

Preliminary Taxonomic Survey and Molecular Documentation of Jellyfish Species (Cnidaria: Scyphozoa and Cubozoa) in Malaysia

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Mohammed Rizman-Idid, Abu Bakar Farrah-Azwa, and Ving Ching Chong (2016) Scientific enquiries into jellyfish blooms and associated problems are often deterred by the lack of taxonomical and ecological studies worldwide. Taxonomic difficulty is attributed to the high degree of morphological variations among and within species. To date, only two scyphozoan jellyfish species have been documented from field surveys in Malaysian waters, whereas another four Malaysian scyphozoan and two cubozoan jellyfish species have been mentioned in toxicological studies. None of these species have; however, been verified. This study thus aimed to document and resolves the uncertainty of earlier identified species in the region using morphology and molecular DNA sequencing. Jellyfish specimens were collected from Malaysian waters in the Straits of Malacca, South-China Sea and the Sulu-Sulawesi Sea over two years (June 2008 to October 2010), and their DNA sequences were compared with those from the Atlantic and Pacific regions. Ten scyphozoan and two cubozoan species were recorded in Malaysian waters (South China Sea and Straits of Malacca). These jellyfish included eight species from the order Rhizostomeae (Rhizostomatidae, Lobonematidae, Mastigiidae, Catostylidae and Cepheidae), two species from Semaestomeae (Pelagiidae and Cyaneidae) and two species from class Cubozoa; one from order Carybdeida (family Carukiidae) and another from order Chirodropida (family Chiropsalmidae). Molecular identification of species using phylogenetic approaches was based on DNA sequences of partial cytochrome oxidase I (COI), 16S and internal transcribed spacer (ITS1) regions. The COI phylogenetic tree of Cubozoa and Scyphozoa species from the Atlantic and Pacific regions showed distinct clustering of six Malaysian jellyfish species. However, most of the deeper divergences and relationships between the families were unresolved, which were also observed in the 16S and ITS1 phylogenetic trees. The Malaysian edible species *Lobonemoides robustus*, *Rhopilema hispidum* and *Rhopilema esculentum* were grouped within Rhizostomeae, whereas other scyphozoans showed phylogenetic affinities to Semaestomeae and Kolpophorae. *Chrysaora* and *Cyanea* appeared non-monophyletic; however their paraphyly was not confirmed. This study has provided the much needed baseline information on the taxonomy of Malaysian jellyfish species which have been substantiated by partial COI, 16S and ITS1 sequences. A total of 12 putative species of jellyfish were identified, which encompassed 12 genera.

Key words: Scyphozoa, Cubozoa, Jellyfish, Phylogenetics, DNA barcoding, Malaysia.

BACKGROUND

Due to the increased awareness on jellyfish blooms in the past two decades, there has been a revival of taxonomical and ecological studies of jellyfish worldwide (Nishikawa et al. 2008; Hopf and Kingsford 2013; Bayha and Graham 2014;

D'Ambra et al. 2015). Jellyfish blooms are known to affect fishing industries, power stations and offshore mining operations by clogging fishing nets and cages, water intake points and vacuum pipes, respectively (Purcell and Arai 2001; Mills 2001; Mullan et al. 2005; Lucas 2001; Lynam et al. 2006). Beach tourism is also affected due to increased

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incidence of jellyfish sting (Lucas 2001; Purcell et al. 2007). The possible causes of the increased jellyfish blooms and swarms include eutrophication (Purcell et al. 2001; Parsons and Lalli 2002; Decker et al. 2004; Harashima et al. 2006; Malej et al. 2007), overfishing (Mullon et al. 2005; Bakun and Weeks 2006), introduction of alien species (Bolton and Graham 2004; Graham and Bayha 2007), installation of artificial substrates in the ocean (Richardson et al. 2009) and climate change (Raskoff 2001; Cai et al. 2005; Attrill et al. 2007; Gibbons and Richardson 2008). Nonetheless, some species of jellyfish are edible and harvested globally for human consumption particularly in Southeast Asia (Omori and Nakano 2001; Hsieh et al. 2001; Pitt and Kingsford 2003; You et al. 2007).

The term 'jellyfish' may be used to name other gelatinous zooplanktons such as comb-jellies (ctenophores) and salps (Haddock 2004). In this study, jellyfish refers to the species in the phylum Cnidaria. Cnidarian jellyfish comprise of three main groups: Scyphozoa, Cubozoa and Hydrozoa. According to the World Register of Marine Species (<http://www.marinespecies.org>), there are 187 and 46 accepted species for Scyphozoa and Cubozoa, respectively. The worldwide distributed scyphozoan jellyfish include four orders; Coronatae (crown jellyfish), Rhizostomeae (true jellyfish), Stauromedusae (stalked jellyfish) and Semaestomeae (sea nettle) (Kramp 1961; Pitt 2000; Brusca and Brusca 2002; Shao et al. 2006; Richardson et al. 2009). In contrast, the cubozoan jellyfish (box jellyfish) species are divided into two orders which are Carybdeida and Chirodropida (Gershwin 2005a, 2005b, 2006a, 2006b; Daly et al. 2007). Hydrozoan jellyfish constitute the most diverse group with 3,676 accepted species in the World Hydrozoa database (Schuchert 2015). However, hydrozoan and stalked jellyfish are not covered in the taxonomic survey of the present study, as there is an urgent need to establish baseline information of the scyphozoan and cubozoan jellyfish towards better understanding of their reoccurring blooms in some coastal areas of Malaysia.

Jellyfish research endeavors are challenged by the problem of species identification due to the high degree of morphological variation among species and often poorly preserved specimens. As a result very few reliable taxonomic keys are available. Despite the advent of molecular genetic techniques such as DNA sequencing which have facilitated identification, jellyfish sequences in the GenBank database are still limited and mostly

comprised of species from temperate regions and the Atlantic Ocean (Häussermann et al. 2009). Few species come from the Pacific Ocean (Dawson 2005b) and almost none from the Southeast Asian region. There are very few records of jellyfish in Malaysian waters, including in the Straits of Malacca and South China Sea. This is because jellyfish species have been overlooked in most of the country's marine biodiversity checklists and surveys and the inability to identify them. Their seasonal presence, which is often unpredictable and highly variable in numbers, confounds jellyfish research. Not surprisingly, the number of species of scyphozoan jellyfish reported in Malaysia has remained in flux. In fact, the only published scyphozoan jellyfish documentation from field surveys were by Daud (1998) and Rumpet (1991), which listed *Rhopilema esculenta* and *Lobonema smithi*. However, toxicological studies have noted four scyphozoan (*Catostylus mosaicus*, *Lychnorhiza lucerna*, *Chrysaora quinquecirrha* and *Chrysaora hysoscella*) and two cubozoan (*Carybdea rastoni* and *Chironex fleckeri*) jellyfish species (Othman and Burnett 1990; Tan et al. 1993; Azila and Othman 1993). Unfortunately, the species identifications of all these studies remain doubtful without satisfactory taxonomic scrutiny and verification. Given the poor information and uncertainty of identified species in the region, this preliminary study aims to document and identify the jellyfish species in Malaysian waters based on their morphological characteristics. Molecular DNA sequence analysis and phylogenetic approaches were also employed to aid identification, especially when morphological features are ambiguous and inadequate to determine the species. Construction of phylogenetic trees that illustrate the affinity of queried sequences to the available reference jellyfish sequences will help to confirm species identification.

