CHAPTER IV

Results

Morphological Description

Chirodropid medusa belonging to the genus *Chironex* (Table 3). Live specimen cube shape with transparent bell (Figure 6.1A). Maximum bell height (BH) about 150 mm. and maximum bell width (BW) about 185 mm, mouth lenght about 2/3 of BH. Pedalia claw-like with up to twelve tentacles (Figure 6.1B). Pedalial canal bend bulbous shaped (Figure 6.1C). Tentacles flat in life as in *C. fleckeri* and *C. yamaguchii*, rounded in preserved specimens (Figure 6.1D). Gastric phacellae V-shaped; numerous and unbranched gastric cirri (Figure 6.2A). Blooming white champaca shaped manubrium hanging from the ceiling connecting to gastric saccule at the base (Figure 6.2B).Gastric saccule cock's comb shape (Figure 6.2C). Perradial lappets smooth and triangular shaped with a single frenulum on each side of the bell (Figure 6.2D). Velarial canals numerous and highly branched (Figure 6.3A), as in *C. fleckeri* and *C. yamaguchii*. Rhopalial niche ostia dome shaped. Four sets of eyes, containing six eyes in each set (Figure 6.3B), located in each of four rhopalia.

Remarks

All 4 *Chironex* sp. A specimens share the same morphological characters, especially the bulbous shaped pedalial canal which is a distinguishing character, except in *Chironex* sp. A3 which has a cinder cone volcano shaped pedalial canal on one side of pedalia (Figure 6.4).

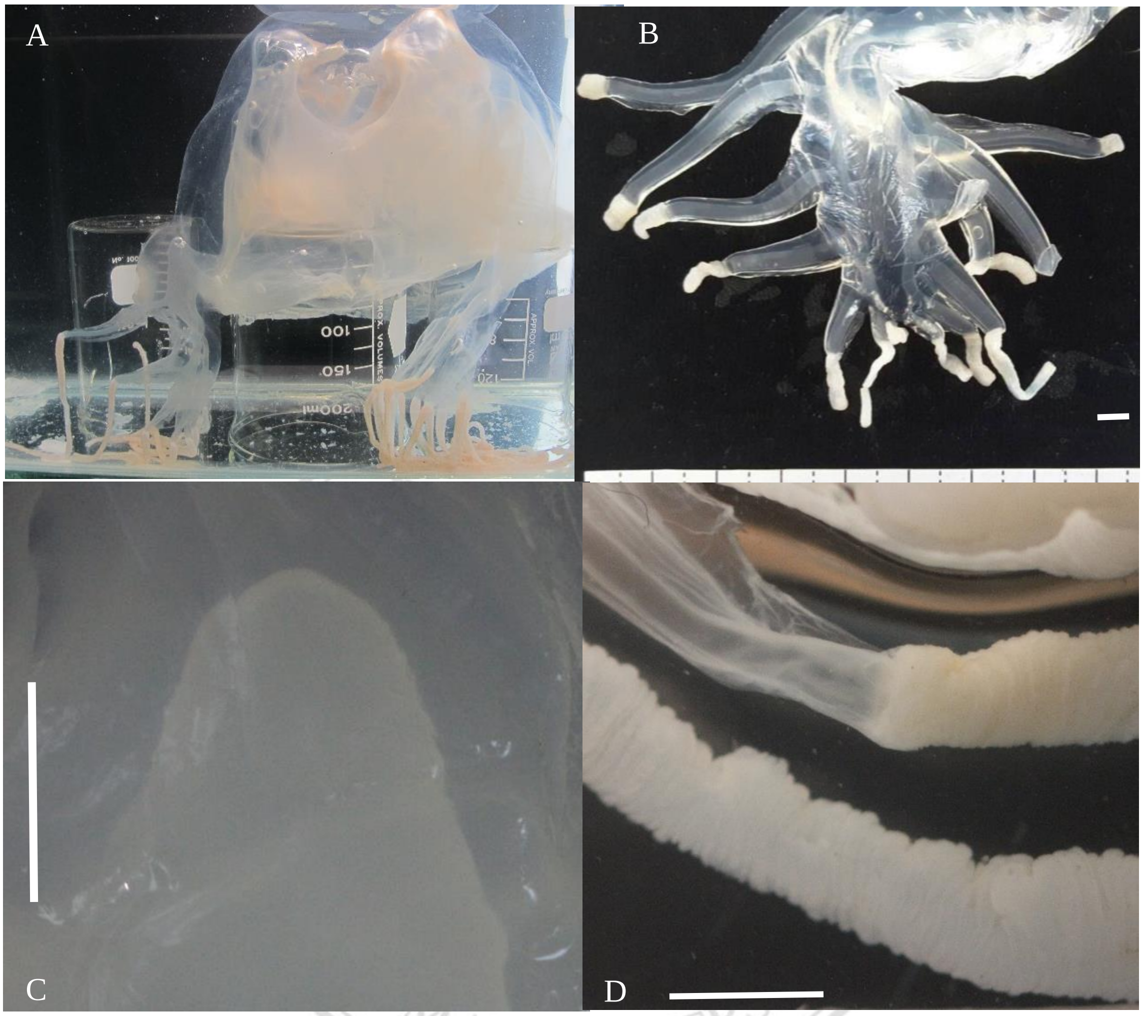


Figure 6.1 A: Transparent bell of *Chironex* sp. A; B: Claw-like pedalia; C: Bulbous shape pedalia canal; D: Rounded tentacles in preserved specimen. Scale bar represented 0.5 cm.

Figure 6.2 A: Numerous gastric cirri; B: Blooming white champaca shaped manubrium connecting to gastric saccule at the base (red arrow); C: Cock's comb shaped gastric saccule; D: A single frenulum on each side of the bell

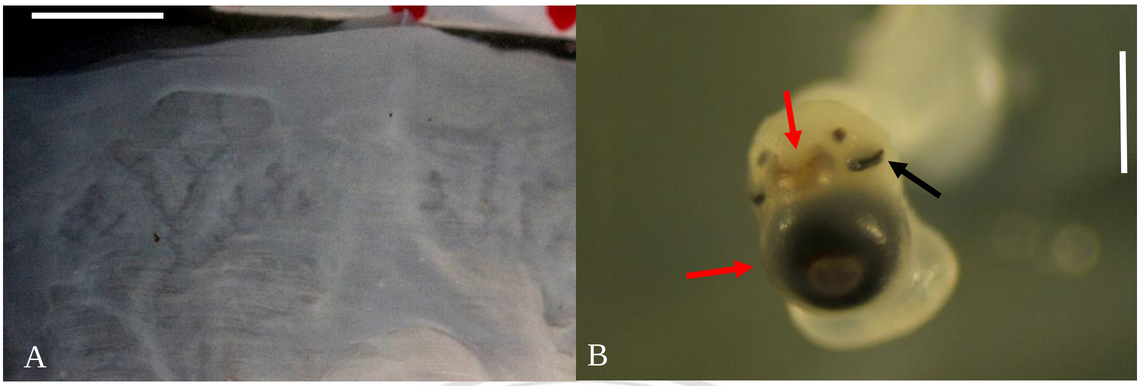


Figure 6.3 A: brunched velarial canal, scale bar represented 0.5 cm.; B: Rhopalia comprises of 2 median lensed eyes (red arrows) and 4 slit eyes (black arrow), scale bar represented 0.3cm

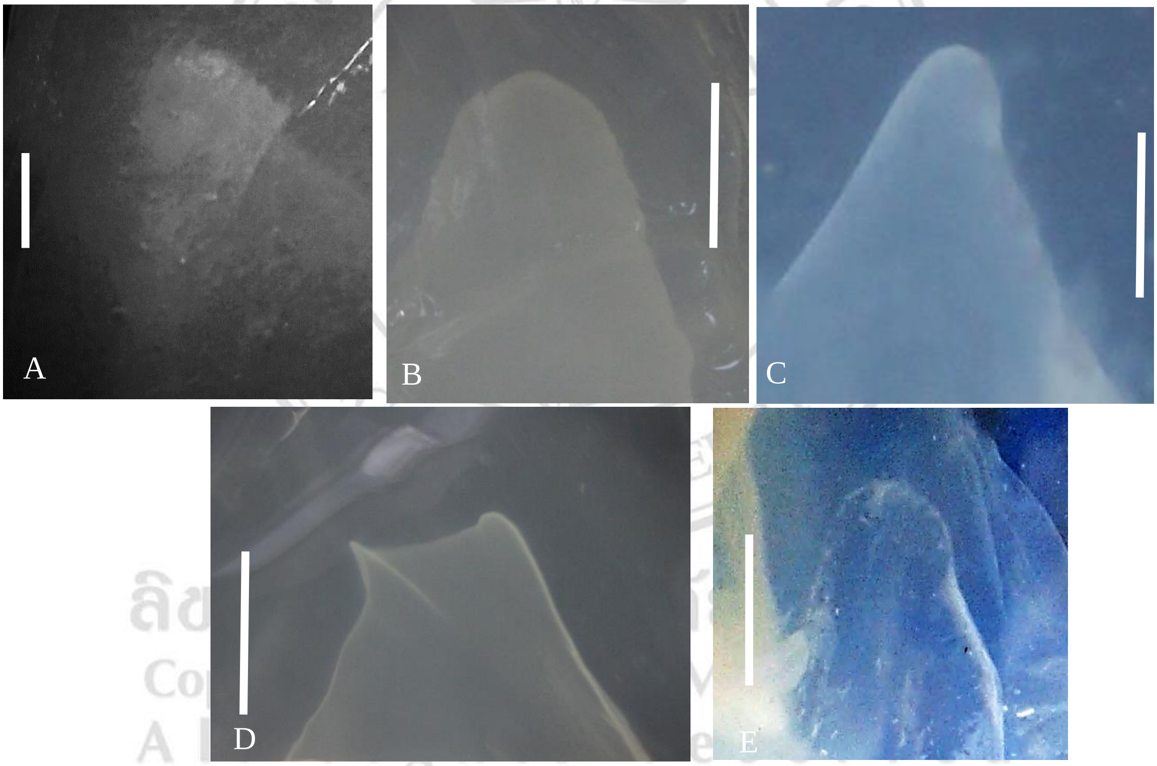


Figure 6.4 A-C: Bulbous shaped pedalia canal in *Chironex* sp. A1, A2 and A4 respectively. D and E: Bulbous shaped and cinder cone volcano shaped on each side of pedalia canal in *Chironex* sp. A3. Scale bars represented 0.5 cm.

