

**Figure S1.** Relative synonymous codon usage (RSCU) in five *P. megacephalus* mitogenomes. .



**Figure S2.** Three-dimensional(3D) structures of the *ATP8* protein of all ten Rhacophoridae.



**Figure S3.** Scatter plots of genetic distance vs. geographical distance for pairwise population comparisons. The horizontal coordinate is Geographical distance (ln Km), and the vertical coordinate is Genetic distance.

**Table S1.** Collection data of samples of *P. megacephalus*.

|  |  |  |  |
| --- | --- | --- | --- |
| Samples | Collecting locality | Latitude | Longitude |
| ZJJH | Wucheng, Jinhua, Zhejiang, China | 29.14 °N | 119.64 °E |
| ZJWZ | Chashan, Wenzhou, Zhejiang, China | 27.92 °N | 120.70 °E |
| GXGL | Xiangshan, Guilin, Guangxi, China | 25.21 °N | 110.25 °E |
| GDQY | Huadu, Guangzhou, Guangdong, China | 23.58 °N | 113.22 °E |
| GDCH | Conghua, Guangzhou, Guangdong, China | 23.65 °N | 113.78 °E |
| GDHD | Huidong, Huizhou, Guangdong,China | 22.985 °N | 114.71 °E |
| FJFQ | Fuqing, Fuzhou, Fujian, China | 25.71 °N | 119.25 °E |
| TGPJ | Phuket Island, Thailand | 7.84 °N | 98.37 °E |

**Table S2.** The other PCR primer pairs, sequences, and used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| PCR Fragments | Primer Names | Primer Sequence(5’ - 3’) | Length of Fragments |
| F5 | P1-219-J | AAAGCATAGTGCTGAAAACGC | ~600 bp |
| P1-818-N | TTGTCGATTATAGAACAGGCTCCTCT |  |
| F6 | P2-1162-J | TAGAAGAGGCAAGTCGTAAC | ~1,800 bp |
| P2-2951-N | GGTGGGTGAGGTAAAGTATT |  |
| F7 | P3-2611-J | GGCTTACGACCTCGATGTTGGATCA | ~1,400 bp |
| P3-4062-N | GGTATGGGCCCAARAGCTT |  |
| F8 | P4-3917-J | GAAAGATAAGGAYCTCCTTGATAG | ~1,400 bp |
| P4-5347-N | AAGTAGAATGAAGCTCGCTGG |  |
| F9 | P5-5169-J | AGCCCGAAGGCCTTCAAAGC | ~2,000 bp |
| P5-7236-N | ATAATAGGGGATGCGGCGTCTTG |  |
| F10 | P6-7069-J | CGAGAAAGGAAGGAGTTGAAC | ~2,500 bp |
| P6-9543-N | GGTCATGGGCTGGGGTTTACTAT |  |
| F11 | P7-7925-J | CGCGACGGCCTTTTAAGCT | ~2,000 bp |
| P7-10724-N | TGAGYCGAAATCAGGTGTCTT |  |
| F12 | P8-10278-J | TCAATTTACTGATGAGGTTT | ~2,300 bp |
| P8-12511-N | TTGCTAACTACTTATTACCGCGGTTC |  |
| F13 | P9-12425-J | AAAACATTAGGCTGTGGCTCTA | ~800 bp |
| P9-13284-N | AGCGATGTGTCTGCAGTGTAGTG |  |
| F14 | P10-13065-J | TAACCTAGACCTATAGTCCGAAAA | ~1,000 bp |
| P10-14136-N | TCTTCTACTGGTTGGCCTCCGAT |  |

