CHAPTER II

Literature Reviews

Phylum Cnidaria derives its name from the Greek word Knide means nettle + L. aria means like or connected with. Comprised more than 10,000 species, cnidarians diverse in forms and colors. The distinguished character of cnidarians is the stinging cell called nematocyst, stinging capsules used for defending and hunting. Their body plan has two variations, polyp and medusa. Polyp is adapted to a sessile life and medusa is adapted for a floating or free-swimming life (Campbell and Reece, 2002; Hickman, 2011; Zhang, 2011).

Phylum Cnidaria can be divided into 5 classes which are Anthozoa, Cubozoa, Hydrozoa, Scyphozoa and Staurozoa (Daly *et al*., 2007). Most of the members in these classes are called jellyfish except Anthozoans which are corals, sea anemones and sea fans. Despite the fact that most of Cnidarians reside in marine biome, they can be found globally in various environment from the icy water to warm water and from the salty seawater to the freshwater (Taylor, 1998). Cnidarians have long been wandered in this planet, even before the Cambrian explosion (Coates, 2003). The first scyphozoan, a true jellyfish, was first emerged during the Cambrian period. Then, came along the first hydrozoan during the Ordovician period. Finally, cubozoan, a box jellyfish, was appeared during the Carboniferous (Young and Hagadorn, 2010).

Box jellyfish catch scientific society and public's attention since they are unique from other jellyfish; for example, they possess complex eyes that allow them to navigate to the preferred habitat and to avoid obstacles (Garm *et al*., 2011) and the potential of their venom that can kill human within minutes after an envenomation (Jouiaei *et al*., 2015). Box jellyfish can be classified into two orders, Carybdeida (Figure 1.1) and Chirodropida (Figure 1.2).

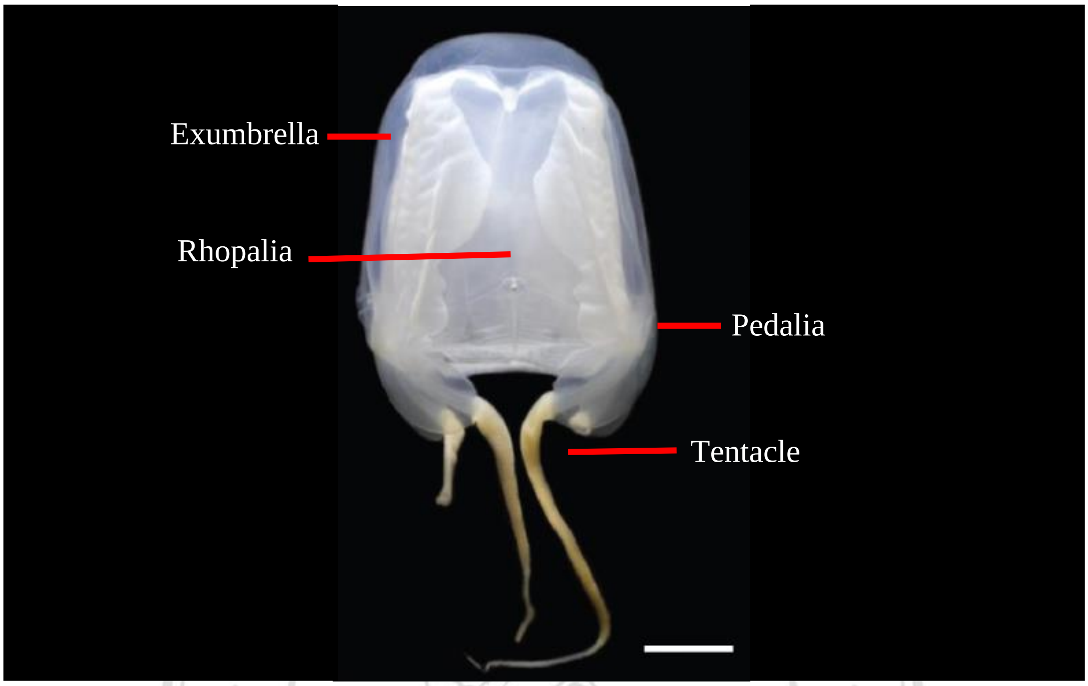

Figure 1.1 General morphology of Carybdeids. Scale bar: 1 cm.
(Adapted from Toshino et al 2015)

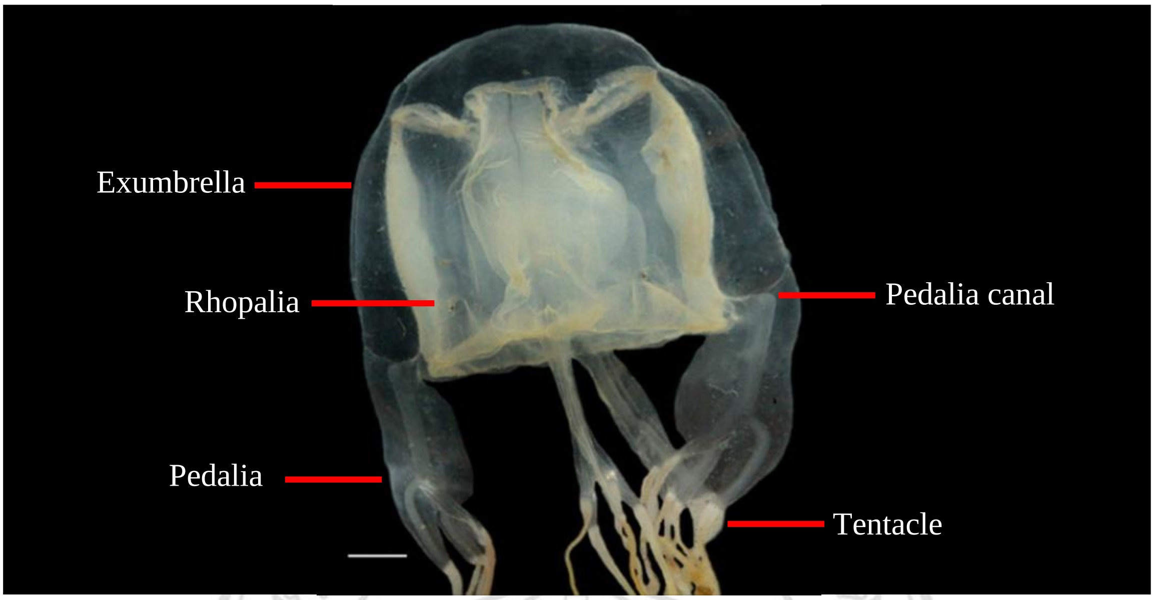

Figure 1.2 General morphology of Chirodropids. Scale bar: 4 cm.
(Adapted from Gershwin and Ekins, 2015)

1. **Order Carybdeida**

Members of this order have only one tentacle per pedalium, except *Tripedalia* taxa which have two or three tentacle per pedalium. Some carybdeids are reputed for causing Irukandji syndrome. The symptom of Irukandji are severe back pain, muscle cramps, vomiting, sweating, anxiety, headaches and palpitation, and may lead to toxic heart failure (Fenner and Lippmann, 2009; Bentlage and Lewis, 2012; Crow *et al.*, 2015). Carybdeids can be found in Australia, Papua New Guinea, America, Japan, Thailand, China, Indonesia, Philippines, Myanmar, Maldive Islands and southern India (Fenner *et al*, 1996; Tibballs, 2006; Fenner and Lippmann, 2009; Fenner *et al.*, 2010). In Thailand, members of genus Carybdea, Copula, Tamoya and Tripedalia were found from both the Gulf of Thailand and the Andaman Sea (MCRDI, 2015).

2. Order Chirodropida

In contrast to Carybdeids, each corner of the Chirodropids' bell bearing more than one tentacles. Envenomation of chirodropids can be lethal to a healthy man within minutes after the sting. Survivors would experience an excruciating pain and would have a significant scars where tentacles made contact (Fenner *et al.*, 1996; Fenner, 2005). One of the most infamous members of this group is *Chironex fleckeri*, the most venomous creature on this planet. Chirodropids can be found in Australia, Indo-pacific region, the Americas, China, Africa, South India, Malaysia and Sri Lanka (Fenner *et al*, 1996; Williamson *et al.*, 1996). In Thailand, Chirodropids genus *Chiropsella*, *Chiropsooides* and *Chironex* have been collected by Department of Marine and Coastal Resources from both the Gulf of Thailand and the Andaman Sea since 2010 (MCRDI, 2015).