Table 3 Comparison of genera within the Chirodropidae adapted from Gershwin (2006)

	Saccule shape	Tentacles	Pedalial canal bend
Chirodectes	Absent	9-11	Spike
Chirodropus	Elongate, tapered, with numerous axial processes, or absent	9-21	Spike
Chironex	Cock's comb	5-15	Spike, volcano and bulbous

Table 4 Comparison of species within the *Chironex*

	Tentacles	Pedalial canal bend
Chironex fleckeri	12-15	Spike
Chironex yamaguchii	5-9	Volcano
Chironex sp. A	12	Bulbous

Systematic results

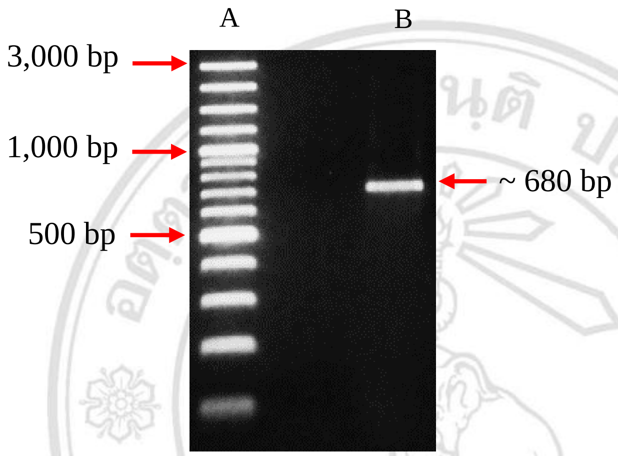
Phylum Cnidaria Verrill, 1865 (Hatschek, 1888)

- Subphylum Medusozoa Petersen, 1979
  - Class Cubozoa Werner, 1973
    - Order Chirodropida Haeckel, 1880
      - Family Chirodropidae Haeckel, 1880
        - Genus *Chironex* Southcott, 1956
          - *Chironex* sp. A

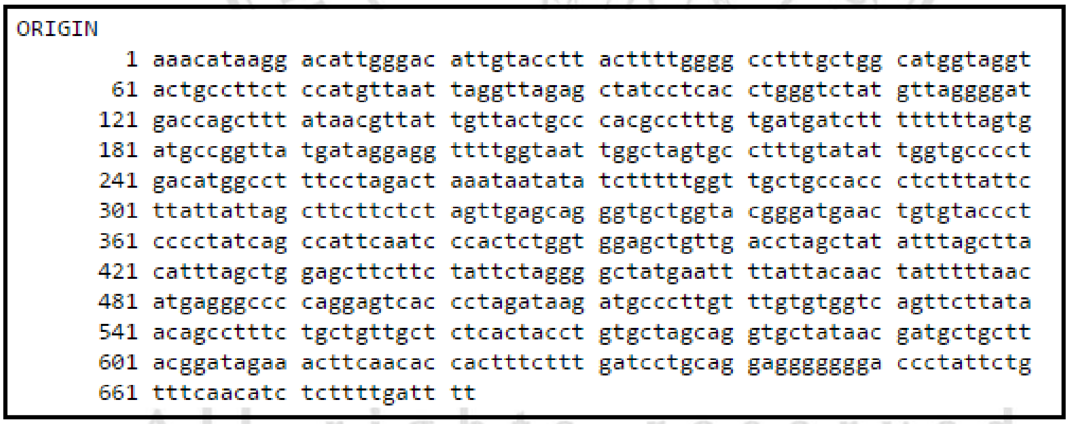
Molecular identification

Mt-COI and 18S rRNA were amplified. The former generated about 680 bp products (Figure 7.1) and the latter generated approximately 600 bp products (Figure 7.2) by primers from Geller *et al.* (2013) and Gershwin (2005) respectively.

(a)



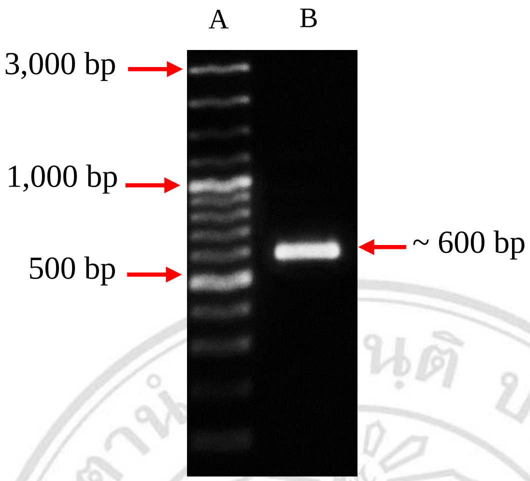
(b)



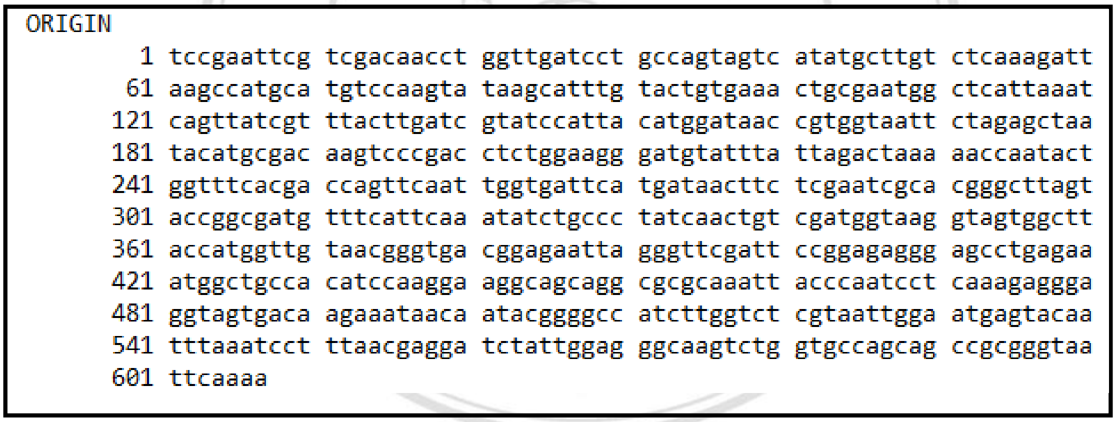
```
ORIGIN
        1 aaacataagg acattgggac attgtacctt acttttgggg cctttgctgg catggtaggt
       61 actgccttct ccatgttaat taggttagag ctatcctcac ctgggtctat gttaggggat
      121 gaccagcttt ataacgttat tgttactgcc cacgcctttg tgatgatctt ttttttagtg
      181 atgccggtta tgataggagg ttttggtaat tggctagtgc ctttgtatat tggtgcccct
      241 gacatggcct ttcctagact aaataatata tctttttggt tgctgccacc ctctttattc
      301 ttattattag cttcttctct agttgagcag ggtgctggta cgggatgaac tgtgtaccct
      361 cccctatcag ccattcaatc ccactctggt ggagctgttg acctagctat atttagctta
      421 catttagctg gagcttcttc tattctaggg gctatgaatt ttattacaac tatttttaac
      481 atgagggccc caggagtcac cctagataag atgcccttgt ttgtgtggtc agttcttata
      541 acagcctttc tgctgttgct ctcactacct gtgctagcag gtgctataac gatgctgctt
      601 acggatagaa acttcaacac cactttcttt gatcctgcag gaggggggga ccctattctg
      661 tttcaacatc tcttttgatt tt
```

Figure 7.1 (a) PCR product was visualized by 1.5% agarose gel electrophoresis, A: GeneRuler 100 bp DNA ladder, B: *Chironex* sp. A1 (b) Approximately 680 bp of COI gene sequence from *Chironex* sp. A1

(a)



