

Figure S1. LSC/IR junction of one leafy and five leafless Neottiae species. The horizontal black and light blue lines represent the LSC/IR_B boundary. The vertical red line represents the boundary shared by autotrophs, whereas the dotted lines show the expansion or contraction of IR boundaries of Neottiae.

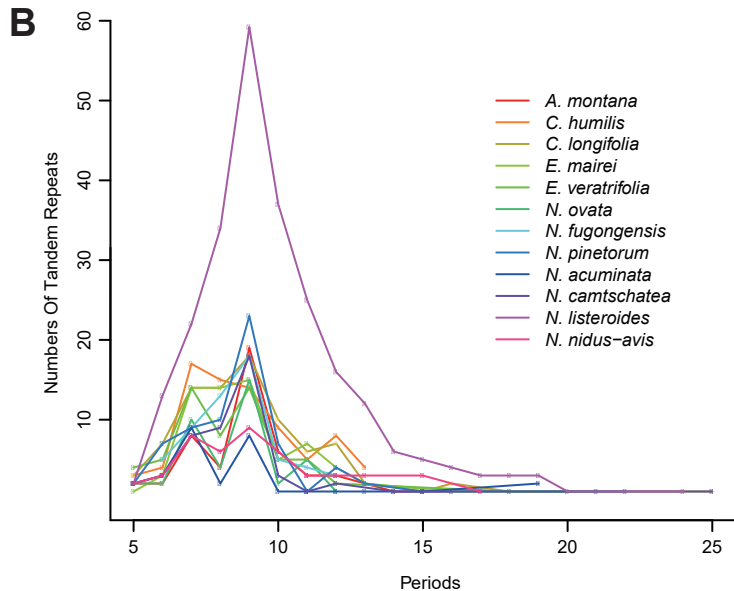
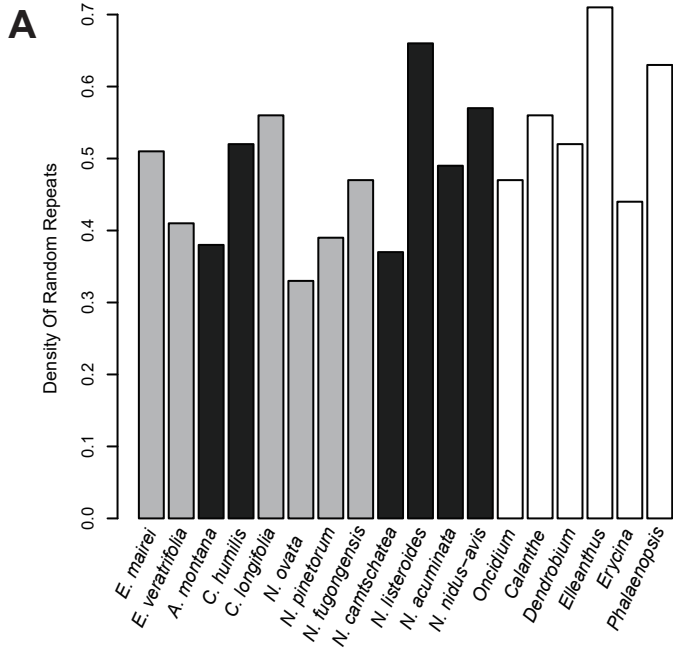


Figure S2. Dispersed and tandem repeats in Neottieae. A. The density of random repeats in Neottieae. Leafy species are shown in gray, leafless species in black, and outgroups in white. B. Tandem repeats in Neottieae.

