

Supplementary Information

Complete chloroplast and ribosomal sequences for 30 accessions elucidate evolution of *Oryza* AA genome species

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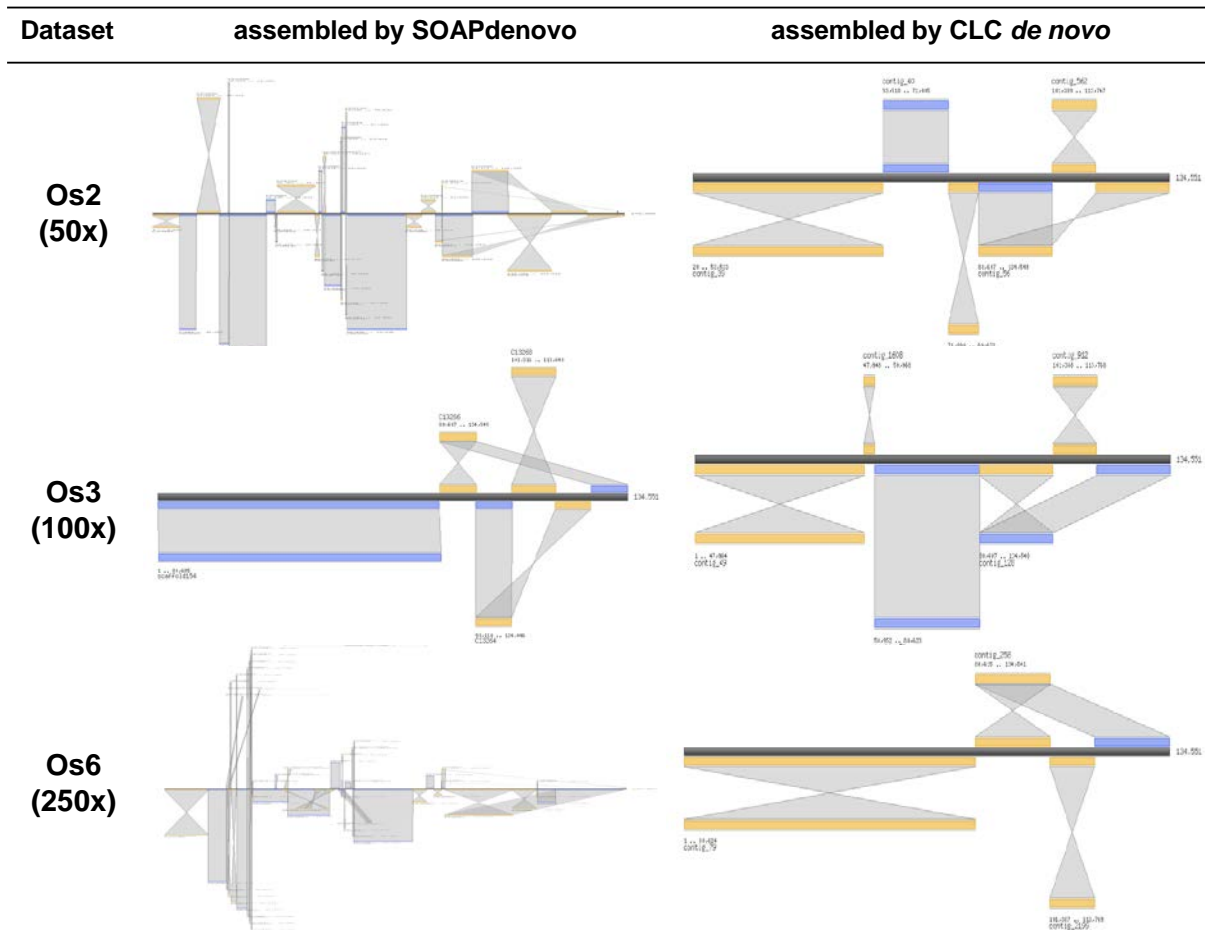
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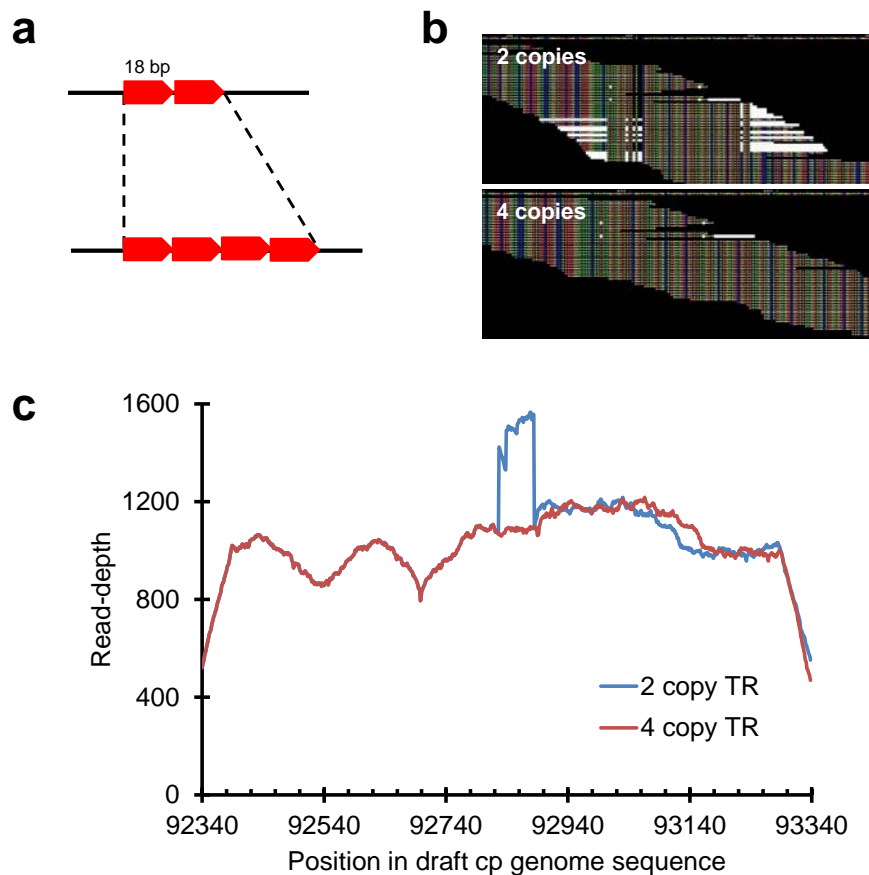
*Corresponding author

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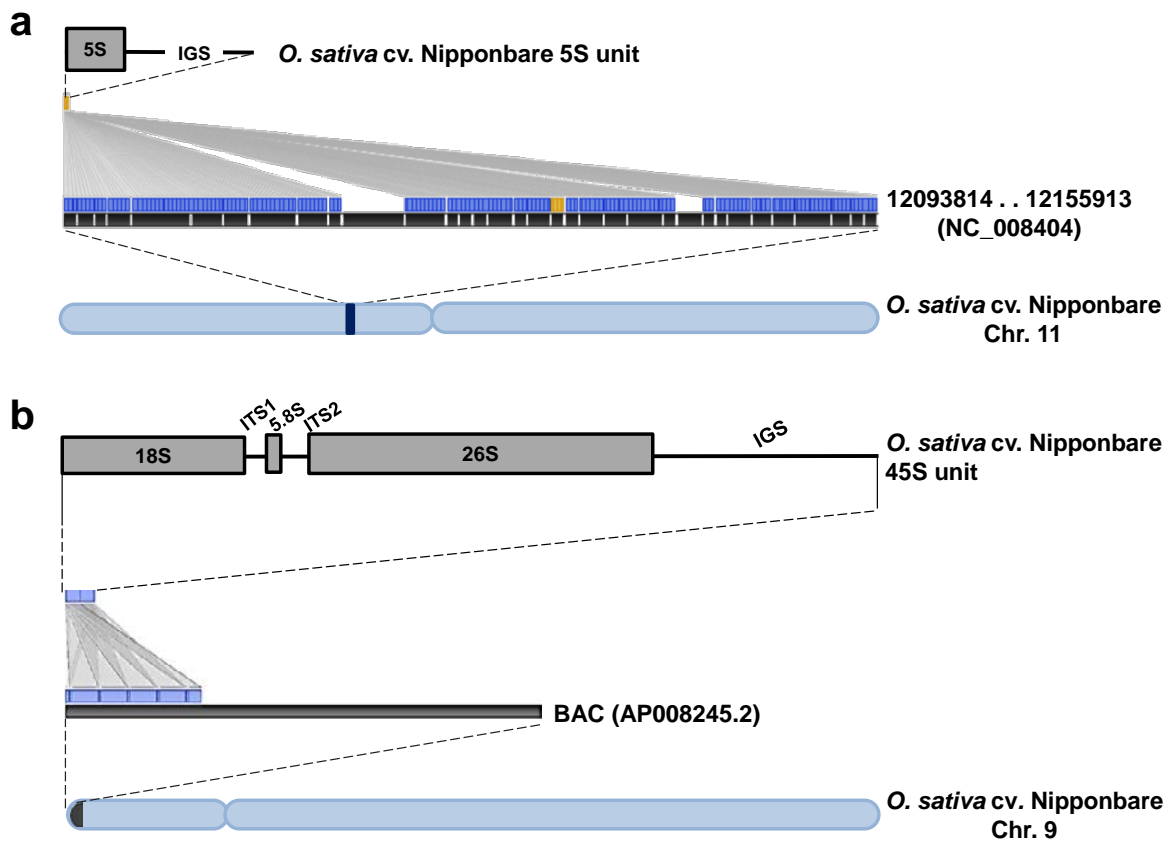
Supplementary Figures



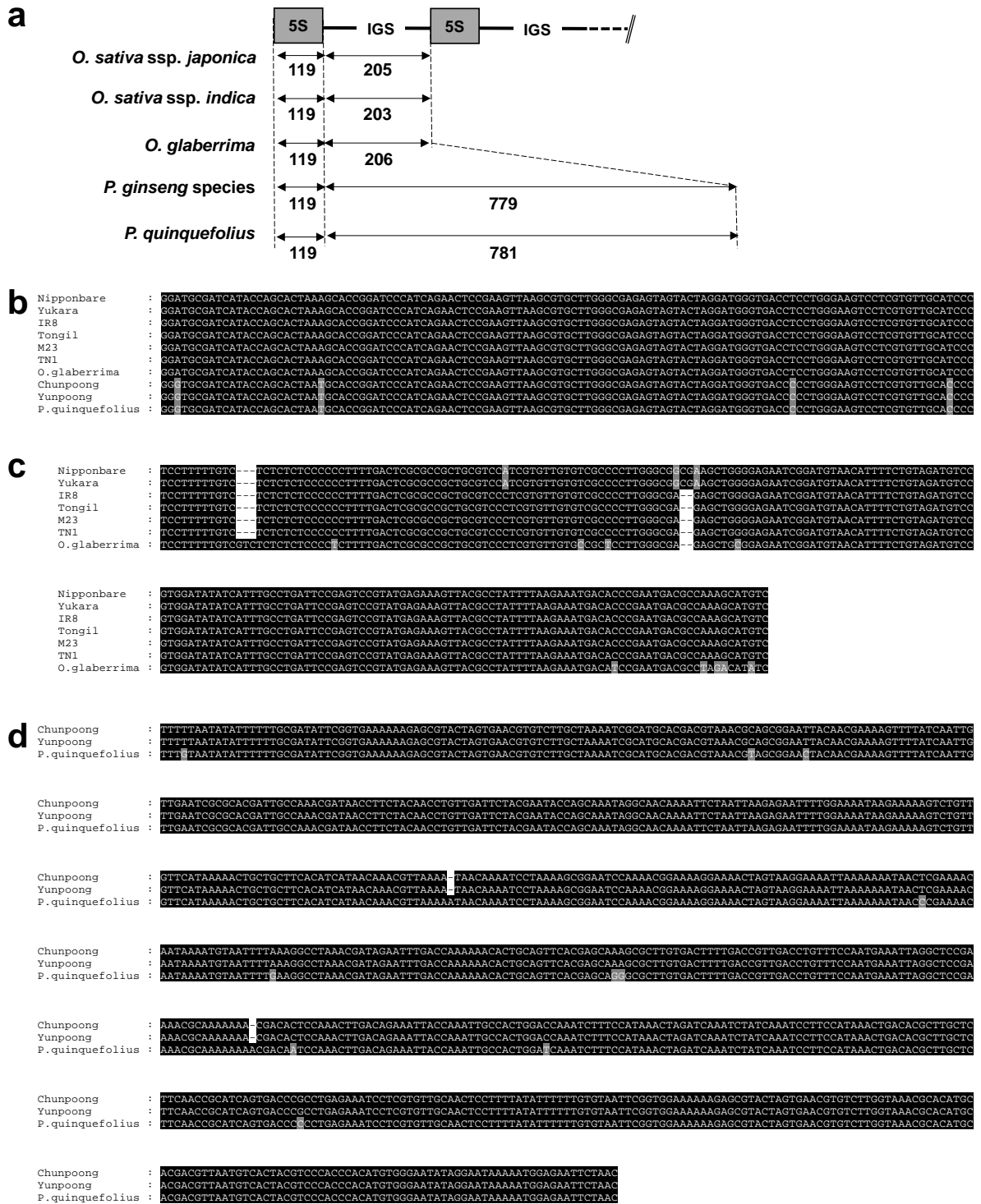
Supplementary Fig. S1. Comparison of rice cp contigs assembled by SOAPdenovo and the CLC *de novo* assembler. Assembled cp contigs from datasets Os2, Os3 and Os6, (Supplementary Table S3) were aligned to the corresponding rice cp genome sequence. Blue and orange bars indicate contigs matching to the reference sequence in forward and reverse orientations, respectively.