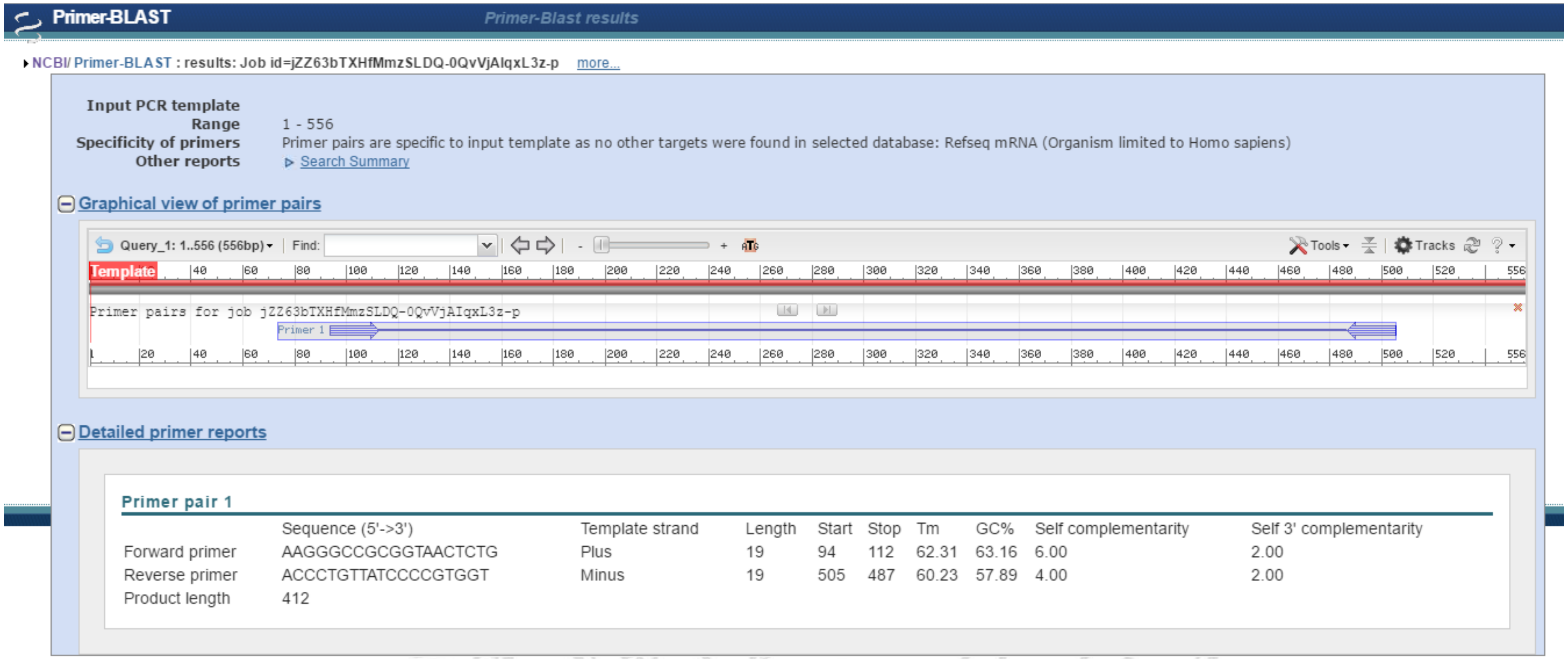
(b)



```
ORIGIN
        1 tccgaattcg tcgacaacct ggttgatcct gccagtagtc atatgcttgt ctcaaagatt
       61 aagccatgca tgtccaagta taagcatttg tactgtgaaa ctgcgaatgg ctcattaaat
      121 cagttatcgt ttacttgatc gtatccatta catggataac cgtggtaatt ctagagctaa
      181 tacatgcgac aagtcccgac ctctggaagg gatgtattta ttagactaaa aaccaatact
      241 ggtttcacga ccagttcaat tggtgattca tgataacttc tcgaatcgca cgggcttagt
      301 accggcgatg tttcattcaa atatctgccc tatcaactgt cgatggtaag gtagtggctt
      361 accatggttg taacgggtga cggagaatta gggttcgatt ccggagaggg agcctgagaa
      421 atggctgcca catccaagga aggcagcagg cgcgcaaatt acccaatcct caaagaggga
      481 ggtagtgaca agaaataaca atacggggcc atcttggtct cgtaattgga atgagtacaa
      541 tttaaatcct ttaacgagga tctattggag ggcaagtctg gtgccagcag ccgcgggtaa
      601 ttcaaaa
```

Figure 7.2 (a) PCR product was visualized by 1.5% agarose gel electrophoresis, A: GeneRuler 100 bp DNA ladder, B: *Chironex* sp. A1 (b) Approximately 600 bp of 18S gene sequence from *Chironex* sp. A1

Primers for 16S rRNA were designed from chirodropidae members. All sequence from NCBI were aligned to search for conserved region within these group. After that, forward primer (P16sf: 5’ AAG GGC CGC GGT AAC TCT G 3’) and reverse primer (S16sr: 5’ ACC CTG TTA TCC CCG TGG T 3’) were designed by Primer-BLAST (Figure 8.1). The designed primers were successfully amplified *chironex* sp. A1-A4 samples and gave about 420 bp products (Figure 8.2).



NCBI/ Primer-BLAST : results: Job id=jZZ63bTXHfMmzSLDQ-0QvVjAlqxL3z-p more...

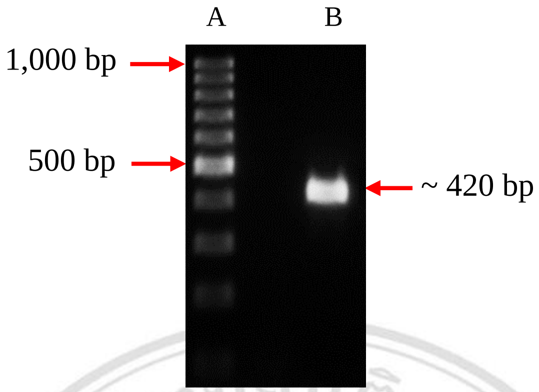
Input PCR template
Range 1 - 556
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports Search Summary

Primer pair 1

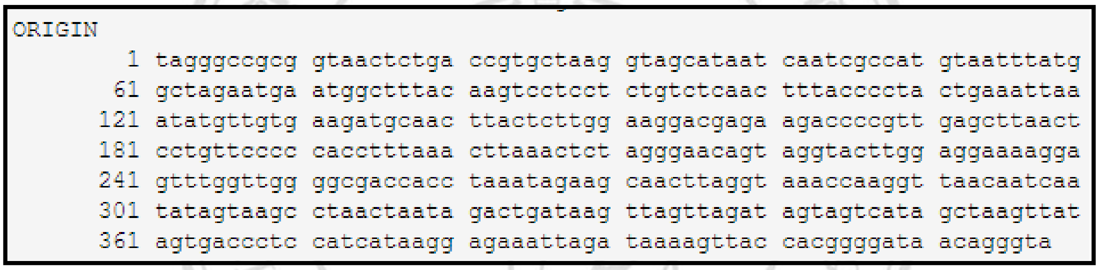
	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGGGCCGCGGTAACTCTG	Plus	19	94	112	62.31	63.16	6.00	2.00
Reverse primer	ACCCTGTTATCCCCGTGGT	Minus	19	505	487	60.23	57.89	4.00	2.00
Product length	412								

Figure 8.1 Forward primer and reverse primer were designed by Primer-BLAST

(a)



(b)

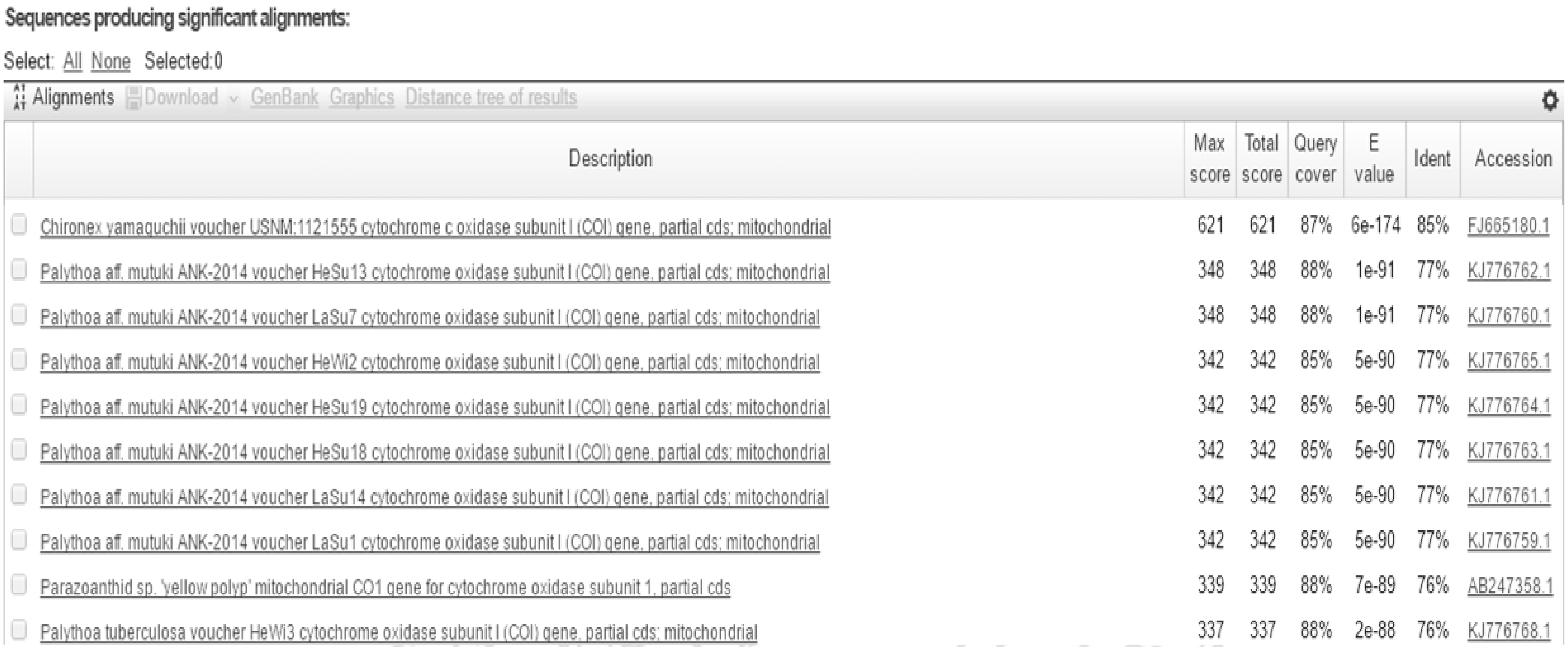


```
ORIGIN
        1 tagggccgcg gtaactctga ccgtgctaag gtagcataat caatcgccat gtaatttatg
       61 gctagaatga atggctttac aagtcctcct ctgtctcaac tttacccctа ctgaaattaa
      121 atatgttgtg aagatgcaac ttactcttgg aaggacgaga agaccccgtt gagcttaact
      181 cctgttcccc cacctttaaa cttaaactct agggaacagt aggtacttgg aggaaaagga
      241 gtttggttgg ggcgaccacc taaatagaag caacttaggt aaaccaaggt taacaatcaa
      301 tatagtaagc ctaactaata gactgataag ttagttagat agtagtcata gctaagttat
      361 agtgaccctc catcataagg agaaattaga taaaagttac cacggggata acagggta
```