Jellyfish identification

For centuries, morphological identification has been performed in order to classify jellyfish specimens (Mayer, 1910; Kramp, 1961). Traditional taxonomy has proved that by utilizing this classical method, specimens can be classified effectively until now (Gershwin and Ekins, 2015). For instance, two new families of carybdeids, which are Alatinidae and Carukiidae, were assigned in 2005 and 2010 respectively (Gershwin, 2005a; Bentlage *et al.*, 2009a). In addition, five new genera, which are *Copula*, *Alatina*, *Morbakka*, *Keesingia* and *Malo*, were proposed (Gershwin, 2005a; Gershwin, 2005c; Bentlage *et al.*, 2009a; Gershwin, 2014). Likewise, in chirodropids, Chiropsellidae fam nov., Chirodectes gen nov., *Chiropsella* gen nov. and *Meteorona* gen nov. were assigned (Gershwin, 2006; Gershwin and Alderslade, 2006; Toshino *et al.*, 2015).

However, not every single specimens could be identified. Some specimens were too difficult to identify because they were damaged or an organ which was used as a key to species was missing during collection according to their gelatinous nature (Lindsay *et al.*, 2015). Due to advanced technologies, nowadays, molecular techniques are adopted in order to accurately identify samples. Proposed by Hebert and colleagues (Hebert *et al.*, 2003), MT-COI gene (Cytochrome oxidase subunit I) is one of the most common genes used as a reference gene for species identification in animals (Bucklin *et al.*, 2011). COI gene is generally used as a 'barcode' for cnidarians' species identification due to its slow evolutionary rate (Stampar *et al.*,2012; Patwardhan *et al.*, 2014; Van Ofwegen *et al.*, 2014; Schwentner and Bosch, 2015). Nevertheless, COI gene's efficiency were reported inferior to 16S rRNA gene in cnidarians (Zheng *et al.*, 2014; Lindsay *et al.*, 2015). 16S ribosomal RNA is a small ribosomal subunit (SSU) which is reported highly conserved in many organisms for it doesn't evolve at the same rate among organisms (Clarridge, 2004; Patwardhan *et al.*, 2014). Likewise, 18S rRNA (SSU) is also widely utilized in many molecular taxonomy and phylogenetic studies as it was also reported that it is a highly conserved region (Patwardhan *et al.*, 2014; Toshino *et al.*, 2015; Sucharitakul *et al.*, 2016). Phylogenetic trees based on these gene are constructed in order to observe relationship within cnidarians in plenty of publications. For instance, Laakmann and Holst (2014) distinguish *Cyanea capillata* from *Cyanea lamarckii*, the species which are closely related (Figure 2.1) (Holst and Laakmann, 2013).

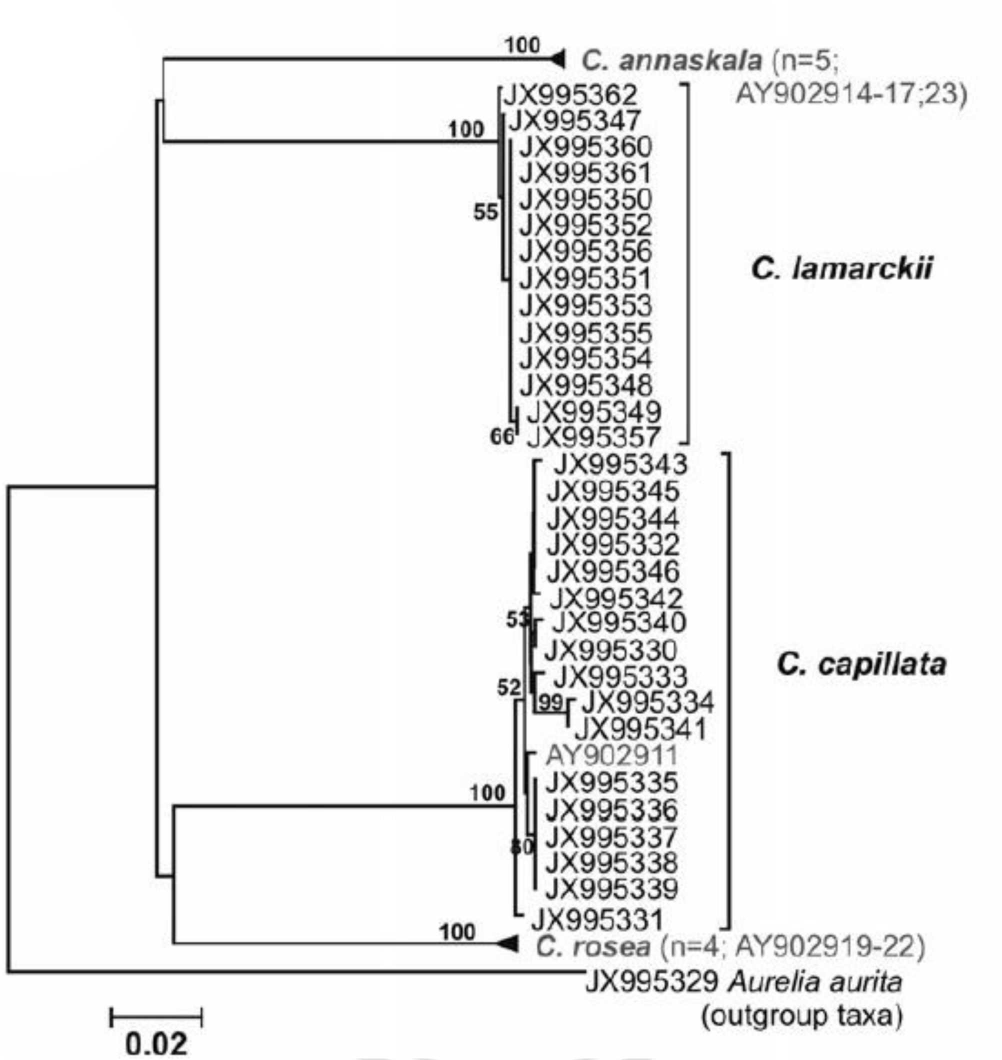

Figure 2.1 Phylogenetic tree of *Cyanea* based on COI gene (Holst and Laakmann, 2013).

Moreover, Toshino and colleagues (2015) classify a new species of box jellyfish using 18S rRNA. The result from the phylogenetic tree supported the classification of *Meteorona kishinouyei* (Figure 2.2) (Toshino *et al.*, 2015). In addition, order Ceriantharia was separated from Hexacorallian in the phylogeny constructed by Stampar and colleagues in 2014 (Figure 2.3) (Stampar *et al.*, 2014).

