

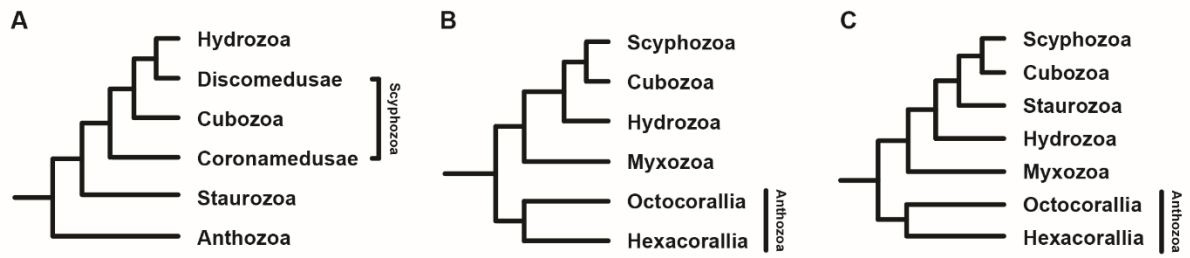
iScience, Volume 26

## **Supplemental information**

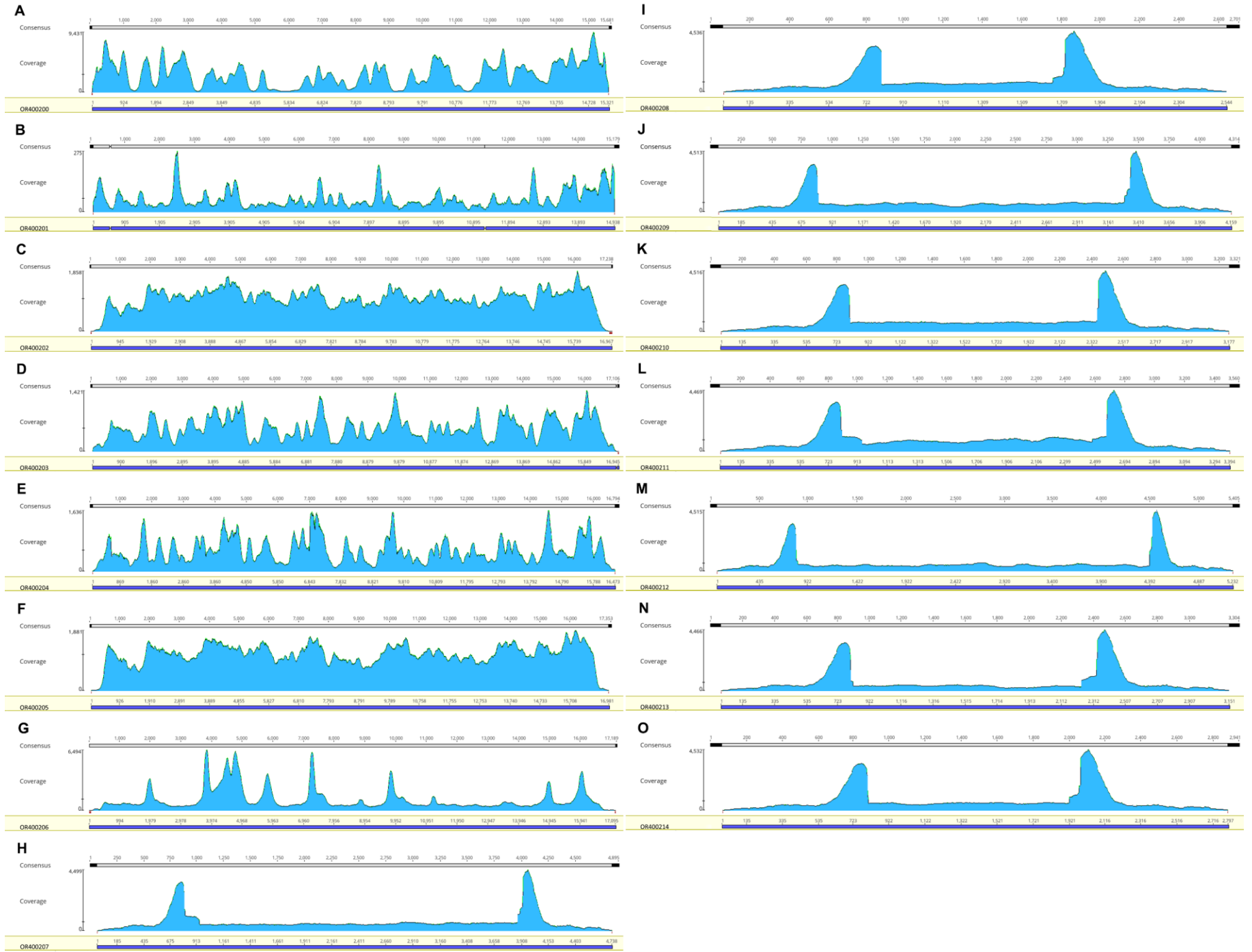
### **Revisiting mitogenome evolution in Medusozoa with eight new mitochondrial genomes**