**Table S3.** List of species used in the phylogenetic analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Family | Genus | Species | Genome length | GenBank No. |
| Rhacophoridae | *Polypedates* | *Polypedates mutus* | 20,056 bp | MN869009 |
| *Polypedates braueri* | 19,904 bp | MK687567 |
| *Polypedates impresus* | 19,720 bp | MN869008 |
| *Polypedates megacephalus* FJFQ | 23,798 bp | OP936085 |
| *Polypedates megacephalus* GDCH | 23,848 bp | OP936086 |
| *Polypedates megacephalus* GXGL | 23,996 bp | OP965715 |
| *Polypedates megacephalus* ZJJH | 24,103 bp | OP965717 |
| *Polypedates megacephalus* TGPJ | 23,881 bp | OP965716 |
| *Polypedates megacephalus* GDHD | 15,353 bp | OP965713 |
| *Polypedates megacephalus* GDQY | 17,012 bp | OP965714 |
| *Polypedates megacephalus* ZJWZ | 15,361 bp | OP965718 |
| *Polypedates megacephalus* 20130003 | 19,952 bp | MH936677 |
| *Polypedates megacephalus* | 16,473bp | AY458598 |
| *Zhangixalus* | *Zhangixalus dennysi* | 18,052 bp | KM035412 |
| *Zhangixalus omeimontis* | 19,604 bp | MN427892 |
| *Zhangixalus schlegelii* | 21,359 bp | AB202078 |
| *Zhangixalus dugritei* | 19,412 bp | MZ712011 |
| *Zhangixalus arboreus* | 22,236 bp | LC565708 |
| *Buergeria* | *Buergeria buergeri* | 19,959 bp | AB127977 |
| Mantellidae | *Mantella* | *Mantella madagascariensis* | 22,874 bp | AB212225 |
| *Mantella baroni* | 20,945 bp | MH141579 |

**Table S4.** The partition schemes and best-fitting models selected for the 11 protein-coding genes and 2 rRNA genes.

|  |  |
| --- | --- |
| **Subset** | **Nucleotide sequence alignments** |
| **Subset partitions** | **Best model** |
| Partition 1 | *ND3*\_codon1, *ND4L*\_codon1, *ND1*\_codon1, *ATP6*\_condon1, *ND4*\_codon1, *ND2*\_codon1  | GTR+I+G |
| Partition 2 | *ND4L*\_codon2*, ND2*\_codon2, *ND4*\_codon2, *ND3*\_codon2*, ATP6*\_codon2  | TVM+I+G |
| Partition 3 | *ATP6*\_codon3*, ND4L*\_codon3, *COI*\_codon3*, ND3*\_codon3, *COII*\_codon3, *COIII*\_codon3  | TRN+G |
| Partition 4 | *Cytb*\_codon1, *COII*\_codon1, *COI*\_codon1, *COIII*\_codon1 | SYM+G |
| Partition 5 |  *COI*\_codon2, *ND1*\_codon2, *Cytb*\_codon2, *COII*\_codon2, *COIII*\_codon2 | HKY+I+G  |
| Partition 6 | *Cytb*\_codon3, *ND2*\_codon3, *ND4*\_codon3, *ND1*\_codon3  | TRN+I+G |
| Partition 7 | *ND6*\_codon1, *ND6*\_codon2 | HKY+G |
| Partition 8 | *ND6*\_codon3  | TRN+G  |
| Partition 9 | *rrnS, rrnL* | GTR+I+G |

|  |
| --- |
| **Table S5.** Base compositions of five *P. megacephalus* mitogenomes. |
| **Species name** | **A+T (%)** | **AT-skew** | **GC-skew** |
| **Mito** | **PCGs** | **rRNAs** | **Contral****region** | **Mito** | **PCGs-H** | **PCGs-L** | **rRNAs** | **Contral****region** | **Mito** | **PCGs-H** | **PCGs-L** | **rRNAs** | **Contral****region** |
| GXGL | 61.9 | 59.9 | 59.5 | 68.5 | -0.009 | -0.056 | -0.353 | 0.146 | -0.018 | -0.252 | -0.293 | 0.464 | -0.102 | 0.247 |
| GDCH | 61.2 | 59.4 | 59.3 | 67.7 | -0.005 | -0.050 | -0.414 | 0.144 | -0.021 | -0.263 | -0.299 | 0.525 | -0.099 | 0.279 |
| ZJJH | 61.4 | 59.2 | 59.2 | 68.3 | -0.003 | -0.048 | -0.427 | 0.146 | -0.015 | -0.267 | -0.301 | 0.520 | -0.101 | 0.289 |
| FJFQ | 61.1 | 59.2 | 59.0 | 67.8 | -0.002 | -0.048 | -0.414 | 0.147 | -0.015 | -0.264 | -0.300 | 0.525 | -0.105 | 0.276 |
| TGPJ | 61.2 | 59.3 | 59.3 | 67.6 | -0.007 | -0.052 | -0.392 | 0.143 | -0.016 | -0.254 | -0.293 | 0.507 | -0.089 | 0.262 |

