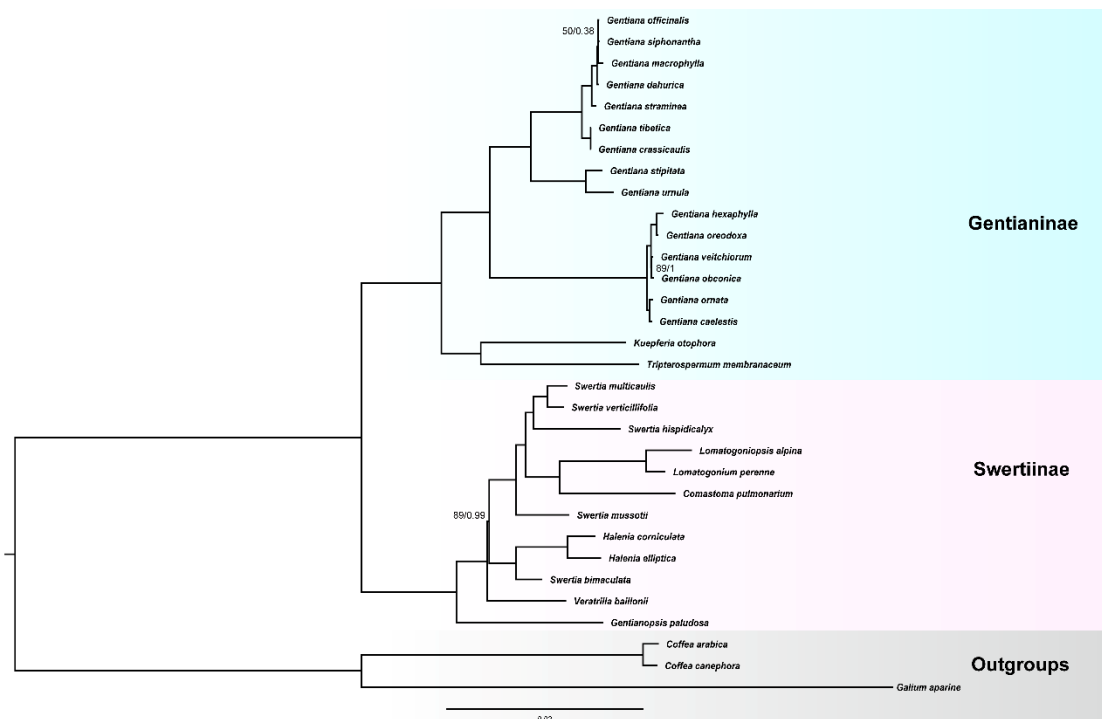
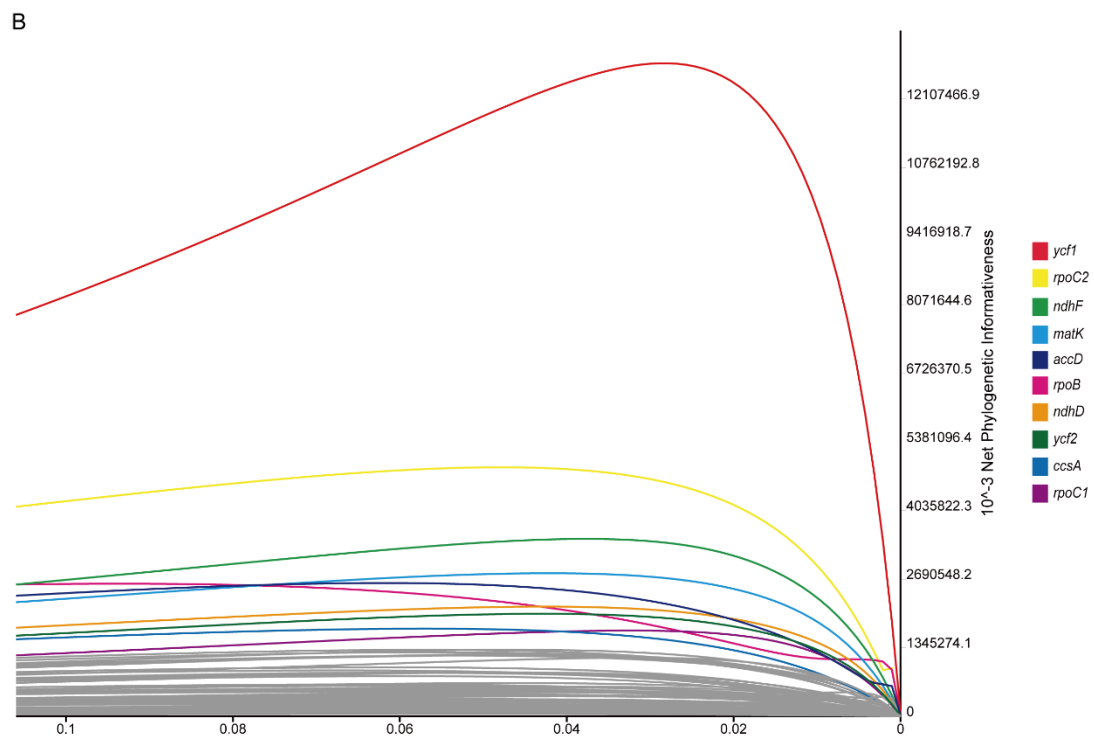
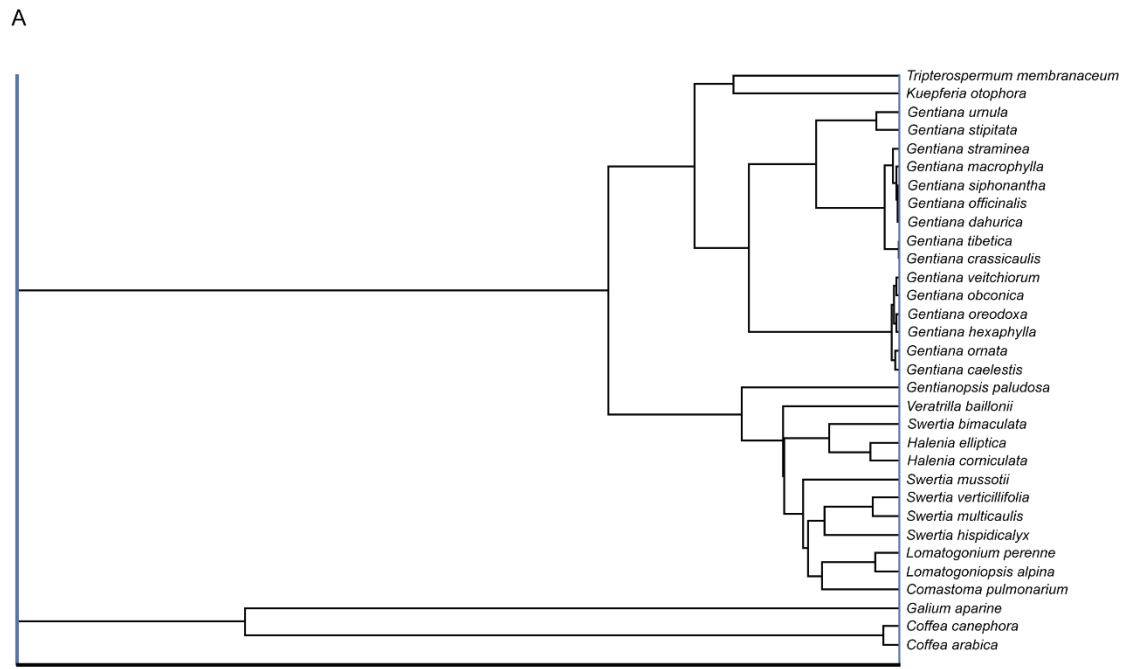


**Figure S1** Loss of *rpl33* coding region in plastomes of *Comastoma pulmonarium* and *Swertia hispidicalyx*. There is a stop codon in coding region of *rpl33* in *Comastoma pulmonarium* due to the change of cytosine (C) to thymine (T) at 22bp, and a small deletion containing the coding region of *rpl33* between the *psaJ* and *rps18* gene in the plastome of *Swertia hispidicalyx*.



**Figure S2** Maximum likelihood phylogram of Gentianeae from unpartitioned concatenated matrix of 76 plastid protein-encoding genes using RAxML. Maximum likelihood bootstrap (BS) values and the PP calculated from MrBayes are shown at nodes, except nodes with 100% BS and 1.0 PP.



**Figure S3** Phylogenetic informativeness profile estimated in PhyDesign. (A) The ultrametric tree of Gentianeae. (B) Net phylogenetic informativeness profile for 76 plastid protein-coding genes. Ten genes with the greatest informativeness are color-coded and indicated at the right. X- and Y-axes represent relative-time and net phylogenetic informativeness, respectively.

