MORPHOLOGICAL CHARACTERISATION OF SELECTED SCYPHOZOAN JELLYFISH SPECIES AND GEOMETRIC MORPHOMETRIC ANALYSIS OF *Chrysaora chinensis* IN PENINSULAR MALAYSIA

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

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Field of Study: **Environmental Science (Marine Sciences)**

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ABSTRACT

Although jellyfish blooms have significant impacts to the coastal environments, the effective management of blooms and understanding of their proliferation are often confounded by the lack of baseline data, which includes species identification, their biology and ecology. In Malaysia, jellyfish blooms can negatively impact human activities such as causing beach closures, damage to fishing nets, threats of stings and blocking power station systems. Therefore, detail characterization and documentation of their morphology would facilitate species identification of jellyfish in the field, especially in Malaysian waters and this region which may harbours many undiscovered species. In this study, the morphology of nine jellyfish species found in Peninsular Malaysia that belong to the class Scyphozoa, namely *Chrysaora chinensis, Cyanea* sp., *Versuriga anadyomene, Rhopilema hispidum, Rhopilema esculentum, Phyllorhiza punctata, Acromitus flagellatus, Lobonemoides robustus* and *Lychnorhiza malayensis* were characterised in detail. Sea nettle jellyfish in Malaysia was verified as *C. chinensis*. The status of *Cyanea* sp. found in Malaysia is uncertain as it may possibly be a new species. This study also reported the first record of *Lychnorhiza malayensis*. A total of 107 specimens of *C. chinensis* were obtained from four coastal areas of Peninsular Malaysia (East-Central, East-North, West-Central, and West-North) to compare the possible morphological variation between populations 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. There were no significant differences in shape among all the specimens based on PCA. However, CVA showed shape variation between populations of the four areas of Peninsular Malaysia.

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ABSTRAK

Walaupun ledakan obor-obor boleh memberi impak yang besar kepada persekitaran pantai, pengurusan yang berkesan dan pemahanam tentang perkembangan mereka sering terjejas disebabkan kekurangan data asas, termasuk pengecaman spesies, biologi dan ekologi mereka. Di Malaysia, ledakan obor-obor boleh memberi kesan negatif kepada aktiviti manusia seperti menyebabkan penutupan pantai, kerosakan kepada jala ikan, ancaman sengatan dan sistem stesyen kuasa tersumbat. Oleh itu, pencirian dan dokumentasi morfologi mereka secara terperinci akan memudahkan pengecaman spesies obor-obor di lapangan, terutamanya di perairan Malaysia dan rantau ini yang mungkin mengandungi pelbagai spesis yang belum ditemui. Dalam kajian ini, morfologi sembilan spesies obor-obor di Semenanjung Malaysia yang tergolong dalam kelas Scyphozoa iaitu *Chrysaora chinensis, Cyanea* sp., *Versuriga anadyomene, Rhopilema hispidum, Rhopilema esculentum, Phyllorhiza punctata, Acromitus flagellatus*, *Lobonemoides robustus*, dan *Lychnorhiza malayensis* telah dicirikan secara terperinci. Obor-obor "sea nettle" di Malaysia telah disahkan sebagai *C. Chinensis*. Status *Cyanea* sp. yang terdapat di Malaysia tidak dapat dipastikan dan mungkin merupakan satu spesies yang baru. Kajian ini juga melaporkan rekod pertama bagi *Lychnorhiza malayensis*. Sejumlah 107 spesimen *C. chinensis* diambil dari empat kawasan persisiran pantai di Semenanjung Malaysia (East-Central, East-North, West-Central, dan West-North) untuk membandingkan variasi morfologi spesies dengan menggunakan analisa "geometric morphometrics". "Procrustes superimposition", "principal component" (PCA) dan *"canonical variate"* (CVA) telah diaplikasikan terhadap imej kantung gastrovaskular *C. chinensis* untuk mendapatkan informasi bentuknya. Keputusan daripada PCA tidak menunjukkan perbezaan bentuk yang signifikan disemua specimen. Tetapi, keputusan CVA menunjukan perbezeaan bentuk diantara populasi dari empat lokasi di Semenanjung Malaysia.

ACKNOWLEDGEMENTS

I would like to express appreciation to my supervisor, Dr. Mohammed Rizman Idid and lab mate Wan Mohd Syazwan for the supports, ideas and guidance. I am grateful to the staff at Institute of Ocean and Earth Sciences (IOES), University of Malaya for providing constant assistance during the study. I thank University of Malaya for providing research grants RU006E-2014 and RG104-11SUS to my supervisor.

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CHAPTER 1: INTRODUCTION

1.1 Jellyfish Studies in Malaysia

The term ‘jellyfish’ generally refers to free-floating gelatinous animals that belong to the phyla Ctenophora and Cnidaria (Figure 1.1). The phylum Ctenaphora consists of organisms such as sea gooseberry and comb jellies, whereas the phylum Cnidaria consists of a wide range of both the sessile and floating forms of organisms that includes jellyfish, corals and sea anemones (http://www.marinespecies.org). All Cnidarian possess cnidae - an organelle-like capsule with eversible tubules, and it is considered as the diagnostic feature of Cnidarian. There are three main classes under the phylum Cnidaria: Cubozoa (46 accepted species), Hydrozoa, and Scyphozoa (187 accepted species) (Figure 1.2). All species under Scyphozoa, Hydrozoa and Cubozoa develop the ‘medusa’ or jellyfish stage in the life cycles. Class Scyphozoa is ascribed with four orders, namely Coronatae (crown jellyfish), Staurozoa (stalked jellyfish), Semaeostome (sea nettle) and Rhizostomae (true jellyfish), with 65 genera and over 187 species (Mayer, 1910; Kramp, 1961; Pitt & Kingsford, 2003; Brusca & Brusca 2002; Shao et al., 2006; Daly, 2007; Richardson et al., 2009; Bayha, 2010).

The most commonly observed jellyfish are usually those from the class Scyphozoa, particularly from the medusa life stage. They begin their life cycle from fertilized eggs that produces planulae. These free swimming planulae (Figure 1.3) then settled at the substrate, becoming scyphistomas, also known as polyps. During this sessile polyp stage, Scyphistomas asexually buds and strobilate to produce ephyra. Strobilation is a process where each layer of the scyphistoma is separated and form a new juvenile jellyfish, resembling a disc being liberated from a stack. This polydisc

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strobilation only found in Scyphozoan. Although most of the scyphozoan have a polyp stage, sometimes a direct development from planula to ephyra is also possible (Arai, 1997; Boero et al., 2008; Ceh, 2015). The adult medusa stages is dominant in the life cycle. Therefore, this free-swimming form is most commonly seen and found stranded on beaches (Figure 1.4).

Jellyfish are 97% water and are semi-transparent. They have two body layers, the outer layer epidermis and the inter layer gastrodermis (Figure 1.5). Between both layers is a thick layer of mesoglea which consists of fibres embedded in a hydrated matrix that contains cells. These layers of tissues make up the umbrella of the jellyfish which is usually bell shape, thus the umbrella is also known as the bell. The scyphozoan jellyfish are tetraradially symmetrical, meaning having many structures in multiples of four. It contains a simple gastrovascular cavity which acts as stomach. They are also characterized by having gastric filament in the stomach. Some scyphozoan jellyfish such as Semaestomeae contain an opening, or mouth at the subumbrella. There are four to eight oral arms near the mouth, which functions as arms to capture and transport food to the gastrovascular cavity. Jellyfish lack eyes, but possess many sensory receptors capable to detect light, pressure, temperature and gravity. These sensory receptors are concentrated in the marginal sense organ that contains the rhopalium (Nakanishi, 2015). Not all jellyfish possess tentacles. For Semaestomeae jellyfish, tentacles can be found at the margin of the bell or at the subumbrella whereas tentacles are absence from the Rhizostomeae jellyfish. Jellyfish contains network of canals that usually anestomoses with each others that formed various patterns (Hamner, 1995; Arai, 1997; Hale, 1999).

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Figure 1.1: An adult scyphozoan jellyfish, *Phyllorhiza punctata*

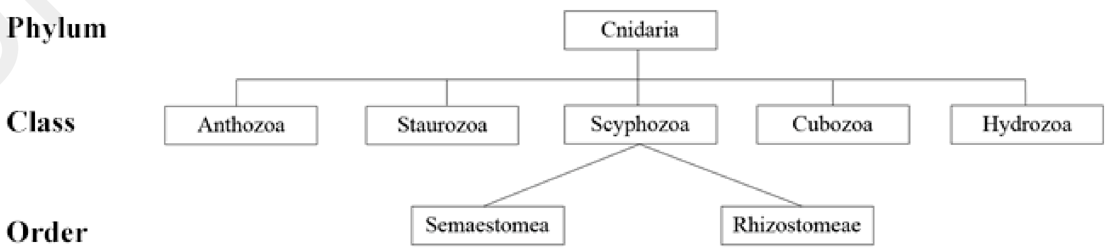

Figure 1.2: Classification of Cnidarian

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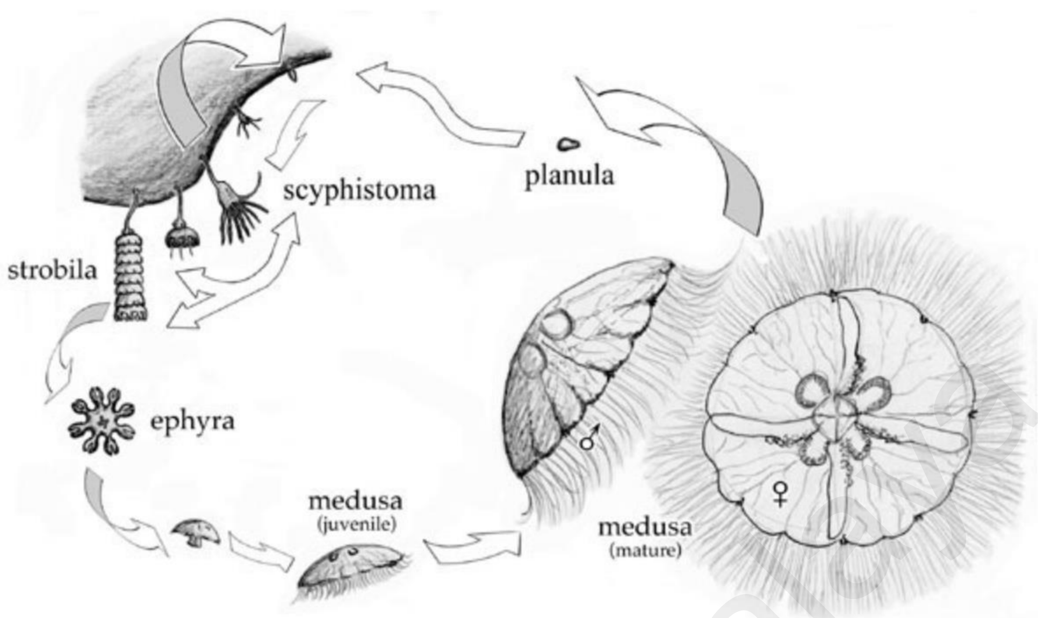

Figure 1.3: Life cycle of jellyfish (http://thescyphozoan.ucmerced.edu/Biol/Ecol/LifeHistory/ScyphozoaLH.html)

18 | 2016年4月20日（星期三）

國內 | 星洲日報

紅水母湧入吉膽

百年一見 島民憂天災前兆

報道 陳烟諭

（巴生19日讯）吉胆岛海域近2周离奇出现大量前所未见的红色水母，让许多渔夫及岛民忧心忡忡，担忧难不成是"大事"发生前的征兆？

他说，五条港附近的海域最多红色水母，所以当地的渔夫最近都不大敢在该处的海域捕鱼。

Figure 1.4: Bloom and stranding of *Crambione mastigophora* in Pulau Ketam in April 2016 (Sin Chew Daily, 20 April 2016)

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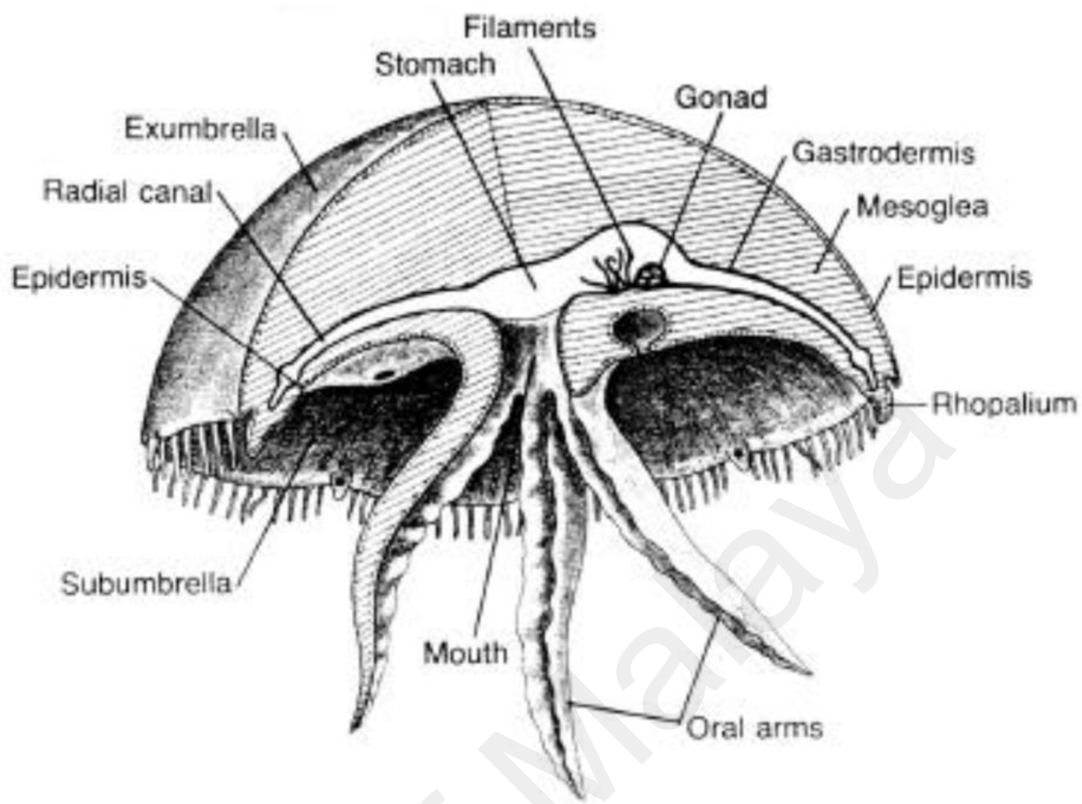

Figure 1.5: Cross section of a jellyfish (Hale, 1999)

The food source generated from the ocean today has been greatly reduced compared to from decades ago, due to factors such as global warming, ocean acidification and over fishing (Morton, 2005; Alison et al., 2009). Jellyfish has long been a delicacy in Asia for centuries and has been increasingly important as a food source (Hsieh et al., 2001; Pitt & Kingsford, 2003; You et al., 2007). But not all jellyfish species are edible, and some are not economically to be produced as food due to its size. A traditionally non edible jellyfish, *Cyanea nozakii* was produced into food in China but because of the low quality and unpleasant taste, the prices of food made from *Cyanea nozakii* fetched a much lower price than the more sought after *Rhopilema esculentum* (Dong et al., 2010). Today, the demand of edible jellyfish is greater than ever with more people from China, Japan, Korea, Singapore, Thailand and Malaysia consuming it. Jellyfish are marinated with a mixture of salt and alum over a period of approximately seven days, and the semi-dried products are sold as food ready for

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consumption. A few commonly sought after edible jellyfish in this region are the "red type" (*Rhopilema esculentum*), "white type" (*Lobonemoides robustus*), "river type" (*Acromitus hardenbergi*), "ball type" or "sunflower type" (*Crambionella annadalei*), "prigi type" (*Crambione mastigophora*), "sand type" (*Rhopilema hispidum*) (Omori & Kitamura, 2004).

Despite its value, jellyfish on the other hand do pose treat to human and the environment (Uye et al., 2010; Graham et al., 2015). Many jellyfish are venomous, and some are even deadly. Beaches are forced to be closed when large quantity of venomous jellyfish swamp the beach, causing lost in tourism revenue (Lucas, 2001; Purcell et al., 2009; Gershwin, 2010). Jellyfish blooms have been reported extensively over the past few decades (Brodeur, 2008; Kogovsek et al., 2010), notably the bloom of *Pelagia noctiluca* throughout the Mediterranean Sea during the 1980s (Doyle et al., 2007), bloom of *Phyllorhiza punctata* in the northern Gulf of Mexico in 2000 (Graham et al., 2003), and the bloom of *Nemopilema nomurai* in the Sea of Japan in 2002 and 2003 (Kawahara et al., 2006). There are increasing evidence indicate that human activities could attribute to the cause of jellyfish bloom, such as eutrophication (Purcell et al., 2001; Parsons & Lalli, 2002; Malej et al., 2007), overfishing (Mullon et al., 2005; Bakun & Weeks, 2006), introduction of alien species (Bolton & Graham, 2004; Graham & Bayha, 2007; Mills, 2001), installation of artificial substrates in the ocean (Richardson et al., 2009) and climate change (Raskoff, 2001; Attrill et al., 2007; Gibbons & Richardson, 2008; Ruiz et al., 2012).

Beside blooms, one of the most alarming problems is the introduction of nonindigenous jellyfish in a new region (Mills, 2001). They are believed to be introduced to the new environment by mean of exchange of ballast water and transport of biofouling polyps. (Richardson et al., 2009). Certain species of jellyfish previously not known to other region has since appearing. These invasive species cause decline in fisheries as they destroy the fishing net (Haddad & Nogueira, 2006).

Limited research was done on jellyfish in Malaysia. Due to the scarcity of research effort in jellyfish, especially the morphological study of jellyfish in Malaysia water, this region may harbor many species that yet to be discovered. Rumpet (1991) and Daud (1998) published a field survey of scyphozoan jellyfish, whereas others studied on their venom and toxinology (Othman & Burnett, 1990; Azila & Othman 1993; Tan et al., 1993), whereby species may have been misidentified. Jellyfish fisheries in Malaysia are focusing on the "red type", as it fetches the highest price due to the high demand from China and Japan, whereas "white type", "river type" and "sand type" fetches relatively lower price (Omori & Nakano, 2001; Omori & Kitamura, 2004), but these studies did not have satisfactory result on morphological descriptions and taxonomy identification as the studies were only done on edible jellyfish. Base on personal observations and reports, jellyfish often swamp and endanger beachgoers of being stung, which sometimes can be lethal. They are often stuck in the cooling intake of the power plant, causing damage to the power plant (Azila & Chong, 2010). It is also notable that when they bloom, they were sometimes caught in the fishing net and destroy them.

Despite their socio-economic importance in fisheries and also its treats, there has been a lack of baseline data and documentation of jellyfish, particularly about their diversity and ecology (Rizman-Idid et al., 2016). Jellyfish diversity studies in general has been confounded by problems of identification. Their morphological examinations are notoriously difficult, due to their fragile bodies, inadequate preservation and lack of sound identification keys.

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More recently, researchers have started using molecular genetic techniques to facilitate the classification of jellyfish and detection of cryptic species (Dawson, 2004). Applications of DNA sequence analysis and phylogenetics have been used to help identify and barcode some of the Malaysian jellyfish species, including *C. chinensis* (Rizman-Idid et al., 2016). Even with molecular techniques, species identification is still based on the description of its morphology. Hence a more detailed morphological evaluation of jellyfish in Malaysia is required.

1.2 Taxonomy Position and Problem of the Malaysia Sea Nettles

Jellyfish has a long history in evolution (Young & Hagadorn, 2010). Fossils have been reported from as early as early Cambrian in China (Hou et al., 2005), and well-preserved medusozoan fossils from the Middle Cambrian was reported in North America (Cartwright et al., 2007). Ever since Linnaeus first described the popular moon jellyfish Aurelia aurita in 1758, many studies have been done on numerous jellyfish species throughout the years, but there are still many problems confounded with their classification, such as the Malaysia sea nettles in the genus Chrysaora. The *Chrysaora* jellyfish are classified under family Pelagidae (order Semaeostomeae) and 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 has a worldwide distribution and have been reported to occur in the South China Sea, North, Central and South America, Africa, Europe and Australia (Morandini & Marques, 2010; Yap & Ong, 2012). According to World Register of Marine Species (http://www.marinespecies.org) there are possibly 15 -18 species, whereby 4 species have been verified; *Chrysaora achlyos*, *Chrysaora hysoscella*, *Chrysaora pacifica* and *Chrysaora quinquecirrha*.

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Although some regions of Southeast Asia have been reported to harbour Chrysaora species such as - *Chrysaora quinquecirrha* and *Chrysaora melanaster* (Kramp, 1961; Yap & Ong, 2012), there is the possibility they were misidentified base on colour variations and 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 complexes that could be detected only by the application of molecular genetic techniques. For example, *Chrysaora 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 & Othman, 1993) and those in Singapore straits as *C. melanaster* (Yap & Ong, 2012) – possibly misidentified due to the colour variations. Some features, such as nematocysts have been used to aid species identification, whereby sea nettles from South China Sea were believed to be *C. chinensis* (Morandini & Marques, 2010; Yap & Ong, 2012).

C. 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 (personal observation). 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 added nutrients to the diet of the jellyfish (Richardson et al., 2009). Therefore, certain areas in Malaysia were reported to have more occurances of *C. chinensis.* Moreover, morphological adaptations to different localities with various ecological and environmental conditions are well documented for jellyfish (Dawson, 2005). Thus, it will be beneficial to study the morphological variation of *C. chinensis* in Malaysia.

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1.3 Introduction to Geometric Morphometric Analysis

Shape analysis has long been an important and fundamental role in biological research (Klingenberg, 2016). Traditionally, taxonomic classification was base mainly on descriptive approach of morphology to formally describe species. Such shape descriptions are sometimes ambiguous, and may not be sufficient to delineate species, especially among closely related species that have high degree of morphological resemblance. In the beginning of the twentieth century, researchers started employing quantitative study of shape by meristic measurement such as length and width, and data collected were subject to statistical analysis, using univariate, bivariate or multivariateanalysis (Webster, 2010; Adams et al., 2013; Chen et al., 2013) to describe the pattern of shape variation (Adams et al., 2004; Bookstein, 1998). With the advancement of computing technology in the late 20th century multivariate morphometrics are preferred, in which multiple measurements are analysed together using Canonical Variates Analysis (CVA), Principal Components Analysis (PCA), and other type of analysis (Polly et al., 2016). In the 1990's, a new approach in studying shape was introduced with the application of Geometric Morphometric Analysis (GMM). GMM is the quantitative study of the biological shape, shape variation, and covariation. Over the years, GMM has been improved and revised, with better effective methods and softwares (Rohlf & Marcus, 1993; Adams et al., 2013). GMM have employed outline and landmark methods.

Landmark coordinates were identified on the shape of the organism, and the non-shape information contained in the data is removed by using the Procrustes superimposition process. The data can then be further analyzed using statistical analysis such as PCA and CVA.

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One of the key advantages of GMM is that shape differences can be visually displayed as illustrations or computer animations, making it the preferred method compared to traditional morphometric which are usually shown as data such as length, width and distance. (Rohlf & Marcus, 1993; Durón-Benítez & Huang, 2016). The various methods of GMM visualization can illustrate even complex morphological changes more effectively, 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 & Marcus, 1993; Adams et al., 2004). Furthermore, these visualizations provide information on morphological changes in their immediate anatomical context (Klingenberg, 2013; Mayer et al., 2014).

GMM have been applied to a variety of organisms and structure such as oak leaf (Viscosi & Cardini, 2011), dog (Drake & Klingenberg, 2010) and cichlid (Maderbacher et al., 2008; Kerschbaumer & Sturmbauer, 2011) (Figure 1.6). Most organisms that were analysed are usually rigid in structure. To date, there have yet to be any study of shape of gelatinous organism such as jellyfish using GMM. 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.

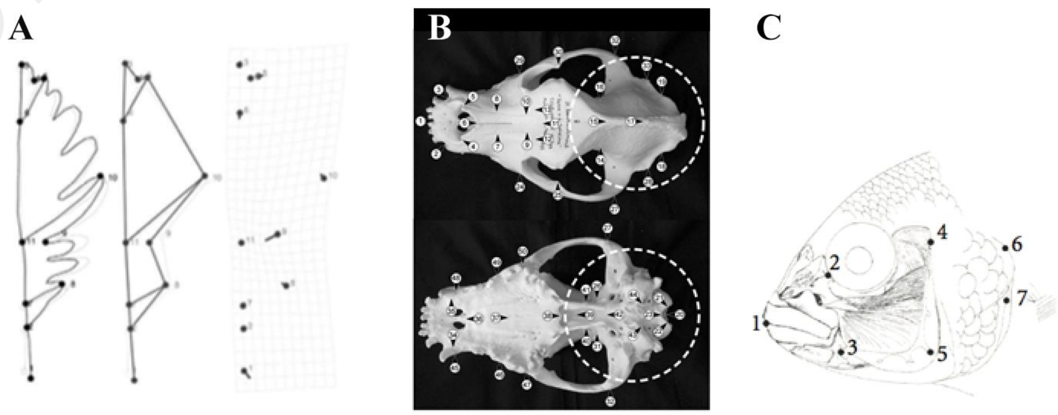

Figure 1.6: GMM used on various organisms. A: Oak leaf. B: Dog. C: Cichlid

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GMM can be performed using mathematical statistical packages such as R, MATHEMATICA© and MATLAB©. There are also other open source integrated software such as MorphoJ (Klingenberg, 2011), MORPHEUS (Slice, 2013) and IMP (Sheets, 2011). R is a comprehensive GMM software because there are a number of packages in it, and one can write script to perform analysis as wish. Thus, complex statistical analysis can be performed in R. Since this study only require the standard statistical analysis such as Procrustes superimposition, Canonical Variates Analysis and Principal Components Analysis, software such as MorphoJ, MORPHEUS and IMP are sufficient to perform the analysis needed for this study. Although these three software have different graphical user interface, they essentially perform the same analysis with the same numerical results. The preference of which software to use then is largely depending on the familiarity and user-friendliness of the software. MorphoJ is choosen to be used in this study because of the extensive training received in using the MorphoJ from the author.

1.4 Research Aims and Questions

The aim of this research is to describe the jellyfish found in Peninsular Malaysia water, and to compare the possible morphological variation within species or genus in Malaysia water. Since this region may harbours many undiscovered species, detailed documentation of morphology is required to allow for better identification and comparison with conspecifics or congenerics in nearby waters. More recently, applications of DNA sequence analysis and phylogenetics have been used to help 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,

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it is important to realize that the efficiency of such DNA barcoding method relies 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 cytochrome oxidase I (COI) sequences to definitively barcode *C. chinensis*. Furthermore, the morphological description of the species in the study was quite simple and preliminary, even lacking photographs, morphological illustrations and have very simple descriptions. Thus many of previous morphological identification needs to be reevaluated. Hence there is need to study them in more detail.

The sea nettle jellyfish of the genus *Chrysaora* has a worldwide distribution and have been reported to occur in the South China Sea, North, Central and South America, Africa, Europe and Australia (Morandini & Marques 2010; Yap & Ong 2012). Although some regions of Southeast Asia have been reported to harbour Chrysaora species such as *Chrysaora quinquecirrha* and *Chrysaora melanaster* (Kramp 1961; Yap & Ong 2012), there is the possibility they were misidentified base on colour variations and warrants verification. In the past, jellyfish species were difficult to identify as there were no reliable taxonomic keys, specimens were badly preserved, and confounded by the existence of cryptic species complexes that could be detected only by the application of molecular genetic techniques. Hence a more detailed morphological evaluation of the Malaysian sea nettle (*Chrysaora chinensis*) is required. Moreover, morphological adaptations to different localities with various ecological and environmental conditions are well documented for jellyfish (Dawson, 2005). Base on personal observation, certain degrees of variations are found. For example, gastrovascular pouches shape of *C. chinensis* are slightly different between

the species found in east coast and west of Peninsular Malaysia. 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 that has relatively better water quality. Thus, this study is the first of its kind to provide a more detailed morphological description of scyphozoan jellyfish in Peninsular Malaysia and to employ geometric morphometric analysis to distinguish populations of *C. chinensis*.

There are two research questions in this project:

Question 1: What are the detailed morphological characteristics of different jellyfish species in Peninsular Malaysia?