Supplementary Fig. S4. Assembly error derived from copy number reduction of tandem repeats (TRs). **a**, Schematic diagram showing incorrect TR copy number reduction from four to two 18-bp unit TRs. **b**, Raw read mapping on cp contigs with two (top) or four (bottom) TRs. Many instances of incorrect read mapping (white reads) are detected on the two-copy TR contig. **c**, Distribution of raw read mapping depth on cp contigs with two (blue) or four (red)-copy TRs. Abnormally high read depth is exhibited in the TR region of the two-copy TR contig because of accumulation of mis-mapping.



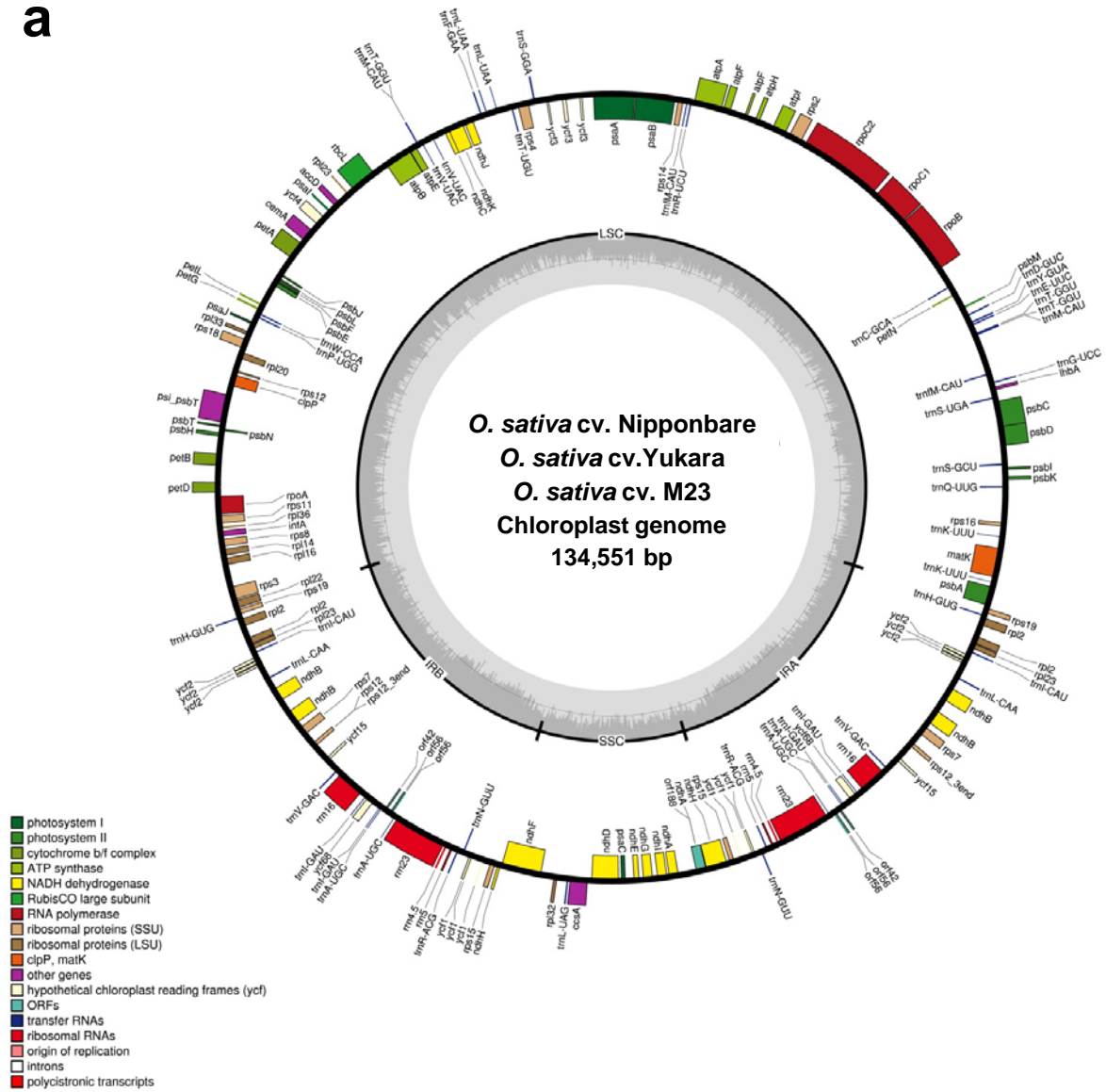
Supplementary Fig. S6. Structure of 5S and 45S contigs and comparison with their homologous sequences in rice pseudo-chromosome sequence. a, Structure of the complete 5S unit assembled in this study and tandem array of the 5S units on a region of rice chromosome 11. **b,** Structure of the complete 45S unit assembled in this study and tandem array of the 45S units on rice BAC sequence located on rice chromosome 9.



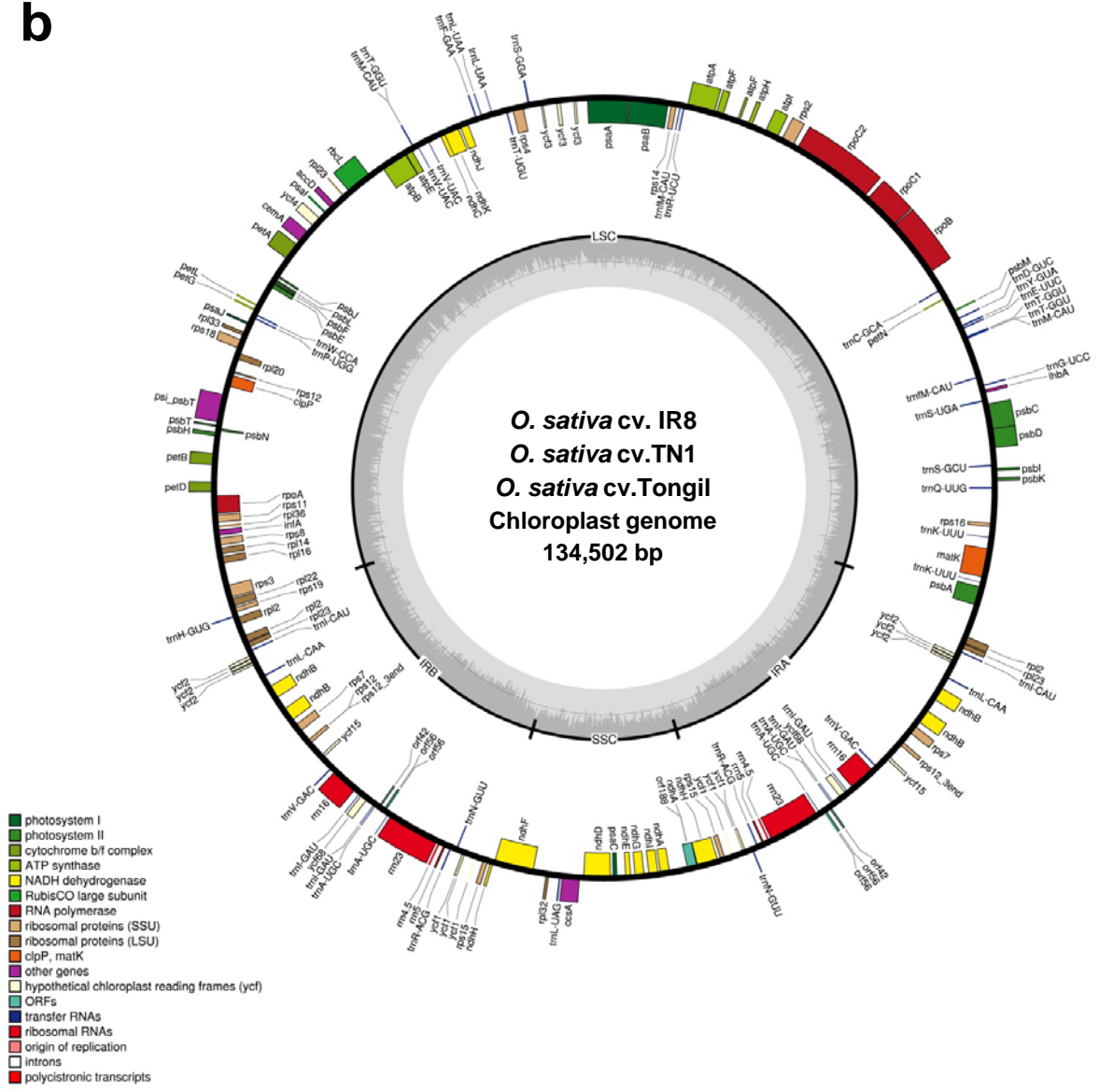
Supplementary Fig. S7. Comparison of complete 5S unit sequences in *Oryza* and *Panax* species. a, Length comparison of 5S units. **b**, Sequence comparison of 5S rRNA coding sequence. **c**, Sequence comparison of IGS of *Oryza* species. **d**, Sequence comparison of IGS of *Panax* species (GenBank nos. KM036312, KM036313, and KM036314). Nucleotide

sequences were aligned and visualized using ClustalW (<http://www.genome.jp/tools/clustalw/>) and GeneDoc software (<http://www.nrbsc.org/gfx/genedoc/>).

