

A morphological evaluation of *Chrysaora chinensis* of Peninsular Malaysia and distinguishing its populations using geometric morphometrics

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Abstract

The morphology of seven specimens of *Chrysaora chinensis* (Scyphomedusae, Semaestomae) obtained from four sites off the coast of Peninsular Malaysia was examined. Morphological characteristics of *C. chinensis* that encompasses structures such as the bell, tentacles, oral arms, stomach, manubrium, radial canals and gonads were described in detail. A total of 107 specimens that represented *C. chinensis* populations of four coastal areas of Peninsular Malaysia (East-Central, East-North, West-Central, and West-North) were also analysed for shape variation using geometric morphometric analysis. Procrustes superimposition, Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were applied to the images of gastrovascular pouches of *C. chinensis* to extract the shape information. Independent contrasts were used for comparisons between shapes. There were no significant differences in shape variation between all the specimens based on the PCA results. However, CVA results showed shape variations between specimens taken from the four areas of Peninsular Malaysia, especially with higher magnitudes of Mahalanobis distances between the east and west coast areas, including between East-Central and East-North, but lower magnitudes were detected between the West-Central and West-North.

Key words: geometric morphometrics, Scyphozoa, jellyfish, morphology, Malaysia

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1 Introduction

The sea nettle jellyfish of the genus *Chrysaora* are classified under the family Pelagidae (order Semaestomae) and are typically recognized by having 32–48 lappets, with eight marginal sense organs, with three or more tentacles per octant, with 16 gastrovascular pouches, and with numerous warts on the exumbrella (Kramp, 1961). Species of sea nettle have a worldwide distribution and have been reported to occur in the South China Sea, North, Central and South America, Africa, Europe and Australia (Morandini and Marques, 2010; Yap and Ong, 2012). According to the World Register of Marine Species (<http://www.marinespecies.org>) there are possibly 15–18 species, whereby 5 species have been verified: *Chrysaora achlyos*, *Chrysaora hysoscella*, *Chrysaora pacifica*, *Chrysaora quinquecirrha* and *Chrysaora chinensis*.

Although some regions of Southeast Asia have been reported to harbour *Chrysaora* species such as *Chrysaora quinquecirrha* and *Chrysaora melanaster* (Kramp, 1961; Yap and Ong, 2012), there is the possibility they were misidentified base on colour variations and this possibility warrants verification. In the past, jellyfish species were notoriously difficult to identify as there were no reliable taxonomic keys, specimens were badly preserved, and confounded by the existence of cryptic species com-

plexes that could be detected only by the application of molecular genetic techniques. For example, *C. chinensis* in Malaysia has been previously identified as *C. hysoscella* or *C. quinquecirrha* based on reports of envenomation and toxicology studies of jellyfish stings (Azila and Othman, 1993) and those in the Singapore Straits as *C. melanaster* (Yap and Ong, 2012)—possibly misidentified due to the colour variations. Some features, such as nematocysts have been used to aid in the species identification, hence sea nettles from the South China Sea were believed to be *C. chinensis* (Morandini and Marques, 2010; Yap and Ong, 2012). More recently, applications of DNA sequence analysis and phylogenetics have helped identify and barcode some of the Malaysian jellyfish species, including *C. chinensis* (Rizman-Idid et al., 2016), which concurred the notion that specimens with different colour morphs are often genetically similar and regarded as the same species. However, it is important to realize that the efficiency of such DNA barcoding method depends on the availability of reference sequences of correctly identified voucher specimens in the GenBank database. Although the study provided 16S and ITS1 sequences, it did not have the required COI sequences to definitively barcode *C. chinensis*. Furthermore, the morphological description of the species in the study was relatively simple and preliminary. Hence a more detailed morphological evalu-

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ation of the Malaysian sea nettle (*C. chinensis*) is required.

Chrysaora chinensis in Malaysia has tendencies to bloom, sometimes causing blockage of cooling systems of coastal power plants, contamination of fishing nets and a nuisance to fishing activities (pers. obs.). Beach tourism is also affected as beach goers are often warned about its stings that are intense with painful burning sensation. In general, jellyfish blooms have been linked to eutrophication and nutrients added to the diet of the jellyfish (Purcell et al., 2007; Richardson et al., 2009). Moreover, morphological adaptations to different localities with various ecological and environmental conditions are well documented for jellyfish (Dawson, 2005). Therefore, this present study aims to distinguish morphologically the populations of Malaysian *C. chinensis* by using geometric morphometrics, especially between populations of the Straits of Malacca that are heavily impacted by anthropogenic activities from those found in the South China Sea where the water quality is somewhat better.

