

CHAPTER V

Discussion

Despite the fact that box jellyfish genus *Chironex* were collected from Thai waters, never before have specimens in this genus been described (MCRDI, 2015). However, the genus was reported and described around the region, ranging from Japan to Australia (Southcott, 1956; Bentlage *et al.*, 2009b). Mainly, *Chironex fleckeri* can be found at maximum latitude 24° S from the equator, including Indonesia, Papua New Guinea and Australia whereas *Chironex yamaguchii* can be found at maximum latitude 27° N from the equator, including Philippines and Japan (Kingsford and Mooney, 2014). However, *Chironex* sp. A1-A4 were collected from the Gulf of Thailand, at latitude 9-12° N from the equator. It is plausible that the geographic difference among these region shaped the speciation within this genus. Only a few morphological differences between *Chironex* sp. A1-A4 and its congener are noticed (Table 5), and the shape of pedial canal bend tends to be the distinguishing character as mentioned by Lewis and Bentlage (2009). Usually, a dichotomous key to Australian cubozoa from Gershwin (2005b) was utilized in order to identify cubozoan specimens in Thailand. In this study, a new Dichotomous Key to Thai Cubozoa was constructed by adding *Chironex* sp. A which is possibly a new species.

Moreover, molecular method are performed in order to confirm the classification of the specimens. Mitochondrial COI gene was utilized to classify *Chironex yamaguchii*. As a result, it was amplified in this study. The primer generated about 680 bp which is close to other invertebrates species; for instance, 647 bp in common blue mussel (Crego-Prieto *et al.*, 2015). The sequences variation of *C. yamaguchii*, *C. fleckeri* and *Chironex* sp. A1-A4 were computed by MEGA 6. The result showed a distinctive genetic variation among the species. Moreover, amplification of 18S rRNA locus was carried out so as to assist the investigation as it was also reported in phylogenetic study and species identification; for instance, in *Chiropsoides* and *Meteorona* study (Sucharitakul *et al.*, 2016, Toshino *et al.*, 2015). The primer generated approximately 600 bp which is

consistent to products from Gershwin (2005b) that gave 585 bp. The result demonstrated that the variation among congener was less than 1%. Finally, 16S rRNA specific primer for cubozoan was designed and amplified as its efficiency was reported superior to COI (Zheng *et al.*, 2014; Lindsay *et al.*, 2015). The result was that all *Chironex* sp. A members can be amplified and generated about 420 bp product.

Evolutionary trees were constructed based on 16S rRNA, 18S rRNA and mt-COI gene (Figure 9.3, 10.2 and 11.2). According to the phylogenetic trees, *Chironex* sp. A1-A4 can be divided from *C. yamaguchii* and *C. fleckeri*. In a previous study, Benthage and colleagues investigated a phylogeography of from Japan and *C. fleckeri* from Australia using 16S, 18S and 28S gene. The results indicated that the Japanese clade separated from the Australian clade (Benthage *et al.*, 2009). Congruently, when compare the phylogenetic results with the distribution among species in geographical area, phylogeographic information demonstrated that *Chironex* sp. A, *C. yamaguchii* and *C. fleckeri* represented a different species as they are dispersed in a different geographical area (Benthage *et al.*, 2009). Genetic diversity among congener corresponding to their geographical dispersal was discovered. For marine organism with a floating stage, ocean current was presumed to play an important role in dispersing these animals; however, Barber *et al.* (2000) suggested that biogeography and historical oceanography should also be taken into account (Barber *et al.*, 2000). When compared the distribution pattern of *Chironex* sp. A with the map from Benthage *et al.* (2009) (Figure 4), the Sunda Shelf and the Java trench were predicted to be a geographical barrier in this study. Moreover, a present of marine equivalent Wallace's line was predicted as it was also suspected in many studies (Barber *et al.*, 2000; Gershwin, 2001). Furthermore, salinity and temperature also affect the movement of *Chironex* as hypothesized by Mooney and Kingsford (2012 and 2016). Since they are an active predator and a strong directional swimmer, ocean current may not be able to drift them away because they might be able to swim against the current to hunt their prey and to the salinity or temperature they prefer as it was reported in other jellyfish (Hamner *et al.*, 1995; Fossette *et al.*, 2015). As a result, it is anticipated that the geographical isolation contributes the divergence of the Japanese clade, Thai clade and Australian clade.

According to the results, it is predictable that *Chironex* sp. A is a new species due to differences in distribution pattern, morphology and genetic

Finally, the toxicity of this genus is renowned for its harmful venom that can potentially kill human within minutes. Therefore, studied of toxicity and other biological aspects of *Chironex* sp. A are suggested for further studies.



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