MATERIALS AND METHODS

Sampling of specimens

Jellyfish medusae were collected from 13 study sites covering the Straits of Malacca, South China Sea and the Sulu-Sulawesi Sea from June 2008 to October 2010 (Table 1). Samples from the Straits of Malacca were obtained off the coast of the states of Kedah, Penang, Perak, Selangor, Malacca and Johore, whereas samples from the South China Sea were collected off Terengganu,

Table 1. Species and location of jellyfish recorded in Malaysian waters with their GenBank accession numbers

Species (n)/ catalog number	Location	Sequences (Accession Number)		
		COI	16S	ITS1
<i>Acromitus flagellatus</i> (22)/ MZUMCS0001-MZUMCS0022	Matang (4°49'42.00"N, 100°31'47.53"E) Klang Strait (03°09'39.95"N, 101°17'28.75"E)	<i>n</i> = 9 JN202973- JN202981	<i>n</i> = 8 JN202929- JN202936	<i>n</i> = N/A
<i>Catostylus townsendi</i> (10)/ MZUMCS0023-MZUMCS0032	Pantai Melawi (5°59'59.23"N, 102°25'36.20"E)	<i>n</i> = N/A	<i>n</i> = N/A	<i>n</i> = N/A
<i>Cephea cephea</i> (15)/ MZUMCS0033-MZUMCS0047	Pulau Kapas (5°12'43.85"N, 103°14'53.43"E)	<i>n</i> = N/A	<i>n</i> = N/A	<i>n</i> = N/A
<i>Chiropsoides buitendijki</i> (2)/ MZUMCC0001-MZUMCC0002	Teluk Bahang (5°27'48.90"N, 100°12'27.51"E)	<i>n</i> = N/A	<i>n</i> = N/A	<i>n</i> = 2 JN202953- JN202954
<i>Chrysaora chinensis</i> (76)/ MZUMCS0048-MZUMCS0123	Teluk Bahang (5°27'48.90"N, 100°12'27.51"E) Klang Strait (03°09'39.95"N, 101°17'28.75"E) Tg. Dawai (5°40'47.60"N, 100°21'48.37"E) Pengkalan Parit Jawa (1°56'0.18"N, 102°36'59.11"E) Pantai Melawi (5°59'59.23"N, 102°25'36.20"E)	<i>n</i> = N/A	<i>n</i> = 6 JN202937- JN202942	<i>n</i> = 3 JN202955- JN202957
<i>Cyanea</i> sp. (39)/ MZUMCS0124-MZUMCS0162	Klang Strait (03°09' 39.95"N, 101°17' 28.75"E) Tg. Dawai (5°40'47.60"N, 100°21'48.37"E) Tanjung Kling (2°10'50.34"N, 102°13'54.26"E)	<i>n</i> = 12 JN202986- JN202997	<i>n</i> = 2 JN202943- JN202944	<i>n</i> = 10 JN202958- JN202967
<i>Lobonemoides robustus</i> (14)/ MZUMCS0163- MZUMCS0176	Klang Strait (03°09' 39.95"N, 101°17' 28.75"E) Pulau Pangkor (4°10'58.33"N, 100°34'40.41"E) Tanjung Kling (2°10'50.34"N, 102°13'54.26"E) Langkawi (6°25'45.60"N, 99°52'18.07"E)	<i>n</i> = 1 JN203013	<i>n</i> = N/A	<i>n</i> = 1 JN202968
<i>Mastigias</i> sp. (3)/ MZUMCS0177-MZUMCS0179	Kudat (7°3'42.30"N, 117°24'46.61"E)	<i>n</i> = N/A	<i>n</i> = N/A	<i>n</i> = N/A
<i>Morbakka</i> sp. (4)/ MZUMCC0003-MZUMCC0006	Klang Strait (03° 09' 39.95"N, 101° 17' 28.75"E)	<i>n</i> = 4 JN202982- JN202985	<i>n</i> = N/A	<i>n</i> = N/A
<i>Phyllorhiza punctata</i> (44)/ MZUMCS0179-MZUMCS0222	Klang Strait (03°09' 39.95"N, 101°17' 28.75"E) Pulau Pangkor (4°10'58.33"N, 100°34'40.41"E) Teluk Bahang (5°27'48.90"N, 100°12'27.51"E) Pengkalan Parit Jawa (1°56'0.18"N, 102°36'59.11"E) Teluk Air Tawar (5°29'14.42"N, 100°22'25.16"E) Pulau Tioman (2°48'23.82"N, 104° 3'10.34"E) Matang (4°49'42.00"N, 100°31'47.53"E)	<i>n</i> = 13 JN202998- JN203010	<i>n</i> = 2 JN202945- JN202946	<i>n</i> = N/A
<i>Rhopilema hispidum</i> (6)/ MZUMCS0223-MZUMCS0228	Klang Strait(03°09' 39.95"N, 101°17' 28.75"E) Pulau Pangkor (4°10'58.33"N, 100°34'40.41"E) Teluk Bahang (5°27'48.90"N, 100°12'27.51"E)	<i>n</i> = 2 JN203011- JN203012	<i>n</i> = 2 JN202947- JN202948	<i>n</i> = 3 JN202969- JN202971
<i>Rhopilema esculentum</i> (5)/ MZUMCS0229-MZUMCS0233	Klang Strait (03°09' 39.95"N, 101°17' 28.75"E)	<i>n</i> = N/A	<i>n</i> = 4 JN202949- JN202952	<i>n</i> = 1 JN202972

n = sample size; N/A – not available

Kelantan and Pahang in Peninsular Malaysia. The Sulu-Sulawesi specimens were sampled off Kudat town, Sabah.

The jellyfish were collected by various fishing gear such as bag nets, beam trawls, gill nets, and scoop nets, as well as scuba diving. Jellyfish collected by bag net and scoop net were in relatively good condition and the least damaged. Initial identification of species was based on live specimens as well as photographs taken in the field. Specimens were photographed immediately after capture to record their live colouration. Exumbrella (bell) diameter and length of oral arms were measured on site while other measurements were recorded from formalin-preserved specimens in the laboratory. Tissue samples of the exumbrella and oral arms were taken and preserved in absolute ethanol for molecular analysis. Whole specimens were preserved in 4% formalin for a week, before being transferred into 10% formalin for better fixation. After a month of formaldehyde fixation, the specimens were transferred into 70% ethanol for long term preservation. Identified specimens were tagged, catalogued and deposited in the Zoological Museum, Institute of Biological Sciences, University of Malaya. Species were identified based mainly on the taxonomic descriptions of Kramp (1961) and Mayer (1910), specifically using the exumbrella shape, live colouration, number of oral arms and exumbrella structures such as patterns of nematocyst warts.

DNA extraction

Approximately 10 mm³ of ethanol-preserved tissue were digested in 600 µl of 2X cetyltrimethylammonium bromide (CTAB) buffer [0.1M Tris-HCL buffer (pH 8.0), 1.2M NaCl, 0.02M EDTA and 2% CTAB] with 60 µl 5M NaCl and 6 µl of 20 mg/ml Proteinase K. Genomic DNA were extracted using the modified CTAB Phenol-Chloroform method (Dawson et al. 1998). Extracted DNA was stored in Tris-HCL buffer. Alternatively, the Qiagen Genra Puregene Tissue Kit was used for DNA extraction following the manufacturer's instructions when extraction using CTAB Phenol-Chloroform method was unsuccessful. The quality of extracted DNA was assessed by gel electrophoresis on 1% TBE agarose gel.

PCR amplification and DNA sequencing

Partial cytochrome oxidase subunit I (COI) gene was amplified using forward and reverse

primers LCOjf (5'-GGTCAACAAATCATAAAGAT ATTGGAAC-3'; Dawson 2005b) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3'; Folmer et al. 1994); partial 16S gene with primers P1 (5'-TCGACTGTTTACCAAAAACATAGC-3') and P3 (5'-GTCGCCCAACTAACTACCAAAC TT-3')(Bridge et al. 1992); and internal transcribed spacers (ITS1) region with primers jfITS1-5f (5'-GGTTTCCGTAGGTGAACCTGCGGAAGGATC -3') and jfITS1-3r (5'-CGCACGAGCCGAGTG ATCCACCTTAGAAG-3') (Dawson and Jacobs 2001). Each 50 µL PCR reaction comprised of 0.5 µM of each primer, 5 µL 10X buffer, 3 mm of MgCl₂, 0.2 mm of dNTPs, 2U of Taq Polymerase (Fermentas, GmbH, Germany) and 0.1 µg template DNA. PCR thermocycling was performed using Eppendorf Thermocycler which began with six steps of 94°C for 8 min, 49°C for 2 min, 72°C for 2 min, 94°C for 4 min, 50°C for 2 min, 72°C for 2 min, followed by 33 cycles of 94°C for 45 s, 51°C for 45 s, and 72°C for 60s, then a final extension at 72°C for 10 min, before ending at 4°C (Dawson 2005). The PCR products (5 µl) were visualized on a 1% agarose gel electrophoresis. PCR products were purified with GeneJET™ PCR Purification Kit (Fermentas). DNA sequencing was done using ABI BigDye® Terminator v3.1 Cycle Sequencing Kit through Applied Biosystems 3730xl DNA Analyzer, USA. Forward and reverse sequences were analysed using Applied Biosystems Sequencing Analysis software v5.