Geometric morphometric analysis is the quantitative study of the biological shape, shape variation and covariation, which until recently have been applied to study the shape of organisms such as oak leaves (Viscosi and Cardini, 2011), dogs (Drake and Klingenberg, 2010) and cichlids (Maderbacher et al., 2008). Traditional multivariate morphometrics usually analyse multiple measurements together by using canonical variates analysis (CVA), principal components analysis (PCA), whereas geometric morphometrics combine multivariate analysis and methods for the direct visualization of biological form, making it appealing to researchers since the results are no longer presented only as a series of statistical data but also as graphical representations of the actual organism being studied (Rohlf and Marcus, 1993; Adams et al., 2004). Furthermore, these visualizations provide information on morphological changes in their immediate anatomical context (Klingenberg, 2013; Bookstein, 2015).

It is noteworthy that the revival of *C. chinensis* was made recently by Morandini and Marques (2010) based on specimens from Philippines and China and assumed the species is distributed throughout the South China Sea. Other than the work by Yap and Ong (2012) on specimens found in the Singapore Straits, there has been no in-depth morphological study of the *C. chinensis* from Malaysia waters, other than the preliminary taxonomic survey and molecular documentation undertaken by Rizman-Idid et al. (2016). Thus, this study is the first of its kind to provide a more detailed morphological description of *C. chinensis* of Peninsular Malaysia and to employ geometric morphometric analysis to distinguish its populations.

2 Materials and methods

2.1 Sample collection

Samples were collected between July 2013 to March 2015 to obtain as many specimens as possible from six sampling sites

that represented four coastal areas; these being West-Central (WC) (Sungai Janggut), West-North (WN) (Pantai Kok and Balik Pulau), East-Central (EC) (Kampung Cempaka), and East-North (EN) (Pantai Sabak and Pantai Melawi) coasts of Peninsular Malaysia (Table 1) (Fig. 1). Fishing equipment such as bag nets and dip nets were employed to collect the samples. Jellyfish specimens that were in relatively good condition and least damaged were chosen and photographed immediately upon acquisition to record their live colouration. 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 bell and oral arms were taken and preserved in absolute ethanol for molecular analysis. Whole specimens were preserved in 3% formalin in sea water for a week and then transferred into new solution of 3% formalin in sea water for improved fixation. The identified specimens were tagged, catalogued and voucher specimens deposited in the Zoological Museum, Institute of Biological Sciences, University of Malaya (MZUM).

2.2 Morphological examination

The preserved specimens and their morphological structures such as bell, lappet, gastrovascular cavity, tentacle, muscle, network of canals, oral arm, scapulae, terminal club, filament and gonad were examined, measured and recorded. Types of nematocysts from the preserved oral arm tissues were identified and photographed under stereo microscope. The morphology of the specimens was also photographed using a digital camera (Olympus PEN Lite E-PL5, lens 18 mm to 105 mm) with additional light sources, scale and colour chart included, following two different methods; firstly, by suspending the specimen with wires in a glass aquarium containing water so as to appear floating and with the specimen positioned as natural as possible; and secondly, by laying the specimen on a flat transparent acrylic stage for dry photography. All morphological examinations and photography procedures were adopted from Daglio and Dawson (2017).

2.3 Geometric morphometric analysis

The gastrovascular pouches of *C. chinensis* (Fig. 2) were chosen for geometric morphometric analysis as these internal structures were consistently intact, as opposed to external structures such as tentacles, oral arms, or exumbrella that are often damaged during sampling. The gastrovascular pouches are located at the underside of the umbrella of the jellyfish with the oral arm extending below it.

A total of 107 specimens were used for geometric morphometric analysis. Each specimen was placed on the acrylic stage with their subumbrella facing up towards the camera, whereby a quadrant of the subumbrella was photographed, which would include three gastrovascular pouches and three rhopalia (Fig. 2). A

Table 1. The sites where samples of *C. chinensis* were collected off the coasts of Peninsular Malaysia, with the sample sizes (*n*) and sampling method

