

Supplementary information

The slow-evolving *Acorus tatarinowii* genome sheds light on ancestral monocot evolution

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The slow-evolving *Acorus tatarinowii* genome sheds light on ancestral monocot evolution

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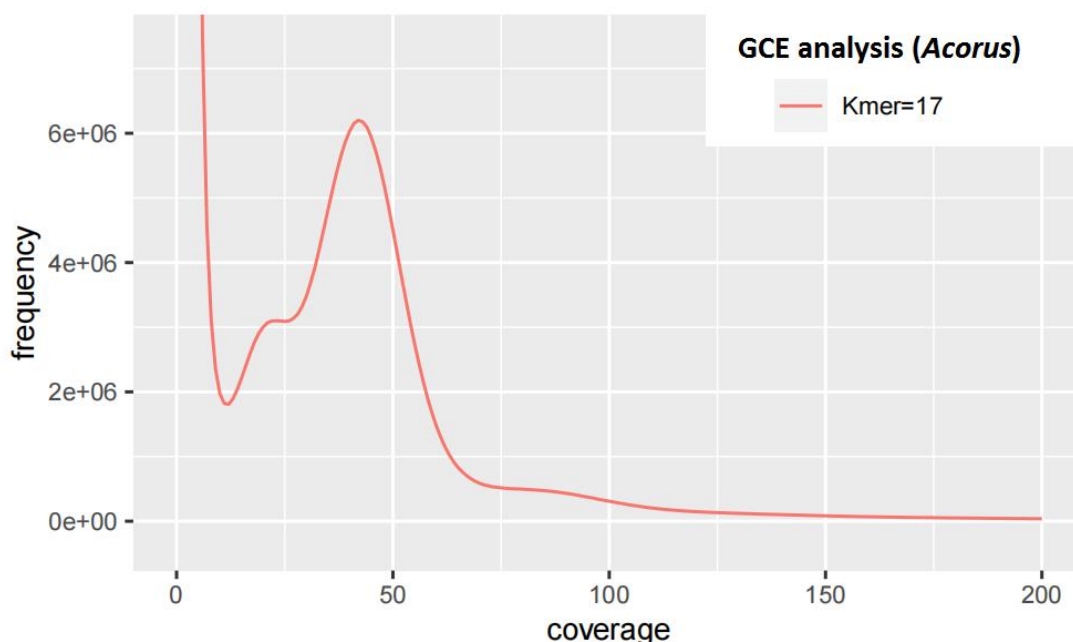
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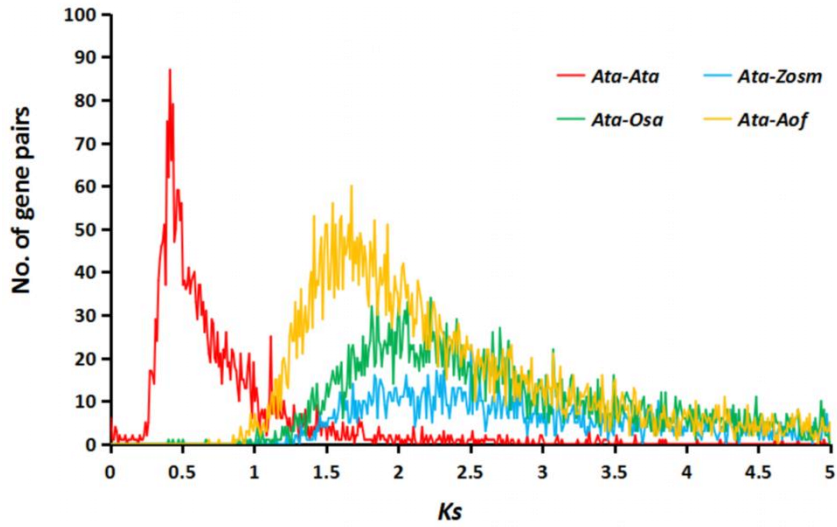
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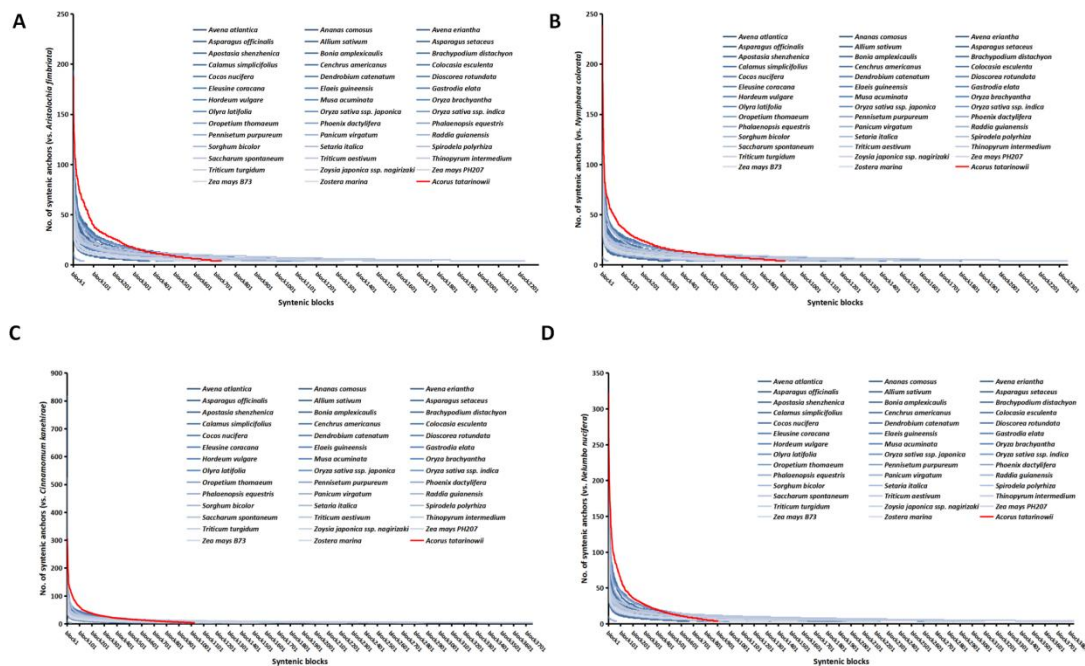
Supplementary Figures



