Complete mitochondrial genome sequence of *Pleurobranchaea novaezealandiae* and *Pleurobranchaea* sp.

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**ABSTRACT**

The complete mitochondrial genome sequences of *Pleurobranchaea novaezealandiae* and *Pleurobranchaea* sp. are first described and analyzed in this study. It is 14,531 bp and 14,709 bp in length, respectively. The base composition of the genome with A + T bias are 66.41% and 68.36%. There are 29 noncoding regions found throughout the mitogenome of *P. novaezealandiae* and 30 noncoding in *Pleurobranchaea* sp., ranging in size from 2 to 294 bp. The phylogenetic tree based on 10 mitogenome, including 1 prosobranchia, 6 opisthobranchia and 3 pulmonata was analyzed in the paper. The results showed that the opisthobranchia and pulmonata were clustered respectively, and the *P. novaezealandiae* and *Pleurobranchaea* sp. were the closest to the *Aplysia californica* in our analysis.

*Pleurobranchaea novaezealandiae* (voucher number: ASTM-Mo-P541) belongs to Mollusc, Gastropoda, Opisthobranchia, Pleurobranchiidae, which has a widespread domestic distribution, such as Bohai Sea, Yellow Sea, Paracel Islands and HongKong, and Japan is its main foreign inhabitancy. *Pleurobranchaea novaezealandiae* is characterized by small body. It presents oblong shape, with a bulge on its back and irregular mastoid processes on its surface. The front of its head extends bilaterally to form flat shape, with small serra- tion at its edge and two pairs of antennae. It has large ovate-oblong feet, which is exposed outside the margin of mantle. It has a blue-yellow back, while its ventral side appears dark maroon.

*Pleurobranchaea* sp. (voucher number: ASTM-Mo-P547) belongs to Mollusc, Gastropoda, Opisthobranchia, Pleurobranchiidae, a small creature inhabiting at shallow. It has oval shape, sharp tail and resembles slug. Besides, with a bulge on its back, its surface emerges white mastoid processes. *Pleurobranchaea* sp. is bilaterally symmetric taking pleo- pod as axle wire, and gills located at the front of head can assist to breathe, together with the secondary gills beneath mantle and the bilateral gills of pleopod.

The complete mitochondrial genome of *P. novaezealandiae* and *Pleurobranchaea* sp. were sequenced and characterized in this article, and the samples of *P. novaezealandiae* and *Pleurobranchaea* sp. were collected from Ganyu, Jiangshu Province, China. Before further processing, specimens were stored in ultra-low temperature freezer.

The complete mitochondrial genome of *P. novaezealandiae* and *Pleurobranchaea* sp. were 14,531 bp and 14,709 bp in length, respectively, and have been deposited in GenBank with accession No. KU365727 and No. KU365728. They consist of 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes as shown in Tables 1 and 2. The ATG, ATT and TTG are used as the start codons, which are very common in invertebrates (Grande et al. 2008), but only ATP8 starts with TTG. Except for COX3 with an incomplete stop codon “T—”, the remaining protein-coding genes stop with the TAG or TAA. Using the tRNA scan-SE 1.21 (Lowe & Eddy 1997), 22 tRNA genes were found to fold into a typical cloverleaf secondary structure. The overall basic composition of the heavy strand in *P. novaezea- landiae* is 36.53% A, 29.88% T, 19.67% G and 13.92% C, with an AT content of 66.41%. Similarly, the overall base composition of the heavy strand in *Pleurobranchaea* sp. is 40.02% A, 28.34% T, 18.46% G and 13.18% C, with an AT content of 68.36%. The AT content is higher than the content of GC, as generally shown in bivalvia mitochondrial genomes (Wang et al. 2010).

The phylogenetic tree (Figure 1) was generated using the MrBayes soft analyses and Maximum Likelihood method (Tamura et al. 2011) from amino acid composition of complete mitochondrial genomes. The results showed that the opisthobranchia and pulmonata were clustered respectively, and the *P. novaezealandiae* and *Pleurobranchaea* sp. were the closest to the *Aplysia californica* in our analysis. It constituted a framework for phylogeny evolution analysis, systematic classification of other euthyneurans (pulmonates and opistho- branchs) (Liu et al. 2015). The comparative mitogenetic analysis of gastropods may provide valuable phylogenetic information.

