**Supplemental materials**

**Table S1.** Base composition and proportion of the four *Ilex* plastid genomes

*Ilex latifolia*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **CDS** | **tRNA** | **rRNA** | **A %** | **T (U) %** | **C (%)** | **G (%)** | **G + C (%)** |
| LSC | 64 | 23 |  | 31.5 | 32.8 | 18.3 | 17.4 | 35.7 |
| SSC | 12 | 1 |  | 34.0 | 34.0 | 16.7 | 15.3 | 31.9 |
| IRs | 19 | 16 | 8 | 28.7 | 28.4 | 20.7 | 22.3 | 43 |
| Total | 95 | 38 | 8 | 30.9 | 31.5 | 19.2 | 18.5 | 37.7 |

*Ilex suaveolens*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **CDS** | **tRNA** | **rRNA** | **A %** | **T (U) %** | **C (%)** | **G (%)** | **G + C (%)** |
| LSC | 64 | 22 |  | 31.5 | 32.8 | 18.3 | 17.4 | 35.7 |
| SSC | 11 | 1 |  | 34.0 | 34.0 | 16.7 | 15.3 | 31.9 |
| IRs | 14 | 16 | 8 | 28.6 | 28.4 | 20.7 | 22.3 | 42.9 |
| Total | 89 | 37 | 8 | 30.8 | 31.5 | 19.2 | 18.5 | 37.6 |

*Ilex viridis*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **CDS** | **tRNA** | **rRNA** | **A %** | **T (U) %** | **C (%)** | **G (%)** | **G + C (%)** |
| LSC | 64 | 22 |  | 31.5 | 32.7 | 18.3 | 17.4 | 35.7 |
| SSC | 11 | 1 |  | 34.0 | 34.1 | 16.6 | 15.3 | 31.9 |
| IRs | 14 | 16 | 8 | 28.6 | 28.4 | 20.7 | 22.3 | 43.0 |
| Total | 89 | 37 | 8 | 30.8 | 31.5 | 19.2 | 18.5 | 37.7 |

*Ilex micrococca*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Region** | **CDS** | **tRNA** | **rRNA** | **A %** | **T (U) %** | **C (%)** | **G (%)** | **G + C (%)** |
| LSC | 64 | 22 |  | 31.5 | 32.8 | 18.3 | 17.4 | 35.7 |
| SSC | 11 | 1 |  | 33.9 | 34.2 | 16.7 | 15.2 | 31.9 |
| IRs | 14 | 16 | 8 | 28.6 | 28.4 | 20.7 | 22.3 | 43.0 |
| Total | 89 | 37 | 8 | 30.8 | 31.5 | 19.2 | 18.5 | 37.6 |

**Table S2.** Statistical analysis on codon usage, RSCU values, and codon-anticodon recognition patterns of four *Ilex* plastid genomes.