matK

<i>Erycina</i>	ATGTATTTCAATAGCAGAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>Calanthe</i>	ATGTATTTAAATGGCAGAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>Elleanthus</i>	ATGTATGTAAATGGCATAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>A. montana</i>	ATATATGTAAATGCCAGAATTACAAGGGTATTTAGATTGAAAAAAGATAGA
<i>C. humilis</i>	ATCTATGTAAATGGCAGAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>C. longifolia</i>	ATCTATGTAAATGGCAGAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>E. mairei</i>	ATCTATGTAAATGGTAGAATTACAAGGATATTTAGATTGAAAAAAGATATA
<i>E. veratrifolia</i>	ATCTATGTAAATGGCAGAATTACAAGGATATTTAGATTGAAAAAAGATAGA
<i>N. ovata</i>	ATCTATGTAAATTGCAGAATTACAAGGAAATTTAGATTGAAAAAAGATAGA
<i>N. pinetorum</i>	ATATATGTAAATTGTAGAATTACAAGGAAATTTTCGATTGAAAAAAGATAGA
<i>N. fugongensis</i>	ATATATGTAAATTGTAGAATTACAAGGAAATTTTGATTGAAAAAAGAGAGA
<i>N. listeroides</i>	ATATATGTAAATTGTAAAATTACAAGGAAATTTTGATTGAAAAAAGAGAGA
<i>N. camtschatea</i>	ATATATTTAAATTGTATAATTACAAGGAAATTTTGATTGAAAAAAGAGAGA
<i>N. acuminata</i>	ATATATGTAAATTGTATAATTAAGGAAATTTTGATTGAAAAAAGAGAGA
<i>N. nidus-avis</i>	ATATATGTAAATTGTAGAATCACAAGGAAATTTTGATTGAAAAAAGATATA

Figure S3. Alternative start codons of the *matK* gene in Neottieae. The 5'-end of the *matK* gene, beginning with the known start codon (in green) is shown for three autotrophic orchids and 12 Neottieae. Red highlights a stop codon, which is followed by another, alternative start codon (green).

