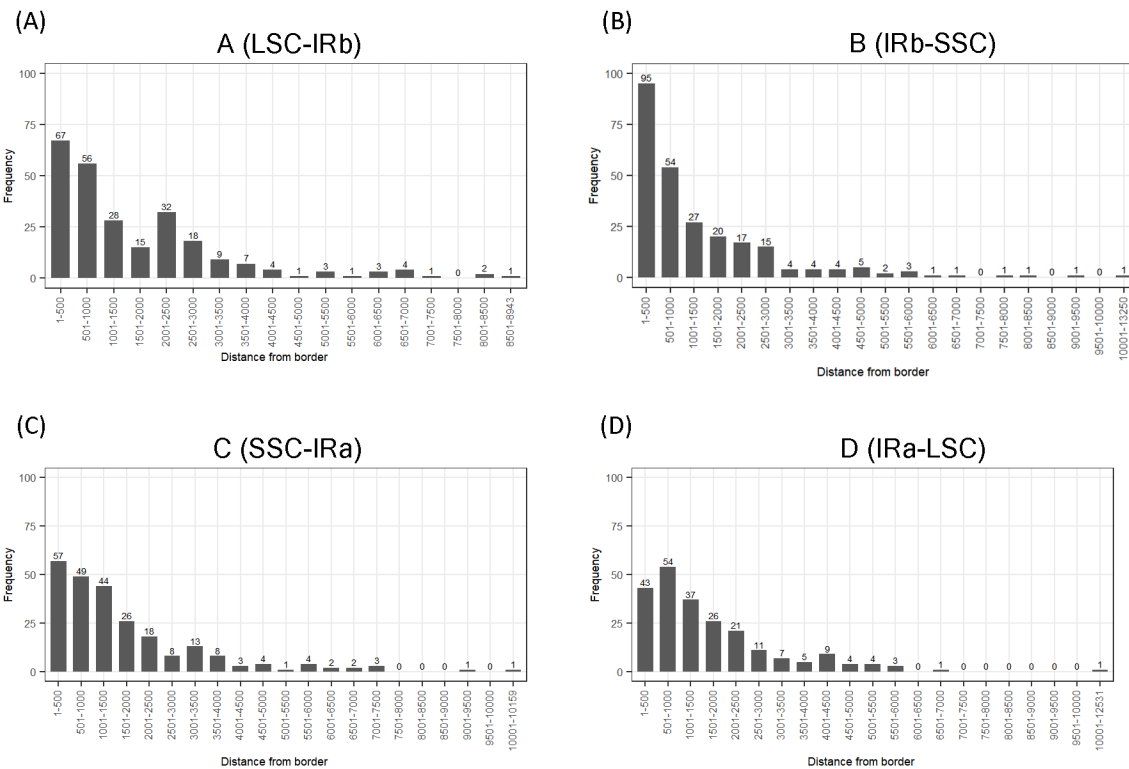




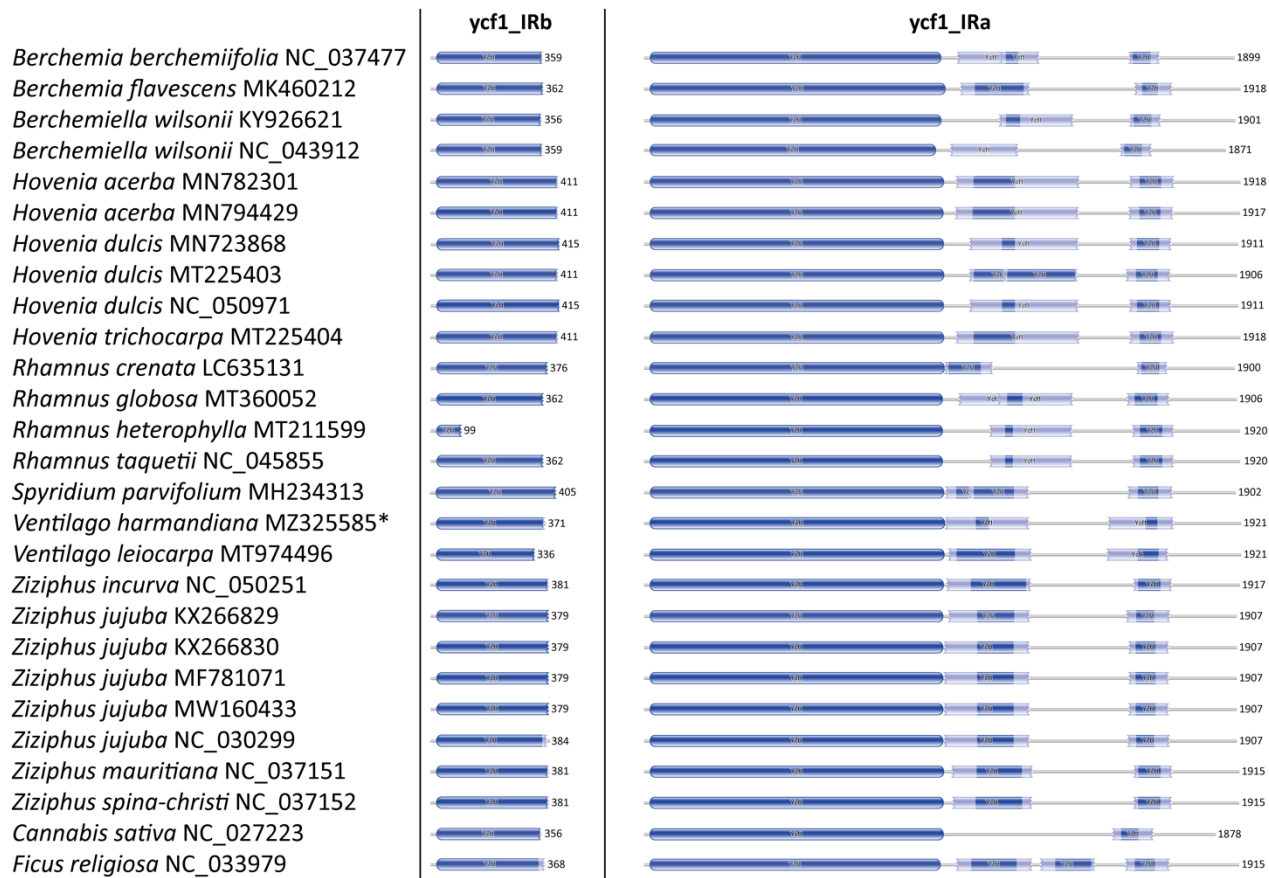
1
2 **Figure S1.** The number of spanning reads over the IR boundaries. (A) Boundary A locates between
3 LSC and IRb. (B) Boundary B locates between IRb and SSC. (C) Boundary C locates between SSC
4 and IRa. (D) Boundary D locates between IRa and LSC. The number of ONT reads spanning over the
5 four IR boundaries at different distances (nt) from the boundaries are illustrated as bar charts. Large
6 single-copy (LSC); inverted repeats (IRs) and small single-copy (SSC).