**Table S6.** Locations of features in the mtDNA of *P. megacephalus* from five regions.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Strand** | **Position** | **Length** | **Initiation** | **Stop** | **Anticodon** | **Intergenic** |
| **(bp)** | **codon** | **codon** | **nucleotide** |
| **tRNAThr** | **H** | **1-71** | 71 |  |  | TGT |  |
| tRNALeu(CUN) | H | 72-143 | 72 |  |  | TAG | 5 |
| tRNAPro | L | 149-217 | 69 |  |  | TGG | 1 |
| tRNAPhe | H | 219-288 | 70 |  |  | GAA | -2 |
| 12S rRNA | H | 287-1216 | 930 |  |  |  |  |
| tRNAVal | H | 1217-1285 | 69 |  |  | TAC |  |
| 16S rRNA | H | 1286-2856 | 1571 |  |  |  | 2 |
| tRNALeu(UUR) | H | 2859-2932 | 74 |  |  | TAA | 3 |
| ND1 | H | 2936-3896. | 961 | ATG | T(AA) |  |  |
| tRNAIle | H | 3897-3967 | 71 |  |  | GAT | -1 |
| tRNAGln | L | 3967-4037 | 71 |  |  | TTG | -1 |
| tRNAMet | H | 4037-4105 | 69 |  |  | CAT |  |
| ND2 | H | 4106-5143 | 1038 | ATT | TAG |  | -2 |
| tRNATrp | H | 5142-5212 | 71 |  |  | TCA |  |
| tRNAAla | L | 5213-5282 | 70 |  |  | TGC | 1 |
| tRNAAsn | L | 5284-5356 | 73 |  |  | GTT | 2 |
| OL | L | 5359-5384 | 26 |  |  |  | -1 |
| tRNACys | L | 5384-5448 | 65 |  |  | GCA |  |
| tRNATyr | L | 5449-5515 | 67 |  |  | GTA | 4 |
| COI | H | 5520-7073 | 1554 | ATA | AGG |  | -13 |
| tRNASer(UCN) | L | 7061-7131 | 71 |  |  | TGA | 1 |
| tRNAAsp | H | 7133-7201 | 69 |  |  | GTC |  |
| COII | H | 7202-7891 | 690 | ATA | TAA |  | 5 |
| tRNALys | H | 7897-7966 | 70 |  |  | TTT |  |
| NC | H | 7967-8679 | 713 |  |  |  |  |
| ATP8 | H | 8680-8832 | 153 | ATG | TAG |  | -4 |
| ATP6 | H | 8829-9507 | 679 | ATA | T(AA) |  |  |
| COIII | H | 9508-10291 | 784 | ATG | T(AA) |  |  |
| tRNAGly | H | 10292-10359 | 68 |  |  | TCC |  |
| ND3 | H | 10360-10699 | 340 | ATG | T(AA) |  |  |
| tRNAArg | H | 10700-10768 | 69 |  |  | TCG |  |
| ND4L | H | 10769-11053 | 285 | ATG | TAA |  | -7 |
| ND4 | H | 11047-12409 | 1363 | GTG | T(AA) |  |  |
| tRNAHis | H | 12410-12478 | 69 |  |  | GTG |  |
| tRNASer(AGY) | H | 12479-12546 | 68 |  |  | GCT | 2 |
| ND6 | L | 12549-13040 | 492 | ATG | AGG |  |  |
| tRNAGlu | L | 13041-13109 | 69 |  |  | TTC | 4 |
| Cytb | H | 13114-14269 | 1156 | ATG | T(AA) |  |  |
| CR1 | H | 14270-15920 | 1651 |  |  |  |  |
| ND5 | H | 15921-17699 | 1779 | ATG | TAA |  |  |
| CR2 | H | 17700-19384 | 1685 | 　 | 　 | 　 | 　 |
| ND5 | H | 19385-21163 | 1779 | ATG | TAA |  |  |
| CR3 | H | 21164-24103 | 2940 |  |  |  |  |

“H”means gene encoded by the H-strand, “L” means gene encoded by the L-strand. Intergenic nucleotide represent noncoding base between genes, a negative number(-) denotes a gene overlapping.