Question 2: Is there any gastrovascular pouch shape variation of *Chrysaora chinensis* across different localities in Peninsular Malaysia?

1.5 Objectives of the Study

The objectives of this study are:

1. To identify, photograph and describe the detailed morphology of selected scyphozoan jellyfish species in Peninsular Malaysia

2. To photograph the gastrovascular pouch of *C. chinensis* and establish its landmark configuration for geometric morphometrics analysis

3. To discriminate between *C.chinensis* populations of Peninsular Malaysia by analyzing the gastrovascular pouch shape variation using geometric morphometrics and statistical analysis base on the configured pouch landmarks.

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1.6 Significance of the Study

The proposed study has the following significance:

1) Some researchers, such as Kramp in his work “Synopsis of the medusae of the world (1961)”, described various species of jellyfish in the Malayan Archipelago. But the Malayan Archipelago encompasses a wide range of region, from Malaysia to Australia. Thus it is unclear whether the specimen is found near Malaysia water. This research could provide more accurate information on the jellyfish found in Malaysia water. The findings of this study will contribute to the Malaysian checklist of marine species, whereby baseline information of jellyfish diversity is still lacking. This study also provide detailed description jellyfish species and relevant photographs of their morphology that would facilitate their identification in the field.

2) This study would verify if sea nettle species in Peninsular Malaysia belong to *C.chinensis* and determine if they consist of morphologically different populations. GMM approach would also be first of its kind to be applied to jellyfish. This part of the study would also contribute to baseline data needed for future management of sea nettle blooms in Peninsular Malaysia.

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CHAPTER 2: MATERIALS AND METHODS

2.1 Collection of Jellyfish Samples

Surveys and samplings in this study were carried out between April 2014 and December 2014 from eight sites to obtain as many specimens possible that may represent different jellyfish species and morphological variations from the east and west coasts of Peninsular Malaysia (Table 2.1). Two sites located in the West-Central (Sungai Janggut and Klang Power Station), three located in West-North (Pantai Kok, Kilim and Balik Pulau), one located in East-Central (Kampung Cempaka), and two located in East-North (Pantai Sabak, Pantai Melawi) coastal areas of Peninsular Malaysia (Table 2.2). These sites were also chosen for the sampling of *C.chinensis* samples for the GMM study. For the purpose of geometric morphometric analysis, a minimum of 32 specimens were required, which was calculated based on 16 landmark configurations used in the GMM.

Table 2.1: The sites where samples of jellyfish were collected off the coasts of Peninsular Malaysia, with the period, GPS coordinates and sampling methods.

Site	Period	GPS	Method
Sungai Janggut	9 Jul 2013* 5 Dec 2013* 29 Apr 2014	N03.16916° E101.29833°	Bag Net
Pantai Kok	14 Apr 2014	N06.34869° E99.64806°	Dip Net
Kilim	14 Apr 2014	N06.4766362° E99.8042679°	Dip Net
Kampung. Cempaka	26 Jun 2014	N03.74365 ° E103.32847 °	Dip Net

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Table 2.1, continue

Pantai Sabak	20 Jul 2014	N06.13712° E102.36976°	Dip Net
Pantai Melawi	21 Jul 2014	N05.99612° E102.43714°	Dip Net
Klang Power Station	26 Jun 2014	N03.3229° E101.30108°	Dip Net
Balik Pulau	14 Dec 2014	N05.53972° E100.34888°	Dip Net

* Museum specimens of that was previously collected (not from this study) but have been used for morphological examination

Table 2.2 The sites where samples of *C. chinensis* were collected off the coasts of Peninsular Malaysia, with the sample sizes (n) and sampling methods. The sites are designated as coastal areas West-North (WN), West-Central (WC), East-North (EN) and East Central (EC) of Peninsular Malaysia.

Area	Site	Period	GPS	Method
West-North (WN) (n = 38)	Pantai Kok (n = 5)	14 Apr 2014	N06.34869° E99.64806°	Dip Net
	Balik Pulau (n = 33)	14 Dec 2014	N05.53972° E100.34888°	Dip Net
West-Central (WC) (n = 27)	Sungai Janggut (n = 10 + 17*)	9 Jul 2013* 5 Dec 2013* 29 Apr 2014	N03.16916° E101.29833°	Bag Net
East-North (EN) (n = 26)	Pantai Sabak (n = 21)	20 Jul 2014	N06.13712° E102.36976°	Dip Net
	Pantai Melawi (n = 5)	21 Jul 2014	N05.99612° E102.43714°	Dip Net
East-Central (EC) (n = 16)	Kampung Cempaka (n = 16)	26 Jun 2014	N03.74365 ° E103.32847 °	Dip Net

* Museum specimens of *C. chinensis* that was previously collected (not from this study) but have been used for GMM in the present study.

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Samples were obtained by using dip nets and bag nets. Dip nets with the mesh size of 5mm were used to scoup out the jellyfish samples from the water surface and kept in containers filled with seawater. Although specimens were less damaged, this method is more labour intensive. Bag nets, also known as Pompang (Figure 2.1), were methods used by fishermen in Sungai Janggut to collect pelagic jellyfish species. Sixteen bag nets were deployed approximately 8m below the water surface during spring tide and left for 6 hours before retrieving the nets. Bag nets were emptied on the boat and jellyfish samples were sorted immediatey from the catch, and placed temporarily into containers filled with seawater.

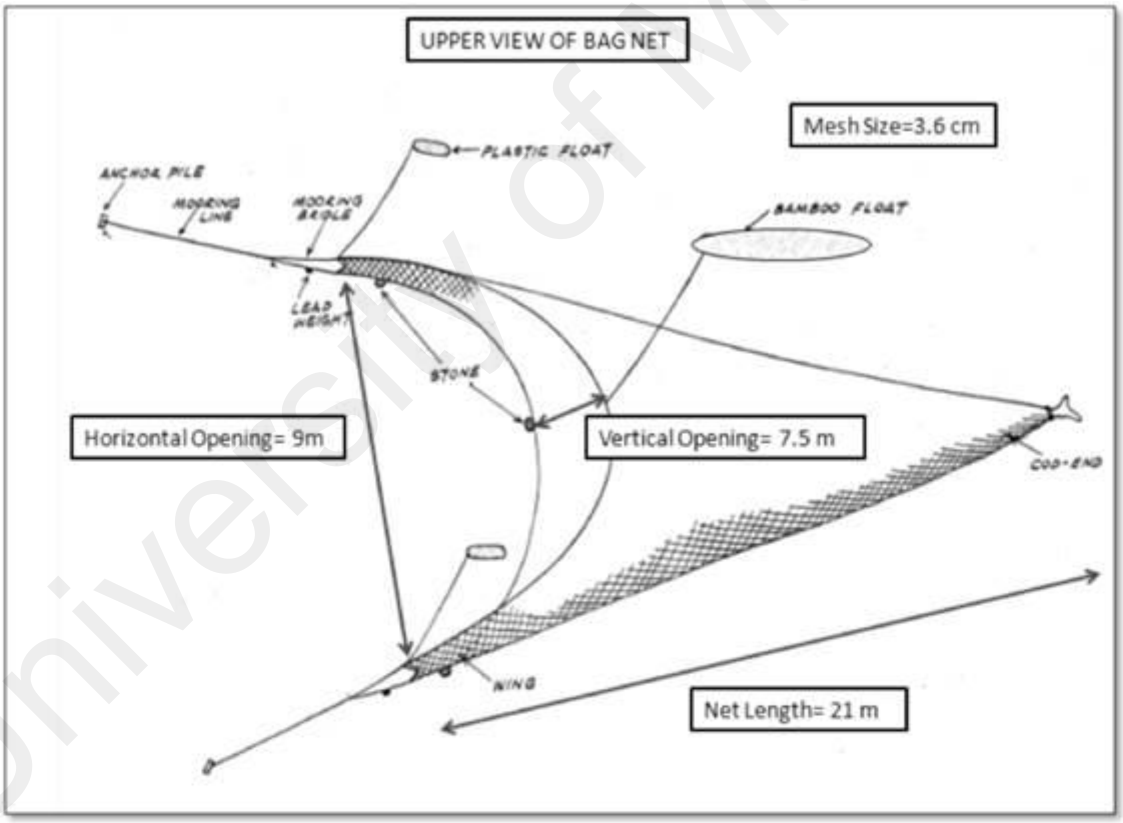

Figure 2.1: Diagram showing pompang (bag net)

Once at the jetty, jellyfish specimens that were relatively in good condition and least damaged were chosen and photographed immediately to record their live colouration by floating them in acrylic aquarium filled with seawater. Species of

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specimens were tentatively identified in the field based on Kramp (1961). Bell diameter and length of oral arms were measured on site using a measuring tape with the exumbrella facing up. Specimens were cataloged and other sampling information such as date and sampling site were recorded.

Tissue samples from oral arm and bell of each specimen were taken using forceps and scissors that were cleaned with alcohol between each sampling to eliminate cross contamination between samples. Tissue samples were rinsed with distilled water and kept in vials containing 100% ethanol, cataloged and stored at 4C° for future molecular genetic studies (not within the scope of the present study).

Each whole specimen was rinsed with distilled water to remove as much debris as possible before being transferred into individual heavy duty plastic bag containing 5% formalin in seawater (Appendix A) and brought back to the laboratory. After seven days of specimens being fixed in formalin, the specimens were rinsed with distilled water to remove any remaining debris and transferred to a new container with 5% formalin in filtered seawater to ensure better fixation.

2.2 Photography of Specimens and Collection of Morphological Data

Species identification of specimens were further verified in the laboratory based on detailed morphological examination and taxonomic classifications of Kramp (1961) and Morandini & Marques (2010). Prior to the examination, each specimen and its catalog paper were removed carefully from its container, place into another container with tap water and soaked for at least five minutes to remove the formalin as it is highly carcinogenic. For safety measure, gloves and mask were used during handling of specimens and examinations done in well ventilated area.

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Morphological examinations of jellyfish specimens were done on major structures such as bell, subumbrella, lappet, gastrovascular cavity, tentacle, muscle, network, oral arm, scapulae, terminal club, filament and gonad. 186 detailed morphological characters of jellyfish specimens were analysed, measured and their respective character states recorded (Appendix B). A set of photographs were also taken for those structures (Appendix C) to facilitate the morphological examination and collection of morphological data. All examinations and photographs were based on a modified data sheet guidelines of how to study the morphological characteristics of scyphozoans, provided by courtesy of Michael Dawson and Liza Gomez Daglio from University of California, Merced.

Morphology of specimens was photographed using a digital camera (Olympus PEN Lite E-PL5, lense 18mm to 105mm) with additional light source, scale and colour chart (Tiffen Q-13 Color Separation Guide) included so that measurements and the colour code (CMYK) can be calibrated appropriately later from the digital images (Figure 2.2). The specimens were photographed in two different methods. The first method was by suspending the specimen using wires in a transparent glass aquarium (50cm x 30cm) that was filled with water against a black background, so that it appears to floating and positioned as natural as possible (Figure 2.3). The placement of the specimen in the glass tank and the camera's angle and distance from the specimen were adjusted depending on the structure to be photographed, whether to obtain closeup or overall images of the structure.

The second method is by laying the specimen on a flat transparent acrylic stage (various sizes depending on the specimens) for dry and more detailed photography of the morphology. A transparent acrylic glass measuring was used as the stage for detailed photography of its morphology. A fluorescent light of 16 watt is placed 15cm

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below the stage. Two extra light sources are placed one on each side of the stage (left and right side) to provide extra light source. (Figure 2.3). In general, the specimen was placed so that the orientation of rhopalia were aligned at 3, 6, 9 and 12 o'clock accordingly, but the positioning of the specimen on the stage, the camera's angle and distance were adjusted depending on the various structures to be photographed.

Microsope and magnifying glass were used to observe minute structures such as the gastric filaments. Visualisation of network of canals, gastrovascular pouches and anastomoses in the lappets were enhanced by carefully injecting food dye (blue, green or red) into the canals.

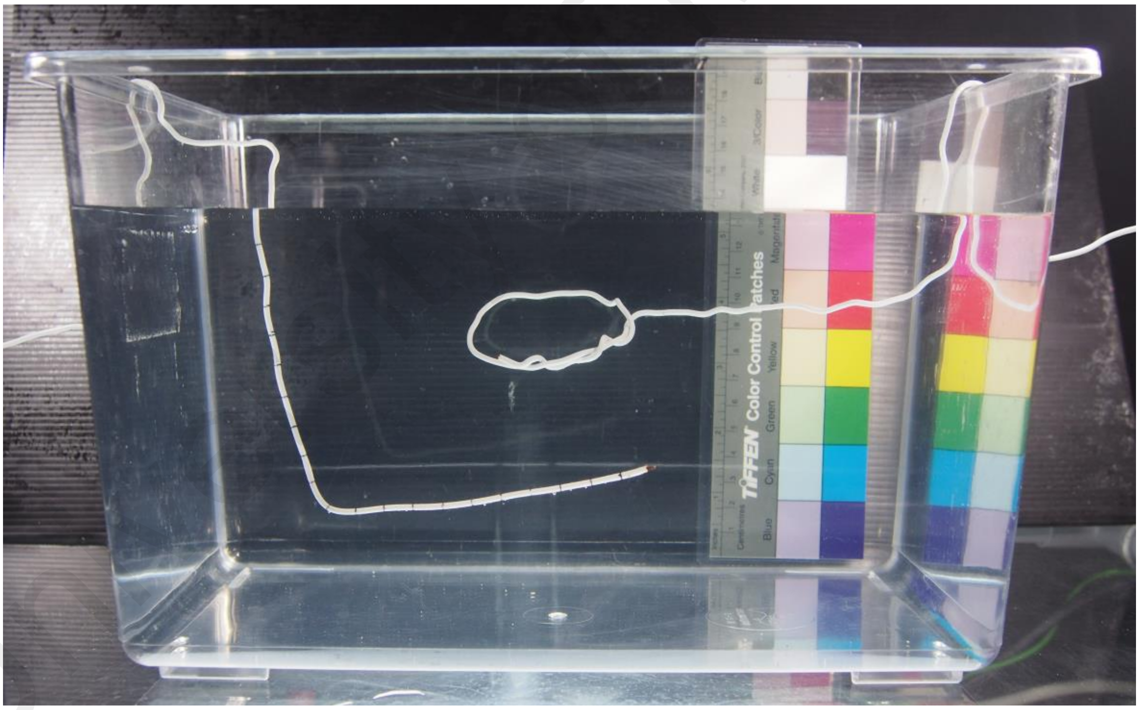

Figure 2.2: Photography setup to float jellyfish in the tank against a black background, with colour chart and scale. Wires were used to suspend the specimens, and a scaled wire was also used to aid recording of measurements.

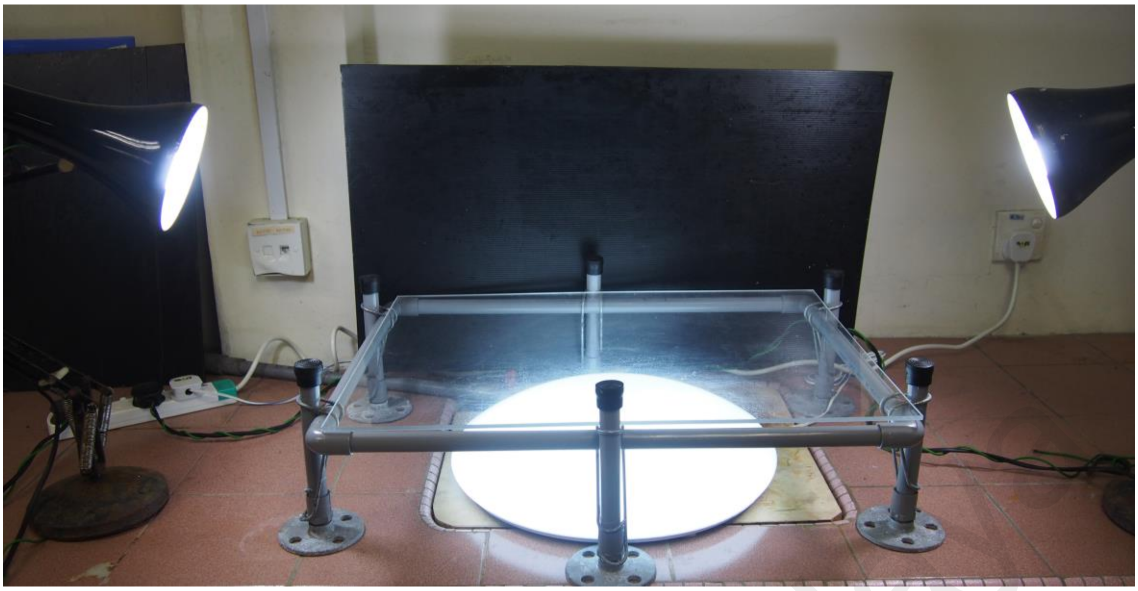

Figure 2.3: Dry photography setup showing theacrylic stage, with light source below and additional light sources at the sides.

2.3 Geometric Morphometric Analysis of Gastrovascular Pouch

Rhopalar gastrovascular pouch of *C. chinen*sis was the chosen structure for the geometric morphometric analysis as this internal structure often remains intact during sampling compared to other external structures. Jellyfish are fragile organisms, and quite often certain structures are destroyed during the sampling, such as detached tentacles and oral arms, deformed exumbrella and others. Gastravascular pouch is located at the underside of the bell of the jellyfish, and with the oral extending below it. Therefore, it is relatively protected during sampling. The other reason for choosing rhopalar gastrovascular pouch is because for Chrysaora species, they generally have two shapes; the Pacific shape and the Atlantic shape. The Pacific septa shape is characterized by first thinning of the tentacular pouch, then enlarging it, thus making an "S" shape. Whereas the Atlantic septa shape is characterized by enlarging of the tentacular pouch but gradually becomes thin, thus appearing pear shaped. Most of the Chrysaora found in the Pacific Ocean such as *C. chinensis*, *C. pacifica* and *C. melancaster* have the Pacific septa shape, whereas the Chrysaora found in the Atlantic

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ocean such as *C. quinquecirrha* and *C. hysoscella* have the Atlantic septa shape (Morandini & Marques, 2010). Therefore, there is reason to believe the biological significance of the gastrovascular pouch variation base on different regions.

2.3.1 Landmark Configurations and Photography of Gastrovascular Pouches

Landmark-based geometric morphometrics methods begin with the identification of the landmarks coordinate, either two or three-dimensional. Landmarks are points on the Cartesian coordinates (x, y, z) that can be identified on each and every specimen in the study to represent the shape. Although these landmark coordinates contains information regarding the shape of the specimen, these coordinates data should not be used directly because the effect of variation in position, orientation and size of the specimens are still present in the coordinate. When studying shape of an organism, the information regarding the position, orientation and size of the specimen is irrelevant. Therefore, this non-shape information must be removed prior to the analysis of the landmark coordinates (Klingenberg, 2013; Mitteroecker et al., 2015). Once the non-shape variables are removed, these variables then can be used to perform statistically analysis. The result of the analysis will be able to tell whether there is any variation in shape comparison, in both statistical scatterplots as well as graphical representation of the shape change (Adams et al., 2004). There are a few criteria in choosing landmarks. Firstly, the landmarks must be of biologically significant. Secondly, landmarks must be able to represent the morphology of the specimen, and all landmarks must be present on all specimens, and must be reliably and repeatedly digitized for each specimen (Klingenberg, 2013).

There are a total of 16 pouches for each specimen of *C. chinensis*, among them eight rhopalar gastrovascular pouches are to the interest of this study. The reason why

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these eight pouches were chosen over the other eight inter-rhopalar gastrovascular pouches is because the rhopalia can be used as the guide to align the pouch more easily along the 3 and 6 o'clock accordingly. Inter-rhopalar pouch could have been chosen to be analised and it would have yield the same result since both pouches neighbouring each other and shape variation on one pouch will also be shown on the neigbouring pouch, but it will take more time to align the axis. 16 points along the edges of the rhopalar grastovascular pouch were chosen as the landmarks for the GMM (Figure 2.4) as those points represent the shape outline of pouch. Point 3 and 15 could have beenomitted but they are used as the semi landmark since they are in the center of the septa. The definition or position of the 16 landmark configurations are given in Table 2.3.

Table 2.3: Position and definition of the 16 landmark configurations.

Landmark	**Position**
LM 1	Proximally, at the center 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

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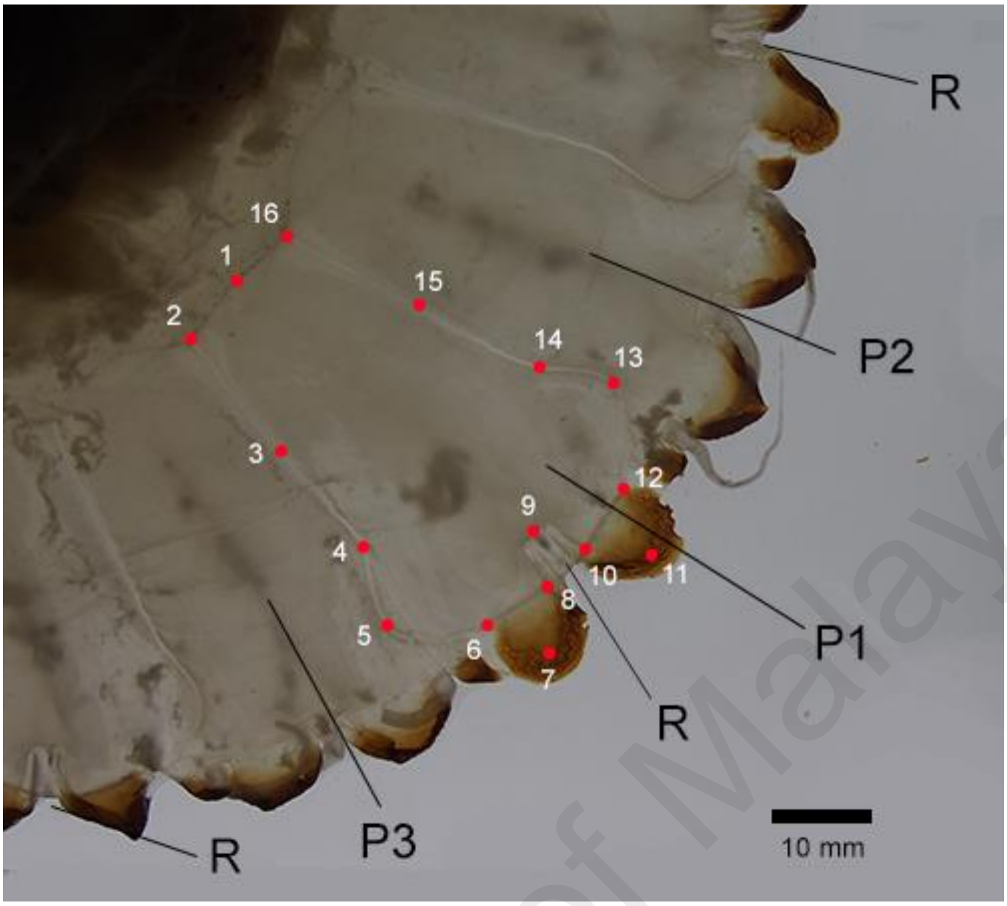

Figure 2.4: An image of a subumbrella quadrant of a *C.chinensis* specimen showing the 16 geometric morphometric landmark points (indicated in red and numbered) of a single gastrovascular pouch analysed (P1). Pouch P1 is flanked by pouches P2 and P3. Rhopalia are indicated as R.

Each specimen was placed on the stage with the subumbrella facing up. Oral arms were moved away from the desired gastrovascular pouch in order to reveal a clear view of the septa. All air bubbles trapped in the pouches were removed by pressing gently on the pouches. The desired pouch is positioned where the rhopalar was aligned at the centre of the lower left quadrant (Figure 2.4). A chosen quadrant of the umbrella containing the pouch to be analysed was photographed, which would include three gastrovascular pouches and three rhopalia. Sometimes, blue dye was injected into the rhopalar pouch to enhance the visualization of the pouch shape when it was difficult to see the edges. Camera was held directly above the specimen at a right angle with a distance of approximately 40cm to 45cm between the camera and the specimen. The pictures of the specimens were taken many times and the clearest pictures were chosen.

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A total of 107 specimens were photographed (Appendix D). The minimum requirement of specimen for a two-dimensional study is twice the number of the landmarks. In this case, with 16 landmarks, the minimum specimen required is 32. Thus a 107 specimen data size is well above the minimum requirement (Klingenberg, 2014). For each specimen, two different pouches were photographed as replicates for each specimen, making it a total of 214 images to be analysed.

2.3.2 Formating of Images Into TPS File

All 214 pouch images representing 107 specimens were converted into data format in order to be processed by MorphoJ (Klingenberg, 2011) which is a software for GMM. Images were saved in a folder in the computer. Images were converted to a TPS file format using tpsUtil version 1.58 (Rohlf, 2010) (Appendix E).

2.3.3 Digitisation of Images

All pouch images were then digitised as an image information file in tpsDIG (Rohlf, 2010). These digitised data contain information of the 16 defined landmarks that were configured on the gastrovasular pouch. During the landmarking process of the pouch in tpsDig software (Appendix F), the scale was adjusted so the sizes of images were standardized. This was done by referring to the scale bar indicated in the images, and calibrating the value of the scale into the tps file. Hence, image information of 214 pouch images representing 107 specimens, were digitized and imported into MorphoJ for geometric morphometric analysis.

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2.3.4 Quantification of Measurement Error

Measurement errors will always occur in any data collection procedure, and errors can be attributed by various factors, such as imprecision of the measuring device, the location of the landmarks, the rigidity of the specimen and the experience of the researchers (Arnqvist & Martensson, 1998; Muñoz-Muñoz & Perpiñán, 2010; Klingenberg, 2014). It is almost impossible to eliminate measurement errors completely, thus it is important to reduce the measurement errors to as minimum as possible.

A pilot study using a relatively small sample size can be performed to determine the relative sizes of errors associated with each step. If the result shows that errors are negligible, then replicate is not needed during the actual process.

One way of quantifying error in geometric morphometrics analysis study involves imaging the specimen twice, and digitize (i.e. measurement) each image twice, which build a 2 stages structure (i.e, imaging and measurement). For certain organisms with symmetrical shape, another stage of “side” may be added to further investigate the error associate with “side”. Procrustes ANOVA then can be performed to compute the deviations of the individual values around the mean value at higher level (Figure 2.5). The value at each level can then be used as relative sizes of errors associated with each step. If the error is small, then replicate is not needed (Viscosi & Cardini, 2011).

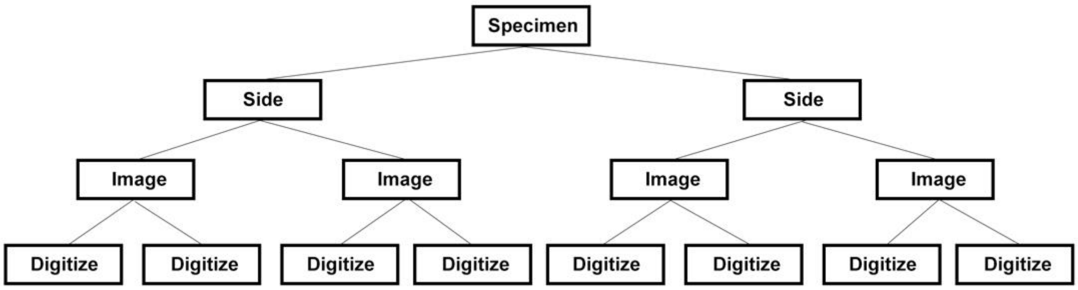

Figure 2.5: The Number of replicate for each level of the process of specimen imaging and digitization.

A preliminary quantification measurement error test was performed on 25 randomly chosen specimens using the Procrustes ANOVA analysis (Appendix G). For each specimen, two gastrovascular pouches were photographed. Each pouch was photographed twice, and each photograph was digitised twice. Therefore, three levels of errors were measured: errors associated with "side", imaging and the digitization of landmarks, respectively. Hence, the total images used in Procrustes ANOVA for each specimen was:

2 pouches (from each specimen) x 2 photographs x 2 digitizations = 8 images.

For 25 specimens, a tps file containing the image information of 200 images was imported into MorphoJ to perform Procrustes ANOVA analysis.