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### Table 1. Mitochondrial genome of the *Pleurobranchaea novaezealandiae*.

| Locus | From | To | Size | Start | Stop | Anti-codon | Intergenic nucleotides* | Strand+ |
|-------|------|----|------|-------|------|-----------|--------------------------|---------|
| tRNA^His | 19 | 85 | 67 | GTG | 2 | L |
| tRNA^Gly | 88 | 154 | 67 | TCC | 27 | L |
| COX2 | 182 | 839 | 658 | ATG | TAA | 1 |
| tRNA^Met | 839 | 904 | 66 | GAA | 7 | L |
| tRNA^Phe | 912 | 978 | 67 | GTC | 83 | L |
| Cytb | 1062 | 2154 | 1093 | ATT | TAG | 6 |
| ND4 | 2161 | 2419 | 259 | ATG | TAA | 20 |
| tRNA^Thr | 2440 | 2507 | 68 | TCA | 3 | L |
| tRNA^Try | 2511 | 2575 | 65 | GTA | 1 | L |
| tRNA^Gln | 5686 | 5754 | 69 | TGC | 1 | L |
| tRNA^Pro | 5836 | 5901 | 66 | TAC | 22 | L |
| l-rRNA | 5901 | 6791 | 956 | 128 | | |
| COX1 | 6109 | 13332 | 7224 | ATT | TAA | 164 |
| tRNA^Lys | 298 | 363 | 66 | TTT | 95 | L |
| ND2 | 459 | 1269 | 811 | TTG | TAG | 108 |
| tRNA^Ile | 1304 | 1371 | 68 | GAT | 52 | L |
| COX3 | 1424 | 2198 | 775 | ATG | TTT | 102 |
| tRNA^Thr | 2199 | 2265 | 67 | TTA | 61 | L |
| ND4 | 2306 | 3115 | 809 | ATT | TAG | 15 |
| ATP6 | 3609 | 3667 | 59 | TAA | 108 |
| tRNA^Met | 3736 | 3803 | 68 | GAT | 97 | H |
| s-rRNA | 4180 | 4975 | 795 | 9H | |
| tRNA^Leu | 4967 | 5034 | 68 | TTC | 3 |
| COX3 | 5038 | 5100 | 63 | TCA | 202 | H |
| tRNA^Glu | 5303 | 5362 | 59 | TAA | 27 |
| COX1 | 5784 | 5850 | 67 | TAA | 3 |
| tRNA^Ala | 5854 | 5920 | 67 | GTG | 13 | H |
| ATP8 | 6008 | 6073 | 66 | TAA | 2 |
| tRNA^Ser | 6072 | 6133 | 61 | TGG | 294 | H |
| COX2 | 6427 | 6489 | 63 | GCA | 12 | L |
| tRNA^His | 6502 | 6564 | 63 | GTG | 20 | L |
| tRNA^Gly | 6585 | 6652 | 68 | TCC | 16 | L |
| Cyth | 6669 | 7329 | 661 | ATT | TAA | 29 | L |
| tRNA^Thr | 7329 | 7395 | 66 | GGA | 5 | L |
| tRNA^Try | 7401 | 7467 | 67 | GTC | 45 | L |
| ND4 | 7513 | 8592 | 1087 | ATT | TAA | 6 |

*Positive numbers indicate the number of nucleotides found in intergenic spacers between different genes. Negative numbers indicate overlapping nucleotides between adjacent genes. H and L indicate genes transcribed on the heavy and light strands, respectively.