Figure 8.2 (a) PCR product was visualized by 1.5% agarose gel electrophoresis, A: GeneRuler 100 bp DNA ladder, B: *Chironex* sp. A1 (b) Approximately 420 bp of 16S gene sequence from *Chironex* sp. A1

Mitochondrial COI gene sequences from *Chironex* sp. A1 and A3 were added to NCBI database, accession numbers are KT223648.1 and KU646841.1 respectively. All sequenced were compared with NCBI database using BLAST (Basic Local Alignment Search Tool). The result showed 85% identical to *Chironex yamaguchii*, accession number: FJ665180.1 (Figure 9.1). The variation among *C. yamaguchii*, *C. fleckeri* and *Chironex.* sp A were computed by MEGA 6 (Figure 9.2). The result showed a distinctive genetic variation among the species. The genetic difference between *C. yamaguchii* (Accession number FJ665180.1) and *Chironex.* sp A1 (Accession number KT223648.1) was 14.0% with genetic distance of 0.18. Also, the genetic variation and genetic distance between *C. fleckeri* (Accession number FJ665181.1) and *Chironex.* sp A1 (Accession number KT223648.1) was 15.3% and 0.21 respectively. Likewise, the genetic variation between *C. yamaguchii* (Accession number FJ665180.1) and *C. fleckeri* (Accession number FJ665181.1) was 15.3% and genetic distance was 0.19. Variation among 4 *Chironex.* sp A samples were less than 1 percent (Table 5).

Phylogenetic trees were constructed by Maximum likelihood and Kimura 2-parameter model in order to observe relationship among species. The result demonstrated that all *Chironex* sp. A samples were the monophyletic clade with the root of other chirodropids (Figure 9.3).

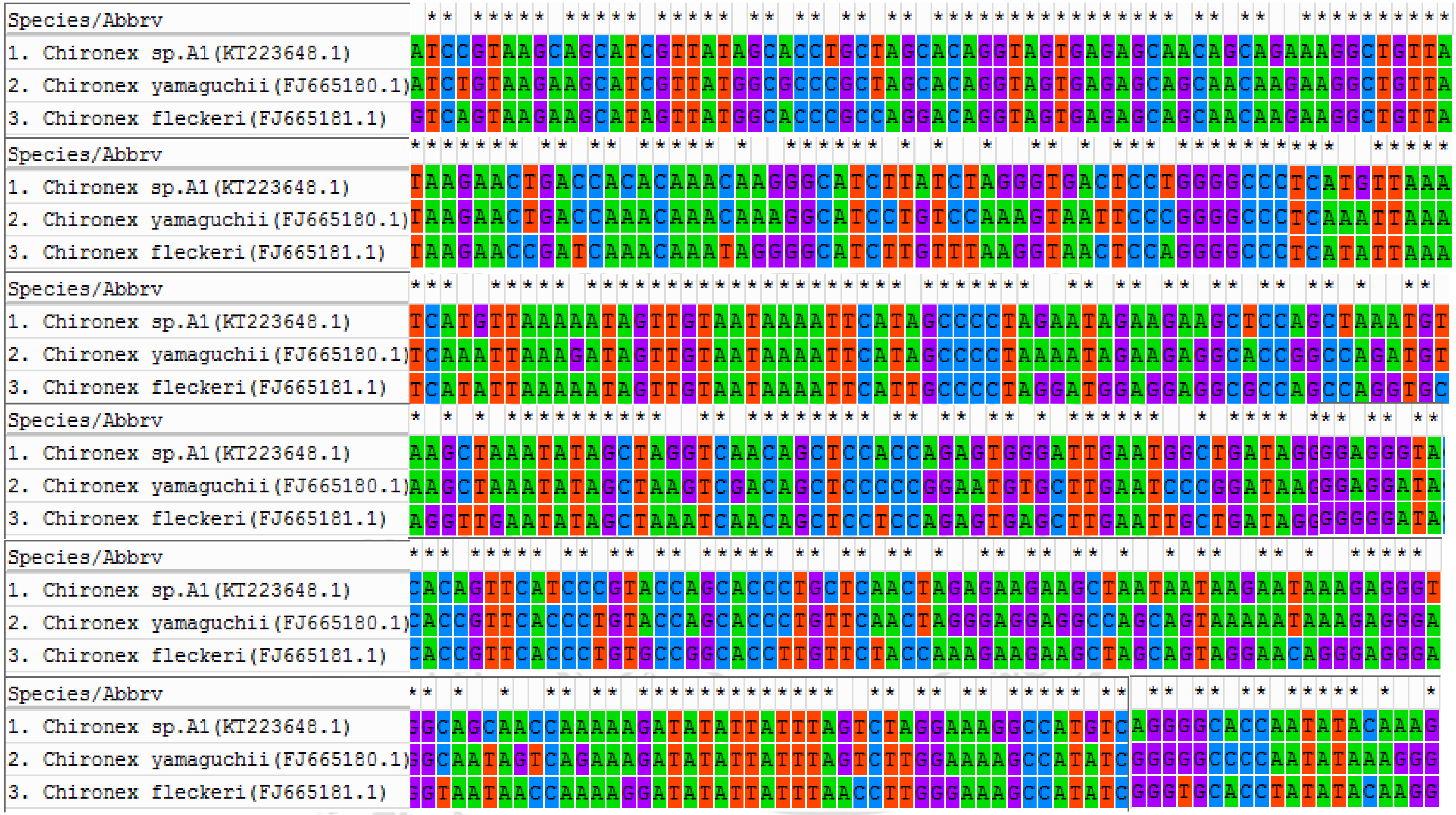


Sequences producing significant alignments:

Select: All None Selected:0

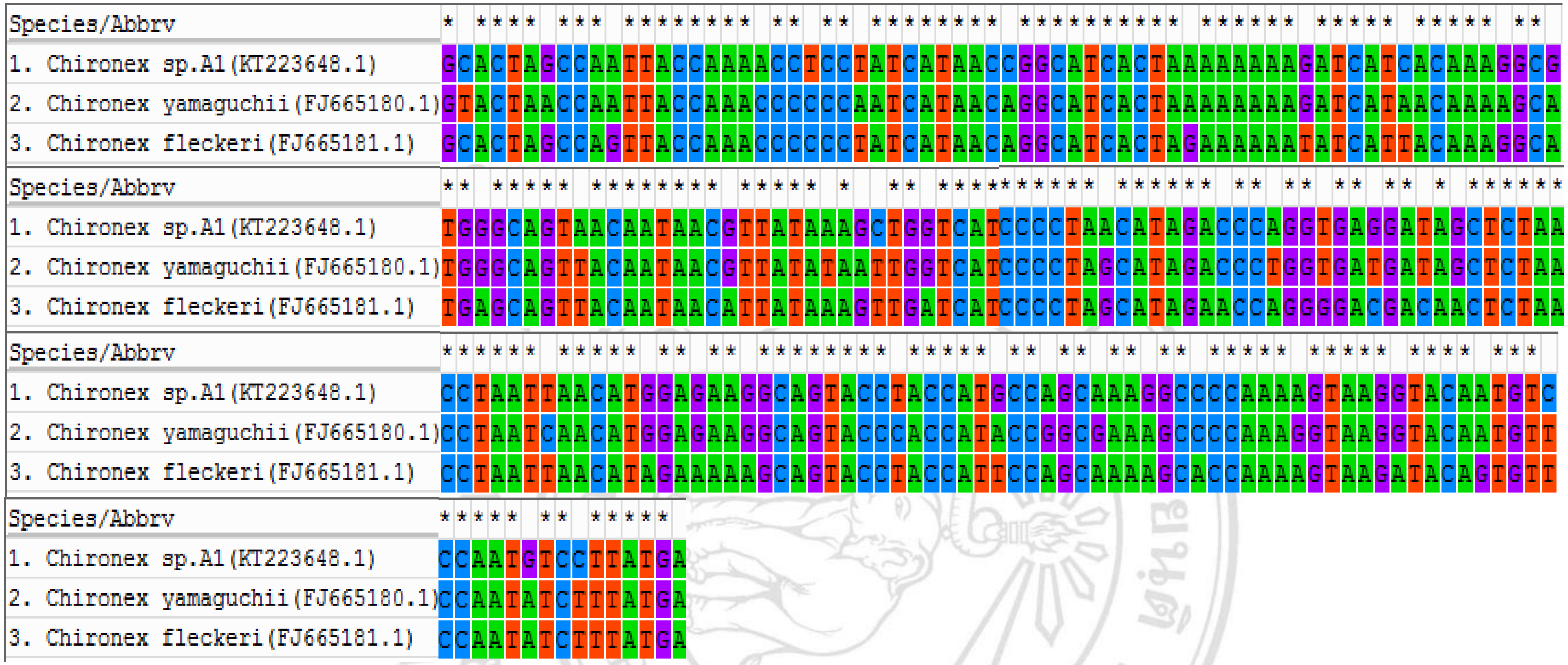
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Chironex yamaguchii voucher USNM:1121555 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	621	621	87%	6e-174	85%	FJ665180.1
☐	Palythoa aff. mutuki ANK-2014 voucher HeSu13 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	348	348	88%	1e-91	77%	KJ776762.1
☐	Palythoa aff. mutuki ANK-2014 voucher LaSu7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	348	348	88%	1e-91	77%	KJ776760.1
☐	Palythoa aff. mutuki ANK-2014 voucher HeWi2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	342	342	85%	5e-90	77%	KJ776765.1
☐	Palythoa aff. mutuki ANK-2014 voucher HeSu19 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	342	342	85%	5e-90	77%	KJ776764.1
☐	Palythoa aff. mutuki ANK-2014 voucher HeSu18 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	342	342	85%	5e-90	77%	KJ776763.1
☐	Palythoa aff. mutuki ANK-2014 voucher LaSu14 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	342	342	85%	5e-90	77%	KJ776761.1
☐	Palythoa aff. mutuki ANK-2014 voucher LaSu1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	342	342	85%	5e-90	77%	KJ776759.1
☐	Parazoanthid sp. 'yellow polyp' mitochondrial CO1 gene for cytochrome oxidase subunit 1, partial cds	339	339	88%	7e-89	76%	AB247358.1
☐	Palythoa tuberculosa voucher HeWi3 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	337	337	88%	2e-88	76%	KJ776768.1