**Table S6.** 2 Location of features in the mtDNA of *P.megacephalus* FJBT.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Strand** | **Position** | **Length** | **Initiation** | **Stop** | **Anticodon** | **Intergenic** |
| **(bp)** | **codon** | **codon** | **nucleotide** |
| **tRNAThr** | **H** | **1-71** | 71 |  |  | TGT | 0 |
| tRNALeu(CUN) | H | 72-143 | 72 |  |  | TAG | 5 |
| tRNAPro | L | 149-216 | 69 |  |  | TGG | 1 |
| tRNAPhe | H | 219-288 | 70 |  |  | GAA | -2 |
| 12S rRNA | H | 287-1216 | 930 |  |  |  | 0 |
| tRNAVal | H | 1217-1285 | 69 |  |  | TAC | 0 |
| 16S rRNA | H | 1286-2864 | 1579 |  |  |  | 2 |
| tRNALeu(UUR) | H | 2867-2940 | 74 |  |  | TAA | 3 |
| ND1 | H | 2944-3904 | 961 | ATG | T(AA) |  | 0 |
| tRNAIle | H | 3905-3975 | 71 |  |  | GAT | -1 |
| tRNAGln | L | 3975-4045 | 71 |  |  | TTG | -1 |
| tRNAMet | H | 4045-4113 | 69 |  |  | CAT | 0 |
| ND2 | H | 4114-5151 | 1038 | ATT | TAG |  | -2 |
| tRNATrp | H | 5150-5220 | 71 |  |  | TCA | 0 |
| tRNAAla | L | 5221-5290 | 70 |  |  | TGC | 1 |
| tRNAAsn | L | 5292-5364 | 73 |  |  | GTT | 2 |
| OL | L | 5367-5392 | 26 |  |  |  | -1 |
| tRNACys | L | 5392-5456 | 65 |  |  | GCA | 0 |
| tRNATyr | L | 5457-5523 | 67 |  |  | GTA | 4 |
| COI | H | 5528-7081 | 1554 | ATA | AGG |  | -13 |
| tRNASer(UCN) | L | 7069-7139 | 71 |  |  | TGA | 1 |
| tRNAAsp | H | 7141-7209 | 69 |  |  | GTC | 0 |
| COII | H | 7210-7899 | 690 | ATA | TAA |  | 5 |
| tRNALys | H | 7905-7974 | 70 |  |  | TTT | 0 |
| NC | H | 7975-8687 | 713 |  |  |  | 0 |
| ATP8 | H | 8688-6640 | 153 | ATA | TAG |  | -4 |
| ATP6 | H | 8837-9515 | 679 | ATA | T(AA) |  | 0 |
| COIII | H | 9516-10299 | 784 | ATG | T(AA) |  | 0 |
| tRNAGly | H | 10300-10367 | 68 |  |  | TCC | 0 |
| ND3 | H | 10368-10707 | 340 | ATG | T(AA) |  | 0 |
| tRNAArg | H | 10708-10776 | 69 |  |  | TCG | 0 |
| ND4L | H | 10777-11061 | 285 | ATG | TAA |  | -7 |
| ND4 | H | 11055-12417 | 1363 | GTG | T(AA) |  | 0 |
| tRNAHis | H | 12418-12486 | 69 |  |  | GTG | 0 |
| tRNASer(AGY) | H | 12487-12554 | 68 |  |  | GCT | 2 |
| ND6 | L | 12557-13048 | 492 | ATG | AGG |  | 0 |
| tRNAGlu | L | 13049-13117 | 69 |  |  | TTC | 4 |
| Cytb | H | 13122-14277 | 1156 | ATG | T(AA) |  |  |
| CR1 | H | 14278-15929 | 1652 |  |  |  |  |
| ND5 | H | 15930-17708 | 1779 | ATG | TAA |  |  |
| CR2 | H | 17709-19280 | 1572 |  |  |  |  |
| ND5 | H | 19281-21059 | 1779 | ATG | TAA |  |  |
| CR3 | H | 21060-23798 | 2739 |  |  |  |  |