**Min Kang Ling, Nicholas Wei Liang Yap, Iffah Binte Iesa, Zhi Ting Yip, Danwei Huang, and Zheng Bin Randolph Quek**

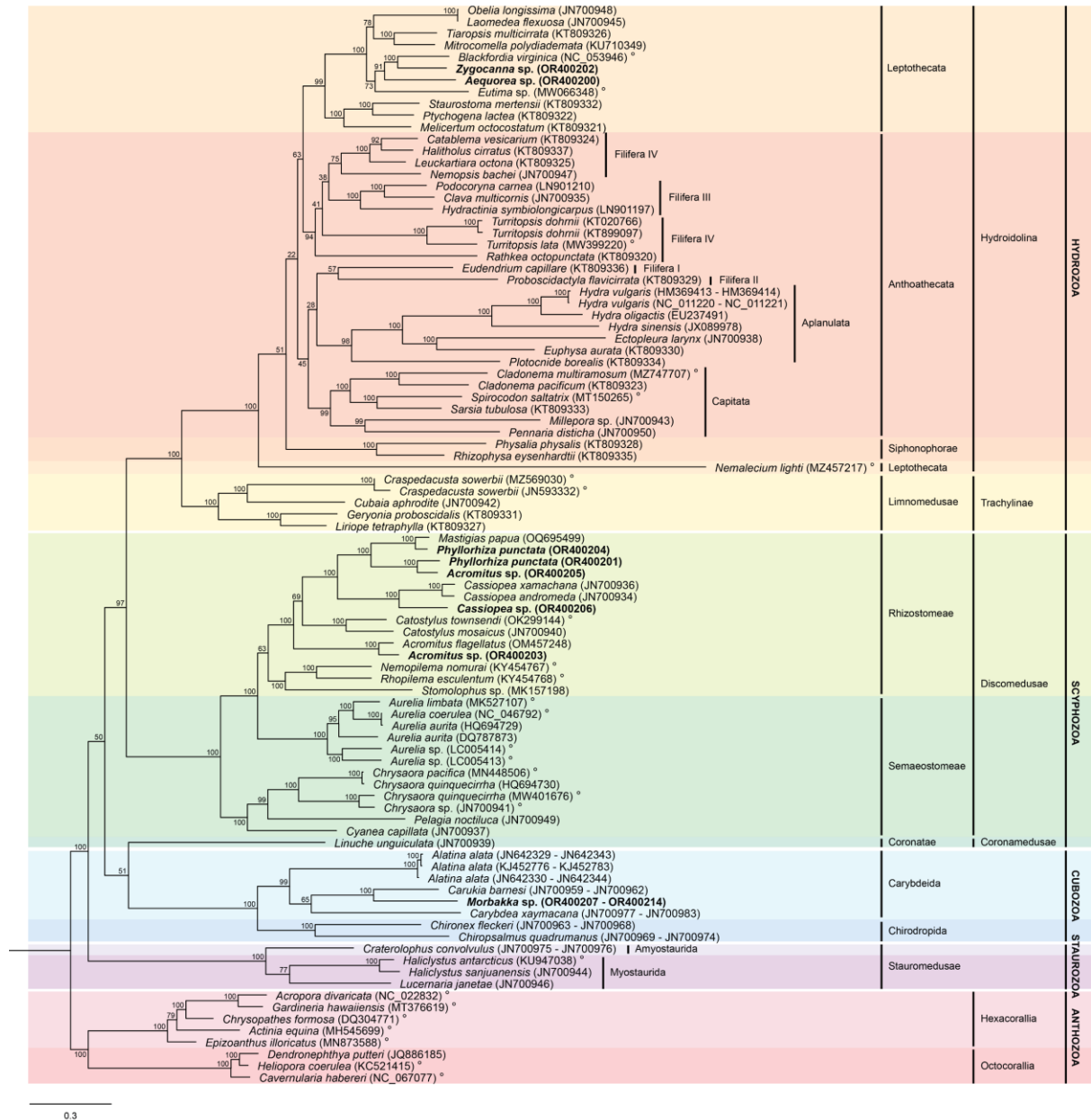
## SUPPLEMENTAL MATERIALS



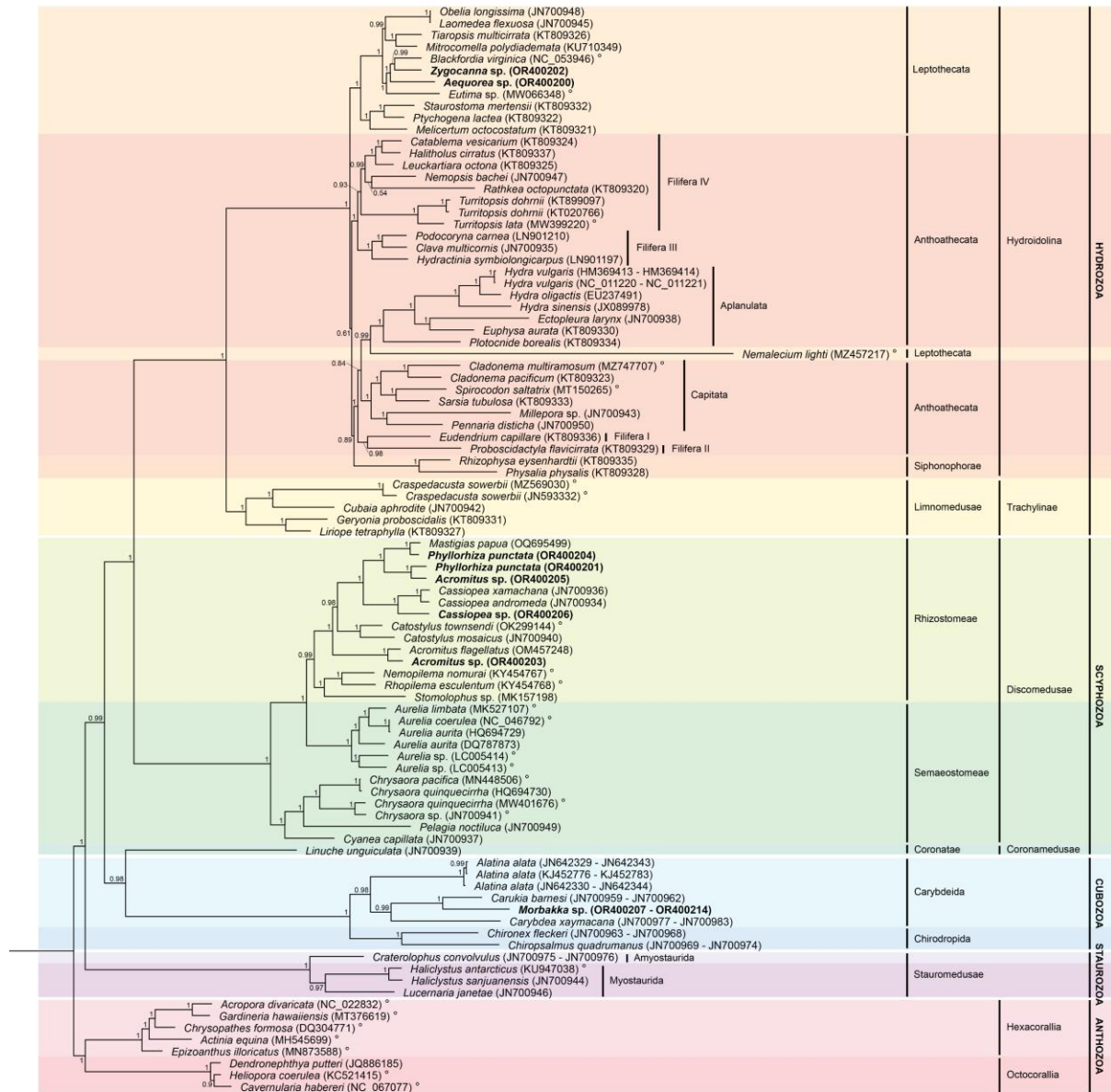
**Figure S1.** Existing hypotheses of cnidarian phylogenetic relationships as reconstructed from previous nuclear genome analyses, related to Figure 1. Phylogenies based on studies from (A) Dawson<sup>1</sup>, (B) Chang et al.<sup>2</sup> and (C) Kayal et al.<sup>3</sup>