Figure 9.1 Sequences from *Chironex* sp. A1-A4 were compared with NCBI database using BLAST (Basic LocalAlignment Search Tool)



```
Species/Abbrv                       ** ***** ***** ***** ** ** ** ** **************** ** **  **********
1. Chironex sp.A1(KT223648.1)      ATCCGTAAGCAGCATCGTTATAGCACCTGCTAGCACAGGTAGTGAGAGCAACAGCAGAAAGGCTGTTA
2. Chironex yamaguchii(FJ665180.1) ATCTGTAAGAAGCATCGTTATGGCGCCCGCTAGCACAGGTAGTGAGAGCAGCAACAAGAAGGCTGTTA
3. Chironex fleckeri(FJ665181.1)   GTCAGTAAGAAGCATAGTTATGGCACCCGCCAGGACAGGTAGTGAGAGCAGCAACAAGAAGGCTGTTA
Species/Abbrv                      ******* ** ** ***** *  ****** * *  *  ** * *** **********  *****
1. Chironex sp.A1(KT223648.1)      TAAGAACTGACCACACAAACAAGGGCATCTTATCTAGGGTGACTCCTGGGGCCCTCATGTTAAA
2. Chironex yamaguchii(FJ665180.1) TAAGAACTGACCAAACAAACAAAGGCATCCTGTCCAAAGTAATTCCCGGGGCCCTCAAATTAAA
3. Chironex fleckeri(FJ665181.1)   TAAGAACCGATCAAACAAATAGGGGCATCTTGTTTAAGGTAACTCCAGGGGCCCTCATATTAAA
Species/Abbrv                      ***  ***** ******************** *******  ** ** ** ** ** ** *  **
1. Chironex sp.A1(KT223648.1)      TCATGTTAAAAATAGTTGTAATAAAATTCATAGCCCCTAGAATAGAAGAAGCTCCAGCTAAATGT
2. Chironex yamaguchii(FJ665180.1) TCAAATTAAAGATAGTTGTAATAAAATTCATAGCCCCTAAAATAGAAGAGGCACCGGCCAGATGT
3. Chironex fleckeri(FJ665181.1)   TCATATTAAAAATAGTTGTAATAAAATTCATTGCCCCTAGGATGGAGGAGGCGCCAGCCAGGTGC
Species/Abbrv                      * * * **********  ** ******** ** ** ** * ******  * **** *** ** **
1. Chironex sp.A1(KT223648.1)      AAGCTAAATATAGCTAGGTCAACAGCTCCACCAGAGTGGGATTGAATGGCTGATAGGGGAGGGTA
2. Chironex yamaguchii(FJ665180.1) AAGCTAAATATAGCTAAGTCGACAGCTCCCCCGGAATGTGCTTGAATCCCGGATAAGGGAGGATA
3. Chironex fleckeri(FJ665181.1)   AGGTTGAATATAGCTAAATCAACAGCTCCTCCAGAGTGAGCTTGAATTGCTGATAGGGGGGGATA
Species/Abbrv                      *** ***** ** ** ** ***** ** ** ** *  ** ** ** *  * **  ** *  *****
1. Chironex sp.A1(KT223648.1)      CACAGTTCATCCCGTACCAGCACCCTGCTCAACTAGAGAAGAAGCTAATAATAAGAATAAAGAGGGT
2. Chironex yamaguchii(FJ665180.1) CACCGTTCACCCTGTACCAGCACCCTGTTCAACTAGGGAGGAGGCCAGCAGTAAAAATAAAGAGGGA
3. Chironex fleckeri(FJ665181.1)   CACCGTTCACCCTGTGCCGGCACCTTGTTCTACCAAAGAAGAAGCTAGCAGTAGGAACAGGGAGGGA
Species/Abbrv                      ** *  *  ** ** *************  ** ** ** ***** ** ** ** ** ***** *  *
1. Chironex sp.A1(KT223648.1)      GGCAGCAACCAAAAAGATATATTATTTAGTCTAGGAAAGGCCATGTCAGGGGCACCAATATACAAAG
2. Chironex yamaguchii(FJ665180.1) GGCAATAGTCAGAAAGATATATTATTTAGTCTTGGAAAAGCCATATCGGGGGCCCCAATATAAAGGG
3. Chironex fleckeri(FJ665181.1)   GGTAATAACCAAAAGGATATATTATTTAACCTTGGGAAAGCCATATCGGGTGCACCTATATACAAGG
```

Figure 9.2 COI gene alignment of *Chironex yamaguchii* (FJ665180.1), *Chironex fleckeri* (FJ665181.1) and *Chironex* sp. A1 (KT223648.1)



```
Species/Abbrv                     * **** *** ******** ** ** ******** ********** ****** ***** ***** ** 
1. Chironex sp.A1(KT223648.1)     GCACTAGCCAATTACCAAAACCTCCTATCATAACCGGCATCACTAAAAAAAAGATCATCACAAAGGCG
2. Chironex yamaguchii(FJ665180.1)GTACTAACCAATTACCAAACCCCCCAATCATAACAGGCATCACTAAAAAAAAGATCATAACAAAAGCA
3. Chironex fleckeri(FJ665181.1)  GCACTAGCCAGTTACCAAACCCCCCTATCATAACAGGCATCACTAGAAAAAATATCATTACAAAGGCA

Species/Abbrv                     ** ***** ******** ***** *  ** ********** ****** ** ** ** ** * ******
1. Chironex sp.A1(KT223648.1)     TGGGCAGTAACAATAACGTTATAAAGCTGGTCATCCCCTAACATAGACCCAGGTGAGGATAGCTCTAA
2. Chironex yamaguchii(FJ665180.1)TGGGCAGTTACAATAACGTTATATAATTGGTCATCCCCTAGCATAGACCCTGGTGATGATAGCTCTAA
3. Chironex fleckeri(FJ665181.1)  TGAGCAGTTACAATAACATTATAAAGTTGATCATCCCCTAGCATAGAACCAGGGGACGACAACTCTAA

Species/Abbrv                     ****** ***** ** ** ******** ***** ** ** ** ** ***** ***** **** *** 
1. Chironex sp.A1(KT223648.1)     CCTAATTAACATGGAGAAGGCAGTACCTACCATGCCAGCAAAGGCCCCAAAAGTAAGGTACAATGTC
2. Chironex yamaguchii(FJ665180.1)CCTAATCAACATGGAGAAGGCAGTACCCACCATACCGGCGAAAGCCCCAAAGGTAAGGTACAATGTT
3. Chironex fleckeri(FJ665181.1)  CCTAATTAACATAGAAAAAGCAGTACCTACCATTCCAGCAAAAGCACCAAAAGTAAGATACAGTGTT

Species/Abbrv                     ***** ** ***** 
1. Chironex sp.A1(KT223648.1)     CCAATGTCCTTATGA
2. Chironex yamaguchii(FJ665180.1)CCAATATCTTTATGA
3. Chironex fleckeri(FJ665181.1)  CCAATATCTTTATGA
```

Figure 9.2 COI gene alignment of *Chironex yamaguchii* (FJ665180.1), *Chironex fleckeri* (FJ665181.1) and *Chironex* sp. A1 (KT223648.1) (continue)

Table 5 Localities and genetic variation among *Chironex* sp. A1-A4 of COI gene (671 bp)

Localities	Specimens	Identity (%)	Nucleotide differences at position					
			5	167	473	663	666	667
Chao Lao beach, Chanthaburi	*Chironex* sp. A1	100	G	C	A	A	G	C
Suan Son beach, Rayong	*Chironex* sp. A2	99.25	A	T	●	T	C	T
Suan Son beach, Rayong	*Chironex* sp. A3	99.55	A	T	G	●	●	●
Pha Ngan island, Surat Thani	*Chironex* sp. A4	99.85	●	T	●	●	●	●

