Evolutionary analysis of Apocynaceae family based on 228 plastomes

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Apocynaceae family consists of about 400 genera and about 5,100 species. Apocynaceae is considered as a family which speciated diversely and successfully adapted to various environment, since it is widely distributed in the five continents except for polar zones and has various morphological traits including herb, shrub, tree and vine. Apocynaceae also has high values in several industrial aspects. Apocynaceae species including *Catharanthus roseus, Rhazya stricta, Asclepias syriaca* and *Calotropis gigantea* were proved to have medicinal effects and *Asclepias* genus occupies large proportion in horticultural market of North America. On previous studies, many plastomes and phylogenetic analyses of Apocynaceae plants were reported, but evolutionary analysis based on every plastome sequence is not conducted yet. In this study, we estimated divergence time of entire Apocynaceae family, predicted geological events highly related to subfamily divergence and suggested structural changes of plastomes and chloroplast genes which are likely to induce speciation. 219 Apocynaceae plastome sequences were collected from NCBI database. Nine Apocynaceae plants were collected and sequenced on Illumina HiSeq platform, and their plastomes were assembled using dnaLCW method. 79 CDS were extracted from each plastome and a phylogenetic tree based on the CDS was constructed using BEAST. Divergence time estimation was conducted using *Coffea* fossil evidence as reference. Divergence time between Rauvolfioideae and Apocynoideae is estimated to be about 60 million years ago (MYA), which corresponds to K-Pg extinction. Divergence time between Apocynoideae and Asclepiadoideae is estimated to be about 30 MYA, which corresponds to drastic temperature drop across the Eocene-Oligocene transition. Ka/Ks values between CDS were calculated through every possible one-to-one inter-subfamilies comparison. Calculated Ka/Ks values were visualized using density plot and ten genes which have high Ka/Ks mean values or extraordinary Ka/Ks tendencies were selected. Amino acid sequences of the ten genes were classified into several types using neighbor-joining methods. The classification is well-matched with a phylogenetic tree and divergence of subfamilies. Among the ten genes, *psaI* gene experienced truncation when Asclepiadoideae diverged, and PsaI subunit is reported to stabilize photosystem I during leaf senescence and chilling stress.

