**Supplementary Materials**

**Phylomitogenomics elucidates the evolution of symbiosis in Thoracotremata (Decapoda: Cryptochiridae, Pinnotheridae, Varunidae)**

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**Supplementary Tables**

**Table S7.** Wilcoxon rank sum test between symbiotic and free-living groups in the free-ratio model, and the average ω ratio (excluding the outliers shown in Fig. 4).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genes | Wilcoxon rank sum test (P-values) | Free-living(Average ω ratio) | Symbiotic(Average ω ratio) | Is ω ratio (free-living >symbiotic) |
| *atp6* | 0.04 | 0.0160 | 0.0208 | Yes |
| *atp8* | 0.62 | 0.0415 | 0.0472 | Yes |
| *cob* | 0.04 | 0.0084 | 0.0106 | Yes |
| *cox1* | 0.27 | 0.0057 | 0.0060 | Yes |
| *cox2* | 0.66 | 0.0112 | 0.0095 | No |
| *cox3* | 0.30 | 0.0119 | 0.0112 | No |
| *nad1* | 0.52 | 0.0099 | 0.0104 | Yes |
| *nad2* | 0.42 | 0.0294 | 0.0274 | No |
| *nad3* | 0.10 | 0.0248 | 0.0314 | Yes |
| *nad4* | 0.29 | 0.0182 | 0.0200 | Yes |
| *nad4l* | 0.45 | 0.0193 | 0.0159 | No |
| *nad5* | 0.02 | 0.0164 | 0.0214 | Yes |
| *nad6* | 0.23 | 0.03016 | 0.0330 | Yes |