**Table S1** Taxa included in present study. NCBI accession numbers and voucher specimens' information are provided for newly sequenced plastomes, “-” indicates no applicable

| Family       | Subtribes   | Species                             | NCBI accession numbers | Voucher specimens information |
|--------------|-------------|-------------------------------------|------------------------|-------------------------------|
| Gentianaceae | Swertiinae  | <i>Comastoma pulmonarium</i>        | MT228723               | FSC-342                       |
| Gentianaceae | Swertiinae  | <i>Gentiana urnula</i>              | MT228724               | ZJW6386                       |
| Gentianaceae | Swertiinae  | <i>Gentianopsis paludosa</i>        | MT228725               | FSC-55                        |
| Gentianaceae | Swertiinae  | <i>Halenia elliptica</i>            | MT228726               | FSC-339                       |
| Gentianaceae | Swertiinae  | <i>Lomatogoniopsis alpina</i>       | MT228728               | ZJW5972                       |
| Gentianaceae | Swertiinae  | <i>Lomatogonium perenne</i>         | MT228729               | ZJW5118                       |
| Gentianaceae | Swertiinae  | <i>Swertia multicaulis</i>          | MT228730               | ZJW5106                       |
| Gentianaceae | Swertiinae  | <i>Veratrilla baillonii</i>         | MT228732               | ZJW6591                       |
| Gentianaceae | Swertiinae  | <i>Halenia corniculata</i>          | MK606372.1             | -                             |
| Gentianaceae | Swertiinae  | <i>Swertia hispidicalyx</i>         | MH321887.1             | -                             |
| Gentianaceae | Swertiinae  | <i>Swertia mussotii</i>             | KU641021.1             | -                             |
| Gentianaceae | Swertiinae  | <i>Swertia verticillifolia</i>      | MF795137               | -                             |
| Gentianaceae | Swertiinae  | <i>Swertia bimaculata</i>           | MH394374               | -                             |
| Gentianaceae | Gentianinae | <i>Kuepferia otophora</i>           | MT228727               | FSC-76                        |
| Gentianaceae | Gentianinae | <i>Tripterospermum membranaceum</i> | MT228731               | KUN1220748                    |
| Gentianaceae | Gentianinae | <i>Gentiana caelestis</i>           | MG192304.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana crassicaulis</i>        | KJ676538.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana dahurica</i>            | MH261259.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana hexaphylla</i>          | MG192305.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana macrophylla</i>         | KY856959.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana obconica</i>            | MG192306.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana officinalis</i>         | MH261261.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana oreodoxa</i>            | MG192307.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana ornata</i>              | MG192308.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana siphonantha</i>         | MH261260.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana stipitata</i>           | MG192309.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana straminea</i>           | KJ657732.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana tibetica</i>            | KU975374.1             | -                             |