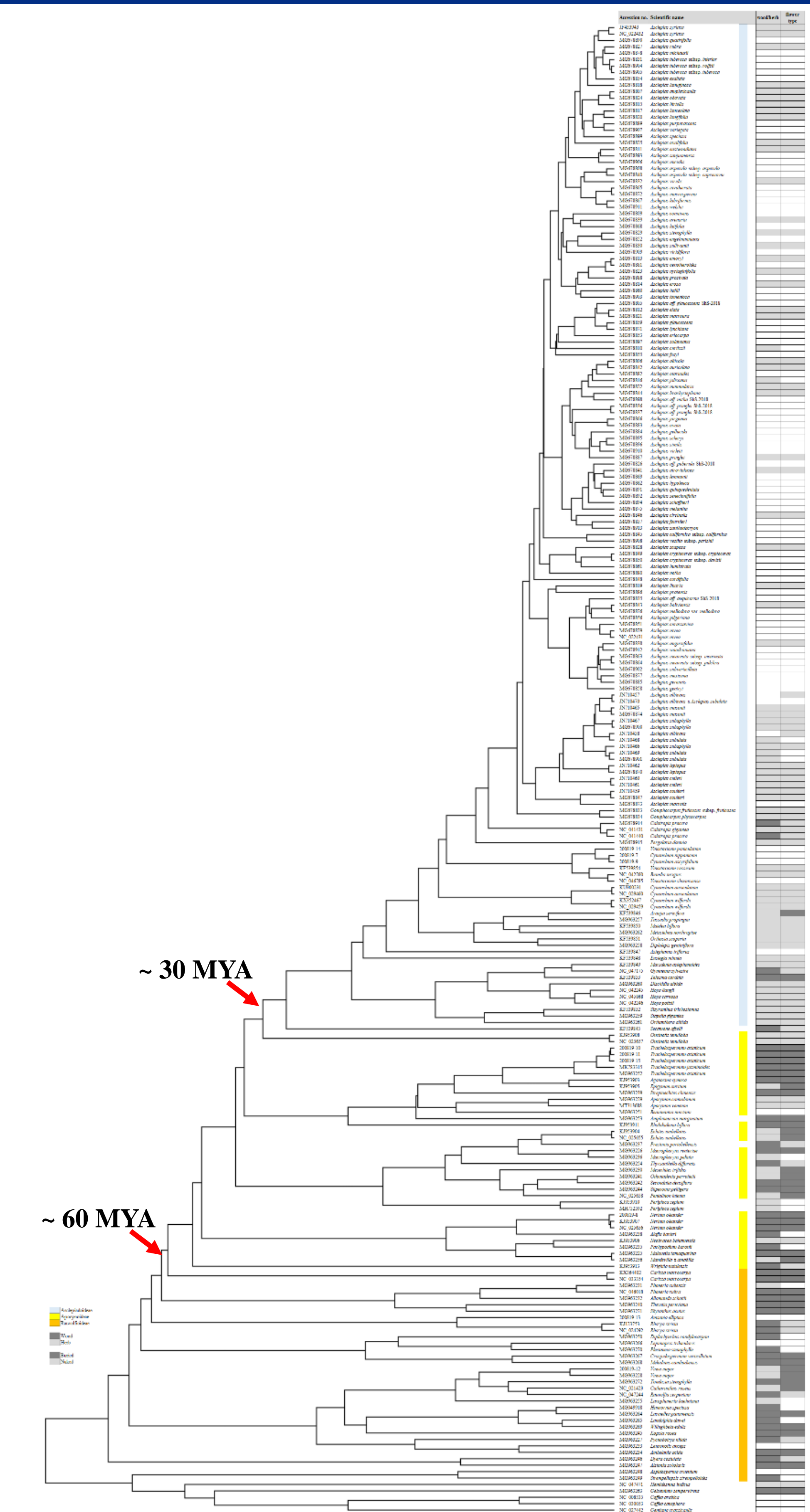## 1. Phylogenetic analysis of plastomes

Figure 1. A phylogenetic tree of 228 plastomes of Apocynaceae plants based on 79 common CDS. The tree was constructed using BEAST and phenotypic traits are indicated on the right.

Figure 2. A phylogenetic tree of 35 complete plastomes of Apocynaceae plants based on 79 common CDS and structural comparisons.

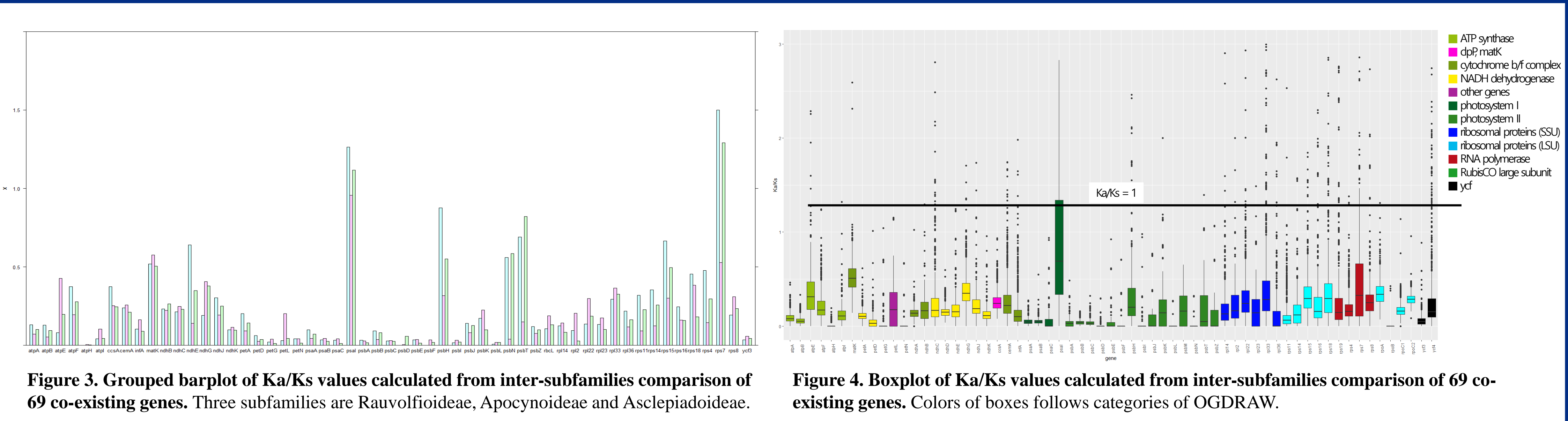## 2. Ka/Ks value visualization – barplot and boxplot

Figure 3. Grouped barplot of Ka/Ks values calculated from inter-subfamilies comparison of 69 co-existing genes. Three subfamilies are Rauvolfioideae, Apocynoideae and Asclepiadoideae.