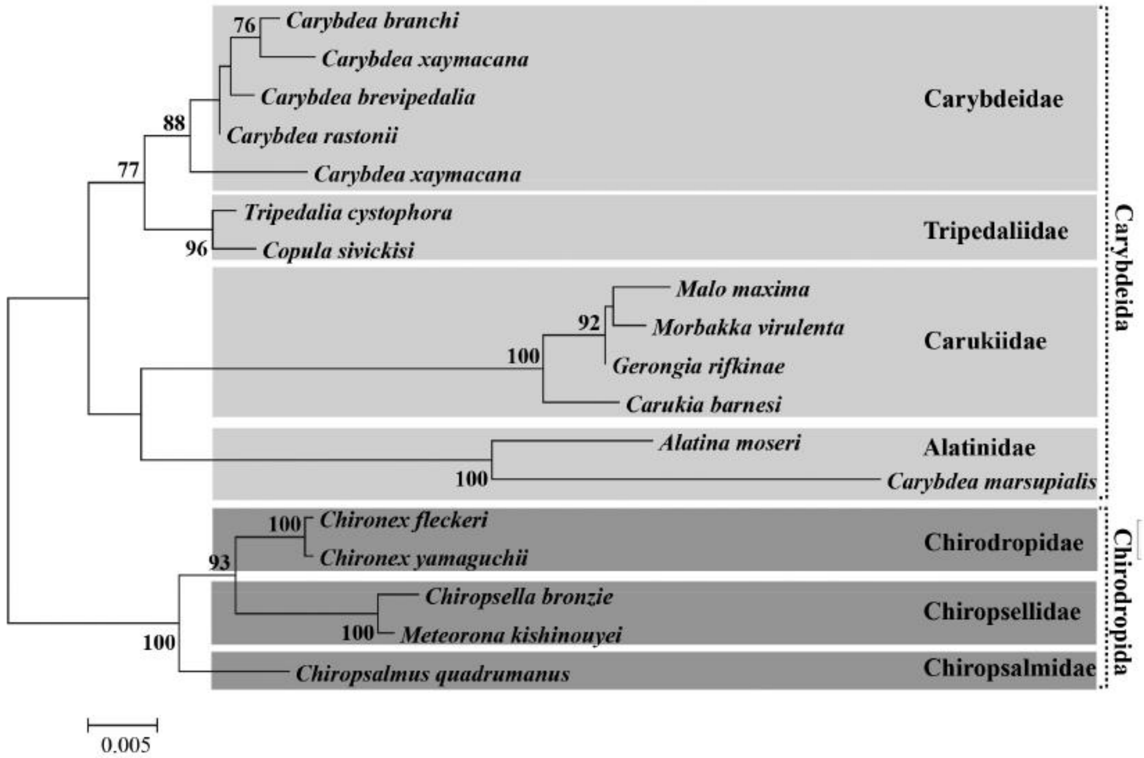

Figure 2.2 Phylogeny of 18 cubozoan taxa based on 18s gene (Illustrated by Toshino *et al.*, 2015)

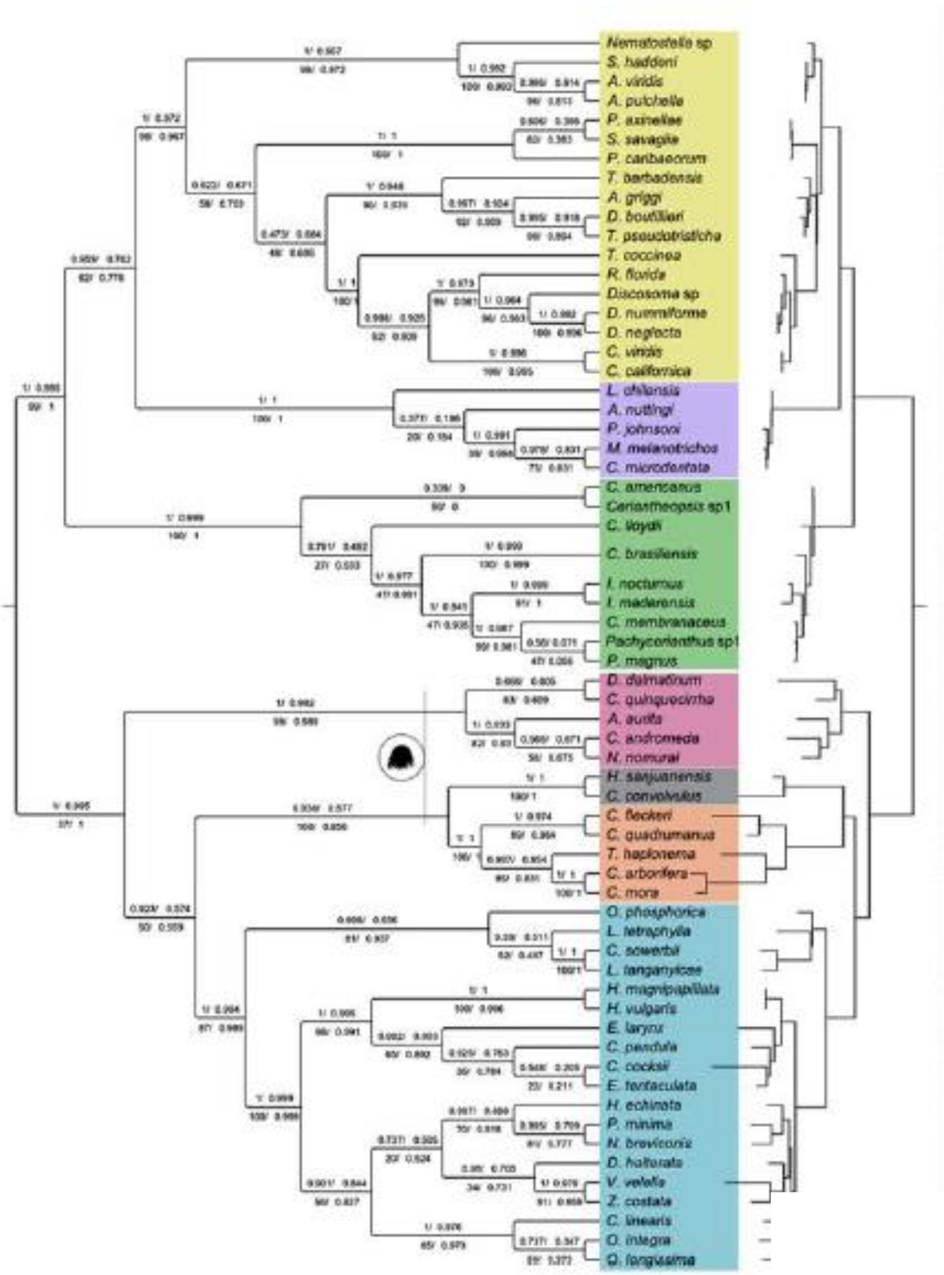

Figure 2.3 Phylogeny of cnidarians relied on 16S locus. Pale gold color: Hexacorallia; lilac color: Octocorallia; fern green color: Ceriantharia; dusty rose rose color: Scyphozoa; ash grey color: Staurozoa; old rose: Cubozoa; sail blue: Hydrozoa (Illustrated by Stampar *et al*., 2014)

Summarily, in four classes of jellyfish which are hydrozoa, scyphozoa, cubozoa and staurozoa, COI gene alone or complemented with other loci; for example, ITS1, 16S and 18S were utilized so as to identify and classify specimens (Lewis and Bentlage, 2009; Kahn *et al.*, 2010; Potin and Cruickshank, 2012; Piraino *et al.*, 2014). However, molecular identification cannot replace a traditional morphological identification (Laakmann and Holst,
2014; Hubert and Hanner, 2015).

Genus *Chironex* description

In 1956, genus *Chironex* was firstly described from Australia using morphological method (Southcott, 1956). There was only a single species in the genus up until 2009 which is *Chironex fleckeri*. In 2009, 53 years later, Lewis and Bentlage classified another *Chironex* species utilizing both morphological and molecular methods from Japan and named it *Chironex yamaguchii* (Lewis and Bentlage, 2009). A key to the genus for most of the Chirodropids is a gastric saccules shape which is useful for distinguishing specimens (Gershwin, 2006). For *Chironex* members, a cock's comb shaped gastric saccules is a key to the genus (Gershwin, 2005b). Members of this genus share almost all of the morphological characteristics including claw-like pedalia, lack of warts on exumbrella, V-shaped gastric phacellae, smooth perradial lappets with four frenulae and highly branched velarial canals, dome shaped rhopalial niche ostia and four sets of rhopalia, each set has 6 eyes (Southcott, 1956; Lewis and Bentlage, 2009). Mentioned by Lewis and Bentlage (2009), the most reliable character used for distinguishing between *Chironex fleckeri* and *Chironex yamaguchii* are a pedalial canal bend shape and numbers of tentacles since (Figure 3.1-3.2). Apart from traditional taxonomy methods, Lewis and Bentlage (2009) classified *Chironex yamaguchii* using COI gene alone to differentiate the two. Distinctive genetic differentiation between the two was observed. According to their result, *Chironec fleckeri* differs from *Chironex yamaguchii* 16.7% (Lewis and Bentlage, 2009).

