# Mitochondrial Genomes Provide Insights into the Phylogeny of Lauxanioidea (Diptera: Cyclorrhapha)

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# Supporting Information

**Table S1. Nucleotide composition of lauxanioidean mitochondrial genome**

| Feature | A+T% | | | | | AT-skew | | | | | GC-skew | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Ces. l* | *Pac. d* | *Cel. o* | *Spa. p* | *Cha. j* | *Ces. l* | *Pac. d* | *Cel. o* | *Spa. p* | *Cha. j* | *Ces. l* | *Pac. d* | *Cel. o* | *Spa. p* | *Cha. j* |
| Whole mitgenome | 76.7 | 76.3 | - | 76.9 | - | 0.007 | 0.004 | - | -0.009 | - | -0.159 | -0.159 | - | -0.174 | - |
| PCGs | 74.6 | 74.3 | 74.3 | 74.5 | 74.6 | -0.155 | -0.166 | -0.147 | -0.157 | -0.155 | 0.031 | 0.031 | 0.000 | 0.027 | 0.031 |
| 1st codon position | 68.7 | 66.8 | 67.2 | 68.2 | 68.7 | -0.105 | -0.108 | -0.071 | -0.085 | -0.106 | 0.251 | 0.251 | 0.227 | 0.253 | 0.249 |
| 2nd codon position | 66.0 | 66.0 | 65.9 | 65.9 | 66.0 | -0.394 | -0.394 | -0.396 | -0.396 | -0.394 | -0.150 | -0.150 | -0.155 | -0.146 | -0.156 |
| 3rd codon position | 89.8 | 90.2 | 90.0 | 89.1 | 89.8 | -0.024 | -0.042 | -0.022 | -0.033 | -0.024 | -0.038 | -0.038 | -0.208 | -0.086 | -0.038 |
| tRNA genes | 77.6 | 76.7 | - | 77.3 | - | -0.008 | -0.014 | - | -0.014 | - | 0.147 | 0.147 | - | 0.114 | - |
| *lrRNA* | 81.8 | 82.0 | 82.2 | 82.0 | 82.0 | -0.017 | -0.002 | 0.022 | -0.007 | -0.068 | 0.308 | 0.308 | 0.274 | 0.330 | 0.359 |
| *srRNA* | 78.7 | 78.4 | 77.1 | 77.2 | 78.7 | 0.014 | 0.010 | 0.012 | 0.047 | -0.060 | 0.283 | 0.283 | 0.263 | 0.246 | 0.333 |
| Control region | 86.8 | 84.7 | - | 88.7 | - | 0.016 | -0.001 | - | 0.030 | - | -0.218 | -0.218 | - | -0.274 | - |

Note: *Ces. l* indicates *Cestrotus liui*, *Pac. d* indicates *Pachycerina decemlineata*, *Cel. o* indicates *Celyphus obtectus*, *Spa. p* indicates *Spanicelyphus pilosus* and *Cha. j* indicates *Chamaemyia juncorum*. The A+T and G+C biases of protein-coding genes were calculated by AT-skew= [A-T]/[A+T] and GC-skew= [G-C]/[G+C], respectively.

**Table S2 Start/stop codons in lauxanioidean mitochondrial genes**

| Taxon | ND2 | | CO2 | | CO1 | | ATP8 | | ATP6 | | CO3 | | ND3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ST | EN | ST | EN | ST | EN | ST | EN | ST | EN | ST | EN | ST | EN |
| *Ces. l* | ATT | TAA | TCG | TAA | ATG | TAA | ATT | TAA | ATG | TAA | ATG | TAA | ATT | TAA |
| *Pac. d* | ATT | TAA | ATG | TAA | ATG | T | ATT | TAA | ATG | TAA | ATG | TAA | ATT | TAG |
| *Cel. o* | ATT | TAA | TCG | TAA | ATG | T | ATC | TAA | ATG | TAA | ATG | TAA | ATC | TAG |
| *Spa. p* | ATT | TAA | ATG | TAA | ATG | T | ATT | TAA | ATG | TAA | ATG | TAA | ATT | TAG |
| *Cha. j* | ATT | TAA | TCG | TAA | ATG | TAA | ATT | TAA | ATG | TAA | ATG | T | ATT | TAG |

| Taxon | ND5 | | ND4 | | ND4L | | ND6 | | CYTB | | ND1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ST | EN | ST | EN | ST | EN | ST | EN | ST | EN | ST | EN |
| *Ces. l* | ATT | TAA | ATG | TAA | ATG | TAA | ATT | TAA | ATG | TAG | ATT | T |
| *Pac. d* | ATT | TAG | ATG | T | ATG | TAA | ATT | TAA | ATG | TAG | TTG | T |
| *Cel. o* | ATC | T | ATG | T | ATG | TAA | ATT | TAA | ATG | TAG | TTG | T |
| *Spa. p* | ATT | T | ATG | T | ATG | TAA | ATT | TAA | ATG | TAG | TTG | T |
| *Cha. j* | ATT | T | ATG | T | ATG | TAA | ATT | TAA | ATG | TAG | ATT | T |

Note: *Ces. l* indicates *Cestrotus liui*, *Pac. d* indicates *Pachycerina decemlineata*, *Cel. o* indicates *Celyphus obtectus*, *Spa. p* indicates *Spanicelyphus pilosus* and *Cha. j* indicates *Chamaemyia juncorum*. ST, start codon; EN, end (stop) codon.

**Table S3. Mismatches in tRNAs.**

| Taxon | A | R | C | E | G | L2 | S2 | T | W | V |
|---|---|---|---|---|---|---|---|---|---|---|
| *Ces. l* | - | U-U | - | U-U | - | A-A | U-U | - | U-U | 2U-U |
| *Pac. d* | U-U | U-U | - | - | U-U | - | - | - | U-U | U-U |
| *Cel. o* | U-U | U-U | U-U | U-U | U-U | - | - | - | U-U | U-U |
| *Spa. p* | U-U | U-U | - | - | U-U | - | - | - | U-U | U-U |
| *Cha. j* | - | U-C | U-U | U-U | - | - | U-C | U-U | - | U-U |

Note: *Ces. l* indicates *Cestrotus liui*, *Pac. d* indicates *Pachycerina decemlineata*, *Cel. o* indicates *Celyphus obtectus*, *Spa. p* indicates *Spanicelyphus pilosus* and *Cha. j* indicates *Chamaemyia juncorum*.

**Table S4. Comparison of different partition schems selected by PartitionFinder**

| **Partition scheme** | **lnL** | **BIC** | **Number of sites** | **Number of subsets** |
|---|---|---|---|---|
| **P12-codon-ML** | -59927.1 | **122096.52** | 7318 | 4 |
| P12-codon-BI | -59953.55 | 122149.42 | 7318 | 4 |
| P12-gene-BI | -60641.96 | 122965.66 | 7318 | 3 |
| P12-gene-ML | -60641.96 | 122965.66 | 7318 | 3 |
| P12-BI | -61861.21 | 123811.4 | 7318 | 1 |
| P12-ML | -61861.21 | 123811.4 | 7318 | 1 |
| **P12R-codon-BI** | -83344.77 | **169016.62** | 10244 | 4 |
| P12R-codon-ML | -83640.21 | 169025.73 | 10244 | 3 |
| P12R-gene-ML | -83820.99 | 169969.05 | 10244 | 4 |
| P12R-gene-BI | -83823.4 | 169973.88 | 10244 | 4 |
| P12R-BI | -85542.81 | 171177.96 | 10244 | 1 |
| P12R-ML | -85542.81 | 171177.96 | 10244 | 1 |
| **P123-codon-BI** | -129006.18 | **261994.29** | 10977 | 7 |
| P123-codon-ML | -128986.15 | 262065.87 | 10977 | 7 |
| P123-gene-BI | -135248.86 | 272842.21 | 10977 | 4 |
| P123-gene-ML | -135248.86 | 272842.21 | 10977 | 4 |
| P123-BI | -138265.31 | 276623.65 | 10977 | 1 |
| P123-ML | -138265.31 | 276623.65 | 10977 | 1 |
| **P123R-codon-BI** | -152250.8 | **308584.67** | 13903 | 7 |
| P123R-codon-ML | -151936.71 | 308662.42 | 13903 | 8 |
| P123R-gene-BI | -158257.59 | 319520.23 | 13903 | 5 |
| P123R-gene-ML | -158257.59 | 319520.23 | 13903 | 5 |
| P123R-BI | -162012.37 | 324120.13 | 13903 | 1 |
| P123R-ML | -162012.37 | 324120.13 | 13903 | 1 |