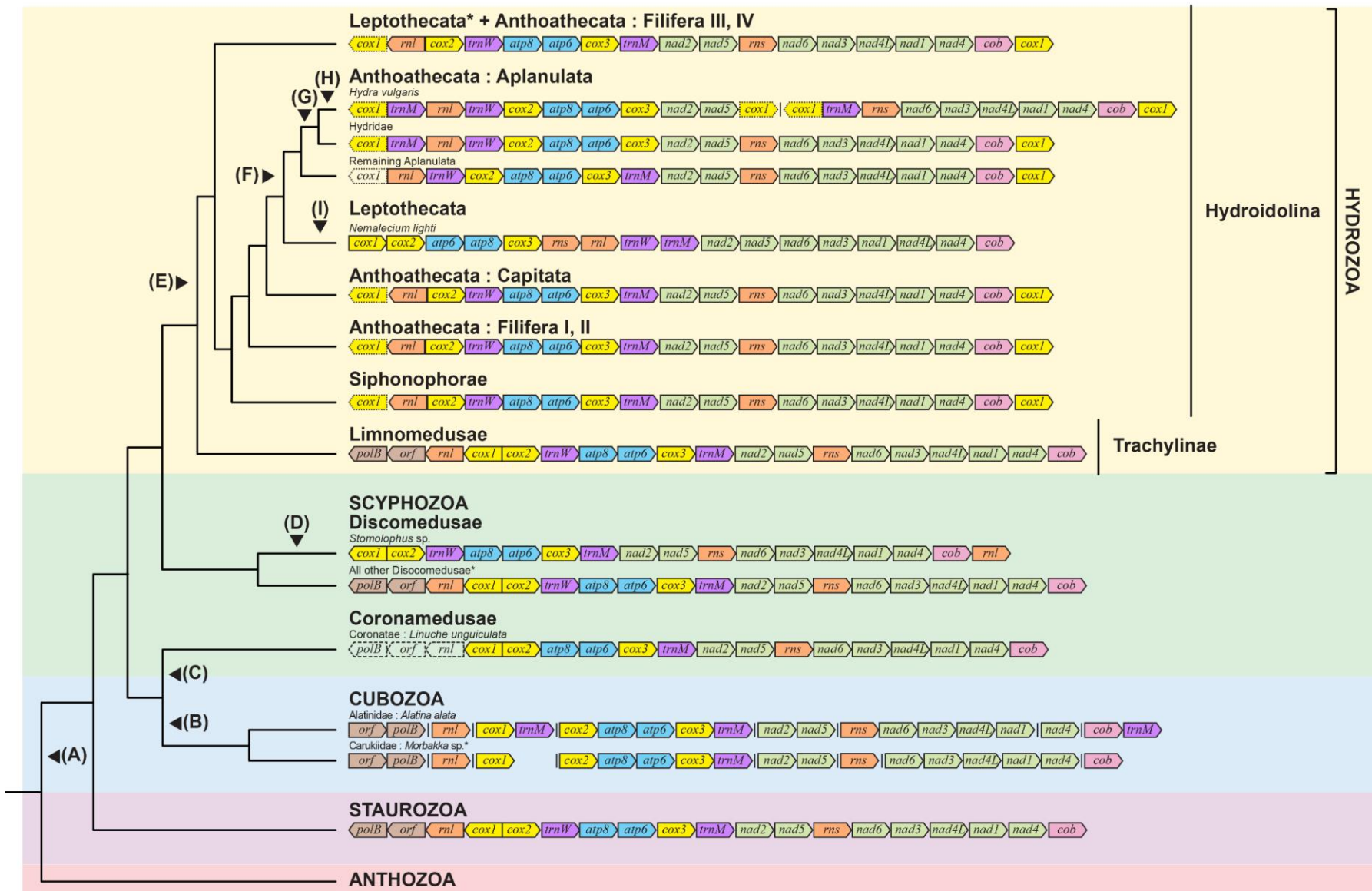
**Figure S2.** DNA mapping figures of assembled mitochondrial genomes in the current study, related to STAR Methods. Low quality reads (MAPQ < q30) were excluded. Visualised in Geneious Prime<sup>4</sup> (v2022.0.1). Scale bar on left denotes maximum coverage and mean coverage (indicated with a marking). (A) OR400200 (*Aequorea* sp.), total reads mapped = 291,414, mean read length = 151 bp, mean read coverage  $\pm$  SD = 2817.1  $\pm$  1922.1; (B) OR400201 (*Phyllorhiza punctata*), total reads mapped = 117,350, mean read length = 151 bp, mean read coverage  $\pm$  SD = 1004.5  $\pm$  308.8; (C) OR400202 (*Zygocanna* sp.), total reads mapped = 6,627, mean read length = 152 bp, mean read coverage  $\pm$  SD = 59.9  $\pm$  42.8; (D) OR400203 (*Acromitus* sp.), total reads mapped = 64,673, mean read length = 151 bp, mean read coverage  $\pm$  SD = 544.6  $\pm$  266.2; (E) OR400204 (*Phyllorhiza punctata*), total reads mapped = 65,620, mean read length = 151 bp, mean read coverage  $\pm$  SD = 562.3  $\pm$  334.8; (F) OR400205 (*Acromitus* sp.), total reads mapped = 131,286, mean read length = 151 bp, mean read coverage  $\pm$  SD = 1121.7  $\pm$  350.1; (G) OR400206 (*Cassiopea* sp.), total reads mapped = 140,616, mean read