<i>Berchemia berchemiifolia</i> NC_037477	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Berchemia flavesces</i> MK460212	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Berchemiella wilsonii</i> KY926621	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Berchemiella wilsonii</i> NC_043912	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia acerba</i> MN782301	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia acerba</i> MN794429	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia dulcis</i> MN723868	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia dulcis</i> MT225403	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia dulcis</i> NC_050971	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Hovenia trichocarpa</i> MT225404	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Rhamnus crenata</i> LC635131	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Rhamnus globosa</i> MT360052	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Rhamnus heterophylla</i> MT211599	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Rhamnus taquetii</i> NC_045855	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Spyridium parvifolium</i> MH234313	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ventilago harmandiana</i> MZ325585*	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ventilago leiocarpa</i> MT974496	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus incurva</i> NC_050251	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus jujuba</i> KX266829	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus jujuba</i> KX266830	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus jujuba</i> MF781071	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus jujuba</i> MW160433	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus jujuba</i> NC_030299	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus mauritiana</i> NC_037151	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ziziphus spina-christi</i> NC_037152	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Cannabis sativa</i> NC_027223	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>
<i>Ficus religiosa</i> NC_033979	IRb revIRa	<u>rps19</u>	rpl2	rpl23	trnI-CAU	ycf2	<u>ycf15</u>	trnL-CAA	ndhB	rps7	rps12	trnV-GAC	rrn16	trnI-GAU	trnA-UGC	rrn23	rrn4.5	rrn5	trnR-ACG	trnN-GUU	<u>ycf1</u>

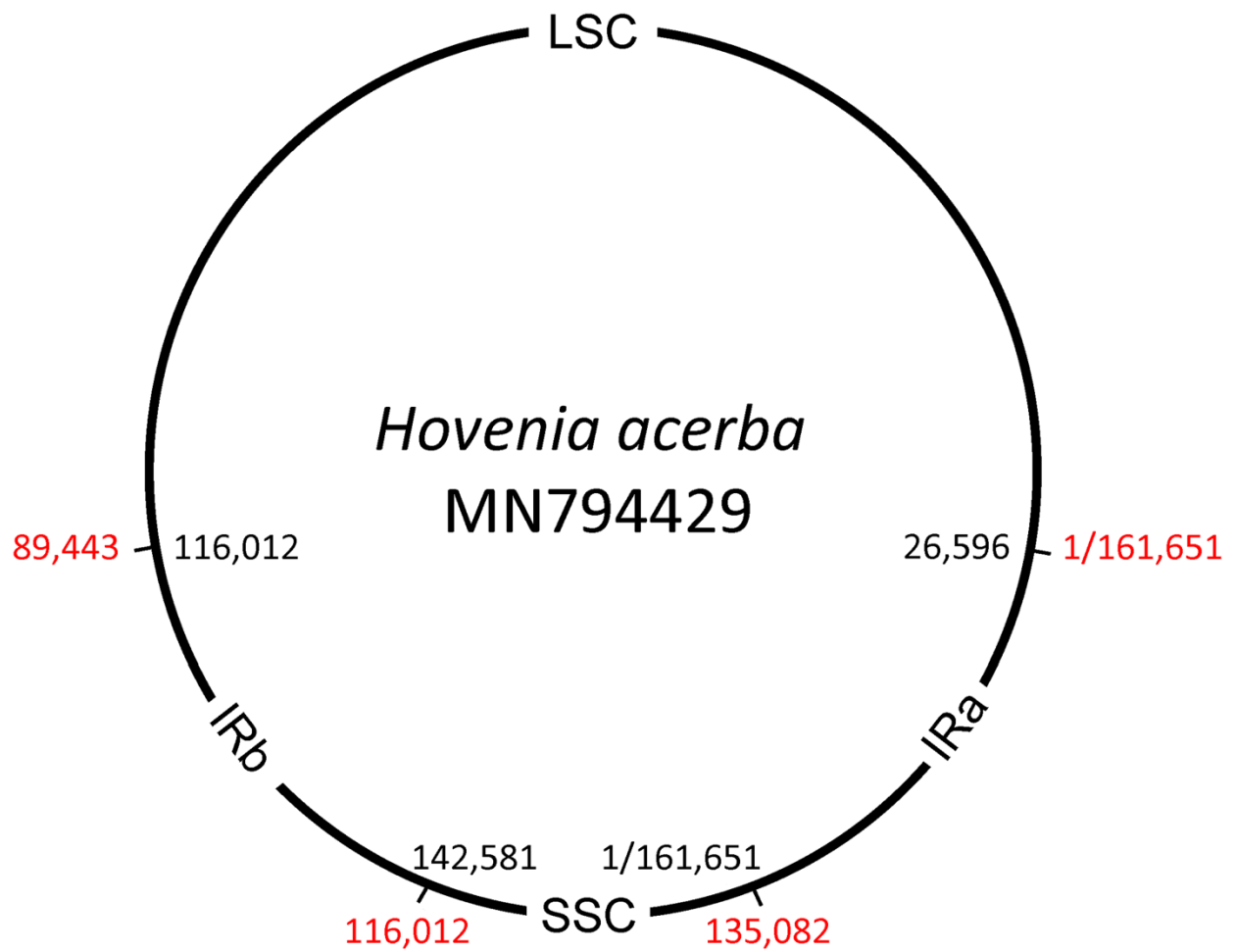
Figure S2. Comparative gene arrangement in IR regions of Rhamnaceae, *C. sativa* and *F. religios*.

