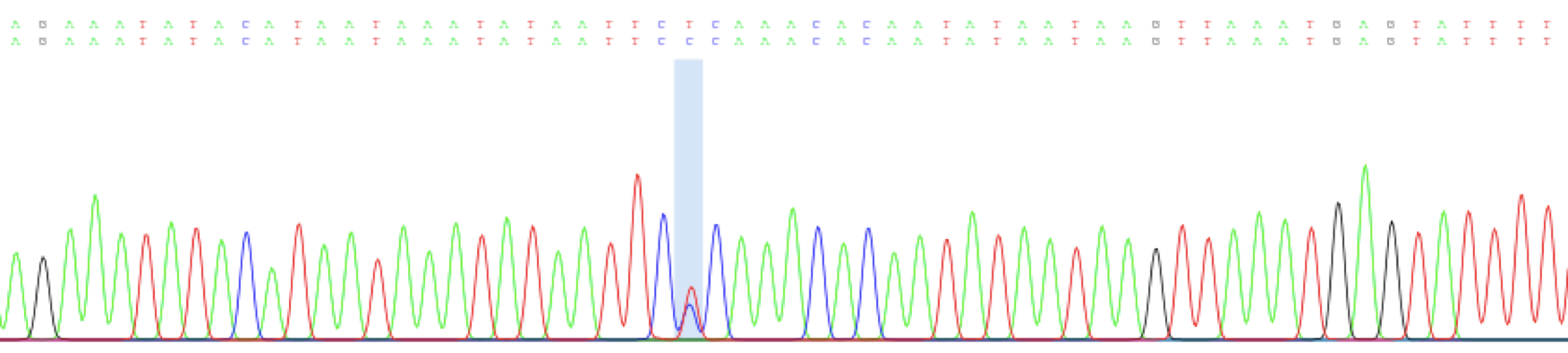
**Supplemental materials **

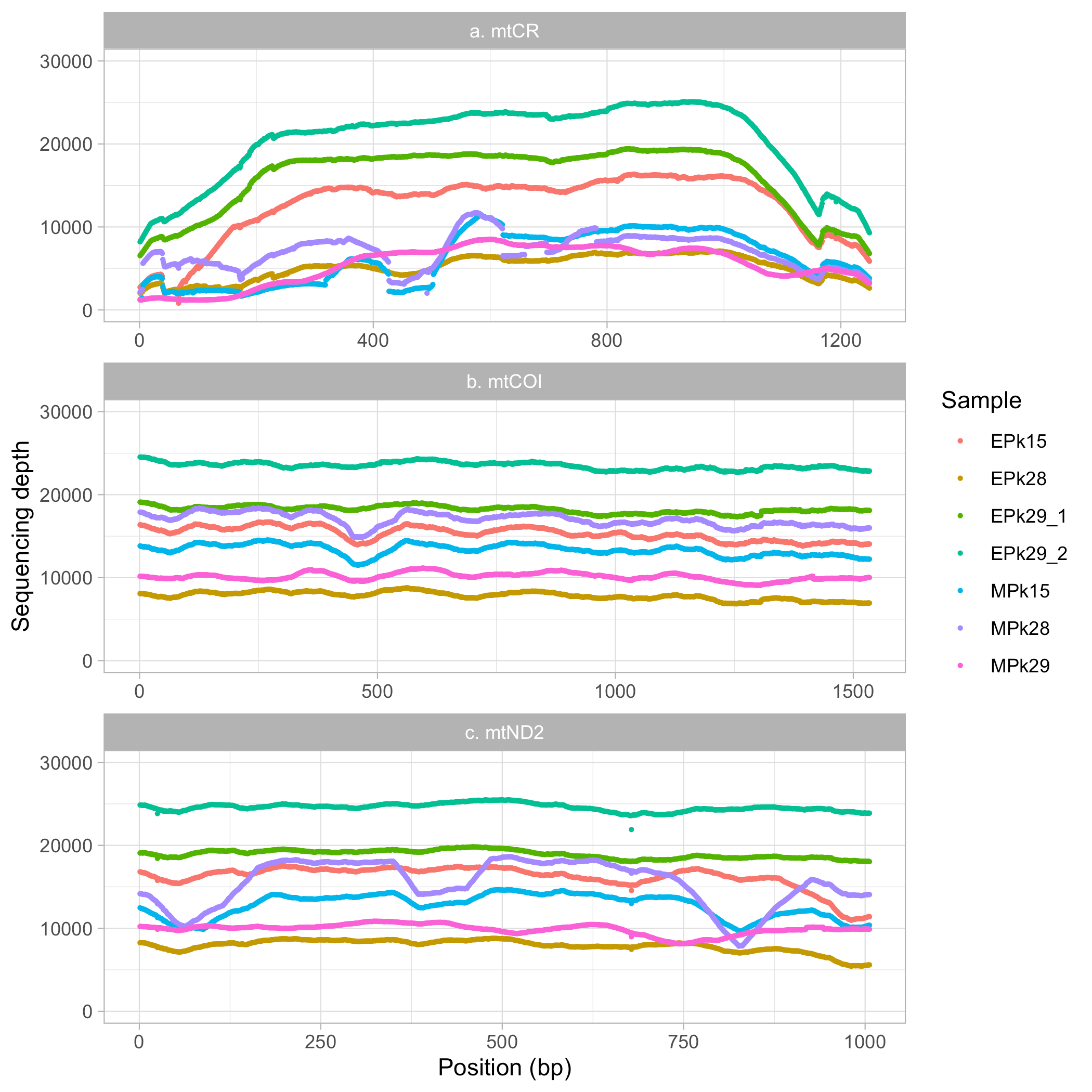
**Supplementary Figure 1:** Chromatogram of partial *mtCR* sequence of MPk29 presenting a clear double peak at position 331 of the reference *mtCR*. The blue band highlighted on this double peak indicated the intra-sample variant called by the Poly Peak Parser [1] with the signal ratio cutoff at 0.33.