Area	Site	Period	GPS	Method
West-North (WN) (<i>n</i> =38)	Pantai Kok (<i>n</i> =5)	14 Apr. 2014	6.348 69°N, 99.648 06°E	Dip Net
	Balik Pulau (<i>n</i> =33)	14 Dec. 2014	5.539 72°N, 100.348 88°E	Dip Net
West-Central (WC) (<i>n</i> =27)	Sungai Janggut (<i>n</i> =27)	9 Jul. 2013	3.169 16°N, 101.298 33°E	Bag Net
		5 Dec. 2013		
		29 Apr. 2014		
East-North (EN) (<i>n</i> =26)	Pantai Sabak (<i>n</i> =21)	20 Jul. 2014	6.137 12°N, 102.369 76°E	Dip Net
	Pantai Melawi (<i>n</i> =5)	21 Jul. 2014	5.996 12°N, 102.437 14°E	Dip Net
East-Central (EC) (<i>n</i> =16)	Kampung Cempaka	26 Jun. 2014	3.743 65°N, 103.328 47°E	Dip Net

Note: The sites are designated as coastal areas West-North (WN), West-Central (WC), East-North (EN) and East Central (EC) of Peninsular Malaysia.

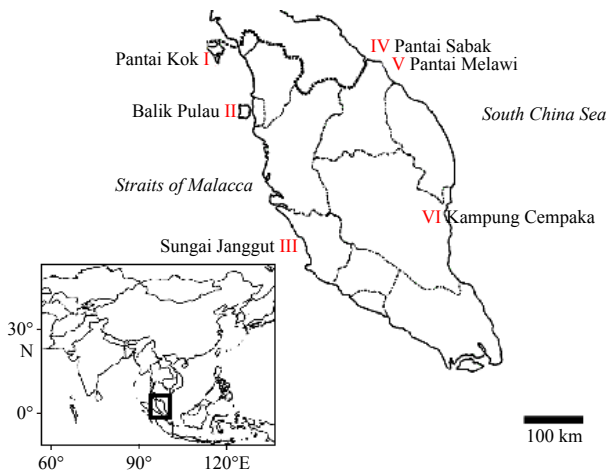


Fig. 1. Map of the sampling sites (I–VI) from the four areas of Peninsular Malaysia where specimens were obtained: East-Central (EC)—Kampung Cempaka (16 specimens), East-North (EN)—Pantai Sabak (21 specimens) and Pantai Melawi (5 specimens), West-Central (WC)—Sungai Janggut (27 specimens) and West-North (WN)—Pantai Kok (5 specimens) and Balik Pulau (33 specimens).

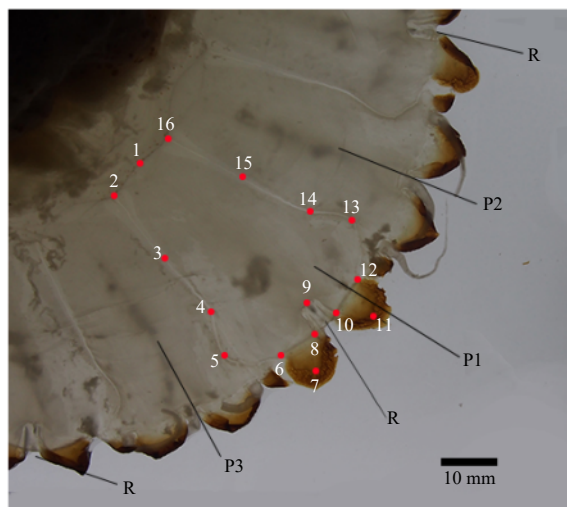


Fig. 2. An image of a subumbrella quadrant of a *C. chinensis* specimen showing the 16 geometric morphometric landmark points (indicated in red and numbered) of a single gastrovascular pouch analysed (P1). Pouch P1 is flanked by pouches P2 and P3. Rhopalia are indicated as R.

scale was placed next to the specimen. Only one of the pouches was chosen for analysis from each image. However, two pouches were photographed as replicates for each specimen.

A total of 214 images representing 107 specimens were converted to a TPS file format using tpsUtil (Rohlf, 2015). TPS images were digitized in tpsDIG (Rohlf, 2015) to produce a digitized image data. These digitized data contain information of 16 defined landmarks (Fig. 2, see Table 2 for landmark definitions) that were configured on the gastrovascular pouch. The image sizes were standardized to a set scale. This digitized image data were imported into MorphoJ (Klingenberg, 2011) for geometric morphometric analysis.

2.4 Procrustes ANOVA analysis

In order to quantify measurement error, 25 specimens were randomly chosen for the Procrustes ANOVA analysis. For each specimen, two gastrovascular pouches were photographed, whereby each pouch was photographed twice, and each photograph was digitized twice. Therefore, two levels of measurement errors were calculated: the errors occurring from imaging and those from digitization of landmarks. A total of eight images were obtained from each specimen for the Procrustes ANOVA analysis (2 pouches × 2 photographs × 2 digitized landmark images). Hence, for 25 specimens, a tps file with 200 raw coordinate data was imported into MorphoJ for the Procrustes ANOVA analysis.

