Genetic Variation among Thai Dugong (*Dugong dugon*) Populations from Cytochrome *C* Oxidase Subunit 1 DNA Sequence Data

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Abstract:

Dugong (*Dugong dugon*) was classified as vulnerable and currently considered rare over most of this range. The population of dugong in Thailand was found in fragmented habitats along the Thai coast. Genetic studies were used in this study to determine the variation of cytochrome c oxidase subunit 1 (COI) DNA sequence of ten dugong samples from Krabi, Phuket, Trang and Satun Province. The DNA extraction result showed that the samples stored in ethanol can maintain the integrity of the genetic material for a long time. The length of COI sequence was 794 to 859 bp and the average length was 823.4 bp. All COI sequences in this study were identical to GenBank accession numbers AY075116.1 which have an average of 91.34%. The lowest genetic distance (0.005) was between dugong 3 and dugong 4, while the highest (0.163) was between dugong 6 and dugong 10 with an average of 0.070. The low genetic distance demonstrated that the gene flow between the dugong population in each area still occurred. The dendrogram constructed from COI sequence comparisons using the Maximum Likelihood method based on the Tamura-Nei model divided ten dugong samples into three subgroups. The major group comprised of seven dugong samples which were collected around Koh Libong and the coastal areas of Trang Province. The clustering corresponds to the area with the largest and abundant seagrass.

Keywords: Dugong, *Dugong dugon*, Genetic variation, Cytochrome C oxidase, COI

Introduction

The dugong (*Dugong dugon*) is unique among mammals in that it is the only fully marine herbivore. It is a member of the genus Dugong, which is the only extant member of the family Dugongidae [1]. The Dugongidae is one of two families in the order Sirenia, the other one is the Trichechidae. All members of the Trichechidae, *Trichechus manatus*, *T. inunguis* and *T. senegalensis*, require fresh water to survive while the dugong is exclusively marine [2]. Dugongs were classified as the most endangered among CITES-listed according to Appendix I of CITES (Convention of International Trade in Endangered Species of Wild Flora and Fauna) [3]. The main causes of the decreasing in dugong populations are inadvertent capture and intentional collision with ships, habitat degradation, hunting for meat or medicinal purposes and slow rate of reproduction.

Thailand has long coastlines of some 2,600 km facing the South China Sea to the east and the Andaman Sea on the west side [4] that is rich in biodiversity. Dugongs were classified as wildlife preservation and were protected in accordance with the Thai Fisheries Act since 1947 [5]. Although the dugong hunting is illegal, the population of both the Andaman Sea and the Gulf of Thailand had declined drastically. In 2017, it is believed that there are no more than 200 dugongs in the Thai waters. Most of them, 130-150, are found in Hat Chao Mai National Marine Park and Mu Ko Libong Non-Hunting Area in Trang province where seagrass is still fertile. Moreover, about 15 are found around Ko Samet islands of Rayong and some areas in Chon Buri, Chanthaburi and Trat provinces, and about 10 others in the Chaiya Bay of Surat Thani [6]. Therefore, data supporting and developing conservation strategies are extremely important to the dugong population in Thailand. Currently, bioinformatics is essential for the manipulation of biological data involving genetic resources conservation, remodeling phylogenetic, assessment of gene

dispersal and search of genomic markers. These genetic techniques can infer parameters like the population structure and movement of their genes. An understanding of the levels of genetic diversity within a population will allow for an understanding of the genetic stability of the population. Higher levels of genetic diversity may allow populations to adapt to environmental changes more efficiently than populations with little genetic diversity [7]. The resulting non-adaptive variation will lead to the greater susceptibility of a population to further environmental change [8].

The analysis of sequence data is one of the bioinformatics used for population studies and measuring genetic diversity is. The most commonly gene used for these approaches is the cytochrome *c* oxidase gene. The cytochrome *c* oxidase plays a central role in the metabolism of eukaryotic aerobic organisms. It is a key enzyme in the electron transport chain which located in the inner mitochondrial membrane. It consists of several subunits, and the catalytic cytochrome *c* oxidase subunit 1 (COI) is encoded in the mitochondrial genome [9]. Due to its mutation rate is fast enough to distinguish closely related species, the COI gene is suitable for the comparative studies of genetic variation among dugong populations. In this study, we amplified and sequenced the COI gene of Thai dugong populations for its genetic variation and phylogenetic analysis. The information obtained may be used to consider that populations of dugong found in Thailand should be managed as connected or as separate stocks and additionally elucidate population structure of dugongs in the region.

Materials and methods

Dugong tissue samples (N = 10) were collected at four localities in the Andaman Sea (Krabi, Phuket, Trang and Satun) by responding to reports of dead specimens (Table 1). Tissue samples were stored in ethanol and then maintained at the Phuket Marine Biological Center from 2010-2017.

