Introduction

The sea cucumber, *Apostichopus japonicus* (Selenka, 1867), is one of the economically important species cultivated in the adjacent seas in East Asian countries, including China [1,2] and Korea [3,4]. It has been used as a source of nutritional foods as well as traditional medical resources widely [5,6]. Due to the importance of this species together with the benefits of next-generation sequencing (NGS) technologies [7-9], various genomics studies of *A. japonicus* have been conducted and published in recent 10 years: three whole genome sequencing projects [10-12], ten complete mitochondrial genomes [13-16], as well as various transcriptome studies [17-29]. This amount of sequence resources is much larger than the other species except model organisms, such as *Arabidopsis thaliana* and *Drosophila melanogaster*, because the cost of generating NGS raw reads of multiple individuals is relatively high enough. Mitochondrial genome sequences have been utilized for understanding phylogenetic relationships [30-33] as well as distinguishing population structures [34-41] because invertebrate mitochondrial genomes have a well-conserved structure [42] and keep a high copy number in the cell [43], which is favorable for assembling the mitochondrial genome from NGS raw reads. In addition, a short length of the mitochondrial genome reduces the costs to investigate intraspecific variations in the mitochondrial genomes [33-36,38-40,44-56]. The current available mitochondrial genomes of *A. japonicus* are also enough to investigate its intraspecific characteristics in detail; however, there is no systematic bioinformatic analysis of all available *A. japonicus* mitochondrial genomes till now.

Here, we presented the investigation of nucleotide diversity of the 17 *A. japonicus* mitochondrial genomes to understand intraspecific characteristics of mitochondrial genomes of certain species in near future.
cific characteristics of *A. japonicus* mitochondrial genomes in detail. The overall trend of nucleotide diversity on *A. japonicus* mitochondrial genomes was investigated with displaying average nucleotide diversity of *A. japonicus* was higher than other species. Moreover, COX1 and 16S rRNA sequences were investigated in detail with the 191 available mitochondrial marker sequences, displaying those different trends of nucleotide diversities between those originated from mitochondrial genomes and marker sequences were identified. Our investigation strategy can be expanded for further understanding of *A. japonicus* mitochondrial genomes, such as the relationship between nucleotide diversity and phenotypic variations, such as body colors [57], in near future.

**Materials and Methods**

**Preparation of mitochondrial genome of *A. japonicus***

All seventeen mitochondrial genomes of *A. japonicus* used in this study were downloaded from the NCBI Nucleotide database and deposited into the GenomeArchive® [58] for conducting nucleotide diversity analyses. 16S ribosomal RNAs and COX1 gene sequences were downloaded from the NCBI Nucleotide database.

**Table 1: List of 17 *A. japonicus* mitochondrial genomes used in this study.**

| No | Strain | Location          | NCBI accession | Length (bp) | GC ratio (%) | Ref.   |
|----|--------|-------------------|----------------|-------------|--------------|--------|
| 1  | GreenQ | Qingdao, China    | NC_012616      | 16,099      | 38           | [14]   |
| 2  | RedO1  | Oita, Japan       | GI557147       | 16,103      | 38.1         | [14]   |
| 3  | RedO2  | Oita, Japan       | GI557148       | 16,103      | 38.1         | [14]   |
| 4  | RedK   | Korea             | FJ594963       | 16,107      | 38.1         | Unpub. |
| 5  | GreenK | Korea             | FJ594968       | 16,102      | 38.1         | Unpub. |
| 6  | BlackK | Korea             | FJ594967       | 16,108      | 38.1         | Unpub. |
| 7  | GreenW | Weihai, China     | EU294194       | 16,096      | 38.1         | [15]   |
| 8  | RedY   | Yamaguchi, Japan  | AB525761       | 16,103      | 38.1         | Unpub. |
| 9  | GreenY | Yamaguchi, Japan  | AB525760       | 16,106      | 38           | Unpub. |
| 10 | BlackY | Yamaguchi, Japan  | AB525437       | 16,105      | 38.1         | Unpub. |
| 11 | GreenD | Dalian, China     | FJ986223       | 16,109      | 38.1         | Unpub. |
| 12 | BlackK2| Geumun-do, Korea  | MK216560       | 16,096      | 38.1         | [13]   |
| 13 | RedK   | Geumun-do, Korea  | MK216561       | 16,107      | 38.1         | [13]   |
| 14 | GreenK2| Geumun-do, Korea  | MK208925       | 16,106      | 38           | [13]   |
| 15 | RedR   | Vladivostok, Russia| KP170616      | 16,100      | 38           | [16]   |
| 16 | GreenL | Lianyungang, China| KP170617      | 16,106      | 38           | [16]   |
| 17 | GreenW | Weihai, China     | KP170618       | 16,106      | 38           | [16]   |