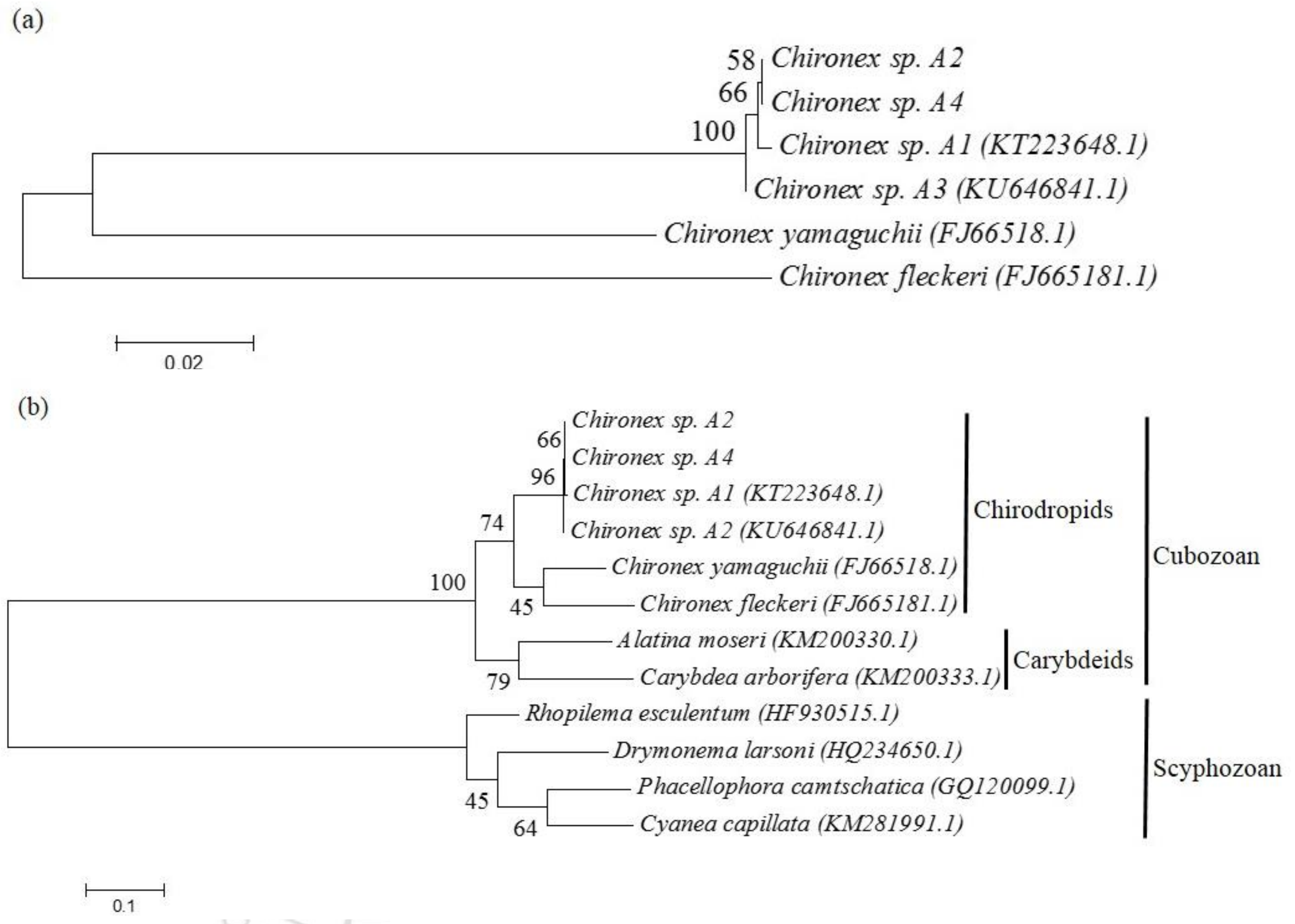
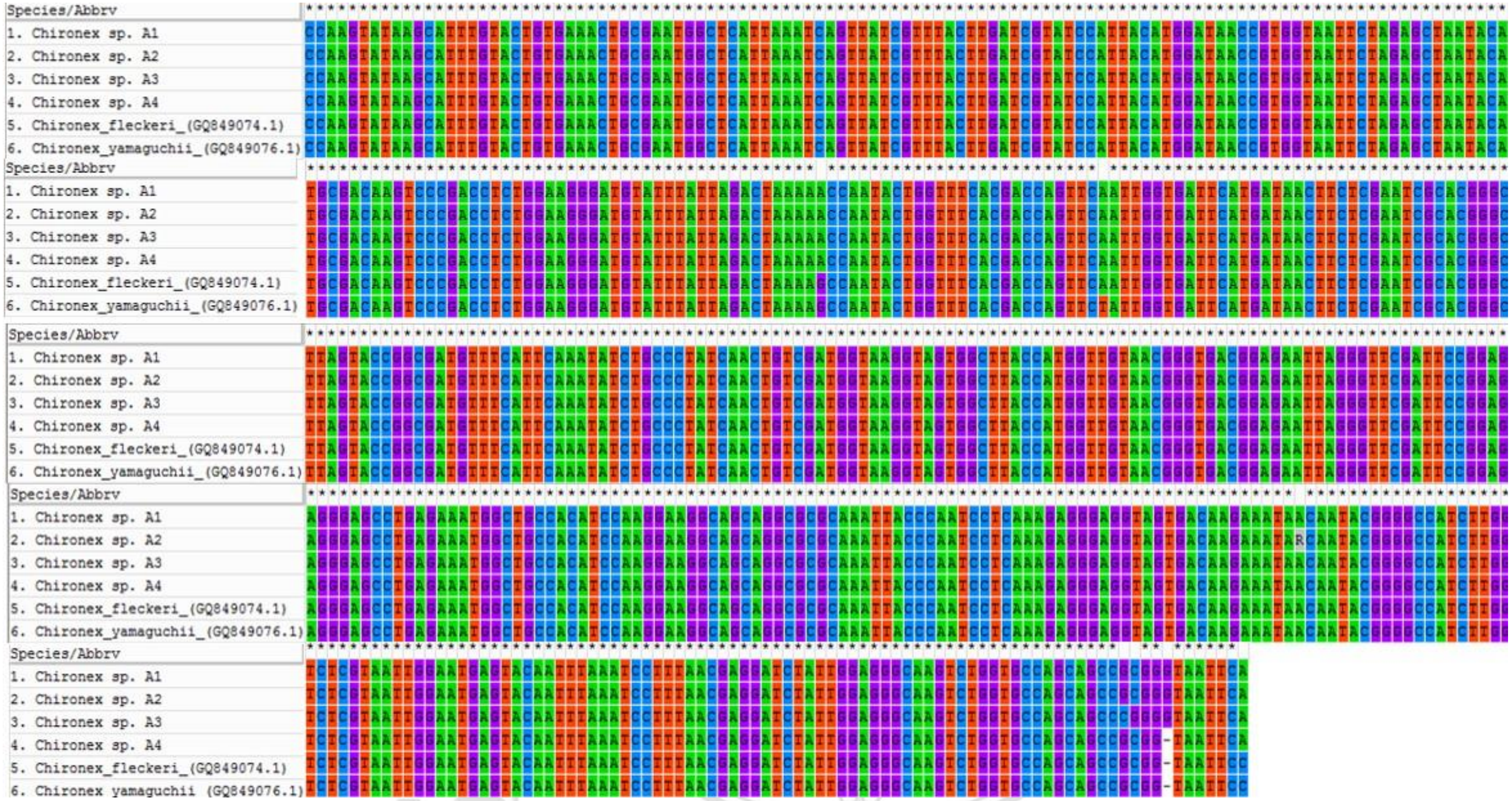


Figure 9.3 (a): Phylogenetic tree of genus *Chironex* based on mt-COI gene; (b): Phylogeny of members from scyphozoa and cubozoa based on mt-COI gene.

For the sequence from 18S rRNA, a sequence from *Chironex* sp. A1 was added into NCBI database, the accession number is KU097000.1. Sequences data from *Chironex yamaguchii* (GQ849076.1), *Chironex fleckeri* (GQ849074.1) and *Chironex* sp. A1-A4 were calculated. The variation among congener calculated by MEGA 6 was less than 1% (Figure 10.1).

Evolutionary trees were constructed by Maximum likelihood and Kimura 2-parameter model. Similar to the result from COI gene, all *Chironex* sp. A samples were the monophyletic clade with the root of other Chirodropids (Figure 10.2).



```
Species/Abbrv                        ***************************************************************************************************************
1. Chironex sp. A1                   CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
2. Chironex sp. A2                   CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
3. Chironex sp. A3                   CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
4. Chironex sp. A4                   CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
5. Chironex_fleckeri_(GQ849074.1)    CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
6. Chironex_yamaguchii_(GQ849076.1)  CCAAGTATAAGCATTTGTACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTACTTGATCGTATCCATTACATGGATAACCGTGGTAATTCTAGAGCTAATACA
Species/Abbrv                        *********************************************** ************************** ************************************
1. Chironex sp. A1                   TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACTGGTTTCACGACCAGTTCAATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
2. Chironex sp. A2                   TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACTGGTTTCACGACCAGTTCAATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
3. Chironex sp. A3                   TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACTGGTTTCACGACCAGTTCAATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
4. Chironex sp. A4                   TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAACCAATACTGGTTTCACGACCAGTTCAATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
5. Chironex_fleckeri_(GQ849074.1)    TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAGCCAATACTGGTTTCACGACCAGTTCAATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
6. Chironex_yamaguchii_(GQ849076.1)  TGCGACAAGTCCCGACCTCTGGAAGGGATGTATTTATTAGACTAAAAGCCAATACTGGTTTCACGACCAGTTCTATTGGTGATTCATGATAACTTCTCGAATCGCACGGGC
Species/Abbrv                        ***************************************************************************************************************
1. Chironex sp. A1                   TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
2. Chironex sp. A2                   TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
3. Chironex sp. A3                   TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
4. Chironex sp. A4                   TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
5. Chironex_fleckeri_(GQ849074.1)    TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
6. Chironex_yamaguchii_(GQ849076.1)  TTAGTACCGGCGATGTTTCATTCAAATATCTGCCCTATCAACTGTCGATGGTAAGGTAGTGGCTTACCATGGTTGTAACGGGTGACGGAGAATTAGGGTTCGATTCCGGAG
Species/Abbrv                        ******************************************************************************************* *******************
1. Chironex sp. A1                   AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATAACAATACGGGGCCATCTTGG
2. Chironex sp. A2                   AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATARCAATACGGGGCCATCTTGG
3. Chironex sp. A3                   AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATAACAATACGGGGCCATCTTGG
4. Chironex sp. A4                   AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATAACAATACGGGGCCATCTTGG
5. Chironex_fleckeri_(GQ849074.1)    AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATAACAATACGGGGCCATCTTGG
6. Chironex_yamaguchii_(GQ849076.1)  AGGGAGCCTGAGAAATGGCTGCCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTCAAAGAGGGAGGTAGTGACAAGAAATAACAATACGGGGCCATCTTGG
Species/Abbrv                        ***************************************************************************  ** ******
1. Chironex sp. A1                   TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGGTAATTCA
2. Chironex sp. A2                   TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGGTAATTCA
3. Chironex sp. A3                   TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGGGTAATTCA
4. Chironex sp. A4                   TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG-TAATTCA
5. Chironex_fleckeri_(GQ849074.1)    TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG-TAATTCC
6. Chironex yamaguchii (GQ849076.1)  TCTCGTAATTGGAATGAGTACAATTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG-TAATTCC
```