Genomic DNA was extracted from dehydrated tissue by phenol-chloroform method with minor modification. This protocol starts with 10 mg of tissue being soaked and washed three times with distilled water. Then the tissue was ground in the isolation buffer (0.075 M NaCl, 0.025 M EDTA, 0.5% SDS) and incubated with protease K at 65ºC overnight. Chloroform was then used to extract the proteins from the digested tissue. DNA precipitate was washed two times with 70% ethanol. The remaining DNA pellet was air-dried and resuspended in TE buffer (10 mM Tris, 1 mM EDTA).

Table 1 Dugong samples in this study, their observation area and date.

Sample No.	Collection Area	Latitude	Longitude	Collection date
1	Ban Phra Muang, Naklua, Kantang, Trang	7.2977300	99.439820	Jan 22, 2010
2	Ao Prao, Koh Libong, Kantang, Trang	7.2634300	99.409940	Jan 24, 2010
3	Koh Nok, Koh Libong, Kantang, Trang	7.2660500	99.464090	Nov 14, 2010
4	Toh Chai Cape, Koh Libong, Kantang, Trang	7.2827500	99.384910	Mar 26, 2011
5	Koh Luk Mai, Koh Libong, Kantang, Trang	7.2514600	99.455900	Apr 11, 2011
6	Thungbulang, Thungbulang, Thunwa, Satun	7.0249900	99.672680	Sep 11, 2011
7	Ao Prao, Koh Libong, Kantang, Trang	7.2634300	99.409940	Dec 23, 2011
8	Yamu Cape, Pa Klok, Maung, Phuket	7.9922600	98.422170	Nov 6, 2012
9	Had Yao Beach, Koh Libong, Kantang, Trang	7.3122310	99.387595	Jul 7, 2013
10	Loh Dalum Beach, Ao Nang, Maung, Krabi	7.7405610	98.770677	Jun 18, 2017

A partial region of COI was amplified using a set of specific primers with sequences as follows: COI-F3, 5'-CCT GCA GGA GGA GGA GGA GAG CC-3' and COI-R3, 5'-AGT ATA AGC GTC TGG GTA GTC-3' [10]. The total volume of 25 uL contains 1X PCR buffer, 0.4 mM dNTP, 2.0 mM $MgCl_2$, 0.25 uM forward primer, 0.25 uM reverse primer, 0.5 unit *Taq* polymerase (Vivantis) and 20 ng DNA template. PCR amplification was performed with the following program: pre-PCR incubation at 95ºC for 15 min, 35 cycles of 95ºC for 20 s, annealing at 60ºC for 45 s, and extension at 72ºC for 30 s, with a final extension at 72ºC for 5 min. PCR products were electrophoresed in 1.5% agarose gels in 1 × TAE buffer. The gel was stained with

ethidium bromide for 30 minutes and then visualized under UV light. For sequencing, PCR products were excised from a 1.5% agarose gel and purified using a PureLink Quick Gel Extraction Kit (Invitrogen) following the manufacturer's instructions. The purified DNA fragments were sequenced on an ABI Prism 3730 automatic sequencer (Gibthai Co., Ltd) using both forward (COI-F3) and reverse primers (COI-R3).

Genetic variations were estimated from COI sequences by using the Maximum Likelihood method based on the Tamura-Nei model [11]. The dendrogram was drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There was a total of 891 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 software [12].

Results and discussion

The extracted DNA fragment by the modified phenol-chloroform method has a large genomic band and locate at the same size as the genomic DNA of the Lambda / *Hin*DIII marker (Figure 1A). Spectrophotometer results showed that the quality of the extracted DNA was good and can be used to amplify the COI gene by polymerase chain reaction (data not showed). This indicates that the samples stored in ethanol can maintain the integrity of the genetic material for a long time, even if collected from dead samples.

Amplification of the COI gene located at approximately size 800 bp on 1.5% agarose gel (Figure 1B) in all the dugong accessions used in this study. The sequencing was carried out 3 times for each sample. The results showed that the nucleotide sequence of most samples was not different. Some examples are slightly different (data not showed). This difference occurs only at the end of the nucleotide sequence, which may be caused by the limitations of the nucleotide sequence analysis system. The sequencing errors can occur from a large number of nucleotides being sequenced and higher error rate at the ends of sequenced [13]. The similarity of these COI sequences compared to the GenBank database by Blastn found that all of them were identical to accession number AY075116.1 with 90.56 -91.59 % Identical (Table 2). These accession number was the *Dugong dugon* mitochondrion, complete genome.