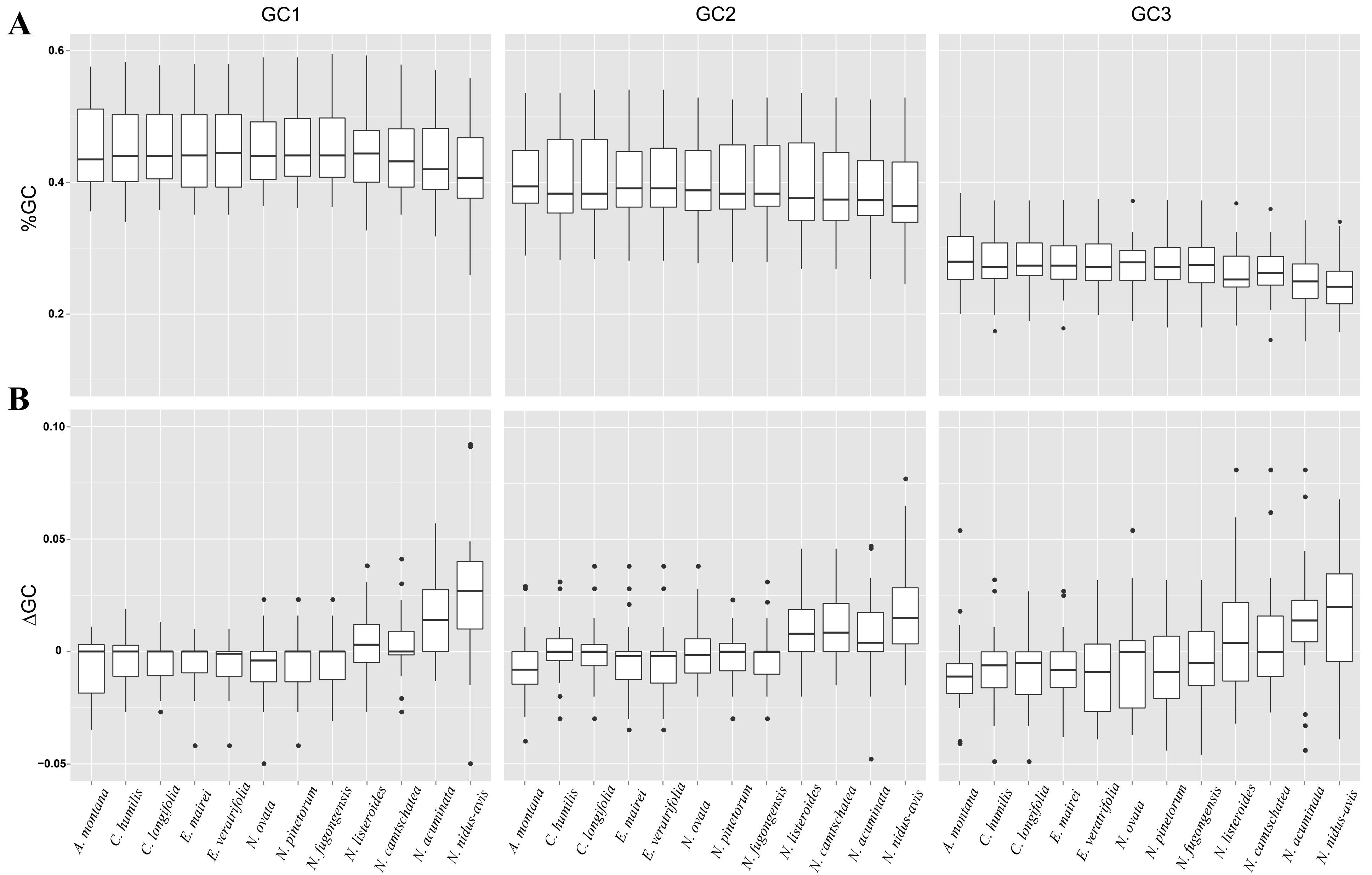


Figure S4. GC content at all codon positions in 27 housekeeping genes. **A.** GC content. **B.** The difference of GC content relative to reference genome *Calanthe*. The line inside each box represents the median, the whisker ends show the 5th and 95th percentiles. Dots mark outliers.