2.5 Procrustes superimposition

A generalized Procrustes superimposition was also performed on the gastrovascular pouch to eliminate the non-shape information (size, position and orientation) of the image. A covariance matrix was initially generated before running the Procrustes analysis to produce a result set. Once the Procrustes analysis was completed, two types of statistical analyses were performed: The Principal Component Analysis (PCA) for the general inspection of shape variation and the Canonical Variate Analysis (CVA) to distinguish populations by coastal areas.

2.6 Visualization of shape outline

An outline file was produced in tpsDIG using 32 landmark co-ordination sets. They were able to represent the shape outline of the gastrovascular pouch that resembles more closely to that of the specimen when compared with outlines based on only 16 landmarks for the geometric morphometric analysis process.

3 Results

3.1 Morphological description

Chrysaora chinensis Vanhoeffen, 1888 (resurrection by Morandini and Marques, 2010)

(Figs 3a–e, 4a–f and 5)

Class Scyphozoa Goette, 1887

Order Semaestomeae Agassiz, 1862

Family Pelagiidae Gegenbaur, 1856

Genus *Chrysaora* Péron and Lesuer, 1809

Materials examined: A total of seven specimens were examined from the sites of Sungai Janggut (MRI 2, MRI 65, MRI 163, and MRI 171: voucher MZUMCS0234– MZUMCS0237), Pantai Sabak (MRI 208: voucher MZUMCS0238), Balik Pulau (MRI 262: voucher MZUMCS0239) and Kampung Cempaka (MRI 190: voucher MZUMCS0240).

Description: Umbrella hemispherical. For adult medusa, ex-umbrella surface finely granulated; in some specimens the ex-umbrella is transparent but some have light reddish spots distributed evenly on the surface. Mesoglea rigid; thick in the centre and thinner towards the bell margin. Six lappets per octant; shapes are semi-circular, with rhopalar lappets. On certain specimens there is a small lappet connecting to the rhopalar lappet. These lappets are loosely connected with the adjacent lappet, and some of them appeared to be separated, therefore making eight lappets per octant on certain specimens. Eight rhopalia present in clefts of the umbrella margin, located at the interradi- al and perradi- al axis. The rhopalia shape is tear-drop like.

Three tentacles per octant (2-1-2, where the numbers represent the ontogenetic order of the development of tentacles) originate at the cleft of the lappet. Tentacles are soft, straight and string-like. Length of tentacles up to four times the diameter of

Table 2. Position of the 16 landmark configurations

Landmark	Position
LM 1	proximally, at the left of the rhopalar pouch nearest to the gastrovascular cavity
LM 2	proximally, at the upper left corner of the pouch
LM 3	centrally, at the left septa of the pouch; half way between the top of the pouch to the point where septa curves
LM 4	at the section where septa starting to curve outward
LM 5	distally at the section where septa is curved at its furthest
LM 6	at the section where septa curves inward
LM 7	distally at the lowest left point of the pouch
LM 8	distally at the lower left of the septa next to the rhopalium
LM 9	centrally at the rhopalium
LM 10	distally at the lower right section of the septa next to the rhopalium
LM 11	distally at the lowest right section of the pouch
LM 12	at the section where septa curves outward
LM 13	distally at the section where septa is curved at its furthest
LM 14	at the section where septa starting to curve inward
LM 15	centrally, at the right septa of the pouch; half way between the top of the pouch to the point where septa curves
LM 16	proximally, at the upper right corner of the rhopalar pouch