**Notes.**“H”means gene encoded by the H-strand, “L” means gene encoded by the L-strand. Intergenic nucleotide represent noncoding base between genes, a negative number(-) denotes a gene overlapping.

**Table S6.** 3 Location of features in the mtDNA of *P.megacephalus* GDCH.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Strand** | **Position** | **Length** | **Initiation** | **Stop** | **Anticodon** | **Intergenic** |
| **(bp)** | **codon** | **codon** | **nucleotide** |
| **tRNAThr** | **H** | **1-71** | 71 |  |  | TGT |  |
| tRNALeu(CUN) | H | 72-142 | 71 |  |  | TAG | 5 |
| tRNAPro | L | 148-216 | 69 |  |  | TGG | 1 |
| tRNAPhe | H | 218-287 | 70 |  |  | GAA | -2 |
| 12S rRNA | H | 286-1216 | 930 |  |  |  |  |
| tRNAVal | H | 1216-1285 | 70 |  |  | TAC | 2 |
| 16S rRNA | H | 1288-2858 | 1571 |  |  |  |  |
| tRNALeu(UUR) | H | 2859-2932 | 74 |  |  | TAA | 3 |
| ND1 | H | 2936-3896. | 961 | ATG | T(AA) |  |  |
| tRNAIle | H | 3897-3967 | 71 |  |  | GAT | -1 |
| tRNAGln | L | 3967-4037 | 71 |  |  | TTG | -1 |
| tRNAMet | H | 4037-4105 | 69 |  |  | CAT |  |
| ND2 | H | 4106-5143 | 1038 | ATT | TAG |  | -2 |
| tRNATrp | H | 5142-5212 | 71 |  |  | TCA |  |
| tRNAAla | L | 5213-5282 | 70 |  |  | TGC | 1 |
| tRNAAsn | L | 5284-5356 | 73 |  |  | GTT | 2 |
| OL | L | 5359-5384 | 26 |  |  |  | -1 |
| tRNACys | L | 5384-5448 | 65 |  |  | GCA |  |
| tRNATyr | L | 5449-5515 | 67 |  |  | GTA | 4 |
| COI | H | 5520-7073 | 1554 | ATA | AGG |  | -13 |
| tRNASer(UCN) | L | 7061-7131 | 71 |  |  | TGA | 1 |
| tRNAAsp | H | 7133-7201 | 69 |  |  | GTC |  |
| COII | H | 7202-7891 | 690 | ATA | TAA |  | 5 |
| tRNALys | H | 7897-7966 | 70 |  |  | TTT |  |
| NC | H | 7967-8679 | 713 |  |  |  |  |
| ATP8 | H | 8680-8832 | 153 | ATG | TAG |  | -4 |
| ATP6 | H | 8829-9507 | 679 | ATA | T(AA) |  |  |
| COIII | H | 9508-10291 | 784 | ATG | T(AA) |  |  |
| tRNAGly | H | 10292-10359 | 68 |  |  | TCC |  |
| ND3 | H | 10360-10699 | 340 | ATG | T(AA) |  |  |
| tRNAArg | H | 10700-10768 | 69 |  |  | TCG |  |
| ND4L | H | 10769-11053 | 285 | ATG | TAA |  | -7 |
| ND4 | H | 11047-12409 | 1363 | GTG | T(AA) |  |  |
| tRNAHis | H | 12410-12478 | 69 |  |  | GTG |  |
| tRNASer(AGY) | H | 12479-12546 | 68 |  |  | GCT | 2 |
| ND6 | L | 12549-13040 | 492 | ATG | AGG |  |  |
| tRNAGlu | L | 13041-13109 | 69 |  |  | TTC | 4 |
| Cytb | H | 13114-14269 | 1156 | ATG | T(AA) |  |  |
| CR1 | H | 14270-15918 | 1649 |  |  |  |  |
| ND5 | H | 15919-17697 | 1779 | ATG | TAA |  |  |
| CR2 | H | 17698-19304 | 1607 | 　 | 　 | 　 | 　 |
| ND5 | H | 19305-21083 | 1779 | ATG | TAA |  |  |
| CR3 | H | 21084-23848 | 2765 | 　 | 　 |  |  |