Sequence and phylogenetic analysis

DNA sequence reads were checked and edited manually using Sequence Scanner (Applied Biosystem™) to remove primer sequences and to construct contiguous sequences. Verification of COI, 16S and ITS1 sequences were done by searching against GenBank sequences using BLAST (Altschul et al. 1990), for preliminary determination of the jellyfish species. Jellyfish sequences generated in this study were aligned with several reference sequences obtained from GenBank. Multiple sequence alignments were done for each gene region using ClustalW (Higgins et al. 1992) and the final alignments were tested for best-fit evolutionary model using Modeltest 3.8 (Posada and Crandall 1998). Analysis of DNA sequence variation, nucleotide composition and genetic distance were performed in Molecular Evolutionary Genetic Analysis (MEGA) version 4.0 (Tamura et al. 2007). Neighbour-Joining (NJ) and

Maximum Parsimony (MP) phylogenetic trees were constructed using PAUP*4.0b10 (Swofford 2003) with 1000 bootstrap replicates. For MP analyses, full heuristic searches were performed with 10 random sequence additions and tree bisection-reconnection (TBR) branch swapping. Evolutionary models were determined and incorporated into the construction of NJ tree and the Bayesian Inference (Bayesian) analyses. Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains (three heated chains and one cold chain) were run for three million generations and the trees were sampled every 100th generation. Twenty five percent of the initial trees were discarded and the remainders were used to construct a consensus tree. Only branches with over 70% bootstrap values or posterior probabilities were considered.

Models of evolution

Prior to the phylogenetic analyses, models of evolution were determined by using Modeltest 3.8 (Posada and Crandall 1998). The program selected GTR + I + G, TVM + I + G, HKY + G and GTR + G as the most suitable evolutionary model for the partial COI, 16S, ITS1 and concatenated (COI + ITS1) sequence alignments, respectively. Parameters of the chosen models are shown in table 2.

RESULTS

Jellyfish species diversity

A total of 12 jellyfish species representing 12 genera were collected. The 12 putative species identified were *Acromitus flagellatus* (Maas 1903), *Cephea cephea* (Forskåll 1775), *Rhopilema hispidum* (Vanhöffen 1888), *Mastigias* sp. (Lesson 1830), *Phyllorhiza punctata* (von Lendenfeld 1884), *Cyanea* sp. (Linne 1758), *Chrysaora chinensis* (Linne 1766), *Lobonemoides robustus* (Stiasny 1920), *Rhopilema esculentum* (Kishinouye 1891), *Catostylus townsendi* (Mayer 1915), *Morbakka* sp. and *Chiropsoides buitendijki*. These species either belong to the class Scyphozoa (order Rhizostomeae, Semaestomeae and family Lobonematidae) or class Cubozoa (order Carybdeida and Chirodropida). Order Rhizostomeae is characterised by the possession of eight oral arms with no marginal tentacles, whereas order Semaestomeae has four oral arms with marginal tentacles (Kramp 1961). The class Cubozoa is characterised by umbrella margin not clefted into lappets and four interradial tentacles or groups of tentacles. The Rhizostomeae specimens represented the families Cepheidae, Mastigiidae, Catostylidae, Lobonematidae and Rhizostomatidae, whereas Semaestomeae comprised of families Cyaneidae and

Table 2. Models of evolution determined by Modeltest 3.8 (Posada and Crandall 1998) for COI, 16S, ITS and concatenated sequence data.

Model parameters	Sequences			
	COI	16S	ITS	COI+ITS
Evolutionary Model	GTR + I + G	TVM+I +G	HKY+G	GTR + G
Base frequency	A= 0.3549 C= 0.1548 G= 0.1080 T= 0.3823	A= 0.3217 C= 0.1873 G= 0.178 T= 0.3130	A= 0.2492, C = 0.2153, G = 0.2411 T = 0.2944	A= 0.261 C= 0.198 G=0.213 T= 0.328
Shape parameter of gamma distribution (α)	0.4607	0.4607	1.6125	0.39
Proportion of invariable site (I)	0.4114	0.3356	-	-
Substitution rates	[A-C] = 0.4999 [A-G] = 8.0157 [A-T] = 0.7523 [C-G] = 1.5555 [C-T] = 16.9377 [G-T] = 1.0000	[A-C] = 2.1343 [A-G] = 6.2854 [A-T] = 2.5423 [C-G] = 0.5589 [C-T] = 6.2854 [G-T] = 1.0000	-	[A-C] = 0.069 [A-G] = 0.200 [A-T] = 0.158 [C-G] = 0.094 [C-T] = 0.452 [G-T] = 0.026
Transition/ Transversion ratio (Ti/Tv)	-	-	1.1000	1.81

Pelagiidae. Box jellyfish from family Carukiidae and Chiropsalmidae were collected also and represented by the order Carybdeida and order Chirodropida, respectively.

Species descriptions

The following describes the species found including records of their exumbrella shape, live colouration, number of oral arms, and exumbrella structures such as patterns of nematocyst warts.

***Morbakka* sp.**

(Fig. 1a)

Class: Cubozoa
Family: Carukiidae
Genus: *Morbakka* (Gershwin 2008)

Description: This species has four tentacles that are simple and long, with nematocysts that cause painful stings. The species lacks gastric phacellae. The transparent exumbrella is taller than it is wide, rectangular, with a flat apex. The bell diameter ranges from 40 - 120 cm. The species possesses “rabbit-ear”-like rhopalial horns which are diagnostic of the genus *Morbakka* (Bentlage and Lewis 2012). The rhopalial horns are swollen rather than pointed at the tips and project from the top centre of the rhopalial niche at a more oblique angle, very similar to those of *Morbakka virulenta* (Bentlage and Lewis 2012). It also has thorn-like extensions at the bases of their pedalial canals and numerous branching velarial canals with lateral diverticula.

Distribution and habitat: The species was found in the mangrove areas of Klang Strait and was caught in a single sampling trip. It is suspected to be more common in the southern Straits of Malacca, although a similar looking but damaged jellyfish specimen was also found in Pantai Melawi, east coast of Peninsular Malaysia. The geographic distribution of *Morbakka* includes Japan (*Morbakka virulenta*), Philippines (*Morbakka* sp.), and Australia (*Morbakka fenneri*).

***Chiropsoides buitendijki* van der Horst, 1907**

(Fig. 1b)

Class: Cubozoa (Werner 1975)
Family: Chirodropidae (Haeckel 1882)
Genus: *Chiropsoides*

Description: The exumbrella is cuboid with rounded edges. The species has gastric saccules

and four rhopalia at the side of the bell. Each pedalium has five or six fingers or tentacles. The gastric saccules are finger-shaped and of similar length as the depth of the bell cavity (Kramp 1961). The species has a painful sting. The exumbrella diameter is 100 mm. Its pedalia show distinct unilateral branching and the pedalial canal possesses a prominent ‘spike’ at the bend. These features are characteristic of *Chiropsoides buitendijki* as described by Gershwin (2006a).

Distribution and habitat: It inhabits the mangrove and coastal areas, and is commonly found in Teluk Bahang. Originally, the species was described under the genus *Chiropsalmus*, until recently revised as *Chiropsoides* (Bentlage et al. 2009).

***Acromitus flagellatus* Maas, 1903**

(Fig. 1c)

Class: Scyphozoa
Family: Catostylidae (Gegenbaur 1857)
Genus: *Acromitus* (Light 1914)

Description: This species has eight long oral arms. The exumbrella is rarely spotless, usually having an uneven distribution of black or dark brown spots. Colour may vary from light red or pink, grey and white or translucent. The distinguishing characteristics of *A. flagellatus* are oral arms as long as or longer than the diameter of the exumbrella, and long distinctive flagellae or “whip-like” terminal clubs. The terminal clubs are usually white. The species has no sting. The average size collected was 100 cm in diameter.

Distribution and habitat: The species is found in mangrove areas and estuaries on the west coast of Peninsular Malaysia. The geographic distribution of *A. flagellatus* includes the Malay Archipelago, Borneo, Java, Indian Ocean, India, Japan and Taiwan (Kramp 1961).