length = 151 bp, mean read coverage  $\pm$  SD = 1192.3  $\pm$  1217.4. (H) OR400207 (*Morbakka* sp. chr. 1), total reads mapped = 25,532, mean read length = 151 bp, mean read coverage  $\pm$  SD = 650.6  $\pm$  727.1; OR400207 (*Morbakka* sp. chr. 2), total reads mapped = 15,916, mean read length = 151 bp, mean read coverage  $\pm$  SD = 753.7  $\pm$  916.5; OR400207 (*Morbakka* sp. chr. 3), total reads mapped = 22,530, mean read length = 151 bp, mean read coverage  $\pm$  SD = 691.7  $\pm$  748.3; OR400207 (*Morbakka* sp. chr. 4), total reads mapped = 19,096, mean read length = 151 bp, mean read coverage  $\pm$  SD = 750.5  $\pm$  831.6; OR400207 (*Morbakka* sp. chr. 5), total reads mapped = 21,582, mean read length = 151 bp, mean read coverage  $\pm$  SD = 819.9  $\pm$  823.3; OR400207 (*Morbakka* sp. chr. 6), total reads mapped = 22,995, mean read length = 151 bp, mean read coverage  $\pm$  SD = 566.6  $\pm$  681.3; OR400207 (*Morbakka* sp. chr. 7), total reads mapped = 15,903, mean read length = 151 bp, mean read coverage  $\pm$  SD = 612.9  $\pm$  859.8; OR400207 (*Morbakka* sp. chr. 8), total reads mapped = 16,246, mean read length = 151 bp, mean read coverage  $\pm$  SD = 706.5  $\pm$  893.9. Abbreviations: chr. – chromosome; SD – standard deviation.