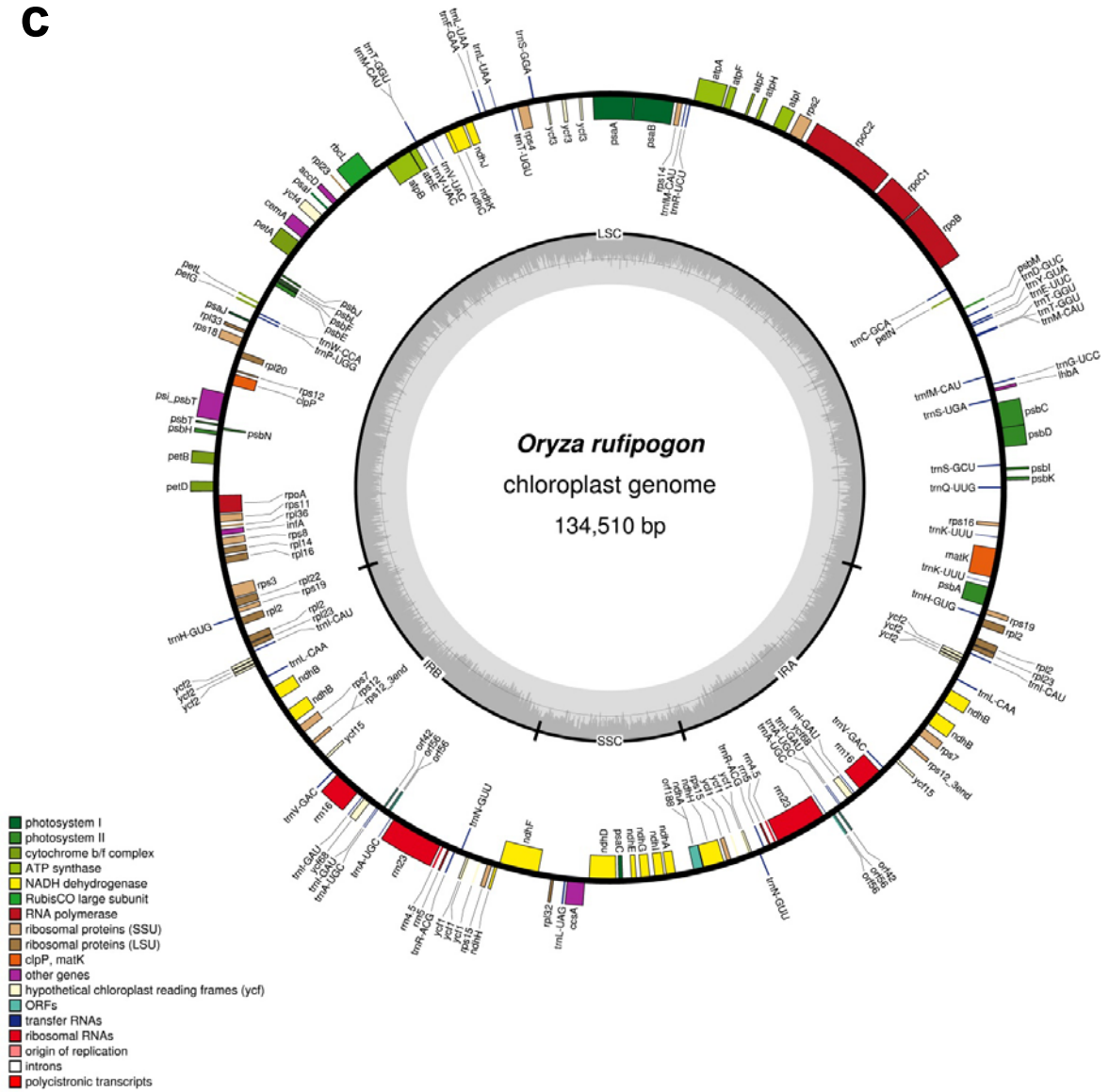
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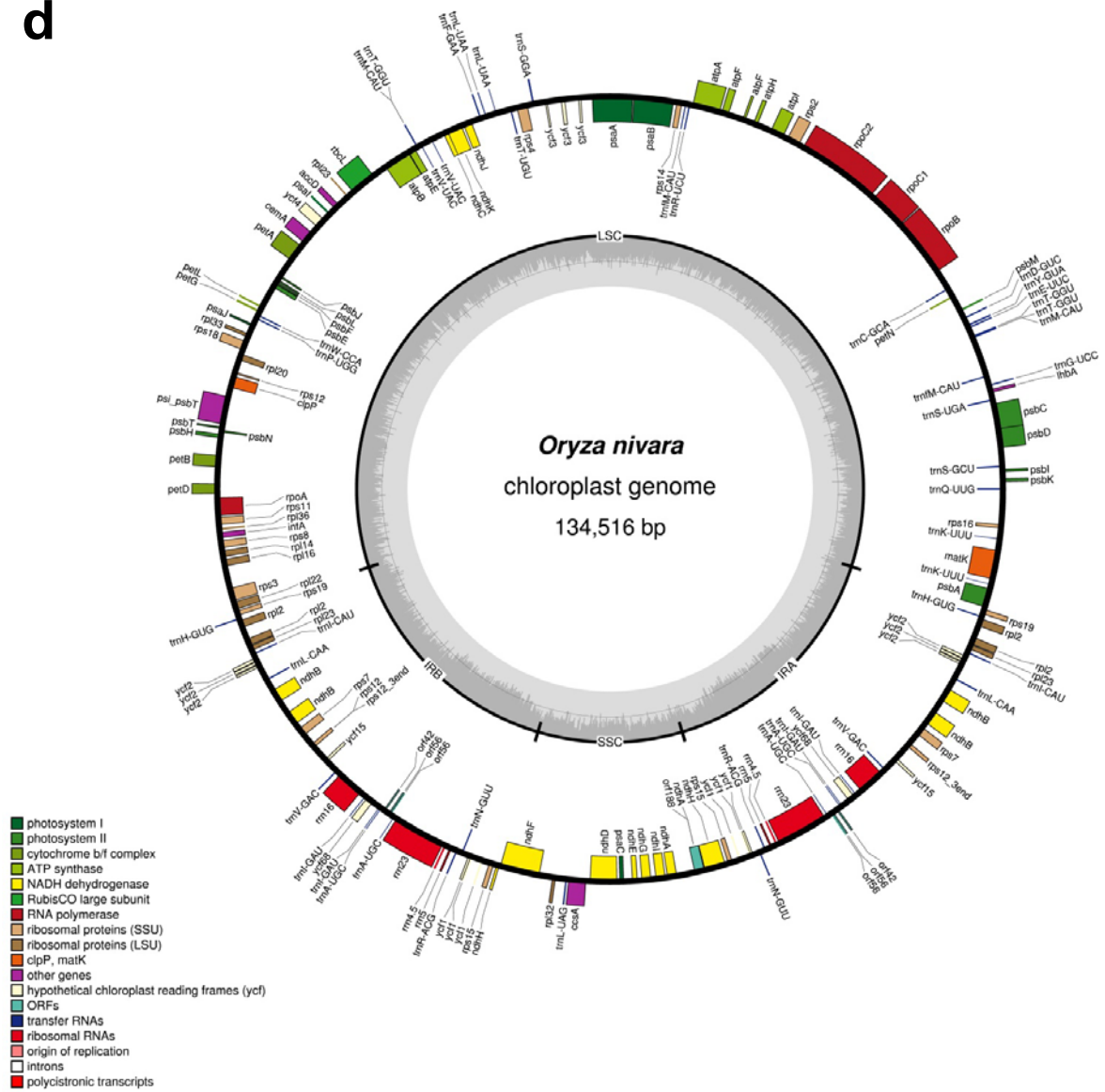
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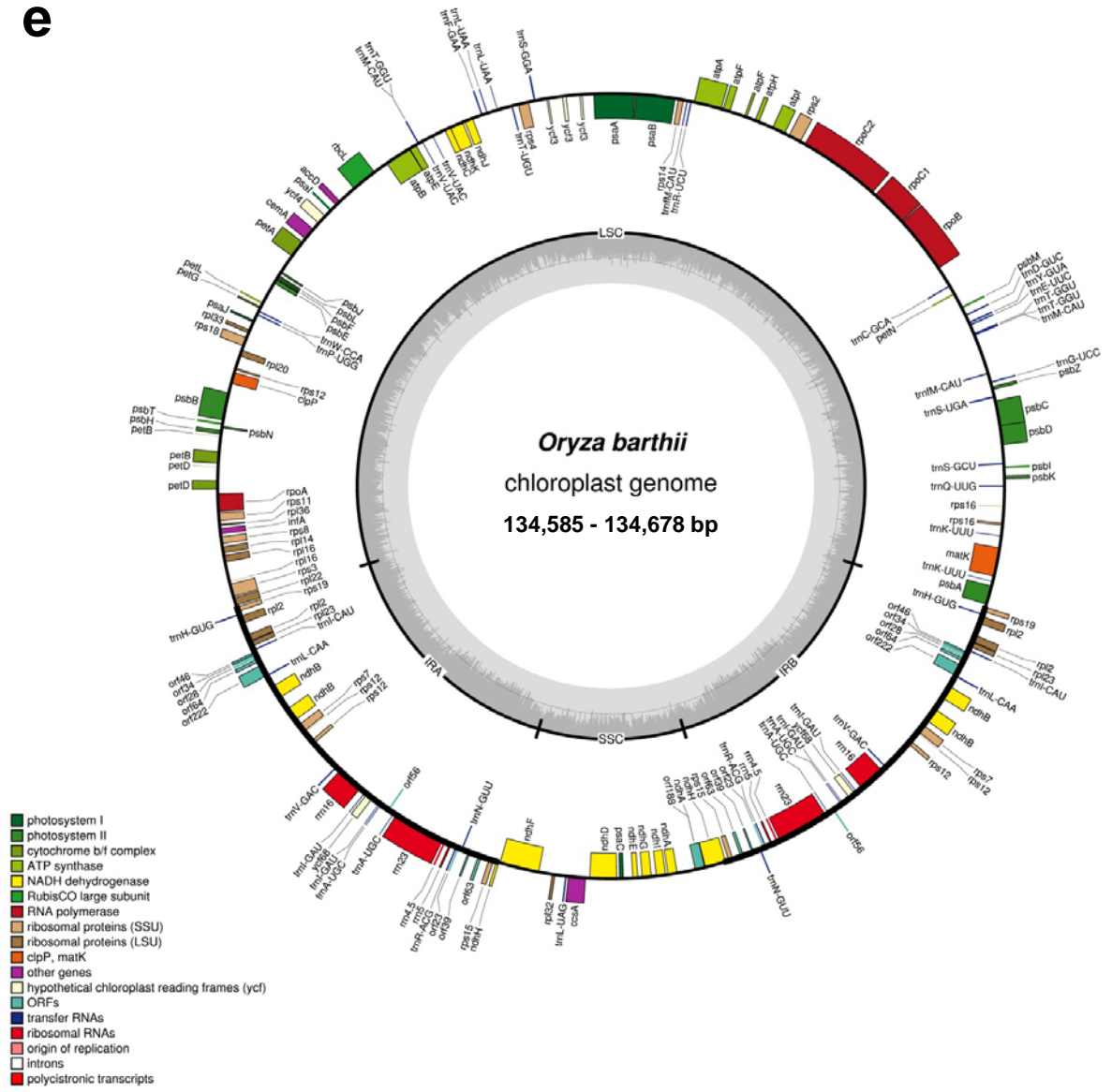
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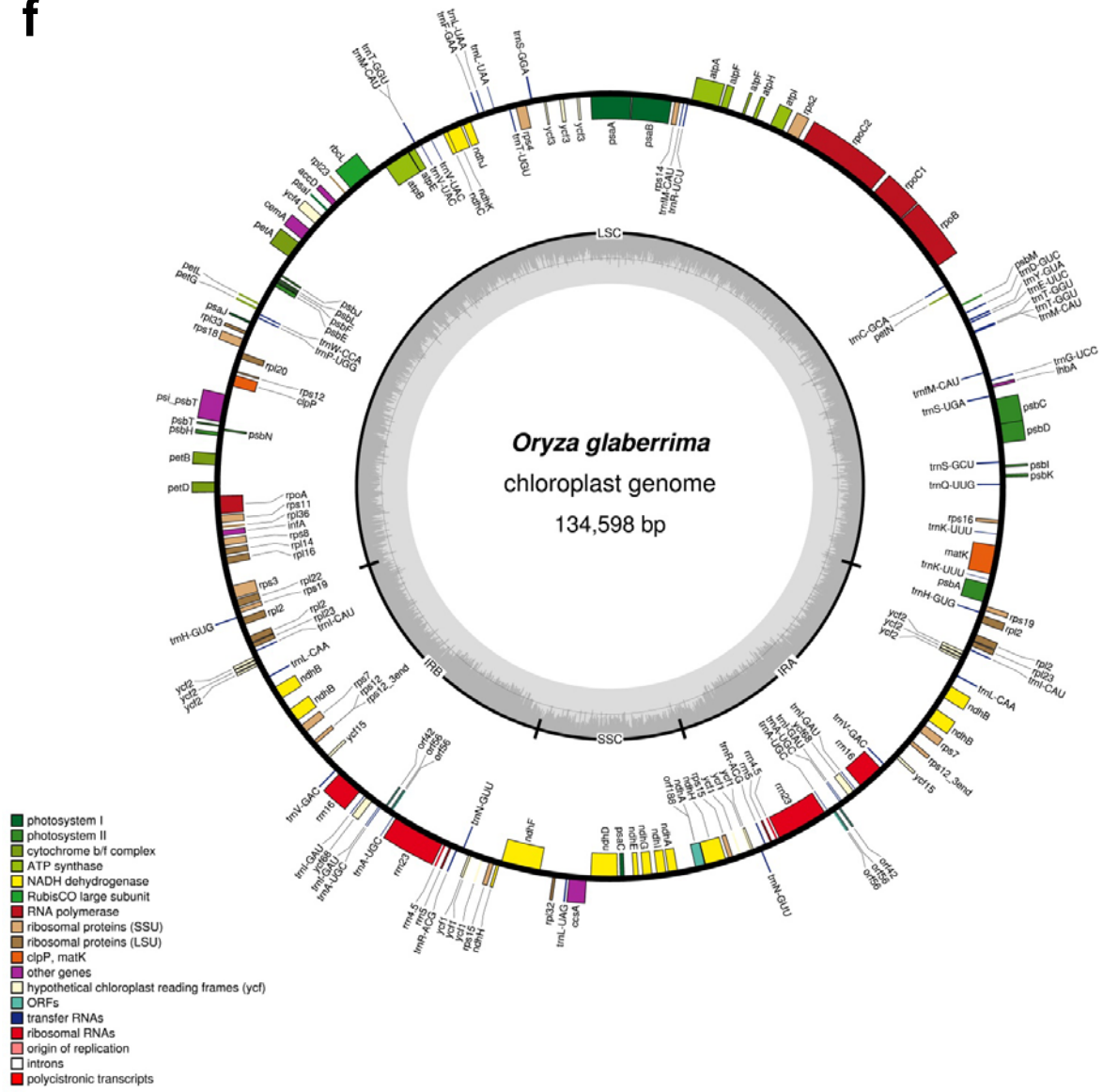
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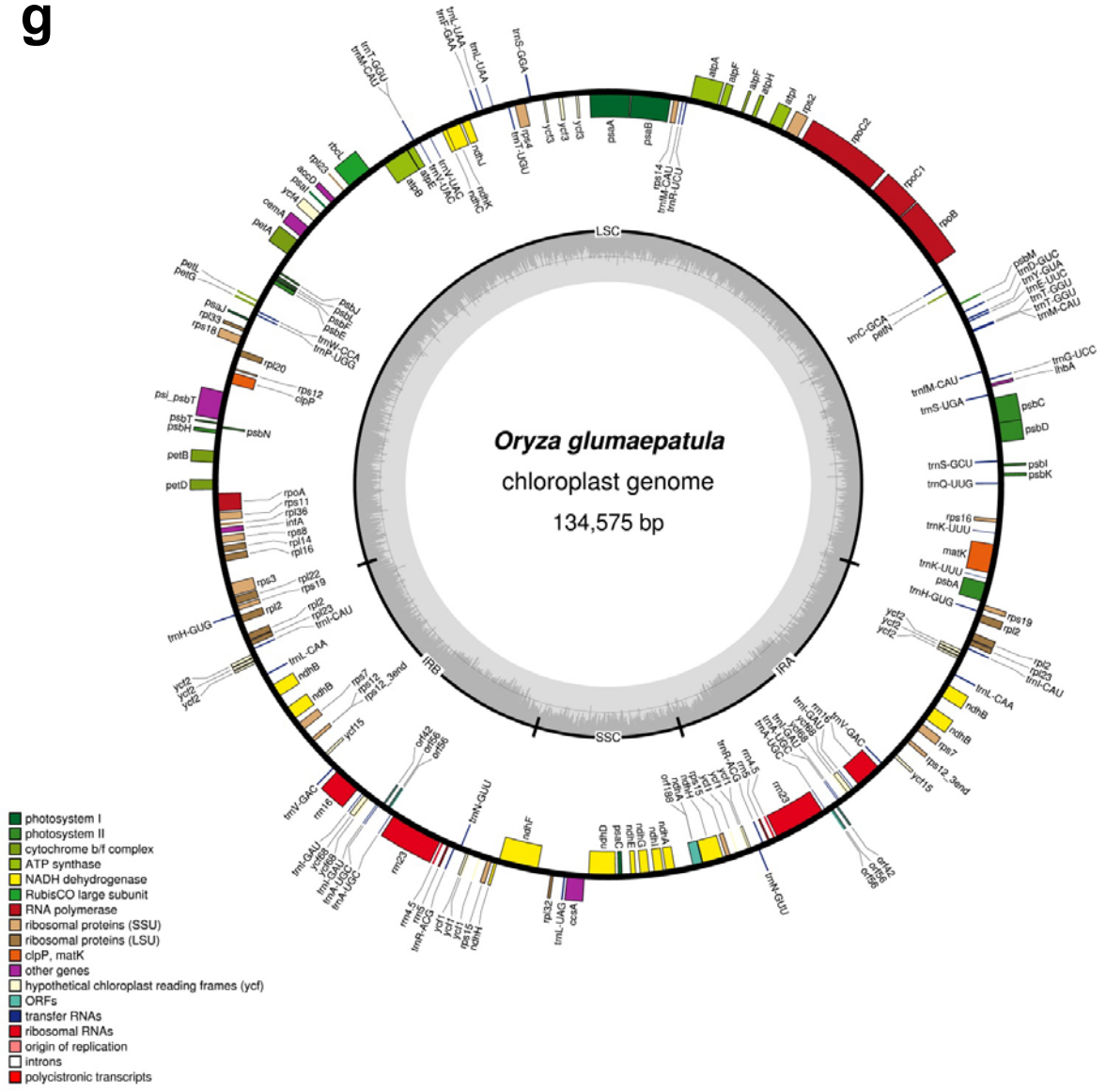