***Catostylus townsendi* Mayer, 1915**

(Fig. 1d)

Class: Scyphozoa
Family: Catostylidae (Gegenbaur 1857)
Genus: *Catostylus* (Mayer 1915)

Description: This species has four pairs of oral arms that appear ribbon-like under the subumbrella without any marginal tentacles and terminal clubs. The number of lappets varies with deep clefts between lappets. Some specimens showed colour variations of the umbrella, mostly

white or light pink with purple margins and only a few specimens with intense blue umbrellas, but all specimens exhibited dark brown spots on the umbrella surface and margin. The species has a mild sting. The average exumbrella diameter ranges from 100-120 cm.

Distribution and habitat: The species was found seasonally between April to June in large numbers at Pantai Melawi, east coast of Peninsular Malaysia. The distribution of *C. townsendi* includes the Malay Archipelago, Gulf of Siam, Borneo, Java and Indochina (Kramp 1961).

Cephea cephea Forskäll, 1775
(Fig. 1e)

Class: Scyphozoa
Family: Cepheidae (L. Agassiz 1862)
Genus: *Cephea* (Péron and Lesueur 1809)

Description: The colouration of the exumbrella is blue to purplish in live specimens, whereby the centre of the exumbrella is heightened and dome-like, surrounded by approximately ten large conical warts and several smaller pointed warts at the centre of the dome. It has eight oral arms and approximately 90 lappets at the umbrella margin. The distinguishing features of *C. cephea* are deep marginal lappets, eight arms that are stout and nearly coalesced at the base. It also has long tapering and brown oral arms (Kramp 1961).



Fig. 1. Jellyfish species collected in Malaysian waters. Cubozoan jellyfish; *Morbakka* sp. (a), *Chiropsoides buitendijki* (b); Scyphozoan jellyfish; *Acromitus flagellatus* (c), *Catostylus townsendi* (d), *Cephea cephea* (e), *Chrysaora chinensis* (f), *Cyanea* sp. (g), *Lobonemoides robustus* (h), *Mastigias* sp. (i), *Phyllorhiza punctata* (j), *Rhopilema hispidum* (k) and *Rhopilema esculentum* (l).

The sting is barely perceptible. The exumbrella diameter is 150 mm and the length of the oral arms is 40 mm.

Distribution and habitat: The specimen was found stranded along the beach nearby a coral reef area at Kapas Island, east coast of Peninsular Malaysia. The distribution of *C. cephea* includes Japan, Indian Ocean, Pacific Ocean, Philippines and the Malay Archipelago (Kramp 1961).

***Chrysaora chinensis* Linne, 1766**

(Fig. 1f)

Class: Scyphozoa
Family: Pelagiidae (Gegenbaur 1856)
Genus: *Chrysaora* (Linne 1766)

Description: The exumbrella surface is finely granulated. Their marginal lappets are slightly elongated, with 24-40 tentacles. The size of oral arms is one to three times longer than the exumbrella diameter. The gonads are semi-circular in shape, and it outlines the gastric filaments which are greatly folded (Kramp 1961). The diameter of exumbrella ranges from 50-150 mm.

Distribution and habitat: According to Morandini and Marques (2010), all *Chrysaora* specimens occurring in the Pacific region such as China, Indonesia and Philippines should be assigned to *C. chinensis*. This species seasonally occur in Klang Straits, Selangor in relatively low abundance. It is non-edible and considered a nuisance to fishing activity. The species was also commonly found in Teluk Bahang, Penang and Muar on the west coast, and Bachok and Pulau Tinggi on the east coast of Peninsular Malaysia.

***Cyanea* sp. Linne, 1758**

(Fig. 1g)

Class: Scyphozoa
Family: Cyaneidae
Genus: *Cyanea* (Péron and Lesueur 1809)

Description: The species has well developed circular muscles, marginal tentacles and several rows of clustered tentacles. The umbrella margin is clefted into lappets. The mesoglea cells are uniformly thick at the subumbrella and thin at the base of the lappets. Their colour is usually yellowish brown or reddish but sometimes almost colourless. The species has four main oral arms and circular muscles at the subumbrella. Exumbrella diameter ranges from 200-300 mm. The species has a perceptible sting.

Distribution and habitat: This non-edible jellyfish occurs throughout the year in the Klang Strait, and found seasonally in Tanjung Kling, west coast of Peninsular Malaysia.

***Lobonemoides robustus* Stiasny, 1920**

(Fig. 1h)

Class: Scyphozoa
Family: Lobonematidae
Genus: *Lobonemoides* (Light 1914)

Description: The exumbrella of this species has conspicuous, sharp papillae. The papillae are long in the apical exumbrella, short in the middle exumbrella and is absent in the marginal exumbrella. The subumbrella muscle is well developed in its intra-circular but weak in its extra-circular part. It has eight oral arms which are not coalesced with each other. Oral arms have numerous, long spindle-shaped and thread-like appendages. The canal system is comprised of 24-32 radial canals. Rhopalar canals are visible from the base to the marginal end, forked and reached the tip of the rhopalar lappets. Inter-rhopalar canals merged into extra-circular anastomosing canals (Kitamura and Omori 2010). The exumbrella diameter ranges from 380 to 460 mm.

Distribution and habitat: The species is commercially known as “white type” jellyfish (Kitamura and Omori 2010), and usually present seasonally in large numbers. It inhabits the coastal fisheries of Klang Strait and is harvested for human consumption.

***Mastigias* sp. Lesson, 1830**

(Fig. 1i)

Class: Scyphozoa
Family: Mastigiidae (Stiasny 1921)
Genus: *Mastigias* (L. Agassiz 1862)

Description: The exumbrella has very fine granulation. Mesoglea is firm with deep furrows between eight velar lappets in each octant. The oral arms terminate naked with club-shaped ends. Mouths not only present along the three edges of the oral arms, but also on their flat and expanded sides. There are numerous small clubs and filaments between the frilled mouths. Oral arms are about half as long as the exumbrella diameter, whereby the simple upper portion is one and half times as long as the three winged lower portion of the oral arm. Each arm usually terminates in club-shaped vesicles between mouths, with less than

10 canal roots in each octant. Rhopalar canals are slender and usually with anastomes (Kramp 1961). It has no sting. The exumbrella diameter measures up to 80 mm.

Distribution and habitat: The specimen was collected from Sabah. The species inhabits the coral reef and coastal areas. *M. papua* Lesson 1830 is distributed in the Malay Archipelago, Japan, Indian and Pacific Ocean, Philippines and the Torres Strait.

***Phyllorhiza punctata* von Lendenfeld, 1884**

(Fig. 1j)

Class: Scyphozoa
Family: Mastigiidae (Stiasny 1921)
Genus: *Phyllorhiza* (L. Agassiz 1862)

Description: This species is also known as the white spotted jellyfish, characterised by white spots that are distributed evenly on the exumbrella, umbrella margin and oral arms. The smaller sized (< 100 mm) specimens, presumably juveniles, have yellow exumbrellas and oral arms, whereas bigger specimens tend to be dark brown. The mesoglea is very thick. The species has eight broad oral arms and sometimes terminal clubs that vary in colour (blue, red or translucent). The end of the oral arms is usually leaf-shaped with many filaments. The species hosts symbiotic zooxanthellae found mostly in the mesoglea, giving the jellyfish body a yellow-brown pigmentation. The species has no perceptible sting. The exumbrella diameter ranges from 30 mm-400 mm.

Distribution and habitat: This non-edible jellyfish occurs in the Klang Strait throughout the year. This species is widely distributed and usually found in coral reefs, mangroves and estuaries. It has been observed to occur also in other parts of Malaysia such as Penang Nature Reserve, Langkawi Island, Lumut, Pangkor Island, Muar, Tanjung Kling and Tioman Island. *P. punctata* is generally distributed in Thailand, Australia, Philippines and Japan.

***Rhopilema hispidum* Vanhöffen, 1888**

(Fig. 1k)

Class: Scyphozoa
Family: Rhizostomatidae (Claus 1883)
Genus: *Rhopilema* (Macri 1778)

Description: This species is locally known as “sand type” jellyfish (trade name) and “ulbu kiburu” (sandpaper jellyfish) in Indonesia. The wide

exumbrella has 16 radial canals and eight rhopalia. The exumbrella has numerous small and sharply pointed warts. The species has eight oral arms with large scapulets and many tentacles (Kramp 1961). The exumbrella diameter ranges from 450-480 mm.

Distribution and habitat: This edible species inhabits the Klang Strait. It also occurs in Penang, Pulau Pangkor and Lumut, on the west coast of Peninsular Malaysia.