2.3.5 Procrustes Superimposition

To remove the information of position, orientation and size of the specimens, a procedure called the generalized Procrustes superimposition is performed. (Klingenberg, 2013; Mitteroecker et al., 2013). The generalized Procrustes superimposition is an iterative procedure that fits each configuration to the mean shape in the sample as closely as possible. The end result of generalized Procrustes superimposition is that only shape related information are extracted from the samples landmark configurations.

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Three stages were involved in the genaralized Procrustes superimposition. First, variation in size was removed by scaling each configuration so that it has a centroid size of 1.0. Secondly, variation in position was removed by shifting the landmark configurations so that they shared the same centroid. The third procedure dealt with rotations to find an optimal orientation for each configuration.

After the generalized Procrustes superimposition, every configuration in the sample is then optimally aligned to the average configuration. Because the configurations were aligned so that position, orientation and size were kept constant according to the criterion for the least-squared fit, the remaining variation in landmark positions will be solely the variation of shape only. The relative position of the landmarks from the mean shape to another shape can then be used to detect the shape variation. These relative position of the landmarks also provide a visualization of the shape change by showing how the landmarks are reposition against each other after the non-shape components variation of position, orientation and size are removed using the Procrustes superimposition. (Klingenberg, 2013).

In this study, a generalized Procrustes superimposition was performed on the gastrovascular pouch to eliminate the non-shape information (size, position and orientation) of the image (Klingenberg, 2016). A covariance matrix was initially generated before running the Procrustes analysis to produce a result set (Appendix H). Once the Procrustes analysis was completed, two types of statistical analyses were performed: the Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA).

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2.3.6 Principle Component Analysis Using MorphoJ

PCA is a multivariate analysis whereby a numbers of factors, or components that can be used to represent relationships among the data set with many possible correlated variables are identified. In PCA, the basic principal is to identify a few major components that explain most of the total variance. The first principal component (PC) accounts for the most variability in the data set, the second PC accounts for the next most variability, and follow by each succeeding PCs. Mathematically, this is done by transforming the data to different axes so that those with the same pattern are aligned to form a PC. In the context of geometric morphometrics analysis, the specimens can be treated as points in a multivariate space where the viewing position of the data are changed so that the most important components of the shape change are able to be identified (Viscosi & Cardini, 2011).

PCA does not assume there any group membership in the data set. PCA will try to maximize the variance on each of the component, and if the between-group variations is bigger than the within-group variations, the scatterplots of PCA may show the group variations. On the other hand, even if PCA failed to show any group varation, it doesn't necessary mean that there is no group variation in the data. (Strauss, 2010). Therefore, PCA can be used as a preliminary review of the data, without making any assumptions of group membership in the data set.

In this study, a PCA is performed for the general inspection of the gastravascular pouch shape of *C. chinensis* (Appendix H).

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2.3.7 Canonical Variate Analysis Using MorphoJ

The primary use of CVA in geometric morphometrics analysis is to determine whether there is a significant difference in shape for pre-defined distinct groups in multivariate data set. Therefore, in contrast to PCA, CVA makes the assumption that there is group membership in the dataset. It tries to maximize the between-group variation relative to the within-group variation. CVA is similar to PCA in the sense that it realigns data to reconstructs new axes to form components, or factors.

There is a possibility that CVA will find distinction between group memberships, even though a preliminary review by PCA may not indicate any distinction between groups. Of course the underlying biological reasons to assign specimens to different groups should be valid, or else the result generated by CVA may not be significant (Sheets et al., 2001; Webster, 2010).

In this study, a CVA was performed on the gastrovascular pouches shape of *C. chinensis* to distinguish populations by four coastal areas (Appendix H).

2.3.8 Visualization of Shape Outline

One of the main advantages of geometric morphometrics analysis is the ability to visualize shape change. One of the widely used method for visualization is the transformation grid. The deformation method is performed by placing a two-dimensional rectangular grid over the first specimen, or the initial form, and the grid was "stretched" to match the morphology of the second specimen, or the target form. All parts of both specimens are still in the same grid cell after the transformation. The change in the grid shows the differences in the shape of both specimens (Richtsmeier et al., 2002). Later on, Bookstein (Bookstein, 1978), successfully adapt D'Arcy

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Thompson's transformation grid into thin plate spline, where he was able to construct the transformation grids using an interpolation technique that fits the grids perfectly on landmarks (Klingenberg, 2013).

Transformation grids by itself with only landmark configurations and grid can be difficult for viewer to understand the morphological structure of the subject. Furthermore, landmarks chosen do not sufficiently describe the morphology of the subject of study. When all the landmarks are connected, the shape will usually not look quite like the shape of the real organism as the connection between each landmark are rather linear and sharp. Thus, to better visualize the shape, an outline drawing is used to represent the organism. Sometimes, two outline drawings are imposed one on top of the other to show the variation in shape. Although outline drawing is visually compelling in displaying shape and variation in shape, it must be reminded that one must not rely solely on outline drawing when analyzing shape and shape variation. Outline file is merely a graphical representation of the shape of the specimen for the purpose of better visualization. The disadvantage with outline file is that the points along the outline are only assumptions, and may not represent the real shape of the specimen (Klingenberg, 2013).

In this study, an outline file was produced in tpsDIG using 56 landmark coordination sets (Figure 2.6, Appendix I). They were able to represent the shape outline of the gastrovascular pouch more closely to that of the specimen when compared with outlines based on only 16 landmarks for the geometric morphometric analysis process.

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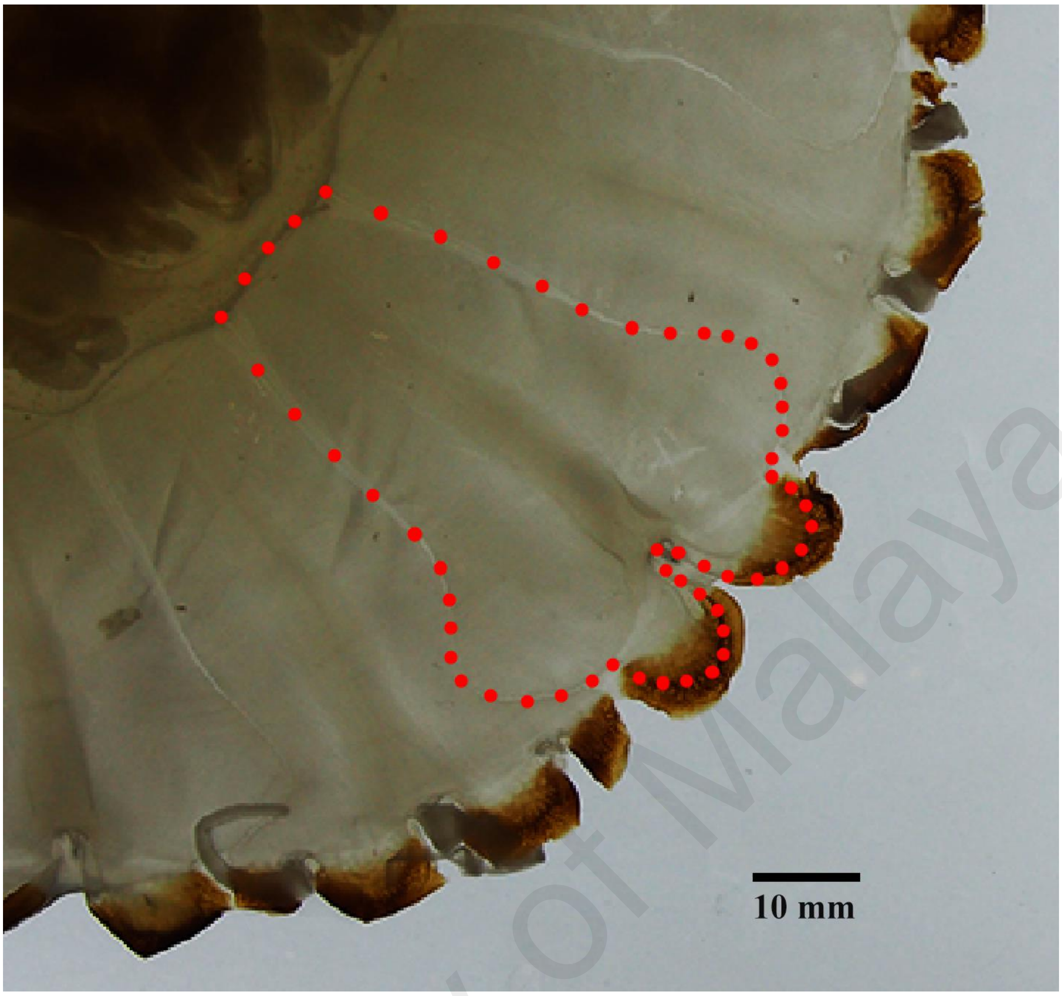

Figure 2.6: 56 landmark configurations used to create the outline of gastrovascular pouch

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CHAPTER 3: RESULTS

3.1 OVERVIEW OF MAJOR MORPHOLOGICAL STRUCTURES OF JELLYFISH

Class Scyphozoa is ascribed with four orders, namely Stauromedusae Coronatae, Semaeostomeae and Rhizostomeae, with 65 genera and over 187 species (Mayer, 1910; Kramp 1961; Daly et al., 2007; Bayha et al., 2010). This study focuces on the order Semaeostome and Rhizostomae only.

3.1.1 Order Semaeostomeae

The order Semaeostomeae composed of three families, four subfamilies, 18 genera and 56 species (Kramp, 1961). Semaestommeae jellyfish are characterized by four oral arms around the mouth. Tentacles are found at the umbrella margin. (Arai, 1997). The two families of Semaeostomeae of the interest in this study are Cyaneidae and Pelagiidae.

FAMILY CYANEIDAE

Family Cyaneidae is Semaeostomeae that central stomach gives rise to radiating pouches, and it in turns gives rise to numerous blind canals extending towards the marginal lappets; without a ring canal; with gonad completely folded; with tentacles arising from the subumbrella distally from the margin.

Genus Cyanea – with eight rhopalia; with eight whorls of adradial tentacles, each contains several rows of tentacles; radial and circular muscles in the subumbrella.

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FAMILY PELAGIIDAE

Family Pelagiidae is Semaeostomeae that central stomach gives rise to radiating pouches divided by a septa; without a ring canal; tentacles are formed at the cleft of the bell margin; oral arms long and folded.

Genus Chrysaora – with 32 – 48 simple marginal lappets; with eight marginal sense organs; with three or more tentacles for each octant; with 16 radial stomach pouches; in the marginal area the eight rhopalar pouches are narrower than the eight tentacular pouches, thus forming an "S" shape; exumbrella with numerous nematocyst warts.

3.1.2 Order Rhizostomeae

The order Rhizostomeae composed of two suborders, 10 families, 25 genera and approximately 89 species (Kramp, 1961). Rhizostomeae jellyfish are characterized by having bell margin cleft into lappet, with no tentacle on the bell margin, without a central mouth, with eight oral arms extended from the subumbrella, where each oral arms are bear numerous secondary mouths. Network of canals are found beyond the stomach. (Kramp, 1961; Arai, 1997). This study focuces on the order Mastigiidae, Versurigidae, Lychnorhizidea, Catostylidea, Lobonematidae and Rhizostomatidae.

FAMILY MASTIGIIDAE

Family Mastigiidae is charaterized with short, pyramidal, three-winged oral arms; with numerous filaments on the oral disk (Kramp, 1961).

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Genus Phyllorhiza - with broad oral arms, leaf-shaped, with window opening in the oral arms, with numerous filaments at the oral arms; network of canals bounded by the ring canal and do not anastomoses with the perradial rhopalar canals.

FAMILY VERSURIGIDAE

Family Versurigidae is charaterized with broad, leaf-shaped oral arms. (Kramp, 1961)

Genus Versuriga – with three-winged oral arms, with scapulaes, with terminal club, with numerous filaments and clubs formed at the oral arms (Kramp, 1961).

FAMILY LYCHNORHIZIDAE

Family Lychnorhizidae is charaterized with centripetal, usually blinded end and not anastomosing with the 16 canals; with broad and folded oral arms (Kramp, 1961).

Genus Lychnorhiza – with three-winged oral arms, without terminal club, with or without filaments; with eight radial canals reaching bell margin, and another eight reaching only the ring canal. (Kramp, 1961)

FAMILY CATOSTYLIDEA

Family Catostylidea is charaterized with intracircular network of canal anastomosing with the ring canal but not always with the 16 radial canals; the eight rhopalar canals reaching bell margin, and inter-rhopalar canals reaching only the ring canal. Oral arms pyramidal (Kramp, 1961).

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Genus Acromitus – Intracircular network of canals anastomosing with the ring canal and the rhopalar canal, but not with the inter-rhopalar canals; oral arm is characterized with a terminal club (Kramp, 1961).

FAMILY LOBONEMATIDAE

Family Lobonematidae is charaterized with intracircular network of canal anastomosing with the ring canal and with some or all of the 16 – 32 radial canals, but not with the stomach; with window openings in the membranes of the oral arms; marginal lappets very elongated, tapering (Kramp, 1961).

Genus Lobonemoides – With large-meshed, intracircular network of canal anastomosing with the ring canal and rhopalar canals.

FAMILY RHIZOSTOMATIDAE

Family Rhizostomatidae is charaterized with oral arms coalesced in proximal portion only; without a mouth opening; with complicated network of canal system; distal portion of the oral arms three winged, and usually with a terminal club (Kramp, 1961).

Genus Rhopilema – With large scapulae and long manubrium; oral arms with numerous club and filaments, usually with a large terminal club; usually without a ring canal; broad network of canal with numerous fine meshes; inter-rhopalar canal wide.

In this study, a total of 146 specimens (nine species from nine genera) were obtained from the coast of Peninsular Malaysia (Figure 3.1 and Appendix J). Among

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them, a total of 44 specimens (eight species from eight genera) were examined which also included museum specimens (Appendix K). The nine species of scyphozoan jellyfish described in this thesis were *Chrysaora chinensis*, *Cyanea* sp., *Rhopilema esculentum*, *Rhopilema hispidum*, *Lobonemoides robustus*, *Versuriga anadyomene*, *Phyllorhiza punctata*, *Lychnorhiza malayensis* and *Acromitus flagellatus*.

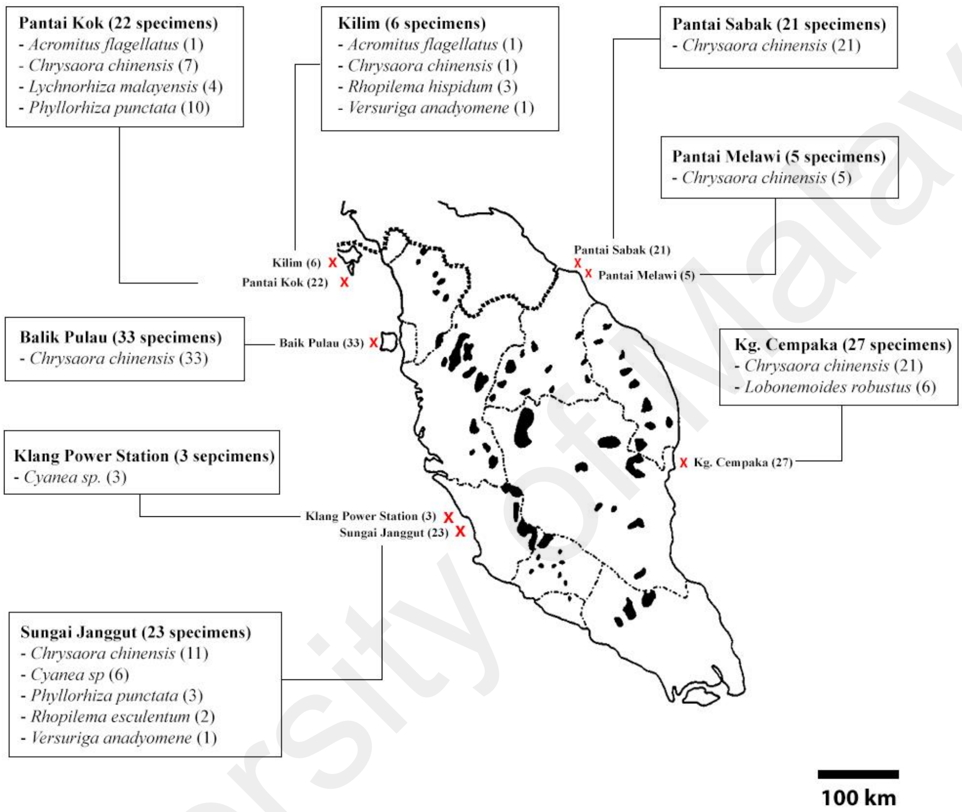

Figure 3.1: Map of eight sampling sites, with number of specimen collected for each species.

Adult medusae of scyphozoan jellyfish (Figure 3.2) consists of a rounded bell attached to a manubrium. Oral arms emerge from below the manubrium. In general, jellyfish in the Semaeostomeae order possesses four oral arms, whereas those in the Rhizostomeae order possess eight oral arms. At the edge of the bell are a series of indentation called lappets. Tentacles are usually formed at the gap between lappets, although not all jellyfish possess tentacle. A central stomach, or gastrovascular cavity is

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found beneath the bell, or the subumbrella. It extends to the margin of the bell, forming a network of canals. The complexity and the shape of the network vary between each species.

Figure 3.2: Whole medusa of an adult jellyfish, *Phyllorhiza punctata*

Below is the definition of several major structure used for the description of the morphology:

BELL - The bell, or the umbrella of adult scyphozoan medusae are usually rounded, forming a hemispherical shape. The outer surface of the bell is the exumbrella, or also known as the aboral surface; whereas the underside of the bell is the subumbrella or the oral surface.

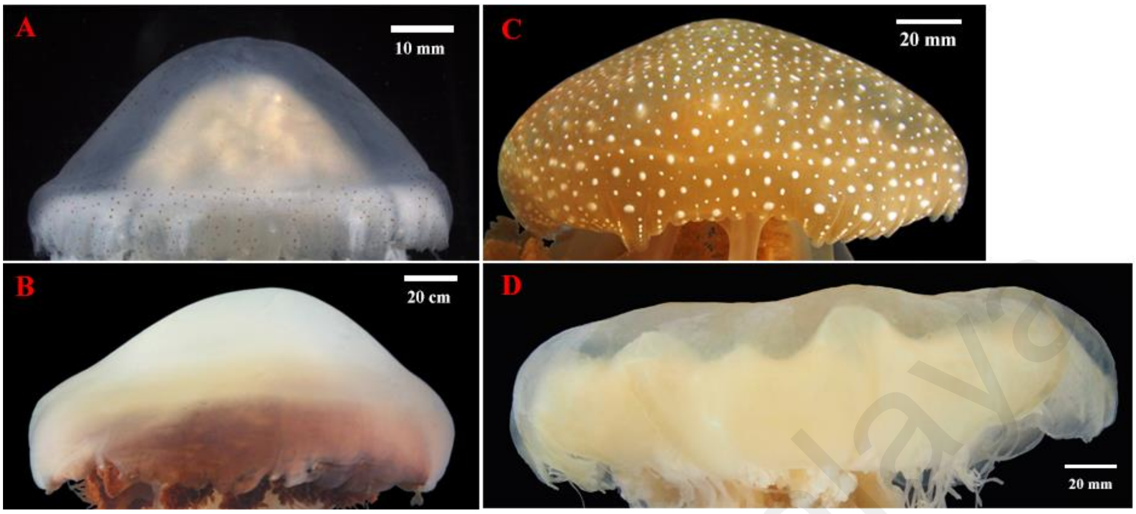

Figure 3.3: Morphology of bell of four different species of scyphozoan jellyfish. *(A)*: *Acromitus flagellates* with smooth surface *(B)*: *Rhopilema esculentum* with smooth surface *(C)*: *Phyllorhiza punctata* with warts on the surface *(D)*: *Cyanea* sp. With smooth surface

LAPPET - The margin of the bell is usually clefted, and each of the indentation is called the lappet. The lappet adjacent to the rhopalium is the rhopalar lappet, and the rest of the lappets are the velar lappets. The lappets can be in many shapes, from rounded, elongated to semi-squared.

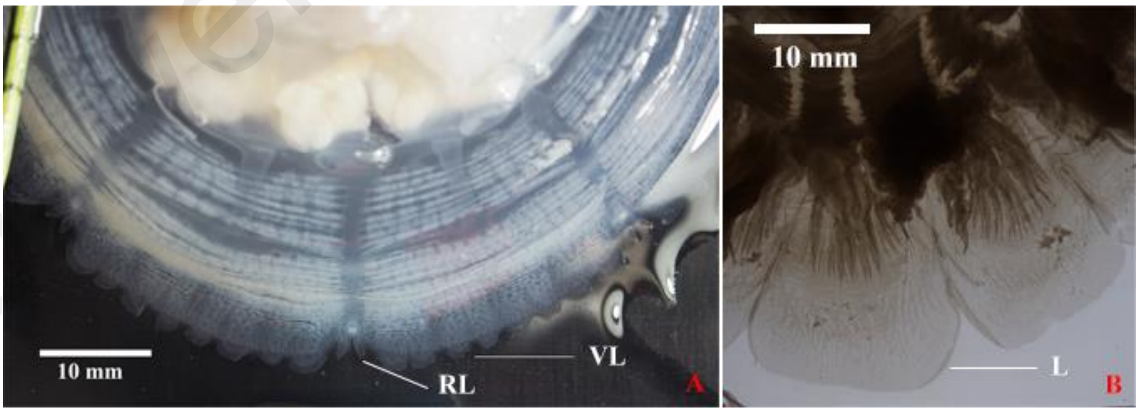

Figure 3.4: Lappet of two different species of scyphozoan jellyfish. *(A)*: Rhopalar lappet (RL) and velar lappet (VL) of *Lychnorhiza malayensis (B)*: Lappet of *Cyanes* sp. (L). Lappet shape broad and semi-square

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MARGINAL SENSORY ORGAN/ RHOPALIUM - Rhopalia (singular: rhopalium): Rhopalia are tear-drop shape structures which contain the sensory organs to detect light (eye spots) or direction (statoliths). Rhopalia are located at the cleft of the bell margin between a pair of lappet. Usually eight to sixteen rhopalia in a jellyfish.

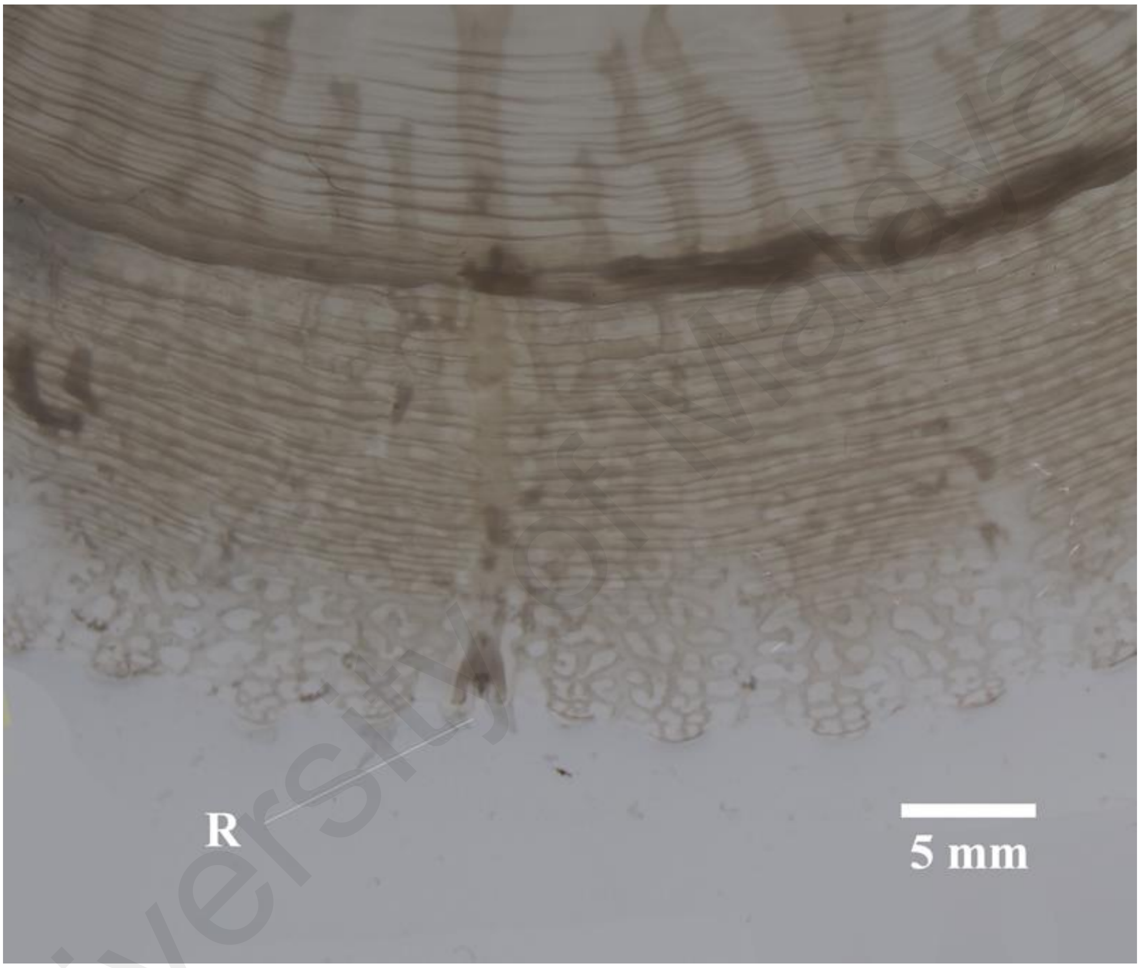

Figure 3.5: Marginal sensory organ / rhopalium (R) of *Lychnorhiza malayensis*

TENTACLE - The tentacles are string-like organs that usually contain cnidae. Tentacles can grow up to several metres in length on certain species. Tentacles are usually not present in Rhizostomeae jellyfish. Tentacles are located at the cleft of the bell margin between a pair of lappet.

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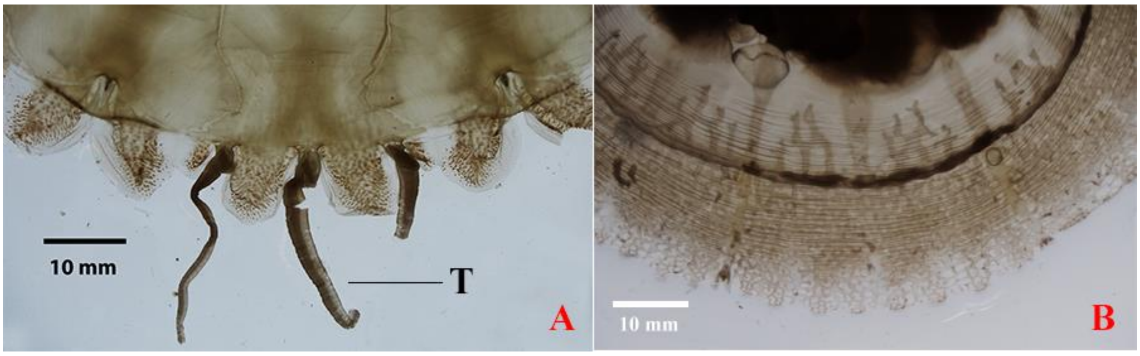

Figure 3.6: Morphology of umbrella. *(A)*: Tentacle (T) of *Chrysaora chinensis* of Semaestomeae. *(B)*: *Lychnorhiza malayensis* of Rhizostomeae without tentacles

MANUBRIUM - The manubrium consists of the under portion of the subumbrella, where the mouth hangs down on a pendulous structure, together with the basal portion of the oral pillar.