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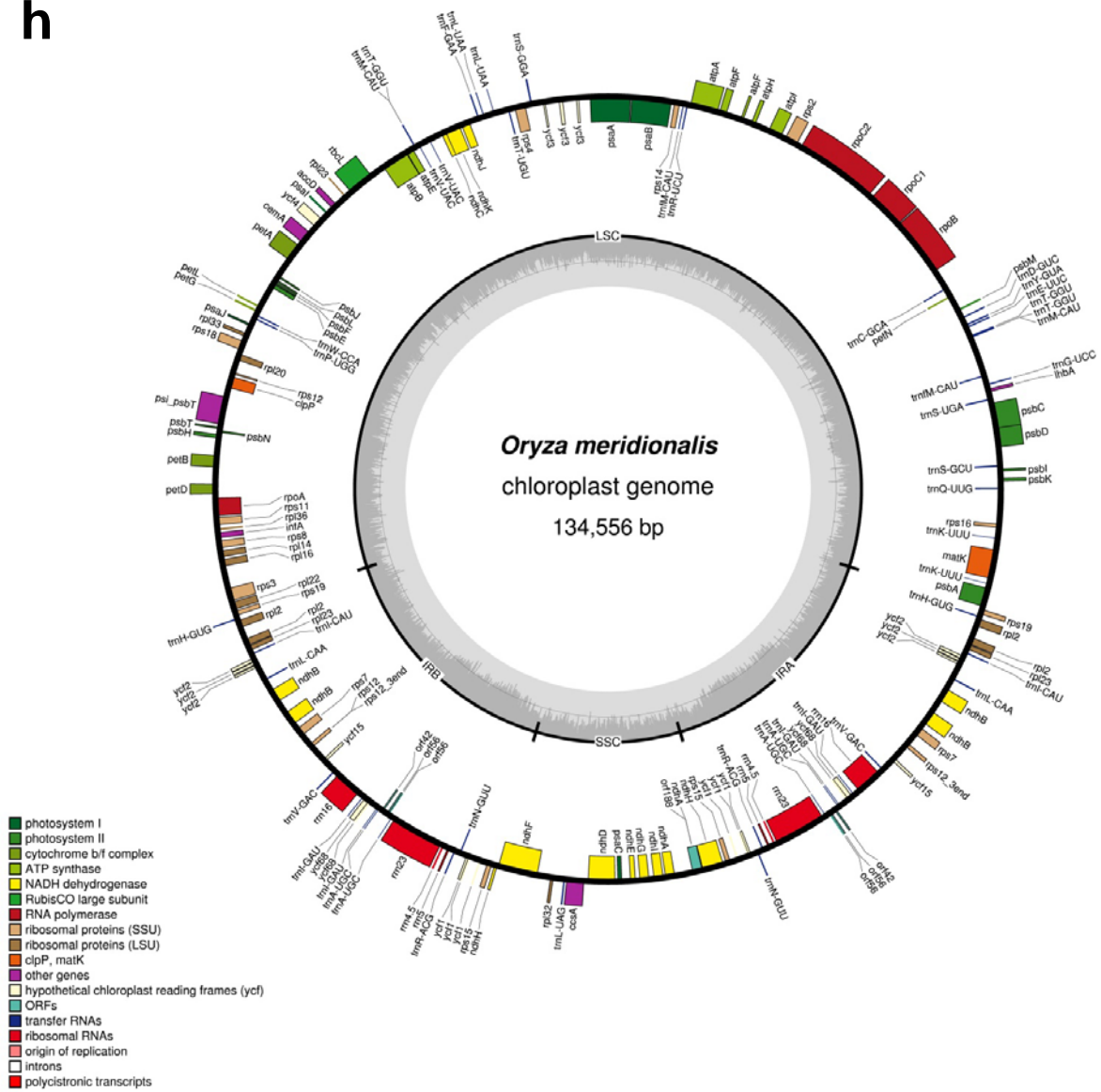
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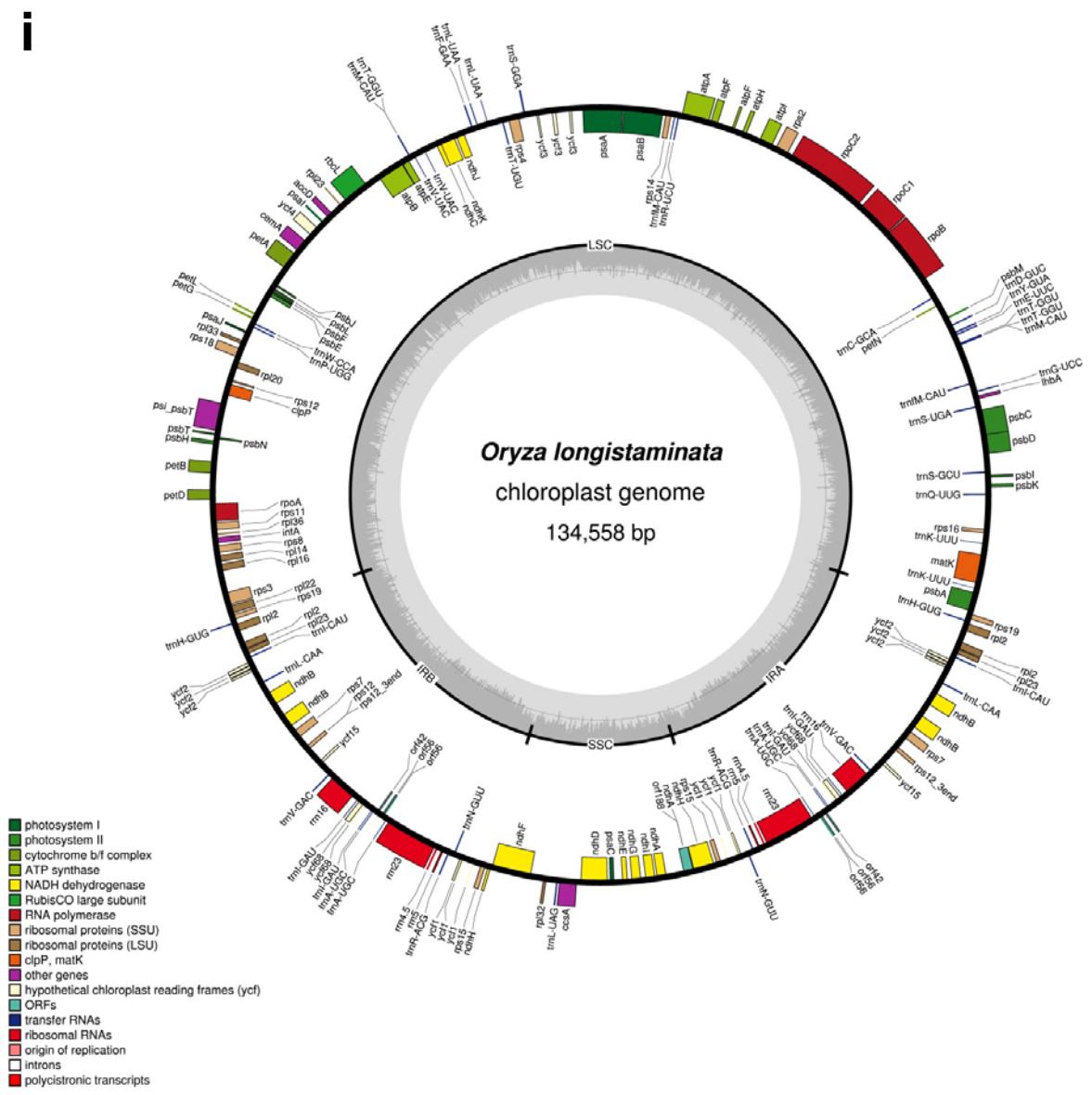
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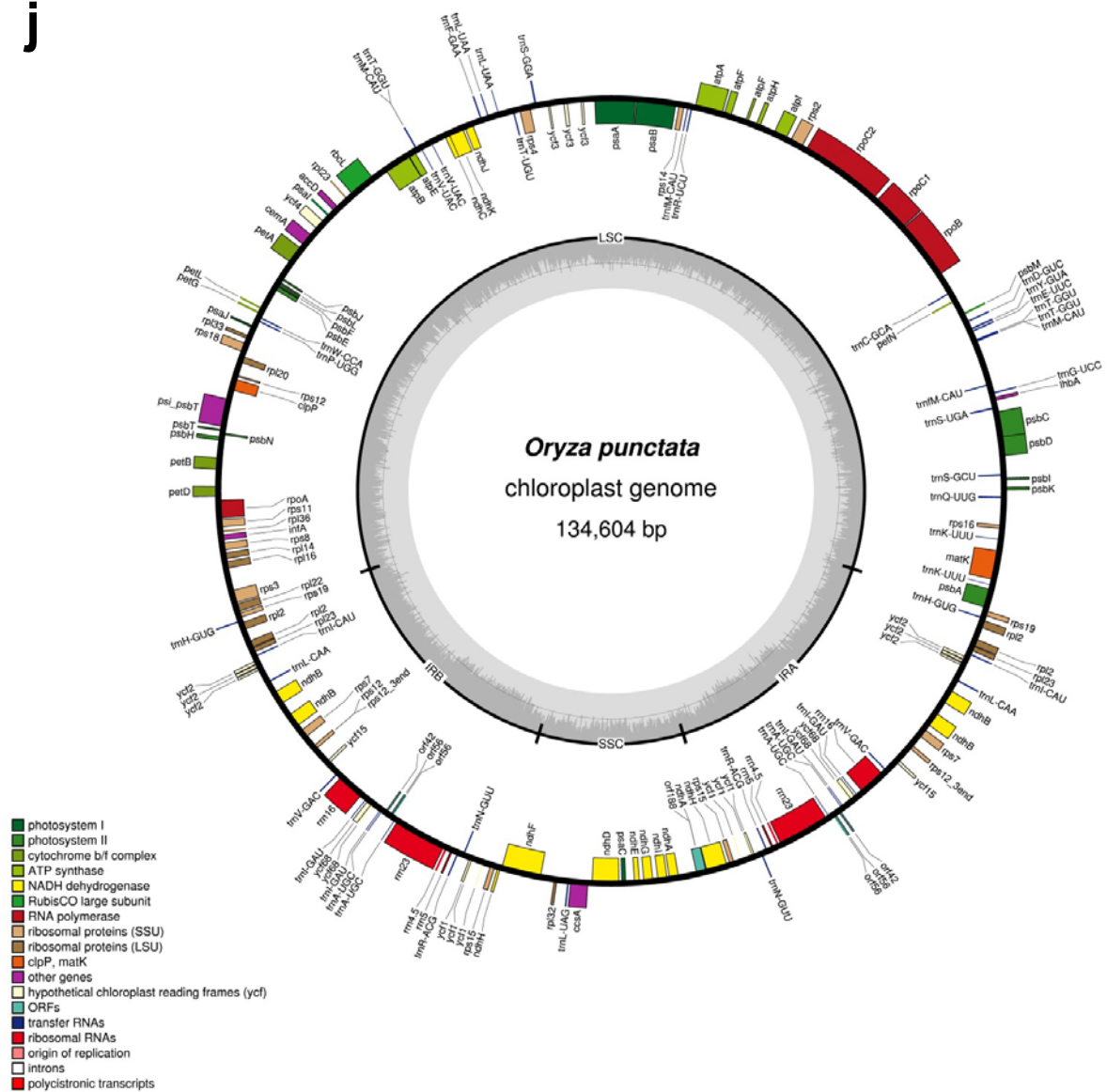
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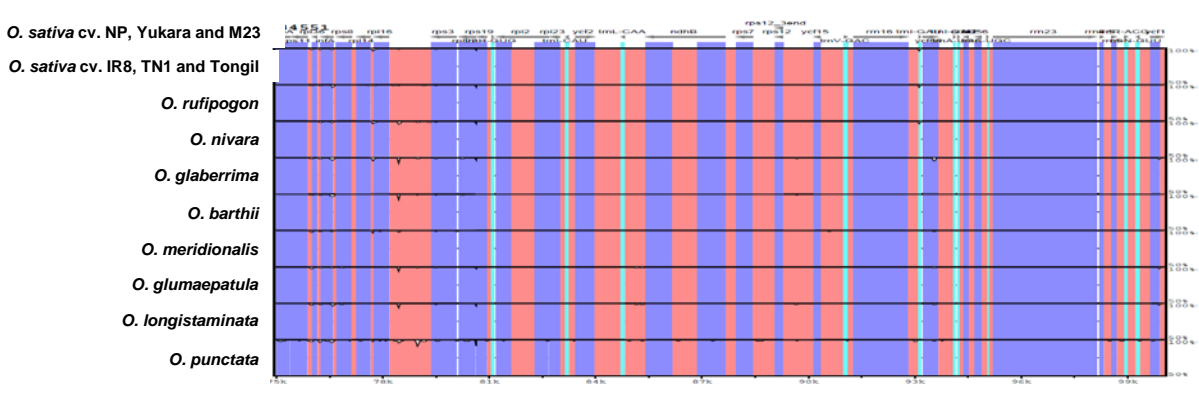
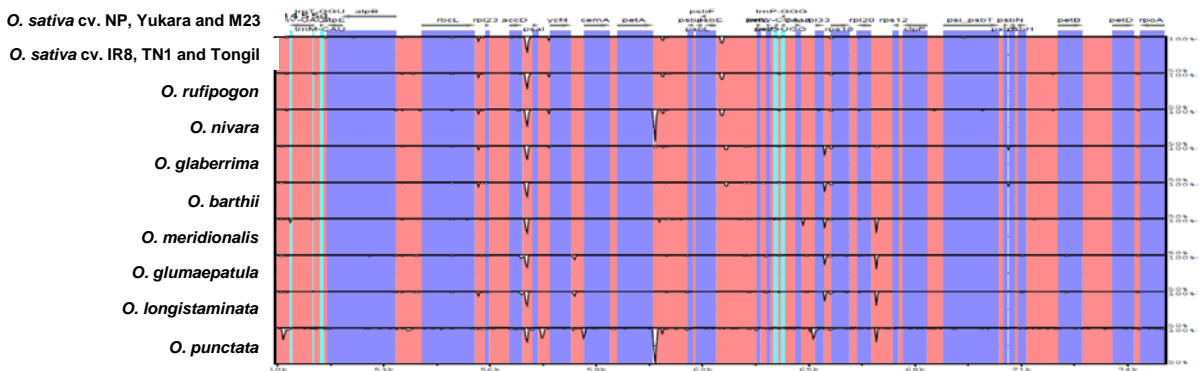
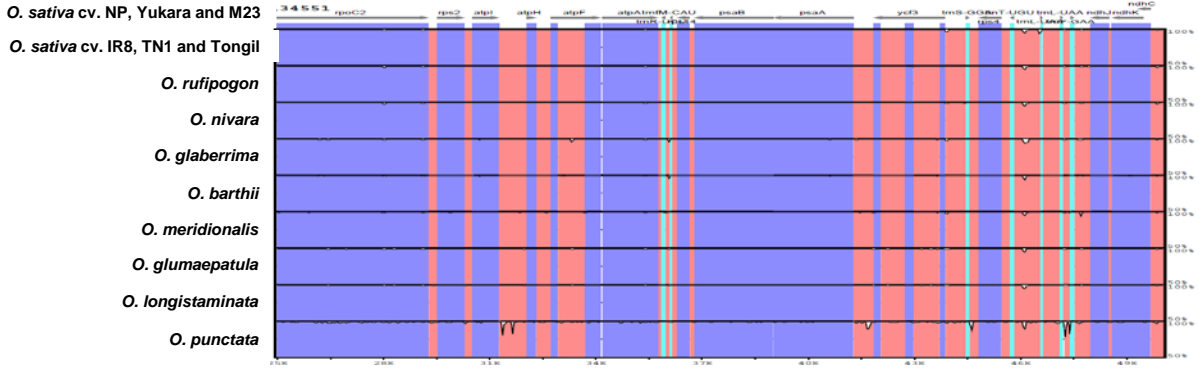
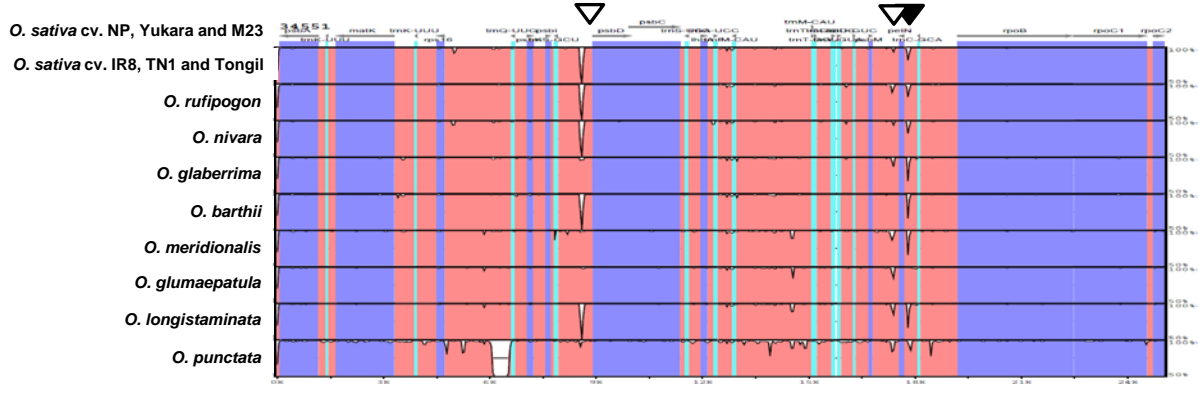