***Rhopilema esculentum* Kishinouye, 1891**

(Fig. 1l)

Class: Scyphozoa
Family: Rhizostomatidae (Cuvier 1799)
Genus: *Rhopilema* (Haeckel 1880)

Description: This species has large scapulets, long manubrium and oral arms with numerous clubs or filaments usually with a large terminal club. The exumbrella is without ring canals (Kramp 1961). The colouration of the umbrella and oral arms is reddish brown. This species may correspond to the “red type” jellyfish as reported by Omori and Nakano (2001). The species has a perceptible sting. The exumbrella diameter of most specimens exceeds 450 mm.

Distribution and habitat: This edible species occurs seasonally in the Klang Strait and is harvested also in Sarawak (Daud 1998). The distribution of *R. esculentum* includes Japan, China and the Bay of Korea. In China, the species is slightly reddish and also commercially harvested.

Molecular characterization

A total of 85 DNA sequences were obtained from the collected jellyfish samples, which comprised of 41 partial COI gene sequences (567-588 bp); 24 partial 16S sequences (254-256 bp); and 20 ITS1 sequences (245-323 bp). The success of PCR and DNA sequencing varied among the species and the targeted region used; the COI, 16S and ITS1 sequences represented only 5 or 6 species. The final COI sequence alignment length was 588 bp which consisted of 76 taxa, inclusive of reference and outgroup species sequences. The COI sequence alignment that translates into 196 amino acid residues contained 301 variable sites and 283 parsimony informative sites. All the taxa showed similar nucleotide composition, with the average of T (thymine) = 35.0, C (cytosine) = 18.9, A (adenine) = 26.5 and G (guanine) = 19.5

percent. The final 16S sequence alignment length was 259 bp which consisted of 41 taxa, contained 156 variable sites and 142 parsimony informative sites. The nucleotide composition was similar across taxa, with an average of T = 27.4, C = 18.2, A = 35.8 and G = 18.6 percent. The final ITS1 sequence alignment length of 36 taxa was 394 bp, which contained 364 variable sites and 326 parsimony informative sites and many indels. The nucleotide composition was similar across taxa, with an average of T = 27.8, C = 22.7, A = 23.8 and G = 25.7 percent.

Phylogenetic analyses and molecular identification

The NJ, MP and Bayesian analyses of the COI sequences produced congruent phylogenetic trees that are rooted with outgroups Anthozoa and Hydrozoa. The COI consensus phylogenetic tree of NJ, MP and Bayesian analyses shows separation of the six Malaysian jellyfish species (*Morbakka* sp., *Rhopilema hispidum*, *Lobonemoides robustus*, *Acromitus flagellatus*, *Phyllorhiza punctata* and the Malaysian *Cyanea* sp.) with high bootstrap values and posterior probabilities at the species nodes (Fig. 2).

All of the mentioned species are also monophyletic and show affinity to four major clades. The Cubozoa clade contains sequences of the Malaysian *Morbakka* sp. that clustered with the reference sequences of *Tamoya* cf. *haplonema* [GenBank: GQ150264] and *Chiropsalmus quadrumanus* [GenBank: GQ120103] with average sequence diversity of 23.6% (± 0.3) and 28.8% (± 0.2), respectively. Although there were no reference COI sequences of *Morbakka* available for verification, the Malaysian *Morbakka* sp. sequences are strongly related to *Carukia barnesi*, suggesting it belongs to family Carukiidae (Fig. 3). Coupled with its "rabbit ear"-like rhopalia horns, it is highly possible that this box jellyfish belongs to the genus *Morbakka*.

Another clade identified is one that consists of the order Semaestomeae (*Aurelia* sp. and *Aurelia limbata*) and Rhizostomeae (*Rhopilema hispidum*, *Rhopilema esculentum*, *Acromitus flagellatus*, *Crambionella orsini*, *Catostylus mosaicus* and *Nemopilema nomurai*). The Malaysian *R. hispidum* sequences do not cluster with *R. esculentum*, but showed weak relationship with *N. nomurai*, another Rhizostomeae jellyfish. The identification of the Malaysian *A. flagellatus* is somewhat confirmed by its clustering with the Indian *Acromitus* sp.,

with a sequence diversity of 2.0% (± 0.2). Besides having no available reference sequences for *Lobonemoides robustus*, the single sequence of this species showed no support for any specific sister group relationship with any other species within this Rhizostomeae and Semaestomeae mixed clade. The Malaysian *Phyllorhiza punctata* showed 8.9% (± 1.6) sequence diversity from the Australian *Phyllorhiza punctata* [GenBank: EU363342], inferring the possibility of being a cryptic species. *P. punctata* showed affinity to the Kolpophorae clade that also contained species members of *Cassiopea*, *Mastigias* and *Cephea*.

A distinctively Semaestomeae clade is also evident and comprises of *Cyanea capillata*, *Cyanea rosea*, *Cyanea annaskala*, *Chrysaora* sp. and *Pelagia noctiluca*. The Malaysian *Cyanea* sp. forms a different cluster from the reference sequences of *C. capillata*, *C. annaskala* and *C. rosea*, with an average sequence diversity of 25.0% (± 1.6). Although the Bayesian analysis appears to suggest paraphyly of *Cyanea* because the *Chrysaora* sequence clusters well with the Malaysian *Cyanea* sp., this is not supported by other NJ and MP analysis.

The 16S phylogeny which is rooted with reference sequences of Cubozoa, designated the Malaysian specimens of *Chrysaora chinensis*, the Malaysian *Cyanea* sp., *Rhopilema esculentum*, *Rhopilema hispidum*, *Acromitus flagellatus* and *Phyllorhiza punctata* into two main clades; Rhizostomeae and Semaestomeae (Fig. 4). The Rhizostomeae clade consists of *Cassiopeia* sp., *P. punctata*, *A. flagellatus*, *R. esculentum* and *R. hispidum*. The tree shows close relatedness between *R. esculentum* and *R. hispidum*. The Malaysian *R. esculentum* clusters with *R. esculentum* from China [GenBank: EU373725 and EU373726] with an average sequence diversity of 4.6% (± 0.5). Within the Semaestomeae clade (*Chrysaora quinquecirrha*, *C. chinensis* and *Cyanea* sp.), the Malaysian *C. chinensis* shows genetic affinity to the Malaysian *Cyanea* sp. rather than to other *Chrysaora* sp. (i.e. reference *C. quinquecirrha*), with an average sequence diversity of 9.5% (± 0.5).

Congruencies between NJ, MP and Bayesian trees are also observed for the ITS1 region, which showed separation of the six Malaysian species (*Chiropsoides buitendijki*, *Chrysaora chinensis*, *Cyanea* sp., *Lobonemoides robustus*, *Rhopilema esculentum* and *Rhopilema hispidum*) with high bootstrap values and posterior probabilities at the species node (Fig. 5). These species are divided