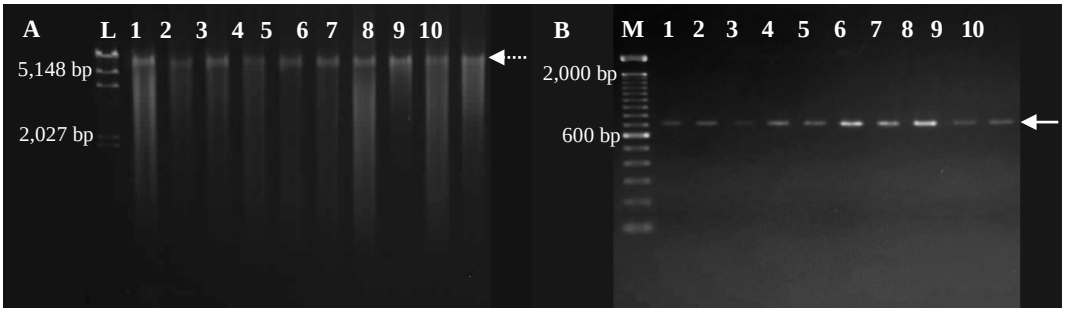
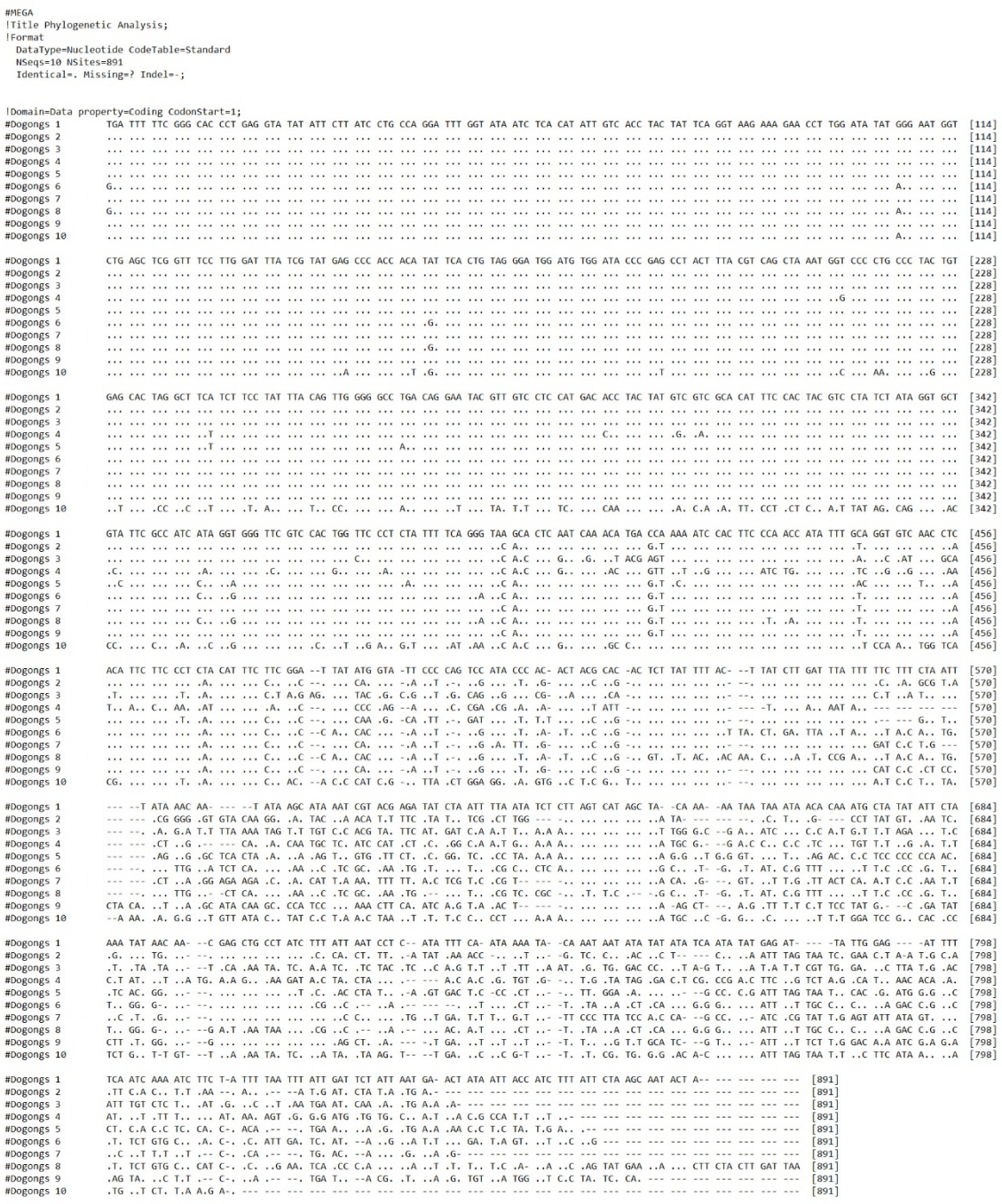


Figure 1 Extracted DNA (A) and amplification products of the COI gene (B) from ten samples of Thai dugong. A 1-10 number of dugong accessions were shown in Table 1. The dash arrow indicates genomic DNA band. The arrow indicates COI amplified fragment. L was Lambda / *Hin*DIII marker and M was a 1kb DNA ladder marker.