Each box represents a gene. The *rps19* and *ycf1* genes (bold and underline) are located at the borders of IR regions. A missing gene that was re-annotated is highlighted in red. A gene in LSC is represented by an orange box. A gene in IR is in a blue box and a gene in both LSC and IR regions is in a white box. The *C. sativa* NC_027223 and *F. religiosa* NC_033979 are the outgroup of phylogenetic analysis. Reversed inverted repeat (revIR).

14



15 **Figure S3.** Domain analysis of YcfI protein sequences. The full-length colored bar represents the
 16 presence of an entire YcfI domain. A shorter colored bar within the grey bar indicates the matching
 17 part of the domain. A truncated domain is shown by a jagged edge. The number indicates the amino
 18 acid length. *YcfI* copy in IRb (ycf1_IRb) and *ycfI* gene in IRa (ycf1_IRa).



19
 20 **Figure S4.** A shift in the origin of *H. acerba* MN794429 chloroplast genome and correction. Suggested
 21 origin is presented in red color.

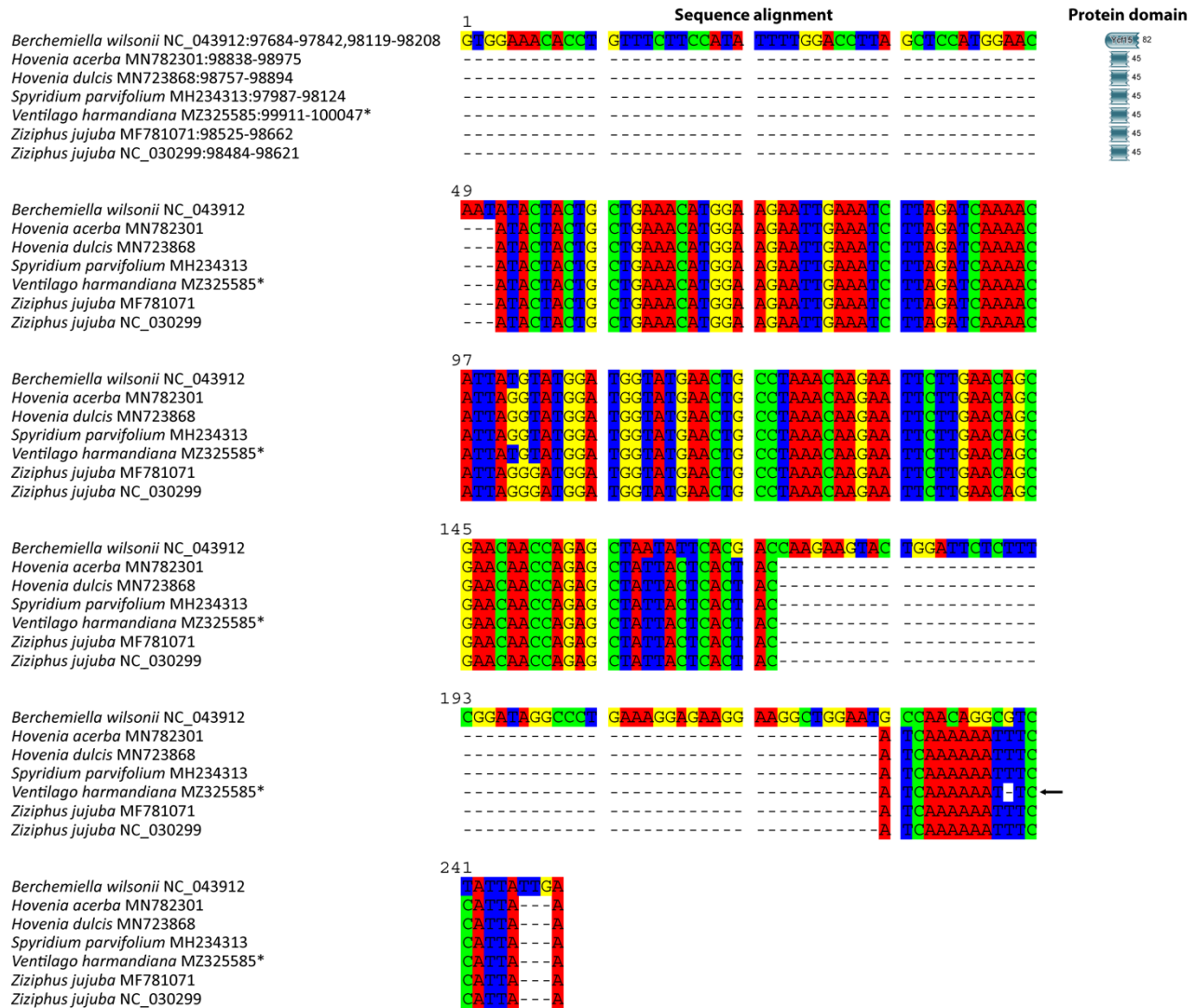


Figure S5. Multiple alignment of the *ycf15* gene sequences and matching domain fragments. This figure shows the alignment of the deposited *ycf15* DNA sequences from six Rhamnaceae organisms and the *ycf15* sequence of *V. harmandiana*. The predicted Ycf15 domain of each *ycf15* is illustrated on the right column. A missing T-base from the *ycf15* sequence of *V. harmandiana* is pointed out by an arrow. The full-length colored bar represents the presence of an entire Ycf15 domain. A truncated domain is shown by a jagged edge. The number indicates the amino acid length. Only the *ycf15* copy in IRb is shown, as its copy in IRa is identical.