**Table S5. Collection information of lauxanioidean flies newly sequenced in the present study.**

| **Taxon** | **Locality** | **Time** | **Collector** |
|---|---|---|---|
| *Ces. l* | Baihualing, Baoshan, Yunnan (N25°17′32.85″ E98°48′23.08″) | 16, July 2013 | Xuankun Li |
| *Pac. d* | Xiayadong, Yadong, Tibet (N27°25′31.99″ E88°55′45.60″) | 19, July 2013 | Xiaoyan Liu |
| *Cel. o* | Bawangling, Changjiang, Hainan (N19°07′21.84″ E109°04′45.64″) | 4, April 2013 | Ziqiang Sun |
| *Spa. p* | Wazidi, Mangshi, Yunnan (N24°26′12.68″ E98°35′16.55″) | 5, July 2013 | Xuankun Li |
| *Cha. j* | Yunwushan Mt., Guyuan, Ningxia (N36°14′20.57″ E106°23′12.51″) | 4, Augeust 2013 | Lei Zhang |

Note: *Ces. l* indicates *Cestrotus liui*, *Pac. d* indicates *Pachycerina decemlineata*, *Cel. o* indicates *Celyphus obtectus*, *Spa. p* indicates *Spanicelyphus pilosus* and *Cha. j* indicates *Chamaemyia juncorum*.

**Table S6. Primers used in this study.**

***Cestrotus liui***

| Number | Primer pairs (F/R) | Sequence (forward and reverse) 5’-3’ |
|---|---|---|
| 1 | TM-J-206/N2-N-732 | GCTAAATAAGCTAACAGGTTCAT/AAGGAAGTTTGGTTTAAACCTCC |
| 2* | Ces-Z1-302/Ces-Z1-1976 | CATGGTTAGGAGCTTGAATAGG/CGGTCAAAAGTAATACCAGTTGATC |
| 3 | TY-J-1460/C1-N-2191 | TACAATCTATCGCCTAAACTTCAGCC/CCCGGTAAAATTAAAATATAAACTTC |
| 4 | C1-J-1751/TL2-N-3014 | GGAGCTCCTGATATAGCATTCCC/TCCATTGCACTAATCTGCCATATTA |
| 5 | Cl-J-2183/C3-N-5460 | CAACATTTATTTTGATTTTTTGG/TCAACAAAGTGTCAGTATCATGC |
| 6 | C3-J-5005/E-rev | CTCCAGCAATTGAATTAGGAGCTA/AGTGATAAGCCTCTTTTTGGCTTC |
| 7 | F-fw/N5-N-7707 | CATTTGATTTGCATTCAAAAAGTATTG/AGGATGAGATGGATTAGGACTAG |
| 8 | H-fw/N4-N-8718 | GAAACAGGAGTAGGAGCTGCTATAGC/GCTTATTCATCGGTTGCTCA |
| 9* | Ces-Z2-8413/Ces-Z2-8893 | CTATTTAATAAAGAAATTTCACC/CCTAAAGCTCATGTTGAAGCTCC |
| 10 | N4-J-8614/N4-N-9061 | TGAGCAACAGAAGAATAAGC/ATCAACCTGAACGATTACAAG |
| 11 | N4-J-8944/I-rev | CAGGAGCTTCAACATGAGCTTTAGG/CTTATTTTTGATTTACAAGACCAATG |
| 12 | N4-J-9511/CB-N-11218 | CCAAAATTGATAACCCTAAAGC/TCAGGTTGAATGTGAATTGG |
| 13 | CB-J-10933/N1-N-12051 | TATGTTCTACCATGAGGACAAATATC/GATTTTGCTGAAGGTGAATCAGA |
| 14* | Ces-Z3-11969/ Ces-Z3-12902 | CAAAACCCCCACTTCTATATTCTAC/GATTGCGACCTCGATGTTGGATTAAG |
| 15 | LR-J-12883/LR-N-13398 | CACCGGTTTGAACTCAGATC/CGCCTGTTTATCAAAAACAT |
| 16 | LR-J-12888/SR-N-14373 | ACGCTGTTATCCCTAAAGTA/AATCCACGATGTACCTTACT |
| 17* | Ces-Z4-14180/ Ces-Z4-14723 | AAGAGCGACGGGCGATGTGTAC/GGCTAAATTTGTGCCAGCAGC |
| 18 | SR-J-14612/SR-N-14922 | AGGGTATCTAATCCTAGTTT/AAGTTTTATTTTGGCTTA |
| 19 | SR-J-14646/N2-N-309 | GCTGGCACAAATTAAATC/CTAAACCTATTCAAGTTCC |

***Pachycerina decemlineata***

| **Number** | **Primer pairs (F/R)** | **Sequence (forward and reverse) 5’-3’** |
|---|---|---|
| 1 | TM-J-206/N2-N-732 | GCTAAATAAGCTAACAGGTTCAT/AAGGAAGTTTGGTTTAAACCTCC |
| 2* | Pac-Z1-555/ Pac-Z1-1672 | GAAGGGCTTAGATGAATTAATTC/CTAATCAATTTCCAAATCC |
| 3 | TY-J-1460/C1-N-2191 | TACAATCTATCGCCTAAACTTCAGCC/CCCGGTAAAATTAAAATATAAACTTC |
| 4 | C1-J-1751/TL2-N-3014 | GGAGCTCCTGATATAGCATTCCC/TCCATTGCACTAATCTGCCATATTA |
| 5 | Cl-J-2183/C3-N-5460 | CAACATTTATTTTGATTTTTTGG/TCAACAAAGTGTCAGTATCATGC |
| 6 | C2-J-3530/A6-N-4493 | AAGTTGATGGAACTCCTGGA/GTAAGTCGAACTGCTAATGT |
| 7 | C3-J-5005/E-rev | CTCCAGCAATTGAATTAGGAGCTA/AGTGATAAGCCTCTTTTTGGCTTC |
| 8* | Pac-Z2-5945/Pac-Z2-7730 | CATTTGATTTGCATTCAAAAAGTATTG/ AGGGTGAGATGGATTGGGGTTGG |
| 8 | H-fw/N4-N-8718 | GAAACAGGAGTAGGAGCTGCTATAGC/GCTTATTCATCGGTTGCTCA |
| 9 | I-fw/N4-N-8924 | CTATTTAATAAAGAAATTTCTCC/CCTAAAGCTCATGTTGAAGCTCC |
| 10 | N4-J-8614/N4-N-9061 | TGAGCAACAGAAGAATAAGC/ATCAACCTGAACGATTACAAG |
| 11 | N4-J-8944/I-rev | CAGGAGCTTCAACATGAGCTTTAGG/CTTATTTTTGATTTACAAGACCAATG |
| 12 | N4-J-9511/CB-N-11218 | CCAAAATTGATAACCCTAAAGC/TCAGGTTGAATGTGAATTGG |
| 13 | CB-J-10933/N1-N-12051 | TATGTTCTACCATGAGGACAAATATC/GATTTTGCTGAAGGTGAATCAGA |
| 14 | N1-J-11891/16S-N-12855 | ATCCTCCTCTTCTATATTCAAT/GATTGCGACCTCGATGTT |
| 15 | LR-J-12883/LR-N-13398 | CACCGGTTTGAACTCAGATC/CGCCTGTTTATCAAAAACAT |
| 16 | LR-J-12888/SR-N-14373 | ACGCTGTTATCCCTAAAGTA/AATCCACGATGTACCTTACT |
| 17 | SR-J-14233/SR-N-14756 | AAGAGCGACGGGCGATGTGT/GACAAAATTCGTGCCAGCAGT |
| 18 | SR-J-14612/SR-N-14922 | AGGGTATCTAATCCTAGTTT/AAGTTTTATTTTGGCTTA |
| 19* | Pac-ZK-14698/Pac-ZK-280 | GCTGGCACAAATTTTGCCAATA/CTAAACCTATTCATGCTCTTAATC |

