 (2-1-2)	present	opened	8, no ocelli	present, small canals
C. quinquecirrha **(Desor, 1848)**	24–40	3-5 (3-2-1-2-3)	absent	closed	8, no ocelli	absent
C. wurlerra **Gershwin and Zeidler, 2008**	24,40	5	not mentioned	-	-	-

Appendix 3. (Continued).

	Radial septa	Gonad
***C. achlyos* Martin, Gershwin, Burnett, Cargo and Bloom, 1997**	Rounded at base; straight up to ¼ of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	Encircling gastric filaments, forming semi-circular ring, heavily folded
***C. caliparea* (Reynaud, 1830)**	Not described	Outlining gastric filaments yellowish
***C. chinensis* Vanhöffen, 1888**	Narrow, widening gradually at proximal end (rounded base); straight up to ca. 1/2, then bent towards each other, then bent towards rhopalia (~45º)	Outlining gastric filaments, semicircular ring, greatly folded
***C. colorata* (Russell, 1964)**	Rounded at base; straight up to ¼ of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	Encircling gastric filaments, forming a semi-circular ring, heavily folded
***C. fungida* (Reynaud, 1830)**	either transparent-white with colourless or brown lappets, dark (usually brown) radiating stripes on transparent-whitish background, light stripes on dark background	Outlining gastric filaments, semicircular ring, greatly folded; non-hermaphroditic
***C. fuscescens* Brandt, 1835**	Thin; rounded at base; straight up to 1/4 of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	Encircling gastric filaments, forming a semi-circular ring, heavily folded
***C. hysoscella* (Linnaeus, 1767)**	narrow, widening gradually (pear-shaped or triangular outline) at proximal end; outer 1/3 to 1/4 bending towards rhopalia (~45º)	outlining gastric filaments, semicircular ring, greatly folded; hermaphrodite, protandric, sperm sacs on gastrovascular cavity
***C. kynthia* Gershwin and Zeidler, 2008**	-	inverted W-shaped gonad
***C. lactea* Eschscholtz, 1829**	-	colour variable: whitish, yellowish-brown to pale pink
***C. melanaster* Brandt, 1835**	Thin; rounded at base; straight up to 1/4 of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	encircling gastric filaments, forming a semi-circular ring, heavily folded
***C. pacifica* (Goette, 1886)**	Thin; rounded at base; straight up to ¼ of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	Outlining gastric filaments, semicircular ring, greatly folded
***C. pentastoma* Péron and Lesueur, 1810**	-	crescentic to M-shaped
***C. plocamia* (Lesson, 1830)**	Thin; rounded at base; straight up to ¼ of margin, then making an "S" (first thinning tentacular pouch, then enlarging it); ending near tentacular base at rhopalar lappet	Outlining gastric filaments, semicircular ring, greatly folded; colour whitish to pale pink

	Radial septa	Gonad
***C. quinquecirrha* (Desor, 1848)**	Narrow, wider at proximal end (pearshaped); outer 1/3 bending towards rhopalia (~45º) ending straight into the margin near tentacle at rhopalar lappet	Outlining gastric filaments, semicircular ring, greatly folded; colour whitish to pale pink
***C. wurlerra* Gershwin and Zeidler, 2008**	-	-