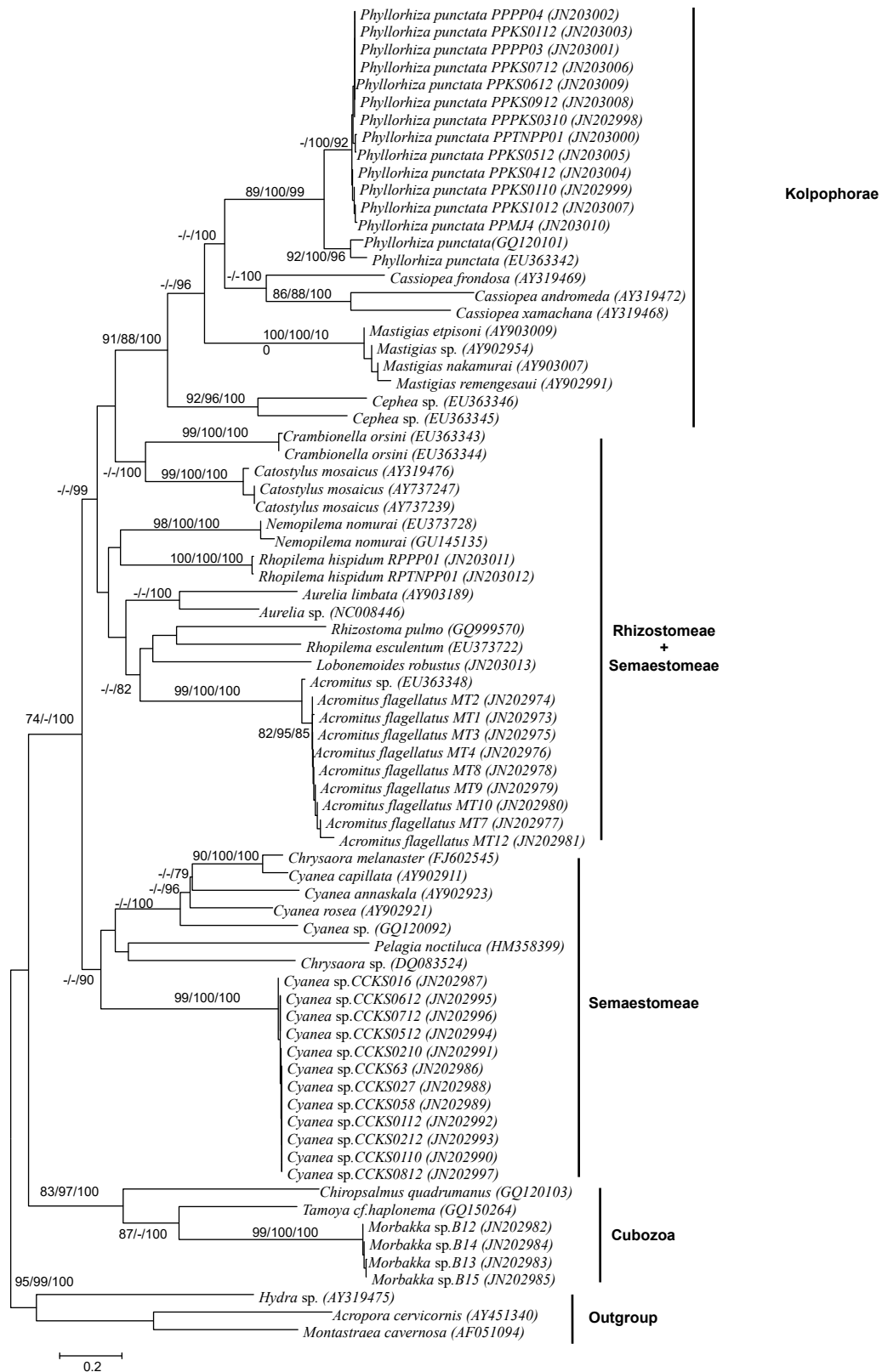


Fig. 2. The partial COI mtDNA sequence Bayesian tree of jellyfish species denoting the major clades. Numbers on the branches indicate bootstrap values for Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian posterior probabilities, respectively. The tree was rooted with outgroups Anthozoa (*Acropora cervicornis* and *Montastraea cavernosa*) and Hydrozoa (*Hydra* sp.).

into five scyphozoan clades when rooted with the Cubozoa sequences (reference *Chironex fleckeri* and Malaysian *C. buitendijki*). These clades represent the Rhizostomeae clade, three *Cyanea* clades and a mixed Semaestomeae and Rhizostomeae clade.

In the Rhizostomeae clade, the Malaysian *Rhopilema esculentum* clusters with the reference *Rhopilema esculentum*, thus confirming its identity. The Malaysian *R. hispidum* is a sister taxon to *R. esculentum*, consistent with being in the same genus. Although there were no reference sequences available to verify *L. robustus*, its distinct sequence showed weak affinity to the Rhizostomeae clade, which is consistent with its classification.

The ITS1 tree appears to show non-monophyly of the genus *Cyanea* as there is distinct separation of the Malaysian *Cyanea* sp., *Cyanea nozakii* and the Australian *Cyanea* species (*C. capillata*, *C. rosea* and *C. annaskala*) into different clades or groups (*Cyanea* Group I-III). Although the genus *Chrysaora* appears paraphyletic, the evidence is weak and circumstantial. Sequences of *Chrysaora chinensis* [GenBank: JN202955-57] from Malaysia are more closely related to *Chrysaora fulgida* [GenBank: HM348774], whereby both species are more related to *Pelagia noctiluca* [GenBank: HM348774] than to another reference sequence of *Chrysaora* sp. [GenBank: DQ083525]. The reference *Chrysaora* sp. is also related unexpectedly to the Japanese *C. nozakii*.

Phylogenetic analyses of concatenated

COI and ITS1 sequences, failed to establish the monophyly of *Cyanea* and *Chrysaora* (Fig. 6). The Australian *Cyanea* species remains separate from the Malaysian *Cyanea* sp. and *Cyanea nozakii*. Only *Chrysaora* sp. and *Chrysaora hysoscella* were available from GenBank for the total evidence molecular analysis. Unfortunately, their sister taxa relationship could not be determined due to poor resolution and support at the deep branches of the tree. Paraphyly of both *Chrysaora* and *Cyanea* remains inconclusive even though they appeared non-monophyletic.

DISCUSSION

Species identifications problems

The COI, 16S and ITS1 phylogenetic trees clearly show that jellyfish sequences are well separated into main clades representing either Class Scyphozoa or Class Cubozoa. Relationships within scyphozoans and cubozoans are mostly unresolved due to polytomy. Although some jellyfish species could be identified from the trees, the general lack of available reference jellyfish sequences made species confirmation impossible. Furthermore, the success of PCR amplifications and sequencing was low in the present study and could not generate sequences for all genetic markers. Significant intra-individual variation could confound sequencing despite successful PCR. Although not tested in the present study, other 16S

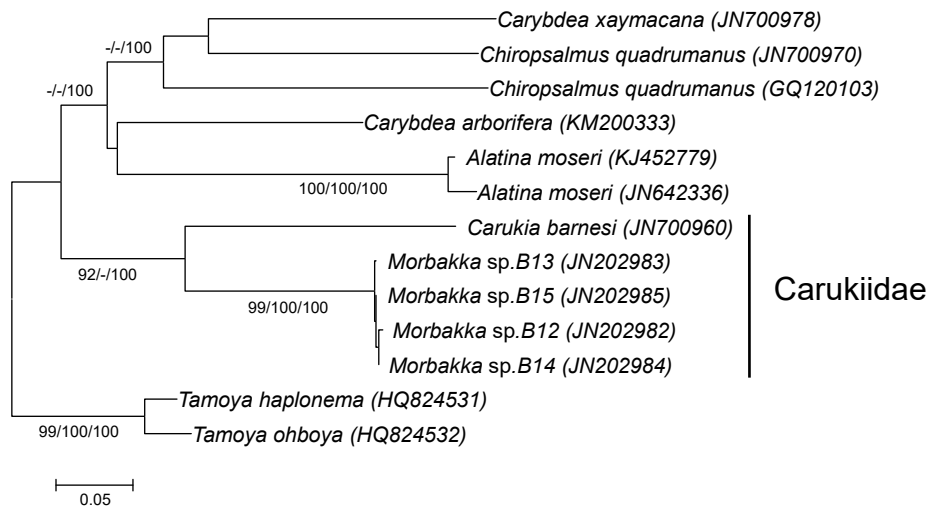


Fig. 3. The partial COI mtDNA sequence Bayesian tree of box jellyfish species denoting the Carukiidae clade. Numbers on the branches indicate bootstrap values for Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian posterior probabilities, respectively.