***Celyphus obtectus***

| **Number** | **Primer pairs (F/R)** | **Sequence (forward and reverse) 5’-3’** |
|---|---|---|
| 1 | TM-J-206/N2-N-732 | GCTAAATAAGCTAACAGGTTCAT/AAGGAAGTTTGGTTTAAACCTCC |
| 2* | Cel-Z1-555/Cel-Z1-1672 | GAAGGGCTCTCTTGAACTAATTC/CCAGTCAATTTCCAAATCC |
| 3 | TY-J-1460/C1-N-2191 | TACAATCTATCGCCTAAACTTCAGCC/CCCGGTAAAATTAAAATATAAACTTC |
| 4 | C1-J-1751/TL2-N-3014 | GGAGCTCCTGATATAGCATTCCC/TCCATTGCACTAATCTGCCATATTA |
| 5* | Cel-Z1-1970/ Cel-Z1-3198 | CAACATCTTTTCTGATTCTTTGG/GAATATTCATAGCTTCAATATC |
| 6* | Cel-Z2-2561/ Cel-Z2-5272 | GTAAATTTAACCTTCTTCCCTCAAC/TCTACAAAATGTCAATATCATGC |
| 7 | C3-J-5005/E-rev | CTCCAGCAATTGAATTAGGAGCTA/AGTGATAAGCCTCTTTTTGGCTTC |
| 8* | Cel-Z3-5787/ Cel-Z3-7548 | CATTTGATTTGCATTCAAAAAGTATTG/AGGATGAGATGGGTTAGGTTTGG |
| 9 | H-fw/N4-N-8718 | GAAACAGGAGTAGGAGCTGCTATAGC/GCTTATTCATCGGTTGCTCA |
| 10 | I-fw/N4-N-8924 | CTATTTAATAAAGAAATTTCTCC/CCTAAAGCTCATGTTGAAGCTCC |
| 11 | N4-J-8614/N4-N-9061 | TGAGCAACAGAAGAATAAGC/ATCAACCTGAACGATTACAAG |
| 12 | N4-J-8944/I-rev | CAGGAGCTTCAACATGAGCTTTAGG/CTTATTTTTGATTTACAAGACCAATG |
| 13* | Cel-Z4-9531/Cel-Z4-10829 | GTTTTCGATTTCTTAC/GTTATTGCTAAAACAATAAAAGG |
| 14 | CB-J-10933/N1-N-12051 | TATGTTCTACCATGAGGACAAATATC/GATTTTGCTGAAGGTGAATCAGA |
| 15 | N1-J-11891/16S-N-12855 | ATCCTCCTCTTCTATATTCAAT/GATTGCGACCTCGATGTT |
| 16 | LR-J-12883/LR-N-13398 | CACCGGTTTGAACTCAGATC/CGCCTGTTTATCAAAAACAT |
| 17 | LR-J-12888/SR-N-14373 | ACGCTGTTATCCCTAAAGTA/AATCCACGATGTACCTTACT |
| 18 | SR-J-14233/SR-N-14756 | AAGAGCGACGGGCGATGTGT/GACAAAATTCGTGCCAGCAGT |
| 19 | SR-J-14612/SR-N-14922 | AGGGTATCTAATCCTAGTTT/AAGTTTTATTTTGGCTTA |

***Spanicelyphus pilosus***

| **Number** | **Primer pairs (F/R)** | **Sequence (forward and reverse) 5’-3’** |
|---|---|---|
| 1 | TM-J-206/N2-N-732 | GCTAAATAAGCTAACAGGTTCAT/AAGGAAGTTTGGTTTAAACCTCC |
| 2 | N2-J-283/C1-N-1740 | CATGACTAGGAACTTGAATAGG/AGAACTAAAGCAGGAGGTAA |
| 3 | TY-J-1460/C1-N-2191 | TACAATCTATCGCCTAAACTTCAGCC/CCCGGTAAAATTAAAATATAAACTTC |
| 4 | C1-J-1751/TL2-N-3014 | GGAGCTCCTGATATAGCATTCCC/TCCATTGCACTAATCTGCCATATTA |
| 5 | Cl-J-2183/C3-N-5460 | CAACATTTATTTTGATTTTTTGG/TCAACAAAGTGTCAGTATCATGC |
| 6 | C3-J-5005/E-rev | CTCCAGCAATTGAATTAGGAGCTA/AGTGATAAGCCTCTTTTTGGCTTC |
| 7 | F-fw/N5-N-7707 | CATTTGATTTGCATTCAAAAAGTATTG/AGGATGAGATGGATTAGGACTAG |
| 8 | H-fw/N4-N-8718 | GAAACAGGAGTAGGAGCTGCTATAGC/GCTTATTCATCGGTTGCTCA |
| 9 | I-fw/N4-N-8924 | CTATTTAATAAAGAAATTTCTCC/CCTAAAGCTCATGTTGAAGCTCC |
| 10 | N4-J-8614/N4-N-9061 | TGAGCAACAGAAGAATAAGC/ATCAACCTGAACGATTACAAG |
| 11 | N4-J-8944/I-rev | CAGGAGCTTCAACATGAGCTTTAGG/CTTATTTTTGATTTACAAGACCAATG |
| 12 | N4-J-9511/CB-N-11218 | CCAAAATTGATAACCCTAAAGC/TCAGGTTGAATGTGAATTGG |
| 13 | CB-J-10933/N1-N-12051 | TATGTTCTACCATGAGGACAAATATC/GATTTTGCTGAAGGTGAATCAGA |
| 14 | N1-J-11891/16S-N-12855 | ATCCTCCTCTTCTATATTCAAT/GATTGCGACCTCGATGTT |
| 15 | LR-J-12883/LR-N-13398 | CACCGGTTTGAACTCAGATC/CGCCTGTTTATCAAAAACAT |
| 16 | LR-J-12888/SR-N-14373 | ACGCTGTTATCCCTAAAGTA/AATCCACGATGTACCTTACT |
| 17 | SR-J-14233/SR-N-14756 | AAGAGCGACGGGCGATGTGT/GACAAAATTCGTGCCAGCAGT |
| 18 | SR-J-14612/SR-N-14922 | AGGGTATCTAATCCTAGTTT/AAGTTTTATTTTGGCTTA |
| 19* | Spa-ZK-14703/Spa-ZK-261 | CGCATCATTTGTGTAACCGCGGC/GCCGAAACTGTAATTATTGTTCCTG |

***Chamaemyia juncorum***

| Number | Primer pairs (F/R) | Sequence (forward and reverse) 5’-3’ |
|---|---|---|
| 1* | Cha-Z1-50/ Cha-Z1-618 | CACTTATTATATTTTG/AAAGAAGTTTGATTTATAGCTCC |
| 2* | Cha-Z2-453/ Cha-Z2-1603 | CCCTTTCATTTTTGATTTCC/GATATAGCCTTTCCCCGAATAAA |
| 3 | TY-J-1460/C1-N-2191 | TACAATCTATCGCCTAAACTTCAGCC/CCCGGTAAAATTAAAATATAAACTTC |
| 4 | C1-J-1751/TL2-N-3014 | GGAGCTCCTGATATAGCATTCCC/TCCATTGCACTAATCTGCCATATTA |
| 5* | Cha-Z3-2026/ Cha-Z3-5312 | CAACATCTTTTTTGATTTTTTGG/TCTACAAAATGTCAATATCAAGC |
| 6 | C2-J-3530/A6-N-4493 | AAGTTGATGGAACTCCTGGA/GTAAGTCGAACTGCTAATGT |
| 7 | C3-J-5005/E-rev | CTCCAGCAATTGAATTAGGAGCTA/AGTGATAAGCCTCTTTTTGGCTTC |
| 8* | Cha-Z4-5822/Cha-Z4-7130 | CATTTGATTTGCATTCAAAAAGTATTG/ATTTTGAGTTTGATTTAAAAAAAATTA |
| 9 | H-fw/N4-N-8718 | GAAACAGGAGTAGGAGCTGCTATAGC/GCTTATTCATCGGTTGCTCA |
| 10 | I-fw/N4-N-8924 | CTATTTAATAAAGAAATTTCTCC/CCTAAAGCTCATGTTGAAGCTCC |
| 11 | N4-J-8614/N4-N-9061 | TGAGCAACAGAAGAATAAGC/ATCAACCTGAACGATTACAAG |
| 12 | N4-J-8944/I-rev | CAGGAGCTTCAACATGAGCTTTAGG/CTTATTTTTGATTTACAAGACCAATG |
| 13* | Cha-Z5-9406/Cha-Z5-11104 | CTAAAATTGAAAGACCTAAAGAAC/TCAGGTTGAATGTGGATAGG |
| 14 | CB-J-10933/N1-N-12051 | TATGTTCTACCATGAGGACAAATATC/GATTTTGCTGAAGGTGAATCAGA |
| 15 | N1-J-11891/16S-N-12855 | ATCCTCCTCTTCTATATTCAAT/GATTGCGACCTCGATGTT |
| 16 | LR-J-12883/LR-N-13398 | CACCGGTTTGAACTCAGATC/CGCCTGTTTATCAAAAACAT |
| 17* | Cha-Z6-12770/Cha-Z6-14228 | ACGCTGTTATCCCTAAAGTA/CGATAATCCACGATGGATCTCAC |
| 18 | SR-J-14233/SR-N-14756 | AAGAGCGACGGGCGATGTGT/GACAAAATTCGTGCCAGCAGT |
| 19 | SR-J-14612/SR-N-14922 | AGGGTATCTAATCCTAGTTT/AAGTTTTATTTTGGCTTA |

* Species-specific primers designed in this study.