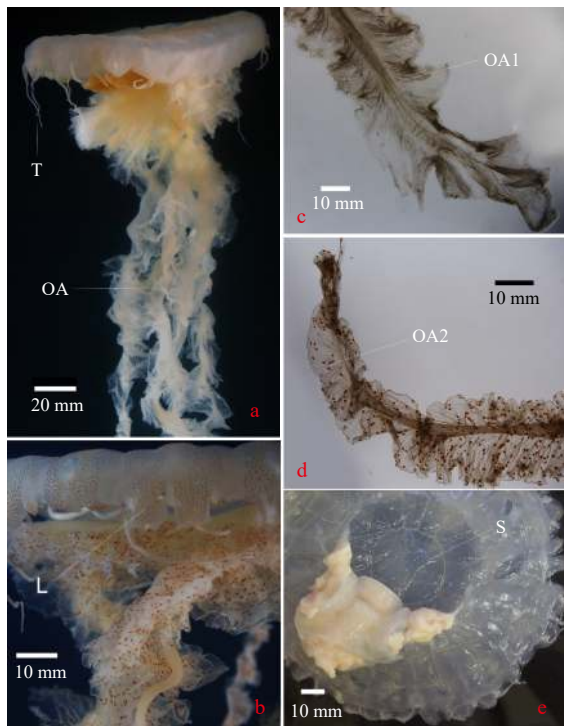


Fig. 3. Gross morphology of *Chrysaora chinensis*. a. Whole medusa floated in a tank, showing four soft and curtain-like oral arms (OA) and tentacles (T); b. side view of the bell (umbrella) with reddish brown pigmentation on the lappet (L); c. oral arms (OA1) without pigmentation; d. oral arms (OA2) with reddish pigmentation; and e. subumbrella view of the central stomach (S).

the bell. Longer tentacles are prone to detachment and damage when handled roughly.

Four oral arms with length up to 200 cm formed at the end of the oral pillar. Oral arms are transparent, soft and curtain like, with a ridge in the centre and intricate folds at the edges. In certain specimens numerous reddish/brownish spots are found all over the oral arm. Scapulae, terminal club and filament absent.

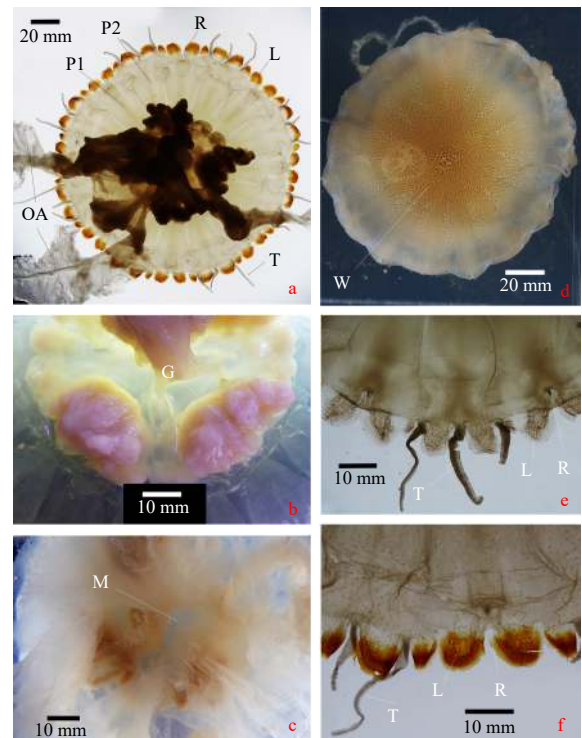


Fig. 4. Morphology of the umbrella. a. Subumbrella view revealing 16 gastrovascular pouches, rhopalar pouch (P1), inter-rhopalar pouch (P2), rhopalium (R), lappet (L), tentacle (T) and oral arms (OA); b. subumbrella view of creamy white gonads (G), whereby each of the four gonads is located inside a subgenital fenestration of the subumbrella; c. subumbrella view showing the mouth (M) bounded by four walls; d. view of exumbrella with numerous warts (W) densely concentrated in the centre; e. view of subumbrella showing lappet (L), rhopalia (R) and three tentacles (T) following a 2-1-2 arrangement per octant, whereby the numbers represent the ontogenetic sequence of tentacles originating from the cleft of the lappet; and f. subumbrella view of another specimen showing distinctly pigmented lappet (L), rhopalia (R) and tentacles (T).

Oral arms nematocysts comprised of heterotrichous microbasic, holotrichous O-isorhiza, holotrichous A-isorhiza and holotrichous a-isorhiza (Fig. 5).

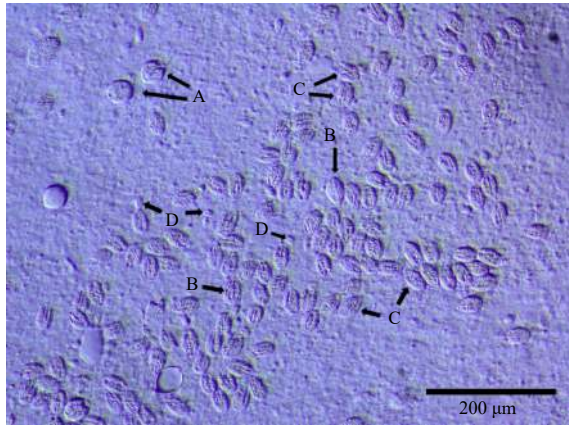


Fig. 5. Nematocysts of *Chrysaora chinensis*. A represents holotrichous O-isorhiza, B holotrichous A-isorhiza, C heterotrichous microbasic, and D holotrichous a-isorhiza.