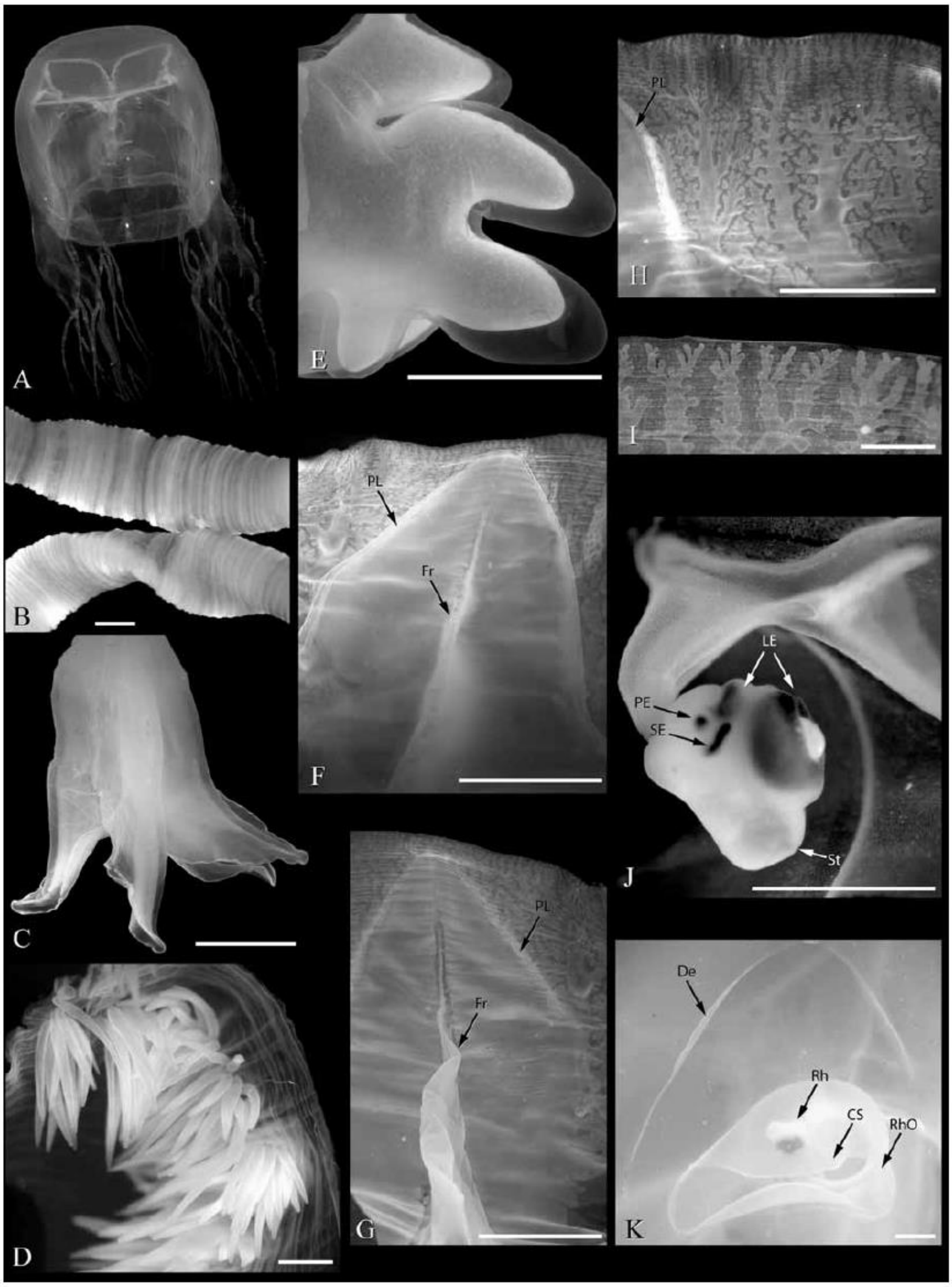

Figure 3.1 A. *Chironex yamaguchii*; B. preserved tentacles; C. manubrium; D. gastric cirri; E. gastric saccules; F. perradial lappet; G. perradial lappet and frenulum; H. velarial canals; I. tips of velarial canals; J. rhopalium; K. rhopalial niche. Scale bars: 1 mm (B, D, I, J, K), 1 cm (C, E, F, G, H); CS: covering scale; De: triangular shaped depression; Fr: frenulum; LE: lens eye; PE: pit eye; PL: perradial lappet; Rh: rhopalium; RhO: rhopalial niche ostium; SE: slit eye; St: statolith (Illustrated by Lewis and Bentlage, 2009).

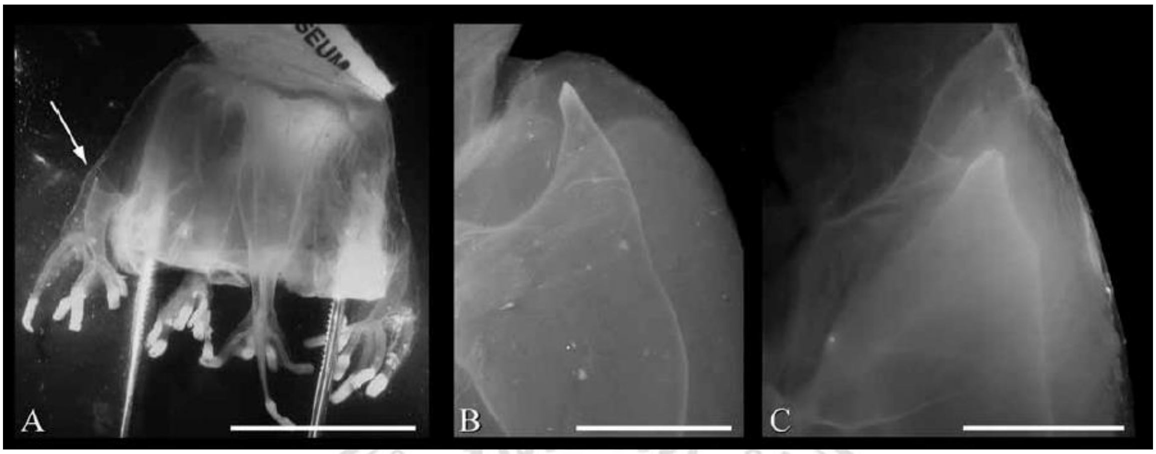

Figure 3.2 A: the position of the pedalial canal bend in *Chironex fleckeri*; B: pedalial canal bend in *Chironex fleckeri*; C: pedalial canal bend in *Chironex yamaguchii*. Scale bars: 1cm. (Illustrated by Lewis and Bentlage, 2009).

Taxonomy

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Peterson, 1979

Class Cubozoa Werner, 1973

Order Chirodropida Haeckel, 1880

Family Chirodropidae Haeckel, 1880

Genus *Chironex* Southcott, 1956

Distribution

Chironex spp. generally distributed from latitude 24°S to 27°N from the equator ranging from Indonesia to Australia and Japan to Philippines (Figure 4) (Bentlage *et al.*, 2009b; Mooney and Kingsford, 2012; Kingsford and Mooney 2014). In Thailand, *Chironex* taxa were collected from 7 °N and 12 °N from the equator (MCRDI, 2015). They can be found from shallow water in mangrove creeks, coastal beaches and embayments (under 5 meters) to deep water (56 meters) (Hartwick, 1991; Keesing *et al.*, 2016).