**Figure S3.** Phylogenetic relationships among Cnidaria mitogenomes based on maximum likelihood (ML) analysis, related to Figure 2. Analysis was conducted with RAXML-NG<sup>5</sup> (v0.8.1). GenBank ascension numbers of mitogenomes are provided in parentheses. Support values correspond to the bootstrap values from the ML phylogeny. Bolded specimens indicate newly assembled mitogenomes from the current study. The ° symbol denotes available mitogenome recorded as circular on GenBank.

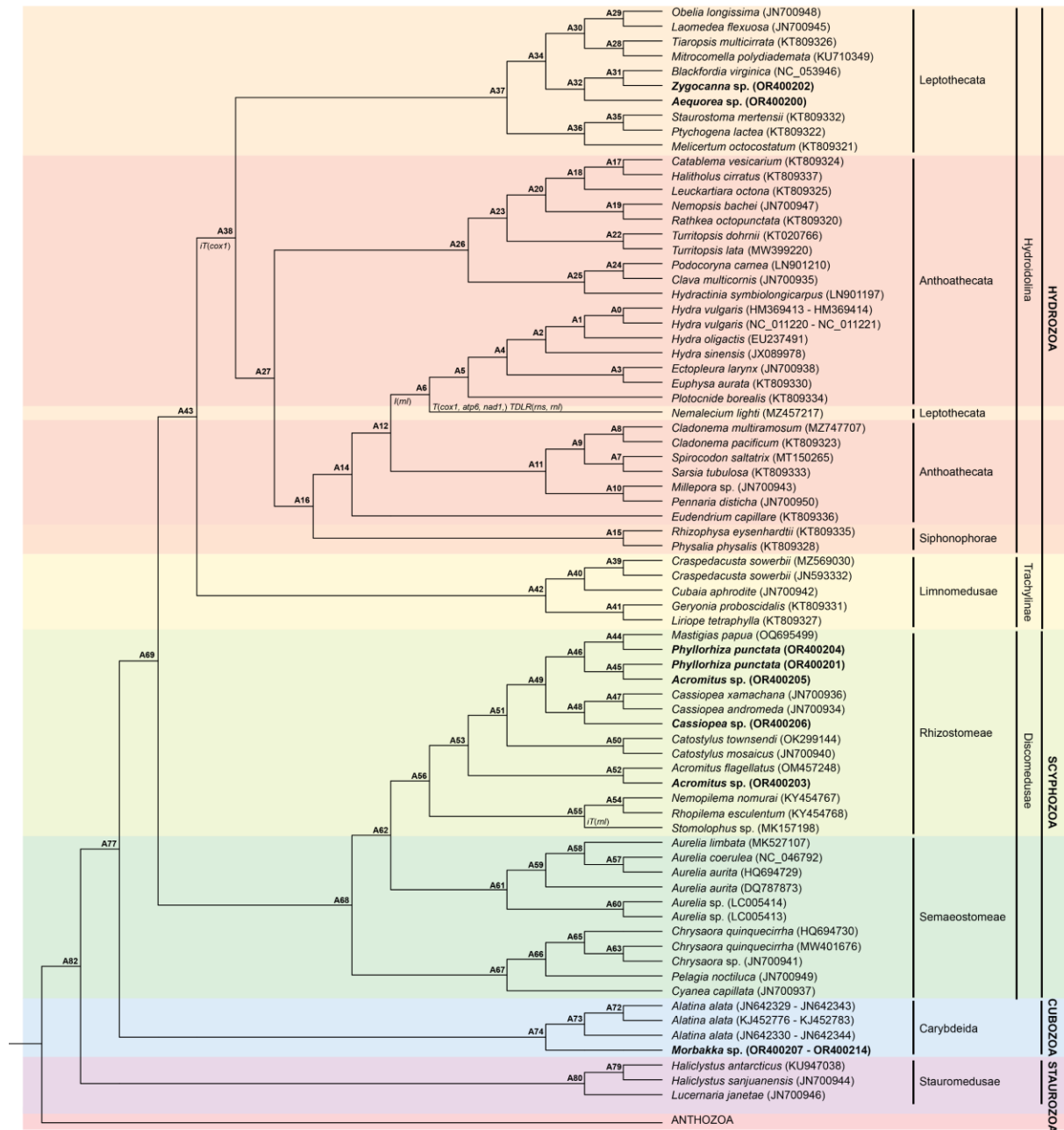


**Figure S4.** Phylogenetic relationships among Cnidaria mitogenomes based on Bayesian (BI) analysis, related to Figure 2. Analysis was conducted with Phylobayes<sup>6</sup> (v3.3e). GenBank ascension numbers of mitogenomes are provided in parentheses. Support values correspond to the posterior probabilities from BI analysis. Bolded specimens indicate newly assembled mitogenomes from the current study. The ° symbol denotes available mitogenome recorded as circular on GenBank.



**Figure S5.** Comparison of gene order of Medusozoa mitochondrial genes with reference to the Bayesian (BI) phylogeny, using complete cnidarian mitogenomes, related to Figure 3. For mitogenomes with multiple chromosomes, vertical lines denote breaks between each chromosomal fragment. Complete mitochondrial genes are indicated in colour, while pseudogenes (as indicated on GenBank) are coloured but outlined with dotted lines. Missing genes are represented by uncoloured boxes outlined with dotted lines. Mitochondrial rearrangements indicated using black arrows: (A) Ancestral medusozoan gene order (AMGO); (B) fragmentation of mitochondrial genome, loss of trnW; (C) loss of trnW, rnl, polB and orf314, (D) inverse transposition of rnl and loss of polB and orf314; (E) loss of gene pair polB-orf314, and duplication, transposition and inversion of cox1; (F) inversion of rnl and transposition of trnW; (G) transposition of trnM; (H) fragmentation and duplication of cox1; (I) transposition of rnl, rns, cox1, atp6 and nad1. Asterisks (\*) denote the phylogenetic positions of specimens assembled in current study: Scyphozoa – *Acromitus* sp., *Cassiopea* sp., *Phyllorhiza punctata*; Hydrozoa – *Zygocanna* sp., *Aequorea* sp.; Cubozoa – *Morbakka* sp.