| Gentianaceae | Gentianinae | <i>Gentiana veitchiorum</i>         | MG192310.1             | -                             |
| Rubiaceae    | -           | <i>Coffea arabica</i>               | NC_008535.1            | -                             |
| Rubiaceae    | -           | <i>Coffea canephora</i>             | KU500324.1             | -                             |
| Rubiaceae    | -           | <i>Galium aparine</i>               | KY562587.1             | -                             |

**Table S2** Genetic characteristics of 76 protein-coding genes used in analyses, including nonsynonymous rate ( $dN$ ), Synonymous rate ( $dS$ ), nucleotide diversity ( $\pi$ ), percent variability (PV), phylogenetic informativeness (PI), gene-tree discordance (GD) and partitioned coalescence support (PCS). Detailed information of functional group is provided in **Table 2**

| Genes       | Functional groups | $dN$   | $dS$   | $dN/dS$ | $\pi$  | PV     | PI       | GD      | PCS |
|-------------|-------------------|--------|--------|---------|--------|--------|----------|---------|-----|
| <i>accD</i> | OG                | 0.3653 | 1.0710 | 0.3411  | 0.0590 | 0.2228 | 67.5645  | 2.1426  | 52  |
| <i>atpA</i> | ATP               | 0.0551 | 1.1528 | 0.0478  | 0.0343 | 0.1808 | 36.1631  | 8.7252  | -56 |
| <i>atpB</i> | ATP               | 0.0514 | 0.7176 | 0.0716  | 0.0270 | 0.1514 | 21.5446  | 2.0286  | -56 |
| <i>atpE</i> | ATP               | 0.1797 | 0.7273 | 0.2471  | 0.0392 | 0.2114 | 8.7099   | 17.6664 | 2   |
| <i>atpF</i> | ATP               | 0.2248 | 0.7315 | 0.3073  | 0.0438 | 0.2250 | 15.2736  | 14.5917 | 27  |
| <i>atpH</i> | ATP               | 0.0056 | 1.1477 | 0.0049  | 0.0291 | 0.1564 | 4.5894   | 32.8122 | 52  |
| <i>atpI</i> | ATP               | 0.0924 | 0.7713 | 0.1197  | 0.0331 | 0.1748 | 13.1140  | 0.9808  | 48  |
| <i>ccsA</i> | OG                | 0.4041 | 1.1645 | 0.3470  | 0.0658 | 0.2811 | 55.2155  | 0.8087  | 56  |
| <i>cemA</i> | OG                | 0.3444 | 0.7356 | 0.4682  | 0.0517 | 0.2727 | 23.1032  | 13.3351 | 27  |
| <i>clpP</i> | OG                | 0.1693 | 0.6523 | 0.2596  | 0.0436 | 0.1881 | 17.6351  | 15.7227 | 0   |
| <i>matK</i> | OG                | 0.5333 | 1.2032 | 0.4433  | 0.0824 | 0.3216 | 87.6416  | 0.9279  | 4   |
| <i>ndhA</i> | NDH               | 0.232  | 0.9876 | 0.2349  | 0.0524 | 0.2423 | 38.8263  | 5.5621  | 0   |
| <i>ndhB</i> | NDH               | 0.0412 | 0.1099 | 0.3748  | 0.0129 | 0.0301 | 6.7568   | 13.6284 | 0   |
| <i>ndhC</i> | NDH               | 0.1199 | 0.9808 | 0.1223  | 0.0361 | 0.1889 | 7.9042   | 22.433  | 0   |
| <i>ndhD</i> | NDH               | 0.1904 | 1.0327 | 0.1844  | 0.0694 | 0.0537 | 62.1701  | 2.6858  | 0   |
| <i>ndhE</i> | NDH               | 0.1535 | 1.0038 | 0.1530  | 0.0448 | 0.1799 | 9.7540   | 17.3181 | 0   |