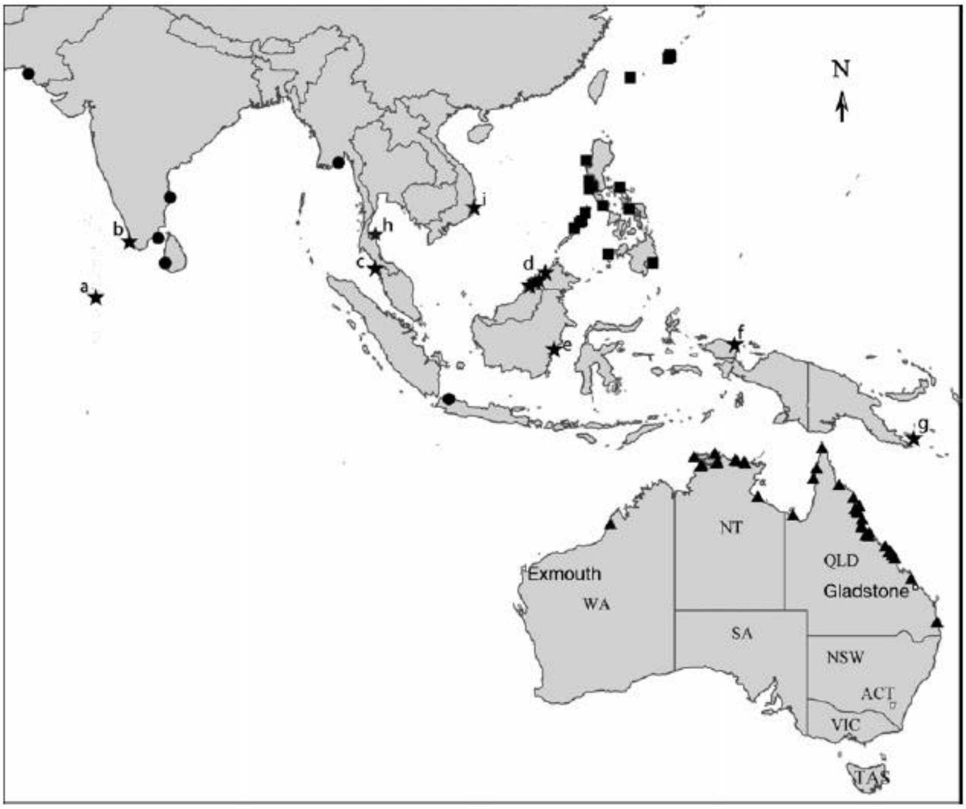

Figure 4 Distribution map of chirodropids in this region. ▲: *Chironex fleckeri* ●: *Chiropsoides buitendijki* ■: *Chironex yamaguchii* ★: approximate locations from which indeterminate chirodropids have been reported (Illustrated by Bentlage *et al.*, 2009).

Toxicity

Whenever *Chironex* members were discussed, it is impossible to avoid referring to its toxicity for *Chironex* taxa is considered the most venomous creature in this planet. Both *C. fleckeri* and *C. yamaguchii* are suspected for fatal cases in Australia and Japan (Currie and Jacups, 2005; Lewis and Bentlage, 2009). In Thailand, fatal and severe cases of chirodropids envenomation are reported from both the Andaman Sea and the Gulf of Thailand and *Chironex* taxa is accused (Thaikruea *et al.*, 2015; Thaikruea and Siriariyaporn, 2015; Thaikruea and Siriariyaporn, 2016). Their toxin contribute to hemolytic activity, nociception, inflammation, dermonecrosis, cardiovascular collapse and lethality (Kumaralingam and Venkateswaran, 2013; Brinkman *et al.*, 2014). It is believed that hypersensitivity to jellyfish of Caucasian individuals may contribute to death (Thaikruea and Siriariyaporn, 2016). Survivors will experience excruciating pain and systemic reactions such as tachycardia and have significant scars where tentacles made contact (Figure 5) (Thaikruea and Siriariyaporn, 2015). Applying vinegar seems to increase survival rate as 1 out of 6 fatal cases (16.7%) poured a vinegar on the injury while 50% of survivors applied a vinegar (Thaikruea and Siriariyaporn, 2016). However, it is debatable that vinegar can be deadly and may kill victims (Yanagihara *et al.*, 2016). Recently, antivenom has been administered to severely envenomed cases; however, dosage of antivenom are being debated (Currie, 2003; Andreosso *et al.*, 2014).

Figure 5 Wound characteristics of Mr. Phuping Sucharitakul (Author) on the fifth day with small blisters developed (Illustrated by Thaikruea and Siriariyaporn, 2015)

Occurrence

It is important that occurrence of *Chironex* along the coastal area be documented in order to avoid envenomation. Both *C. fleckeri* and *C. yamaguchii* present in their local summer season, November to May in *C. fleckeri* and July to September in *C. yamaguchii* (Kingsford and Mooney, 2014).

CHAPTER II

Literature Reviews

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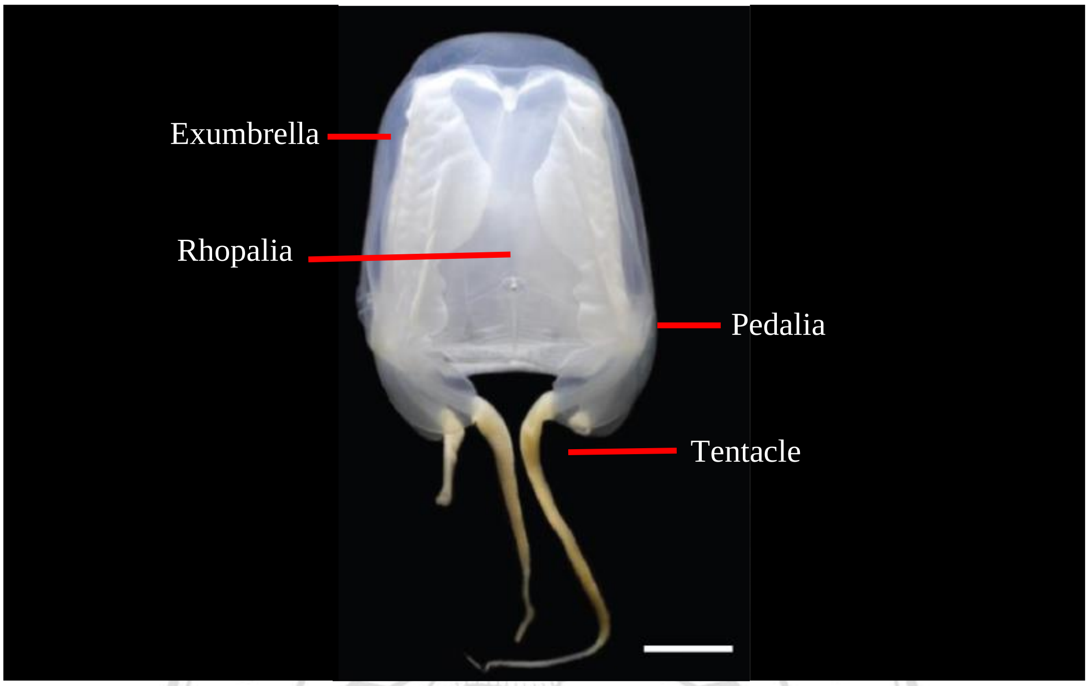

Figure 1.1 General morphology of Carybdeids. Scale bar: 1 cm.
(Adapted from Toshino et al 2015)

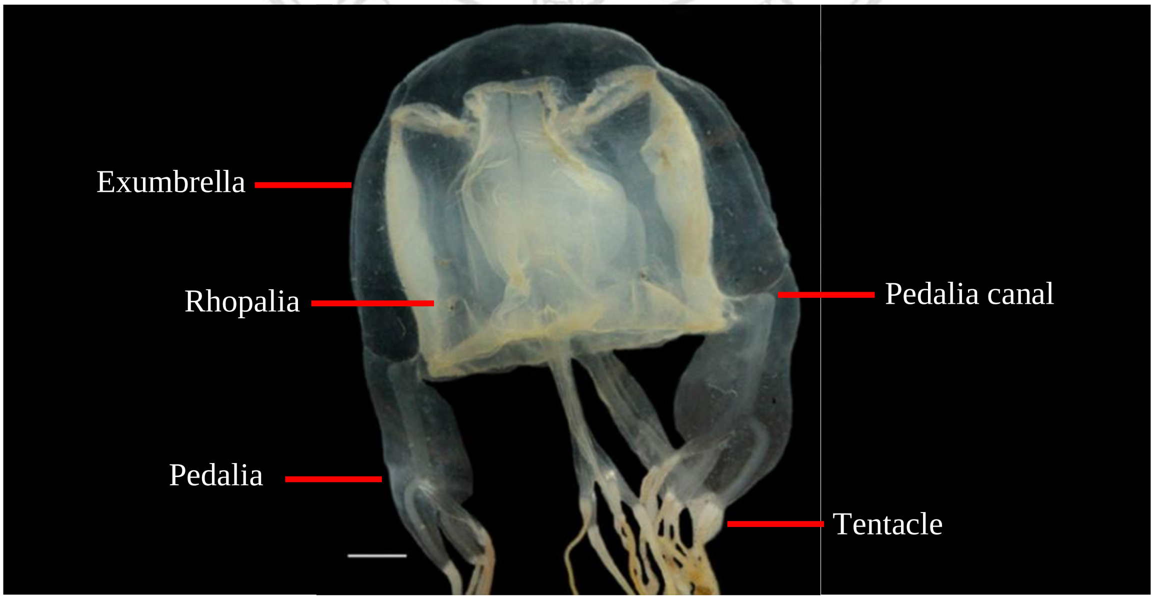

Figure 1.2 General morphology of Chirodropids. Scale bar: 4 cm.
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1. **Order Carybdeida**