**Notes.**“H”means gene encoded by the H-strand, “L” means gene encoded by the L-strand. Intergenic nucleotide represent noncoding base between genes, a negative number(-) denotes a gene overlapping.

**Table S6.** 4 Location of features in the mtDNA of *P.megacephalus* TGPJ.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Strand** | **Position** | **Length** | **Initiation** | **Stop** | **Anticodon** | **Intergenic** |
| **(bp)** | **codon** | **codon** | **nucleotide** |
| **tRNAThr** | **H** | **1-71** | 71 |  |  | TGT | 0 |
| tRNALeu(CUN) | H | 72-143 | 72 |  |  | TAG | 5 |
| tRNAPro | L | 149-217 | 69 |  |  | TGG | 1 |
| tRNAPhe | H | 219-288 | 70 |  |  | GAA | -2 |
| 12S rRNA | H | 287-1216 | 930 |  |  |  | 0 |
| tRNAVal | H | 1217-1285 | 69 |  |  | TAC | 0 |
| 16S rRNA | H | 1286-2856 | 1571 |  |  |  | 2 |
| tRNALeu(UUR) | H | 2859-2932 | 74 |  |  | TAA | 3 |
| ND1 | H | 2936-3896 | 961 | ATG | T(AA) |  | 0 |
| tRNAIle | H | 3897-3967 | 71 |  |  | GAT | -1 |
| tRNAGln | L | 3967-4037 | 71 |  |  | TTG | -1 |
| tRNAMet | H | 4037-4105 | 69 |  |  | CAT | 0 |
| ND2 | H | 4106-5143 | 1038 | ATT | TAG |  | -2 |
| tRNATrp | H | 5142-5212 | 71 |  |  | TCA | 0 |
| tRNAAla | L | 5213-5282 | 70 |  |  | TGC | 1 |
| tRNAAsn | L | 5284-5356 | 73 |  |  | GTT | 2 |
| OL | L | 5359-5384 | 26 |  |  |  | -1 |
| tRNACys | L | 5384-5448 | 65 |  |  | GCA | 0 |
| tRNATyr | L | 5449-5515 | 67 |  |  | GTA | 4 |
| COI | H | 5520-7073 | 1554 | ATA | AGG |  | -13 |
| tRNASer(UCN) | L | 7061-7131 | 71 |  |  | TGA | 1 |
| tRNAAsp | H | 7133-7201 | 69 |  |  | GTC | 0 |
| COII | H | 7202-7891 | 690 | ATA | TAA |  | 5 |
| tRNALys | H | 7897-7966 | 70 |  |  | TTT | 0 |
| NC | H | 7967-8679 | 713 |  |  |  | 0 |
| ATP8 | H | 8680-8832 | 153 | ATG | TAG |  | -4 |
| ATP6 | H | 8829-9507 | 679 | ATA | T(AA) |  | 0 |
| COIII | H | 9508-10291 | 784 | ATG | T(AA) |  | 0 |
| tRNAGly | H | 10292-10359 | 68 |  |  | TCC | 0 |
| ND3 | H | 10360-10699 | 340 | ATG | T(AA) |  | 0 |
| tRNAArg | H | 10700-10768 | 69 |  |  | TCG | 0 |
| ND4L | H | 10769-11053 | 285 | ATG | TAA |  | -7 |
| ND4 | H | 11047-12409 | 1363 | GTG | T(AA) |  | 0 |
| tRNAHis | H | 12410-12478 | 69 |  |  | GTG | 0 |
| tRNASer(AGY) | H | 12479-12546 | 68 |  |  | GCT | 2 |
| ND6 | L | 12549-13040 | 492 | ATG | AGG |  | 0 |
| tRNAGlu | L | 13041-13109 | 69 |  |  | TTC | 4 |
| Cytb | H | 13114-14269 | 1156 | ATG | T(AA) |  | 0 |
| CR1 | H | 14270-15908 | 1639 |  |  |  | 0 |
| ND5 | H | 15909-17693 | 1785 | ATG | TAA |  | 0 |
| CR2 | H | 17694-19266 | 1573 |  |  |  | 0 |
| ND5 | H | 19267-21051 | 1785 | ATG | TAA |  | 0 |
| CR3 | H | 21052-23881 | 2830 |  |  |  |  |