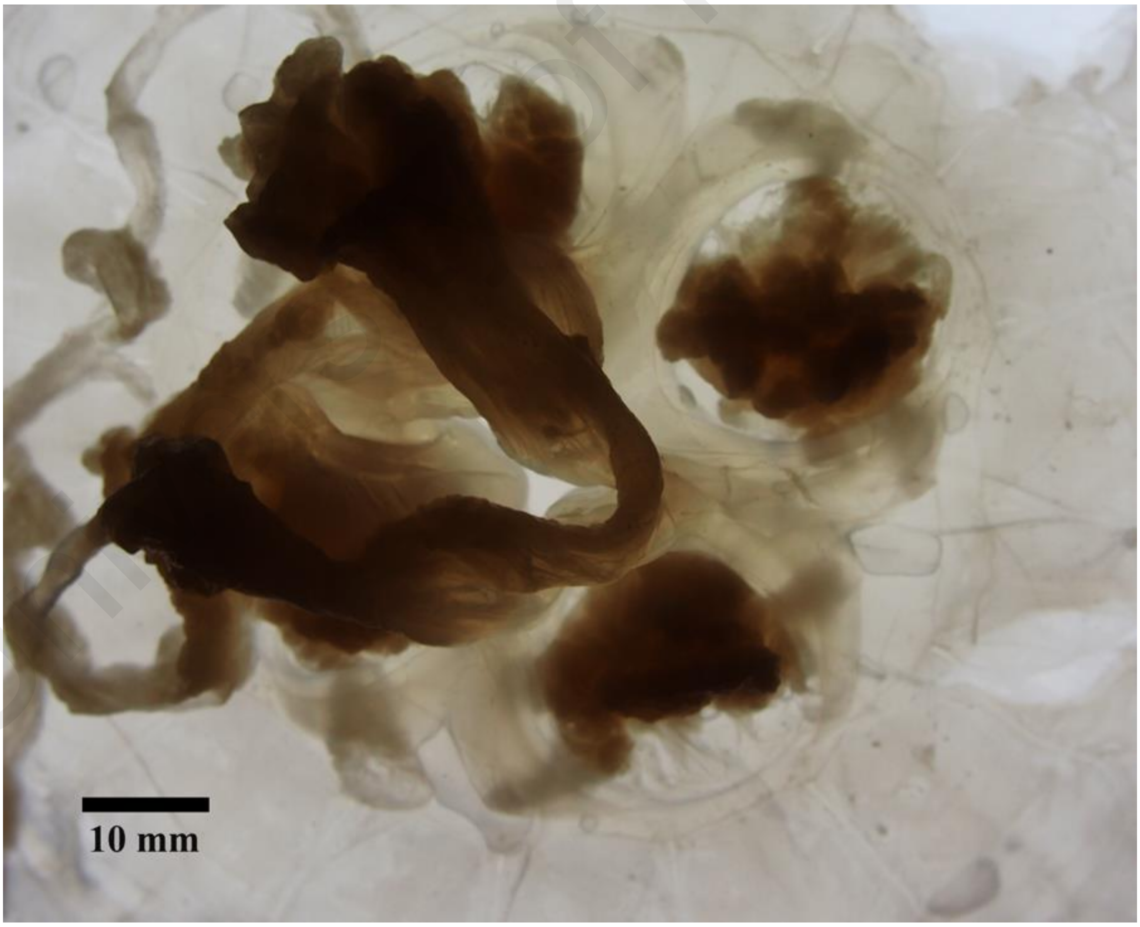

Figure 3.7: Morphology of the manubrium of *C. chinensis*

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ORAL ARM - Oral arm, also known as mouth arm is the organ extending from the base of the manubrium, connected by the oral pillar. There are four oral arms for the order Semaestomeae, and eight for the order Rhizostomeae. Oral arms are used to catch prey for the former order, whereas the latter, food are transferred from the oral arms into the stomach through second arms (or mouthlets) on the oral arms.

Figure 3.8: Gross morphology of oral arm. *(A)*: Whole medusa of *Chrysaora chinensis* floated in a tank, showing four soft and curtain-like oral arms (OA). *(B)*: Oral arm (OA) of *Rhopilema esculentum*. *(C)*: Oral arm (OA) of *Acromitus flagellatus*

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SCAPULAE - Scapulae are leaf-like organs attached to the manubrium. Usually two scapulae are attached to each oral arm. Scapulae, like the oral arm, consist of numerous mouth openings and stomach filaments.

Figure 3.9: Morphology of scapulae. *(A)*: Blade shape scapulae of *Rhopilema hispidum*. *(B)*: Scapulae (S) attached to oral arms of *Rhopilema esculentum*

TERMINAL CLUB - Terminal club is the organ that originates at the margin of the oral arm. It dangles at the tip of the oral arm.

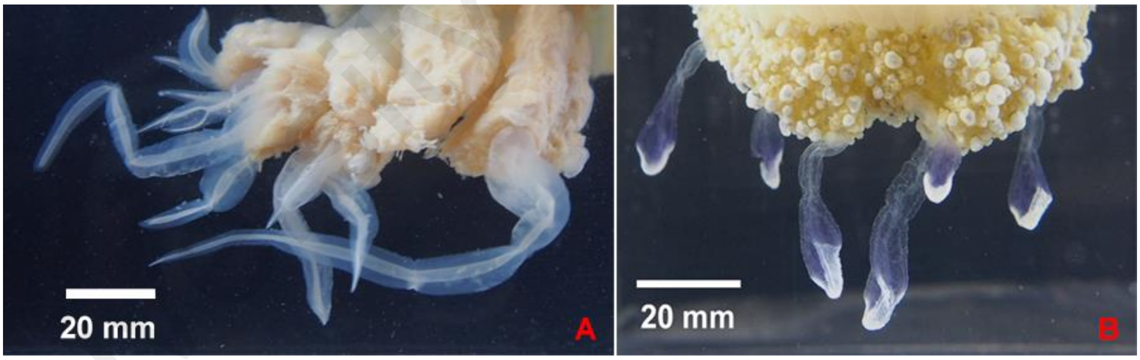

Figure 3.10: Morphology of oral arm with terminal club. *(A)*: Terminal club (C) at the margin of the oral arm of *Rhopilema esculentum*. *(B)*: Terminal club (C) at the margin of the oral arm of *Phyllorhiza punctata*

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FILAMENT - Filaments can be found on the oral disk and the oral arms. Filaments are primarily used for swimming and feeding. Some species even have nematocyst on filaments, and also used filaments to externally transport food to the mouth

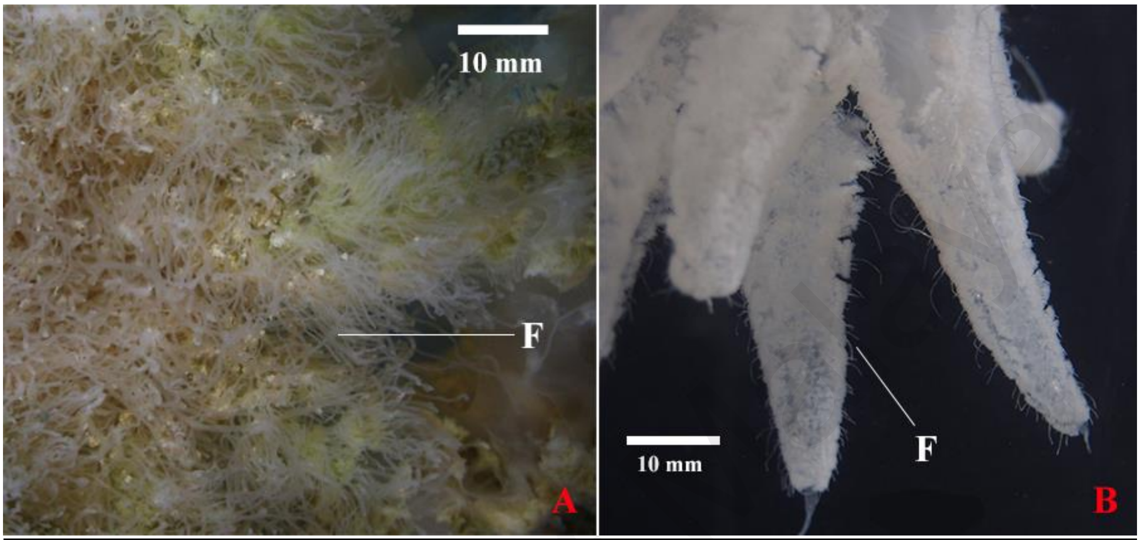

Figure 3.11: Morphology of filaments. *(A)*: Filament (F) at the oral disk of *Phyllorhiza punctata*. *(B)*: Filament (F) at the oral arms of *Acromitus flagellatus*

GASTROVASCULAR CAVITY - Stomach or gastrovascular cavity is the space between the exumbrella and subumbrella that would contain products of digestions which are distributed to the rest of the gastrovascular system. (Arai, 1997). The distribution is done through a network of canal (see also the Network of Canal section below). The gastravascular cavity in scyphozoan jellyfish is usually divided into four compartments. Each compartment is separated from each other by a radial septa.

Figure 3.12: Morphology of subumbrella of *Chrysaora chinensis* revealing the gastrovascular cavity

SUBGENITAL FENESTRATION - Also known as ostia, it is the opening formed at the base of the subumbrella, fusioned between a pair of oral arms. It is usually oval in shape. In certain mature medusae, gonads can be seen protruding from within the subgenital fenestrations.

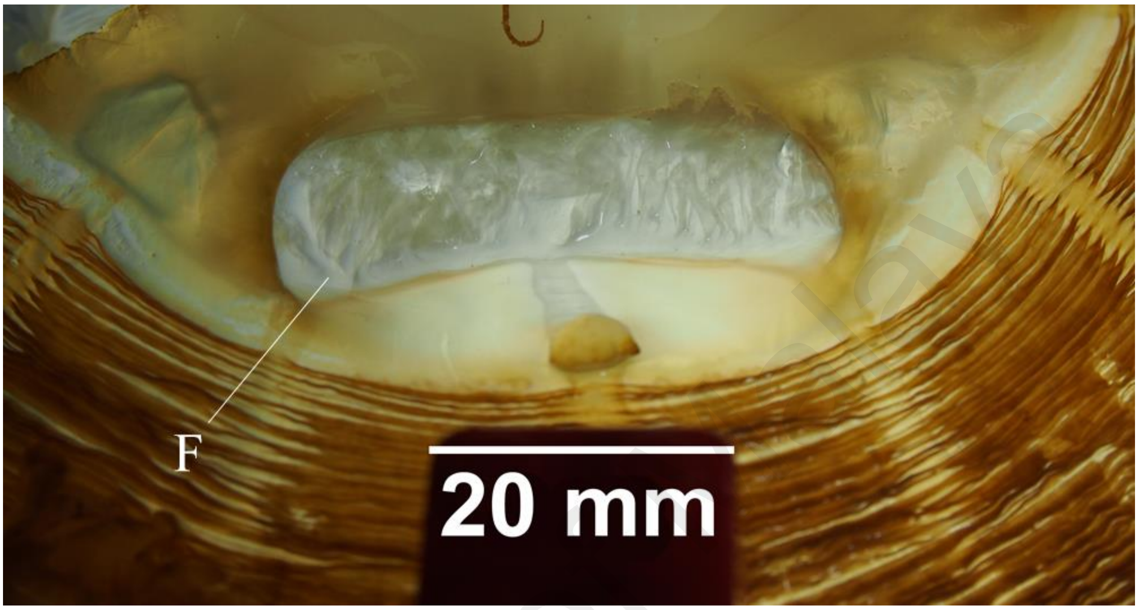

Figure 3.13: Subumbrella morphology of subgenital fenestration (F) of *Rhopilema esculentum*

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PAPILLAE - Papillae are protuberances located in the front or around the subgenital fenestration. Some species usually have three papillaes for each subgenital fenestration. One, usually a larger one, located at the central, and one smaller papillae one on each side the subgenital fenestration.

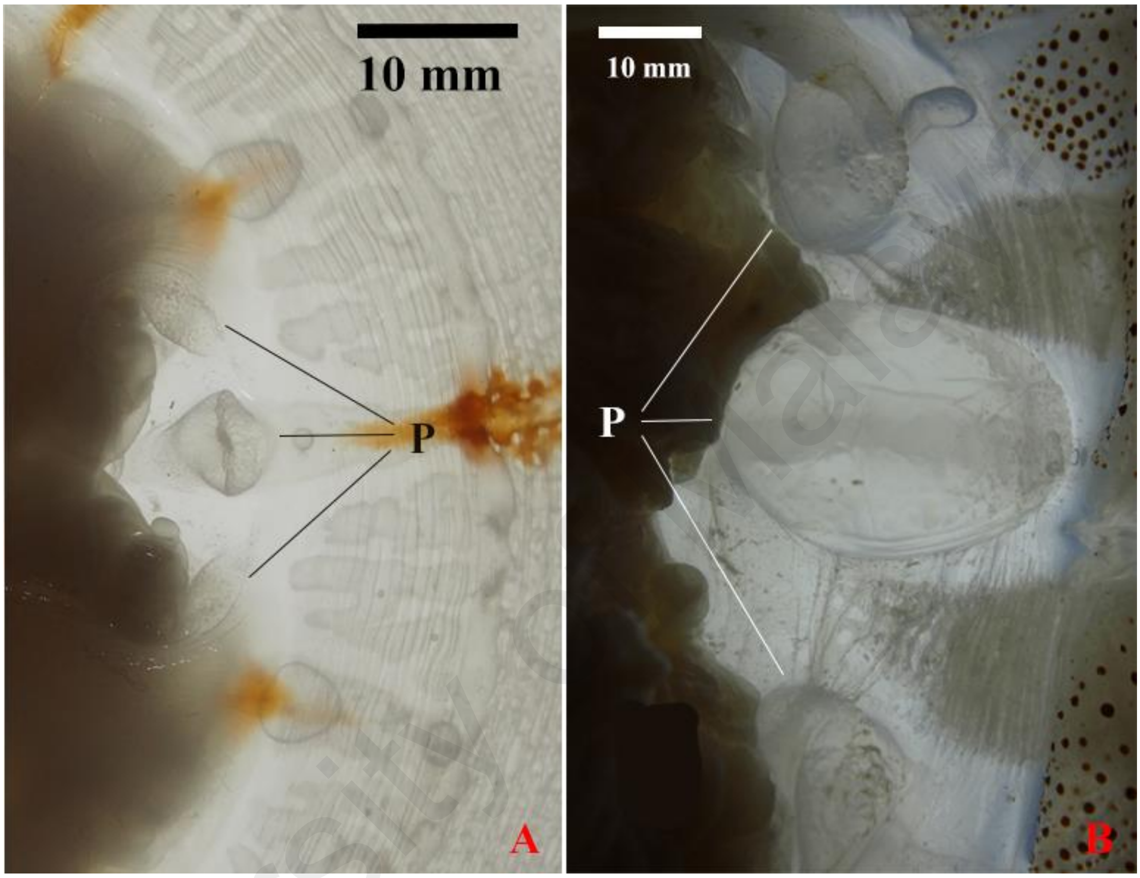

Figure 3.14: Subumbrella morphology of papillae. (A): Three papillae (P) at the subumbrella of *Lychnorhiza malayensis*. (B): Three papillae (P) at the subumbrella of *Rhopilema hispidum*

NETWORK OF CANALS - Numerous tubes that radiating from the gastrovascular cavitiy form a network of canals. The canals leading from the axis where gonads are located are called the interradial canal. The canals leading from the axis between gonads are called perradial canal. The canals between the interradial and perradial canals are called the adradial canals. The complexity and the shape of the network of canals vary between each species and can be useful for species identifcation.

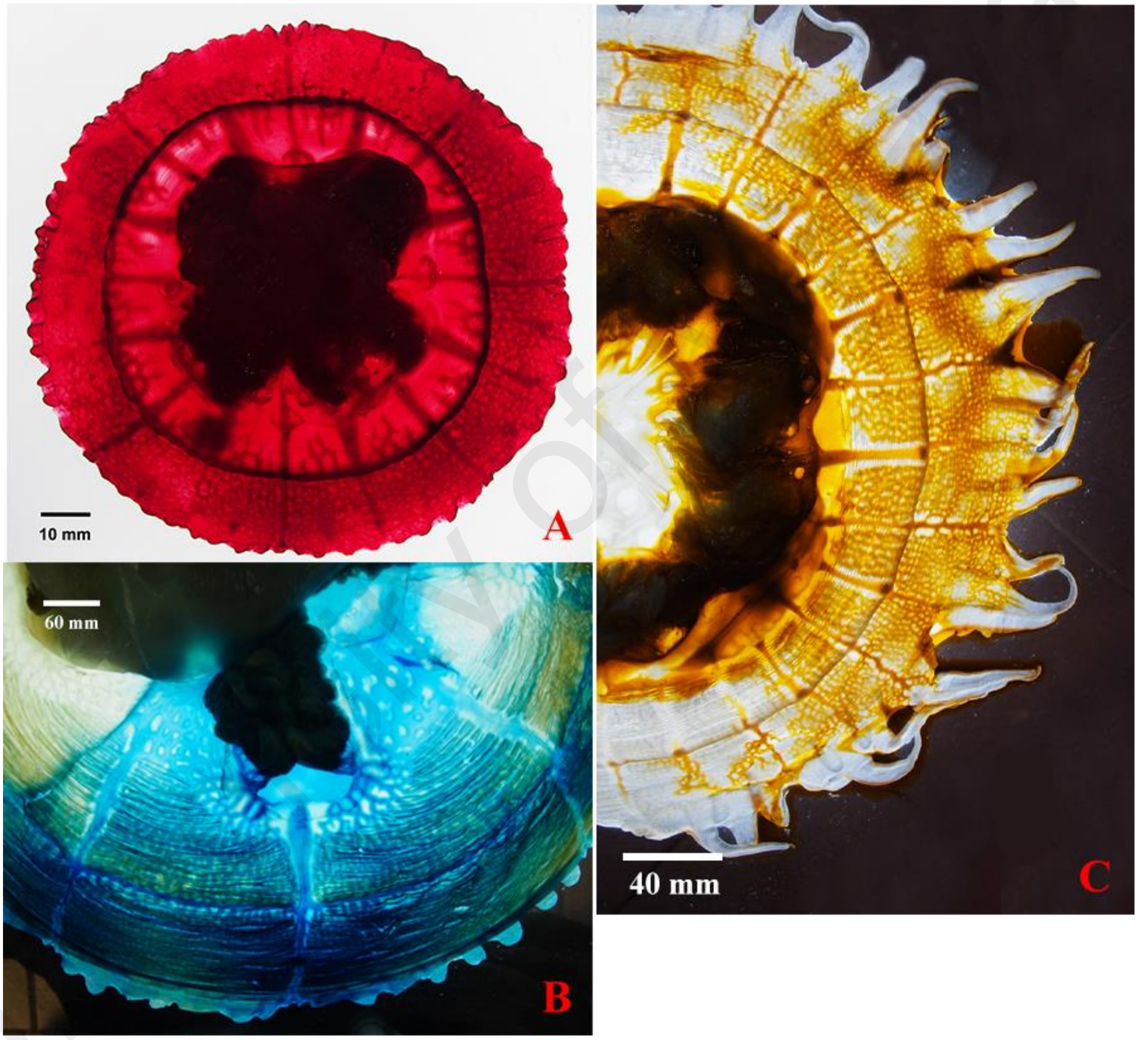

Figure 3.15: Network of canals of *(A)*: *Lychnorhiza malayensi*s injected with red dye. *(B)*: *Phyllorhiza punctata* injected blue dye. *(C)*: *Lobonemoides robustus* injected with yellow dye

MUSCLE - There are two types of muscles in Scyphozoan jellyfish; the radial muscles that extend radially on the subumbrella and the coronal muscles are found as concentric rings around the subumbrella. Sometimes the coronal muscles may or may not overlap with the radial canals underneath.

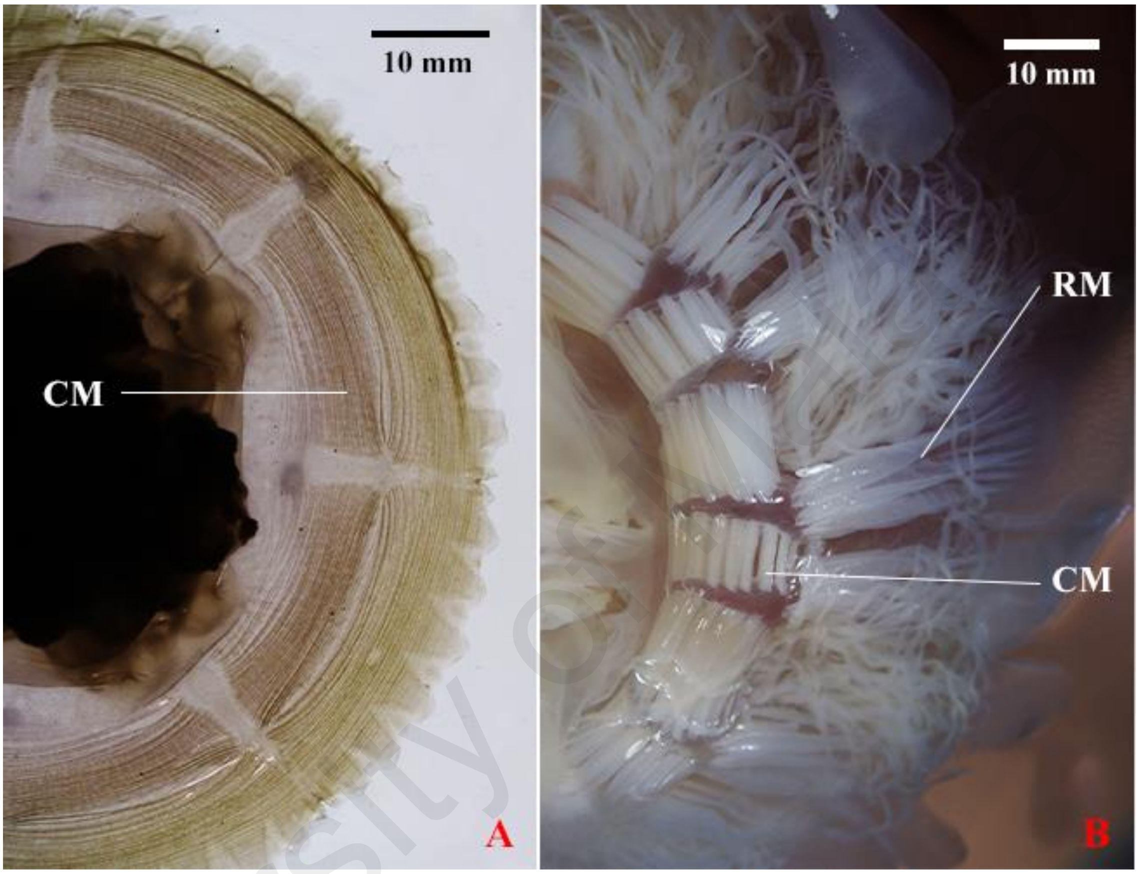

Figure 3.16: Subumbrella morphology of muscle. *(A)*: Coronal muscle (CM) of *Phyllorhiza punctata*. *(B)*: Coronal muscle (CM) and radial muscle (RM) of *Cyanea* sp.

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GONAD - Gonads of the scyphozoan jellyfish arise from the gastrodermis. In medusae, the gonads are usually situated on the base of the gastrovascular cavity, peripheral to the gastric filaments (Arai, 1997). Gonads are usually milky pink to yellowish. In adult medusae, gonads can be seen protruding from the subgenital fenestrations

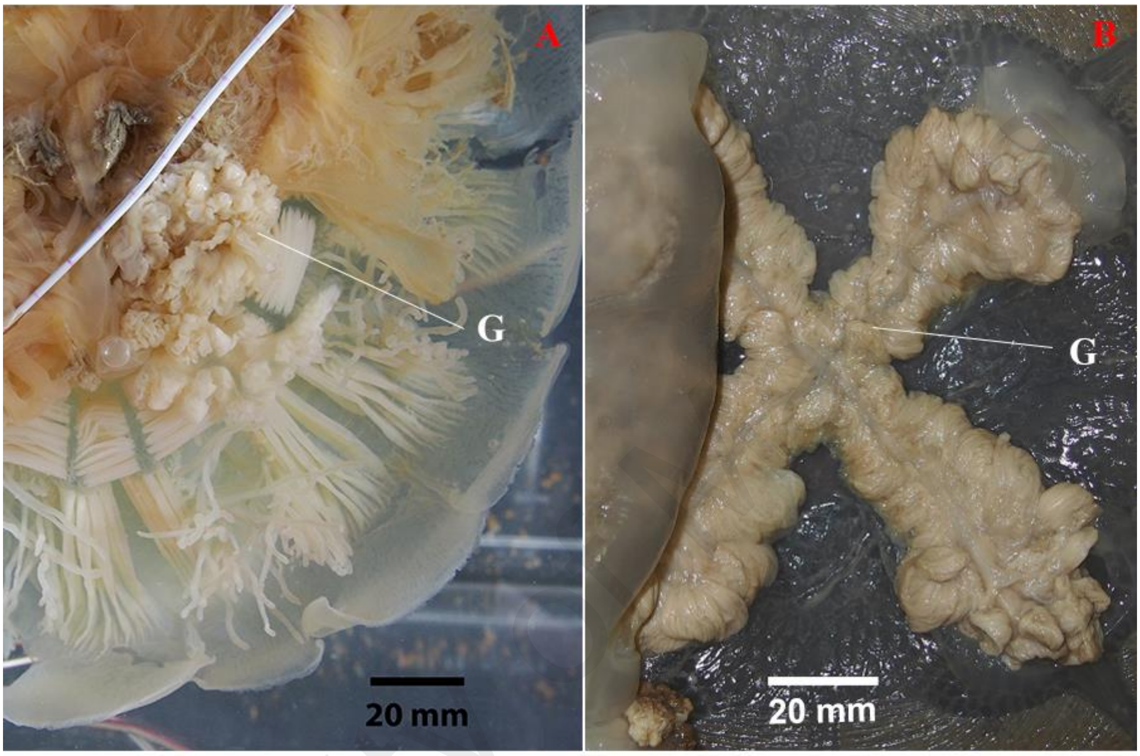

Figure 3.17: Subumbrella morphology of gonad. *(A)*: Gonad (G) protruding from the subgenital fenestration of *Cyanea* sp. *(B)*: Gonad (G) at the base of the gastrovascular cavity of *Versuriga anadyomene.*

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3.2 Morphological Description of *Chrysaora chinensis* Vanhöffen 1888

Class SCYPHOZOA (Goette, 1887)

Order SEMAEOSTOMEAE (Agassiz, 1862)

PELAGIIDAE (Gegenbaur, 1856)

***Chrysaora* (Péron & Lesueur, 1809)**

***Chrysaora chinensis* (Vanhöffen, 1888)** resurrection by Morandini & Marques (2010) (Figure 3.18 – 3.19)

Material Examined - A total of seven specimens were examined from sites Sungai Janggut (MRI 2, MRI 65, MRI 163, MRI 171), Pantai Sabak (MRI 208), Balik Pulau (MRI 262) and Kampung Cempaka (MRI 190).

Description of specimens- Bell: Umbrella hemispherical. For adult medusa, exumbrella surface finely granulated; in some specimens the exumbrella 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; shape 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 interradial and perradial axis. Rhopalia shape is tear-drop like.

Tentacles: Three tentacles per octant (2-1-2, where the numbers represent the ontogenetic order of the development of tentacles), originated at the cleft of the lappet. Tentacles soft, straight and string-like. Tentacle length up to four times the diameter of the bell. Personal field observations of the tentacles were much longer, more than four times of the bell diameter, but sometimes detached when specimens were caught or handled roughly.

Oral arms: Four oral arms, up to 200cm in length in adult medusa; oral arms 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 absent. Terminal club absent. Filament absent.

Stomach, manubrium and radial canals: Central stomach shape slightly rounded, as it is formed by the boundary of the 16 gastrovascular pouches, where eight are rhopalar pouches and another eight are inter-rhopalar pouches. Radial septa present; extending distally from stomach toward bell margin. For rhopalar pouches, septa starts 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 absent. Network of canal absent. Radial and coronal musculatures not prominent. Manubrium is not prominent. Oral disk at the base is about 1/3 as wide as the bell diameter, and the base of oral arm is less than 1/3 as wide as the bell diameter. Mouth present with four walls around the mouth. Quadralinga absent.