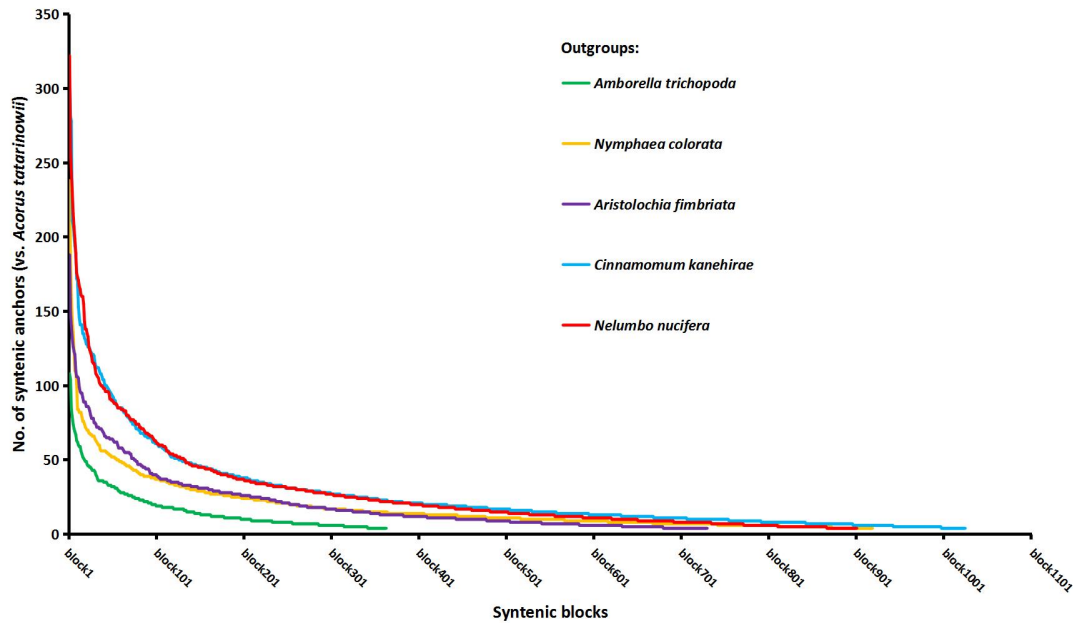
Supplementary Figure S1. K-mer (k = 17) analysis for estimating the genome size of *Acorus* based on Illumina reads. X-axis is the depth of read coverage. Y-axis is the frequency at that depth divided by the total frequency of all depths. Genome size = K-mer number / peak depth. The secondary peak at a position half of that of the main peak represents the rate of heterozygosity.



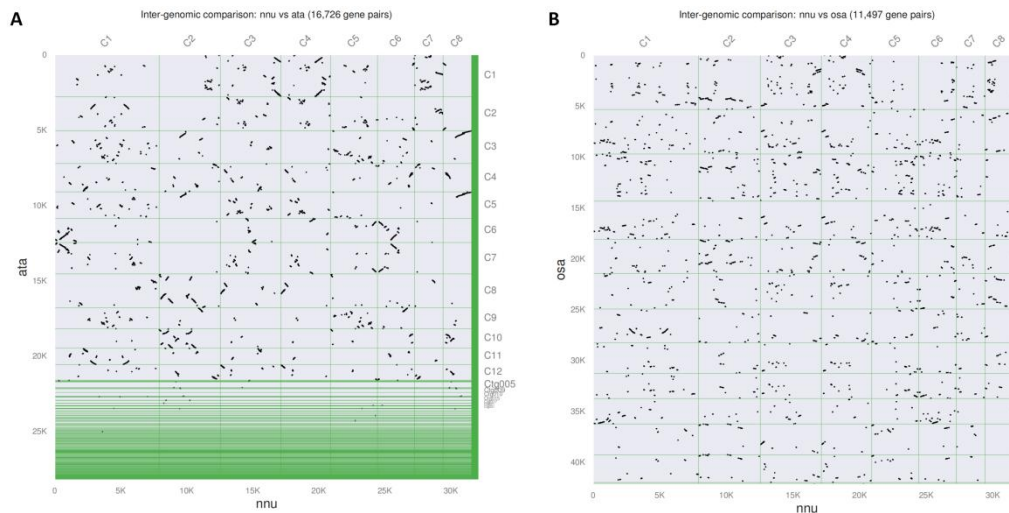
Supplementary Figure S2. Density distributions of syntenic paralogs of *Acorus* and syntenic orthologs between *Acorus* and other monocots according to Ks divergence. Ata: *Acorus*; Zosm: seagrass; Osa: rice; Aof: asparagus.