Figure 10.1 18S gene alignment of *Chironex* spp. by MEGA 6

]

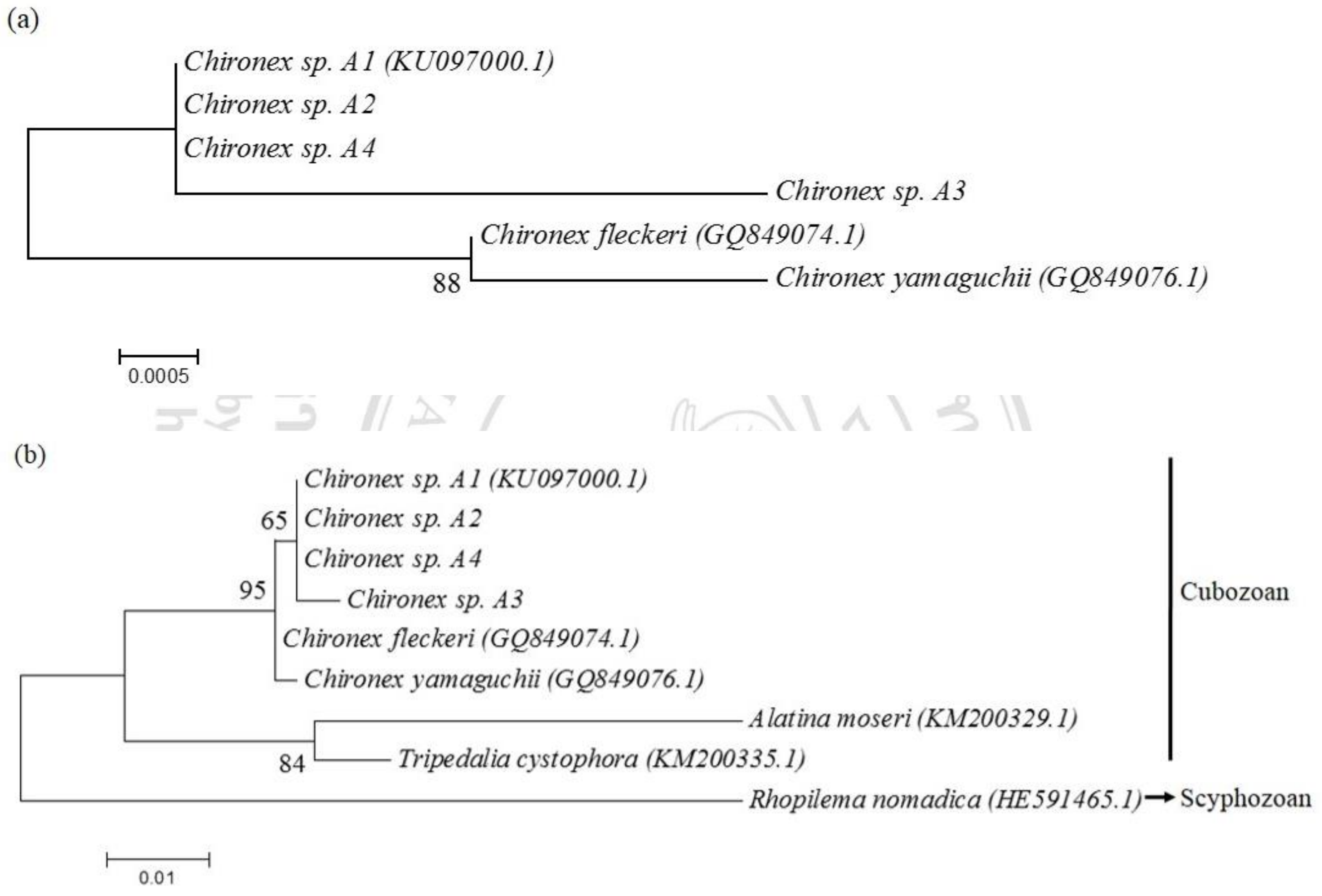
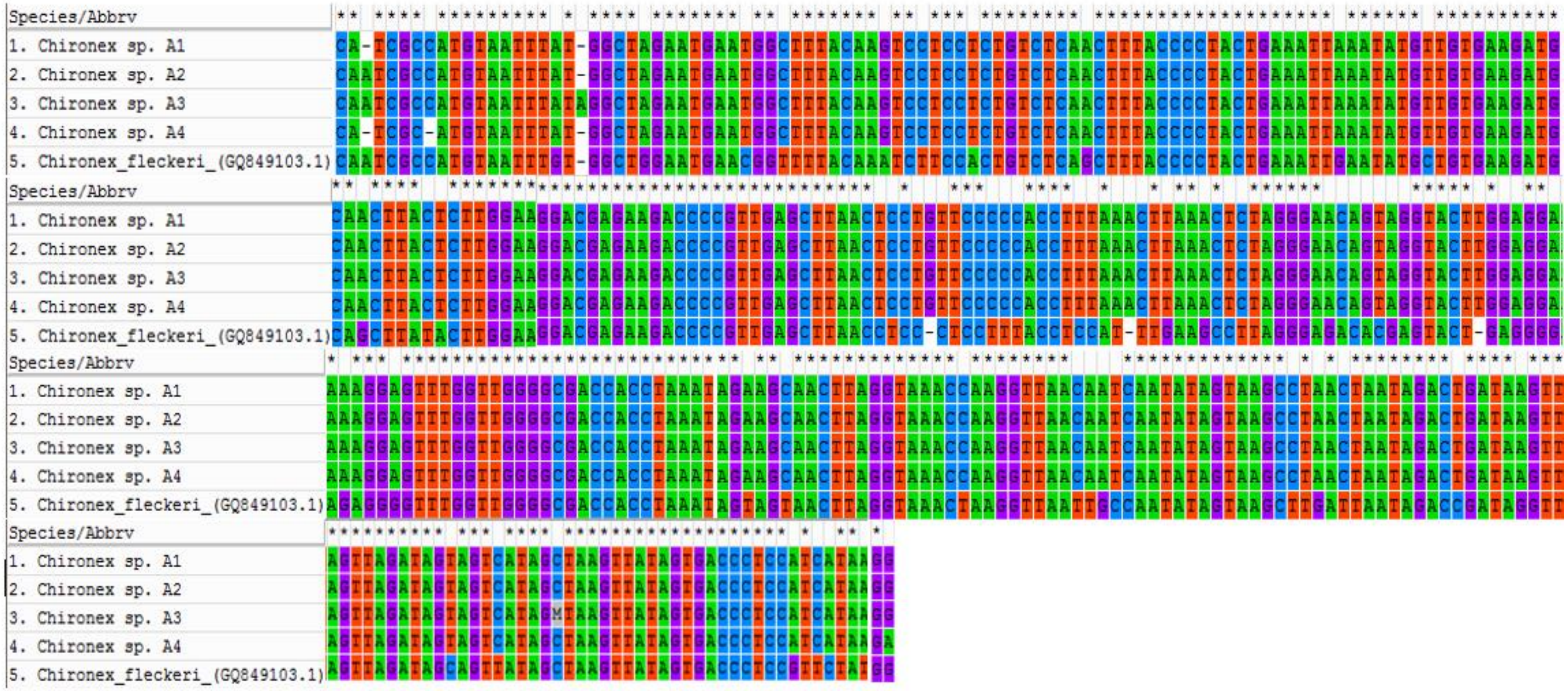


Figure 10.2 (a): Phylogenetic tree of genus *Chironex* based on 18S rRNA; (b): Phylogeny of members from scyphozoa and cubozoa based on 18S rRNA

For 16S sequences, sequences from *Chironex yamaguchii* (from Dr. Allen Collins by personal contact), *Chironex fleckeri* (GQ849103.1) and *Chironex* sp. A1 (KX065499) *Chironex* sp. A4 were computed by MEGA 6. Genetic difference between *Chironex fleckeri* and *Chironex* sp. A1 was 17% while the variation within Chironex sp. A1-A4 was relatively low (0.0%) (Figure 11.1).