Central stomach shape slightly rounded, as it is formed by the boundary of the 16 gastrovascular pouches, where eight are rho-

palar pouches and eight are inter-rhopalar pouches. Radial septa present; extending distally from stomach toward bell margin. For rhopalar pouches, septa begin widening at approximately 1/3 from the centre, and thinning again, making an “S” shape that ends at the base of the lappet. Subgenital fenestration shape oval, 1/8 of umbrella diameter. Papillae and network of canal absent. Radial and coronal musculatures are not prominent. Manubrium is not prominent. Oral disk at the base is about 1/3 of bell diameter, and the base of oral arm is less than 1/3 of the bell diameter. Mouth present surrounded by four walls. Quadrilinga absent.

Four gonads present, each located inside a subgenital fenestration of the subumbrella; each gonad is separated from the neighbouring gonad by a septum; associates with the subumbrella and manubrium. Colour creamy white. Gastric filaments present.

3.2 Geometric morphometric analysis

The measurement error of the gastrovascular pouch was computed from the Procrustes ANOVA (Table 3). The error caused by digitization and imaging was 0.5% and 1.64% of the individual variation, respectively. Both errors were negligible as they were much smaller than the individual variation. The error caused by individual by pouch (i.e., two different gastrovascular pouches per specimen) was quite large at 45.8% of the individual variation. Therefore, it was necessary to use more than one gastrovascular pouch per specimen to average out the errors.

Table 3. Result of Procrustes ANOVA

Effect	SS	MS	Percentage of error/%	df	F	P
Individual	0.356 088 89	0.000 529 89	-	672	2.18	<0.000 1
Pouch	0.009 046 13	0.000 323 07	-	28	1.33	0.119 2
Individual×Pouch	0.163 039 50	0.000 242 62	45.80	672	27.96	<0.000 1
Imaging	0.012 149 61	0.000 008 68	1.64	1 400	3.29	<0.000 1
Digitize	0.007 382 85	0.000 002 64	0.50	2 800	-	-

PCA was performed on a total of 107 specimens. Most of the total variance was explained by the first two PCs. PC1 was accounted for 33.86% of the total variance of the shape change based on the widening of both sides of the gastrovascular pouch. PC2 was accounted for 19.41% of the total variance of the shape change following the retraction or shortening of the distal left and right points of the pouch, thus protuberance of the pouch appears less prominent and more blunt. The shape space scatter plot between PC1 and PC2 (Fig. 6) indicates that most of the specimens overlap, with a few exceptions of outliers. Hence, there were no significant differences in shape between all the specimens based on PCA.

CVA was performed on a total of 107 specimens representing populations of four coastal areas of the Peninsular Malaysia: East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN). CV1 accounts for 47.46%, CV2 accounts for 32.71%, and CV3 accounts for 19.83% of the amount of relative between-group variation (Fig. 7). Populations of EC and EN are separated from those of WC and WN. Populations of WC and WN overlap and are not distinguishable (Fig. 8). The magnitude of Mahalanobis distance is lowest between populations of WC and WN populations but higher between east and west coast comparisons, the highest between EC and EN (Table 4).

4 Discussion

The detailed morphological description of *C. chinensis* in the present study represents the first of its kind in Malaysia.

Chrysaora chinensis was originally described by Vanhöffen (1888), and known to occur in South China Sea (Vanhöffen,

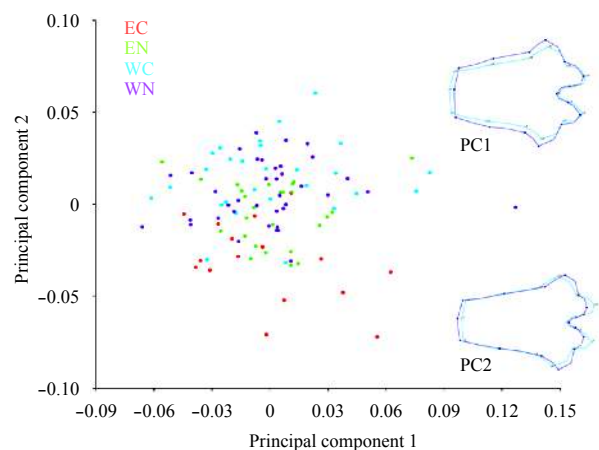


Fig. 6. Scatter plots of PC1 vs. PC2. PC1 accounted for 33.86% and PC2 accounted for 19.41% of the total variance of the shape change of gastrovascular pouch of specimens from East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia. Two independent contrasts of the gastrovascular pouch shape of the two main components PC1 and PC2 are illustrated whereby, light blue outline indicates the mean shape and the dark blue outline indicates the shape change.