**Supplementary Table 1:** Summary statistics of the assembled partial 5kb mitogenome

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Number of paired-end readsa** | | | | **Average sequencing depth** |
| **After quality control** | **Mapped to the referenceb** | **Retained after Deduplicationb** | **Retained after Filteringb** |
| MPk15 | 1,732,080 | 1,110,795 | 719,316 | 697,775 | 9,405.19 |
| EPk15 | 1,689,562 | 1,309,156 | 845,532 | 834,491 | 11,635.10 |
| MPk28 | 1,986,746 | 1,420,300 | 908,931 | 870,940 | 11,719.37 |
| EPk28 | 1,451,262 | 644,755 | 448,185 | 440,951 | 6,120.60 |
| MPk29 | 2,121,238 | 1,645,209 | 554,664 | 513,890 | 7,382.55 |
| EPk29\_1 | 7,971,184 | 3,042,463 | 1,143,980 | 970,089 | 14,293.89 |
| EPk29\_2 | 8,181,462 | 3,795,905 | 1,418,271 | 1,242,741 | 18,501.28 |

a: Number of reads taken both ends into account

b: Number of mapped reads calculated by flagstat in samtools [2]



**Supplementary Figure 2:** Distribution of sequencing depth supporting *mtCR* (a), *mtCOI* (b) and *mtND2* (c) of seven *P. pelagicus* samples: EPk15, EPk28, EPk29\_1, EPk29\_2, MPk15, MPk28 and MPk29*.*

**Supplementary Table 2:** The statistical results carried out on the differences in the heteroplasmy levels between offspring and their corresponding mothers (O-M). The O-M values were separated into sub-datasets based on the type of mutation, mtDNA region and maternal relationship.