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

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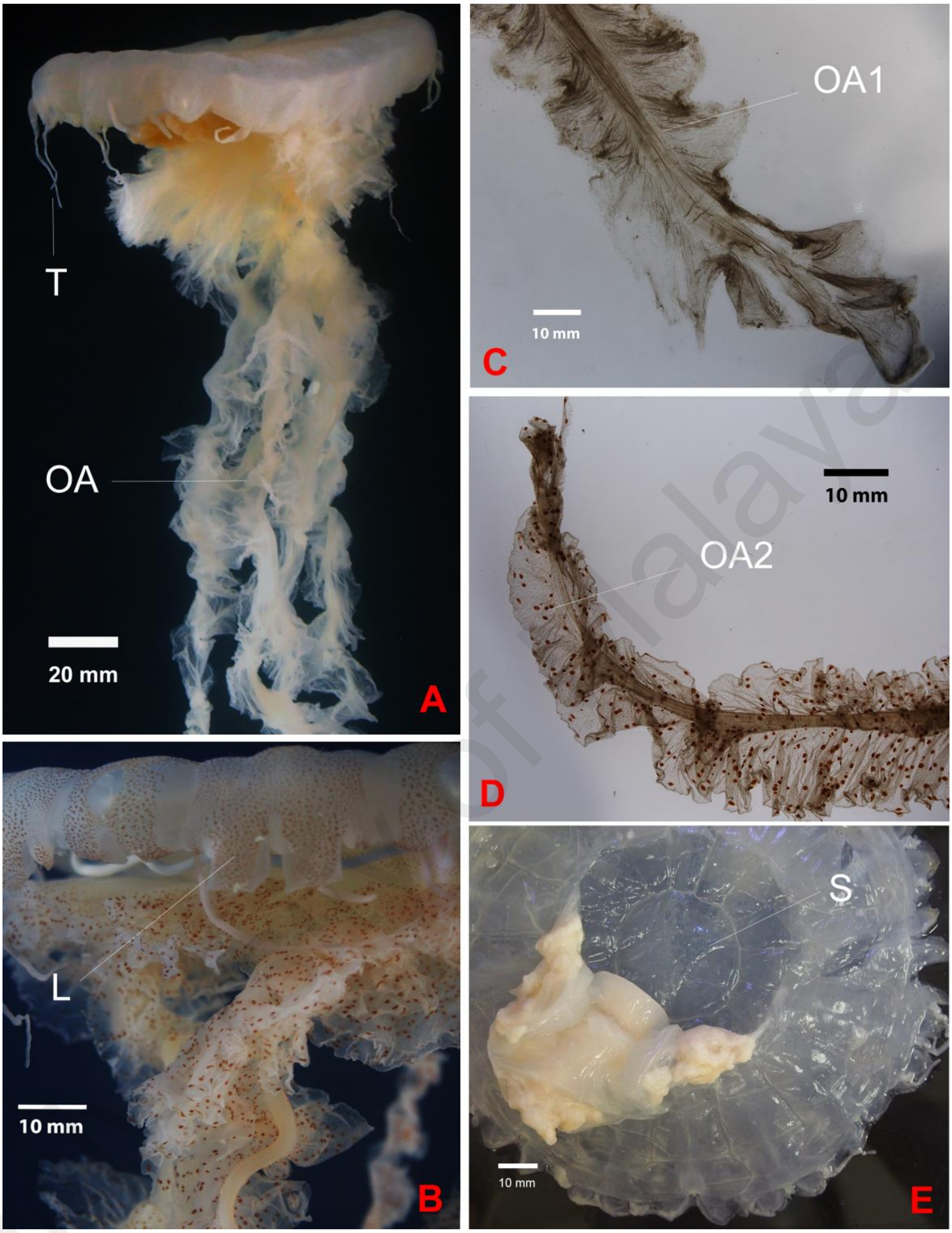

Figure 3.18 Gross morphology of *Chrysaora chinensis*. *(A)*: Whole medusa floated in a tank, showing four soft and curtain-like oral arms (OA) and tentacles (T). *(B)*: Side view of the bell (umbrella) with reddish brown pigmentation on the lappet (L). *(C)*: Oral arm (OA1) without pigmentation; *(D)*: Oral arm (OA2) with reddish pigmentation; (E): Subumbrella view of the central stomach (S).

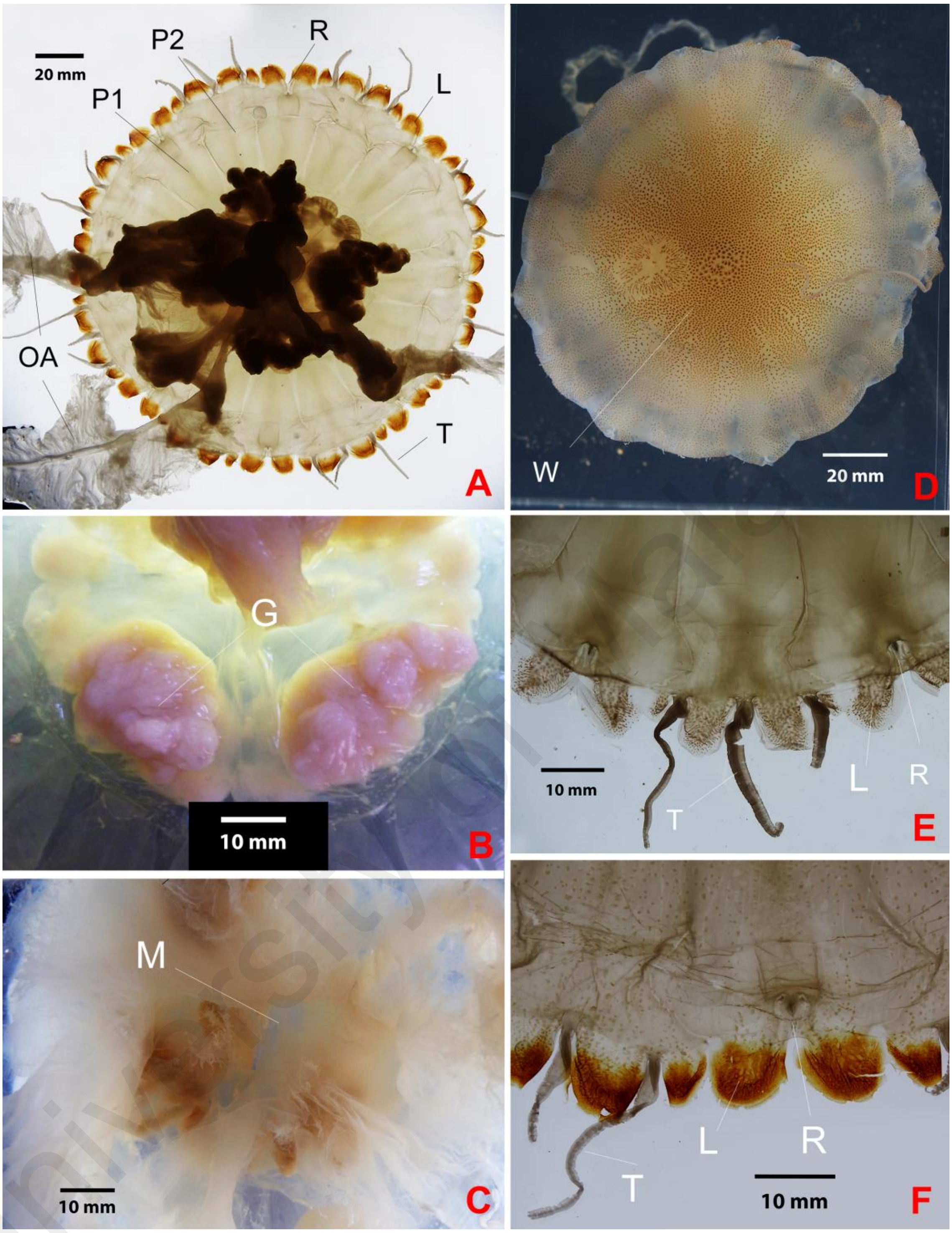

Figure 3.19: Umbrella morphology of *C.chinensis*. *(A)*: Subumbrella view revealing 16 gastrovascular pouches with rhopalar pouch (P1), inter-rhopalar pouch (P2), rhopalium (R), lappet (L), tentacle (T) and oral arms (OA). *(B)*: Subumbrellla view of creamy white gonads (G), whereby each of the four 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.

Figure 3.19, Continue

(F): Subumbrella view of another specimen showing distinctly pigmented lappet (L), rhopalia (R) and tentacles (T).

3.3 Morphological Description of *Cyanea* Sp.

Class SCYPHOZOA (Goette, 1887)

Order SEMAEOSTOMEAE (Agassiz, 1862)

Cyaneidae (Agassiz, 1862)

***Cyanea* (Péron & Lesueur, 1810)**

***Cyanea* Sp.** (Figure 3.20 – 3.21)

Material Examined - A total of seven specimens were examined from sites Sungai Janggut (MRI 55, MRI 56, MMRI 95, MRI 96, MRI 97, MRI 127 SJG 10)

Description of specimens - Bell: Flattened hemispherical. Mesoglea is rigid, thick in the centre and thin towards the bell margin. Numerous faint warts are distributed on exumbrella surface, denser at the centre of the bell and sparce towards the cleft of the lappet. Sixteen equally broad sized lappets, semi square shaped along the margin of the umbrella. All lappets overlap each other at the cleft. Rhopalar cleft is deeper than the inter-rhopalar cleft. Eight tear drop shaped rhopalia, present in clefts of the umbrella margin, located at the interradial and perradial axis. .

Tentacle - Eight whorls of tentacles originating from the coronal muscle at the subumbrella. Each tentacle whorl is horseshoe shaped, consisting of two to three layers, located between two groups of inter-rhopalar radial muscles. Tentacles are soft, straight and string-like; up to 200 tentacles per whorl.

Oral arm - Four oral arms, up to four times as long as the diameter of the bell although most were detached during the sampling process; oral arms formed at the end of the oral pillar. Oral arms are transparent, soft and curtain like. Scapulae, terminal clubs and filaments were absent.

Stomach and Manubrium - The digestive system of this species consists of a gastrovascular cavity or central stomach, which is slightly round, as it is formed by the boundary of the 16 coronal muscle groups. Sixteen pouches in the umbrella are comprised of eight rhopalar pouches and eight tentacular pouches. Subgenital fenestration is oval, 1/9 of the bell diameter. Papillae absent. Between six to seven transverse "ridges" connecting both pouches below their surface. Both rhopalar pouches and tentacular pouches also anastomoses with the lappets on both side of the cleft, forming dense network on the lappet. Sixteen groups of coronal muscles originating from the outer margin of the central stomach at the subumbrella, extending towards the rim of the oral disk. Each coronal muscle group consists of six to eight muscle folds. Bigger coronal muscle groups alternate with slightly smaller coronal muscle groups. Muscle groups are not connected, with a ridge in between the two coronal muscle groups. Gastrovascular pit absent. Sixteen groups of radial muscle originate at the underside of the lappet, perpendicular to the coronal muscle. Each radial muscle group consists of nine to eleven radiating muscle folds with different sizes and lengths, with the longest muscle folds located in the centre of the muscle field. Manubrium thick and smooth; oral disk at the base is about 1/3 as wide as the bell diameter.

Gonad - Gonads form below the subumbrella and attached with the subumbrella and manubrium; each gonad is separated from the neighbouring gonads. Colour creamy white. Gonads are big and mostly protruding out of the subgenital fenestration. Gastric filaments present.

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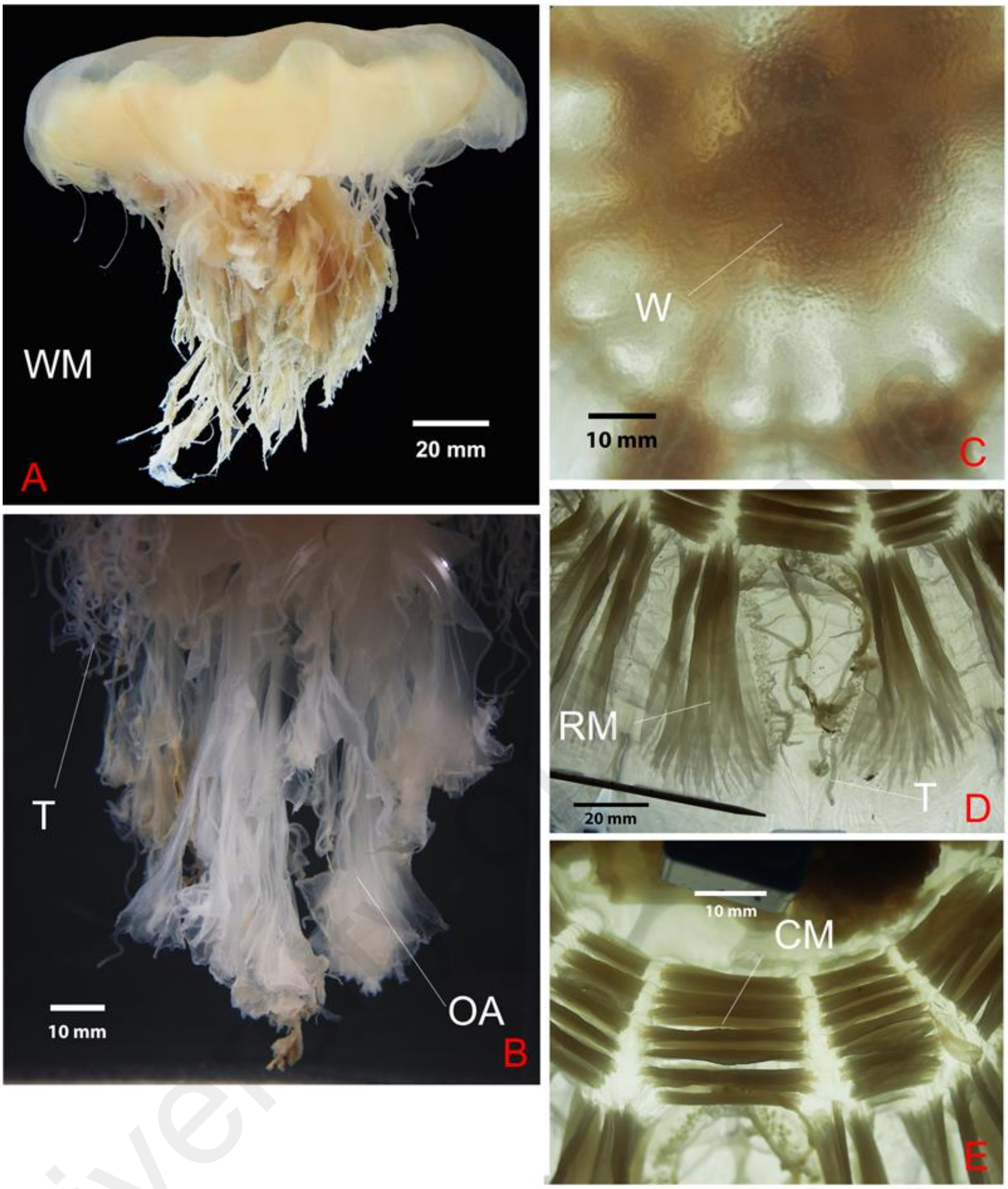

Figure 3.20: Gross morphology of *Cyanea* sp. (*A*): Whole medusa. (*B*): Numerous tentacles (T) and curtain like oral arms (OA). (*C*): View of exumbrella with numerous warts (W). (*D*): Subumbrella showing a group of radial muscle (RM) and a horseshoe shaped whorl of tentacles (T) between each group of radial muscle. (*E*) Subumbrella view showing a group of coronal muscle (CM).

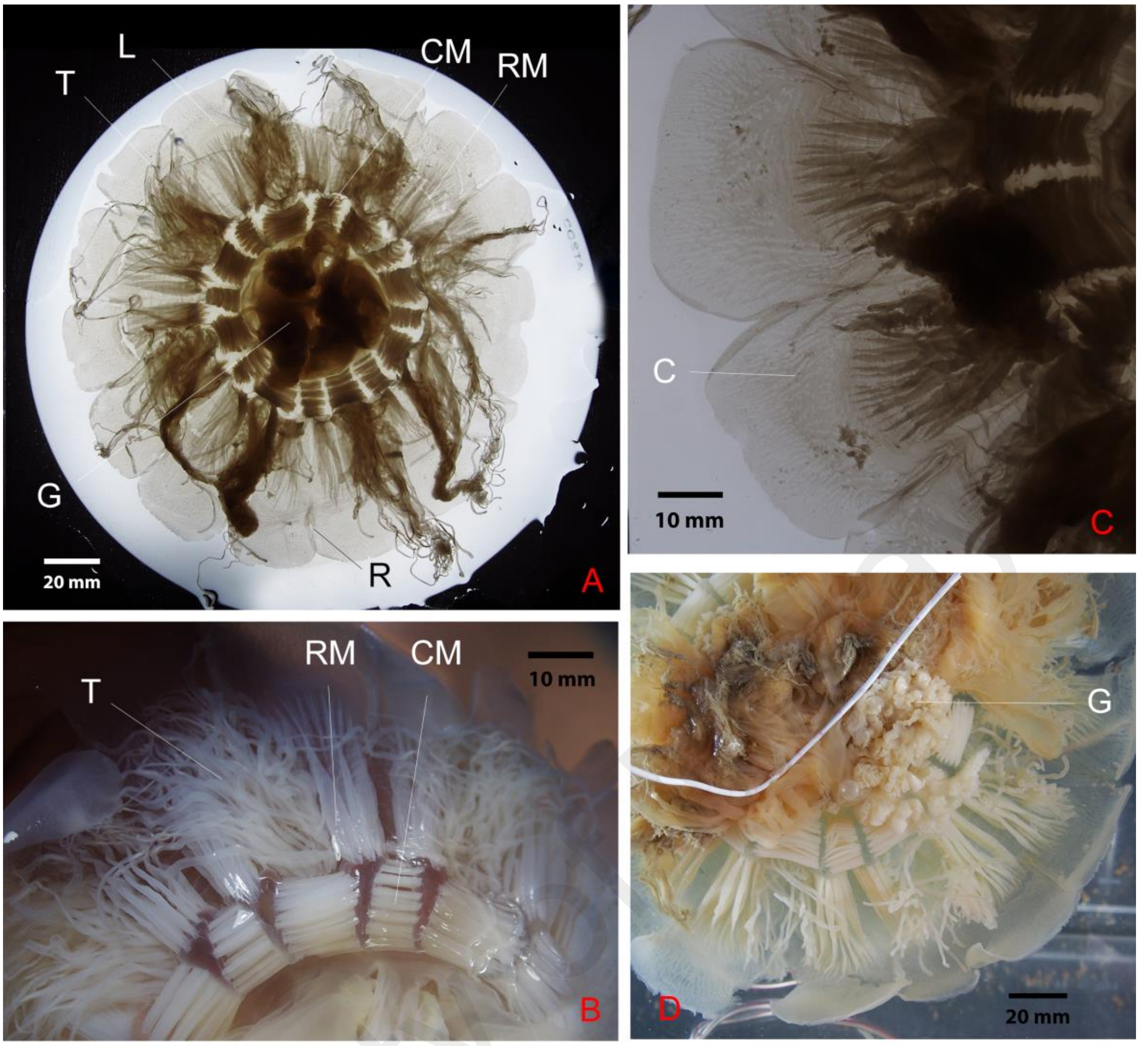

Figure 3.21: Subumbrella morphology and structures of *Cyanea* sp. (*A*): Subumbrella view of the medusa. (R) Rhopalium. (G) Gonad. (T) Tentacle. (L) Lappet. (CM) Coronal muscle. (RM) Radial muscle. (*B*): A whorl of tentacle (T). Radial muscle (RM). Coronal muscle (CM), alternating between a longer group and a shorter group. (*C*): Lappet shape broad and semi-circular, with network of canal (C). (*D*): Gonad (G) creamy white.

3.4 Morphological Description of *Rhopilema esculentum* Kishinouye 1891

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

RHIZOSTOMATIDAE (Cuvier, 1799)

Rhopilema (Haeckel, 1880)

***Rhopilema esculentum* (Kishinouye, 1891)** (Figure 3.22 – 3.23)

Material Examined - A total of three specimens were examined from site Sungai Janggut (MRI 87, MRI 98, IND 3)

Description of specimens - Bell: Flattened hemispherical. Umbrella surface is smooth and reddish. Mesoglea is rigid, thick in the centre and thin towards the bell margin. Eight lappets per octant; shape are slightly elongated with pointy rhopalar lappets. Eight tear-drop shaped rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Tentacles absent.

Oral arm - Eight oral arms, as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop into three wings at the distal section (approximately at the distal 1/5 of the oral arm). Two wings face away from the central axis (outer wings) and one wing faces the central axis (inner wing). Numerous mouthlets located at the frills of the wings. One to three spindle-shape appendages form at the frills of the wings. Numerous filaments found at the frill of the wings. For each oral arm, two blade-shaped but flat scapulae developed approximately 1/3 distally from the base of each oral pillar, then develop into two wings for each scapulae. Winged portion of the scapulae is smaller than the winged portion of the oral arms. Numerous mouthlets located at the frill of the scapulae. Numerous short filaments located at the frills of the scapulae. Terminal club absent.

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Stomach and Manubrium - Gastovascular cavity or central stomach is floret shaped. Each subgenital fenestration has one papillae located at the base of the manubrium and near the edge of the subgenital fenestration. Papille dome shape. Papillae surface is rough with numerous warts. Sixteen radial canals on the subumbrella are comprised of eight rhopalar canals and eight inter-rhopalar canals; all canals fully extend to the margin of the bell, where rhopalar canals split at the distal end and branch toward the neighbouring lappets. Ring canal present. Rhopalar canals start anastomosing with the neighbouring canals at about 1/3 from the centre, whereas interhopalar canals start anastomosing with the neighbouring canals at about 1/2 from the centre. Radial muscle absent. Sixteen coronal muscle fields cover the network mesh underneath. Coronal muscle interrupted on the rhopalar canals but not on the inter-rhopalar canals. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and the base of oral arm is less than 1/6 as wide as the bell diameter.

Gonad - Gonad floret shape, formed at the subumbrella; each gonad is separated from the neighbouring gonad; attached with the subumbrella and manubrium. Colour white. Gastric filaments present.

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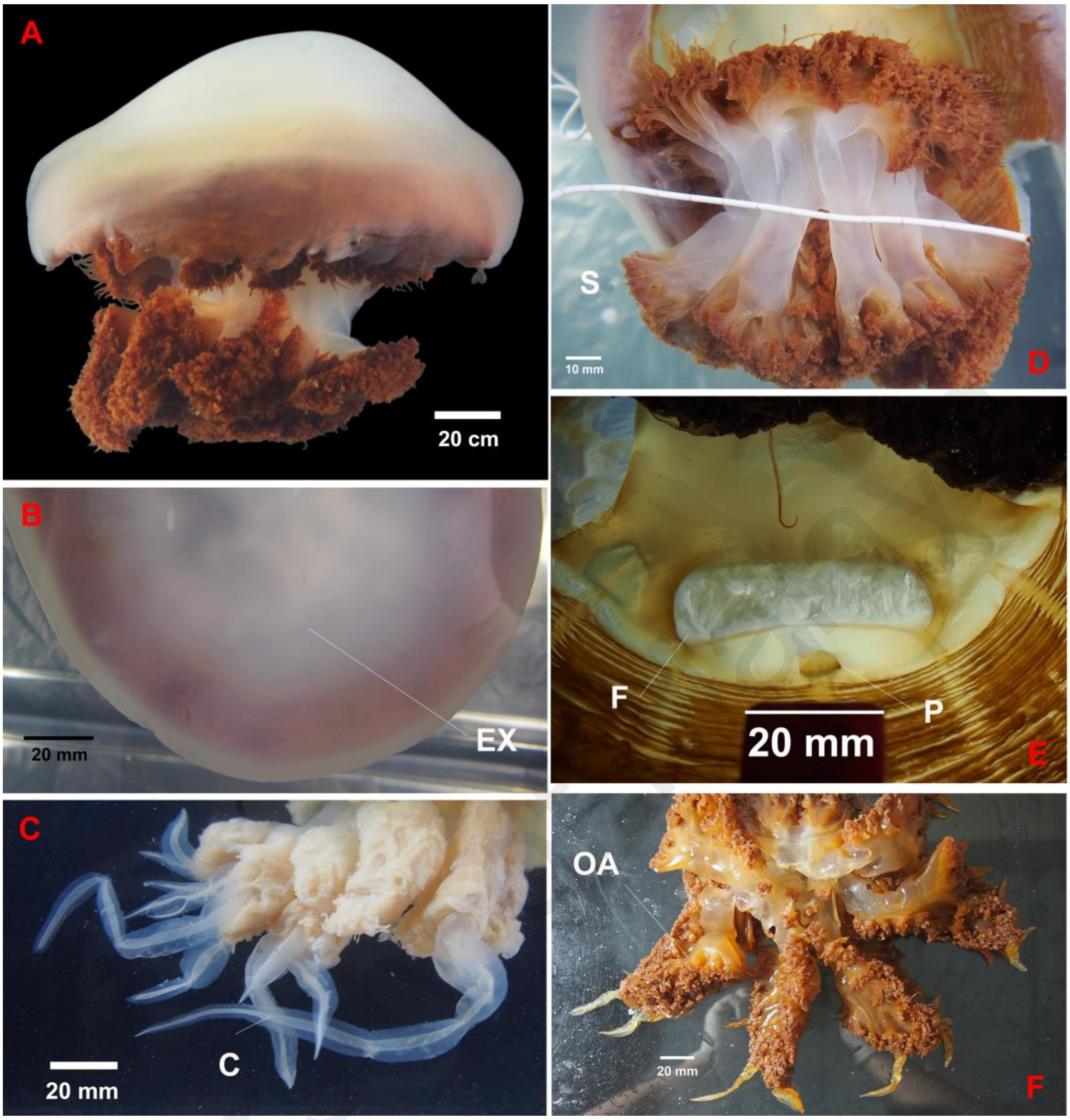

Figure 3.22: Gross morphology of *Rhopilema esculentum* (*A*): Whole medusa. (*B*): Exumbrealla (EX) smooth. (*C*): Terminal club (C) attached to oral arm. (*D*): 16 scapulae (S) in each medusa. (*E*): One subgenital fenestration (F) for each quadrant. One papilae (P) at the centre of the fenestration. (*F*): Oral arm (OA).

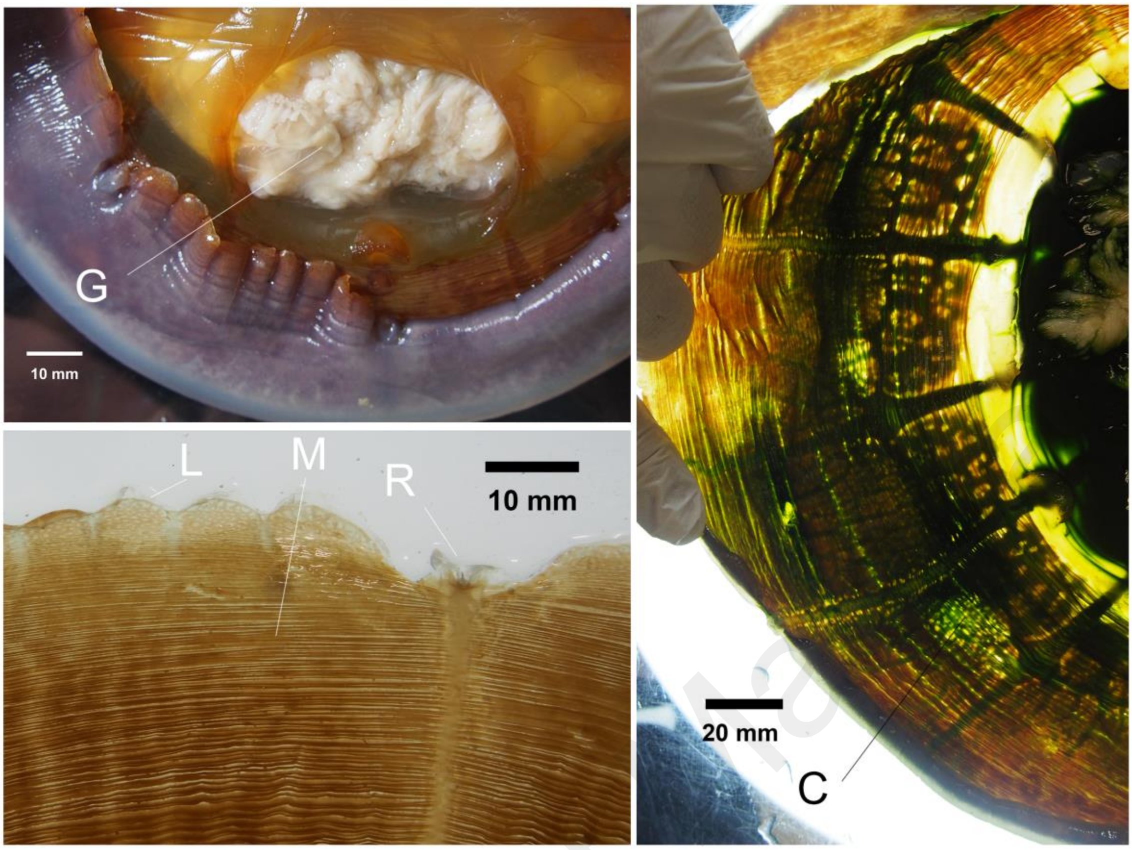

Figure 3.23: Subumbrella morphology and structures of *Rhopilema esculentum* (*A*): Gonad (G) creamy white. (*B*): Lappet (L). Coronal muscle (M). Rhopalia (R). (*C*): Network of canals injected with green dye.