**Table S8.** The results of selective pressure analysis of each mitochondrial gene based on the branch-site model (Model A null vs Model A) for the gall crabs (Cryptochiridae), pea crabs (Pinnotheridae), and two symbiotic varunids. In the Bayes Empirical Bayes (BEB), one star represents sites with greater posterior probability (PP ≥ 95%), two stars (PP ≥ 99%) are positively selected.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Protein | Model A null (lnL) | Model A (lnL) | df | Chi-square (p) | Positively selected site (BEB: PP≥95%) |
| Cryptochiridae | *atp6* | -15128.656897 | -15128.656897 | 1 | 1 |  |
| *atp8* | -4589.891381 | -4588.217852 | 1 | 0.067 |  4 M 0.999\*\* 8 F 0.999\*\* 10 L 0.998\*\* 16 F 0.981\* 26 E 1.000\*\* |
| *cob* | -23324.546950 | -23324.546950 | 1 | 1 |  25 L 0.530 207 S 0.923 276 T 0.969\* 321 I 0.658 |
| *cox1* | -29862.398315 | -29862.398315 | 1 | 1 |  |
| *cox2* | -11900.142779 | -11900.142779 | 1 | 1 |  |
| *cox3* | -14890.249110 | -14890.249110 | 1 | 1 |  |
| *nad1* | -18850.470119 | -18850.470119 | 1 | 1 |  |
| *nad2* | -28428.159441 | -28428.159441 | 1 | 1 |  |
| *nad3* | -7663.856629 | -7663.856629 | 1 | 1 |  |
| *nad4* | -28653.448974 | -28653.448974 | 1 | 1 |  |
| *nad4l* | -5739.354145 | -5739.354145 | 1 | 1 |  |
| *nad5* | -33283.990859 | -33283.990859 | 1 | 1 |  5 F 0.976\* 13 V 0.701 14 V 0.594 25 S 0.689 350 I 0.518 354 M 0.998\*\* 400 V 0.825 404 V 0.617 |
| *nad6* | -12872.787310 | -12957.575664 | 1 | 0 |  63 F 0.999\*\* 78 L 0.589 79 T 0.877 81 S 0.929 89 P 1.000\*\* |
| Pinnotheridae | *atp6* | -16057.911731 | -16057.911731 | 1 | 1 |  |
| *atp8* | -4883.178500 | -4883.178500 | 1 | 1 |  26 S 0.955\* 28 I 0.904 |
| *cob* | -24752.871004 | -24752.871004 | 1 | 1 |  310 D 0.999\*\* |
| *cox1* | -31822.239128 | -31822.239128 | 1 | 1 |  |
| *cox2* | -12797.444979 | -12797.444979 | 1 | 1 |  |
| *cox3* | -16116.700486 | -16116.700486 | 1 | 1 |  |
| *nad1* | -19947.072622 | -19947.072622 | 1 | 1 |  |
| *nad2* | -30077.074307 | -30077.074307 | 1 | 1 |  50 S 0.976\* 55 I 0.988\* 106 V 0.843 |
| *nad3* | -8263.625226 | -8263.625226 | 1 | 1 |  |
| *nad4* | -30340.873416 | -30340.873416 | 1 | 1 |  125 L 0.995\*\* 126 V 0.993\*\* 130 G 0.938 131 V 1.000\*\* 318 S 0.913 |
| *nad4l* | -6087.498550 | -6087.498550 | 1 | 1 |  |
| *nad5* | -35214.671347 | -35214.671347 | 1 | 1 |  149 L 0.521 158 S 1.000\*\* 214 A 0.998\*\* 215 S 0.843 371 S 0.890 400 T 0.947 426 I 1.000\*\* |
| *nad6* | -13673.134214 | -13673.134214 | 1 | 1 |  35 F 0.999\*\* 71 S 0.951\* 75 F 1.000\*\* 80 T 0.692 82 P 0.882 91 N 0.714 |
| *A. inaequipes* and *T. horvathi* (Varunidae) | *atp6* | -14377.525391 | -14377.288921 | 1 | 0.492 |  |
| *atp8* | -4322.020065 | -4320.835179 | 1 | 0.124 |  10 L 0.883 18 L 0.943 26 N 0.999\*\* |
| *cob* | -22528.738913 | -22528.738913 | 1 | 1 |  |
| *cox1* | -28799.697975 | -28799.697975 | 1 | 1 |  |
| *cox2* | -11377.588529 | -11377.588529 | 1 | 1 |  |
| *cox3* | -14347.446919 | -14346.507202 | 1 | 0.170 |  |
| *nad1* | -18111.752616 | -18111.445638 | 1 | 0.433 |  |
| *nad2* | -27172.619495 | -27172.619495 | 1 | 1 |  |
| *nad3* | -7374.039532 | -7374.039532 | 1 | 1 |  |
| *nad4* | -27452.136684 | -27452.136684 | 1 | 1 |  |
| *nad4l* | -5427.607573 | -5427.607573 | 1 | 1 |  |
| *nad5* | -31973.682602 | -31973.408566 | 1 | 0.459 |  4 G 0.723 21 A 0.909 37 L 0.602 112 S 0.997\*\* 380 V 0.869 404 C 0.993\*\* 423 S 0.893 436 I 0.925 |
| *nad6* | -12434.494402 | -12434.494402 | 1 | 1 |  |

Note: When the evidence for positive selection is not very strong, the Likelihood-Ratio Test (LRT) and BEB may show conflicting results. In particular, when p>0.05, BEB can still detect several positive selection sites (i.e. genes *atp8*, *cob and nad5* in Cryptochiridae; genes *atp8*, *nad2*, *nad4*, *nad5* and *nad6* in Pinnotheridae; genes *atp8* and *nad5* insymbiotic Varunidae*.*).

**Supplementary Figures**

**Figure S1.** The Relative Synonymous Code Usage (RSCU) for concatenated Protein Coding Genes (PCGs) of: A) *Kroppcarcinus siderastreicola*; B) *Opecarcinus hypostegus*; C) *Troglocarcinus corallicola*; and D) *Hapalocarcinus marsupialis* s.l..

**Figure S2.** Secondary structures of 22 transfer RNA genes of: A) *Kroppcarcinus siderastreicola*; B) *Opecarcinus hypostegus*; C) *Troglocarcinus corallicola*; and D) *Hapalocarcinus marsupialis* s.l.. The names of the mitochondrial transfer RNA genes can be found in Table S2.

**Figure S3.** Bayesian inference tree based on a concatenated dataset including 13 Protein Coding Genes (PCGs) and two ribosomal RNA genes (16s rRNA and 12s rRNA) by MrBayes.