| Sample | N | Median(Mean) | H0: Mean(O-M) = 0 | | Kolmogorov-Smirnov testa | Multiple comparisonb | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Student’s  *t* test | Wilcoxon test | ANOVA | Kruskal-Wallis test |
| **Segregating heteroplasmic variants** | | | | | | | |
| All | 48 | -0.116  (0.029) | 0.579 | 0.883 | < 0.001\*\*\* | NA | NA |
| Type of mutation | | | | | | | |
| Noncoding | 7 | 0.666  (0.488) | 0.017\* | 0.078 | 0.709 | < 0.001\*\*\* | < 0.001\*\*\* |
| Silence | 38 | -0.128  (-0.059) | 0.219 | 0.153 | < 0.001\*\*\* |
| Replacement | 3 | -0.093  (0.066) | 0.726 | 1.000 | 0.653 |
| mtDNA region | | | | | | | |
| *mtCR* | 7 | 0.666  (0.488) | 0.017\* | 0.078 | 0.709 | < 0.001\*\*\* | 0.001\*\* |
| *mtCOI* | 22 | -0.128  (-0.037) | 0.549 | 0.353 | 0.002\*\* |
| *mtND2* | 19 | -0.117  (-0.064) | 0.359 | 0.332 | 0.026\* |
| Maternal relationship | | | | | | | |
| EPk15 | NA | NA | NA | NA | NA | NA | NA |
| EPk28 | 25 | 0.393  (0.163) | 0.088 | 0.016\* | 0.115 | 0.021\* | 0.485 |
| EPk29\_1 | 4 | -0.132  (-0.112) | 0.015\* | 0.125 | 0.450 |
| EPk29\_2 | 19 | -0.117  (-0.118) | < 0.001\*\*\* | < 0.001\*\*\* | 0.748 |
| **Non-segregating heteroplasmic variants** | | | | | | | |
| All | 85 | -0.067  (0.015) | 0.705 | < 0.001\*\*\* | < 0.001\*\*\* | NA | NA |
| Type of mutation | | | | | | | |
| Noncoding | 26 | -0.011  (0.350) | 0.001\*\* | 0.075 | 0.012\* | < 0.001\*\*\* | < 0.001\*\*\* |
| Silence | 55 | -0.127  (-0.134) | < 0.001\*\*\* | < 0.001\*\*\* | 0.291 |
| Replacement | 4 | -0.084  (-0.114) | 0.120 | 0.125 | 0.924 |
| mtDNA region | | | | | | | |
| *mtCR* | 26 | -0.011  (0.350) | 0.001\*\* | 0.075 | 0.012\* | < 0.001\*\*\* | < 0.001\*\*\* |
| *mtCOI* | 26 | -0.146  (-0.163) | < 0.001\*\*\* | < 0.001\*\*\* | 0.191 |
| *mtND2* | 33 | -0.082  (-0.109) | < 0.001\*\*\* | < 0.001\*\*\* | 0.123 |
| Maternal relationship | | | | | | | |
| EPk15 | 32 | -0.206  (-0.025) | 0.743 | 0.032\* | 0.001\*\* | 0.001\*\* | < 0.001\*\*\* |
| EPk28 | 18 | -0.015  (0.290) | 0.010\* | 0.417 | 0.009\*\* |
| EPk29\_1 | 25 | -0.120  (-0.103) | < 0.001\*\*\* | < 0.001\*\*\* | 0.341 |
| EPk29\_2 | 10 | -0.062  (-0.059) | < 0.001\*\*\* | 0.002\*\* | 0.999 |
| **All inherited heteroplasmic variants** | | | | | | | |
| All | 133 | -0.095  (0.020) | 0.523 | 0.003\*\* | < 0.001\*\*\* | NA | NA |
| Type of mutation | | | | | | | |
| Noncoding | 33 | 0.472 (0.379) | < 0.001\*\*\* | 0.009\*\* | 0.009\*\* | < 0.001\*\*\* | < 0.001\*\*\* |
| Silence | 93 | -0.128  (-0.103) | < 0.001\*\*\* | < 0.001\*\*\* | < 0.001\*\*\* |
| Replacement | 7 | -0.093  (-0.037) | 0.650 | 0.297 | 0.340 |
| mtDNA region | | | | | | | |
| *mtCR* | 33 | 0.472 (0.379) | < 0.001\*\*\* | 0.009\*\* | 0.009\*\* | < 0.001\*\*\* | < 0.001\*\*\* |
| *mtCOI* | 48 | -0.130  (-0.105) | 0.001\*\* | < 0.001\*\*\* | 0.001\*\* |
| *mtND2* | 52 | -0.100 (-0.093) | 0.001\*\* | < 0.001\*\*\* | 0.001\*\* |
| Maternal relationship | | | | | | | |
| EPk15 | 32 | -0.206  (-0.025) | 0.743 | 0.032\* | 0.001\*\* | < 0.001\*\*\* | < 0.001\*\*\* |
| EPk28 | 43 | -0.015  (0.216) | 0.003\*\* | 0.010\* | 0.038\* |
| EPk29\_1 | 29 | -0.124  (-0.104) | < 0.001\*\*\* | < 0.001\*\*\* | 0.161 |
| EPk29\_2 | 29 | -0.107  (-0.097) | < 0.001\*\*\* | < 0.001\*\*\* | 0.389 |

a: Kolmogorov-Smirnov test was applied to test whether the distribution of O-M was normal.

b: ANOVA and Kruskal-Wallis test were applied to compare mean of normally distributed datasets and median of non-normally distributed datasets, respectively.