**Table S7. The best partitioning scheme selected by PartitionFinder for different dataset.**

| Dataset | Subset Partitions | Best Model |
|---|---|---|
| P123R-codon:<br>7 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, ATP8_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | HKY+I+G |
| | P4: (ATP8_pos1, Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | HKY+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | HKY+I+G |
| P123R-codon:<br>8 partitions (ML) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |

| | | |
|---|---|---|
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | GTR+I+G |
| | P4: (ATP8_pos1, ATP8_pos3, ND2_pos1, ND3_pos1, ND6_pos1) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND4L_pos1, ND5_pos1, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| | P6: (ND1_pos3, ND4L_pos3, ND5_pos3) | GTR+I+G |
| | P7: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P8: (ND4_pos3) | GTR+I+G |
| P123-codon: 7 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | HKY+I+G |
| | P4: (ATP8_pos1, ATP8_pos3, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | HKY+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | HKY+I+G |

| | | |
|---|---|---|
| P123-codon:<br>7 partitions (ML) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | GTR+I+G |
| | P4: (ATP8_pos1, ATP8_pos3, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | GTR+I+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | GTR+I+G |
| P12R-codon:<br>4 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND4_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP8_pos1, Asn, Asp, Glu, ND2_pos1, ND3_pos1, ND6_pos1) | GTR+I+G |
| | P4: (Ala, Arg, Cys, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND4L_pos1, ND4_pos1, ND5_pos1, Phe, | GTR+I+G |

| | | |
|---|---|---|
| | Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | |
| P12R-codon: 3 partitions (ML) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND4_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP8_pos1, Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND4_pos1, ND5_pos1, ND6_pos1, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| P12-codon: 4 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos1, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P4: (ND4_pos1, ND4_pos2) | GTR+I+G |
| P12-codon: | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, | GTR+I+G |

| | | |
|---|---|---|
| 4 partitions (ML) | CytB_pos1) | |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP8_pos1, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P4: (ND4_pos1, ND4_pos2) | GTR+I+G |
| P123R-Al-codon: 7 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, ATP8_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | HKY+I+G |
| | P4: (ATP8_pos1, Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | HKY+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | HKY+I+G |

| | | |
|---|---|---|
| P123R-Al-codon:<br>8 partitions (ML) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | GTR+I+G |
| | P4: (ATP8_pos1, ATP8_pos3, ND2_pos1, ND3_pos1, ND6_pos1) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, ND1_pos1, ND4L_pos1, ND5_pos1, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| | P6: (ND1_pos3, ND4L_pos3, ND5_pos3) | GTR+I+G |
| | P7: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P8: (ND4_pos3) | GTR+I+G |
| P123-Al-codon:<br>7 partitions (BI) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | HKY+I+G |

| | | |
|---|---|---|
| | P4: (ATP8_pos1, ATP8_pos3, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | HKY+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | HKY+I+G |
| P123-Al-codon: 7 partitions (ML) | P1: (ATP6_pos1, CO1_pos1, CO2_pos1, CO3_pos1, CytB_pos1) | GTR+I+G |
| | P2: (ATP6_pos2, ATP8_pos2, CO1_pos2, CO2_pos2, CO3_pos2, CytB_pos2, ND1_pos2, ND2_pos2, ND3_pos2, ND4L_pos2, ND5_pos2, ND6_pos2) | GTR+I+G |
| | P3: (ATP6_pos3, CO1_pos3, CO2_pos3, CO3_pos3, CytB_pos3, ND2_pos3, ND3_pos3, ND6_pos3) | GTR+I+G |
| | P4: (ATP8_pos1, ATP8_pos3, ND1_pos1, ND2_pos1, ND3_pos1, ND4L_pos1, ND5_pos1, ND6_pos1) | GTR+I+G |
| | P5: (ND1_pos3, ND4L_pos3, ND5_pos3) | GTR+I+G |
| | P6: (ND4_pos1, ND4_pos2) | GTR+I+G |
| | P7: (ND4_pos3) | GTR+I+G |
| P123R-gene: 7 partitions (BI) | P1: (ATP6, CO1, CO2, CO3, CytB, ND3) | GTR+I+G |
| | P2: (ATP8, ND2, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, | GTR+I+G |

| | | |
|---|---|---|
| | Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | |
| P123R-gene: 7 partitions (ML) | P1: (ATP6, CO1, CO2, CO3, CytB, ND3) | GTR+I+G |
| | P2: (ATP8, ND2, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| P123-gene: 4 partitions (BI) | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| | P2: (ATP8, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| P123-gene: 4 partitions (ML) | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| | P2: (ATP8, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| P12R-gene: 4 partitions (BI) | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| | P2: (ATP8, Asn, Asp, Glu, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4, ND4L, ND5) | GTR+I+G |
| | P4: (Ala, Arg, Cys, Gly, His, Leu1, Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |

| | | |
|---|---|---|
| P12R-gene: | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| 4 partitions (ML) | P2: (ATP8, Glu, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4, ND4L, ND5) | GTR+I+G |
| | P4: (Ala, Arg, Asn, Asp, Cys, Gly, His, Leu1, Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| P12-gene: | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| 4 partitions (BI) | P2: (ATP8, ND1, ND2, ND3, ND4L, ND5, ND6) | GTR+I+G |
| | P3: (ND4) | GTR+I+G |
| P12-gene: | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| 3 partitions (ML) | P2: (ATP8, ND1, ND2, ND3, ND4L, ND5, ND6) | GTR+I+G |
| | P3: (ND4) | GTR+I+G |
| P123R-Al-gene: | P1: (ATP6, ATP8, ND2, ND3, ND6) | GTR+I+G |
| 7 partitions (BI) | P2: (CO1, CO2, CO3, CytB) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| P123R-Al-gene: | P1: (ATP6, ATP8, ND2, ND3, ND6) | GTR+I+G |
| 7 partitions (ML) | P2: (CO1, CO2, CO3, CytB) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |

| | | |
|---|---|---|
| | P4: (ND4) | GTR+I+G |
| | P5: (Ala, Arg, Asn, Asp, Cys, Glu, Gly, His, Leu1, Leu2, Lys, Phe, Pro, Ser1, Ser2, Thr, Trp, Tyr, Val, lrRNA, srRNA) | GTR+I+G |
| P123-Al-gene: | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| 4 partitions (BI) | P2: (ATP8, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |
| P123-Al-gene: | P1: (ATP6, CO1, CO2, CO3, CytB) | GTR+I+G |
| 4 partitions (ML) | P2: (ATP8, ND2, ND3, ND6) | GTR+I+G |
| | P3: (ND1, ND4L, ND5) | GTR+I+G |
| | P4: (ND4) | GTR+I+G |