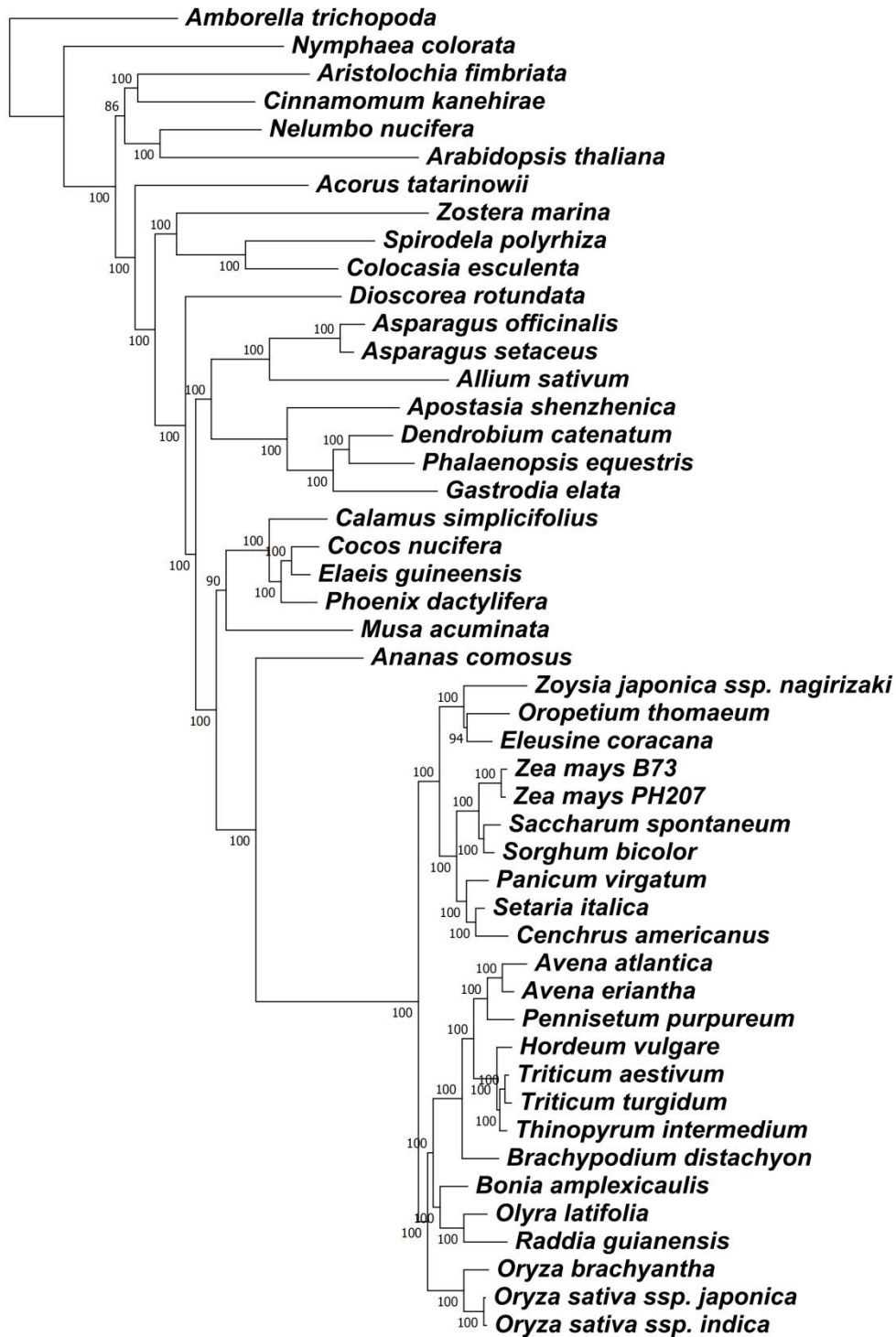
Supplementary Figure S3. Comparison of ‘syntenic block size’ decay among different monocot species when compared to the outgroups *Aristolochia* (A), *Nymphaea* (B), *Cinnamomum* (C) and *Nelumbo* (D).