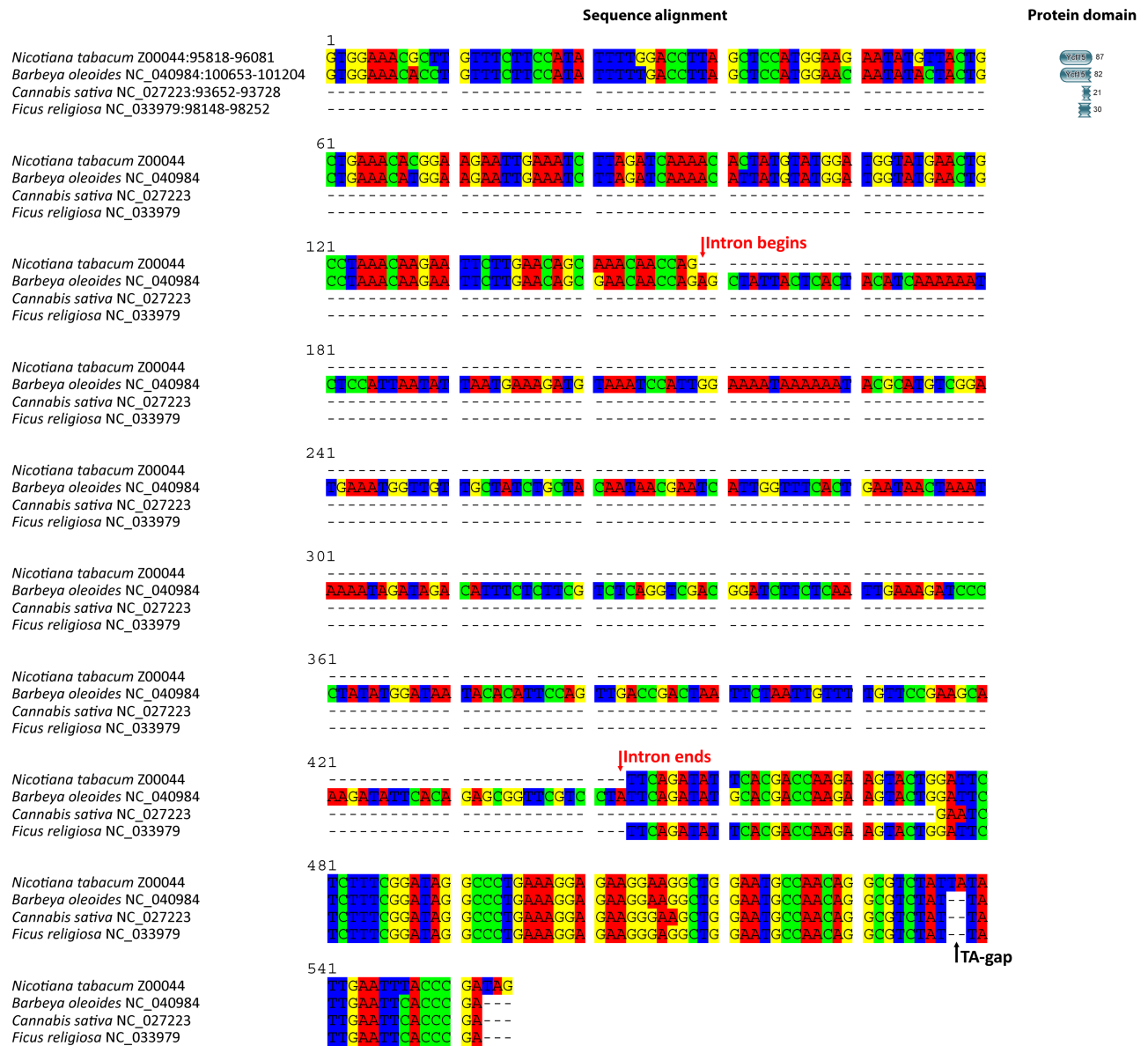


Figure S6. Re-annotation of the *ycf15* gene in *Barbeya oleoides* NC_040984 and the outgroup. The alignment of the *ycf15* gene sequences of *Barbeya oleoides*, *C. sativa* and *F. religiosa* to the *ycf15* gene sequence of *N. tabacum* Z00044 is illustrated. The TA-gap is pointed out by a black arrow. The predicted Ycf15 domain of each *ycf15* is illustrated on the right column. The full-length colored bar represents the presence of an entire Ycf15 domain. A truncated domain is shown by a jagged edge. The number indicates the amino acid length. Only the *ycf15* copy in IRb is shown, as its copy in IRa is identical.

Nicotiana tabacum Z00044:95818-96081
Berchemia berchemiifolia NC 037477:97981-98134
Berchemia flavescens NC K466212:98441-98589
Berchemiella wilsonii KY926621:97936-98094
Berchemiella wilsonii NC 043912:97864-97837
Hovenia acerba MN782301:98787-98980
Hovenia acerba MN794429:125348-125501
Hovenia dulcis MN723668:98706-98859
Hovenia dulcis MT225403:98788-98941
Hovenia dulcis NC 050971:100165-100318
Hovenia trichocarpa MT225404:98789-98942
Rhamnus crenata LC635131:98082-98235
Rhamnus globosa MT360055:98175-98328
Rhamnus heterophylla MT211599:95304-95457
Rhamnus parvifolia NC 045855-98654-98807
Spyridium taquetii MLH234131:97936-98089
Ventilago harmalandiana MT225446:98585-98860-100013*
Ventilago leioleuca MT974496:93999-99552
Ziziphus incurva NC 0052051:98073-98226
Ziziphus jujuba KC266829:98227-98380
Ziziphus jujuba KC266830:98192-98345
Ziziphus jujuba MF781071:98474-98627
Ziziphus jujuba MW160433:98587-98740
Ziziphus jujuba NC 030299-98433-98586
Ziziphus mauritiana NC 037151:98468-98621
Ziziphus spina-christi NC 0373152:98548-98701