**Figure S6.** Results from the TreeRex analysis of the Bayesian (BI) Medusozoa phylogeny, related to Figure 4. Where applicable, inferred mitochondrial genome rearrangements are listed at the corresponding nodes. Gene order rearrangements are as follows: I – inversion; T - transposition; iT – inverse transposition; TDRL – tandem duplication random loss. Genes undergoing rearrangements are indicated in parentheses. Labels at nodes denote the inferred gene order for the common ancestor as listed in the BI TreeRex analysis output (see Supplementary Data S2).

**Table S2.** Sampling localities of the eight medusozoan specimens used for mitogenome assembly in the current study, deposited in Lee Kong Chian Natural History Museum (LKCNHM), related to STAR Methods. Abbreviations: ID – Identity; No. – Number.

| <b>Morphological ID</b>     | <b>Accession No.</b> | <b>LKCNHM Catalogue No.</b> | <b>Locality</b>                         | <b>Coordinates</b> |
|-----------------------------|----------------------|-----------------------------|---|--------------------|
| <i>Aequorea</i> sp.         | OR400200             | ZRC.CNI.1440                | Offshore of People Association campsite | 01°18'N, 103°57'E  |
| <i>Phyllorhiza punctata</i> | OR400201             | ZRC.CNI.1437                | St. John's Island                       | 01°12'N, 103°51'E  |
| <i>Zygocanna</i> sp.        | OR400202             | ZRC.CNI.1433                | Chek Jawa, Pulau Ubin                   | 01°24'N, 103°59'E  |
| <i>Acromitus</i> sp.        | OR400203             | ZRC.CNI.1396                | OBS Camp 1, Pulau Ubin                  | 01°25'N, 103°55'E  |
| <i>Phyllorhiza punctata</i> | OR400204             | ZRC.CNI.1439                | Pulau Jong                              | 01°12'N, 103°47'E  |
| <i>Acromitus</i> sp.        | OR400205             | ZRC.CNI.1436                | Terumbu Raya                            | 01°12'N, 103°44'E  |
| <i>Cassiopea</i> sp.        | OR400206             | ZRC.CNI.3022/3*             | St. John's Island                       | 01°12'N, 103°51'E  |
| <i>Morbakka</i> sp.         | OR400207 – OR400214  | ZRC.CNI.1418                | ONE °15 Marina Sentosa Cove             | 01°14'N, 103°50'E  |

**Table S3.** List of medusozoan mitochondrial sequences assembled in the current study (Morphological ID), as well as the reference medusozoan mitochondrial genome sequences obtained from GenBank used for identifying assembled mitochondrial chromosomes (Blast Reference ID) and annotating identified mitochondrial genes (Mt Gene Alignment Reference ID), related to STAR Methods. The corresponding accession numbers for all reference mitogenomes used have also been provided. For the *Cassiopea* sp. used in the current study (\*), subsamples of the specimen used for mitogenome reconstruction were preserved in ethanol (ZRC.CNI.3022) and formalin (ZRC.CNI.3023). Abbreviations: ID – Identity; Mt – Mitochondrial; No. – Number.