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

NA: Not applicable

**Supplementary Table 3:** The multiple pairwise comparison p-values of O-M calculated from segregating and non-segregating variants categorized by type of mutation. The p-values computed by Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) of segregating and non-segregating variants were shown above and below the diagonal line, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Noncoding | Silence | Replacement |
| Noncoding |  | 0.001\*\* | 0.205 |
| Silence | < 0.001\*\*\* |  | 0.311 |
| Replacement | 0.048\* | 1.000 |  |

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

**Supplementary Table 4:** The multiple pairwise comparison p-values of O-M calculated from combined segregating and non-segregating variants categorized by type of mutation. The p-values computed by the Turkey (the ANOVA post-hoc test) and Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) were shown above and below the diagonal line, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Noncoding | Silence | Replacement |
| Noncoding |  | < 0.001\*\*\* | 0.002\*\* |
| Silence | < 0.001\*\*\* |  | 0.829 |
| Replacement | 0.013\* | 0.361 |  |

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

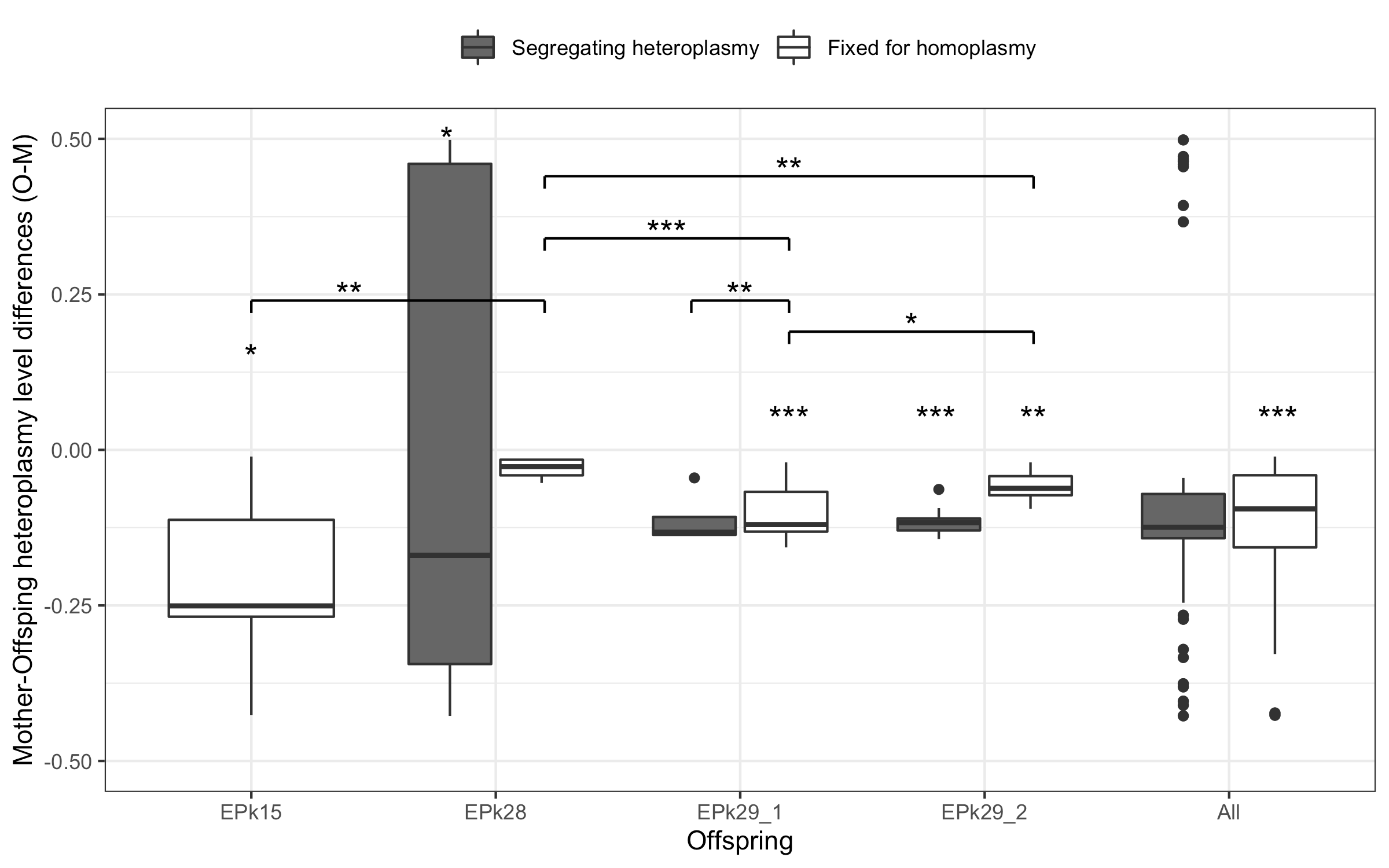
**Supplementary Table 5:** The multiple pairwise comparison p-values of O-M calculated from segregating and non-segregating variants categorized by mtDNA region. The p-values computed by the Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) for segregating and non-segregating variants were shown above and below the diagonal line, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
|  | *mtCR* | *mtCOI* | *mtND2* |
| *mtCR* |  | 0.002\*\* | 0.004\*\* |
| *mtCOI* | < 0.001\*\*\* |  | 1.000 |
| *mtND2* | < 0.001\*\*\* | 0.076 |  |