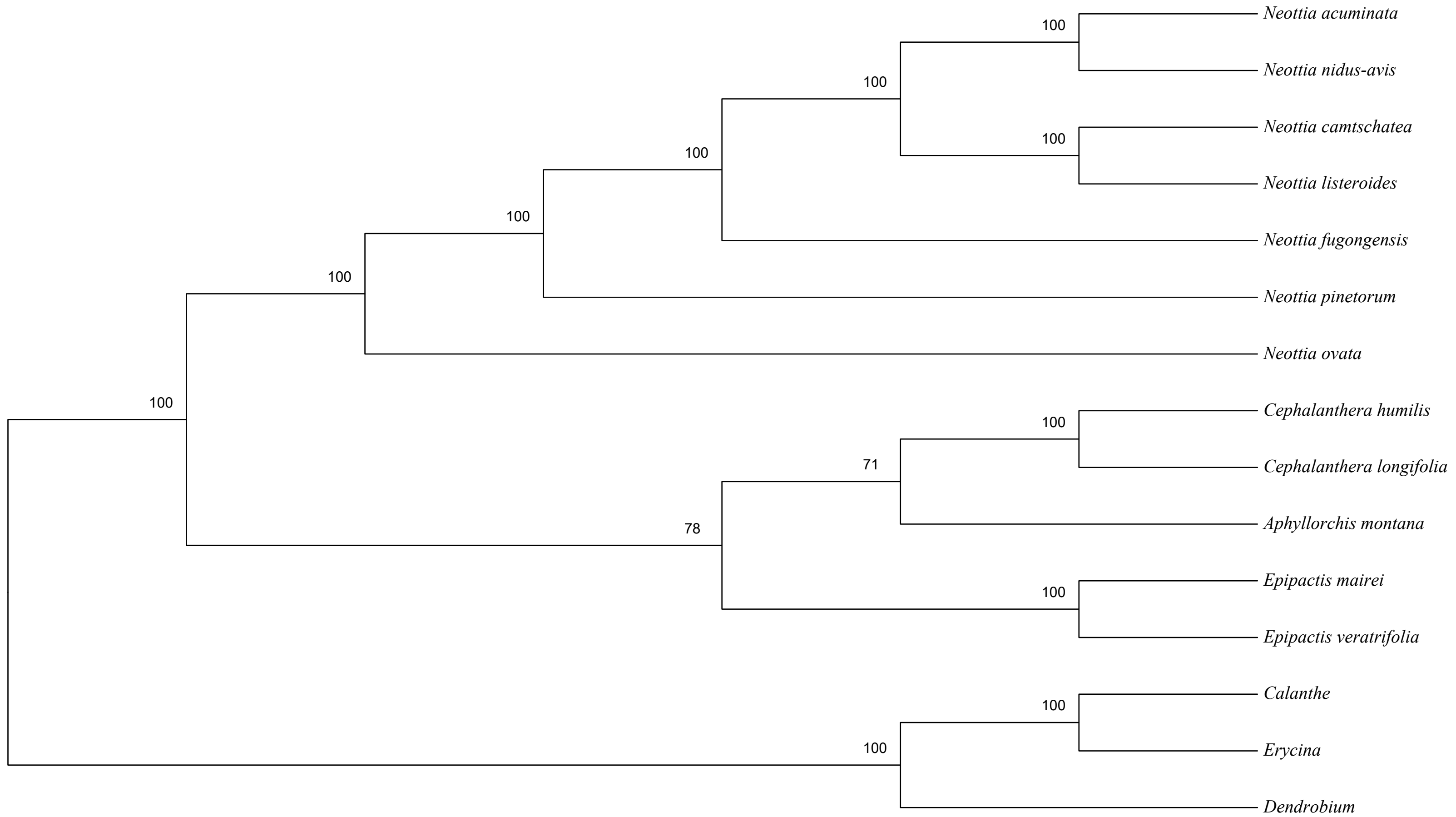


Figure S5. Phylogenetic tree based on Maximum Parsimony analyses of chloroplast. Number above branch, BP support.

0.9

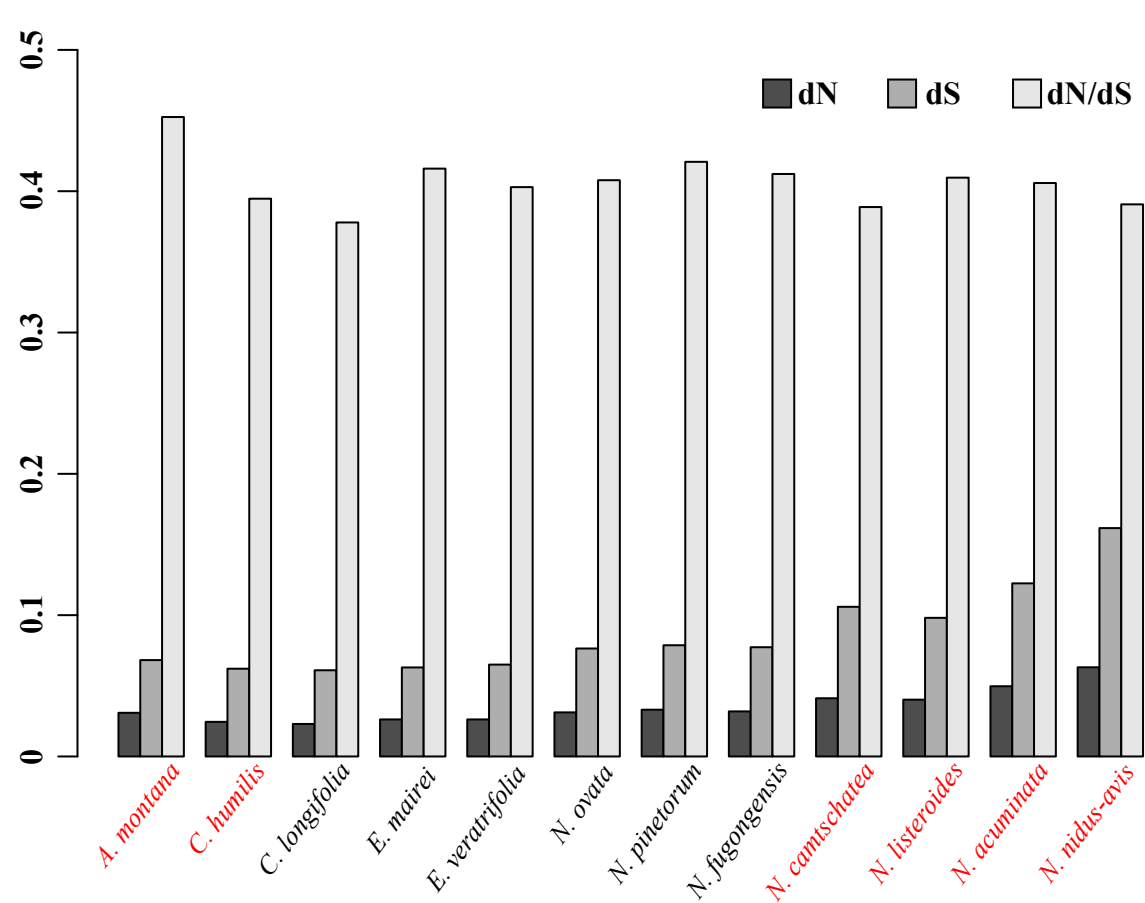


Figure S6. Synonymous and nonsynonymous substitution rates of concatenated housekeeping genes.