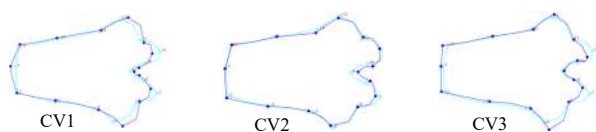


Fig. 7. Independent contrast of component using canonical variate analysis of the gastrovascular pouch of specimens from East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia, whereby CV1, CV2 and CV3 accounts for 47.46%, 32.72% and 19.83% of the amount of relative between-group variation, respectively. Light blue outline indicates the mean shape and the dark blue outline the shape change. CV1 denotes changes with blunt protuberance at the margin and enlarging of the distal end, CV2 changes with blunt protuberance at the margin and CV3 changes with blunt protuberance at the margin and enlarging of the distal end.

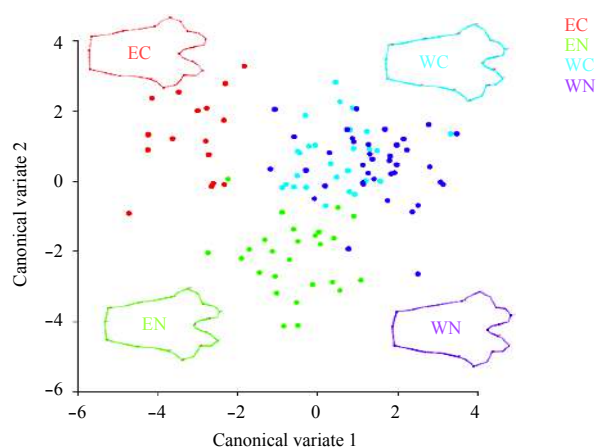


Fig. 8. Scatter plot of CV1 vs. CV2 and illustration of mean shape of gastrovascular pouch of specimens from four coastal areas East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia. Plot shows shape differences mainly between specimens of the east and west coasts, even between those from EN and EC, but with no distinct differences between those from WN and WC.

Table 4. Mahalanobis distances among four coastal areas designated as West-North (WN), West-Central (WC), East-North (EN) and East-Central (EC) of Peninsular Malaysia

	WC	EN	EC
WN	2.648 5	3.403 4	4.609 0
WC	-	3.401 4	4.215 1
EN	-	-	4.329 6

1911). The species is commonly found in all beaches in Malaysia. Based on personal observations and interviews with fishermen, they occur more abundantly at more polluted areas, particularly during the dry season (April to October). Prior to the resurrection of *C. chinensis* by Morandini and Marques (2010), many *Chrysaora* specimens found in the South China Sea and Malay Archipelago have been identified as either *C. melanaster* (Yap and Ong, 2012) or *C. helvola* (Kramp, 1961). However, Morandini and Marques (2010) emphasized that *C. chinensis* is very different from *C. helvola* (valid name *C. fuscescens*) and *C. melanaster* due to the differences in tentacle and lappet numbers. Both of the latter species also are larger than their observed specimens of

C. chinensis. Recent studies by Yap and Ong (2012) ascribed the specimens from St. John's Island in Singapore as *C. chinensis*. Likewise, findings from this present study, which focused on specimens obtained from the east and west coasts of Peninsular Malaysia confirms that the sea nettles found in these waters were morphologically identified as *C. chinensis*. Furthermore, DNA sequences from some of the specimens obtained in the present study (unpublished data) revealed that they are *C. chinensis* and genetically similar and phylogenetically clustered to those reported by Rizman-Idid et al. (2016). Although there is no correction to the species description by Morandini and Marques (2010), the morphological description of *C. chinensis* from this study is more detailed than that by Rizman-Idid et al. (2016). It is noteworthy that the colourations of *C. chinensis* can vary greatly. Some specimens are transparent whereas some are reddish with radiating stripes on the exumbrella with pigmentations on oral arms and reddish-brown lappets. Nonetheless, this colouration is corroborated by the colouration diagnosis of *C. chinensis* by Morandini and Marques (2010) and Yap and Ong (2012).