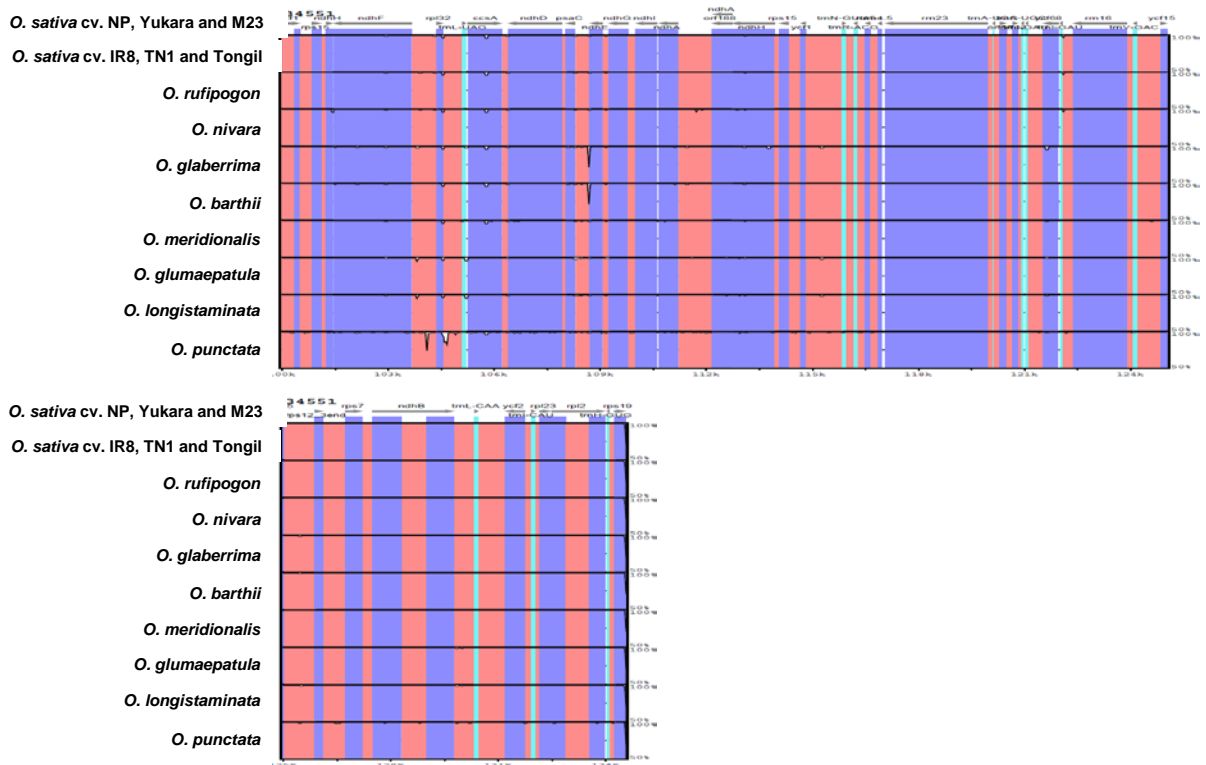
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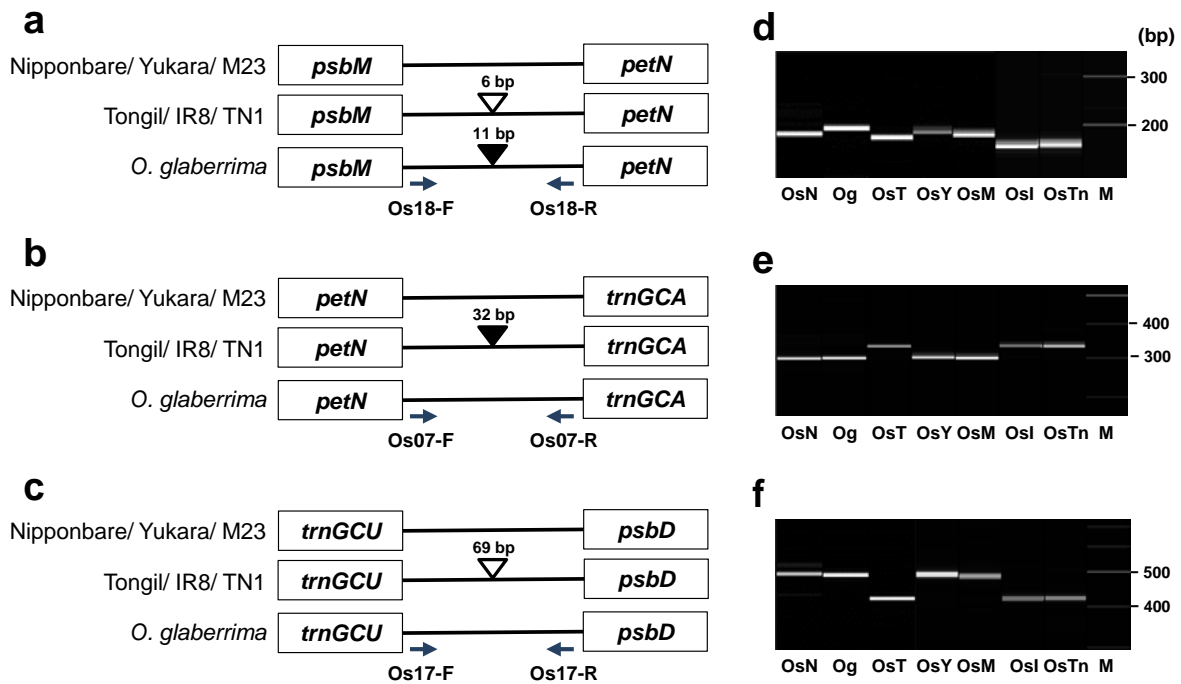
Supplementary Fig. S8. Chloroplast genome map of nine *Oryza* species. The complete cp genome sequence was generated by the dnaLCW method and annotated using the DOGMA program (<http://dogma.cccb.utexas.edu/>). The map was prepared using OGDRAW (<http://ogdraw.mpimp-golm.mpg.de/>). Genes transcribed clockwise and counterclockwise are indicated on the outside and inside of the large circle, respectively. The four parts of the cp genome and GC content are indicated on the inner circle. **a**, *O. sativa* cv. Nipponbare, cv. Yukara, and cv. M23; **b**, *O. sativa* cv. IR28, cv. TN1, and cv. Tongil; **c**, *O. rufipogon* accessions; **d**, *O. nivara* accessions; **e**, *O. barthii* accessions; **f**, *O. glaberrima* accessions; **g**, *O.*