### Table 2. Mitochondrial genome of the *Pleurobranchaea* sp.

| Locus | From | To | Size | Start | Stop | Anti-codon | Intergenic nucleotides* | Strand+ |
|-------|------|----|------|-------|------|-----------|--------------------------|---------|
| COX1 | 13 | 133 | 121 | ATT | TAA | 164 |
| tRNA^Lys | 298 | 363 | 66 | TTT | 95 | L |
| ND2 | 459 | 1269 | 811 | TTG | TAG | 34 |
| tRNA^Ile | 1304 | 1371 | 68 | GAT | 52 | L |
| COX3 | 1424 | 2198 | 775 | ATG | TTT | 102 |
| tRNA^Thr | 2199 | 2265 | 67 | TTA | 61 | L |
| ND4 | 2306 | 3115 | 809 | ATT | TAG | 15 |
| ATP6 | 3609 | 3667 | 59 | TAA | 108 |
| tRNA^Met | 3736 | 3803 | 68 | GAT | 97 | H |
| s-rRNA | 4180 | 4975 | 795 | 9H | |
| tRNA^Leu | 4967 | 5034 | 68 | TTC | 3 |
| COX3 | 5038 | 5100 | 63 | TCA | 202 | H |
| tRNA^Glu | 5303 | 5362 | 59 | TAA | 27 |
| COX1 | 5784 | 5850 | 67 | TAA | 3 |
| tRNA^Ala | 5854 | 5920 | 67 | GTG | 13 | H |
| ATP8 | 6008 | 6073 | 66 | TAA | 2 |
| tRNA^Ser | 6072 | 6133 | 61 | TGG | 294 | H |
| COX2 | 6427 | 6489 | 63 | GCA | 12 | L |
| tRNA^His | 6502 | 6564 | 63 | GTG | 20 | L |
| tRNA^Gly | 6585 | 6652 | 68 | TCC | 16 | L |
| Cyth | 6669 | 7329 | 661 | ATT | TAA | 29 | L |
| tRNA^Thr | 7329 | 7395 | 66 | GGA | 5 | L |
| tRNA^Try | 7401 | 7467 | 67 | GTC | 45 | L |
| ND4 | 7513 | 8592 | 1087 | ATT | TAA | 6 |

*Positive numbers indicate the number of nucleotides found in intergenic spacers between different genes. Negative numbers indicate overlapping nucleotides between adjacent genes. H and L indicate genes transcribed on the heavy and light strands, respectively.

(continued)
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Table 2 Continued

| Locus     | From | To   | Size | Start | Stop | Anti-codon | Intergenic nucleotides* | Strand+ |
|-----------|------|------|------|-------|------|------------|-------------------------|---------|
| tRNA^{Tt} | 8885 | 8950 | 66   |       |      | TCA        | 2                       | L       |
| tRNA^{Ty} | 8953 | 9006 | 54   |       |      | GTA        | 63                      | L       |
| ND1       | 9070 | 9934 | 865  | ATT   | TAA  |            | 132                     | L       |
| ND5       | 10,067 | 11,576 | 1510 | ATTG  | TAG  |            | 69                      | L       |
| ND6       | 11,646 | 12,060 | 415  | ATT   | TAA  |            | 0                       | L       |
| tRNA^{Tto} | 12,061 | 12,125 | 65   |       |      | TGG        | 10                      | L       |
| tRNA^{Auu} | 12,136 | 12,203 | 68   |       |      | TGC        | –1                      | L       |
| tRNA^{Auu2} | 12,203 | 12,265 | 63   | ATT   |      |            | 12                      | L       |
| L-rRNA    | 12,278 | 13,235 | 58   |       |      |            | 150                     | L       |
| tRNA^{Tal} | 13,386 | 13,450 | 65   |       |      | TAC        | 46                      | L       |
| COX1      | 13,497 | 14,706 | 1210 | ATT   | TAA  |            | 0                       | L       |

*Positive numbers indicate the number of nucleotides found in intergenic spacers between different genes. Negative numbers indicate overlapping nucleotides between adjacent genes. +H and L indicate genes transcribed on the heavy and light strands, respectively.

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Figure 1. Phylogenetic tree generated using the MrBayes analyses and Maximum Likelihood method from amino acid composition of the complete mitochondrial genomes.