*Ilex latifolia*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** | **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** |
| Phe | UUU | 958 | 1.27 |  | Tyr | UAU | 806 | 1.61 |  |
|  | UUC | 556 | 0.73 |  |  | UAC | 197 | 0.39 | trnY-GUA |
| Leu | UUA | 878 | 1.83 |  | TER | UAA | 45 | 1.42 |  |
|  | UUG | 590 | 1.23 | trnL-CAA |  | UAG | 28 | 0.88 |  |
|  | CUU | 617 | 1.29 |  | His | CAU | 498 | 1.54 |  |
|  | CUC | 205 | 0.43 |  |  | CAC | 147 | 0.46 | trnH-GUG |
|  | CUA | 400 | 0.83 |  | Gln | CAA | 730 | 1.51 | trnQ-UUG |
|  | CUG | 190 | 0.40 |  |  | CAG | 240 | 0.49 |  |
| Ile | AUU | 1113 | 1.45 |  | Asn | AAU | 1026 | 1.55 |  |
|  | AUC | 471 | 0.61 |  |  | AAC | 301 | 0.45 | trnN-GUU |
|  | AUA | 718 | 0.94 |  | Lys | AAA | 1046 | 1.48 | trnK-UUU |
| Met | AUG | 647 | 1.00 |  |  | AAG | 365 | 0.52 |  |
| Val | GUU | 514 | 1.41 |  | Asp | GAU | 911 | 1.62 |  |
|  | GUC | 208 | 0.57 | trnV-GAC |  | GAC | 214 | 0.38 | trnD-GUC |
|  | GUA | 534 | 1.46 |  | Glu | GAA | 1044 | 1.50 | trnE-UUC |
|  | GUG | 207 | 0.57 |  |  | GAG | 350 | 0.50 |  |
| Ser | UCU | 619 | 1.74 |  | Cys | UGU | 235 | 1.52 |  |
|  | UCC | 345 | 0.97 | trnS-GGA |  | UGC | 74 | 0.48 |  |
|  | UCA | 437 | 1.23 |  | TER | UGA | 22 | 0.69 |  |
|  | UCG | 190 | 0.53 |  | Trp | UGG | 477 | 1.00 |  |
| Pro | CCU | 444 | 1.54 |  | Arg | CGU | 348 | 1.28 | trnR-ACG |
|  | CCC | 217 | 0.75 |  |  | CGC | 107 | 0.39 |  |
|  | CCA | 329 | 1.14 | trnP-UGG |  | CGA | 372 | 1.37 |  |
|  | CCG | 161 | 0.56 |  |  | CGG | 139 | 0.51 |  |
| Thr | ACU | 569 | 1.64 |  | Ser | AGU | 430 | 1.21 |  |
|  | ACC | 250 | 0.72 | trnT-GGU |  | AGC | 116 | 0.33 | trnS-GCU |
|  | ACA | 431 | 1.24 | trnT-UGU | Arg | AGA | 504 | 1.86 | trnR-UCU |
|  | ACG | 137 | 0.40 |  |  | AGG | 159 | 0.59 |  |
| Ala | GCU | 642 | 1.82 |  | Gly | GGU | 587 | 1.27 |  |
|  | GCC | 215 | 0.61 |  |  | GGC | 176 | 0.38 | trnG-GCC |
|  | GCA | 427 | 1.21 | trnA-UGC |  | GGA | 764 | 1.66 | trnG-UCC |
|  | GCG | 125 | 0.35 |  |  | GGG | 319 | 0.69 |  |

(total number of universal genetic code: 27, 121)

*Ilex viridis*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** | **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** |
| Phe | UUU | 951 | 1.27 |  | Tyr | UAU | 799 | 1.61 |  |
|  | UUC | 545 | 0.73 |  |  | UAC | 194 | 0.39 | trnY-GUA |
| Leu | UUA | 867 | 1.84 |  | TER | UAA | 42 | 1.45 |  |
|  | UUG | 578 | 1.23 | trnL-CAA |  | UAG | 22 | 0.76 |  |
|  | CUU | 602 | 1.28 |  | His | CAU | 495 | 1.54 |  |
|  | CUC | 202 | 0.43 |  |  | CAC | 146 | 0.46 | trnH-GUG |
|  | CUA | 382 | 0.81 |  | Gln | CAA | 727 | 1.53 | trnQ-UUG |
|  | CUG | 192 | 0.41 |  |  | CAG | 224 | 0.47 |  |
| Ile | AUU | 1096 | 1.45 |  | Asn | AAU | 1017 | 1.54 |  |
|  | AUC | 459 | 0.61 |  |  | AAC | 301 | 0.46 | trnN-GUU |
|  | AUA | 705 | 0.94 |  | Lys | AAA | 1045 | 1.48 | trnK-UUU |
| Met | AUG | 639 | 1 |  |  | AAG | 370 | 0.52 |  |
| Val | GUU | 509 | 1.41 |  | Asp | GAU | 902 | 1.62 |  |
|  | GUC | 196 | 0.54 | trnV-GAC |  | GAC | 212 | 0.38 | trnD-GUC |
|  | GUA | 528 | 1.47 |  | Glu | GAA | 1050 | 1.51 | trnE-UUC |
|  | GUG | 208 | 0.58 |  |  | GAG | 337 | 0.49 |  |
| Ser | UCU | 602 | 1.73 |  | Cys | UGU | 231 | 1.56 |  |
|  | UCC | 336 | 0.97 | trnS-GGA |  | UGC | 66 | 0.44 |  |
|  | UCA | 427 | 1.23 |  | TER | UGA | 23 | 0.79 |  |
|  | UCG | 186 | 0.54 |  | Trp | UGG | 477 | 1 |  |
| Pro | CCU | 435 | 1.56 |  | Arg | CGU | 344 | 1.28 | trnR-ACG |
|  | CCC | 206 | 0.74 |  |  | CGC | 106 | 0.4 |  |
|  | CCA | 318 | 1.14 | trnP-UGG |  | CGA | 372 | 1.39 |  |
|  | CCG | 157 | 0.56 |  |  | CGG | 137 | 0.51 |  |
| Thr | ACU | 559 | 1.64 |  | Ser | AGU | 423 | 1.22 |  |
|  | ACC | 238 | 0.7 | trnT-GGU |  | AGC | 110 | 0.32 | trnS-GCU |
|  | ACA | 431 | 1.26 | trnT-UGU | Arg | AGA | 494 | 1.84 | trnR-UCU |
|  | ACG | 139 | 0.41 |  |  | AGG | 157 | 0.59 |  |
| Ala | GCU | 636 | 1.81 |  | Gly | GGU | 581 | 1.28 |  |
|  | GCC | 214 | 0.61 |  |  | GGC | 179 | 0.4 | trnG-GCC |
|  | GCA | 421 | 1.2 | trnA-UGC |  | GGA | 744 | 1.64 | trnG-UCC |
|  | GCG | 131 | 0.37 |  |  | GGG | 307 | 0.68 |  |