**Table S8. Phylogenetic trees generated from different datasets and methods.**

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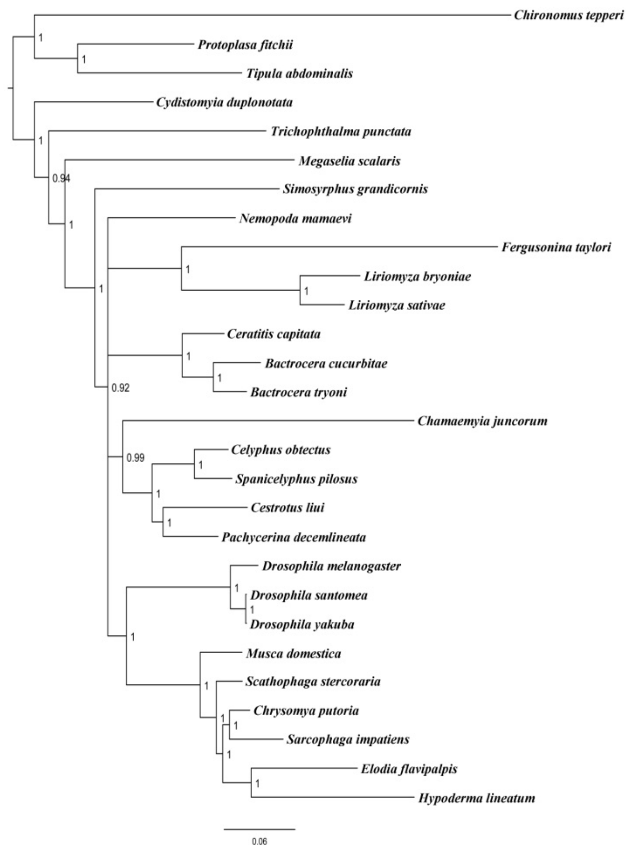
<td>
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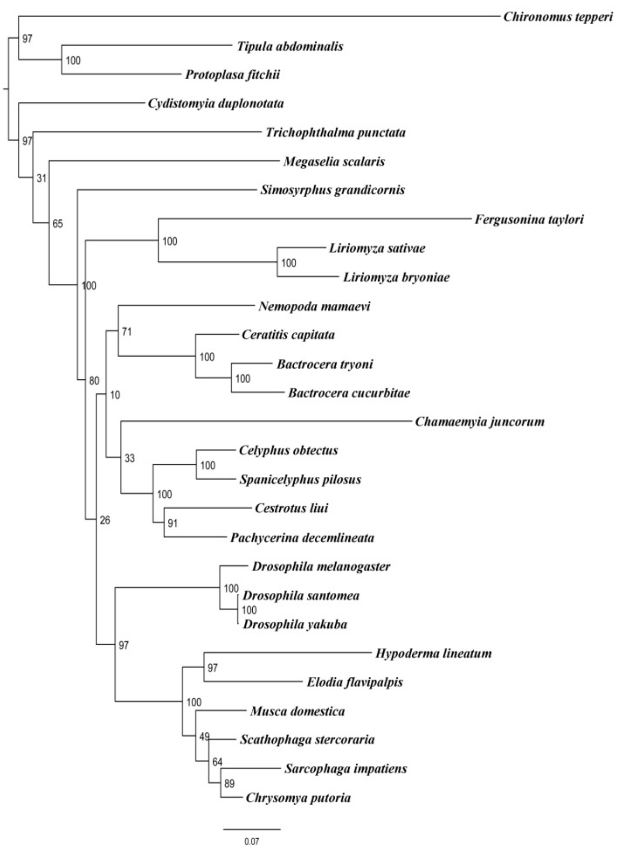
<td>
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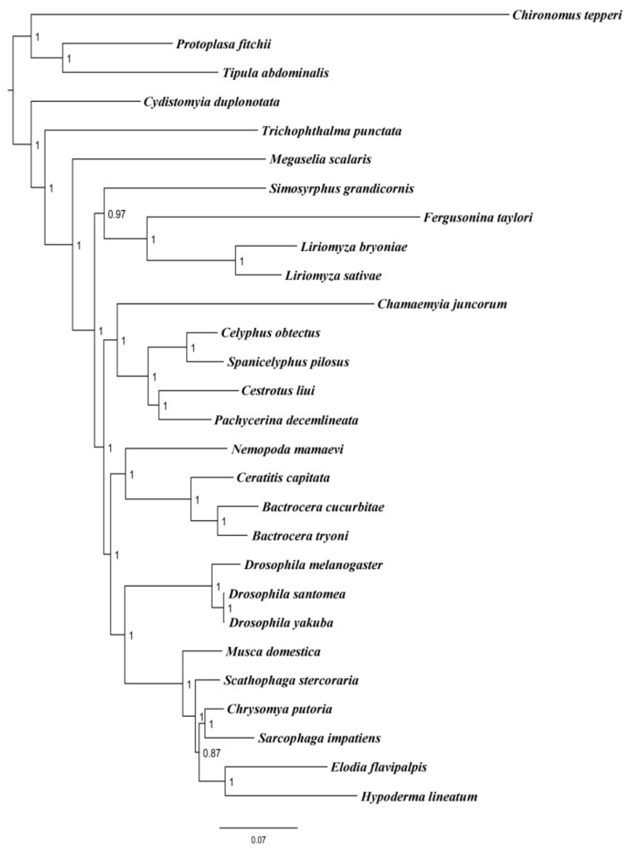
<td>
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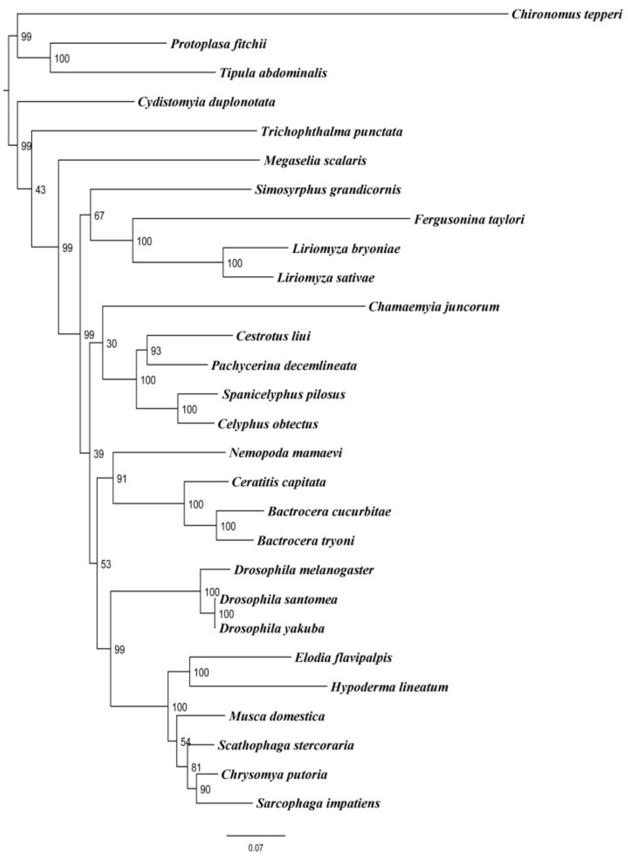
<td>
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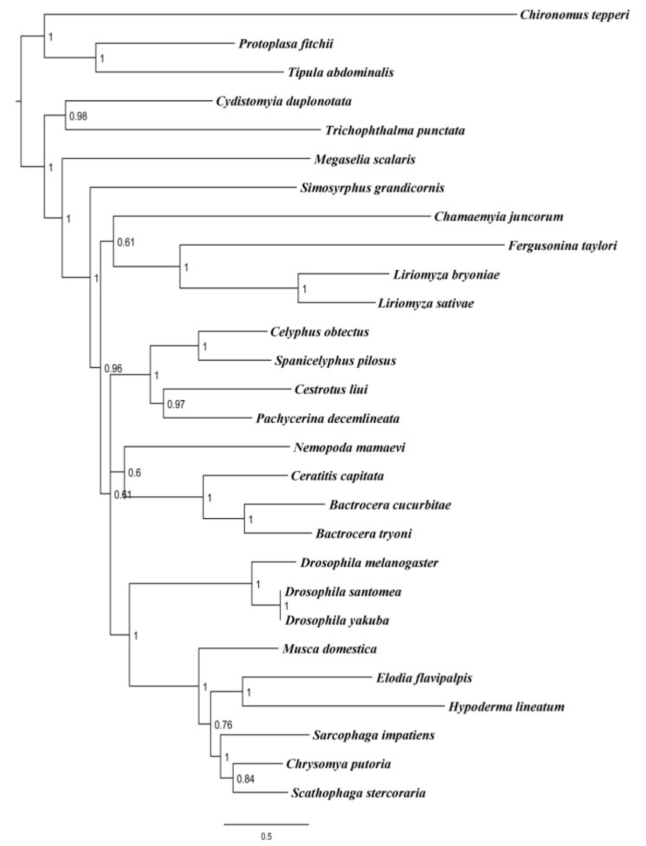

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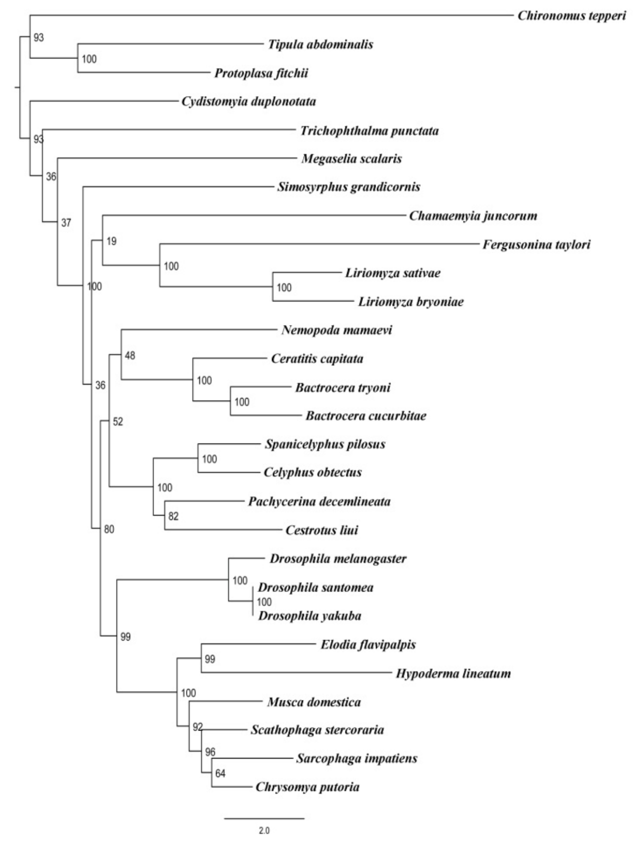