Nicotiana tabacum Z00044
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Berchemia flavescens MK0212
Berchemia wilsonii NC_026621
Berchemia wilsonii NC_043912
Hovenia acerba MN782301
Hovenia acerba MN794429
Hovenia dulcis MN723868
Hovenia dulcis NC_043912
Hovenia dulcis NC_050971
Hovenia trichocarpa MT225404
Rhamnus crenata LC635131
Rhamnus globosa MT360052
Rhamnus heterophylla MT121599
Rhamnus taquetii NC_045855
Spyridium parvifolium HM234313
Ventilago harmadoniana MT235585*
Ventilago leiocarpa MT974496
Ziziphus incurva NC_052051
Ziziphus jujuba KX266829
Ziziphus jujuba KX266830
Ziziphus jujuba MF781071
Ziziphus jujuba MW160313
Ziziphus jujuba NC_030299
Ziziphus mauritiana NC_037151
Ziziphus sapina-christi NC_037152

Nicotiana tabacum Z00044
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Berchemia flavescens MK460212
Berchemiella wilsonii KY926611
Berchemiella wilsonii NC_043912
Hovenia acerba MN782301
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Hovenia dulcis MN723868
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Rhamnus globosa MT360052
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Ventilago harmadionia MT235585*
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Ziziphus incurva NC_050251
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Ziziphus jujuba NC_030299
Ziziphus mauritiana NC_037151
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Nicotiana tabacum Z00044
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Berchemia florescens HK60212
Berchemia wilsonii KY926621
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Hovenia dulcis MN723868
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Rhamnus globosa MT360052
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Spyridium parvifolium MK234313
Ventilago harmadomandica MT235585*
Ventilago leiocarpa MT974496
Ziziphus incurva NC_050251
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Ziziphus jujuba NC_030299
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Ziziphus sapota-christi NC_037152

Sequence alignment

[illegible][illegible][illegible][illegible]

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Ventilogia leiodora MT974446-9928-99391
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Nacitaciona tabacoa 200044
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Nicotiana tabacum 200044
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Nacitona tabacum C00044
Berchemia berchemiifolia NK_037477
Berchemia flavescens MK466012
Berchemia utilis NK052611
Berchemiella wilsonii NK_043912
Hovenia acerba MN782301
Hovenia acerba MN794429
Hovenia dulcis MN723968
Hovenia dulcis MT225403
Hovenia dulcis NK_050971
Hovenia trichocarpa MT225404
Rhamnus crenata LC635311
Rhamnus glabrescens NK040525
Rhamnus heterophylla MT211599
Rhamnus taquetii NK_045855
Spyridium parvifolium MK1234813
Ventrigia harmadendri MT255885*
Veitrobia leiocarpa MT974496
Ziziphus incurve NK_050251
Ziziphus jujuba KX266289
Ziziphus jujuba KX266290
Ziziphus jujuba MF781071
Ziziphus jujuba MW160433
Ziziphus jujuba NK_030299
Ziziphus ururiatica NK_037151
Ziziphus spina-christi NK_037152