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3.5 Morphological Description of *Rhopilema hispidum* Vanhöffen 1888

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

RHIZOSTOMATIDAE (Cuvier, 1799)

Rhopilema (Haeckel, 1880)

***Rhopilema hispidum* (Vanhöffen, 1888)** (Figure 3.24)

Material Examined – A total of four specimens were examined from site Sungai Janggut (MRI 13, MRI 89, IND 9, IND 10).

Description of specimens - Bell: Umbrella hemispherical with a rough surface that feels like sand paper. Numerous colourless warts and larger reddish brown circular warts are found on the exumbrella and lappets. Warts are bigger and more concentrated at the centre of the bell, decreasing in size towards the lappets. Eight slightly elongated and pointy lappets located in each octant. The velar lappets adjacent to rhopalar lappets are slightly bigger than the rest of the lappets. Short furrow forms between lappets, whereas larger furrow forms between two pairs of velar lappets. Numerous warts are concentrated on the lappets. Eight tear-drop shaped rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Tentacles absent.

Oral arm - Eight oral arms, as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop to three wings at the distal section (approximately at the distal 1/5 of the oral arm). Two wings face away from the central axis (outer wings) and one wing faces the central axis (inner wing). Numerous mouthlets located at the frill of the wings. Sixteen blade-shape but flat scapulae for each medusa. Four scapulae developed approximately 1/3 distally from the base of each oral pillar, then develop to two wings for each scapulae. Winged portion of the scapulae is smaller than the winged

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portion of the oral arms. Numerous mouthlets located at the frill of the scapulae. Numerous short filaments located at the frills of the scapulae. Terminal club absent from the specimens observed, although there is a segment of left over hanging from the end of the oral arm that may be the origin of the missing terminal club. In the field, terminal clubs are observed. According to Omori (2001), a club shape terminal club is found at the tip of the oral arm. Filaments are found at the scapulae.

Stomach and Manubrium - Gastrovascular cavity, or central stomach is floret shape. Subgenital fenestration shape oval. 1/7 of the size of the bell diameter. Each subgenital fenestration has three papillae. The largest papillae located at the upper side of the subgenital fenestration, aligning with the rhopalar canal. One smaller on each side of the subgenital fenestration. Papillae shape oval with rough surface. Sixteen radial canals on the subumbrella comprised eight rhopalar canals and eight inter-rhopalar canals; all canals fully extend to the margin of the bell, where rhopalar canals split at the distal end and branch toward the neighbouring lappets. Inter-rhopalar doesn't branch toward the neighbouring lappets. Ring canal absent. A mesh of network formed between the canals, where the proximal part of the network anastomosing more densely but decrease in size towards the distal part. Rhopalar canal starts anastomosing with the neighbouring canals at about 1/3 from the gastovascular cavity, whereas interhopalar canals start anastomosing with the neighbouring canals at about ½ from the gastovascular cavity. Three canals (two adradial, one perradial) connecting to the oral arm. Radial muscles absent. Sixteen prominent coronal muscle fields is interrupted at the sixteen radii; each field forms a pyramid shape. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and the base of oral arm is less than half as wide as the bell diameter.

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Gonad- Gonads are floret shape and form below the subumbrella; each gonad is separated from the neighbouring gonads and attached with the subumbrella and manubrium. Colour yellowish. Gastric filaments present.

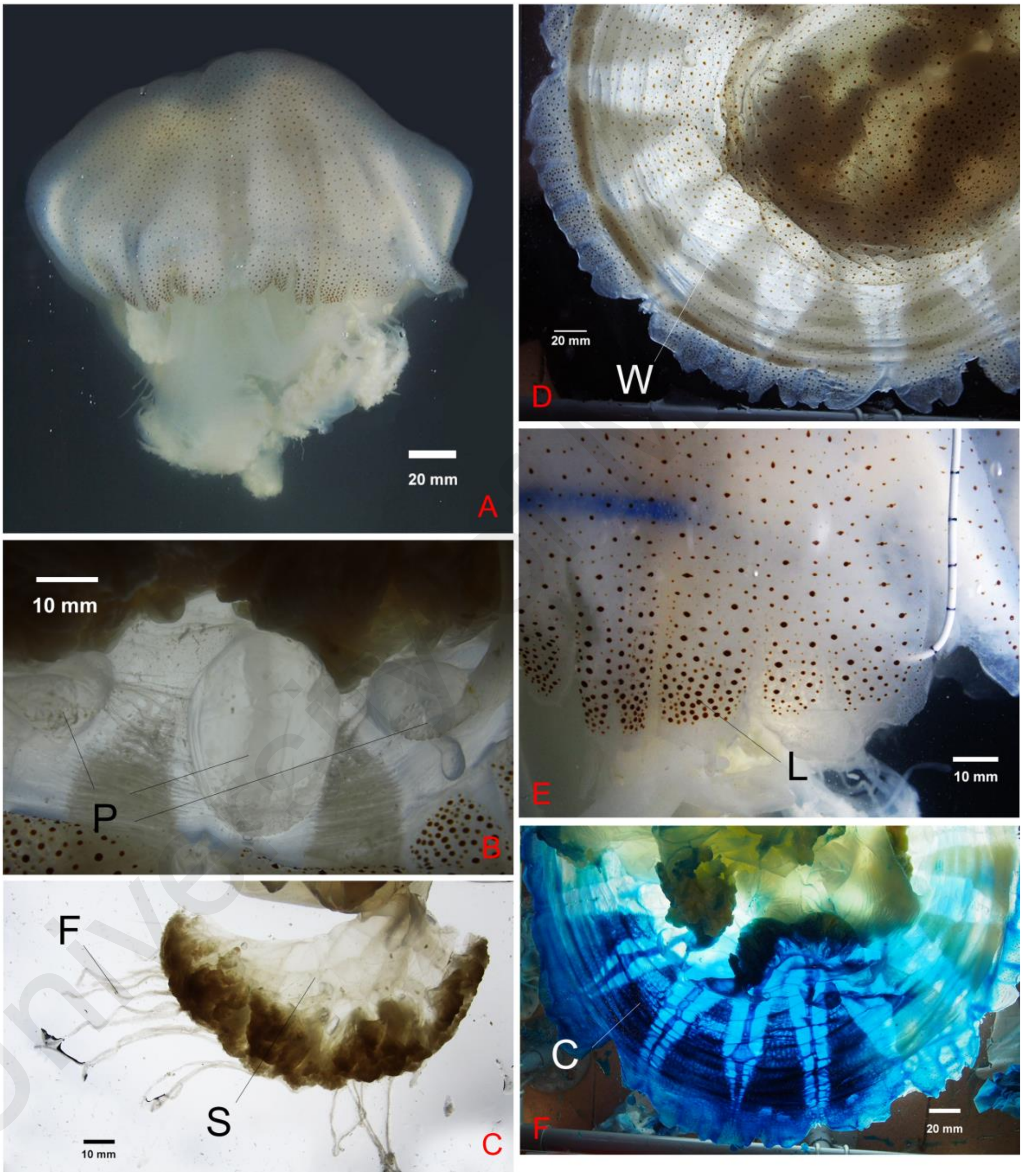

Figure 3.24: Gross morphology of *Rhopilema hispidum* (*A*): Whole medusa. (*B*): Three papilae (P) for each quadrant where the largest is in the centre. (F) Filaments. (*C*): Blade shape scapulae (S) with numerous filaments (F). (*D*): Exumbrella surface rough, with numerous warts (W). (*E*): Lappet (L). (*F*): Network of canal (C) injected with blue dye, forming a pyramid shape of network.

3.6 Morphological Description of *Lobonemoides robustus* Stiasny 1920

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

Lobonematidae (Stiasny, 1921)

***Lobonemoides* (Light, 1914)**

***Lobonemoides robustus* (Stiasny, 1920)** (Figure 3.25 – 3.26)

Material Examined – A total of five specimens were examined from site Sungai Janggut (IND 6, IND 7, IND 8, SJ 130) and Kampung Cempaka (MRI 204).

Description of specimens - Bell: Umbrella hemispherical. Numerous soft, long conical papillae on the exumbrella, where size and density of the protuberance decreasing towards the bell margin. Eight elongated lappets in each octant. Rhopalar lappets are small and pointy. Short furrow between each lappets. A formation of larger furrow between two pairs of velar lappet. 16 tear-drop shaped rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Tentacles absent.

Oral arm – Eight oral arms, as long as the bell diameter; base of oral arm is less than 1/3 as wide as the bell diameter oral arms begin at the end of the oral pillar, then develop into three wings at the distal section (approximately at the distal 1/5 of the oral arm). Two wings facing away from the central axis (outer wings) and one wing faces the central axis (inner wing). Numerous mouthlets located at the frills of the wings. Numerous filaments present at the frills of the oral arms. Fenestrations, or windows are found in the oral arm. Scapulae absent. A definite terminal club absent, but four to nine spindle shape clubs on each oral arm that are easily detached. Numerous pigmented spots on the clubs. A canal is found in the centre of the club, extending proximately

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from the base of the club towards the distal end of the club. String like filaments are found on oral arm.

Stomach and Manubrium – Almost floret shaped gastrovascular cavity, or central stomach Subgenital fenestration is oval; 1/5 the length of the bell diameter. Three small and non-prominent dome shaped papillae located at the lower side of the subgenital fenestration; the papillae in the centre is smaller than the two on each side; the centre papillae forms a ridge that extends towards the inner subgenital fenestration. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter. Thirty two radial canals on the subumbrella comprising of16 rhopalar canals and 16 inter-rhopalar canals; all canals efface beyond the ring canal, towards fully to the margin of the bell, where rhopalar canals split into two at the distal end and branch toward the neighbouring lappets. Canals are found in the lappets. Within the ring canal, three anastomoses connecting both sides of the rhopalar. Most interrhopalar canals are without anastomoses except a few exceptions where one anastomos exist. Beyond the ring canal, rhopalar canals anastomoses with the neighbouring canals through approximately twelve anastomoses, whereas interophalar canal fuses with the neighbouring canals completely. Networks are also found in the oral arm, where a main canal connects with the oral arm from the oral disk. Radial muscles absent. Each quadrant has one coronal muscle fieldt, comprising 80 to 96 muscle folds. The coronal muscle folds are interrupted at the sixteen rhopalar radii. Purple spots are found in the muscle field and the surface of the canals and lappets. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and the base of oral arm is less than 1/3 as wide as the bell diameter.

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Gonad - Floret shape and bluish gonads found below the subumbrella; each gonad is separated with the neighbouring gonad; attaches to the subumbrella and manubrium. Gonad protruding out of the subgenital fenestration. Gastric filaments present.

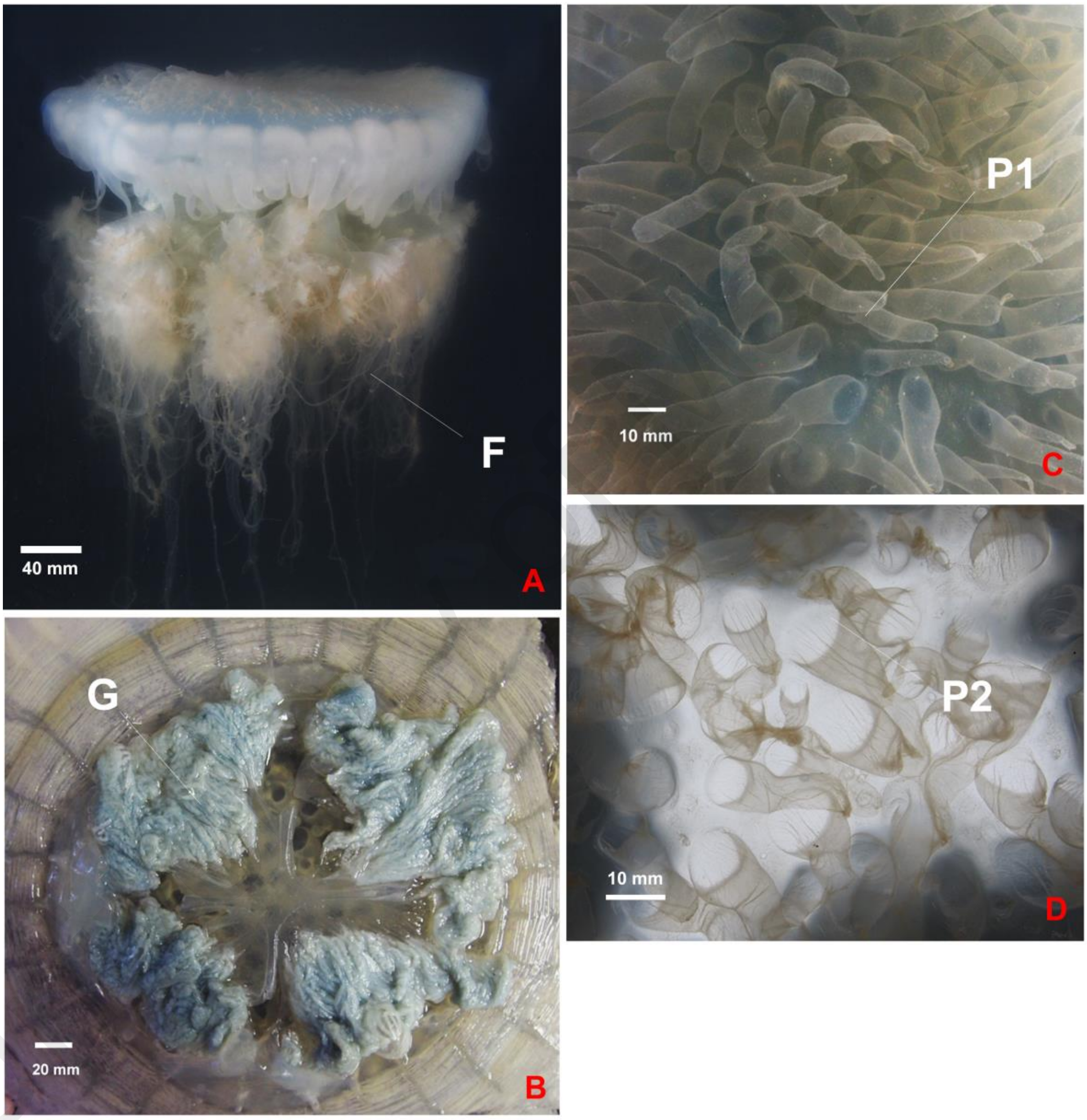

Figure 3.25: Gross morphology of *Lobonemoides robustus (A)*: Whole medusa. (F) Filaments. *(B)*: Gonad (G). (*C, D*): Conical papillae on the exumbrella in water (P1), and on dry stage (P2).

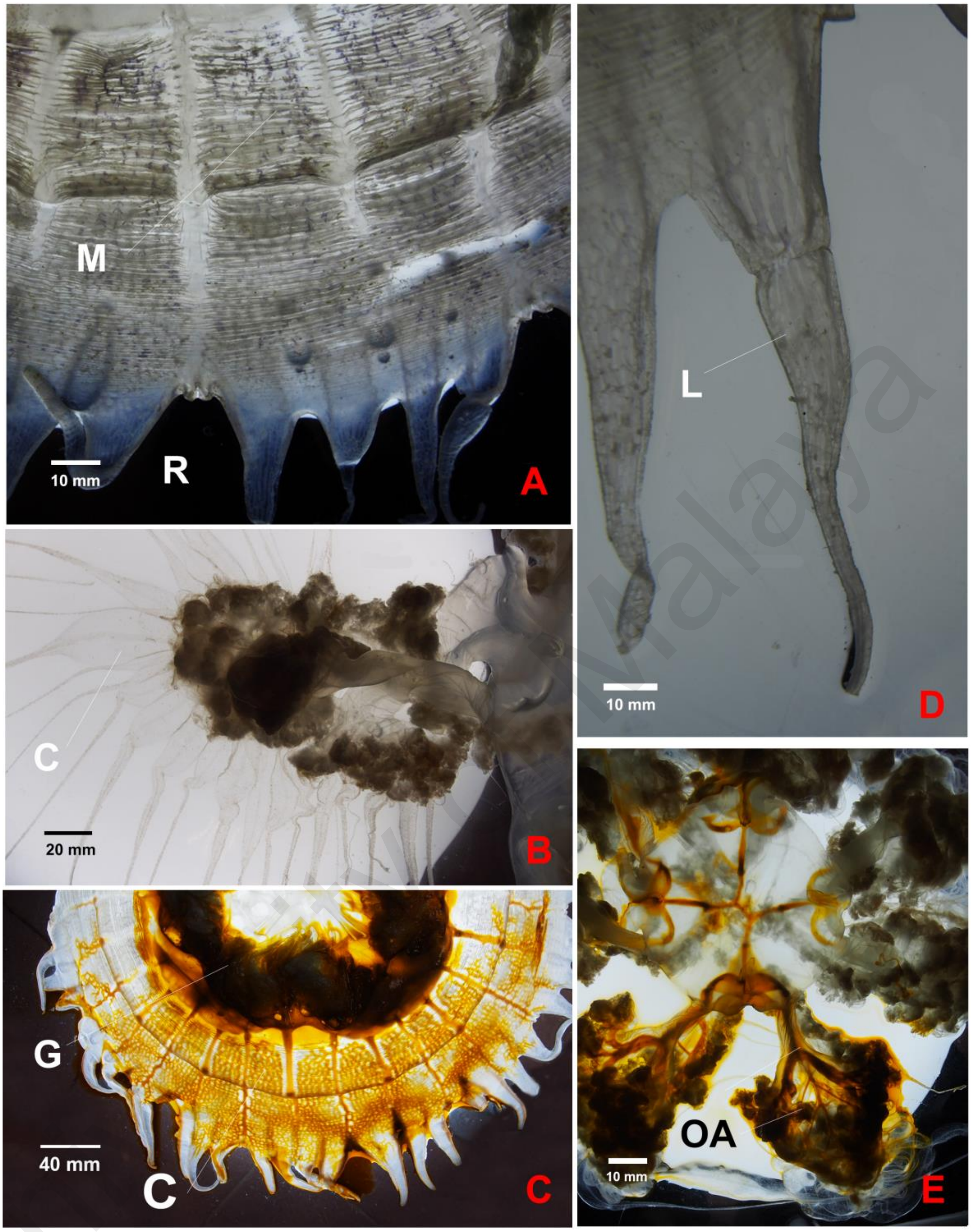

Figure 3.26: Morphology of *Lobonemoides robustus* (*A*): Coronal musculatures (M) with colouration. Rhopalium (R). (*B*): Spindle shape club (C). (*C*): Network of canal (C) with yellow dye injected. (G) Gonad. (*D*): Lappet (L) elongated. (*E*): Oral arm (OA) with yellow dye injected.

3.7 Morphological Description of *Versuriga anadyomene* Maas 1903

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

Mastiigidae (Stiasny, 1921)

Versuriga

***Versuriga anadyomene* (Maas, 1903)** (Figure 3.27 – 3.28)

Material Examined – A total of two specimens were examined from sites Kilim (MRI 153) and Sungai Janggut (MRI 176).

Description – Bell - Umbrella hemispherical. Exumbrella suface grooved irregularly where the size and quantity of the grooves decreased from the centre towards the margin. Eight lappets in each octant; shape are slightly elongated. The lappet neighbouring rhopalar lappets are slightly bigger than the rest of the velar lappets. Rhopalar lappets small and pointy. Short furrow between each velar lappets. A larger furrow formed between two pairs of velar lappet. Sometimes a small lappet formed between two lappets. Eight rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Rhopalia shape is tear-drop like. Tentacles absent.

Oral arm - Eight oral arms, as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop to three wings at the distal section (approximately at the distal 1/5 of the oral arm). Numerous mouthlets at the frills of the wings. Numerous appendages are found at the frills of the wings. Fenestrations, or windows are found on the oral arms. Scapulae absent. Terminal club absent. Some club shape structures are found at the margin of the oral arms. Filaments are found at the oral disk. Filament shape string-like.

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Stomach and Manubrium - Gastrovascular cavity, or centre stomach shape floret. Subgenital fenestration shape oval; 1/5 the length of the bell diameter. Papillae absent. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and oral disk at the mouth is 1/3 as wide as the bell diameter. Eight radial canals on the subumbrella. All eight rhopalar canals extending almost all the way to the margin of the bell, where canals split at the distal end and branch toward the neighbouring velar lappets. Ring canal present. Interradial rhopalar canals connect with the neighbouring canals proximally, whereas perradial rhopalar canals only connected with the neighbouring canals beyond the ring canal. Network becomes denser and smaller towards the margin of the bell. Radial muscles absent. Eight coronal muscle fields, each is interrupted at the eight radii. Coronal musculature starts forming approximate 1/3 distally from the centre, covering 2/3 of the subumbrella until the margin.

Gonad - Floret shaped and yellowish gonads found formed below the subumbrella; each gonad is separated with the neighbouring gonads; attaches with the subumbrella and manubrium. Gastric filaments present.

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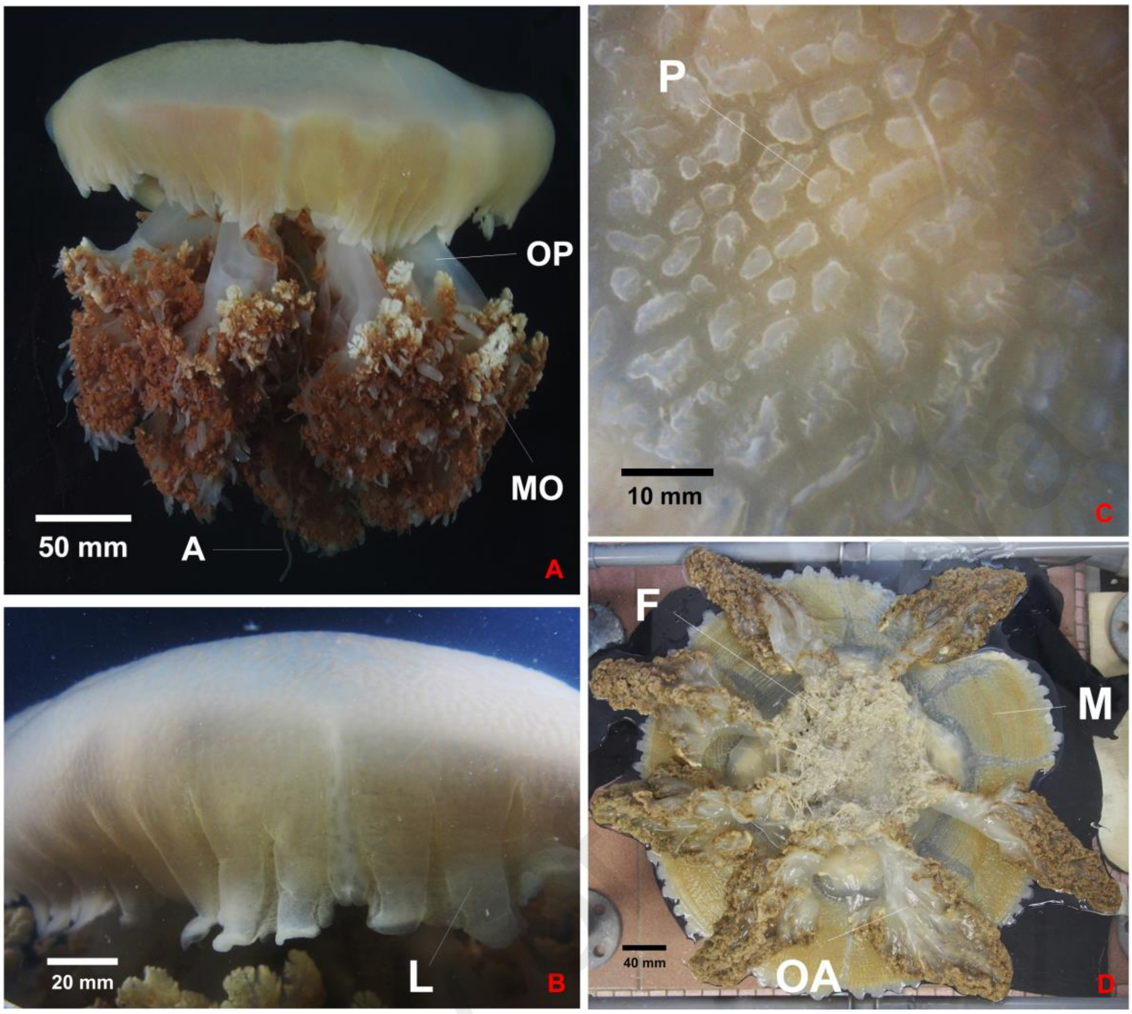

Figure 3.27: Gross morphology of *Versuriga anadyomene* (*A*): Whole medusa. Oral pillar (OP). Numerous mouthlets (MO) on oral arms. Appendage (A). (*B*): Lappet (L). (*C*): Portuberances or irregular grooves (P) on exumberalla. (*D*): Numerous filaments on the oral disk. Coronal muscle (M). (OA) Oral arm.

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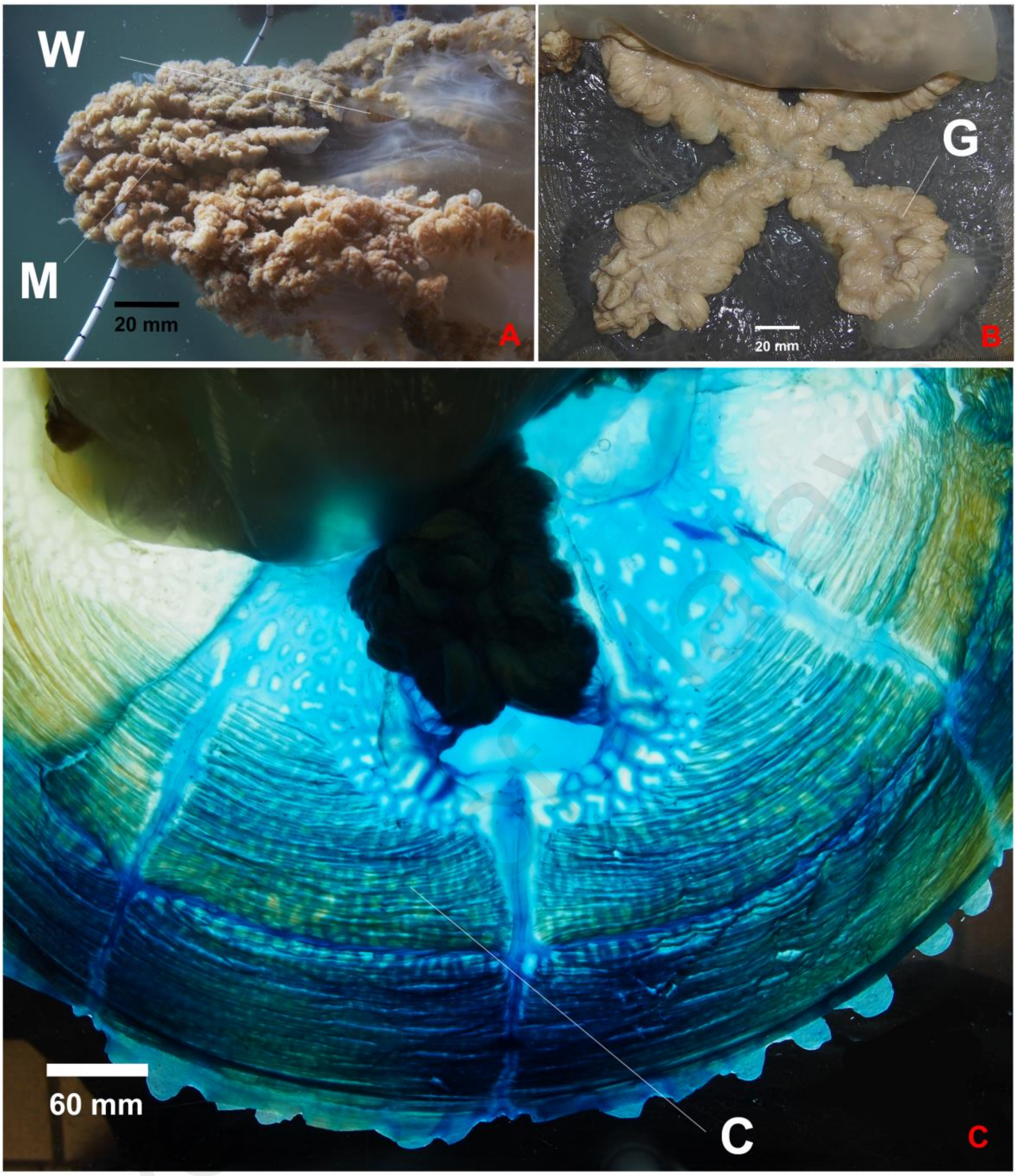

Figure 3.28: Morphology of *Versuriga anadyomene* (*A*): Oral arm with numerous mouthlets (M). A window on the oral arm (W). (*B*): Gonad (G) brownish. (*C*): Network of canals injected with blue dye.