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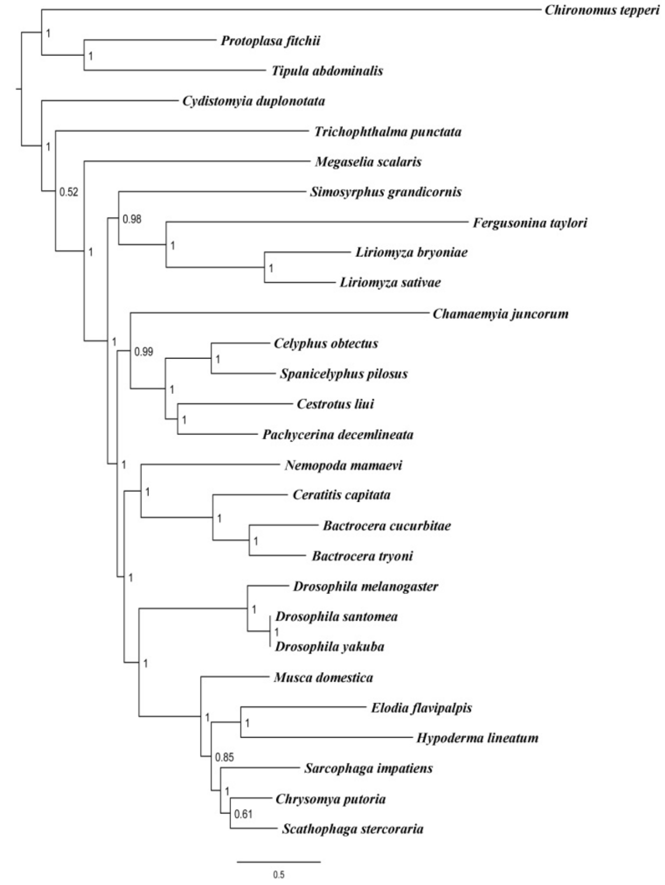

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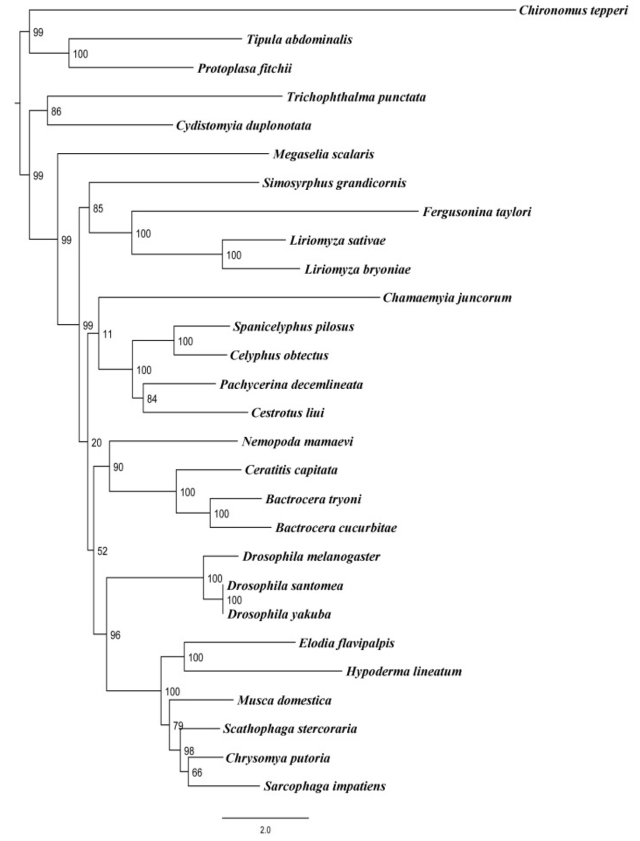
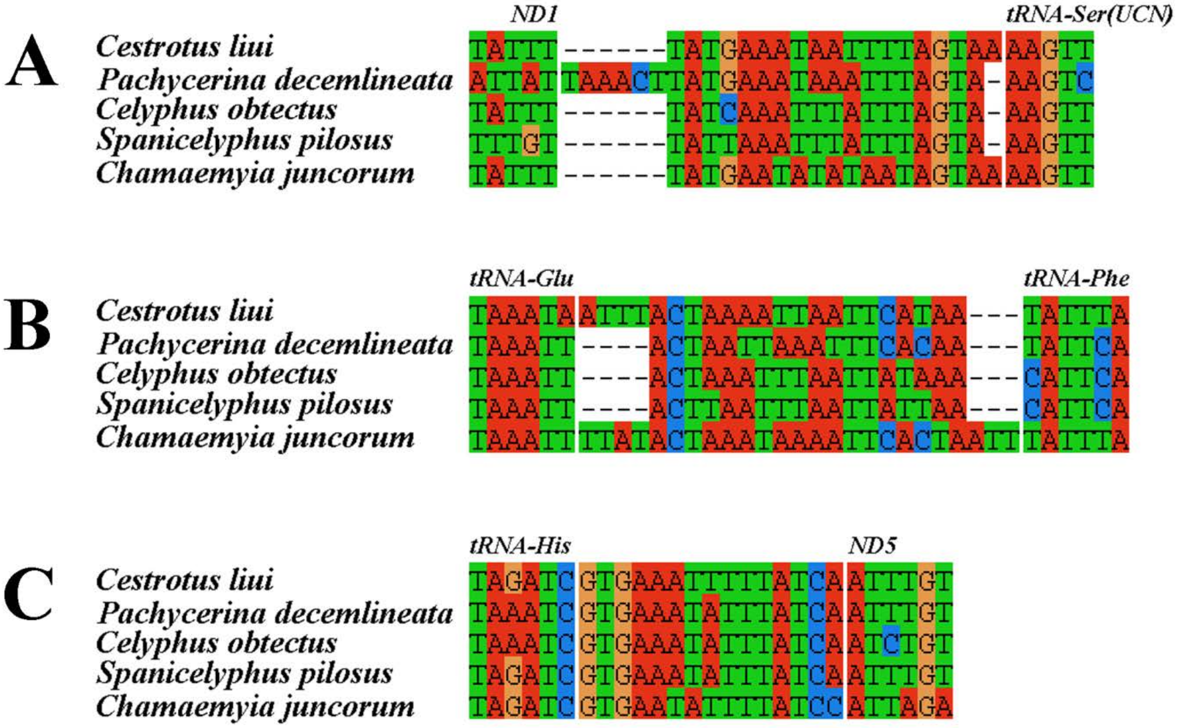


P123R_ML

**Figure S1. Sequence aliments of three intergenic sequences among five lauxanioidean flies.** A, the intergenic sequences between *ND1* and *tRNA*$^{Ser(UCN)}$, reversed sequences; B, the intergenic sequences between *tRNA*$^{Glu}$ and *tRNA*$^{Phe}$, forward sequences; C, the intergenic sequences between *tRNA*$^{His}$ and *ND5*, reversed sequences.



```
A                             ND1                                 tRNA-Ser(UCN)
Cestrotus liui                TATTT ------TATGAAATAATTTTAGTAA AAGTT
Pachycerina decemlineata      ATTAT TAAACTTATGAAATAAATTTAGTA- AAGTC
Celyphus obtectus             TATTT ------TATCAAATTTATTTAGTA- AAGTT
Spanicelyphus pilosus         TTTGT ------TATTAAATTTATTTAGTA- AAGTT
Chamaemyia juncorum           TATTT ------TATGAATATATAATAGTAA AAGTT

B                             tRNA-Glu                              tRNA-Phe
Cestrotus liui                TAAATA ATTTACTAAAATTAATTCATAA--- TATTTA
Pachycerina decemlineata      TAAATT ----ACTAATTAAATTTCACAA--- TATTCA
Celyphus obtectus             TAAATT ----ACTAAATTTAATTATAAA--- CATTCA
Spanicelyphus pilosus         TAAATT ----ACTTAATTTAATTATTAA--- CATTCA
Chamaemyia juncorum           TAAATT TTATACTAAATAAAATTCACTAATT TATTTA

C                             tRNA-His               ND5
Cestrotus liui                TAGATC GTGAAATTTTTATCA ATTTGT
Pachycerina decemlineata      TAAATC GTGAAATATTTATCA ATTTGT
Celyphus obtectus             TAAATC GTGAAATATTTATCA ATCTGT
Spanicelyphus pilosus         TAGATC GTGAAATATTTATCA ATTTGT
Chamaemyia juncorum           TAGATC GTGAATATTTTATCC ATTAGA
```

**Figure S2. Putative secondary structures of tRNAs found in cyclorrhaphan mt genomes.** Red filled circle, nucleotide conserved in cyclorrhaphan mt genomes; Green filled circle, nucleotide conserved in lauxanioidean mt genomes; hollowed circle, nucleotide not conserved. The cyclorrhaphan substitution pattern for each tRNA was modeled using as reference the structure determined for *Cestrotus liui*.