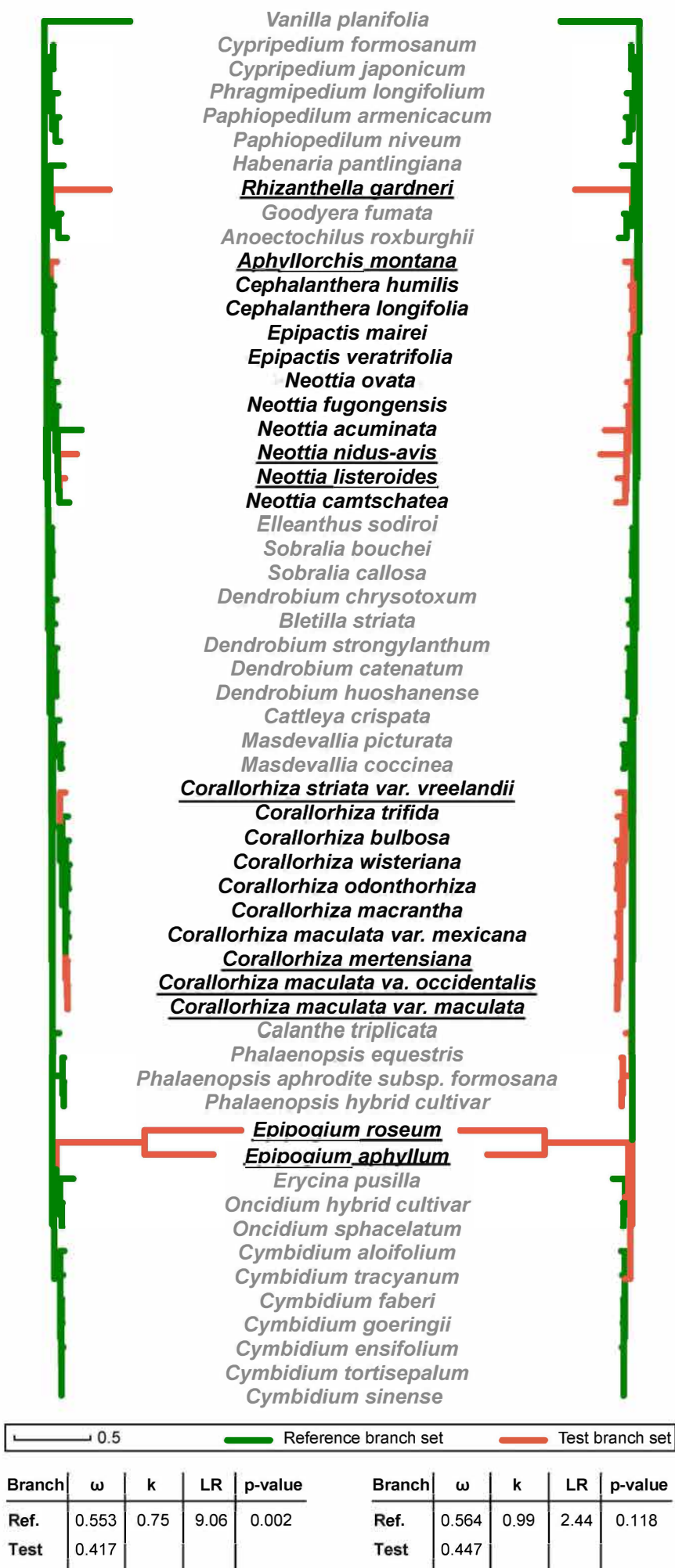


Figure S7. Summary of the RELAX selection tests in green and nongreen orchids. Phylo-genetic trees to the left and right illustrate the test branch sets (achlorophyllous mycoheterotrophs – left; chlorophyllous and achlorophyllous mycoheterotrophs – right) in relation to the references (chlorophyllous taxa – left; autotrophs – right). Autotrophic species are shown in gray, chlorophyllous mycoheterotrophs in black, and achlorophyllous mycoheterotrophs are underlined. The results of the RELAX selection tests are summarized at the bottom. ω – mean omega per branch set, k – selection intensity parameter after Wertheim *et al.*, 2015 LR – likelihood ratio.