**Notes.** “H”means gene encoded by the H-strand, “L” means gene encoded by the L-strand. Intergenic nucleotide represent noncoding base between genes, a negative number(-) denotes a gene overlapping.

**Table S6.** 5 Location of features in the mtDNA of *P.megacephalus* GXGL.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Strand** | **Position** | **Length** | **Initiation** | **Stop** | **Anticodon** | **Intergenic** |
| **(bp)** | **codon** | **codon** | **nucleotide** |
| **tRNAThr** | **H** | **1-71** | 71 |  |  | TGT |  |
| tRNALeu(CUN) | H | 72-143 | 72 |  |  | TAG | 5 |
| tRNAPro | L | 149-217 | 69 |  |  | TGG | 1 |
| tRNAPhe | H | 219-288 | 70 |  |  | GAA | -2 |
| 12S rRNA | H | 287-1216 | 930 |  |  |  |  |
| tRNAVal | H | 1217-1285 | 69 |  |  | TAC |  |
| 16S rRNA | H | 1286-2860 | 1575 |  |  |  | 2 |
| tRNALeu(UUR) | H | 2863-2936 | 74 |  |  | TAA | 3 |
| ND1 | H | 2940-3900 | 961 | ATG | T(AA) |  |  |
| tRNAIle | H | 3901-3971 | 71 |  |  | GAT | -1 |
| tRNAGln | L | 3971-4041 | 71 |  |  | TTG | -1 |
| tRNAMet | H | 4041-4109 | 69 |  |  | CAT |  |
| ND2 | H | 4110-5147 | 1038 | ATT | TAG |  | -2 |
| tRNATrp | H | 5146-5216 | 71 |  |  | TCA |  |
| tRNAAla | L | 5217-5286 | 70 |  |  | TGC | 1 |
| tRNAAsn | L | 5288-5360 | 73 |  |  | GTT | 2 |
| OL | L | 5363-5388 | 26 |  |  |  | -1 |
| tRNACys | L | 5388-5452 | 65 |  |  | GCA |  |
| tRNATyr | L | 5453-5519 | 67 |  |  | GTA | 4 |
| COI | H | 5524-7077 | 1554 | ATA | AGG |  | -13 |
| tRNASer(UCN) | L | 7065-7135 | 71 |  |  | TGA | 1 |
| tRNAAsp | H | 7137-7205 | 69 |  |  | GTC |  |
| COII | H | 7206-7895 | 690 | ATA | TAA |  | 5 |
| tRNALys | H | 7901-7970 | 70 |  |  | TTT |  |
| NC | H | 7971-8678 | 708 |  |  |  |  |
| ATP8 | H | 8679-8831 | 153 | ATG | TAG |  | -4 |
| ATP6 | H | 8828-9506 | 679 | ATA | T(AA) |  |  |
| COIII | H | 9507-10290 | 784 | ATG | T(AA) |  |  |
| tRNAGly | H | 10291-10358 | 68 |  |  | TCC |  |
| ND3 | H | 10359-10698 | 340 | ATG | T(AA) |  |  |
| tRNAArg | H | 10699-10767 | 69 |  |  | TCG |  |
| ND4L | H | 10768-11052 | 285 | ATG | TAA |  | -7 |
| ND4 | H | 11046-12408 | 1363 | GTG | T(AA) |  |  |
| tRNAHis | H | 12409-12477 | 69 |  |  | GTG |  |
| tRNASer(AGY) | H | 12478-12545 | 68 |  |  | GCT | 2 |
| ND6 | L | 12548-13039 | 492 | ATG | AGG |  |  |
| tRNAGlu | L | 13040-13108 | 69 |  |  | TTC | 4 |
| Cytb | H | 13113-14268 | 1156 | ATG | T(AA) |  |  |
| CR1 | H | 14269-15999 | 1731 |  |  |  |  |
| ND5 | H | 16000-17778 | 1779 | ATG | TAA |  |  |
| CR2 | H | 17779-19471 | 1693 | 　 | 　 | 　 | 　 |
| ND5 | H | 19472-21250 | 1779 | ATG | TAA |  |  |
| CR3 | H | 21251-23996 | 2746 | 　 | 　 |  |  |