(total number of universal genetic code: 26, 729 )

*Ilex micrococca* (total number of universal genetic code: 26, 743)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** | **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** |
| Phe | UUU | 960 | 1.28 |  | Tyr | UAU | 798 | 1.61 |  |
|  | UUC | 542 | 0.72 |  |  | UAC | 194 | 0.39 | trnY-GUA |
| Leu | UUA | 863 | 1.84 |  | TER | UAA | 42 | 1.45 |  |
|  | UUG | 578 | 1.23 | trnL-CAA |  | UAG | 23 | 0.79 |  |
|  | CUU | 598 | 1.27 |  | His | CAU | 494 | 1.54 |  |
|  | CUC | 202 | 0.43 |  |  | CAC | 146 | 0.46 | trnH-GUG |
|  | CUA | 384 | 0.82 |  | Gln | CAA | 729 | 1.53 | trnQ-UUG |
|  | CUG | 192 | 0.41 |  |  | CAG | 223 | 0.47 |  |
| Ile | AUU | 1102 | 1.46 |  | Asn | AAU | 1014 | 1.54 |  |
|  | AUC | 451 | 0.6 |  |  | AAC | 303 | 0.46 | trnN-GUU |
|  | AUA | 710 | 0.94 |  | Lys | AAA | 1042 | 1.47 | trnK-UUU |
| Met | AUG | 640 | 1 |  |  | AAG | 371 | 0.53 |  |
| Val | GUU | 512 | 1.42 |  | Asp | GAU | 901 | 1.62 |  |
|  | GUC | 198 | 0.55 | trnV-GAC |  | GAC | 210 | 0.38 | trnD-GUC |
|  | GUA | 526 | 1.46 |  | Glu | GAA | 1052 | 1.52 | trnE-UUC |
|  | GUG | 209 | 0.58 |  |  | GAG | 335 | 0.48 |  |
| Ser | UCU | 608 | 1.75 |  | Cys | UGU | 233 | 1.56 |  |
|  | UCC | 333 | 0.96 | trnS-GGA |  | UGC | 66 | 0.44 |  |
|  | UCA | 424 | 1.22 |  | TER | UGA | 22 | 0.76 |  |
|  | UCG | 183 | 0.53 |  | Trp | UGG | 477 | 1 |  |
| Pro | CCU | 434 | 1.55 |  | Arg | CGU | 341 | 1.27 | trnR-ACG |
|  | CCC | 209 | 0.75 |  |  | CGC | 105 | 0.39 |  |
|  | CCA | 320 | 1.15 | trnP-UGG |  | CGA | 370 | 1.37 |  |
|  | CCG | 154 | 0.55 |  |  | CGG | 140 | 0.52 |  |
| Thr | ACU | 560 | 1.63 |  | Ser | AGU | 422 | 1.22 |  |
|  | ACC | 240 | 0.7 | trnT-GGU |  | AGC | 112 | 0.32 | trnS-GCU |
|  | ACA | 432 | 1.26 | trnT-UGU | Arg | AGA | 500 | 1.86 | trnR-UCU |
|  | ACG | 140 | 0.41 |  |  | AGG | 159 | 0.59 |  |
| Ala | GCU | 639 | 1.82 |  | Gly | GGU | 580 | 1.28 |  |
|  | GCC | 216 | 0.62 |  |  | GGC | 177 | 0.39 | trnG-GCC |
|  | GCA | 419 | 1.19 | trnA-UGC |  | GGA | 747 | 1.65 | trnG-UCC |
|  | GCG | 129 | 0.37 |  |  | GGG | 308 | 0.68 |  |