3.8 Morphological Description of *Phyllorhiza punctata* von Lendenfeld 1884

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

Mastiigidae (Stiasny, 1921)

***Phyllorhiza* (Agassiz, 1862)**

***Phyllorhiza punctata* (von Lendenfeld, 1884)** (Figure 3.29 – 3.30)

Material Examined – A total of six specimens were examined from sites Sungai Janggut (IND 2, IND 11, IND 14, MRI 14), Pantai Kok (MRI 131, MRI 139).

Description – Bell - Umbrella hemispherical. Transparent white in the centre of the bell, gradually brownish orange towards the margin of the bell. Numerous round, white warts are found evenly distributed on the exumbrella surface. Eight lappets in each octant; shape are slightly elongated. Rhopalar lappets small and pointy. Short furrow between each lappets. A larger furrow formed between two pairs of velar lappet. Eight rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Rhopalia shape is tear-drop like. Tentacle absent.

Oral arm - Eight oral arms, as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop to three wings at the distal section (approximately at the distal 1/3 of the oral arm). Numerous mouthlets at the frills of the wings. Numerous appendages with bulbs are found at the frills of the wings. Scapulae absent. One terminal club on each oral arm; half as long as the bell diameter, with a bulb at the end. Colour light brown / purple. Numerous filaments are found on oral disk.

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Stomach and manubrium - Gastrovascular cavity, or centre stomach shape floret. Papillae absent. Eight radial canals on the subumbrella, all eight canals extending all the way to the margin of the bell, where rhopalar canals split at the distal end and branch toward the neighbouring lappets. Ring canal present. Interradial rhopalar canals connected with the neighbouring canals proximally, whereas perradial rhopalar canals only connected with the neighbouring canals beyond the ring canal. Network becomes denser and smaller towards the margin of the bell. Radial muscles absent. Eight coronal muscle fields, each is interrupted at the eight radii. Up to 96 muscle folds per quadrant. Coronal musculature starts forming approximate 1/3 distally from the centre, covering 2/3 of the subumbrella until the margin. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and oral disk at the mouth is 1/4 as wide as the bell diameter.

Gonad - Floret shaped and yellowish gonads found formed below the subumbrella; each gonad is separated with the neighbouring gonads; attaches with the subumbrella. Gastric filaments present.

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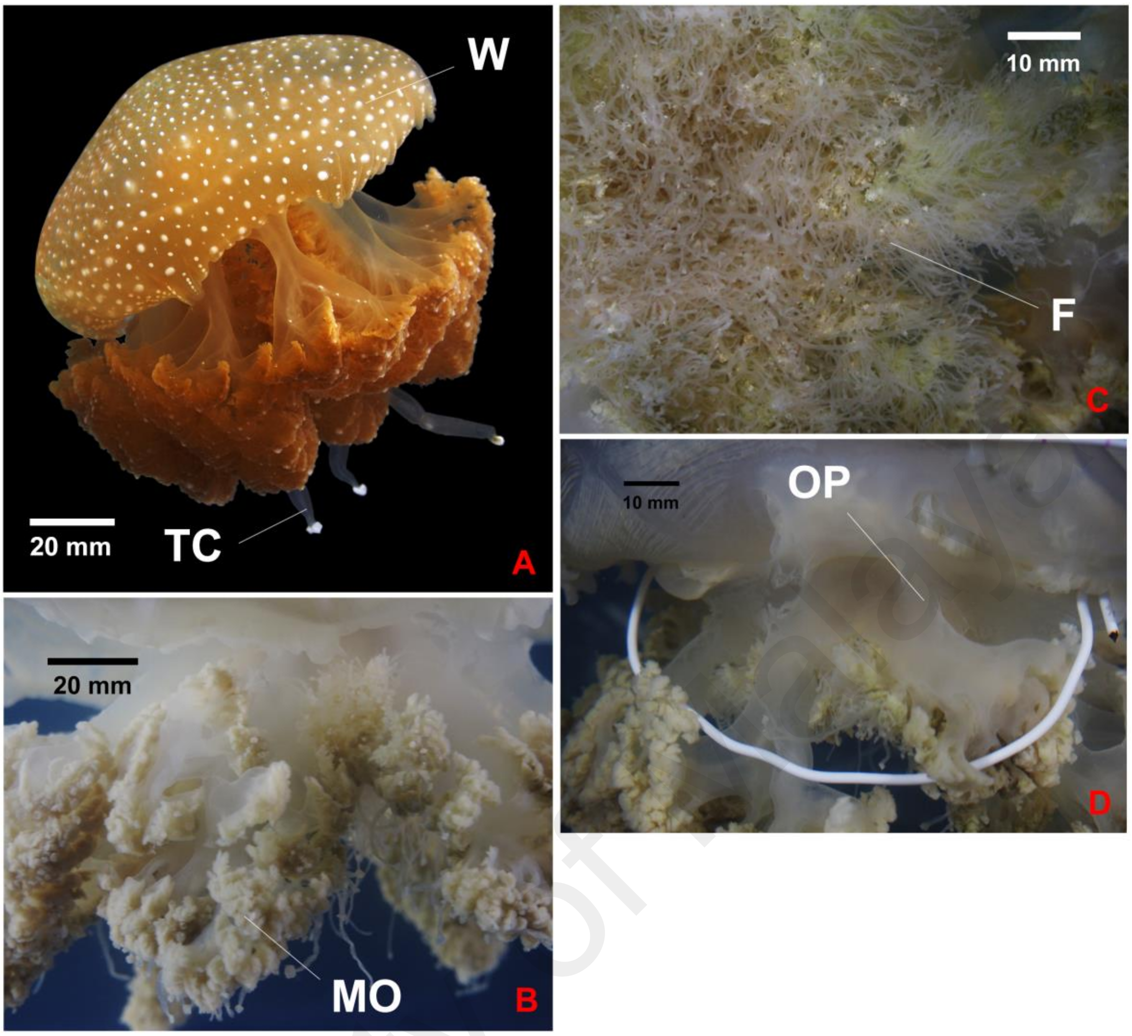

Figure 3.29: Gross morphology of *Phyllorhiza punctata* (*A*): Whole mesusa. Numerous warts (W) on the exumbrella. Terminal club (TC). (*B*): Numerous mouthlets (MO) on the oral arm. (*C*): Filaments (F) on oral disk. (*D*): Oral pillar (OP). The white ring is a wire to suspend the jellyfish and it is not part of the jellyfish structure.

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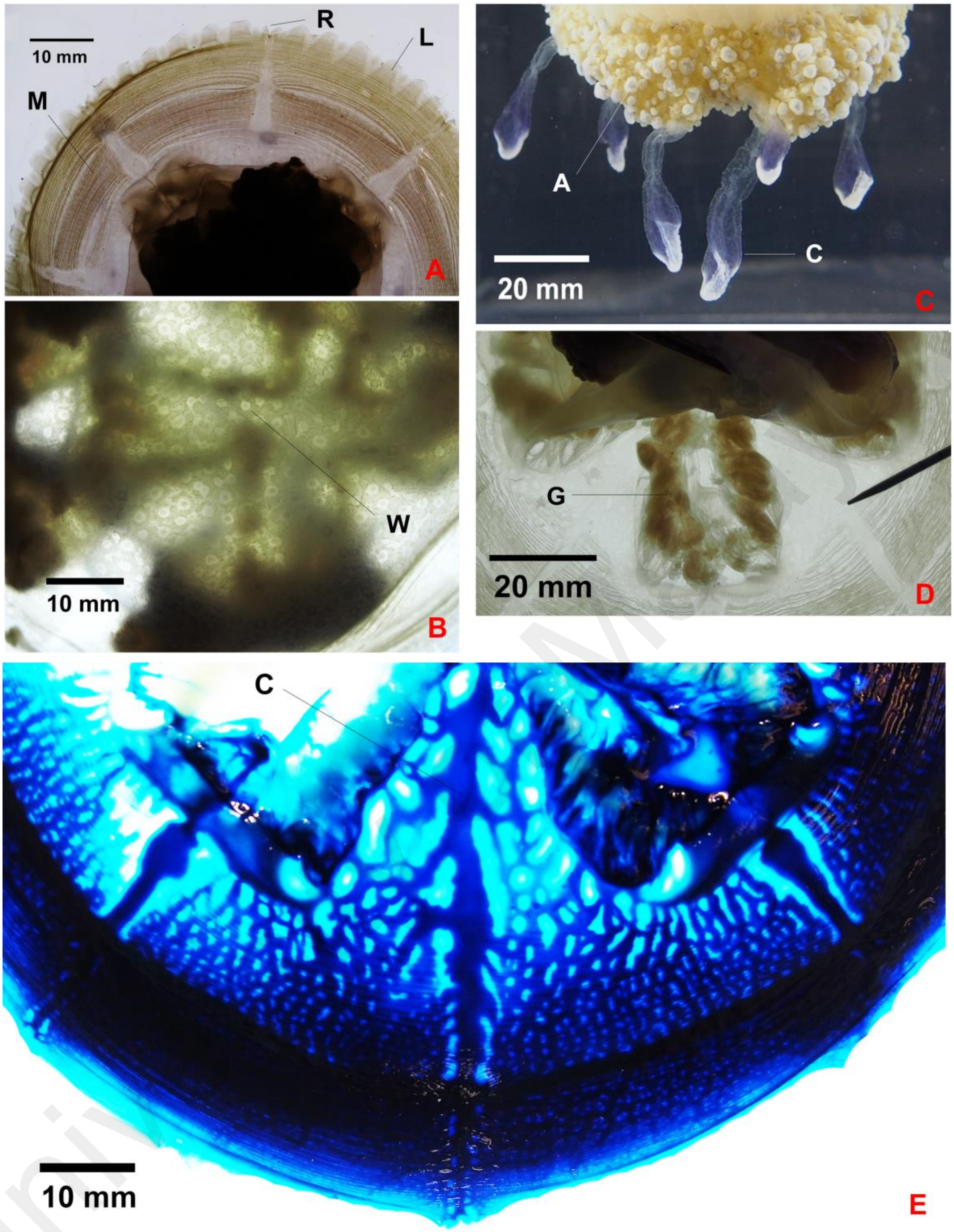

Figure 3.30: Morphology of *Phyllorhiza punctata* (*A*): Coronal musculatures (M). (R) Rhopalia (L) Lappet. (*B*): Warts (W) on exumbrella. (*C*): Numerous appendages (A) on the oral arms. Terminal club (C). (*D*): Gonad (G) brownish. (*E*): Network of canals (C) injected with blue dye.

3.9 Morphological Description of *Lychnorhiza malayensis* Stiasny 1920

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

Lychnorhizidae (Haeckel, 1880)

***Lychnorhiza* (Haeckel, 1880)**

***Lychnorhiza malayensis* (Stiasny, 1920)** (Figure 3.31)

First record of species in Malaysia

L. malayensis has a wide distribution in the Malayan Archipelago and the Indian Ocean), where it was previously found by stiassny in 1920s & 30s in Batavia, Java, by Menon in 1930 in Madras, India, by Nair in 1951 in Trivandrum, India, but so far have yet to be documented or found in Malaysia. It has recently been documented in Andaman Sea and Gulf of Thailand (http://marinegiscenter.dmcr.go.th) and in 2014 sampling by Jeyabaskaran et al (2016) from Thiruvananthapuram to Goa, India.

Material Examined - A total of six specimens were examined from sites Sungai Janggut (IND 13, MRI 35, MRI 36, MRI 37, MRI 38, MRI 39)

Description – Bell - Umbrella hemispherical. Exumbrella surface smooth. Eight slightly pointy lappets in each octant. Rhopalar lappets small and pointy. Eight tear-drop shaped rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Tentacles absent.

Oral arm - Eight oral arms, as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop to three wings at the distal section (approximately at the distal 1/3 of the oral arm). Numerous mouthlets at the frills of the wings. Scapulae absent. Terminal club absent. Filaments absent.

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Stomach and manubrium - Stomach shape floret. Subgenital fenestration shape oval; 1/10 the length of the bell diameter. Five papillaes around each subgenital fenestration. Three papillae located at the upper side of the margin subgenital fenestration, aligning with the rhopalar canal. One smaller on each side of the subgenital fenestration. Papillae surface decorated with fine dots, but smooth. Sixteen radial canals on the subumbrella. Eight rhopalar canals extending fully to the margin of the bell. Eight inter-rhopalar canals (perradial rhopalar canals) do not extend beyond the ring canal. Ring canal present. Between four to five centripetal canals within the ring canal blindly ended (except a few are not blindly ended) and do not anastomoses with the neighbouring rhopalar nor interrhopalar canals. Canals becomes denser and smaller towards the margin of the bell. Radial muscles absent. One coronal muscle fields not interrupted at the eight radii. Between 80 to 96 muscle folds per quadrant. Coronal musculature starts forming approximate 1/3 distally from the centre, covering 2/3 of the subumbrella till the margin. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and oral disk at the mouth is 1/4 as wide as the bell diameter.

Gonad – Gonads are floret shaped gonads and form below the subumbrella; each gonad is separated from the neighbouring gonads; attaches with the subumbrella and manubrium. Colour yellowish. Gastric filaments present. Gonad is completely protected within the fenestration with outfold.

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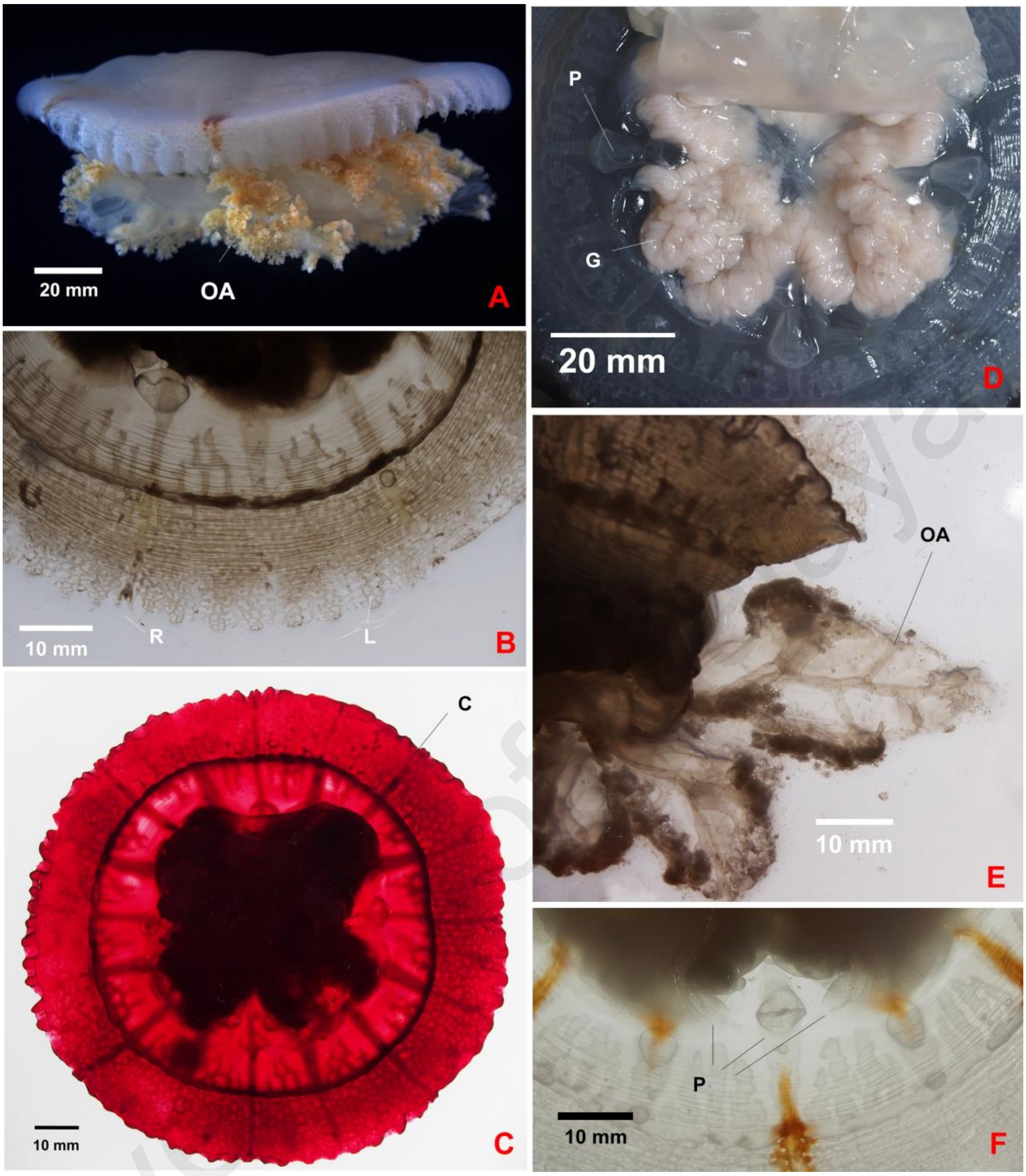

Figure 3.31: Gross morphology of *Lychnorhiza malayensis* (*A*) Whole medusa. (OA) Oral arm. (*B*) Rhopalium (R). Lappet (L). (*C*) Network of canals (C) injected with red dye. (*D*) Gonad (G) creamy white. (P) Papillae. (*E*) Oral arm (OA). (*F*) Three papillae (P).

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3.10 Morphological Description of *Acromitus flagellatus* Maas 1903

Class SCYPHOZOA (Goette, 1887)

Order RHIZOSTOMEAE (Agassiz, 1862)

Catostylidae (Gegenbaur, 1857)

***Acromitus* (Light, 1914)**

***Acromitus flagellatus* (Maas, 1903)** (Figure 3.32)

Material Examined – A total of three specimens were examined from sites Sungai Buluh (MRI 21, MRI 23, MRI 24).

Description – Bell - Umbrella hemispherical. Exumbrella surface smooth. Numerous red dots are distributed on the exumbrella. Eight lappets in each octant; shape are slightly elongated. Rhopalar lappets small and pointy. Short furrow between each lappets. A larger furrow formed between two pairs of velar lappet. Eight rhopalia present in clefts of the umbrella margin, located at the interradial and perradial axis. Rhopalia shape is tear-drop like. Tentacle absent.

Oral arm - Eight oral arms, 2/3 as long as the bell diameter; oral arms formed at the end of the oral pillar, then develop to three wings at the distal section (approximately at the distal 2/3 of the oral arm). Numerous mouthlets at the frills of the wings. Numerous filaments at the frills of the wing. Scapulae Absent. Terminal club absent, but a single thread-like filament is formed at the distal end of each oral arm. Numerous string-like filaments on the oral arms.

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Stomach and manubrium - Gastrovascular cavity, or centre stomach shape floret. Subgenital fenestration shape oval; 1/7 the length of the bell diameter. A triangular shape papillae is found at the centre of the subgenital fenestration. Papillae surface smooth. 16 radial canals on the subumbrella. Eight rhopalar canals extending fully to the margin of the bell. Eight inter-rhopalar canals do not extend beyond the ring canal. Ring canal present. Between three to five centripetal canals within the ring canal anastomoses with each other, and anastomoses with the neighbouring rhopalar canals, but not with the inter-rhopalar canals. Canals becomes denser and smaller towards the margin of the bell. Radial muscles absent. Eight coronal muscle fields covering the network mesh underneath, each interrupted by the four interradial canals and the four perradial canals. Coronal musculature starts forming 1/3 distally from the centre, covering 2/3 of the subumbrella up to the margin. Muscle fields become denser beyond the ring canal. Manubrium thick and smooth; oral disk at the base is about half as wide as the bell diameter, and oral disk at the mouth is 1/4 as wide as the bell diameter.

Gonad – Gonads are floret shaped gonads and form below the subumbrella; each gonad is separated from the neighbouring gonads; attaches with the subumbrella and manubrium. Colour yellowish. Gastric filaments present.

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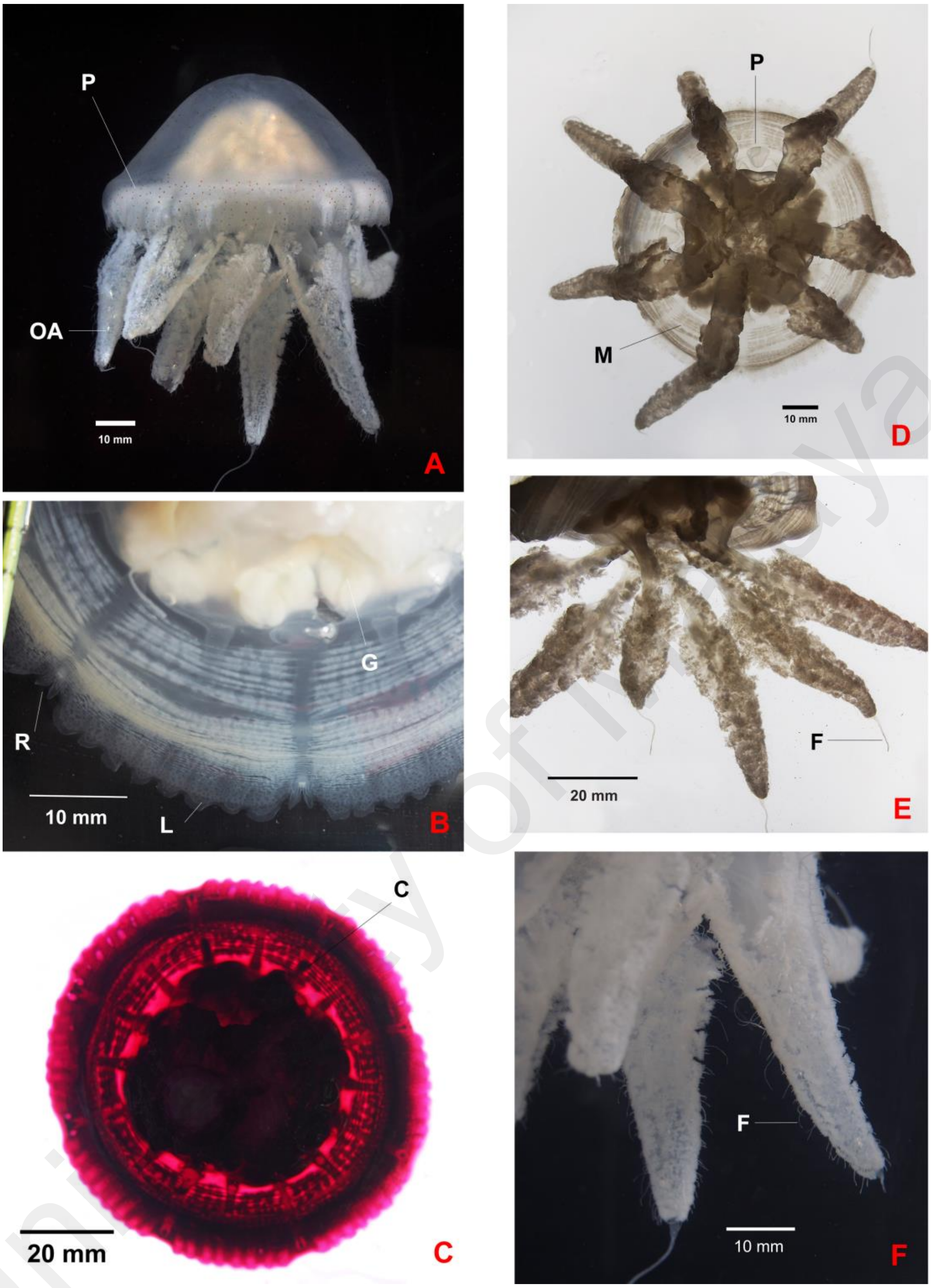

Figure 3.32: Gross morphology of *Acromitus flagelatus* (*A*): Whole medusa. Oral arm (OA). (*B*): Lappet (L) pointy. Rhopalium (R). (*C*): Network of canals (C) injected with red dye. (*D*): Papilae (P). Coronal muscle (M). (*E*): A single terminal filament. (F): on oral arm. (*F*) Numerous filaments (F) on oral arms.

3.11 Geometric Morphometric Analysis

A total of 107 specimens of *Chrysaora chinensis* were used in this study. 16 are from the East-Central of Peninsular Malaysia, 26 from East-North, 27 from West-Central and 38 from West-North. Among them, 17 specimens were from the museum (Appendix D, Figure 3.33).

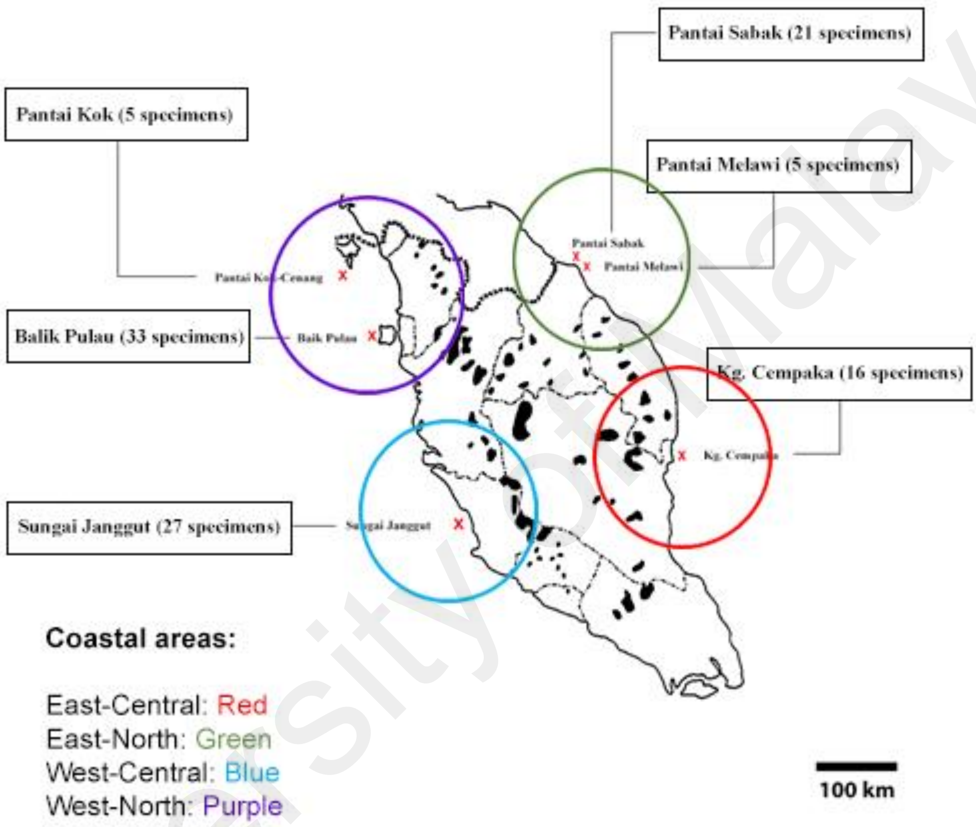

Figure 3.33 Map of the sampling locations for geometric morphometric analysis, with the number of specimens obtained. Green circle represents East-North (EN), red circle represent East-Centre (EC), blue represents West-Central (WC), and purple represents West-North (WN) coastal areas of Peninsular Malaysia.

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3.11.1 Procrustes ANOVA

The measurement error of the gastrovascular pouch was computed from the Procrustes ANOVA (Table 3.1). 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 differences between pouches in individual specimen (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 error, but there is no need to digitize an image twice.