Figure 4. Boxplot of Ka/Ks values calculated from inter-subfamilies comparison of 69 co-existing genes. Colors of boxes follows categories of OGDRAW.

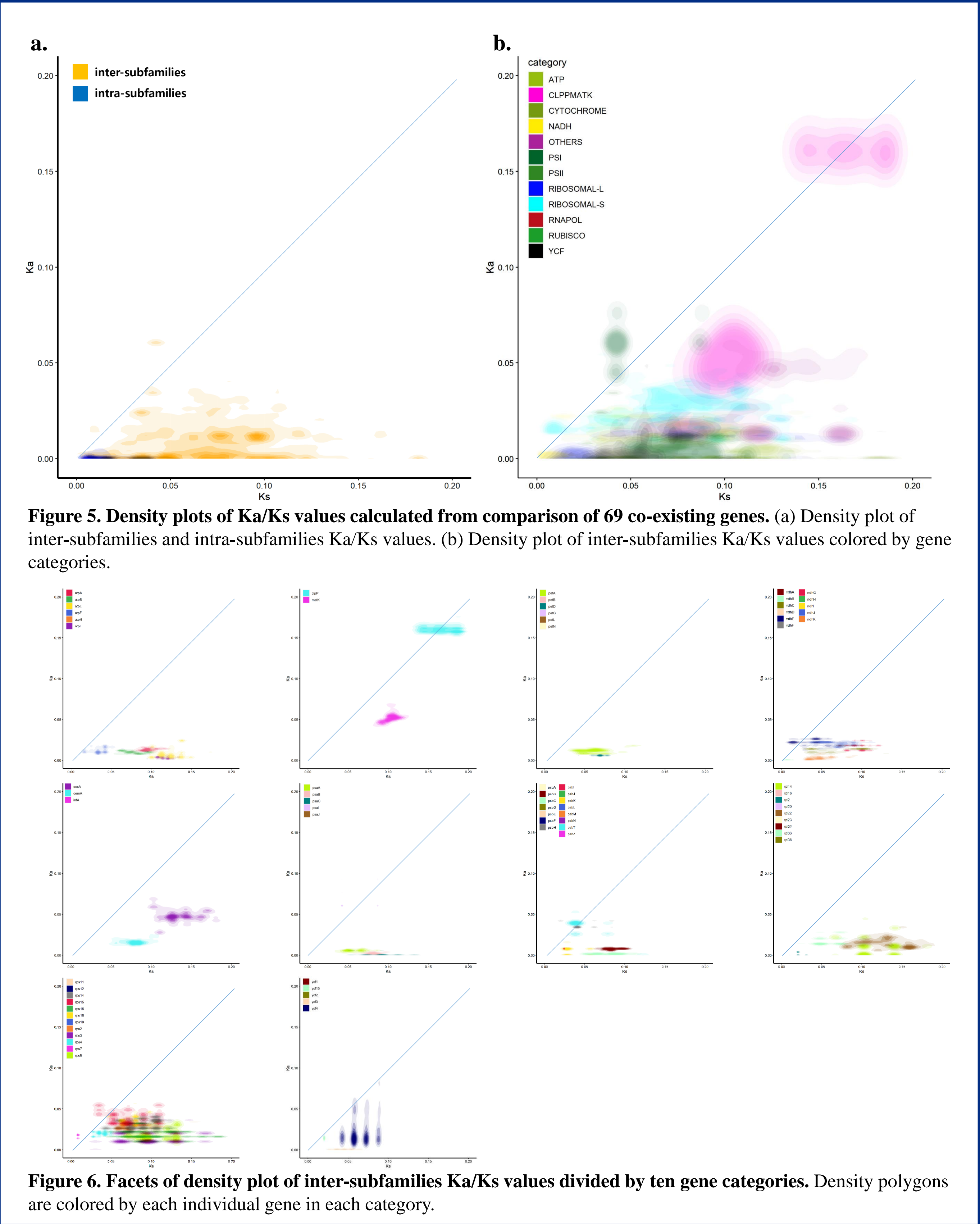## 3. Ka/Ks value visualization – density plot

Figure 5. Density plots of Ka/Ks values calculated from comparison of 69 co-existing genes. (a) Density plot of inter-subfamilies and intra-subfamilies Ka/Ks values. (b) Density plot of inter-subfamilies Ka/Ks values colored by gene categories.