| Specimen No.    | Morphological ID            | Blast Reference ID             | Accession No.       | Mt Gene Alignment Reference ID | Accession No.       |
|-----------------|-----------------------------|--------------------------------|---------------------|--------------------------------|---------------------|
| ZRC.CNI.1440    | <i>Aequorea</i> sp.         | <i>Aequorea coerulescens</i>   | MN066550            | <i>Blackfordia virginica</i>   | MW376866            |
| ZRC.CNI.1437    | <i>Phyllorhiza punctata</i> | <i>Phyllorhiza punctata</i>    | OM764500            | <i>Pelagia noctiluca</i>       | JN593332            |
|                 |                             |                                |                     | <i>Cassiopea xamachana</i>     | JN700936            |
|                 |                             |                                |                     | <i>Catostylus townsendi</i>    | OK299144            |
|                 |                             |                                |                     | <i>Mastigias papua</i>         | OQ695499            |
|                 |                             |                                |                     | <i>Stomolophus</i> sp.         | MK157198            |
| ZRC.CNI.1433    | <i>Zygocanna</i> sp.        | <i>Zygocanna apapillatus</i>   | MW528704            | <i>Blackfordia virginica</i>   | MW376866            |
| ZRC.CNI.1396    | <i>Acromitus</i> sp.        | <i>Acromitus flagellatus</i>   | NC061659            | <i>Eutima</i> sp.              | MW066348            |
|                 |                             |                                |                     | <i>Acromitus flagellatus</i>   | OM457248            |
|                 |                             |                                |                     | <i>Catostylus mosaicus</i>     | JN700940            |
|                 |                             |                                |                     | <i>Catostylus townsendi</i>    | OK299144            |
|                 |                             |                                |                     | <i>Phyllorhiza punctata</i>    | OR400201            |
| ZRC.CNI.1439    | <i>Phyllorhiza punctata</i> | <i>Phyllorhiza punctata</i>    | OM764500            | <i>Phyllorhiza punctata</i>    | OR400204            |
| ZRC.CNI.1436    | <i>Acromitus</i> sp.        | <i>Acromitus flagellatus</i>   | NC061659            | <i>Cassiopea xamachana</i>     | JN700936            |
|                 |                             |                                |                     | <i>Catostylus townsendi</i>    | OK299144            |
|                 |                             |                                |                     | <i>Acromitus flagellatus</i>   | OM457248            |
|                 |                             |                                |                     | <i>Cassiopea xamachana</i>     | JN700936            |
|                 |                             |                                |                     | <i>Catostylus mosaicus</i>     | JN700940.1          |
|                 |                             |                                |                     | <i>Catostylus townsendi</i>    | OK299144            |
|                 |                             |                                |                     | <i>Phyllorhiza punctata</i>    | OR400201            |
| ZRC.CNI.1418    | <i>Morbakka</i> sp.         | <i>Alatina alata</i>           | KJ452776 – KJ452783 | <i>Alatina alata</i>           | KJ452776 – KJ452783 |
|                 |                             | <i>Alatina alata</i>           | JN642329 – JN642343 | <i>Alatina alata</i>           | JN642329 – JN642343 |
|                 |                             | <i>Carybdea xaymacana</i>      | JN700977 – JN700983 | <i>Alatina alata</i>           | JN642330 – JN642344 |
|                 |                             | <i>Carukia barnesi</i>         | JN700959 – JN700962 |                                |                     |
|                 |                             | <i>Chironex fleckeri</i>       | JN700963 – JN700968 |                                |                     |
|                 |                             | <i>Chiropsalmus quadumanus</i> | JN700969 – JN700974 |                                |                     |
| ZRC.CNI.3022/3* | <i>Cassiopea</i> sp.        | <i>Cassiopea xamachana</i>     | JN700936            | <i>Cassiopea andromeda</i>     | JN700934            |
|                 |                             |                                |                     | <i>Cassiopea xamachana</i>     | JN700936            |

**Table S5.** List of evolutionary models used for Maximum Likelihood (ML) and Bayesian (BI) phylogenetic analyses of mitochondrial genes in medusozoan mtDNA, related to STAR Methods. Models for the ML analysis were determined using ModelTest-NG<sup>7</sup> (v0.2.0) while those for the BI analysis were carried out based on the default settings of Phylobayes<sup>6</sup> (v3.3e).

| Mitochondrial Gene | ModelTest-NG Evolutionary Model |                 |
|--------------------|---------------------------------|-----------------|
|                    | RAxML                           | Phylobayes      |
| <i>atp6</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>atp8</i>        | TPM3uf+I+G4                     | CAT-Poisson +G4 |
| <i>cob</i>         | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>cox1</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>cox2</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>cox3</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>nad1</i>        | TVM+I+G4                        | CAT-Poisson +G4 |
| <i>nad2</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>nad3</i>        | TVM+G4                          | CAT-Poisson +G4 |
| <i>nad4</i>        | TVM+I+G4                        | CAT-Poisson +G4 |
| <i>nad4L</i>       | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>nad5</i>        | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>nad6</i>        | TVM+I+G4                        | CAT-Poisson +G4 |
| <i>orf314</i>      | TPM3uf+I+G4                     | CAT-Poisson +G4 |
| <i>polB</i>        | TPM3uf+I+G4                     | CAT-Poisson +G4 |
| <i>rns</i>         | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>rnl</i>         | GTR+I+G4                        | CAT-Poisson +G4 |
| <i>trnM</i>        | TPM2uf+G4                       | CAT-Poisson +G4 |
| <i>trnW</i>        | TVMef+G4                        | CAT-Poisson +G4 |

## SUPPLEMENTAL REFERENCES

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