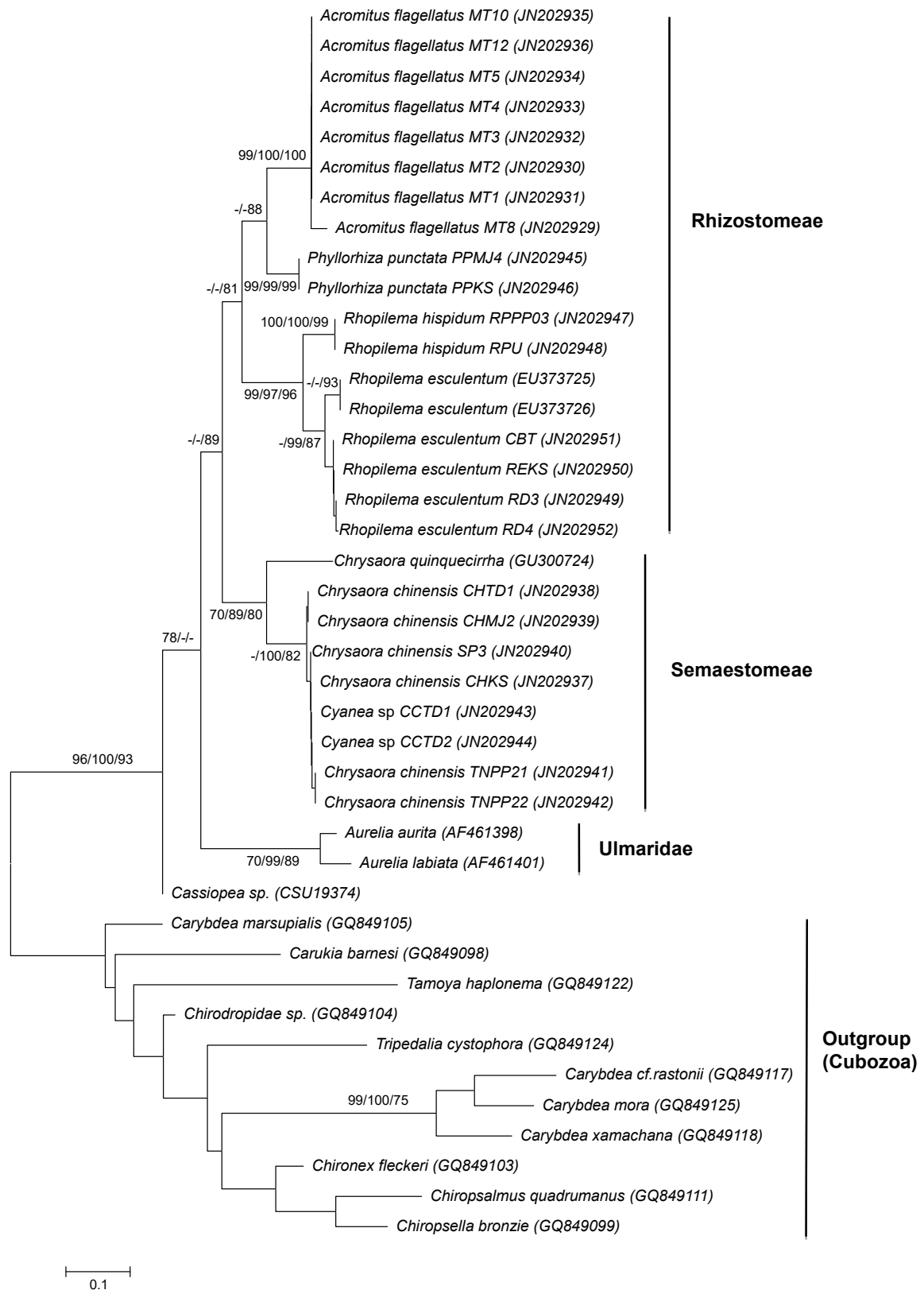


Fig. 4. The partial 16S mtDNA sequence Bayesian tree for jellyfish species which denotes major clades. The tree was rooted with outgroup Cubozoa. Numbers on the branches indicate bootstrap values of Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian posterior probabilities, respectively.

and COI primers (Cunningham and Buss 1993, Geller et al. 2013), may have better potential in future jellyfish barcoding studies. Nevertheless, the application of COI, 16S, and ITS1 as barcoding genes has been successful in confirming and identifying some of the jellyfish species, or at least provided some very much needed baseline data and reference sequences for comparative studies (Matsumura et al. 2005; Ki et al. 2009a; Hamner and Dawson 2009; Bayha and Graham 2009).

Various evolutionary and systematic studies have employed the mtDNA COI (Dawson and Jacobs 2001; Schroth et al. 2002; Herbert et al. 2003; Holland et al. 2004; Dawson 2005a; Dawson 2005b) due to its maternal mode of inheritance, limited recombination, rapid evolution and the robustness of mtDNA against degradation (Avise 1994). Previous studies have also employed these genetic markers and detected cryptic species (Dawson and Jacobs 2001, Dawson 2003).

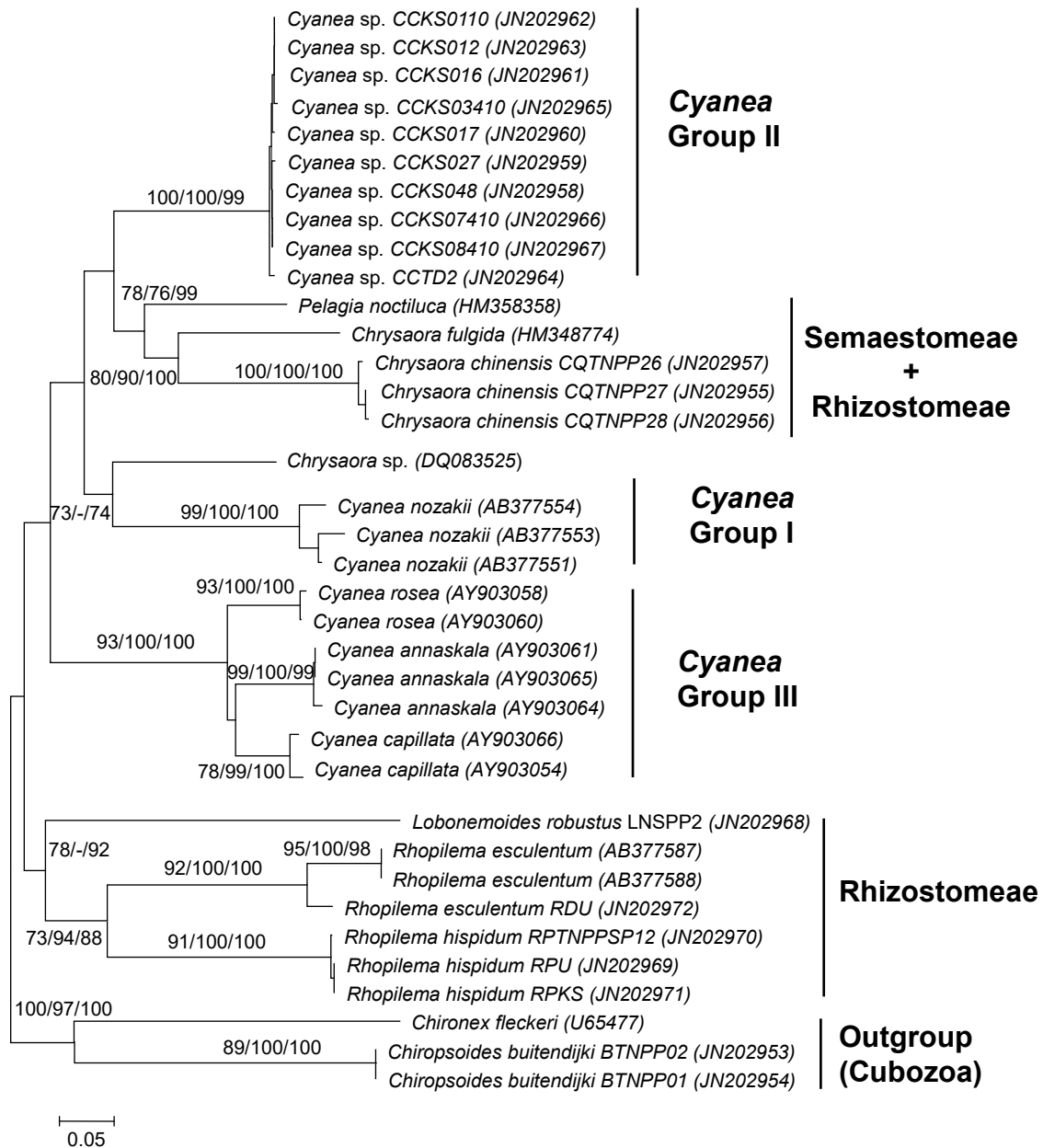


Fig. 5. The partial ITS1 sequence Bayesian tree for jellyfish species which was rooted with outgroup Cubozoa. The tree denotes major scyphozoan clades and distinct *Cyanea* species groupings (*Cyanea* Group I-III). Numbers on the branches indicate bootstrap values of Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian posterior probabilities, respectively.