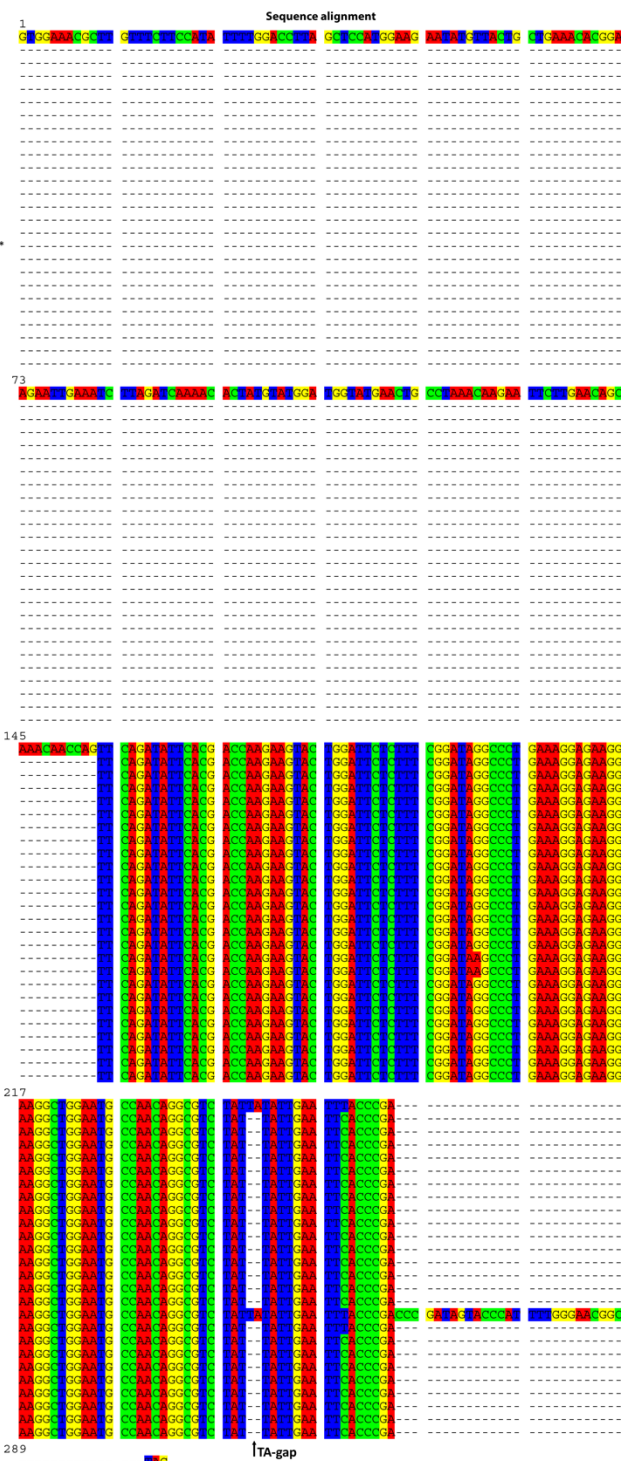


Figure S7. Re-annotation of the *ycf15* gene in Rhamnaceae. (A) The alignment of the reference gene sequence to the first exon and (B) to the second exon of the *ycf15* gene in Rhamnaceae organisms. The figure shows the multiple sequence alignment using *N. tabacum* Z00044 as a reference to re-annotate the *ycf15* gene of the organisms in this study. TA-gaps in the *ycf15* sequences of the Rhamnaceae are pointed out by a black arrow. The predicted Ycf15 domain of each *ycf15* is illustrated on the right column. The full-length colored bar represents the presence of an entire Ycf15 domain. A truncated domain is shown by a jagged edge. The number indicates the amino acid length. Only the *ycf15* copy in IRb is shown, as its copy in IRa is identical.

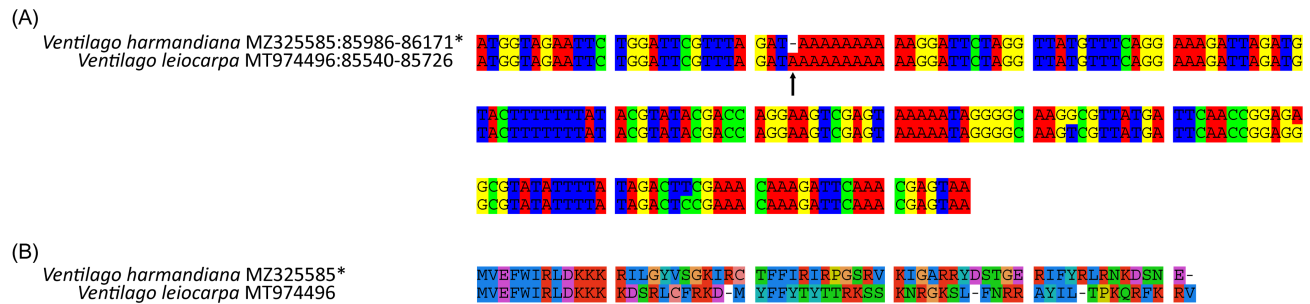


Figure S8. Re-annotation of *infA* gene in *V. leiocarpa*. (A) The alignment of *infA* DNA sequences between *V. harmandiana* and *V. leiocarpa*. A-gap is pointed out by an arrow. (B) Comparing amino acid sequences of the *infA* between *V. harmandiana* and *V. leiocarpa*. A dashed line (-) indicates a stop codon.