glumaepatula accessions; **h**, *O. meridionalis* accessions; **i**, *O. longistaminata* (IRGC110404);
j, *O. punctata* (IRGC105690).

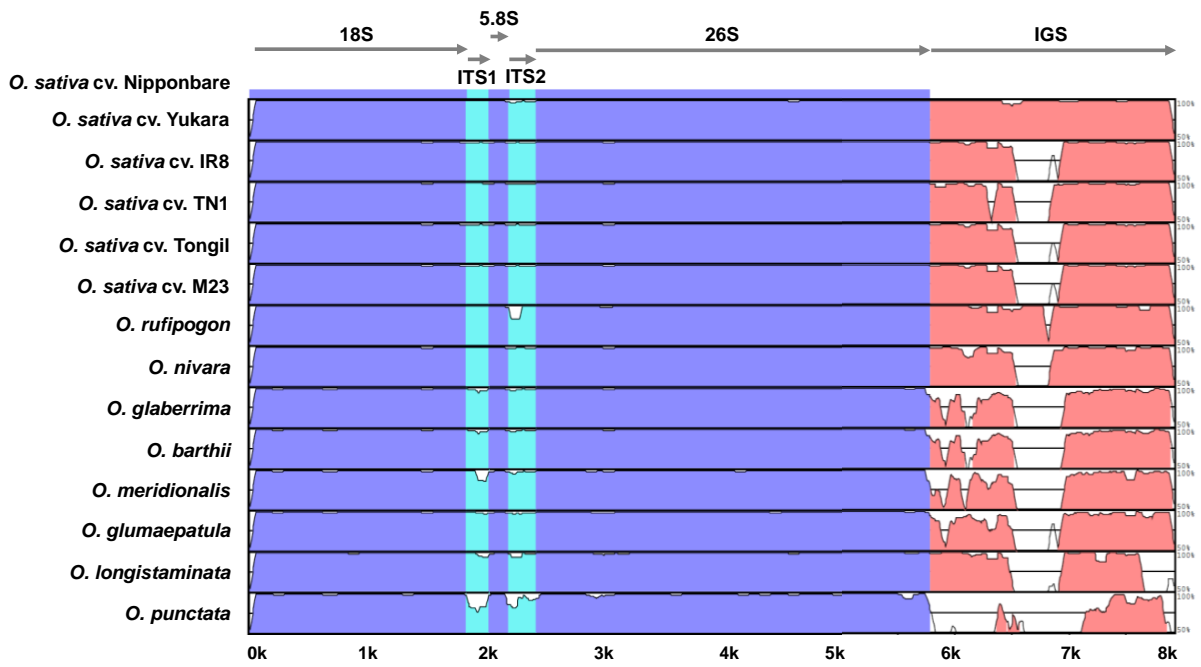




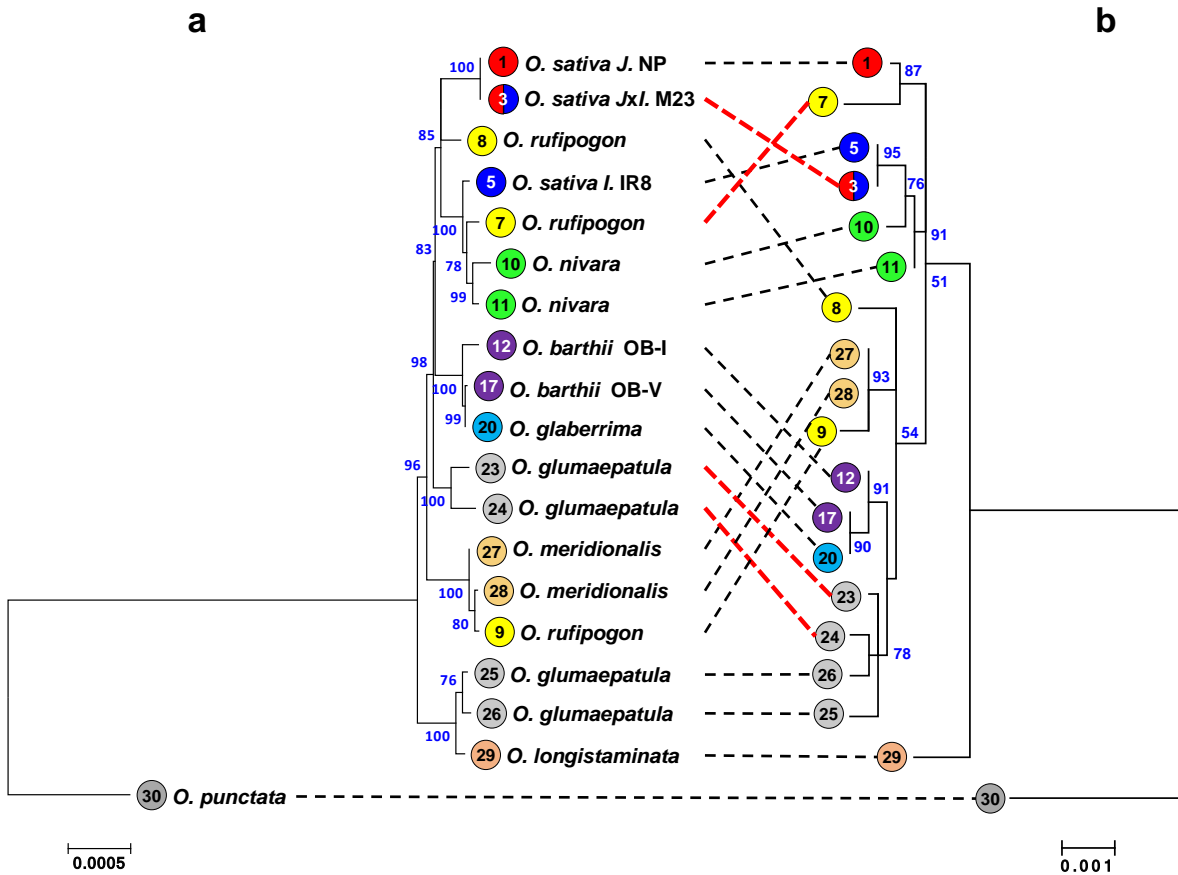
Supplementary Fig. S9. Comparison of cp genome sequences of *Oryza* species. Complete cp genomes of *Oryza* species were generated by the dnaLCW method. Genic regions were identified using the DOGMA program (<http://dogma.cccb.utexas.edu/>) and the comparative map was prepared using mVISTA (<http://genome.lbl.gov/vista/mvista/submit.shtml>). Empty and filled arrowheads indicate deletion and insertion of nucleotides, respectively, which were analyzed in Supplementary Fig. S10.