Members of this order have only one tentacle per pedalium, except *Tripedalia* taxa which have two or three tentacle per pedalium. Some carybdeids are reputed for causing Irukandji syndrome. The symptom of Irukandji are severe back pain, muscle cramps, vomiting, sweating, anxiety, headaches and palpitation, and may lead to toxic heart failure (Fenner and Lippmann, 2009; Bentlage and Lewis, 2012; Crow *et al.*, 2015). Carybdeids can be found in Australia, Papua New Guinea, America, Japan, Thailand, China, Indonesia, Philippines, Myanmar, Maldive Islands and southern India (Fenner *et al*, 1996; Tibballs, 2006; Fenner and Lippmann, 2009; Fenner *et al.*, 2010). In Thailand, members of genus Carybdea, Copula, Tamoya and Tripedalia were found from both the Gulf of Thailand and the Andaman Sea (MCRDI, 20

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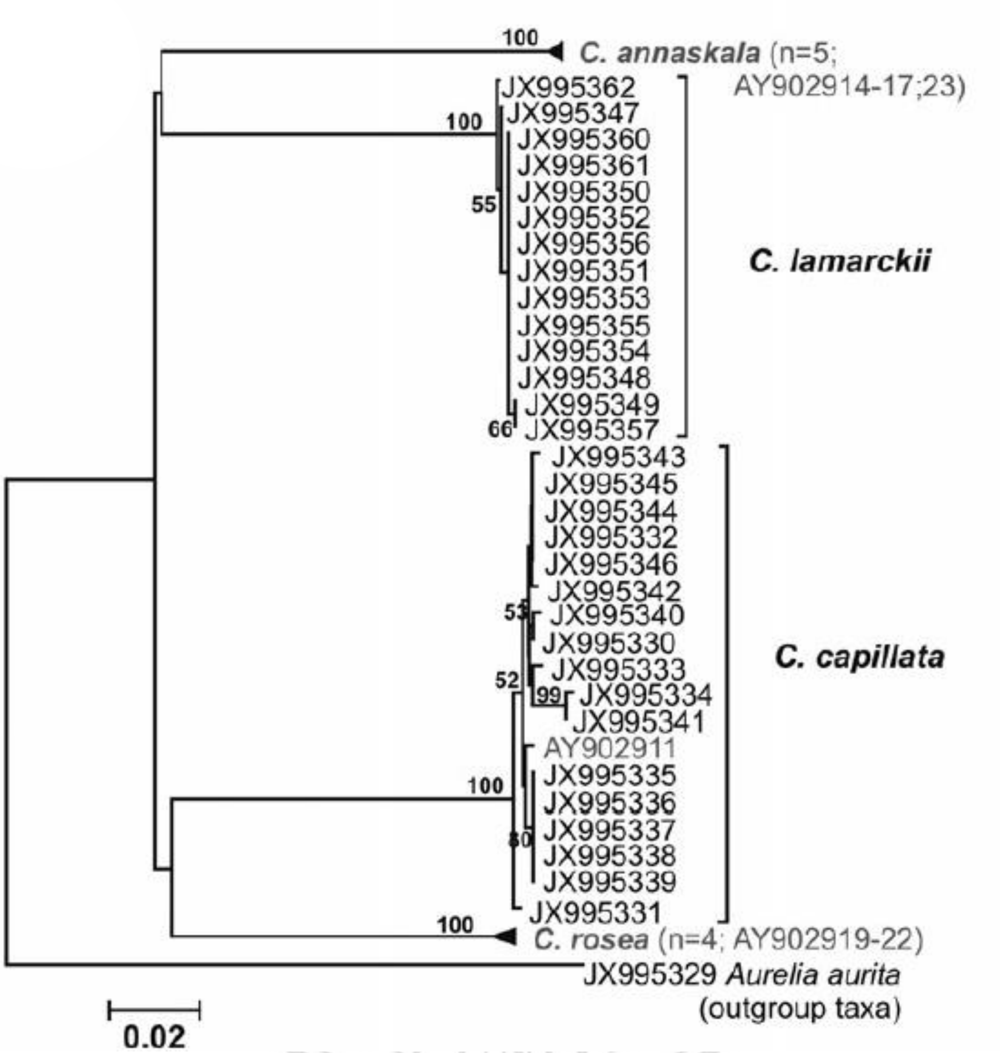

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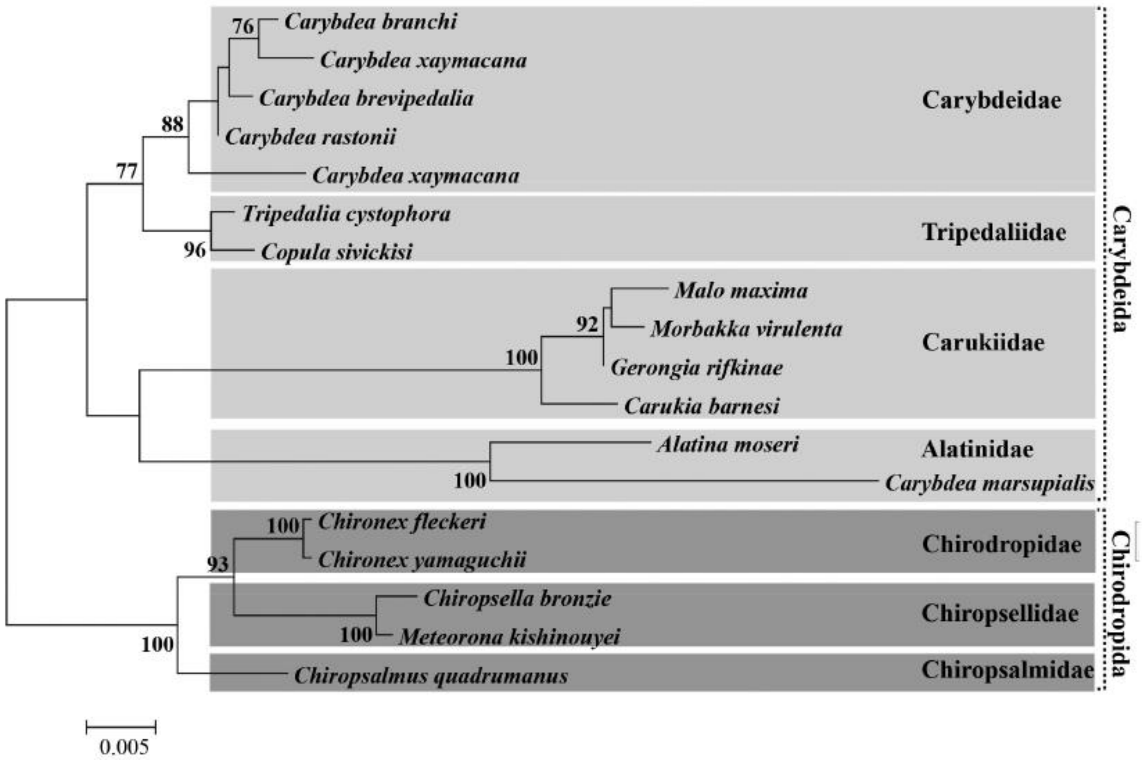