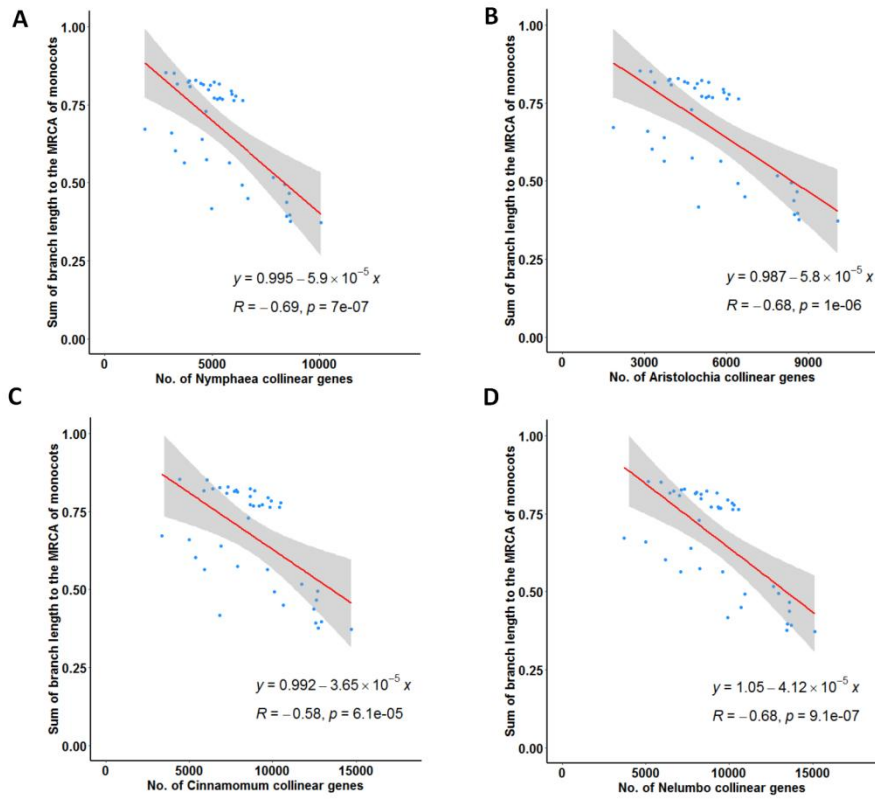
Supplementary Figure S4. Comparison of ‘syntenic block size’ decay among different outgroup species (*Amborella*, *Nymphaea*, *Aristolochia*, *Cinnamomum* and *Nelumbo*) when compared to *Acorus*.



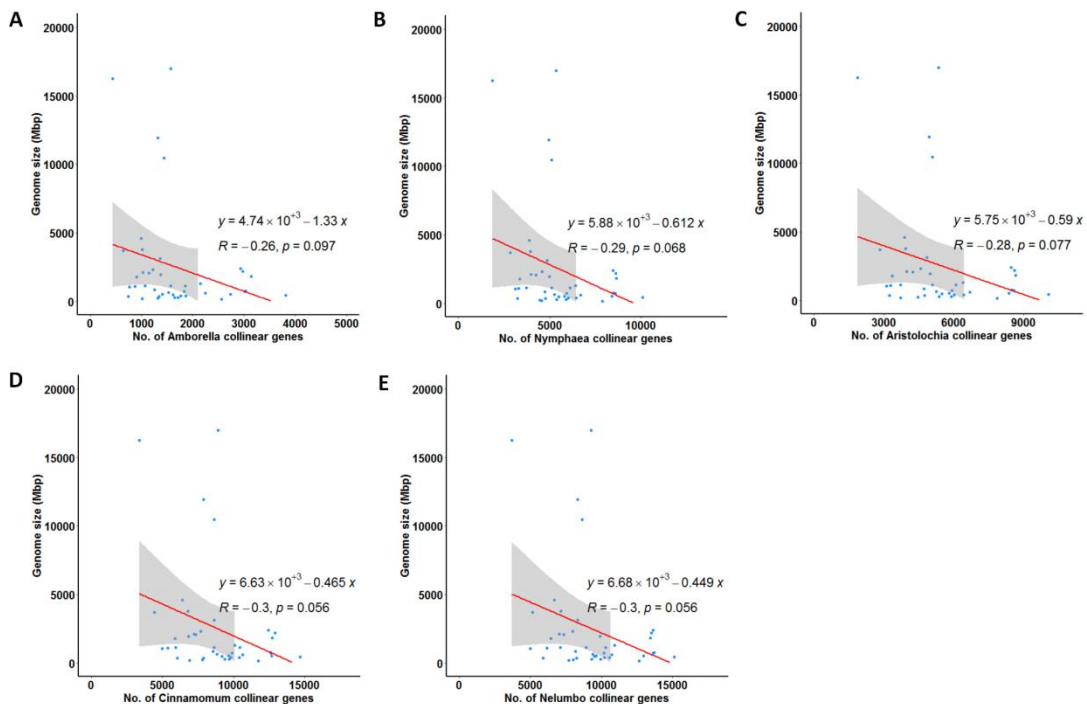
Supplementary Figure S5. Scatter plot of between-species syntenies. A. Synteny between *N. nucifera* (nnu) and *Acorus* (ata). B. Synteny between *N. nucifera* (nnu) and rice (osa).