Table 3.1: Result of Procrustes ANOVA (SS=Sum of square, MS=Mean Squre, df=degree of freedom).

Effect	SS	MS	% of error	df	F	P
Individual	0.35608889	0.00052989	-	672	2.18	< 0.0001
Pouch	0.00904613	0.00032307	-	28	1.33	0.1192
Ind x Pouch	0.16303950	0.00024262	45.8	672	27.96	< 0.0001
Imaging	0.01214961	0.00000868	1.64	1400	3.29	< 0.0001
Digitize	0.00738285	0.00000264	0.50	2800	-	-

3.11.2 Principal Component Analysis (PCA)

PCA was performed on a total of 107 specimens. Most of the total variance was explained by the first two PCs (Figure 3.34). PC1 accounted for 33.86% of the total variance of the shape change based on the widening of both sides of the gastrovascular pouch. PC2 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

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protuberance of the pouch appear less prominent and more blunt (Figure 3.34, Appendix L). The shape space scatter plot between PC1 and PC2 (Figure 3.35) indicates that most of the specimens overlaps, with a few exception of outliers. Hence, there were no significant differences in shape among all the specimens based on PCA.

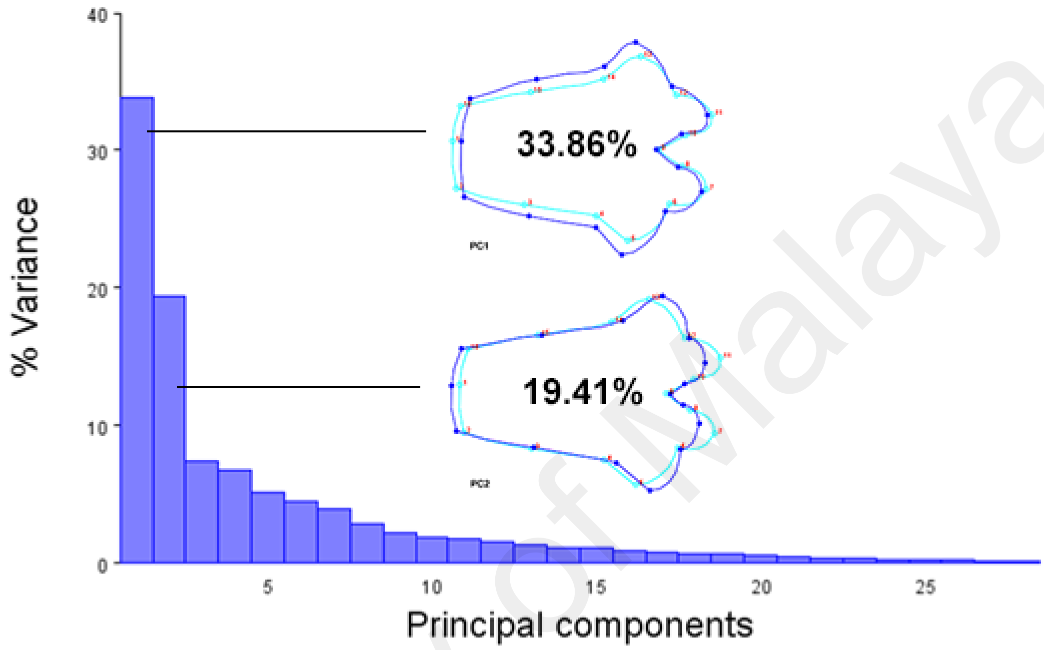

Figure 3.34: PCA Result – % of variation explained by components. Two independent contrasts of the gastrovascular pouch shape of the two main components PC1 and PC2 are illustrated whereby light blue outline indicates the mean shape and the dark blue outline indicates the shape change.

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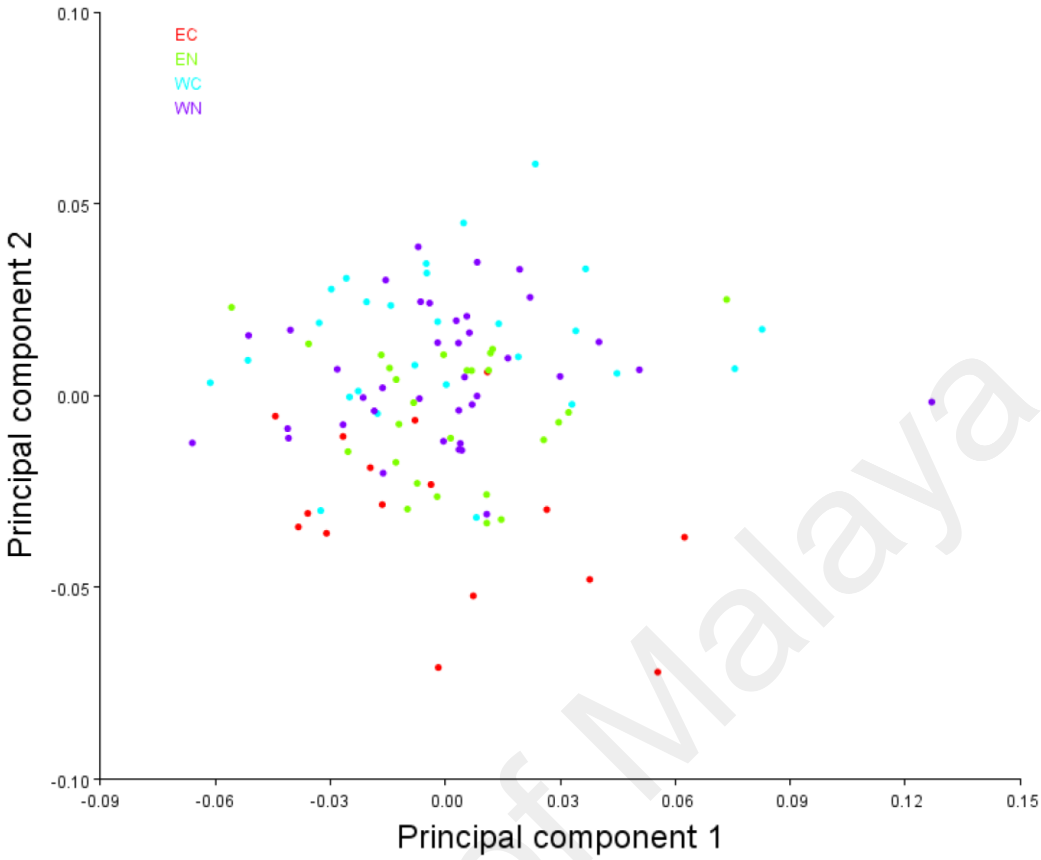

Figure 3.35: Scatter plots of PC1 vs PC 2. PC1 accounted for 33.86% and PC2 accounted for 19.41% of the total variance of the shape change of gastrovascular pouch of specimens from East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia.

3.11.3 Canonical Variate Analysis (CVA)

CVA was performed on pouches of *C.chinensis* populations from four coastal areas of the Peninsular Malaysia: East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN), with a total of 107 specimens. CV1 accounts for 47.46%, CV2 accounts for 32.72%, and CV3 accounts for 19.83% of the amount of relative between-group variation (Figure 3.36). Specimens from East-Central and East-North are separated from the other two groups (West-Central and West-North). There are considerable amount of overlapping among the group West-Central and West-North

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(Figure 3.30). The magnitude of Mahalanobis distances is lower between West-Central and West-North but higher between east and west coast areas, whereas East-Central and West-North has the highest Mahalabonis distance. (Table 3.2).

The outline drawing of the visualization of the gastrovascular pouch shape of *C.chinensis* populations from the four coastal areas of Peninsular Malaysia show some variations in shape (Figure 3.37). Pairwise comparisons among the four areas show that:

- EC vs WC: Protuberance at the margin of WC is more blunt
- EC vs WN: Protuberance at the margin of WN is more blunt, whereas the EC's pouch is slightly enlarged
- EC vs EN: EC's pouch is slightly enlarged
- WC vs EN: Protuberance at the margin of WC is more blunt
- WC vs WN: Protuberance at the margin of WC is more blunt
- WN vs EN: Protuberance at the margin of WN is more blunt

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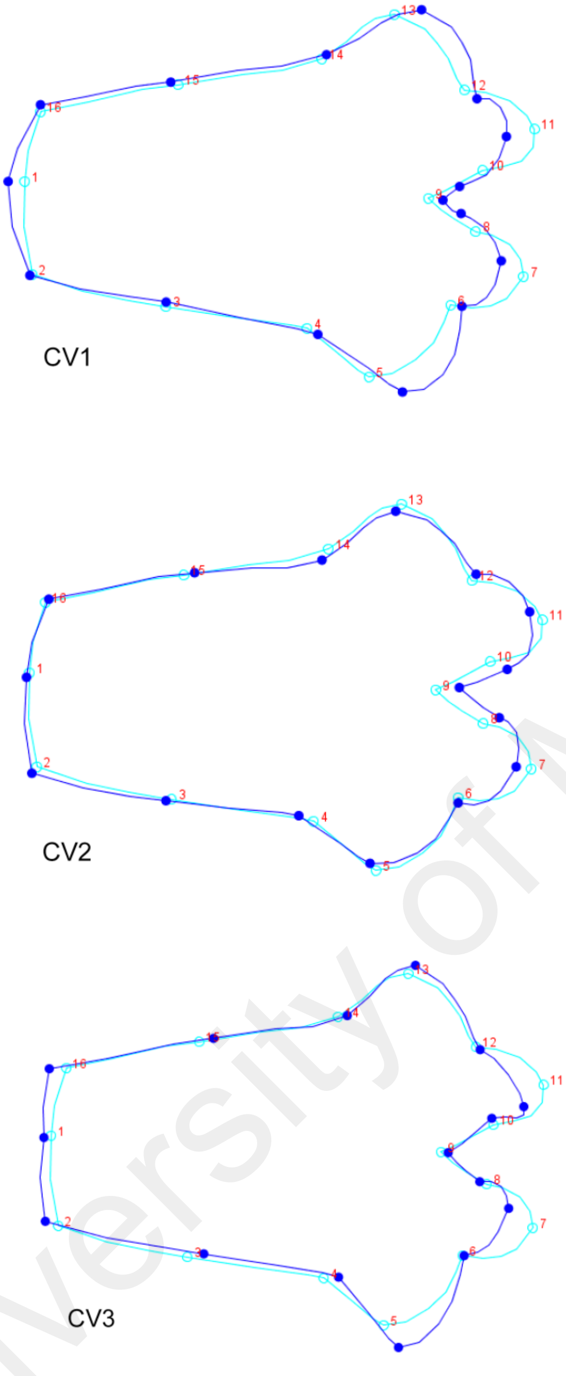

Fig. 3.36: Independent contrast of component using canonical variate analysis of the gastrovascular pouch of specimens from East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia, whereby CV1, CV2 and CV3 accounts for 47.46%, 32.72% and 19.83% of the amount of relative between-group variation, respectively. Light blue outline indicates the mean shape and the dark blue outline indicates the shape change. CV1 denotes changes with blunt protuberance at the margin and enlarging of the distal end, CV2 denotes changes with blunt protuberance at the margin and CV3 denotes changes with blunt protuberance at the margin and enlarging of the distal end.

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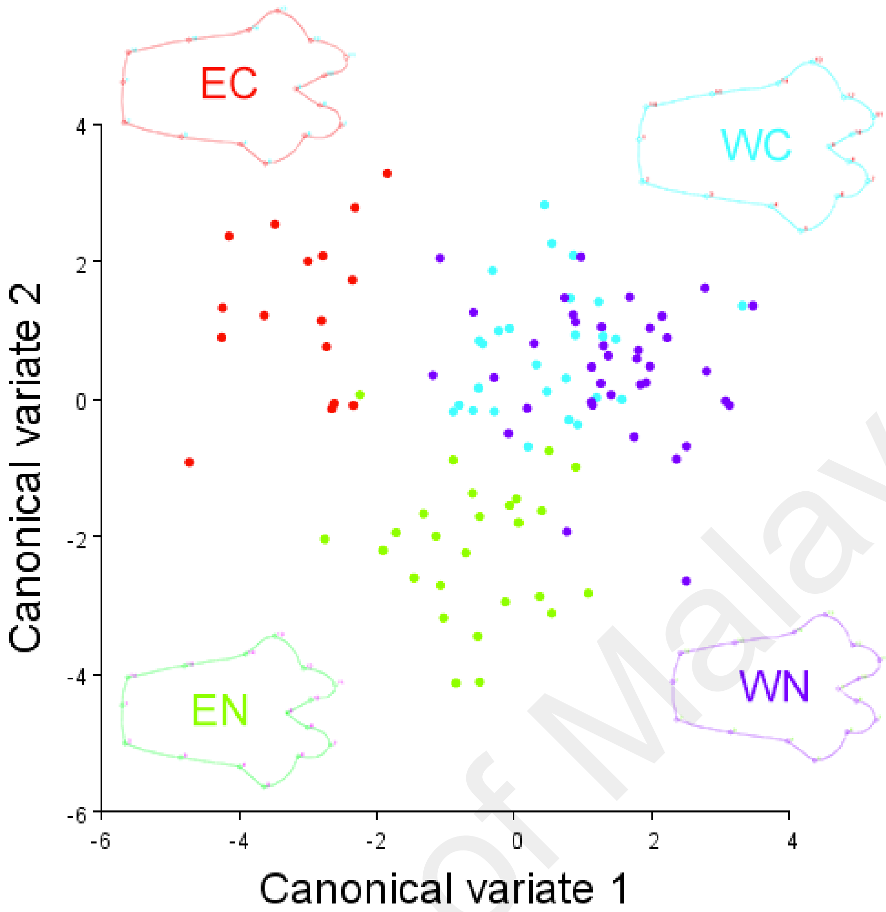

Figure 3.37: Scatter plot of CV1 vs CV2 and illustration of mean shape of gastrovascular pouch of specimens from four coastal areas East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia. Plot shows shape differences mainly between specimens of the east and west coasts, even between those from EN and EC, but with no distinct differences between those from WN and WC.

Table 3.2: Mahalanobis distances among the pouch shape of *C. chinensis* populations from four coastal areas designated as West-North (WN), West-Central (WC), East-North (EN) and East Central (EC) of Peninsular Malaysia.

	EC	EN	WC
EN	4.3296	-	-
WC	4.2151	3.4014	-
WN	4.6090	3.4034	2.6485

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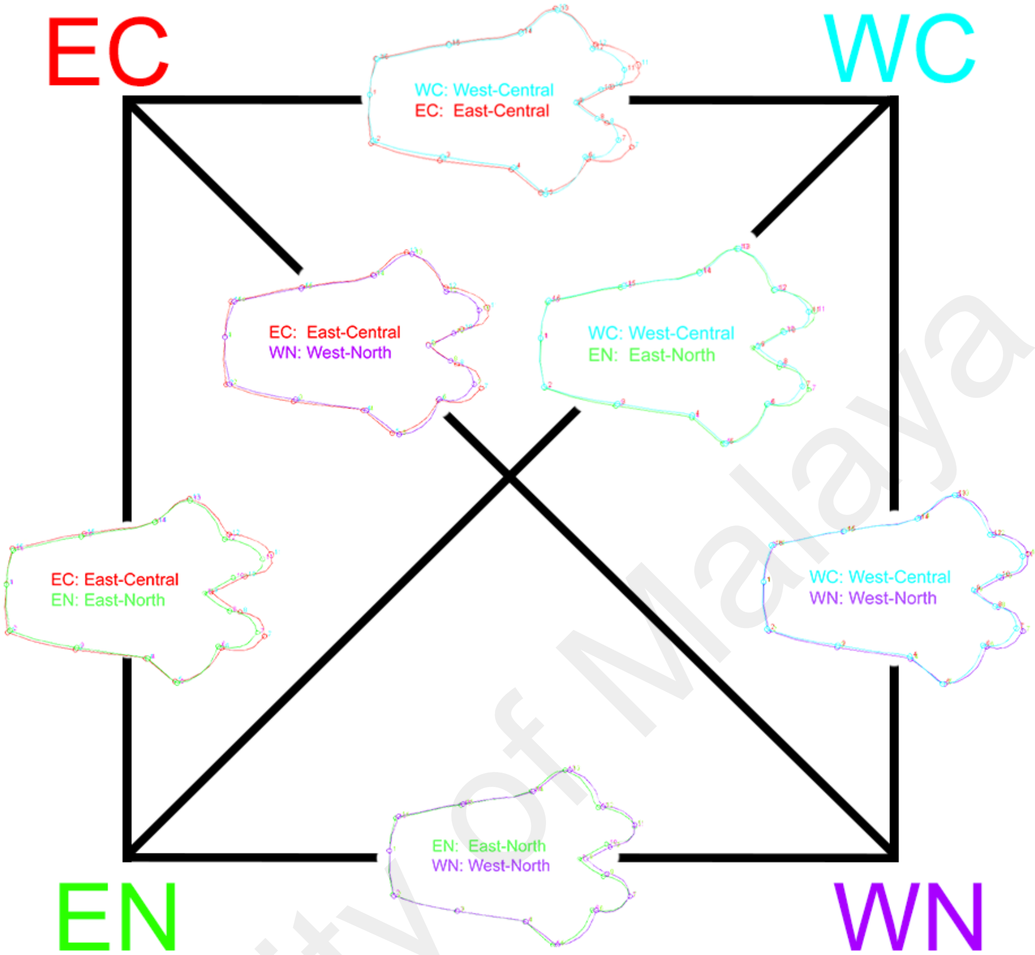

Figure 3.38: Pairwise comparisons between the pouch shape of C.chinensis populations from East-Central (EC), East-North (EN), West-Central (WC) and West-North (WN) of Peninsular Malaysia

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CHAPTER 4: DISCUSSION

4.1 Morphology, Diversity and Importance of Jellyfish Species of Peninsular Malaysia

Although the demand for jellyfish has increased, the catch had actually decreased. Annual catch in 2000 was 179,086 metric tons (wet weight) from Philippines, Thailand, Indonesia, Myanmar and Malaysia, and dropped drastically to only 13,402 metric tons in 2004 (Kitamura & Omori, 2010). Personal communication with local fishermen indicate that the supply of edible jellyfish is not enough to meet the high demand. Despite its importance, jellyfish study in Malaysia is still relatively scarce. Light (1914) on board the steamer Albatross to the Philippines and recorded several jellyfish species he found during the trip (including Chrysaora, Lychnorhiza and Acromitus species) but unfortunately he did not make his stop in Pensinsular Malaysia. This study is aimed to try to fill in the gaps from previous studied. This studies not only report a new record of *Lychnorhiza malayensis* in Malaysia, but also provided detail description and photographs of eight scyphozoan jellyfish species previously reported but without detail information.

Ever since Linnaeus first described the popular moon jellyfish *Aurelia aurita* in 1758, jellyfish taxonomy has long been subjected to disagreements and revision. Two particular jellyfish that subject to such scrutiny are *Chrysaora* sp. and *Cyanea capillata*. Since the description of the Genus Chrysaora in 1810 by Peron & Leseur, a number of revisions had been done by various researchers. Eschscholtz (1829) listed six species in the genus, Lesson (1843) listed 13, Agassiz (1862) listed nine, Haeckel (1880) listed 10, Mayer (1910) listed 15, Kramp (1961) listed 11, and most recently Morandini & Marques (2010) listed 15.

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C. capillata poses another problem to the taxonomy position. It has been revised by Linnaeus et al (1758), Mayer (1910), Kramp (1961), Dawson (2005), Sparmann (2012) and Kolbasova et al. (2015). It was believed to be a cosmopolitan species but recent research by Dawson (2005) has shown that it is in fact not. Thus it will be beneficial to study jellyfish species from this region to ascertain its taxonomy position.

4.2 Lion's Mane Jellyfish (*Cyanea* sp.) of Malaysia

The Cyanea species obtained in the west coast of Peninsular Malaysia were without gastrovascular pit, and the exumbrella surface is lightly granulated with warts. Gastrovascular pit is a prominent feature of *Cyanea capillata*, thus the Malaysian specimen cannot be ascribed to be *C. capillata*. By injecting colour dye to the rhopalar and tentacular pouches of Cyanea species obtained in Peninsular Malaysia show up to five transverse "ridges" connecting both pouches, and the peripheral canals at the lappet contains numerous networks of anastomoses, forming a dense network, which according to Kramp (1961) is one of the characteristic of *C. nozakii*. But according to Kramp (1961), the colour of *C. nozakii* is white, but the species obtained are brownish and yellowish. Thus, the Cyanea species obtained in Peninsular Malaysia cannot be ascribed to *C. nozakii*. *C. lamarki* does not seem to fit the characteristic of Cyanea species obtained in Peninsular Malaysia because the locality of *C. lamarki* was reported to be in Europe. Furthermore, its rhopalar and tentacular stomach pouches are completely separated.

Another Cyanea species, *C. barkeri* from Australia (Gershwin et al., 2010) is similar to the Cyanea species from Peninsular Malaysia in that they both lack gastrovascular pit. But it is not clear whether *C. barkeri*'s rhopalar and tentacular pouches are connected, and whether the exumbrella surface is smooth or granulated.

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Kramp (1961) recorded *C. buitendijki* in Malay Archipelago but he did not describe the exumbrella surface nor the existence of musculature intrusion. Furthermore, Malay Archipelago encompasses a wide region ranging from Malaysia, Singapore, Indonesia to Philippines. Thus cannot ascribe the Cyanea species from Peninsular Malaysia to *C.barkeri* nor *C. buitendijki.*

Molecular phylogenetic evidence of Rizman-Idid et al. (2016) showed that Malaysian Cyanea did not cluster with either *C. nozakii*, *C. capillatta*, *C. rosacea* nor *C. annaskala* and it is very distinct from any available Cyanea sequences. Hence, with molecular and morphological evidence it is plausible that Malaysian Cyanea is a new species.

Table 4.1: Comparison of *Cyanea* sp. from Peninsular Malaysia with other Cyanea species based on Kramp (1961).

	C. capillata	***C. buitendijki***	***C. lamarki***	***C. nozakii***	***Cyanea* sp.**
Musculature intrusion	Y	?	N	?	N
Exumbrella papillose	N	?	Y	N	Y
Locality	Australia, NW Pacific, China, Mutsu Bay, Japan, Trivandrum Coast India	Malay Archipelago	Bay of Biscay, Norway, Holland, W. Coast of Sweden, Denmark, Iceland	Japan, NW Pacific, Indochina, Indian Ocean	Malaysia
Size	up to 1000mm	up to 310mm	up to 1000mm	up to 260mm	up to 250mm

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Table 4.1, continue

Pouch	Rhopalar and tentacular stomach pouches completely separated	Rhopalar and tentacular stomach pouches connected by several broad transversed anastomoses	Rhopalar and tentacular stomach pouches completely separated	Rhopalar and tentacular stomach pouches connected by several broad transversed anastomoses	Rhopalar and tentacular stomach pouches connected by several broad transversed anastomoses
Canal	Peripheral canals more or less curved	Peripheral canals without anastomoses	Peripheral canals more or less curved	Peripheral canals with numerous anastomoses, forming a dense network	Peripheral canals with numerous anastomoses, forming a dense network
Colour	Reddish brown or yellowish		Blue	Milky white	Brownish, yellowish

4.3 Sea Nettles (*C. Chinensis*) of Peninsular Malaysia

The detailed morphological description of *C. chinensis* in the present study represents the first of its kind in Malaysia. *C. chinensis* is commonly found in most beaches in east and west coast of Peninsular Malaysia (Rizman-Idid et al., 2016). Base on personal observation and interviews with fishermen, they occur more abundantly at the polluted areas such as Balik Pulau, Pantai Kok and Kilim, particularly during the dry season (April to October). Previous study reported the occurrence of *C. chinensis* in South China Sea, (Vanhöffen, 1911), but Kramp (1961) reported *C. chinensis* as *C. helvola*. Prior to the resurrection of *C. chinensis* by Morandini & Marques (2010), many Chrysaora specimens found in the South China Sea and Malay Archipelago have been identified as either *C. melanaster* (Yap & 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 difference in tentacle and lappet number. Both the latter species are larger than in their observed *C. chinensis* specimens. Recent study by Yap & Ong (2012) ascribed the specimens from St. John's Island in Singapore as *C. chinensis*. Likewise, findings from the 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 (data not shown) revealed that they are *C. chinensis* and genetically similar and phylogenetically clustered to those reported by Rizman-Idid et al., (2016). It is noteworthy that the colouration of *C. chinensis* varies greatly. Some specimens are transparent whereas some are reddish with radiating stripes on the exumbrella, with numerous pigmentations on oral arms and exumbrella, and reddish brown lappets. Nonetheless, this colouration corroborated with the colouration diagnosis of *C. chinensis* by Yap & Ong (2012) and Morandini & Marques (2010).

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 and transport nutrients from the central stomach 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 vessel (Ibrahim & Khalid, 2007). The low water quality of Straits of Malacca is due to pollution from shipping industries, aquaculture, densely populated coastal areas with nutrients and effluent discharged into the straits, causing eutrophication (Rezai et al., 2003; Praveena et al., 2011). According

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to Bong & Lee (2008), the effects of development at nearshore increases the total suspended solids and decreases the dissolved oxygen. Human activities that lead to eutrophication have been associated with the bloom of the jellyfish (Arai 2001; Parsons & Lalli, 2002; Malej et al., 2007; Richardson et al., 2009), and jellyfish appear to be able to adapt to fluctuations of various levels of water quality and environmental parameters, such as salinity, temperature, food source (Purcell et al., 2012). A few geographical variation studies have shown that morphology affects the jellyfish distribution, pulse rate and swimming speed (Dawson & Hamner, 2003).

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 coastal areas (East-Central, East-North, West-Central, and West-North) 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 350km, which may not be of significant magnitude to explain the isolation by distance for these populations. Nonetheless, these populations may have been kept separated due to occurrence of eddies and different water circulations at 5°N 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.

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4.4 Potential Application of GMM in Jellyfish Studies

Although geometric morphometric analysis have been proven useful in analysing and detecting even very minute shape variations in various organisms (Klingenberg, 2013, Li et al., 2016), its application as demonstrated in the present study on gelatinous organism such as jellyfish, may still be informative albeit 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 of measurements due to mishandling of specimens. Up until now, 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 cause damage to 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 time (Kapiris et al., 1997). Therefore, using geometric morphometric analysis to detect shape variation in jellyfish poses a higher degree of difficulties compared to rigid organisms, since even slight false variation in the shape due to sampling or handling error can lead to the conclusion as real variation.

The practical usage may be improved by choosing a different structure for landmark configurations, depending on the species. For example, 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. On the other hand, certain structures make poor landmarks. Filaments and tentacles are easily detached and damaged. They tend to move rather easily and it is difficult to set its

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orientation and identify landmarks. Other jellyfish species, such as *Rhopilema esculentum* of the Rhizostomeae family which is bigger in size and with more rigid structures, may be a better target for landmark configuration and geometric morphometric study.

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CHAPTER 5: CONCLUSION

Morphological studies are crucial for species identification of jellyfish. Even if molecular techniques are available, identification is firstly based on morphology. Currently there is a revival in the taxonomy of jellyfish, with many revisions and efforts to obtain samples world wide for comparisons. Nonetheless, there is still a lack of jellyfish taxonomist worldwide. Hence studies like this would be helpful in providing baseline information, including detailed morphology characterization and photographs that could aid identification in the field for biologist and marine enthusiasts.

Below are the major findings of this study:

1) Morphology of nine jellyfish species (from eight families and eight genera) found in Peninsular Malaysia that belong to the class Scyphozoa, namely *Chrysaora chinensis, Cyanea* sp., *Versuriga anadyomene, Rhopilema hispidum, Rhopilema esculentum, Phyllorhiza punctata, Acromitus flagellatus, Lobonemoides robustus* and *Lychnorhiza malayensis* were characterised in details

2) *Cyanea* sp. found in the Malaysia water may be new species as it could not be morphologically ascribed to other known Cyanea species

3) A new record of *Lychnorhiza malayensis* in Malaysia was reported

4) The Malaysian sea nettle jellyfish was verified as *C. chinensis*

5) GMM analysis of the gastrovascular pouch of specimens of *C. chinensis* indicates shape variation between the four coastal areas of Peninsular Malaysia,

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especially with higher magnitudes of Mahalanobis distances between east and west coast areas, East-Central and East-North comparisons, but lower differences between the West-Central and West-North areas.

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