The COI sequences of the ten dugong samples were aligned and resulted in 891 positions with 466 variable sites (52.30%) as show in Figure 2. The alignment result indicated that each sample has a different length of the COI sequence. The length was 794 to 859 bp and the average length was 823.4 bp. The COI gene has an average CG content of 39.05%. The highest was sample dugong No. 2 and the lowest was dugong No. 1 with CG content of 40.13% and 38.07%, respectively. The AT content of the COI sequence is high across the dugong sample, it does not have a simple explanation but often use the transcription hypothesis of codon usage as an explanation instead [14]. The cell has a high availability of ATP and relatively low availability of the other three rNTPs, so the transcription efficiency can be increased by using A in the third codon position of the protein encoding gene [15]. The third codon position, the usage tends toward A or U

because not only do they pair well with optimal codons, they also cloud pair with other synonymous codons. On the other hand, if the third codon base is G or C, they cannot completely pair with optimal codons [14].

The genetic distance matrix is showed in Table 3. The average genetic distance between the ten dugong samples from the Kimura 2-parameter model was 0.070. The minimum value was between dugong 3 and dugong 4, while the maximum value was between dugong 6 and dugong 10 with genetic distance value 0.005 and 0.163, respectively. The minimum and maximum genetic distance between the specimens of the dugong corresponds to the location of the collecting area. Distance from the location of dugong 6 to dugong 10 is approximately 125 kilometers apart, while dugong 3 and dugong 4 are only about 9 kilometers away. Close distance makes it easy to communicate with each other. The gene flow between populations is always occurring, resulting in minor variations in population genetics. Gene flow within a population can increase the genetic variation of the population, whereas gene flow between genetically distant populations can reduce the genetic difference between the populations [16]. For dugong 6 and dugong 10, geographical isolation could increase opportunities for genetic divergence. A similar result was reported by Bushell (2013) [17], which found a strong genetic differentiation between the dugong populations form the Gulf of Thailand and the North Andaman Sea and between the Gulf of Thailand and Trang Province through pairwise comparison of microsatellite alleles. However, there is no significant difference between Trang Province and the North Andaman Sea populations.



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#MEGA
!Title Phylogenetic Analysis;
!Format
  DataType=Nucleotide CodeTable=Standard
  NSeqs=10 NSites=891
  Identical=. Missing=? Indel=-;

!Domain=Data property=Coding CodonStart=1;
#Dogongs 1        TGA TTT TTC GGG CAC CCT GAG GTA TAT ATT CTT ATC CTG CCA GGA TTT GGT ATA ATC TCA CAT ATT GTC ACC TAC TAT TCA GGT AAG AAA GAA CCT TGG ATA TAT GGG AAT GGT  [114]
#Dogongs 2        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 3        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 4        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 5        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 6        G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ...  [114]
#Dogongs 7        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 8        G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ...  [114]
#Dogongs 9        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [114]
#Dogongs 10       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ...  [114]

#Dogongs 1        CTG AGC TCG GTT TCC TTG GAT TTA TCG TAT GAG CCC ACC ACA TAT TCA CTG TAG GGA TGG ATG TGG ATA CCC GAG CCT ACT TTA CGT CAG CTA AAT GGT CCC CTG CCC TAC TGT  [228]
#Dogongs 2        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 3        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 4        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ...  [228]
#Dogongs 5        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 6        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 7        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 8        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 9        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]
#Dogongs 10       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [228]

#Dogongs 1        GAG CAC TAG GCT TCA TCT TCC TAT TTA CAG TTG GGG GCC TGA CAG GAA TAC GTT GTC CTC CAT GAC ACC TAC TAT GTC GTC GCA CAT TTC CAC TAC GTC CTA TCT ATA GGT GCT  [342]
#Dogongs 2        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 3        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 4        ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... .G. .A. ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 5        ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 6        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 7        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 8        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 9        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [342]
#Dogongs 10       ..T ... .CC ..C ..T ... .T. A.. ... T.. CC. ... ... A.. ... ..T ... TA. T.T ... TC. ... CAA ... ... .A. C.A .A. TT. CCT .CT C.. A.T TAT AG. CAG ... .AC  [342]