| <i>ndhF</i> | NDH               | 0.2168 | 1.1928 | 0.1818  | 0.0603 | 0.0271 | 109.9486 | 3.9719  | 0   |
| <i>ndhG</i> | NDH               | 0.2711 | 1.0581 | 0.2562  | 0.0551 | 0.2689 | 19.9161  | 10.7515 | 0   |
| <i>ndhH</i> | NDH               | 0.1388 | 1.1791 | 0.1177  | 0.0490 | 0.2152 | 36.1738  | 4.5341  | 0   |
| <i>ndhI</i> | NDH               | 0.1511 | 1.2747 | 0.1185  | 0.0501 | 0.2032 | 17.8783  | 5.1046  | 0   |
| <i>ndhJ</i> | NDH               | 0.1261 | 0.8440 | 0.1494  | 0.0410 | 0.1983 | 11.5506  | 9.5790  | 0   |
| <i>ndhK</i> | NDH               | 0.1310 | 0.9647 | 0.1358  | 0.0376 | 0.1856 | 15.4820  | 4.9396  | 0   |
| <i>petA</i> | PET               | 0.1236 | 0.9438 | 0.1309  | 0.0436 | 0.2090 | 21.9469  | 3.5395  | -56 |
| <i>petB</i> | PET               | 0.0344 | 1.0109 | 0.0340  | 0.0279 | 0.1376 | 14.6061  | 8.4695  | 2   |
| <i>petD</i> | PET               | 0.0554 | 0.8376 | 0.0662  | 0.0321 | 0.1303 | 10.2926  | 23.1140 | -52 |
| <i>petG</i> | PET               | 0.0270 | 0.5722 | 0.0473  | 0.0207 | 0.1351 | 1.1984   | 31.7335 | 8   |
| <i>petL</i> | PET               | 0.1336 | 0.7772 | 0.1719  | 0.0303 | 0.2151 | 1.3856   | 35.6260 | 0   |
| <i>petN</i> | PET               | 0.0961 | 0.5843 | 0.1645  | 0.0293 | 0.1379 | 1.5554   | 29.4858 | -24 |
| <i>psaA</i> | PSA               | 0.0326 | 0.7722 | 0.0422  | 0.0249 | 0.1409 | 35.2175  | 6.7164  | 0   |
| <i>psaB</i> | PSA               | 0.0292 | 0.8319 | 0.0351  | 0.0268 | 0.1367 | 34.2358  | 3.2928  | -56 |
| <i>psaC</i> | PSA               | 0.0273 | 1.1449 | 0.0238  | 0.0257 | 0.1276 | 5.2226   | 17.3406 | 54  |
| <i>psaI</i> | PSA               | 0.1990 | 0.3948 | 0.5040  | 0.0266 | 0.1759 | 1.8664   | 16.8629 | -27 |
| <i>psaJ</i> | PSA               | 0.0566 | 0.7561 | 0.0748  | 0.0326 | 0.1778 | 4.3983   | 28.5362 | 30  |
| <i>psbA</i> | PSB               | 0.0151 | 0.8314 | 0.0182  | 0.0252 | 0.1246 | 16.5847  | 15.3867 | 28  |
| <i>psbB</i> | PSB               | 0.0569 | 0.8398 | 0.0678  | 0.0278 | 0.1594 | 25.213   | 7.2645  | 1   |
| <i>psbC</i> | PSB               | 0.0281 | 0.7164 | 0.0393  | 0.0260 | 0.1438 | 21.5323  | 9.8471  | 28  |
| <i>psbD</i> | PSB               | 0.0205 | 0.6772 | 0.0303  | 0.0223 | 0.118  | 14.3902  | 10.2727 | -56 |

|              |         |        |        |        |        |        |          |         |     |
|--------------|---------|--------|--------|--------|--------|--------|----------|---------|-----|
| <i>psbE</i>  | PSB     | 0.0376 | 0.5819 | 0.0646 | 0.0244 | 0.1245 | 2.8053   | 17.5954 | -25 |
| <i>psbF</i>  | PSB     | 0.0484 | 0.6442 | 0.0751 | 0.0229 | 0.1453 | 1.5568   | 34.5233 | -2  |
| <i>psbH</i>  | PSB     | 0.2929 | 0.6900 | 0.4245 | 0.0471 | 0.2237 | 8.1256   | 17.1520 | 56  |
| <i>psbI</i>  | PSB     | 0.0388 | 0.6232 | 0.0622 | 0.0288 | 0.1389 | 1.4368   | 26.5306 | 54  |
| <i>psbJ</i>  | PSB     | 0.0565 | 0.4395 | 0.1286 | 0.0165 | 0.1000 | 1.2624   | 32.8013 | 53  |
| <i>psbL</i>  | PSB     | 0.0509 | 0.3419 | 0.1487 | 0.0156 | 0.0877 | 1.1146   | 34.6862 | -27 |
| <i>psbM</i>  | PSB     | 0.0917 | 0.5728 | 0.1601 | 0.0295 | 0.1471 | 1.3478   | 28.6185 | 26  |