**Notes.** “H”means gene encoded by the H-strand, “L” means gene encoded by the L-strand. Intergenic nucleotide represent noncoding base between genes, a negative number(-) denotes a gene overlapping.

**Table S7.** Features of *P. megacephalus* control regions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **species** | **Length of CR1** | **copy number of 38 bp tandem repeat in CR1** | **Length of CR2** | **copy number of 38 bp tandem repeat in CR2** | **Length of CR3** | **copy number of 38 bp tandem repeat in CR3** | **copy number of 100 bp tandem repeats in CR3** | **Length of similar regions/similarity** |
| GDCH | 1649 | 8.6 | 1607 | 7.5 | 2765 | 7.5 | 11.6 | 99%(99.32%) |
| FJFQ | 1652 | 8.6 | 1572 | 6.5 | 2739 | 9.5 | 10.6 | 99%(98.92%) |
| GXGL | 1731 | 9.6 | 1693 | 8.5 | 2746 | 9.5 | 10.7 | 99%(99.76%) |
| ZJJH | 1651 | 8.6 | 1685 | 9.5 | 2940 | 9.5 | 12.6 | 99%(99.45%) |
| TGPJ | 1639 | 7.6 | 1573 | 6.5 | 2830 | 6.5 | 12.6 | 99%(98.98%) |

**Table S8.** Pairwise genetic distance (below diagonal) and natural logarithm of geographical distance (km) (above diagonal) between geographical populations of *P. megacephalus* based on the mitogenomes. The specific collection locations of the two published sequences (MH936677 and AY458598) retrieved from the NCBI database are unknown, and geographical distances to other samples were not calculated.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Samples** | **ZJJH** | **ZJWZ** | **GXGL** | **GDQY** | **GDCH** | **GDHD** | **FJFQ** | **TGPJ** | **MH936677** | **AY458598** |
| ZJJH |  | 5.140 | 6.934 | 6.788 | 6.737 | 6.735 | 5.945 | 8.084 | **-** | **-** |
| ZJWZ | 0.002 |  | 6.988 | 6.792 | 6.734 | 6.701 | 5.649 | 8.081 | **-** | **-** |
| GXGL | 0.054 | 0.054 |  | 5.861 | 5.984 | 6.247 | 6.810 | 7.740 | **-** | **-** |
| GDQY | 0.055 | 0.054 | 0.002 |  | 4.040 | 5.113 | 6.483 | 7.764 | **-** | **-** |
| GDCH | 0.002 | 0.000 | 0.054 | 0.054 |  | 4.794 | 6.396 | 7.783 | **-** | **-** |
| GDHD | 0.055 | 0.054 | 0.001 | 0.002 | 0.054 |  | 6.311 | 7.792 | **-** | **-** |
| FJFQ | 0.003 | 0.002 | 0.054 | 0.055 | 0.002 | 0.055 |  | 7.996 | **-** | **-** |
| TGPJ | 0.039 | 0.039 | 0.057 | 0.058 | 0.038 | 0.058 | 0.039 |  | **-** | **-** |
| MH936677 | 0.003 | 0.002 | 0.054 | 0.055 | 0.002 | 0.055 | 0.002 | 0.039 |  | - |
| AY458598 | 0.055 | 0.055 | 0.001 | 0.003 | 0.055 | 0.002 | 0.055 | 0.058 | 0.056 |  |