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

**Supplementary Table 6:** The multiple pairwise comparison p-values of O-M calculated from combined segregating and non-segregating variants categorized by mtDNA region. The p-values computed by the Turkey (the ANOVA post-hoc test) and Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) were shown above and below the diagonal line, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
|  | *mtCR* | *mtCOI* | *mtND2* |
| *mtCR* |  | < 0.001\*\*\* | < 0.001\*\*\* |
| *mtCOI* | < 0.001\*\*\* |  | 0.976 |
| *mtND2* | < 0.001\*\*\* | 0.240 |  |

****

**Supplementary Figure 3:** The distribution of the differences in heteroplasmy levels between offspring and their corresponding mothers (O-M) of segregating and non-segregating variants. The O-M values were further separated into groups based on the familial relationship. The height of each box represents the inter-quartile range (IQR). The upper and lower whiskers extend 1.5 IQR from the 75th and 25th percentiles, respectively. The line at the middle of the box represents the median value. The stars presented above the box represent that the median of O-M values was significantly different from zero, which \*, \*\* and \*\*\* represent p-values of the Wilcoxon test that are less than 0.05, 0.01, and 0.001, respectively. The significant difference of the median O-M between datasets that were computed by the Wilcoxon rank sum test was also shown using the same symbol.

**Supplementary Table 7:** The multiple pairwise comparison p-values of O-M calculated from segregating and non-segregating variants categorized by maternal relationship. The p-values computed by the Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) for segregating and non-segregating variants were shown above and below the diagonal line, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | EPk15 | EPk28 | EPk29\_1 | EPk29\_2 |
| EPk15 |  | NA | NA | NA |
| EPk28 | 0.005\*\* |  | 1.000 | 0.830 |
| EPk29\_1 | 0.636 | < 0.001\*\*\* |  | 1.000 |
| EPk29\_2 | 0.571 | 0.003\*\* | 0.039\* |  |

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

**Supplementary Table 8:** The multiple pairwise comparison p-values of O-M calculated from combined segregating and non-segregating variants categorized by maternal relationship. The p-values computed by the Turkey (the ANOVA post-hoc test) and Wilcoxon rank sum test (the Kruskal-Wallis post-hoc test) were shown above and below the diagonal line, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | EPk15 | EPk28 | EPk29\_1 | EPk29\_2 |
| EPk15 |  | 0.012\* | 0.790 | 0.831 |
| EPk28 | 0.153 |  | < 0.001\*\*\* | < 0.001\*\*\* |
| EPk29\_1 | 0.625 | 0.003\*\* |  | 0.999 |
| EPk29\_2 | 0.461 | 0.003\*\* | 1.000 |  |

\*: p-value is less than 0.05. \*\*: p-value is less than 0.01. \*\*\*: p-value is less than 0.001.

**References**

1. Hill JT, Demarest BL, Bisgrove BW, Su YC, Smith M, Yost HJ. Poly peak parser: Method and software for identification of unknown indels using sanger sequencing of polymerase chain reaction products. Developmental Dynamics. 2014;243:1632-6.

2. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25:2078-9.