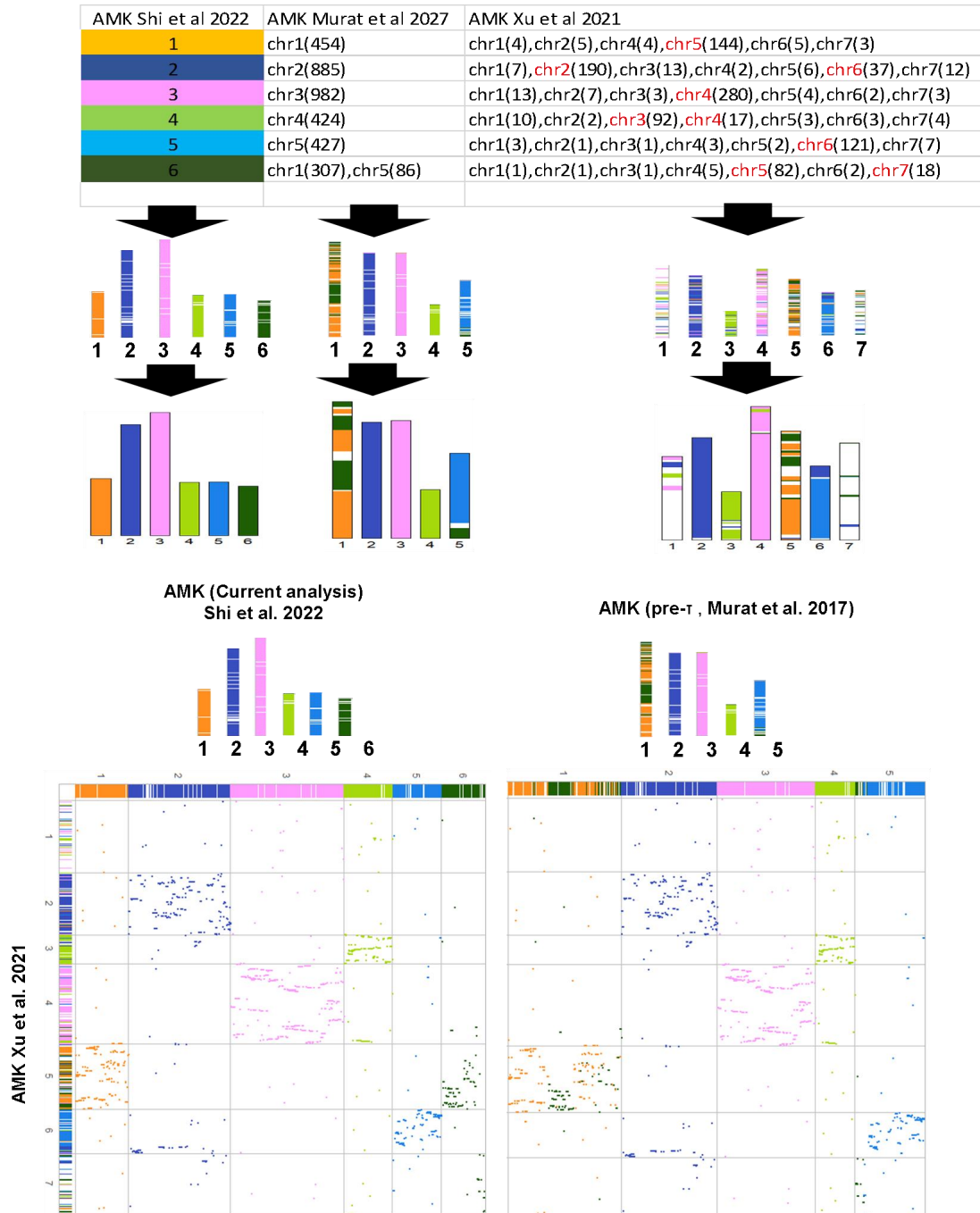
Supplementary Figure S6. Phylogenetic tree of monocots and outgroups based on concatenated single-copy gene alignments. Branch length: number of amino acid substitutions per site. Nodes: bootstraps.



Supplementary Figure S7. Significantly negative correlation (calculated by Pearson's correlation) between the number of syntenic genes in *Nymphaea* (A)/ *Aristolochia* (B)/ *Cinnamomum* (C)/ *Nelumbo* (D) and the sum of branch length to the MRCA of monocots in the concatenated single-copy gene tree. The error bands represent 95% confidence intervals based on a binomial model.