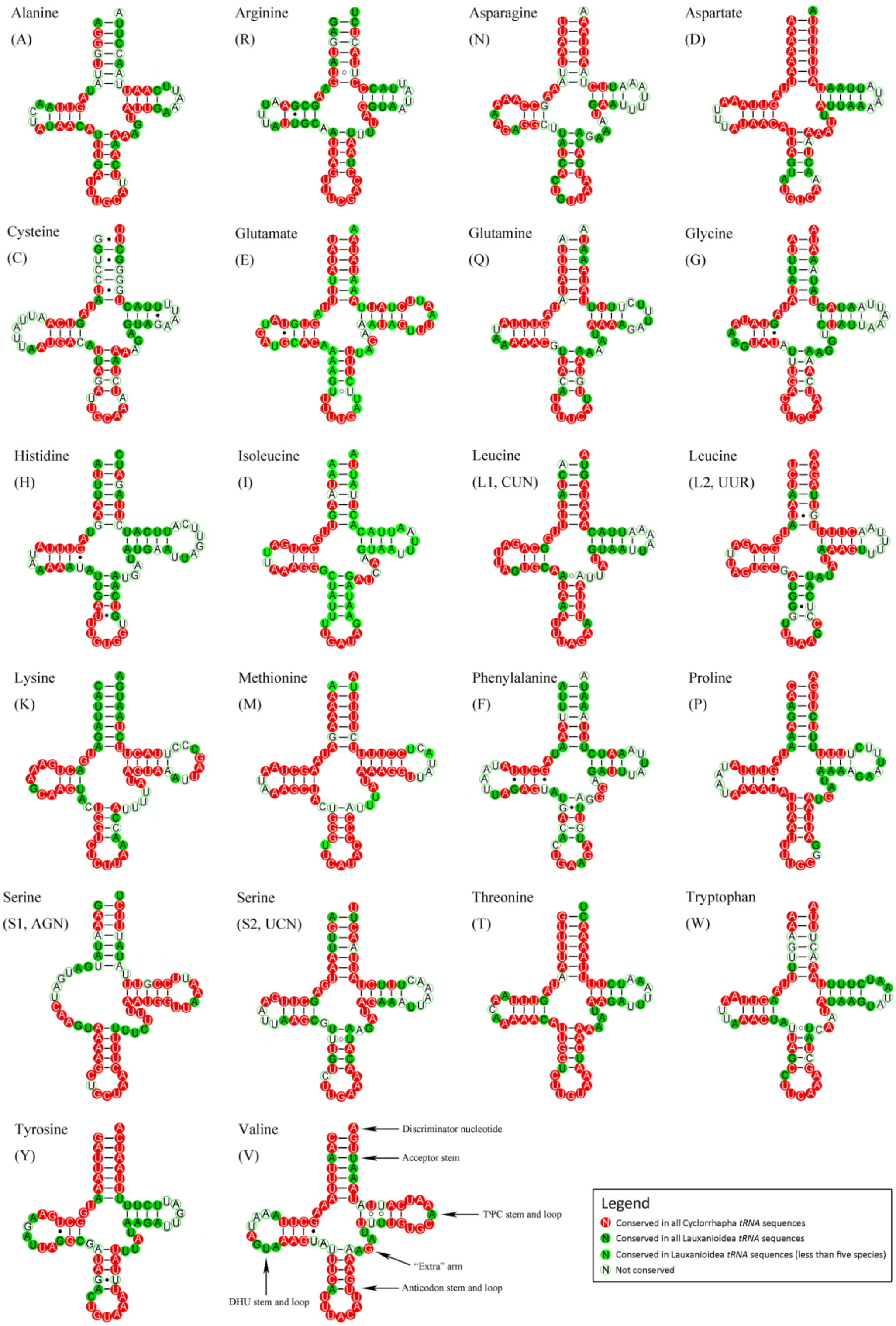


**Figure S3. Conservation and A+T contents of tRNAs in cyclorrhaphan mt genomes.** The percentage of identical nucleotides for each tRNA family was inferred from a multiple alignment produced with MEGA 5.0 (Tamura et al., 2011) and refined manually, taking into account the secondary structure.

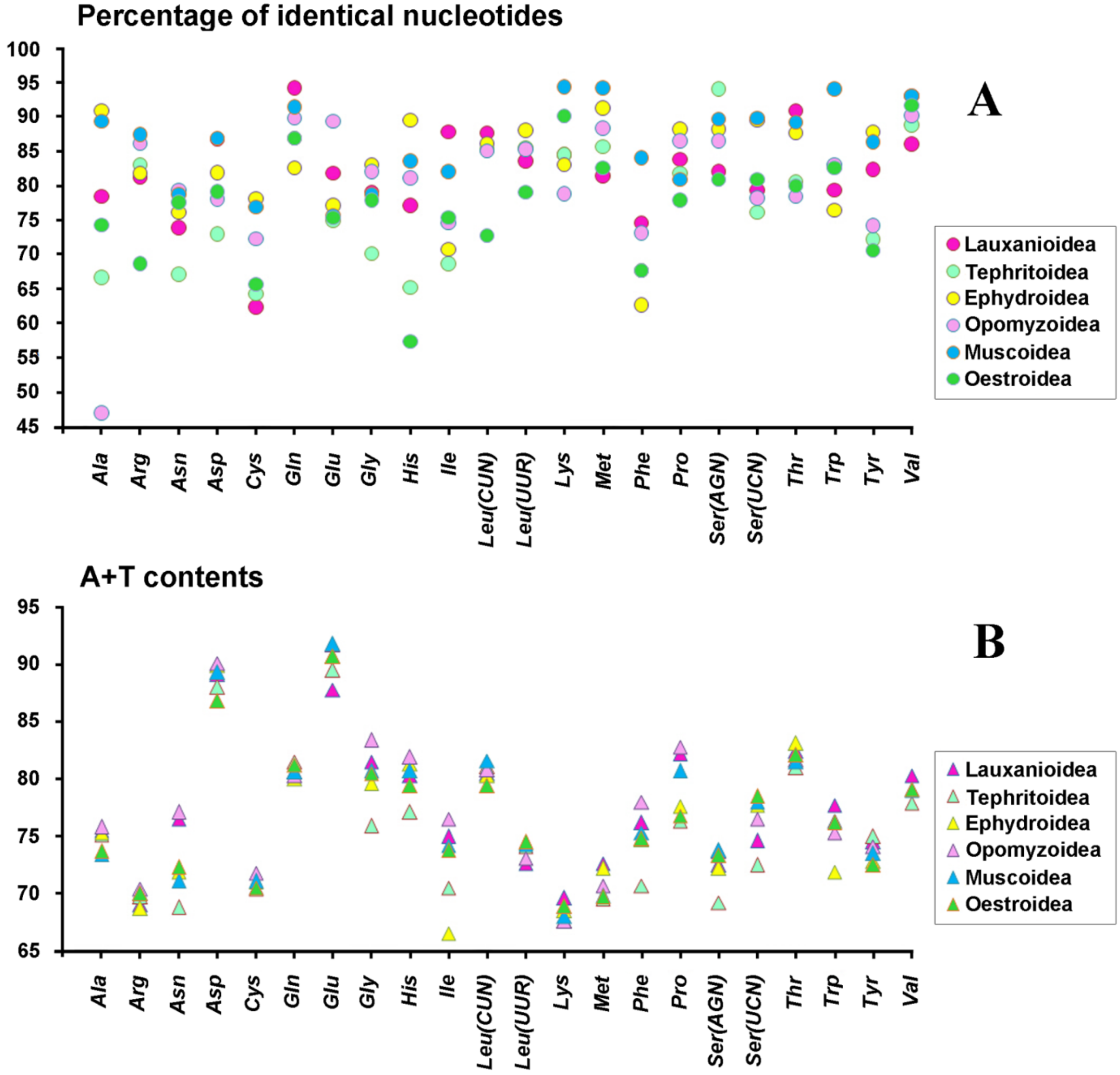


**Figure S4. Predicted secondary structure of the *lrRNA* gene in *Cestrotus liui*.** Red filled circle, nucleotide conserved in cyclorrhaphan mt genomes; Green filled circle, nucleotide conserved in lauxanioidean mt genomes; hollowed circle, nucleotide not conserved.