(total number of universal genetic code: 26, 743)

*Ilex suaveolens*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** | **Amino Acid** | **Codon** | **No.** | **RSCU** | **tRNA** |
| Phe | UUU | 954 | 1.27 |  | Tyr | UAU | 798 | 1.61 |  |
|  | UUC | 543 | 0.73 |  |  | UAC | 196 | 0.39 | trnY-GUA |
| Leu | UUA | 865 | 1.84 |  | TER | UAA | 42 | 1.45 |  |
|  | UUG | 581 | 1.23 | trnL-CAA |  | UAG | 22 | 0.76 |  |
|  | CUU | 599 | 1.27 |  | His | CAU | 494 | 1.54 |  |
|  | CUC | 202 | 0.43 |  |  | CAC | 146 | 0.46 | trnH-GUG |
|  | CUA | 383 | 0.81 |  | Gln | CAA | 729 | 1.53 | trnQ-UUG |
|  | CUG | 193 | 0.41 |  |  | CAG | 222 | 0.47 |  |
| Ile | AUU | 1100 | 1.46 |  | Asn | AAU | 1018 | 1.54 |  |
|  | AUC | 457 | 0.61 |  |  | AAC | 303 | 0.46 | trnN-GUU |
|  | AUA | 707 | 0.94 |  | Lys | AAA | 1046 | 1.48 | trnK-UUU |
| Met | AUG | 638 | 1 |  |  | AAG | 368 | 0.52 |  |
| Val | GUU | 508 | 1.41 |  | Asp | GAU | 904 | 1.62 |  |
|  | GUC | 197 | 0.55 | trnV-GAC |  | GAC | 211 | 0.38 | trnD-GUC |
|  | GUA | 527 | 1.46 |  | Glu | GAA | 1049 | 1.51 | trnE-UUC |
|  | GUG | 208 | 0.58 |  |  | GAG | 338 | 0.49 |  |
| Ser | UCU | 603 | 1.74 |  | Cys | UGU | 230 | 1.55 |  |
|  | UCC | 336 | 0.97 | trnS-GGA |  | UGC | 67 | 0.45 |  |
|  | UCA | 426 | 1.23 |  | TER | UGA | 23 | 0.79 |  |
|  | UCG | 187 | 0.54 |  | Trp | UGG | 475 | 1 |  |
| Pro | CCU | 436 | 1.57 |  | Arg | CGU | 342 | 1.27 | trnR-ACG |
|  | CCC | 205 | 0.74 |  |  | CGC | 107 | 0.4 |  |
|  | CCA | 317 | 1.14 | trnP-UGG |  | CGA | 368 | 1.37 |  |
|  | CCG | 156 | 0.56 |  |  | CGG | 139 | 0.52 |  |
| Thr | ACU | 557 | 1.63 |  | Ser | AGU | 421 | 1.21 |  |
|  | ACC | 240 | 0.7 | trnT-GGU |  | AGC | 112 | 0.32 | trnS-GCU |
|  | ACA | 430 | 1.26 | trnT-UGU | Arg | AGA | 497 | 1.85 | trnR-UCU |
|  | ACG | 140 | 0.41 |  |  | AGG | 158 | 0.59 |  |
| Ala | GCU | 636 | 1.81 |  | Gly | GGU | 579 | 1.28 |  |
|  | GCC | 215 | 0.61 |  |  | GGC | 178 | 0.39 | trnG-GCC |
|  | GCA | 421 | 1.2 | trnA-UGC |  | GGA | 747 | 1.65 | trnG-UCC |
|  | GCG | 130 | 0.37 |  |  | GGG | 309 | 0.68 |  |

(total number of universal genetic code: 26, 735)

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**Figure S1.** Analysis of SSRs in four *Ilex* plastid genomes*.* (**a**) The numbers of three different types of SSRs. (**b**) The numbers of different SSRs nucleotide sequence.

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**Figure S2.** Analysis of the Long Repeated Sequences in Four *Ilex* Plastid Genomes*.* (**a**) The numbers of different types of the long repeats sequence. (**b**) The numbers and compositions of the forward repeats. (**c**) The numbers and compositions of the reverse repeats. (**d**) The numbers and compositions of the complement repeats. (**e**) ) The numbers and compositions of the palindrome repeats.

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**Figure S3.** The phylogenetic analyses of fourteen *Ilex* species using the CDS sequences. The evolutionary tree was constructed with MEGA X by the Maximum Likelihood (ML) method and Tamura-Nei model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analyses involved 19 members in Figure 6.