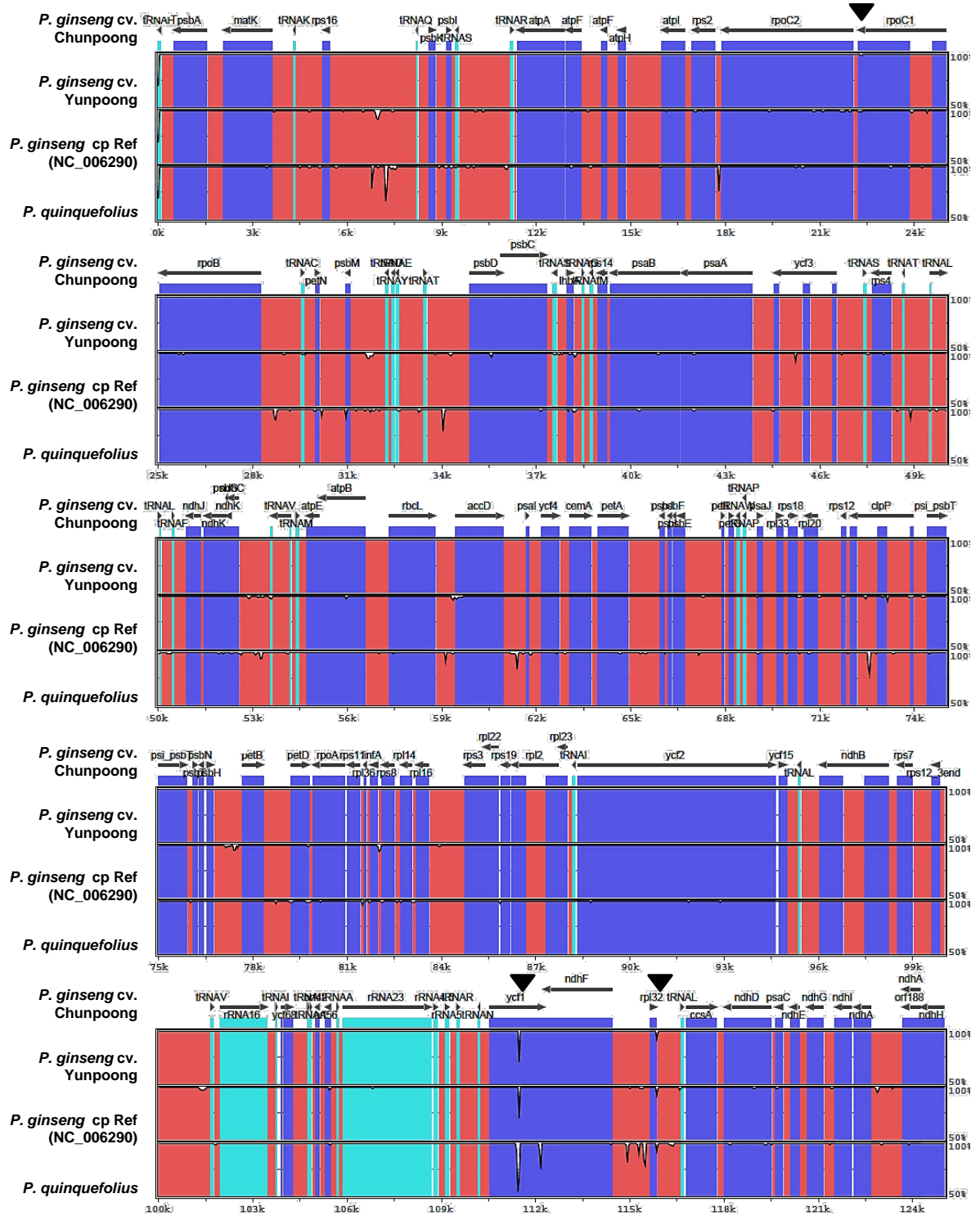
Supplementary Fig. S10. Schematic representation and validation of polymorphism for three InDel regions between *Oryza* subspecies. Complete cp genomes of seven *Oryza* species were generated and compared to identify polymorphic regions. Three InDel regions found in *psbM-petN* (**a** and **d**), *petN-trnGCA* (**b** and **e**), and *trnGCU-psbD* (**c** and **f**) were confirmed by PCR analysis. Empty and filled arrowheads indicate deletion and insertion of nucleotides, respectively, when compared with the cp genome sequence of cv. Nipponbare. Arrows indicate primer positions (Supplementary Table S6). OsN, *O. sativa* cv. Nipponbare; Og, *O. glaberrima*; OsT, cv. Tongil; OsY, cv. Yukara; OsM, cv. M23; OsI, cv. IR8; OsTn, cv. TN1; M, DNA size marker.

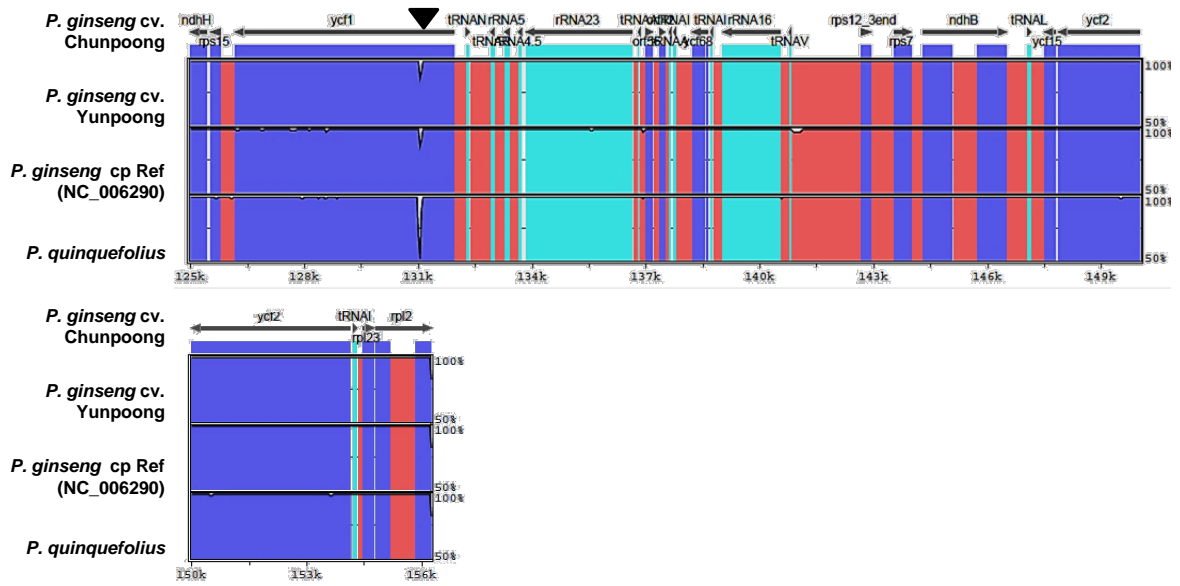


Supplementary Fig. S11. Comparison of 45S units of *Oryza* species. Complete 45S units of *Oryza* species were generated by method developed in this study and the comparative map was constructed using mVISTA (<http://genome.lbl.gov/vista/mvista/submit.shtml>).

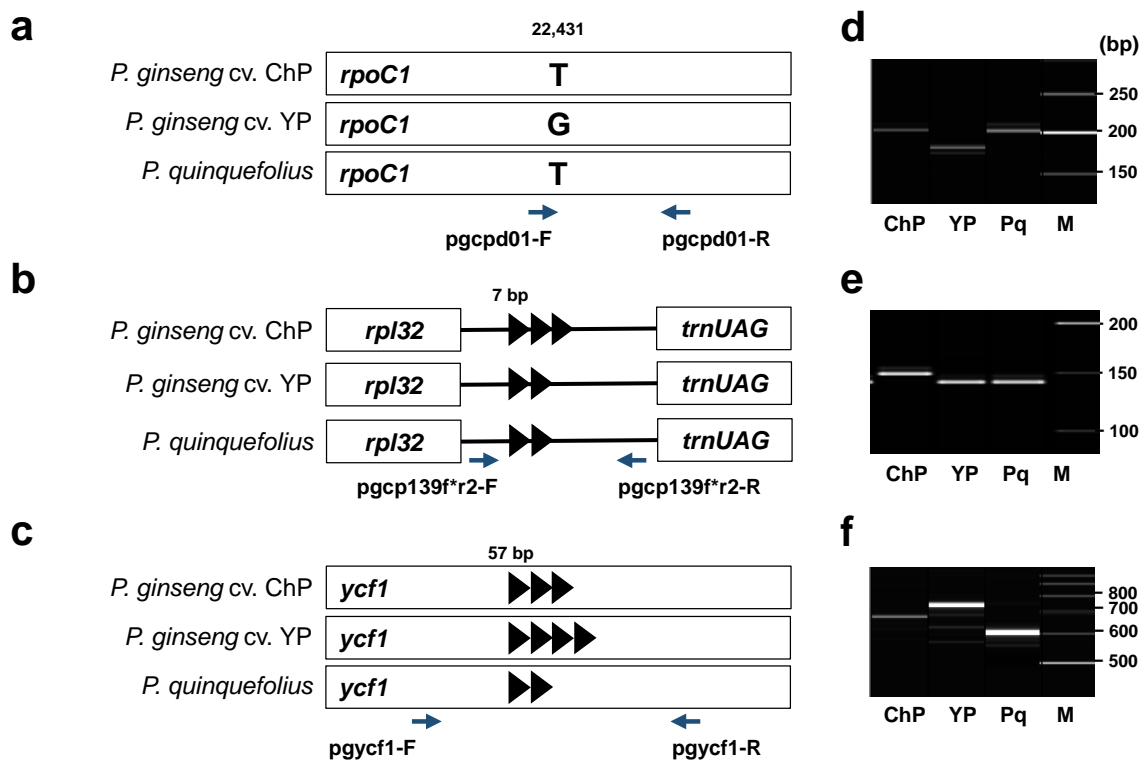


Supplementary Fig. S12. Phylogenomic tree of *Oryza* species using the maximum likelihood (ML) method. (a,b) Phylogenetic trees were built based on the complete cp genome (a) and 45S cistron sequences (b). *O. sativa* ssp. *japonica* and *indica* groups are denoted as *J* and *I*, respectively. Different species/subspecies are indicated with different colored labels. Numbers in colored circles represent accessions labeled in Table 1. Dashed lines connect the positions of each accession/cultivar in the two trees; red highlights major differences between trees. The tree was generated by the maximum likelihood (ML) method with 1,000 bootstrap replicates using MEGA6.

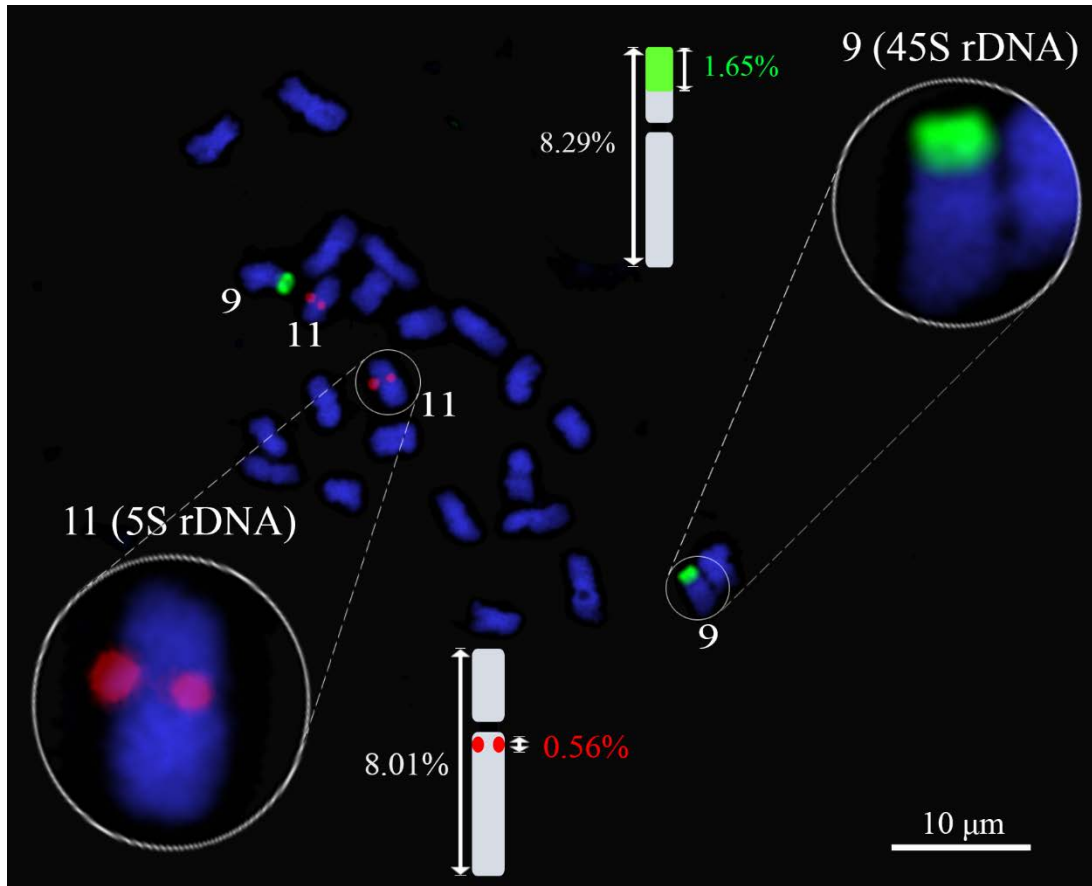




Supplementary Fig. S13. Comparison of cp genome sequences of *Panax* species. Complete cp genomes of *P. ginseng* cv. Chunpoong (GenBank Accession No. KM088019)⁵⁵, cv. Yunpoong (KM088020)⁵⁵, and *P. quinquefolius* (KM088018) were generated by the dnaLCW method and the cp genome sequence in GenBank (*P. ginseng* cv. Nees; NC_006290) was used for comparison. Genic regions were identified using the DOGMA program (<http://dogma.cccb.utexas.edu/>) and the comparative map was prepared using mVISTA (<http://genome.lbl.gov/vista/mvista/submit.shtml>). Filled arrowheads indicate polymorphic regions analyzed in Supplementary Fig. S14.