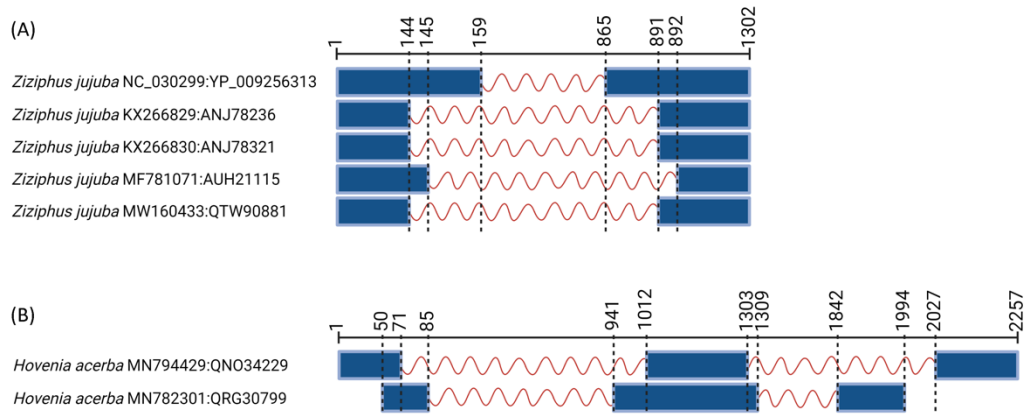
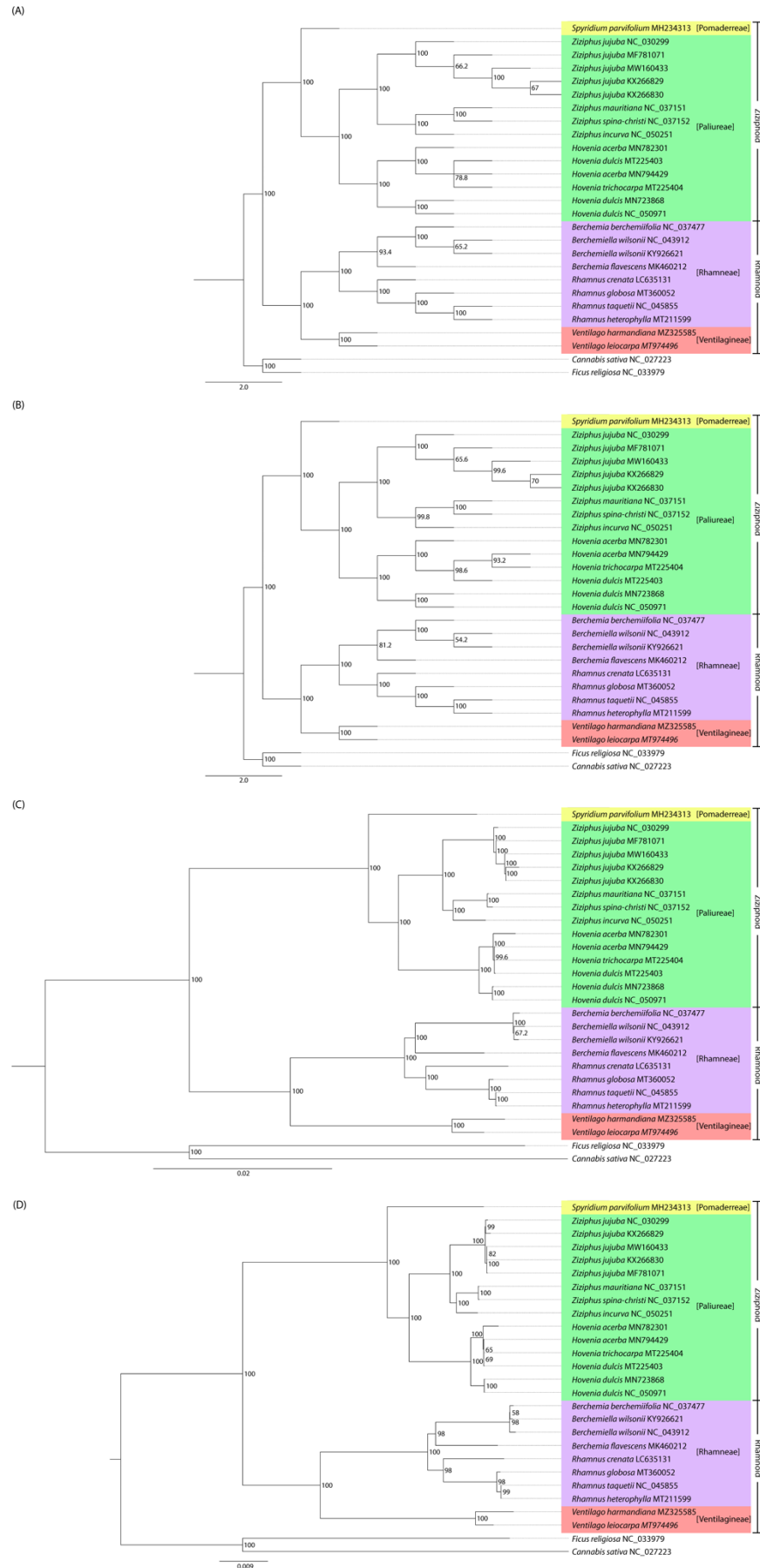
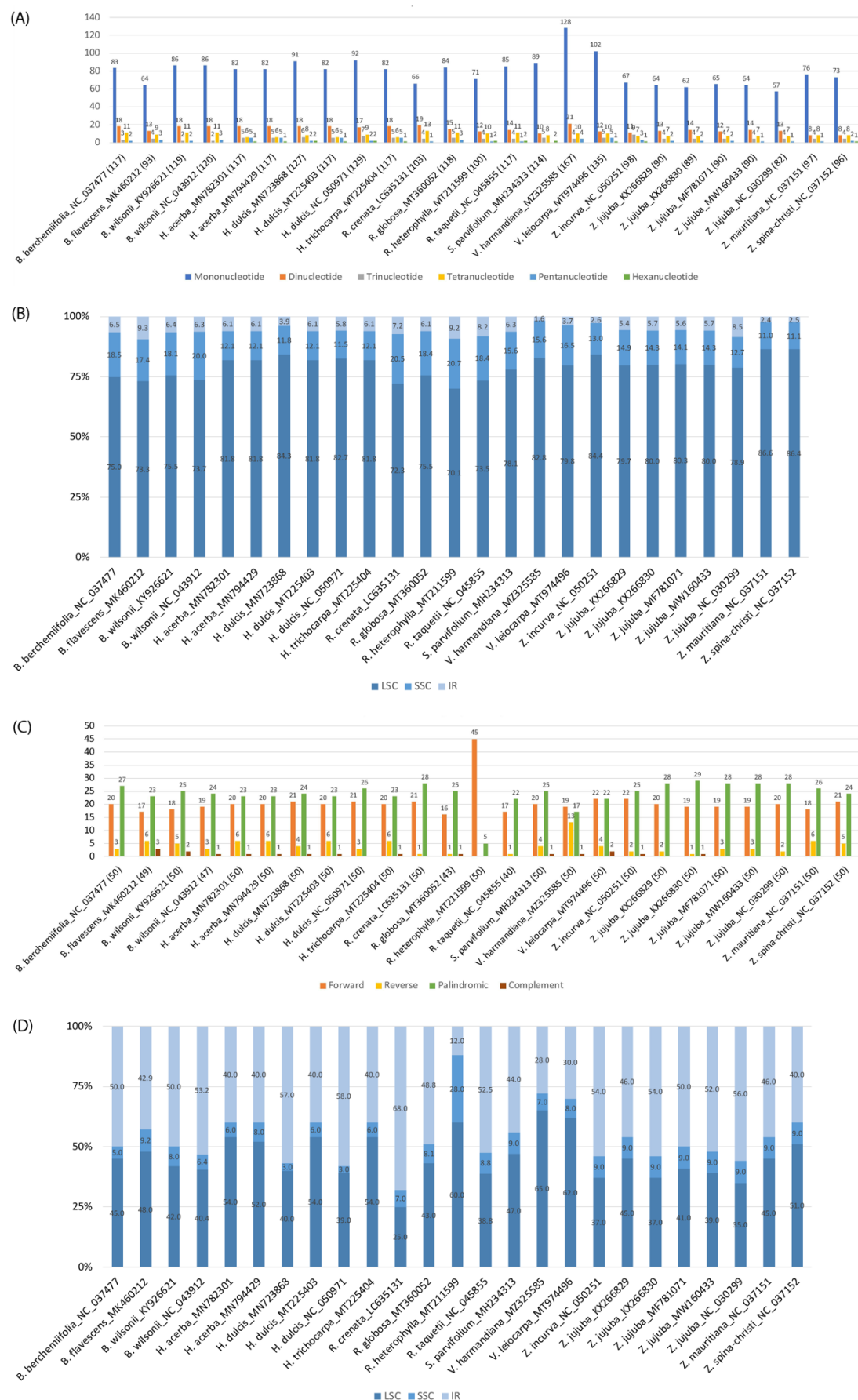