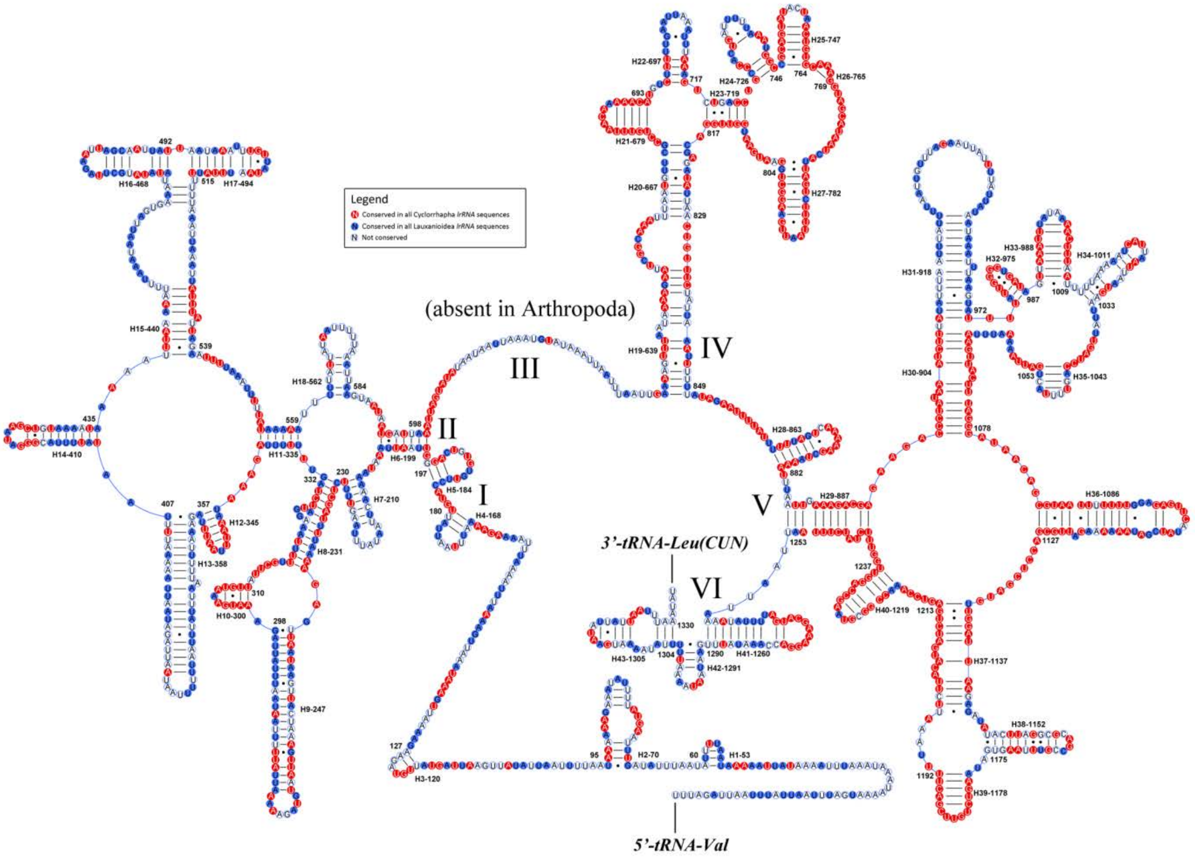


**Figure S5. Predicted secondary structure of the *srRNA* gene in *Cestrotus liui*.** Red filled circle, nucleotide conserved in cyclorrhaphan mt genomes; Green filled circle, nucleotide conserved in lauxanioidean mt genomes; hollowed circle, nucleotide not conserved.

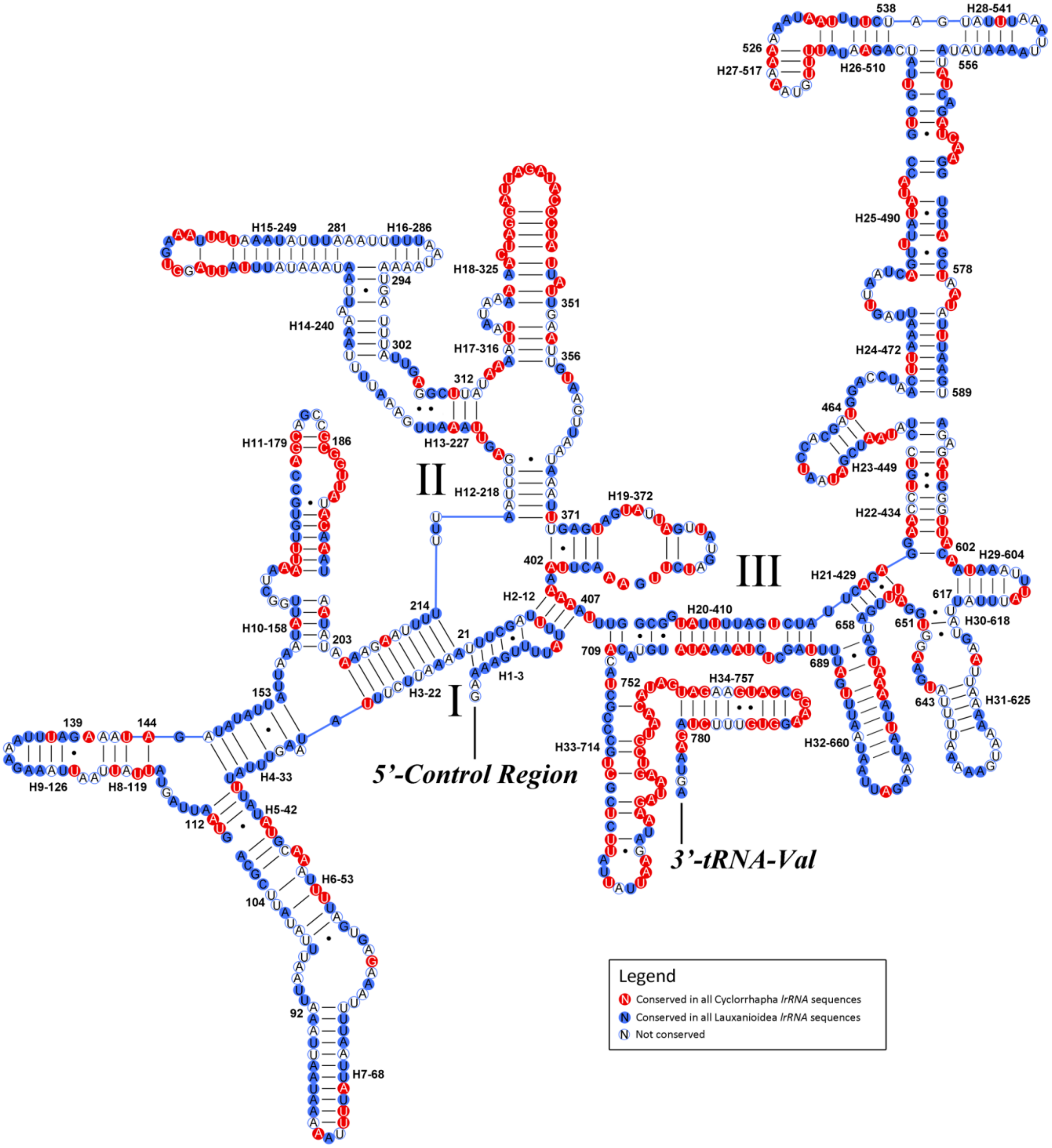


**Figure S6. Nucleotides conservation and A+T contents of rRNAs in cyclorrhaphan mt genomes.** Blue circle, percent of identical nucleotides; Red triangle, A+T contents.