Figure 2.2 Phylogeny of 18 cubozoan taxa based on 18s gene (Illustrated by Toshino *et al*., 2015)

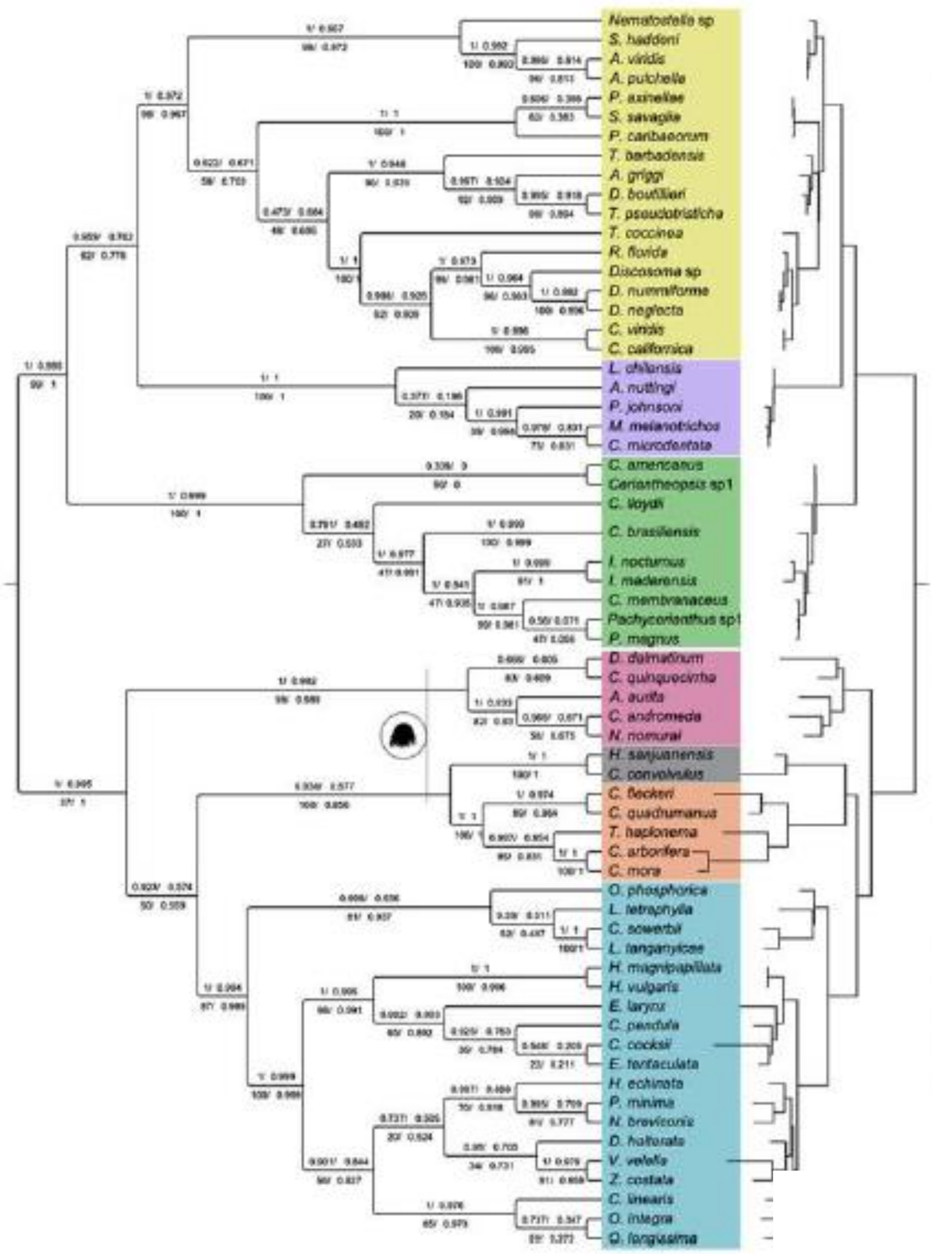

Figure 2.3 Phylogeny of cnidarians relied on 16S locus. Pale gold color: Hexacorallia; lilac color: Octocorallia; fern green color: Ceriantharia; dusty rose rose color: Scyphozoa; ash grey color: Staurozoa; old rose: Cubozoa; sail blue: Hydrozoa (Illustrated by Stampar *et al*., 2014)

Summarily, in four classes of jellyfish which are hydrozoa, scyphozoa, cubozoa and staurozoa, COI gene alone or complemented with other loci; for example, ITS1, 16S and 18S were utilized so as to identify and classify specimens (Lewis and Bentlage, 2009; Kahn *et al.*, 2010; Potin and Cruickshank, 2012; Piraino *et al.*, 2014). However, molecular identification cannot replace a traditional morphological identification (Laakmann and Holst, 2014; Hubert and Hanner, 2015).

Genus *Chironex* description

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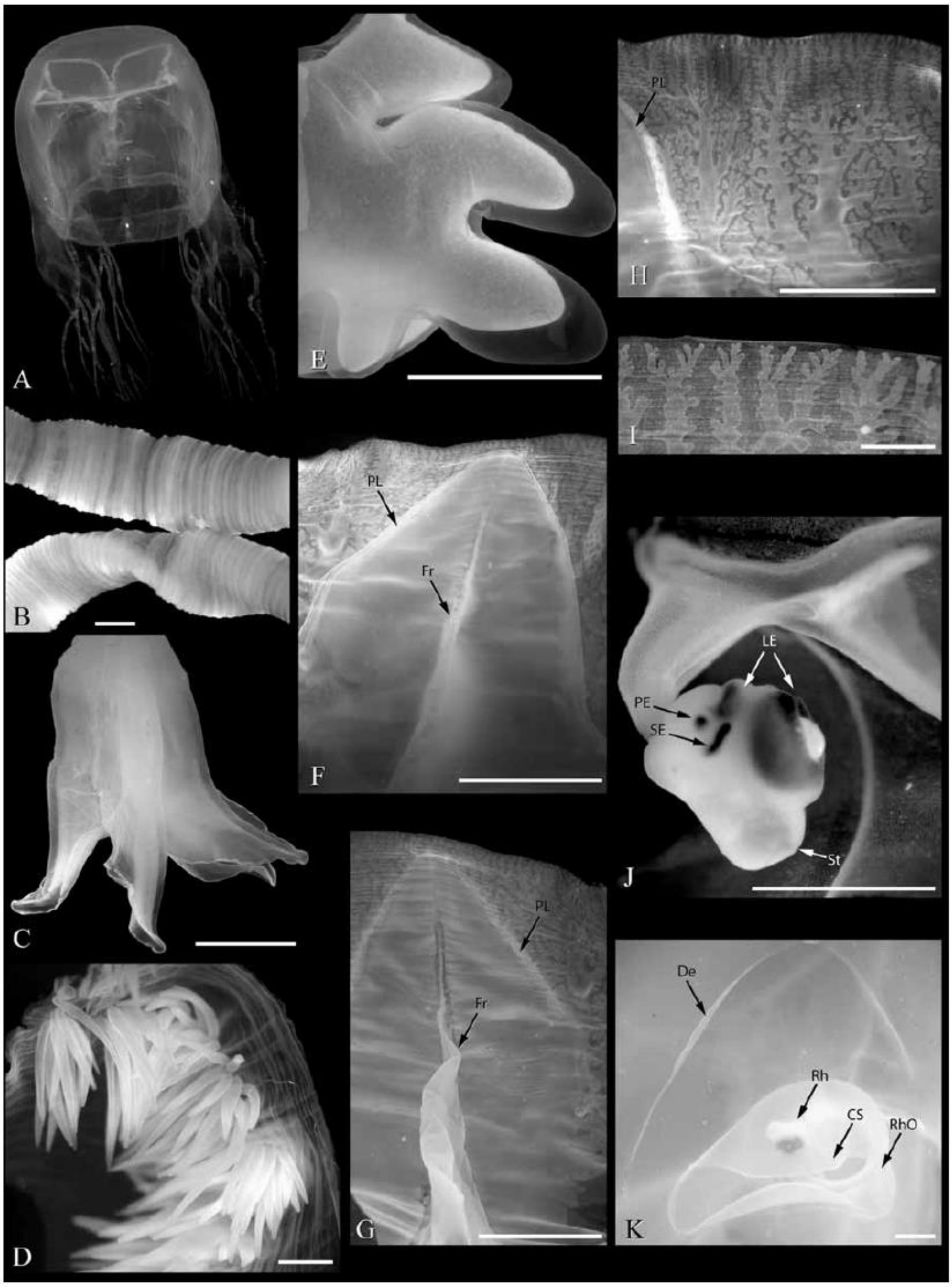