Molecular genetic techniques may improve the taxonomical studies of jellyfish as identification based on morphological features alone is usually confounded by the 1) high degree of morphological polymorphism including colour variations, 2) difficulty in preserving and identifying specimens, 3) limited distinguishing characters and 4) incomplete taxonomic sampling (Haddock 2004; Bayha et al. 2010). According to You et al. (2007), different jellyfish populations may have different colourations/pigmentations, which are useful for distinguishing the populations. In the present study, *P. punctata* collected from three study sites do have different colourations and shapes of terminal clubs. *P. punctata* specimens collected from Matang are red and have long terminal clubs, whereas specimens from Tioman Island are blue and have round terminal clubs. Teluk Bahang specimens of *P. punctata* are transparent with round terminal club. Additionally, colour variations were also observed within specimens of *R. hispidum*, *C. chinensis* and *C. townsendi* from one sampling. These colour variations within species are possibly attributed to the zooxanthellae present in the mesoglea of some jellyfish species. Nonetheless, recent study on the upside-down jellyfish, *Cassiopea andromeda* showed no association between colour morphs and zooxanthella (Lampert et al. 2012).

While there is an increase in jellyfish research globally, rigorous jellyfish research could not be achieved if their basic biology and taxonomy remain elusive. Although better taxonomic keys for jellyfish are made available, jellyfish taxonomic expertise are limited globally. Therefore

collaborative research and taxonomic training should be encouraged, coupled with the use of molecular genetic techniques that could greatly improve taxonomical studies of this group.

Jellyfish Phylogenetics

Although a phylogenetic approach was taken to facilitate identification of the Malaysian jellyfish species, it is not the aim of the present study to infer phylogenetic relationships at taxonomic levels for the classification of jellyfish, as this would require many more taxonomically representative sequences. However, several phylogenetic relationships, albeit weakly supported, were observed and warrant explanation.

The present study suggests possible non-monophyletic or paraphyletic grouping of *Cyanea* and *Chrysaora*. It is interesting that *Cyanea* species from Malaysian (*Cyanea* sp.), Japan (*C. nozakii*) and Australia (*C. capillata*, *C. rosea* and *C. annaskala*) that *Cyanea* did not form a single clade. Perhaps this is a phylogenetic analysis artifact, as ITS sequences are notoriously difficult to align. However, paraphyly has been observed between Semaestomes and Rhizostomes (Collins 2002; Collins et al. 2006; Hamner and Dawson 2009). According to Dawson (2004), such paraphyletic and polyphyletic grouping of families are common. Although the present study could not conclude the paraphyly of *Cyanea*, the genetically distinct Malaysian *Cyanea* sp. may indicate the possibility of a new or cryptic species.

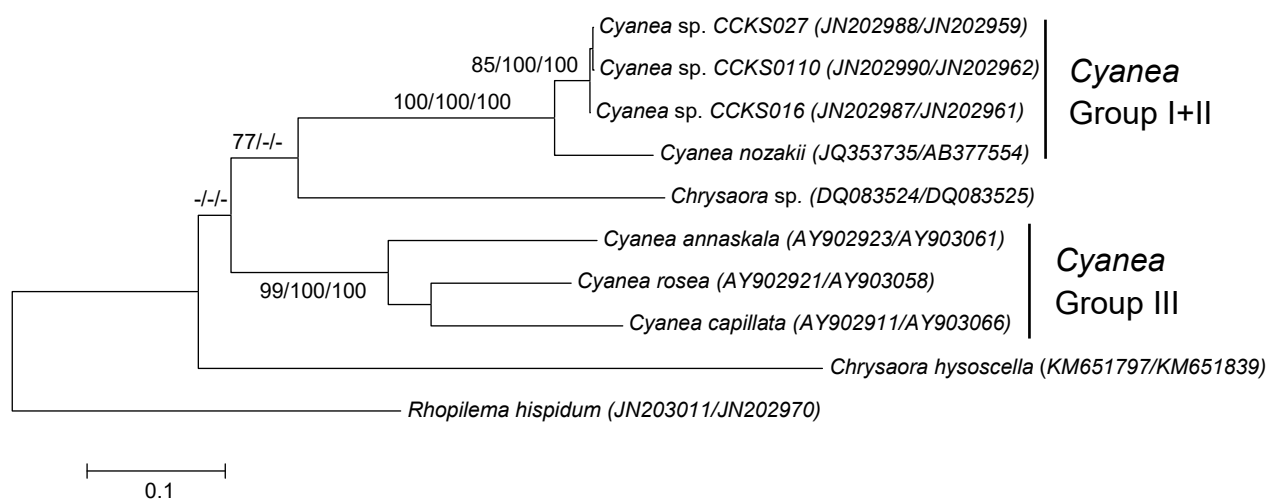


Fig. 6. The Bayesian tree of concatenated sequences (partial COI and ITS1) for jellyfish species which was rooted with *Rhopilema hispidum*. The tree denotes separation of *Cyanea* species into different groups (*Cyanea* Group I + II and III). Numbers on the branches indicate bootstrap values of Neighbour Joining (NJ), Maximum Parsimony (MP) and Bayesian posterior probabilities, respectively.

Diversity and distribution

There are few scientific studies on Malaysian jellyfish and the general information on jellyfish remains patchy and unverified. Even the commercially exploited edible species have been poorly studied (Rumpet 1991; Daud 1998) and only a few species have been identified recently (Omori and Nakano 2001; Kitamura and Omori 2010). The early research on jellyfish in Malaysia were mostly on the envenomation and toxicology of jellyfish stings, particularly from species that were believed to be *Catostylus mosaicus*, *Lychnorhiza lucerna*, *Chrysaora quinquecirrha*, *Chrysaora hysoscella*, *Carybdea rastoni*, and *Chironex fleckeri* (Othman and Burnett 1990; Tan et al. 1993; Azila and Othman 1993). Recently, more effort in the monitoring of jellyfish have been carried out, including the present study, in highly jellyfish affected areas such as in the Penang National Park. Some of the edible jellyfish in Malaysia such as “white type” jellyfish may refer to *Lobonemoides robustus* which was previously misidentified as *Lobonema smithii* (Daud 1998; Omori and Nakano 2001; Kitamura and Omori 2010; Kondo et al. 2014), whereas the “red type” edible jellyfish refers to *R. esculentum* (Daud 1998; Omori and Nakano 2001). From the present study, the edible species, *L. robustus* and *R. esculentum* contribute significantly to the jellyfish catch in Klang Strait (pers. obs.). Other edible jellyfish such as *R. hispidum* is also known to be harvested (Omori and Nakano 2001).

Monthly surveys in common fishing grounds in Klang Strait have indicated seasonal variation in jellyfish species composition. For example, *Cyanea* sp. and *P. punctata* were caught in high numbers every month, whereas *R. esculentum* and *L. robustus* were present in high numbers from July to September, i.e. during the relatively drier period of the southwest monsoon (pers. obs.). In Malaysia, *R. hispidum* is not commonly harvested for consumption, but is commercially exploited in Pakistan, where it was previously misidentified as *Rhizostoma pulmo* (Muhammed and Sultana 2008). Other than the 12 species of Scyphozoa and Cubozoa reported in this study, Malaysian waters may harbour many more jellyfish species that have yet to be recorded. Based on previous records of jellyfish in Southeast Asia, it is probable that similar species could also be found in Malaysia. Species that have been previously sighted by scuba divers include *Chironex fleckeri*, *Aequorea* sp. (Hydrozoa), *Cassiopea* sp., *Versuriga* sp. and

Vellela sp. (pers. orb.). Many jellyfish species are therefore yet to be recorded and described while awaiting taxonomic identification.

CONCLUSIONS

This study has provided the much needed baseline information on the diversity of Malaysian jellyfish species which have been substantiated by partial cytochrome oxidase I (COI), 16S and internal transcribed spacer (ITS1) sequences. A total of 12 putative species of jellyfish were identified, which encompassed 12 genera. Most of these species, particularly the edible species, occurred in high abundance in the coastal waters of the west coast as compared to the east coast of Peninsular Malaysia. More studies of Malaysian jellyfish are needed including species identification, trophic ecology and reproduction to tackle the problem of jellyfish blooms, and to further exploit jellyfish for commercial purposes. The current revision on the taxonomic of jellyfish seems to suggest different species composition in the Atlantic and Pacific Ocean (Dawson, pers. comm.). Issues such as invasive species (Perry et al. 2000; Graham et al. 2003) may further confound identification and alter local ecology. Concerted global action and collaboration in jellyfish studies, including global initiatives to document jellyfish such as jellywatch.org, cnidtol.com and JEDI (Jellyfish Database Initiative) to compile jellyfish information are therefore needed to understand jellyfish blooms and invasions.

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