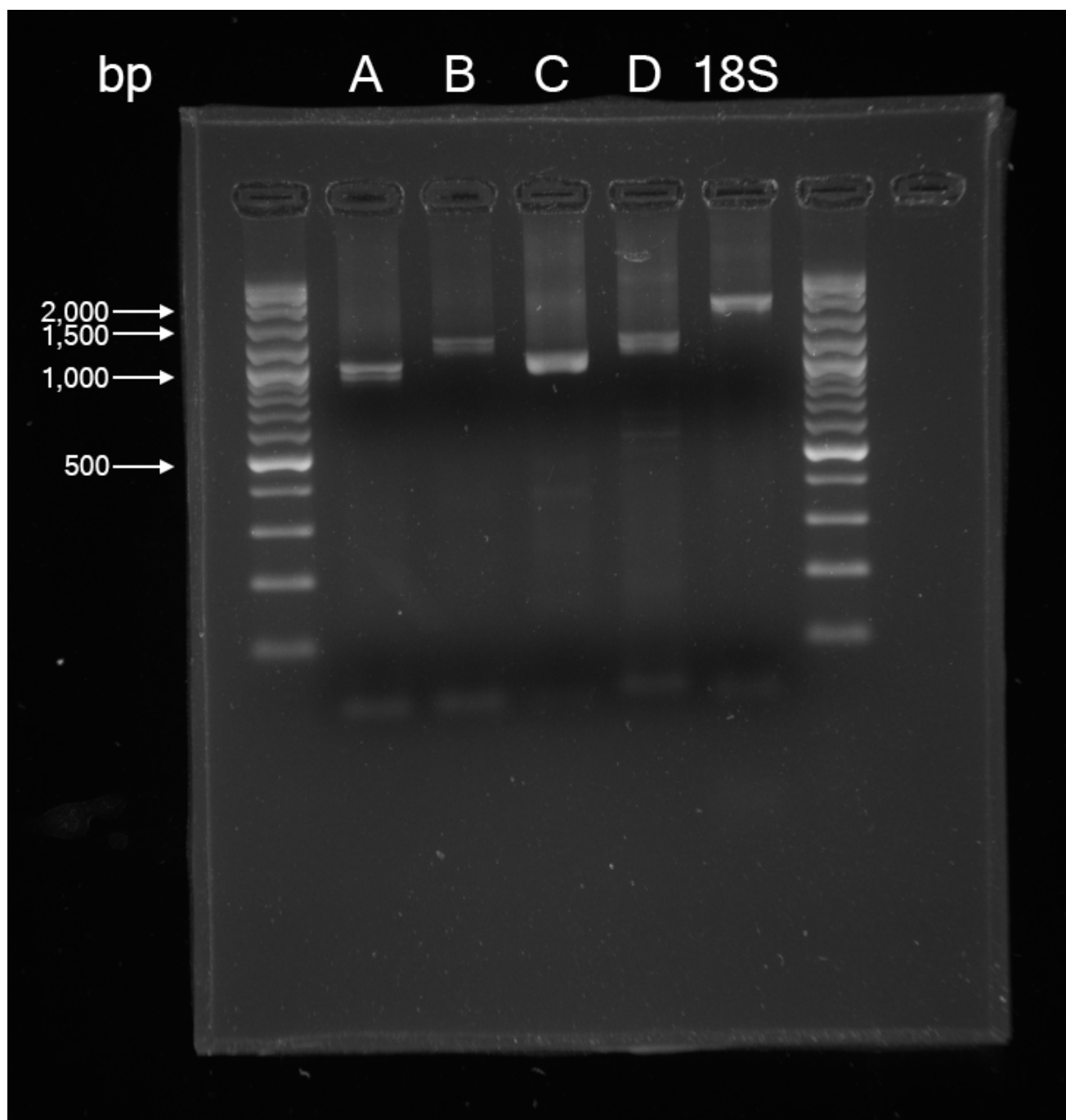
Figure S9. Comparisons of the *atpF* and *clpP* gene structures. (A) Comparing the *atpF* gene structure of *Z. jujuba* species. (B) Comparing the *clpP* gene structure of *H. acerba* species. A blue box represents a coding region and the red wavy line represents an intergenic region. The base position of the *atpF* in *Z. jujuba* NC_030299 and the *clpP* in *H. acerba* MN794429 is used as a reference for each gene.



61 **Figure S10.** Phylogenetic trees of Rhamnaceae reconstructed from the orthologous proteins (A) using
62 ML method, (B) using MP method, (C) using BI method and (D) the tree reconstructed from the
63 orthologous genes using RAxML method. Different colors correspond to tribes labelled in square
64 brackets. The outgroup branch is not colored. Two major groups of Rhamnaceae: ziziphoid and
65 rhamnoid are presented on the rightmost text. A number in each tree indicates the ML bootstrap value,
66 the MP bootstrap value, the posterior probability of BI, the ML bootstrap value, respectively.



68 **Figure S11.** Characteristics and comparisons of Rhamnaceae chloroplast genome features. (A)
69 Comparison of the SSR types. (B) Distribution of SSRs in LSC, SSC and IR regions. (C) Comparison
70 of the long repeat types. (D) Distribution of long repeats in LSC, SSC and IR regions. The total number
71 of SSRs and long repeat sequences in each species is in the parenthesis.



72
73 **Figure S12.** The original image of gel in Figure 1C before cropping and inverting color.