#Dogongs 1        GTA TTC GCC ATC ATA GGT GGG TTC GTC CAC TGG TTC CCT CTA TTT TCA GGG TAA GCA CTC AAT CAA ACA TGA CCA AAA ATC CAC TTC CCA ACC ATA TTT GCA GGT GTC AAC CTC  [456]
#Dogongs 2        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C A.. ... ... ... ... ... ... ... G.T ... ... ... ... ... ... ... ... ..T. ... ... ... ..A  [456]
#Dogongs 3        ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... ..C A.C ... G.. .G. ..T ACG AGT ... ... ... ... ... ... ... ... ... .A. ..C .AT ... GCA  [456]
#Dogongs 4        .C. ... ... ... .A. ... ... ... .C. ... ... G.. ... .A. ... ... ... ... ... ..C A.C ... G.. ... .AC ... GTT ..T ..G ... ... ATC TG. ... ... .TC ..G ..G ... .AA  [456]
#Dogongs 5        ..C ... ... ... C.. ..A ... ... ... ... ... ... ... .A. ... ... ... ... ... ..C A.. ... ... ... ... ... ... G.T .C. ... ... ... ... ... ... ... .AC ... ... T.. ..A  [456]
#Dogongs 6        ... ... ... ... C.. ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ..C A.. ... ... ... ... ... ... G.T ... ... ... ... ... ... ... .T. ... ... ... ..A  [456]
#Dogongs 7        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C A.. ... ... ... ... ... ... G.T ... ... ... ... ... ... ... .T. ... ... ... ..A  [456]
#Dogongs 8        ... ... ... ... C.. ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ..C A.. ... ... ... ... ... ... G.T ... ... ... ... ... ... ... .T. ... ... ... ..A  [456]
#Dogongs 9        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C A.. ... ... ... ... ... ... G.T ... ... ... ... ... ... ... .T. ... ... ... ..A  [456]
#Dogongs 10       CC. ... C.. .A. ..C ..G ... ... ... .C. ..T ..G A.. G.T ... .AT .AA ..C A.C ... G.. ... .GC C.. ... ... ... ... ... ... ... ..T CCA A.. TGG TCA  [456]

#Dogongs 1        ACA TTC TTC CCT CTA CAT TTC TTC GGA --T TAT ATG GTA -TT CCC CAG TCC ATA CCC AC- ACT ACG CAC -AC TCT TAT TTT AC- --T TAT CTT GAT TTA TTT TTC TTT CTA ATT  [570]
#Dogongs 2        ... ... ... ... .A. ... ... C.. ..C --. ... CA. ... --A ..T .-. ..G ... .T. .G- ... ..C ..G -.. ... ... ... ... -- --. ... ... ... ... ... .C. .A. GCG T.A  [570]
#Dogongs 3        .T. ... ... .T. .A. ... ... C.T A.G AG. ... TAC .G. C.G ..T .G. CAG ..G ... CG- ..A ... .CA -.. ... ... ... ... -- --. ... ... ... ... ... C.T ..A T.. ...  [570]
#Dogongs 4        T.. A.. C.. AA. .AT ... ... .A. ..C --. ... CCC .AG --A ... .C. CGA .CG .A. .A- ... ..T ATT -.. ... ... ... ... -- --- -T. ... A.. AAT A.. --- --- --- ---  [570]
#Dogongs 5        ... ... ... .T. .A. ... ... C.. ..C --. ... CAA .G. -CA .TT .-. GAT ... .T. T.T ... ..C ..G -.. ... ... ... ... -- --. ... ... ... ... ... --- --- G.. T..  [570]
#Dogongs 6        ... ... ... ... .A. ... ... C.. ..C --C A.. CAC ... --A ..T .-. ..G ... .T. .A- .T. ..C ..G -.. ... ... ... ..T TA. CT. GA. TTA ..T A.. ..T A.C A.. TG.  [570]
#Dogongs 7        ... ... ... ... .A. ... ... C.. ..C --. ... CA. ... --A ..T .-. ..G .A. TT. .G- ... ..C ..G -.. ... ... ... ... -- --. ... ... ... ... ... GAT C.C T.G ---  [570]
#Dogongs 8        ... ... ... ... .A. ... ... C.. ..C --C A.. CAC ... --A ..T .-. ..G ... .T. .A- .T. ..C ..G -.. GT. .T. AC. .AC AA. C.. ..A .T. CCG A.. ..T A.C A.. TG.  [570]
#Dogongs 9        ... ... ... ... .A. ... ... C.. ..C --. ... CA. ... --A ..T .-. ..G ... .T. .G- ... ..C ..G -.. ... ... ... ... -- --. ... ... ... ... ... CAT C.C .CT CC.  [570]
#Dogongs 10       CG. ... ... .T. .A. ... ... C.. AC. --A C.C CAT C.G -.. TTA .CT GGA GG. .A. GTG ..C T.C G.. T.. ... ... ... ... -- --. ... ... ... ... ... A.T C.C T.. TA.  [570]