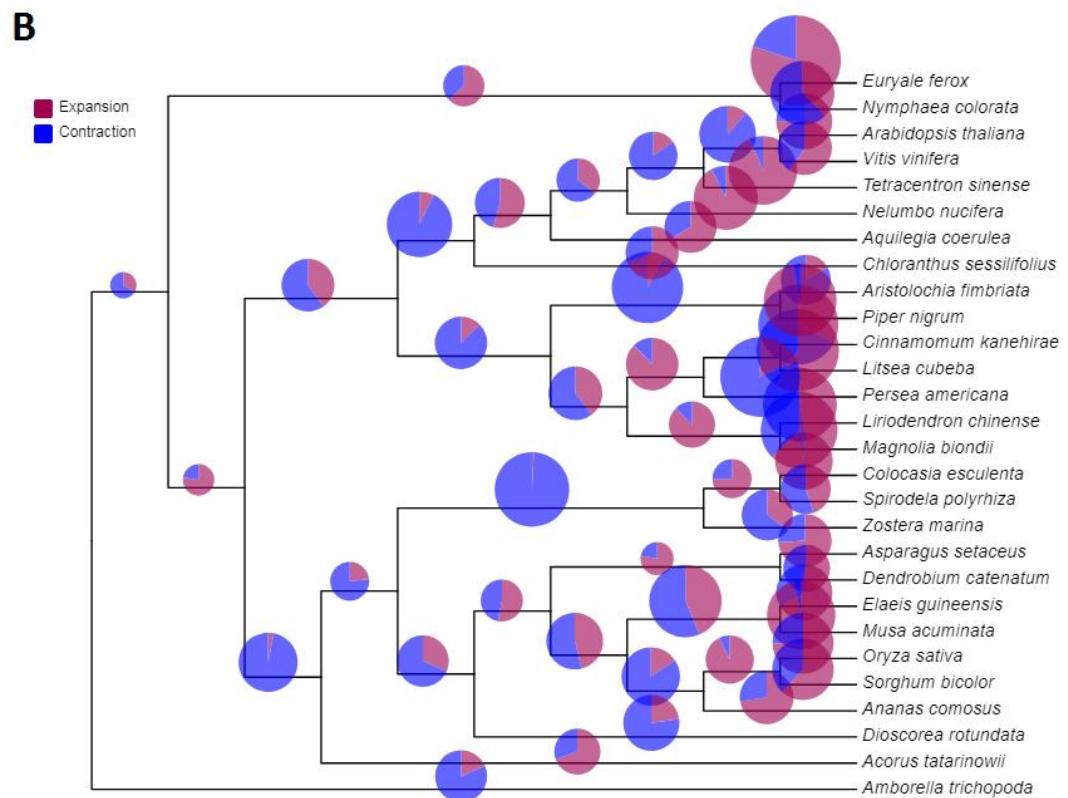
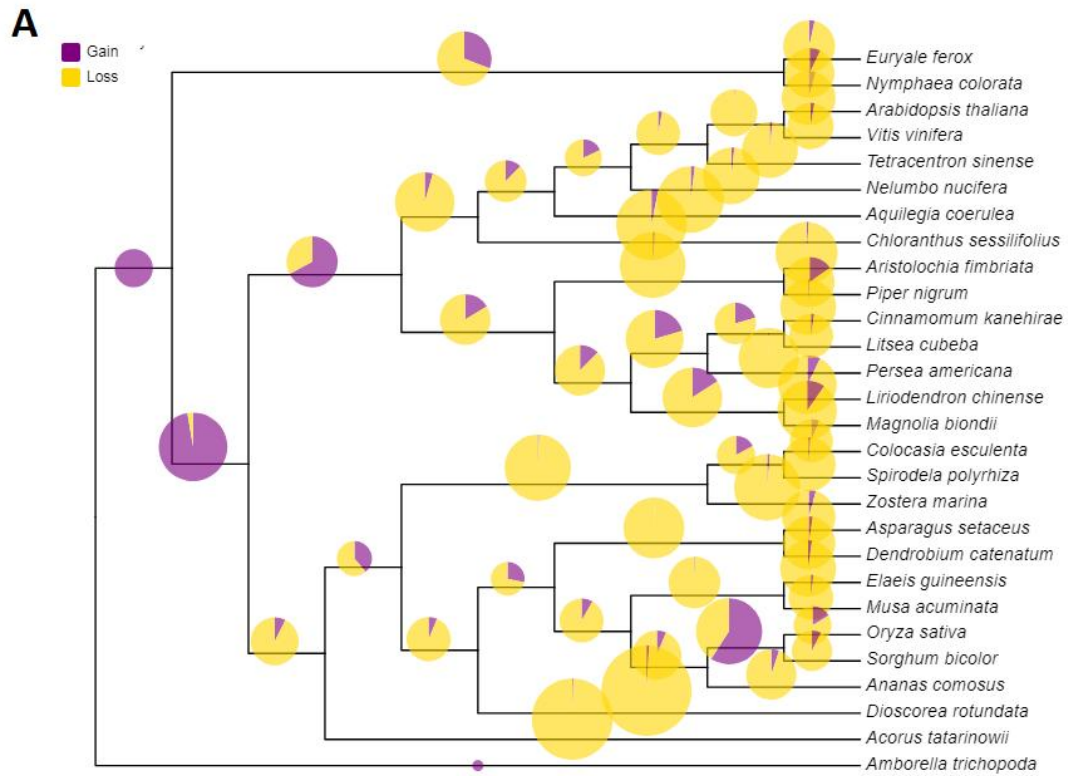


Supplementary Figure S8. No significantly negative correlation (calculated by Pearson's correlation) between the number of syntenic genes in *Amborella* (A)/ *Nymphaea* (B)/ *Aristolochia* (C)/ *Cinnamomum* (D) and *Nelumbo* (E) genome size. The error bands represent 95% confidence intervals based on a binomial model.