| <i>psbN</i>  | PSB     | 0.0565 | 0.4743 | 0.1191 | 0.0192 | 0.1163 | 1.4453   | 28.3032 | 2   |
| <i>psbT</i>  | PSB     | 0.0001 | 0.9968 | 0.0001 | 0.0336 | 0.1143 | 2.4292   | 29.2809 | 48  |
| <i>psbZ</i>  | PSB     | 0.0737 | 0.8185 | 0.0900 | 0.0223 | 0.1882 | 2.4807   | 29.7000 | 0   |
| <i>rbcL</i>  | Rubisco | 0.0816 | 0.8366 | 0.0976 | 0.0294 | 0.1505 | 37.4035  | 2.2135  | 56  |
| <i>rpl14</i> | RPL     | 0.0878 | 0.8567 | 0.1025 | 0.0380 | 0.1995 | 5.4226   | 9.7891  | -52 |
| <i>rpl16</i> | RPL     | 0.1220 | 1.2469 | 0.0978 | 0.0390 | 0.2034 | 9.1429   | 16.4541 | 55  |
| <i>rpl2</i>  | RPL     | 0.0295 | 0.1926 | 0.1529 | 0.0098 | 0.0624 | 3.0633   | 25.9169 | 50  |
| <i>rpl20</i> | RPL     | 0.2510 | 0.9705 | 0.2586 | 0.0449 | 0.2164 | 16.7373  | 15.1967 | 0   |
| <i>rpl22</i> | RPL     | 0.3859 | 1.7402 | 0.2218 | 0.0683 | 0.2952 | 25.7191  | 2.4413  | 4   |
| <i>rpl23</i> | RPL     | 0.0236 | 0.2666 | 0.0885 | 0.0079 | 0.0609 | 0.9822   | 32.11   | -26 |
| <i>rpl32</i> | RPL     | 0.2438 | 1.4596 | 0.1670 | 0.0535 | 0.2222 | 7.6006   | 21.9948 | 28  |
| <i>rpl33</i> | RPL     | 0.4289 | 1.3109 | 0.3249 | 0.0498 | 0.1324 | 9.2624   | NA      | -24 |
| <i>rpl36</i> | RPL     | 0.0609 | 1.2482 | 0.0488 | 0.0335 | 0.2342 | 1.7339   | 31.2538 | 54  |
| <i>rpoA</i>  | RPO     | 0.2720 | 1.0983 | 0.2476 | 0.0547 | 0.2653 | 34.6309  | 16.6631 | -56 |
| <i>rpoB</i>  | RPO     | 0.1229 | 0.8775 | 0.1401 | 0.0357 | 0.2036 | 69.2034  | 3.2748  | -28 |
| <i>rpoC1</i> | RPO     | 0.1382 | 0.9017 | 0.1533 | 0.0381 | 0.3872 | 46.1063  | 8.1058  | -56 |
| <i>rpoC2</i> | RPO     | 0.2508 | 0.9633 | 0.2603 | 0.0526 | 0.2509 | 147.8433 | 0       | 56  |
| <i>rps11</i> | RPS     | 0.1891 | 1.154  | 0.1638 | 0.0498 | 0.2160 | 13.9206  | 17.0221 | 56  |
| <i>rps12</i> | RPS     | 0.0423 | 0.3696 | 0.1144 | 0.0187 | 0.0780 | 4.0176   | 27.9498 | -56 |
| <i>rps14</i> | RPS     | 0.158  | 0.8777 | 0.1800 | 0.0435 | 0.2400 | 5.9692   | 12.1429 | 0   |
| <i>rps15</i> | RPS     | 0.2841 | 1.4808 | 0.1919 | 0.0627 | 0.2500 | 11.7144  | 5.4910  | 0   |
| <i>rps18</i> | RPS     | 0.1622 | 0.7838 | 0.2070 | 0.0386 | 0.1883 | 6.5995   | 19.0925 | -28 |
| <i>rps19</i> | RPS     | 0.1347 | 0.9256 | 0.1456 | 0.0408 | 0.2174 | 4.8625   | 21.3507 | 56  |
| <i>rps2</i>  | RPS     | 0.1390 | 0.9439 | 0.1472 | 0.0365 | 0.2076 | 15.7626  | 10.3947 | 0   |
| <i>rps3</i>  | RPS     | 0.2297 | 1.2682 | 0.1811 | 0.0548 | 0.2861 | 19.7711  | 7.1380  | 27  |
| <i>rps4</i>  | RPS     | 0.1255 | 0.8958 | 0.1401 | 0.0394 | 0.2085 | 13.7031  | 6.4906  | 0   |
| <i>rps7</i>  | RPS     | 0.0196 | 0.1704 | 0.1152 | 0.0077 | 0.0473 | 1.2825   | 20.4554 | 50  |
| <i>rps8</i>  | RPS     | 0.2600 | 1.0960 | 0.2372 | 0.0568 | 0.2990 | 13.6557  | 15.5247 | -24 |
| <i>ycf1</i>  | OG      | 0.7634 | 1.2515 | 0.6100 | 0.0884 | 0.3460 | 438.8369 | 0.9808  | -56 |
| <i>ycf2</i>  | OG      | 0.1163 | 0.1621 | 0.7173 | 0.0191 | 0.0974 | 58.8030  | 1.0214  | -28 |
| <i>ycf3</i>  | OG      | 0.0429 | 0.6762 | 0.0635 | 0.0243 | 0.1310 | 6.1739   | 14.5601 | 4   |
| <i>ycf4</i>  | OG      | 0.2270 | 0.7662 | 0.2963 | 0.0393 | 0.2283 | 14.4875  | 7.2522  | -52 |