Phylogeny based on 16S gene were constructed using Maximum likelihood and Kimura 2-parameter model. According to the trees, *Chironex* sp. A1-A4 were separated from *Chironex fleckeri*. Furthermore, the result revealed that *Chironex* sp. A1- A4 samples, were the monophyletic clade with the root of other chirodropids and separated from other scyphozoan (Figure 11.2).



```
Species/Abbrv                     ** **** ********* * **** ******* ** ******* ** *** ******** ******************* ****** **********
1. Chironex sp. A1                CA-TCGCCATGTAATTTAT-GGCTAGAATGAATGGCTTTACAAGTCCTCCTCTGTCTCAACTTTACCCCTACTGAAATTAAATATGTTGTGAAGATG
2. Chironex sp. A2                CAATCGCCATGTAATTTAT-GGCTAGAATGAATGGCTTTACAAGTCCTCCTCTGTCTCAACTTTACCCCTACTGAAATTAAATATGTTGTGAAGATG
3. Chironex sp. A3                CAATCGCCATGTAATTTATAGGCTAGAATGAATGGCTTTACAAGTCCTCCTCTGTCTCAACTTTACCCCTACTGAAATTAAATATGTTGTGAAGATG
4. Chironex sp. A4                CA-TCGC-ATGTAATTTAT-GGCTAGAATGAATGGCTTTACAAGTCCTCCTCTGTCTCAACTTTACCCCTACTGAAATTAAATATGTTGTGAAGATG
5. Chironex_fleckeri_(GQ849103.1) CAATCGCCATGTAATTTGT-GGCTGGAATGAACGGTTTTACAAATCTTCCACTGTCTCAGCTTTACCCCTACTGAAATTGAATATGCTGTGAAGATG
Species/Abbrv                     ** ****  **********************************  *   ***   ****  *   * ** *  ******       ***** *  **
1. Chironex sp. A1                CAACTTACTCTTGGAAGGACGAGAAGACCCCGTTGAGCTTAACTCCTGTTCCCCCACCTTTAAACTTAAACTCTAGGGAACAGTAGGTACTTGGAGGA
2. Chironex sp. A2                CAACTTACTCTTGGAAGGACGAGAAGACCCCGTTGAGCTTAACTCCTGTTCCCCCACCTTTAAACTTAAACTCTAGGGAACAGTAGGTACTTGGAGGA
3. Chironex sp. A3                CAACTTACTCTTGGAAGGACGAGAAGACCCCGTTGAGCTTAACTCCTGTTCCCCCACCTTTAAACTTAAACTCTAGGGAACAGTAGGTACTTGGAGGA
4. Chironex sp. A4                CAACTTACTCTTGGAAGGACGAGAAGACCCCGTTGAGCTTAACTCCTGTTCCCCCACCTTTAAACTTAAACTCTAGGGAACAGTAGGTACTTGGAGGA
5. Chironex_fleckeri_(GQ849103.1) CAGCTTATACTTGGAAGGACGAGAAGACCCCGTTGAGCTTAACCTCC-CTCCTTTACCTCCAT-TTGAAGCCTTAGGGAGACACGAGTACT-GAGGGG
Species/Abbrv                     * *** *************************** ** ************* ********    ************* * * ******** **** ***
1. Chironex sp. A1                AAAGGAGTTTGGTTGGGGCGACCACCTAAATAGAAGCAACTTAGGTAAACCAAGGTTAACAATCAATATAGTAAGCCTAACTAATAGACTGATAAGTT
2. Chironex sp. A2                AAAGGAGTTTGGTTGGGGCGACCACCTAAATAGAAGCAACTTAGGTAAACCAAGGTTAACAATCAATATAGTAAGCCTAACTAATAGACTGATAAGTT
3. Chironex sp. A3                AAAGGAGTTTGGTTGGGGCGACCACCTAAATAGAAGCAACTTAGGTAAACCAAGGTTAACAATCAATATAGTAAGCCTAACTAATAGACTGATAAGTT
4. Chironex sp. A4                AAAGGAGTTTGGTTGGGGCGACCACCTAAATAGAAGCAACTTAGGTAAACCAAGGTTAACAATCAATATAGTAAGCCTAACTAATAGACTGATAAGTT
5. Chironex_fleckeri_(GQ849103.1) AGAGGGGTTTGGTTGGGGCGACCACCTAAATAGTAGTAACTTAGGTAAACTAAGGTTAATTGCCAATATAGTAAGCTTGATTAATAGACCGATAGGTT
Species/Abbrv                     ********** *** **** ******************* *  ** *
1. Chironex sp. A1                AGTTAGATAGTAGTCATAGCTAAGTTATAGTGACCCTCCATCATAAGG
2. Chironex sp. A2                AGTTAGATAGTAGTCATAGCTAAGTTATAGTGACCCTCCATCATAAGG
3. Chironex sp. A3                AGTTAGATAGTAGTCATAGMTAAGTTATAGTGACCCTCCATCATAAGG
4. Chironex sp. A4                AGTTAGATAGTAGTCATAGCTAAGTTATAGTGACCCTCCATCATAAGA
5. Chironex_fleckeri_(GQ849103.1) AGTTAGATAGCAGTTATAGCTAAGTTATAGTGACCCTCCGTTCTATGG
```

Figure 11.1 Alignment of *Chironex* members based on 16S gene

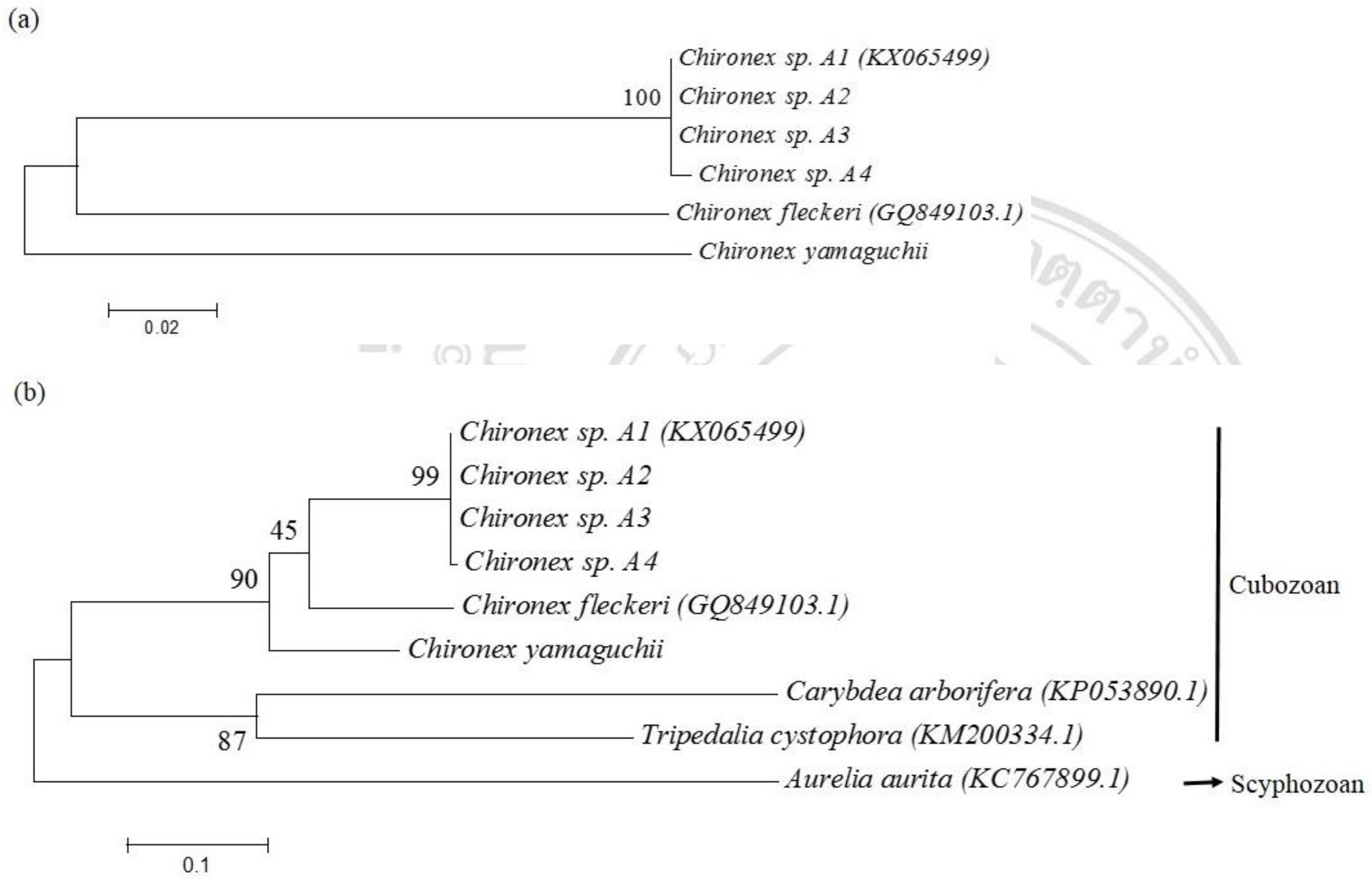


Figure 11.2 (a): Phylogeny of genus *Chironex* based on 16S rRNA; (b): Phylogeny of members from scyphozoa and cubozoa based on 16S rRNA

DICHOTOMOUS KEY TO THAI CUBOZOA

1A. Cubomedusae with more than one tentacle arise from each corner of the bell ……....4

1B. Cubomedusae with only one tentacle arise from each corner of the bell……………..2

2A. Frown-shaped rhopalial niche ostium, prominent thorn pedalial canal bend, exumbrella nematocyst warts present…………………...………………………..3

2B. Orange and brown tentacles, bright orange gonads, exumbrella nematocyst warts absent, phacellae present………….……………………………..*Carybdea sivickisi*

3A. Phacellae absent, 2 eyes per rhopalium, pedalial lappets present…..*Morbakka fenneri*

3B. Phacellae present, 6 eyes per rhopalium, pedalial lappets absent...*Tamoya haplonema*

4A. Each corner bearing 3 tentacles, frown shaped rhopalial niche ostium, gastric saccule absent, perradial lappet absent, exumbrella nematocyst present …………………. ………………………………………..…….……………..*Tripedalia cystophora*

4B. Each corner bearing more than 3 tentacles, gastric saccule present, perradial lappets present, exumbrella nematocyst absent………………….……….…………………..5

5A. Finger-shaped gastric saccule, unilaterally branched pedalia, Thorn shaped pedalial canal, each side of the corner bearing 5-6 tentacles…..….*Chiropsoides buitendijki*

5B. Cock's comb shaped gastric saccule, claw-like pedalia, doubly uniserial branched pedalia…………………………………………...6

6A. Volcano shaped pedalial canal bend, 5-9 tentacles arise from the rim …..…………………………………………………...*Chironex yamaguchii*

6B. More than 11 tentacles arise from each corner of the bell……………………….…...7

7A. Upward-pointing thorn pedalial canal bend, up to 15 tentacles arise from each corner of the bell……………………………...……….…………………..*Chironex fleckeri*

7B. Dome shaped pedalial canal, 11-12 tentacle arise from each corner of the bell………. ……………………..…………………………………...……*Chironex* sp. A1-A4