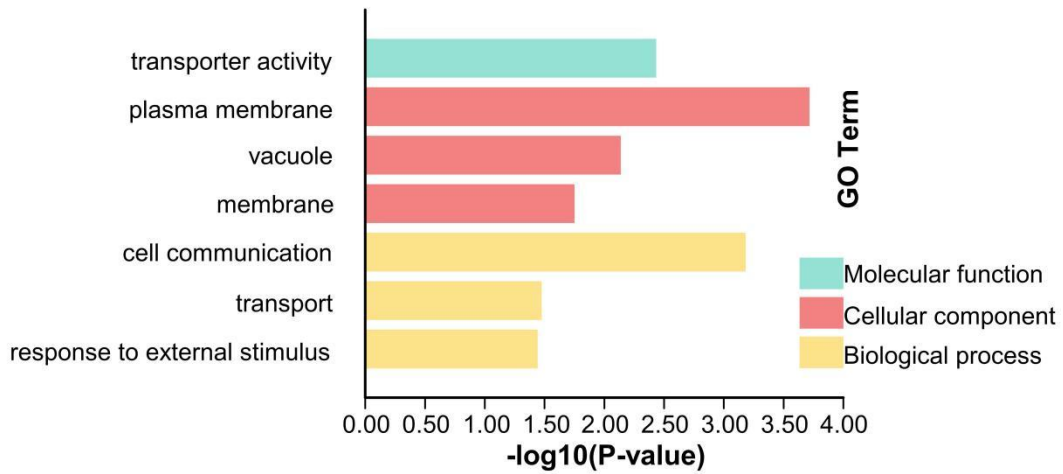


Supplementary Figure S9. Comparison of the structures of the current n=5 AMK to the n=6 AMK from Murat et al. (2017) and n=7 AMK from Xu et al. (2021). The

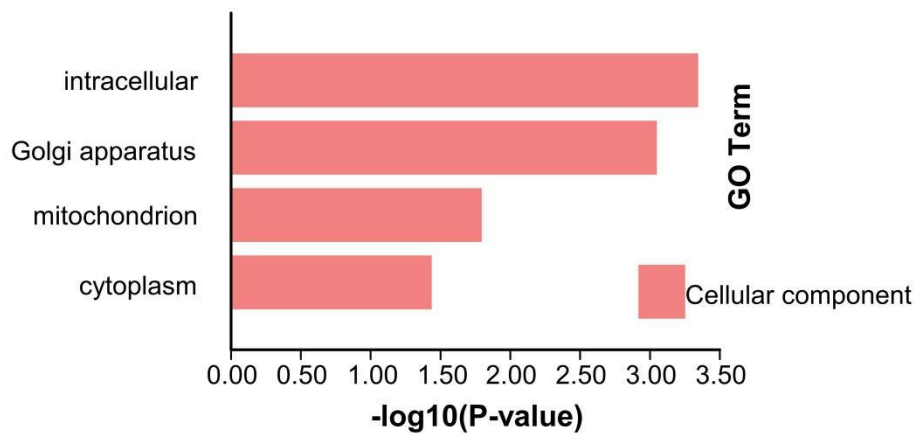
chromosome-to-chromosome relationships between the current n=6 AMK (Shi et al. 2022), n=5 AMK from Murat et al. (2017) and n=7 AMK from Xu et al. (2021), with the orthologous chromosomes highlighted in red for the n=7 AMK from Xu et al. (2021), defining the following chromosome correspondence of 1-1-5, 3-3-4, 5-5-6, 2-2-(2-6), 4-4-(3-4), 6-(1-5)-(5-7), respectively in the current n=6 AMK, n=5 AMK from Murat et al. (2017) and n=7 AMK from Xu et al. (2021). Dotplot-based comparison of the chromosomal relationship between the current n=5 AMK and n=6 AMK from Murat et al. (2017), in x-axis, and the n=7 AMK from Xu et al. (2021), in y-axis illustrating the previous chromosome synteny relationships between the proposed ancestors.



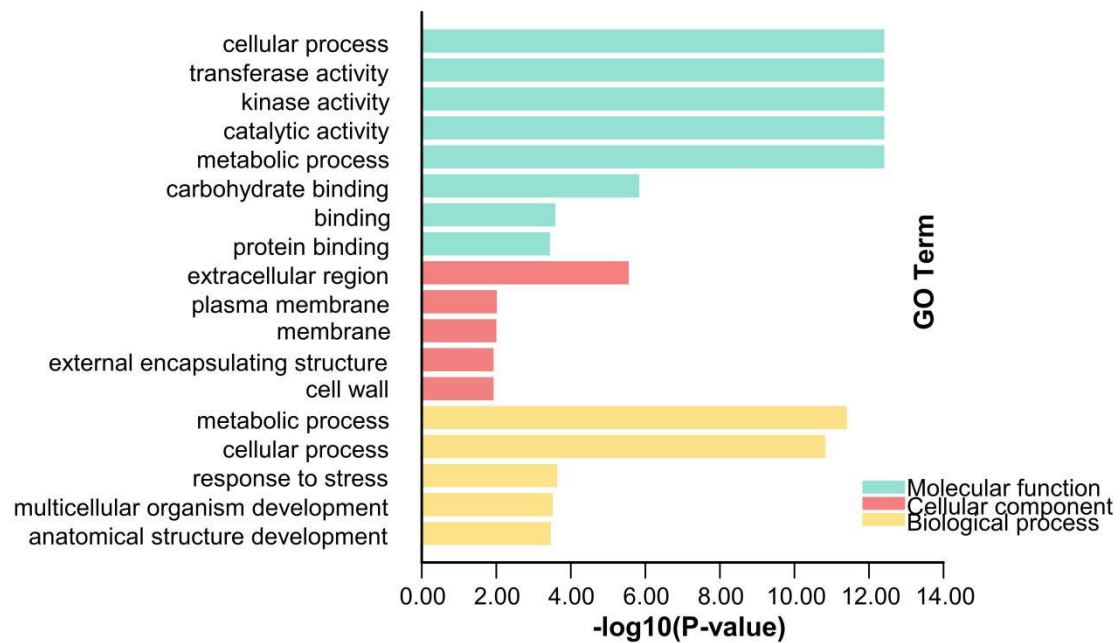
Supplementary Figure S10. Ortholog group (OG) evolution of representative plants. A. OG gain and loss based on Dollo parsimony. B. Significant expansion and contraction of OGs by CAFÉ analysis. Only OGs with at least two members are analyzed.