#Dogongs 1        --- --T ATA AAC AA- --- --T ATA AGC ATA AAT CGT ACG AGA TAT CTA ATT TTA ATA TCT CTT AGT CAT AGC TA- -CA AA- -AA TAA TAA ATA ACA CAA ATG CTA TAT ATT CTA  [684]
#Dogongs 2        --- --- .CG GGG .GT GTA CAA GG. .A. TAC ..A ACA T.T TTC .TA T.. TCG .CT TGG --- -.. ... ... ... ..A TA- --- --- --. .C. T.. .G- --- CCT TAT GT. .AA TC.  [684]
#Dogongs 3        --- --. .A. G.A T.T TTA AAA TAG T.T TGT C.C ACG TA. TTC AT. GAT C.A A.T T.. A.A A.. ... ... ... ..T TGG G.C --G A.. ATC ... C.C A.T G.T T.T AGA ... T.C  [684]
#Dogongs 4        --- --- .CT ..G .-- --- CA. .A. CAA TGC TC. ATC CAT .CT .C. .GG C.A A.T G.. A.A A.. ... ... ... ..A TGC G.- --G A.C C.. C.C .TC ... TGT T.T ..G .A. T.T  [684]
#Dogongs 5        --- --- .AG ..G .GC TCA CTA .A. ..A .AG T.. GTG .TT CT. .C. GG. TC. .CC TA. A.A A.. ... ... ... ..A G.G ..T G.G GT. ... T.. .AG AC. C.C TCC CCC CCA AC.  [684]
#Dogongs 6        --- --. ... TTG ..A TCT CA. ... .AA ..C .TC GC. .AA .TG .T. ... T.. .CG C.. CTC A.. ... ... ... ..G C.. .T- -G. .T. AT. C.G TTT ... ..T T.C .CC .G. T..  [684]
#Dogongs 7        --- --- .CT ..A .GG AGA AGA .C. .A. CAT T.A AA. TTT TT. A.C TCG T.C .CG T-- --- -.. ... ... ... ..A CA. .G- --. GT. ..T T.G .TT ACT CA. A.T C.C .AA T.T  [684]
#Dogongs 8        --- --. ... TTG ... -CT CA. ... .AA ..C .TC GC. .AA .TG .-- ... T.. .CG TC. CGC -.. .T. T.C .-- -.G C.. .T- -G. .T. AT. C.G TTT ... ..T T.C .CC .G. T..  [684]
#Dogongs 9        CTA CA. ..T ..A .GC ATA CAA GC. CCA TCC ... AAA CTT CA. ATC A.G T.A .AC T-- --- -.. ... ... ... ..A -AG CT- --. A.G .TT T.T C.T TCC TAT G.- --C .GA TAT  [684]
#Dogongs 10       --A AA. .A. G.G ..T GTT ATA C.. TAT C.C T.A A.C TAA ..T .T. T.C C.. CCT ... A.A A.. ... ... ... ..A TGC ..C -G. G.. .C. ... ..T T.T GGA TCC G.. CAC .CC  [684]

#Dogongs 1        AAA TAT AAC AA- --C GAG CTG CCT ATC TTT ATT AAT CCT C-- ATA TTT CA- ATA AAA TA- -CA AAT AAT ATA TAT ATA TCA ATA TAT GAG AT- --- -TA TTG GAG --- -AT TTT  [798]
#Dogongs 2        .G. ... TG. ..- --. ... ... ... ... .C. CA. CT. TT. ..A TAT .AA ACC -.. ..T ..- -G. TC. C.. .AC ..C T-- --- C.. ..A ATT TAG TAA TC. GAA C.T A-A T.G C.A  [798]
#Dogongs 3        .T. .TA .TA ..- --T .CA .AA TA. TC. A.A TC. .TC TAC .TC ..C A.G T.T ..T .TT ..A AT. .G. TG. GAC CC. ..T A-G T.. ..A T.A T.T CGT TG. GA. ..C TTA T.G .AC  [798]
#Dogongs 4        C.T AT. ..T ..A TG. A.A G.. .AA GAT A.C TA. CTA ... .-- --- A.C A.C .G. TGT .G- -.. T.G .TA TAG .GA C.T CG. CCG A.C TTC ..G TCT A.G .CA T.. AAC ACA .A.  [798]
#Dogongs 5        .TC AC. GG. ..- --. ... ... ... ..T .C. .AC CTA T.. ..A .GT GAC T.C -CC .CT ..- -.. TT. GGA .A. ... ..- --G CC. C.G ATT TAG TAA T.. CAC .G. ATG G.G ..C  [798]
#Dogongs 6        T.. GG. G-. ..- --. ... ... ... ... .CG ..C .-- ..A .-- --- --. ..T ... .CT ..- -T. .TA ..A .CT .CA ... G.G G.. ... ATT ..T TGC C.. C.. ..A GAC C.G ..C  [798]
#Dogongs 7        ..C .T. .G. ..- --. ... ... ... ... ..C C.. ... .TG ..T GA. T.T T.. G.T ..- -TT CCC TTA TCC A.C CA- --G CC. ..- ATC .CG TAT T.G AGT ATT ATA GT. ...  [798]
#Dogongs 8        T.. GG. G-. ..- --G A.T .AA TAA ... .CG ..C .-- ..A .-- ... AC. A.T ... .CT ..- -T. .TA ..A .CT .CA ... G.G G.. ... ATT ..T TGC C.. C.. ..A GAC C.G ..C  [798]
#Dogongs 9        CTT .T. GG. ..- --G ... ... ... ... ... .AG CT. .A. --- -.T GA. ..T ..T ..T ..- -T. T.. ..G T.T GCA TC- --G T.. ..- ATT ..T TCT T.G GAC A.A ATC G.A G.A  [798]
#Dogongs 10       TCT G.. T-T GT- --T ..A .AA TA. TC. ..A TA. .TA AG. T-- --T GA. ..C ..C G-T ..- -T. .T. CG. TG. G.G .AC A-C ... ... ATT TAG TAA T.T ..C TTC ATA A.. ..A  [798]