Figure 6. Facets of density plot of inter-subfamilies Ka/Ks values divided by ten gene categories. Density polygons are colored by each individual gene in each category.
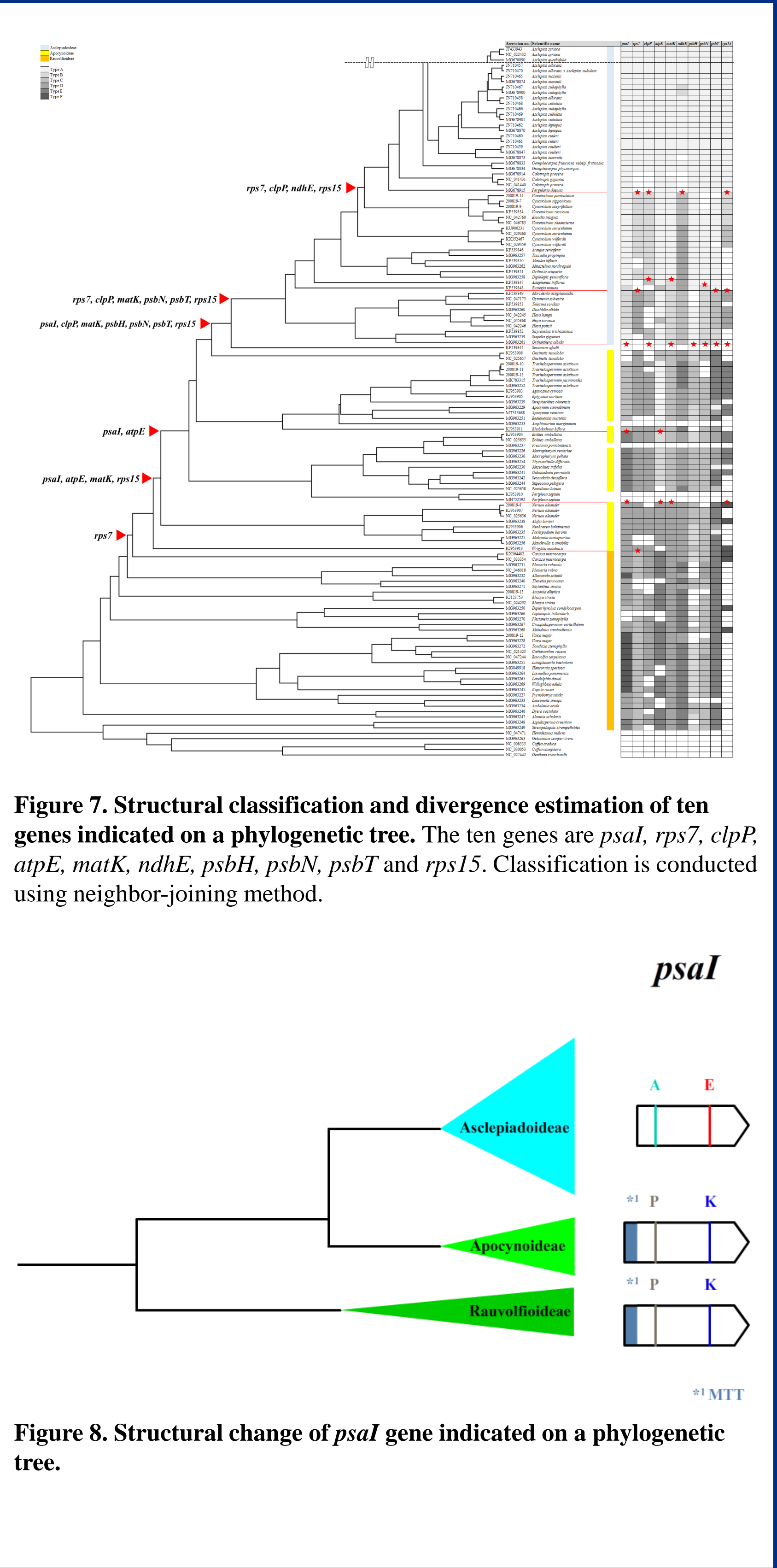
4. Structural changes of chloroplast genes

Figure 7. Structural classification and divergence estimation of ten genes indicated on a phylogenetic tree. The ten genes are *psaI, rps7, clpP, atpE, matK, ndhE, psbH, psbN, psbT* and *rps15*. Classification is conducted using neighbor-joining method.

psaI

Figure 8. Structural change of *psaI* gene indicated on a phylogenetic tree.

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