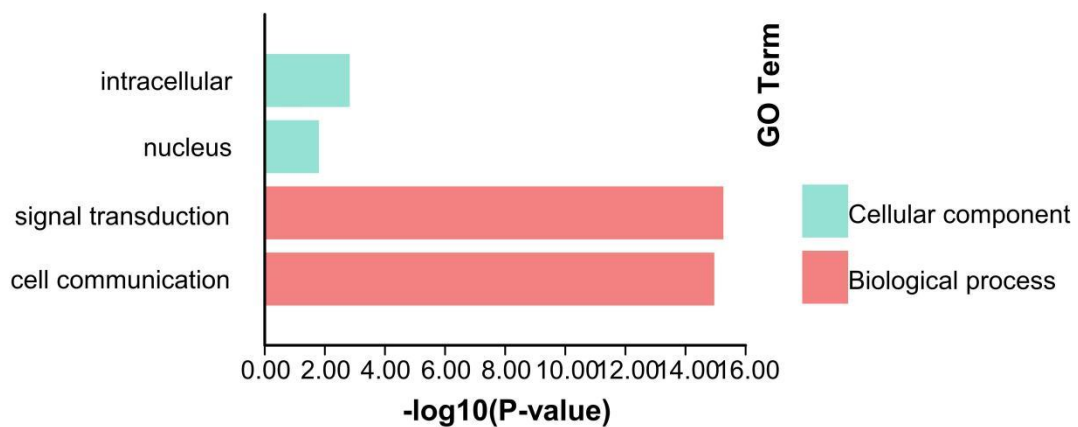
Supplementary Figure S11. Significantly enriched Gene Ontology (GO) terms for OGs gained in the ancestral monocot. P-values were obtained using an one-sided hypergeometric test.



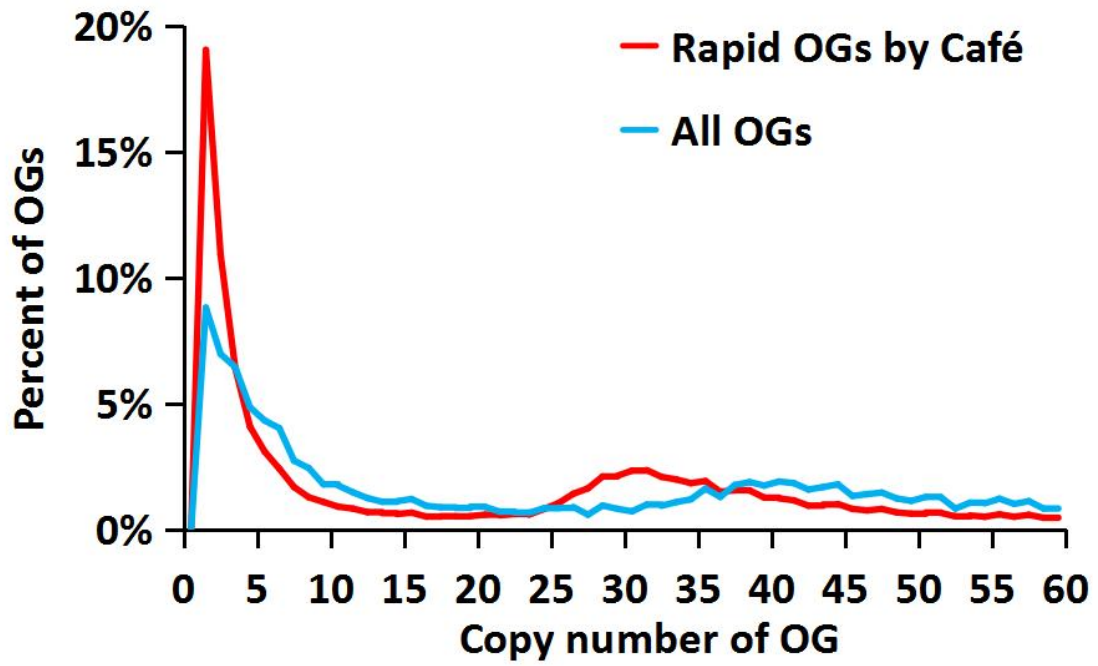
Supplementary Figure S12. Significantly enriched Gene Ontology (GO) terms for OGs lost in the ancestral monocot. P-values were obtained using an one-sided hypergeometric test.



Supplementary Figure S13. Significantly enriched Gene Ontology (GO) terms for OGs expanded in ancestral monocot by CAFÉ analysis. P-values were obtained using an one-sided hypergeometric test.



Supplementary Figure S14. Significantly enriched Gene Ontology (GO) terms for OGs contracted in ancestral monocot by CAFÉ analysis. P-values were obtained using an one-sided hypergeometric test.



Supplementary Figure S15. Density distribution of all ortholog groups (OGs) and rapidly evolving OGs by CAFÉ analysis according to an incremental OG size.