**Results and Discussions**

Till now, the seventeen *A. japonicus* mitochondrial genomes were sequenced from the three-color variants, red, green, and black sea cucumbers (Table 1) and the samples for mitochondrial genomes were captured in various regions of China, Korea, Japan, and Russia (Table 1). Their lengths range from 16,096 bp (MK216560) to 16,109 bp (FJ986223), of which the difference is only 14 bp (0.087% against the longest mitochondrial genome). In the order Aspidochirotida including *A. japonicus*, two *Holothuria* species, *H. leucospliota* and *H. spinifera*, contain more than one mitochondrial genome, displaying that intraspecific differences of the three *H. leucospliota* mitochondrial genomes (GenBank accessions are MK801674, NC_046849, and MN276190) [75, 76] and two *H. spinifera* mitochondrial genomes (GenBank accessions are MW218896 and NC_046508) [77] are 67 bp and 23 bp, respectively. It indicates that the intraspecific length difference of *A. japonicus* mitochondrial genome is relatively small. In addition, This intraspecific difference is also smaller than those of several insect spe-
cies, *Sogatella furcifera* (43 bp from three mitochondrial genomes; Park et al., in submission) [78], *Laodelphax striatellus* (155 bp from eighty-seven mitochondrial genomes) [34, 35, 37], and *Aphis gossypii* (186 bp from five mitochondrial genomes) [46, 48, 52, 55, 56] except that of *Nilaparvata lugens* (14 bp from the five mitochondrial genomes excluding them without control regions) [38-40, 79] (Park et al., in submission). It implies that intraspecific differences of *A. japonica* mitochondrial genome with considering the geographical distribution of the seventeen samples are not so high.

GC ratio of the seventeen *A. japonicus* mitochondrial genome ranges from 38.0% to 38.1% (Table 1). There are no differences in the number of genes and gene orders among the seventeen mitochondrial genomes, displaying the conserved manner of mitochondrial genomes, which is also same to those of *S. furcifera*, *L. striatellus*, *A. gossypii*, and *N. lugens*.

Sequence-level intraspecific variations are also important, especially for distinguish the origin of samples. One useful way to investigate intraspecific variations is nucleotide diversity. Nucleotide diversity of 17 *A. japonicus* displays the highest peak in the control regions and the lowest in 16S ribosomal RNA (rRNA) and 12S rRNA (Figure 1). Usually, control regions display high level of nucleotide diversity [33]. Average nucleotide diversity was calculated as 0.00374, which is much larger than those of nine *S. furcifera* mitochondrial genomes (0.000246; Choi et al., in preparation; Park et al. in preparation) [78] and *L. striatellus* (0.00188) [34, 35, 37], indicating that there is relatively high level of intraspecific variations on *A. japonica* mitochondrial genomes.

In detail, the 3’ regions of COX1, COX2, and COX3 and the 5’ regions of CYTB and ND1 display the lower nucleotide diversity of the average nucleotide diversity (Figure 1), indicating that these regions are conserved on *A. japonicus* mitochondrial genomes. In addition, tRNA cluster between the control region and ND1 gene also present lower nucleotide diversity than the average (Figure 1). In addition, nucleotide diversity of COX1 gene displays the differences between 5’ region and 3’ region: 5’ region of COX1 shows higher nucleotide diversity than average, while the 3’ region presents lower (Figure 1). It indicates that the resolution of molecular marker based on COX1 gene will be different along with the region of COX1.

Currently available 191 mitochondrial molecular marker sequences of *A. japonicus* in NCBI consist of 96 16S ribosomal RNA and 95 COX1 genes. Three out of 95 COX1 genes contain 16S rRNA sequences so that these three COX1 genes cover 5’ region. The remaining COX1 genes also cover 5’ region but after the coverage of the three genes. Nucleotide diversity of COX1 genes together with 17 complete COX1 genes originated from the complete mitochondrial genomes display that the highest peak of nucleotide diversity is 0.013 at 351 to 450 bp region of the COX1 gene; while 17 COX1 genes from the mitochondrial genome show the valley point, showing opposite trend (Figure 2A). Moreover, the region between 451 bp to 651 bp shows the reverse manner: 17 COX1 genes from the mitochondrial genome show a high nucleotide diversity than that of all COX1 genes (Figure 2A). It indicates that the coverage of the samples used in complete mitochondrial genomes is different from that of all COX1 genes as well as the 5’ region of COX1 provides enough resolution to conduct phylogeny studies.

Nucleotide diversity of 16S rRNA sequences show a much higher value than that of 16S rRNA from 17 mitochondrial genomes.

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**Figure 1:** Nucleotide diversity of 17 *A. japonicus* mitochondrial genome.

The x-axis indicates nucleotide position of multiple sequence alignment of 17 *A. japonicus* mitochondrial genomes. Y-axis presents nucleotide diversity. The thin blue line on the graph indicates average nucleotide diversity. Below the graph, 37 genes were drawn with colored arrow diagrams: blue arrows indicate protein coding-genes, orange arrows indicate transfer RNAs, yellow arrows mean ribosomal RNAs, and grey arrows show control region.
The region of which nucleotide diversity is high (601 bp to 1301 bp region in Figure 2B) was sequenced in most of 16S rRNA marker sequences. It is interesting that the average nucleotide diversity of 16S rRNA (0.04691) is higher than that of COX1 markers (0.007155), suggesting that rRNA sequences may have better resolution than COX1 in *A. japonicus*.

Through this investigation of nucleotide diversity of 17 *A. japonicus* mitochondrial genome, overall features of intraspecific variations of *A. japonicus* mitochondrial genomes and 16S rRNA and COX1 marker sequences were identified. Even though the cost to obtain complete mitochondrial genomes using NGS technologies is still not cheap, enough mitochondrial genomes in certain species can provide more information of intraspecific characteristics. In near future, this method of investigation can be applied to the species which have more mitochondrial genome sequences for selecting the best marker sequences of that species as well as for understanding intraspecific characteristics of mitochondrial genomes.

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**Conflict of Interest**

No conflict of interest.

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