#Dogongs 1        TCA ATC AAA ATC TTC T-A TTT TAA TTT ATT GAT TCT ATT AAT GA- ACT ATA ATT ACC ATC TTT ATT CTA AGC AAT ACT A-- --- --- --- ---  [891]
#Dogongs 2        .TT C.A C.. T.T .AA --. A.. .-- --A T.G AT. CTA T.A .TG A.- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [891]
#Dogongs 3        ATT TGT CTC T.. .AT .G. ..C ..T .AA TGA AT. CAA .A. .TG A.A .A- --- --- --- --- --- --- --- --- --- --- --- --- ---  [891]
#Dogongs 4        AT. ..T .TT T.. ... AT. AA. AGT .G. G.G ATG .TG TG. C.. A.T ..A C.G CCA T.T ..T ..- --- --- --- --- --- --- --- ---  [891]
#Dogongs 5        CT. C.A C.C TC. CA. C-. ACA .-- --. TGA A.. ..A .G. .TG A.A .AA C.C T.C TA. T.G A.. .-- --- --- --- --- --- --- ---  [891]
#Dogongs 6        .T. TCT GTG C.. .A. C-. .C. ATT GA. TC. AT. --A ..G ..A T.T ... GA. T.A GT. ..T ..C ..G --- --- --- --- --- --- ---  [891]
#Dogongs 7        ..C ..T T.T ..T .-- C-. .CA .-- --. TG. AC. --A ... .G. ..A .G- --- --- --- --- --- --- --- --- --- --- --- --- ---  [891]
#Dogongs 8        .T. TCT GTG C.. CAT C-. .C. ..G AA. TCA .CC C.A ... ..A ..T .T. T.. T.C .A- ..A ..C .AG TAT GAA ..A ... CTT CTA CTT GAT TAA  [891]
#Dogongs 9        .AG TA. ..C T.T .-- C-. ..A .-- --. TGA T.. --A CG. .T. ..A .G. TGT ..A TGG ..T C.C TA. TC. CA. --- --- --- --- --- --- ---  [891]
#Dogongs 10       .TG ..T CT. T.A A.G A-. --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---  [891]
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Figure 2 Sequence comparisons of COI sequence from ten dugong samples. Dots (.) indicate the nucleotides and dashes (-) are introduced to gap.

Table 2 The length and nucleotide composition of the COI gene of the dugong specimens in this study and their identical GenBank accession number.

Sample No.	Length (bp)	Base Content					Identical GenBank Acc. No.	% Identical
		A	C	G	T	%GC		
Dugongs 1	838	236	191	128	283	38.07	AY075116.1	91.59%
Dugongs 2	795	207	187	132	269	40.13	AY075116.1	91.59%
Dugongs 3	830	228	185	133	284	38.31	AY075116.1	91.59%
Dugongs 4	821	223	192	131	275	39.34	AY075116.1	91.59%
Dugongs 5	830	224	187	140	279	39.40	AY075116.1	91.59%
Dugongs 6	833	222	193	132	286	39.02	AY075116.1	90.56%
Dugongs 7	804	214	185	132	273	39.43	AY075116.1	91.59%
Dugongs 8	859	242	194	135	288	38.30	AY075116.1	90.85%
Dugongs 9	830	218	198	132	282	39.76	AY075116.1	91.59%
Dugongs 10	794	218	183	125	268	38.79	AY075116.1	90.85%
Average	823.4	223.2	189.5	132	278.7	39.05		91.34%

Table 3 Estimates of genetic distance matrix between dugong COI sequences.