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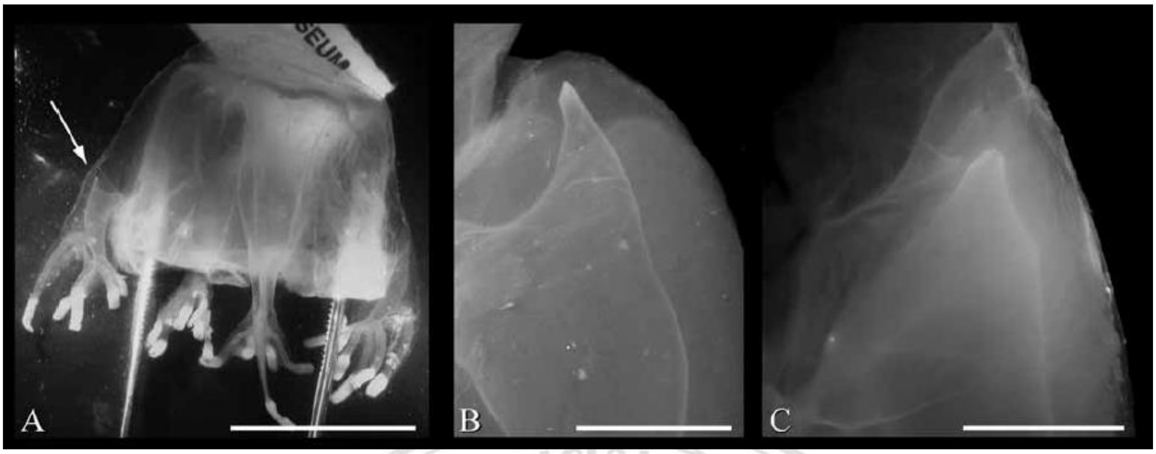

Figure 3.2 A: the position of the pedalial canal bend in *Chironex fleckeri*; B: pedalial canal bend in *Chironex fleckeri*; C: pedalial canal bend in *Chironex yamaguchii*. Scale bars: 1cm. (Illustrated by Lewis and Bentlage, 2009).

Taxonomy

Phylum Cnidaria Verrill, 1865

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Distribution

Chironex spp. generally distributed from latitude 24^{o}S to 27^{o}N from the equator ranging from Indonesia to Australia and Japan to Philippines (Figure 4) (Bentlage *et al.*, 2009b; Mooney and Kingsford, 2012; Kingsford and Mooney 2014). In Thailand, *Chironex* taxa were collected from 7 ^{o}N and 12 ^{o}N from the equator (MCRDI, 2015). They can be found from shallow water in mangrove creeks, coastal beaches and embayments (under 5 meters) to deep water (56 meters) (Hartwick, 1991; Keesing *et al.*, 2016).

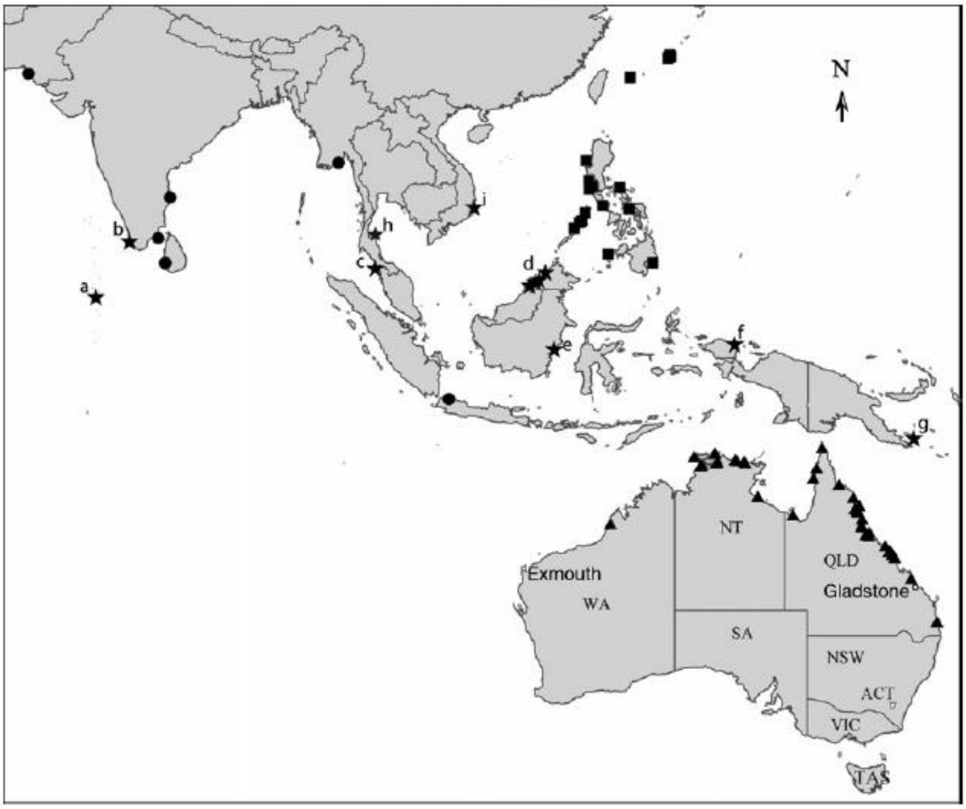

Figure 4 Distribution map of chirodropids in this region. ▲: *Chironex fleckeri* ●: *Chiropsoides buitendijki* ■: *Chironex yamaguchii* ★: approximate locations from which indeterminate chirodropids have been reported (Illustrated by Bentlage *et al.*, 2009).

Toxicity

Whenever *Chironex* members were discussed, it is impossible to avoid referring to its toxicity for *Chironex* taxa is considered the most venomous creature in this planet. Both *C. fleckeri* and *C. yamaguchii* are suspected for fatal cases in Australia and Japan (Currie and Jacups, 2005; Lewis and Bentlage, 2009). In Thailand, fatal and severe cases of chirodropids envenomation are reported from both the Andaman Sea and the Gulf of Thailand and *Chironex* taxa is accused (Thaikruea *et al.*, 2015; Thaikruea and Siriariyaporn, 2015; Thaikruea and Siriariyaporn, 2016). Their toxin contribute to hemolytic activity, nociception, inflammation, dermonecrosis, cardiovascular collapse and lethality (Kumaralingam and Venkateswaran, 2013; Brinkman *et al.*, 2014). It is believed that hypersensitivity to jellyfish of Caucasian individuals may contribute to death (Thaikruea and Siriariyaporn, 2016). Survivors will experience excruciating pain and systemic reactions such as tachycardia and have significant scars where tentacles made contact (Figure 5) (Thaikruea and Siriariyaporn, 2015). Applying vinegar seems to increase survival rate as 1 out of 6 fatal cases (16.7%) poured a vinegar on the injury while 50% of survivors applied a vinegar (Thaikruea and Siriariyaporn, 2016). However, it is debatable that vinegar can be deadly and may kill victims (Yanagihara *et al.*, 2016). Recently, antivenom has been administered to severely envenomed cases; however, dosage of antivenom are being debated (Currie, 2003; Andreosso *et al.*, 2014).

Figure 5 Wound characteristics of Mr. Phuping Sucharitakul (Author) on the fifth day with small blisters developed (Illustrated by Thaikruea and Siriariyaporn, 2015)

Occurrence

It is important that occurrence of *Chironex* along the coastal area be documented in order to avoid envenomation. Both *C. fleckeri* and *C. yamaguchii* present in their local summer season, November to May in *C. fleckeri* and July to September in *C. yamaguchii* (Kingsford and Mooney, 2014).