Both PCA and CVA were used for data analysis of geometric morphometrics, but the result of CVA better illustrates the shape variation between the different geographical. CVA is a type of analysis that efficiently summarizes the maximal difference between more than two pre-defined groups (relative to within-group variance), unlike PCA which is a quick way to explore overall variation across the dataset (such as outliers and clusters of specimens) and may not be able to detect differences between groups (Webster and Sheets, 2010). In this study, the result of CVA shows that the shape difference is mainly between the east and west coast, where the shape of the gastrovascular pouch of specimens from the east coast is slightly elongated compared to those from the west coast. Unlike Rhizostomeae jellyfish, *C. chinensis* do not have well developed canal. The gastrovascular pouches of *C. chinensis* are used to store nutrients from the central stomach that permeates to various parts of the jellyfish. The east coast of Peninsular Malaysia is facing the South China Sea, whereas the west coast is facing the Straits of Malacca, one of the busiest sea routes in the world, hosting annually about 65 000 vessels for international navigation, and up to another additional 15 000 fishing vessels (Ibrahim and Khalid, 2007). The low water quality of the Straits of Malacca is due in a large part to pollution from the shipping industry, aquaculture, a densely populated coastal areas resulting in nutrients and effluent discharged into the straits, increasing the total suspended solids and decreases the dissolved oxygen, thus causing eutrophication (Rezai et al., 2003; Bong and Lee, 2008; Praveena et al., 2011). Human activities that lead to eutrophication have been associated with the bloom of the jellyfish (Arai, 2001; Parsons and Lalli, 2002; Malej et al., 2007; Purcell et al., 2007; Richardson et al., 2009). Jellyfish appear to be capable of adapting to fluctuating levels of water quality and other environmental parameters, such as salinity, temperature, food source (Purcell et al., 2012). According to Brotz (2011), there were different trends of jellyfish populations in the coastal ecosystems and oceans worldwide. Using data that were aggregated and analysed at the scale of Large Marine Ecosystem (LME), he showed there is an increase in native and invasive jellyfish populations in the Bay of Bengal, which included the Straits of Malacca in the LME's definition. In contrast, the abundance of such jellyfish populations in the South China Sea was stable or variable.

Since the main function of the gastrovascular system is for the circulation of nutrients (Arai, 1997), these differences in gastrovascular pouch shapes observed in the present study may be a

form of morphological adaptation of *C. chinensis* populations to the different conditions of the coastal areas of Peninsular Malaysia. The landmass of Peninsular Malaysia acts as the main physical barrier which separates the east from the west populations. The EC population also appears distinct from the EN population. Although the distance between the sampling areas of both populations is only approximately 350 km; a distance that may not be of significant magnitude to explain the isolation by distance for these populations; these populations may have been kept separated due to occurrences of eddies and differing water circulations at 5°N, just off the east coast of Peninsular Malaysia (Daryabor et al., 2016). According to Daryabor et al. (2016), such water circulations may influence the distribution of the nutrient balance in regulating primary productivity and the changes in the marine ecosystem.

The present study has successfully demonstrated that internal structure such as the gastrovascular pouch is suitable, as the result appears to be robust with low measurement errors. Although geometric morphometric analysis has been proven useful in analysing and detecting even very minute shape variations in various organisms (Klingenberg, 2013), its application as demonstrated in the present study on gelatinous organism such as jellyfish, may still be informative albeit with a few practical challenges. Geometric morphometric analysis seems more suited for organisms with rigid structures, as landmarks are more easily identified and configured, thus detection of shape variation would be attributed due to real morphological differences rather than inconsistencies in measurements due to mishandling of specimens. To date, there has been no shape variation study using geometric morphometric analysis on soft or gelatinous organisms. Jellyfish, being gelatinous, can be quite easily distorted, either from bad sampling methods that can damage their delicate structures or even from prolonged preservation of specimens. In fact, formalin can contribute to the change of shape of jellyfish, especially after a long period of immersion (Kapuris et al., 1997). Therefore, using geometric morphometric analysis to detect shape variation in jellyfish poses a higher degree of difficulty compared to rigid organisms.

The practical usage may be improved by choosing a different structure for landmark configurations, depending on the species. As for other jellyfish species, such as *Rhopilema esculentum* of the Rhizostomeae family are bigger in size and generally more robust, such that their external or internal rigid structures may be chosen as a better target for geometric morphometric study.

5 Conclusions

Although the study of jellyfish is still new in Malaysia, the present study contributes much needed baseline data for the country and region. The present study has successfully ascribed the sea nettles found in Peninsular Malaysia as *C. chinensis* and demonstrated that its populations are morphologically varied between the different coastal areas base on gastrovascular pouch shape. Application of geometric morphometric analysis may also facilitate future studies of jellyfish to either delineate species or distinguish populations.

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