Sample No.	1	2	3	4	5	6	7	8	9	10
Dugongs 1	0.000									
Dugongs 2	0.010	0.000								
Dugongs 3	0.011	0.008	0.000							
Dugongs 4	0.011	0.007	0.005	0.000						
Dugongs 5	0.015	0.016	0.019	0.016	0.000					
Dugongs 6	0.151	0.153	0.151	0.151	0.151	0.000				
Dugongs 7	0.008	0.010	0.011	0.008	0.011	0.150	0.000			
Dugongs 8	0.089	0.092	0.092	0.092	0.097	0.165	0.091	0.000		
Dugongs 9	0.008	0.007	0.010	0.008	0.015	0.155	0.010	0.092	0.000	
Dugongs 10	0.111	0.116	0.119	0.119	0.112	0.163	0.109	0.101	0.116	0.000
Average	0.070									

The dendrogram constructed from COI sequence comparisons using the Maximum Likelihood method based on the Tamura-Nei model demonstrated that the 10 taxa were divided into three subgroups (Figure 3). The first major group (I) comprised of seven dugong samples; 1, 2, 3, 4, 5, 7 and 9. The second group (II) comprised of two dugong samples; 8 and 10. The last group (III) has only one member; dugong 6. The dugong 6 was separated from the other members, which was not consistent with the location of the sampling. Because the distance from Koh Libong, the collection area of a major group, was only 40 kilometers away from the location of dugong 6 sampling. While the location of dugong 8 sampling was 140 kilometers away and along with many islands that are blocked such as Koh Yoa Yai, Koh Yao Noi, Phi Phi Island and Koh Lanta. However, the clustering of dugongs 8 and dugongs 10 (II) corresponds to the sampling location. The distance of about 50 kilometers between Yamu Cape, Pa Klok, Maung, Phuket and Loh Dalum Beach, Phi Phi Island, Ao Nang, Maung, Krabi resulting in both populations being able mating, thus forming a gene flow. However, the average genetic distance in this study was 0.07, which is considered very low. This demonstrated that the gene flow between the dugong population in each area still occurred. The genetic differences are likely caused by the variation of each individual. Furthermore, genetic grouping was not differentiated by region indicating maternal dispersal over long distances [17].

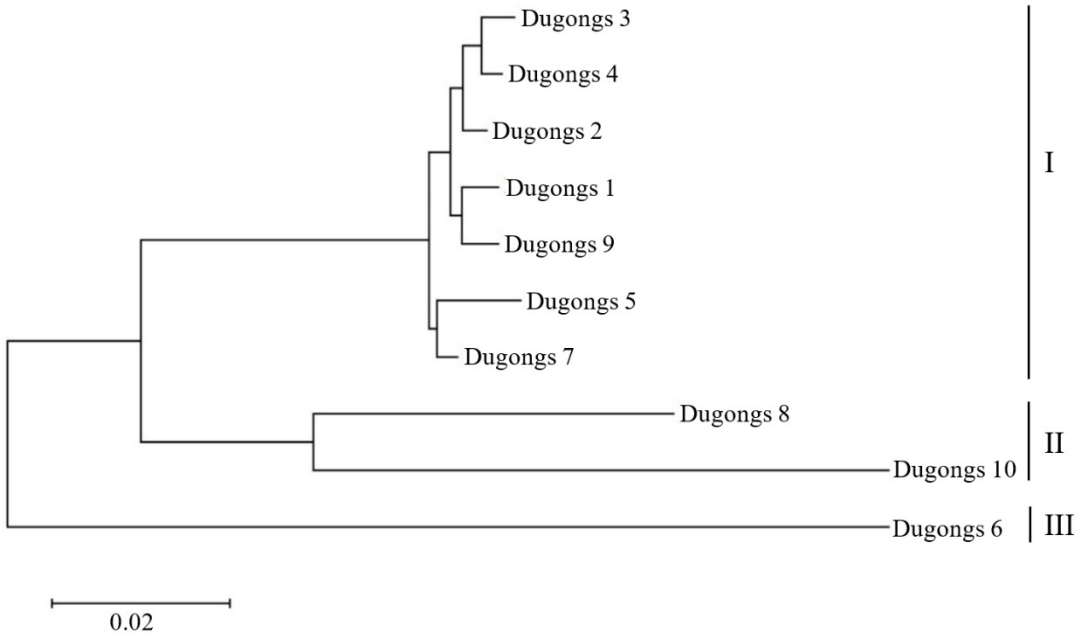

Figure 3 A dendrogram of the 10 dugong samples constructed from sequence comparisons of the COI gene using the Maximum Likelihood method based on the Tamura-Nei model.

The sampling area of the major group was around Koh Libong and the coastal areas of Trang Province. This is because Trang Province has the largest existing population of dugong and largest and healthiest seagrass meadows in the country with at least 11 species present [18]. The seagrass beds and islands near Trang possibly house the largest group of dugongs remaining in Southeast Asia [19]. Therefore, the conservation of dugongs must be conserved along the sea-grass.

Conclusions

The average length of the COI sequence of ten dugong samples was 823.4 bp. All COI sequence in this study were identical to GenBank accession number AY075116.1 with an average of 91.34%. The average genetic distance from the Kimura 2-parameter model was 0.070. The dendrogram divided ten dugong samples into three subgroups. The major group contains seven dugong samples from around Koh Libong and the coastal areas of Trang Province. The low genetic distance demonstrated a gene flow between populations, especially in areas with the largest and abundant seagrass.

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