Supplementary Fig. S14. Schematic representation and validation of three polymorphic regions between cp genome sequences of two *P. ginseng* cultivars. Complete cp genomes of *P. ginseng* cv. Chunpoong (ChP), cv. Yunpoong (YP), and *P. quinquefolius* (Pq) were compared to identify SNP (a) and copy number variation (CNV) sites (b and c). a and d, SNP analysis using dCAPS primers designed for the SNP site of the *rpoC1* gene. *Xba*I digestion of the amplicon produced a digested fragment only in YP but not in ChP and Pq. b and e, CNV analysis of a 7-bp tandem repeat in the *rpl32-trnUAG* region. PCR amplification confirmed 2 copies of the repeat in YP and Pq, and 3 copies in ChP. c and f, CNV analysis of a 57-bp tandem repeat in the *ycf1* gene. PCR amplification confirmed 2, 3, and 4 copies of the repeat in Pq, ChP, and YP, respectively. Arrows indicate primer sites previously reported⁵⁶. M indicates DNA size marker.



Supplementary Fig. S15. FISH analysis of 45S and 5S in *O. sativa* genome. nR content genome was estimated based on the ratio of the area covered by probe hybridization signals to the total chromosome area (Supplementary Tables S4). Values outside parentheses represent the areas of nR-bearing chromosomes or FISH signals, while values inside parentheses represent estimated genomic contents.

Supplementary Tables

Supplementary Table S1. Characterization of the 30 longest contigs in the *de novo* assembly of the rice Os2 dataset (1x genome coverage; 50x cp genome coverage).

Ctg_no.	Ctg length (bp)	Ctg Coverage (x)	Best hit in GenBank and TE DB					Description
			Acc. no.	Length (bp)	Match begin	Match end	E-value	
ctg_39	53,713	48.89	GU592207.1	134,551	18	53,535	0.0	<i>O. sativa japonica</i> chloroplast genome
ctg_56	20,802	97.14	GU592207.1	134,551	80,605	101,406	0.0	<i>O. sativa japonica</i> chloroplast genome
ctg_911	19,415	10.76	BA000029.3	490,520	173,036	183,181	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_40	18,492	48.63	GU592207.1	134,551	53,516	72,007	0.0	<i>O. sativa japonica</i> chloroplast genome
ctg_619	16,859	12.57	BA000029.3	490,520	214,506	228,529	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_596	16,024	10.00	BA000029.3	490,520	343,504	359,495	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_562	12,383	47.71	GU592207.1	134,551	101,387	113,769	0.0	<i>O. sativa japonica</i> chloroplast genome
ctg_380	11,412	9.89	BA000029.3	490,520	36,168	47,528	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_135	8,628	53.43	GU592207.1	134,551	71,988	80,624	0.0	<i>O. sativa japonica</i> chloroplast genome
ctg_72	8,112	154.94	MUDR1_OS	8,052	966	7,715	0.0	<i>O. sativa</i> MuDR-type DNA transposon
ctg_948	7,982	8.40	BA000029.3	490,520	80,797	88,762	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_1284	7,882	8.33	BA000029.3	490,520	382,357	390,429	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_567	7,173	29.88	MDR2	6,967	76	6,516	0.0	<i>O. sativa</i> MuDR-type DNA transposon
ctg_183	7,166	12.39	SZ-55_I	7,062	1	6,973	0.0	<i>O. sativa</i> retrotransposon SZ-55
ctg_173	6,889	342.62	M11585.1	3,377	2	3,377	0.0	<i>O. sativa</i> 25S ribosomal RNA gene
ctg_180	6,776	24.93	RIRE7_I	5,899	1,394	5,899	0.0	RIRE7 gypsy-like endogenous retrovirus
ctg_316	6,622	95.83	MuDR3_OS	8,604	97	6,666	0.0	<i>O. sativa</i> MuDR-type DNA transposon
ctg_580	6,607	8.34	BA000029.3	490,520	73,247	79,345	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_319	6,403	108.69	RIRE1_I	5,276	800	5,275	0.0	RIRE1, a copia-like retrotransposon
ctg_343	6,331	69.87	SZ-37_I	8,831	1	5,674	0.0	<i>O. sativa</i> retrotransposon SZ-37
ctg_265	6,210	44.78	CRM-I_OS	5,933	689	5,933	0.0	<i>O. sativa</i> centromeric LTR retrotransposon
ctg_3958	6,059	7.67	BA000029.3	490,520	207,511	213,320	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_570	5,760	9.18	BA000029.3	490,520	163,967	169,688	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_1105	5,713	7.93	BA000029.3	490,520	256,888	262,540	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_2119	5,635	7.67	BA000029.3	490,520	397,094	400,264	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_675	5,512	40.40	MDR1	7,383	867	6,361	0.0	<i>O. sativa</i> MuDR-type DNA transposon
ctg_4531	5,341	8.03	BA000029.3	490,520	299,598	304,451	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_1460	5,281	9.83	BA000029.3	490,520	53,451	58,496	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_2770	5,280	10.04	BA000029.3	490,520	66,550	70,862	0.0	<i>O. sativa japonica</i> mitochondrial genome
ctg_1612	5,236	9.26	BA000029.3	490,520	65,707	70,862	0.0	<i>O. sativa japonica</i> mitochondrial genome

The best hit sequences were found by BlastN searches using contig sequences as queries. Contigs similar to sequences from chloroplasts, mitochondria, and ribosome are indicated by green, orange, and blue, respectively.

Supplementary Table S2. Characterization of the 30 longest contigs among the *de novo* assembly of the ginseng Pg2 dataset (0.05x genome coverage; 50x cp genome coverage).

Ctg_no.	Ctg length (bp)	Ctg Coverage (x)	Best hit in GenBank					Description
			Acc. no.	Length (bp)	Match begin	Match end	E-value	
ctg_3	86,351	49.12	AY582139.1	156,318	1	86,125	0.0	<i>P. ginseng</i> chloroplast genome
ctg_8	26,153	103.15	AY582139.1	156,318	86,107	112,177	0.0	<i>P. ginseng</i> chloroplast genome
ctg_15	18,122	45.15	AY582139.1	156,318	112,159	130,266	0.0	<i>P. ginseng</i> chloroplast genome
ctg_30	9,423	150.85	GQ178077.1	3,362	1	3,362	0.0	<i>P. ginseng</i> cv Yunpoong 26S ribosomal RNA gene
ctg_671	5,196	8.55	HQ874649.1	502,773	224,443	225,174	0.0	<i>Ricinus communis</i> mitochondrial genome
ctg_49	4,446	87.98	Copia-74_ALY-I	5,483	1,540	5,043	0.0	<i>Arabidopsis lyrata</i> LTR retrotransposon
ctg_347	4,234	12.01	Gypsy18-PTR_I	7,283	640	4,969	0.0	<i>Populus trichocarpa</i> LTR retrotransposon
ctg_269	3,671	7.49	GQ856147.1	3,792,376	277,261	278,161	0.0	<i>Citrullus lanatus</i> mitochondrial genome
ctg_41	3,555	21.38	Copia-88_VV-I	7,228	1,627	2,612	4.00E-174	<i>Vitis vinifera</i> LTR retrotransposon
ctg_658	3,462	4.13	EU365401.1	509,941	103,101	103,722	0.0	<i>Bambusa oldhamii</i> mitochondrial genome
ctg_258	3,447	4.27	HQ874649.1	502,773	223,813	224,397	0.0	<i>Ricinus communis</i> mitochondrial genome
ctg_181	3,350	43.54	EnSpm-6_STu	6,594	3,865	4,636	3.00E-50	<i>Solanum tuberosum</i> EnSpm DNA transposon
ctg_321	3,346	4.86	JQ248574	281,132	118,398	119,277	0.0	<i>Daucus carota</i> ssp. <i>sativus</i> mitochondrial genome
ctg_428	3,242	6.19	JQ248574	281,132	225,651	227,064	0.0	<i>Daucus carota</i> ssp. <i>sativus</i> mitochondrial genome
ctg_256	3,189	23.66	EnSpm2_PTr	12,422	2,262	2,393	3.00E-28	<i>Populus trichocarpa</i> EnSpm-type DNA transposon
ctg_34	3,147	6.08	JQ248574	281,132	152,185	153,281	0.0	<i>Daucus carota</i> ssp. <i>sativus</i> mitochondrial genome
ctg_359	3,142	13.20	Copia-3_CP-I	4,073	1,463	2,198	1.00E-140	<i>Carica papaya</i> LTR retrotransposon
ctg_407	3,077	16.79	MuDR-1_STu	9,408	1,590	1,687	2.00E-09	<i>Solanum tuberosum</i> MuDR-type DNA transposon
ctg_93	2,900	4.55	EU431224.1	476,890	136,810	136,903	2.00E-29	<i>Carica papaya</i> mitochondrial genome
ctg_352	2,893	39.53	GYPOT1_I	5,099	2,780	3,366	3.00E-87	<i>Populus trichocarpa</i> internal sequence of GYPOT LTR
ctg_157	2,637	4.77	JN375330.1	715,001	704,715	705,342	0.0	<i>Phoenix dactylifera</i> mitochondrial genome
ctg_101	2,609	10.09	Copia-72_VV-I	4,168	2,384	3,690	1.00E-153	<i>Vitis vinifera</i> LTR retrotransposon
ctg_935	2,556	4.15	BA000042.1	430,597	127,414	127,860	0.0	<i>Nicotiana tabacum</i> mitochondrial genome
ctg_833	2,534	5.41	Copia-4_PD-I	4,256	1,437	1,511	4.00E-07	<i>Phoenix dactylifera</i> LTR retrotransposon
ctg_631	2,466	5.16	AY061993.1	4,584	824	1,259	0.0	<i>Daucus carota</i> mitochondrial genome
ctg_420	2,460	30.33	PSAT6	607	456	553	3.00E-11	<i>Pisum sativum</i> dispersed repetitive DNA, PSAT6
ctg_240	2,385	19.22	MuDR-5_ALy	9,235	2,468	2,573	3.00E-13	<i>Arabidopsis lyrata</i> MuDR-type DNA transposon
ctg_26	2,380	34.72	Gypsy22-VV_I	5,609	1,978	2,480	1.00E-64	<i>Vitis vinifera</i> LTR retrotransposon
ctg_687	2,338	5.31	FR714868.1	396,947	135,650	136,768	0.0	<i>Malus x domestica</i> mitochondrial genome
ctg_75	2,240	120.50						No hit

The best hit sequences were found by BlastN searches using contig sequences as queries. Contigs similar to sequences from chloroplasts, mitochondria, and ribosomes are indicated by green, orange, and blue, respectively.

Supplementary Table S3. Summary of cp genome assembly using different amounts of WGS data from *O. sativa* (cv. Nipponbare) and *P. ginseng* (cv. Chunpoong)

Species	Dataset	Amount of WGS used	Coverage (x) to		No. of cp contigs ^c	No. of errors in the initial assembly				
			Genome ^a	Cp ^b		False gaps	False SNPs	Tandem repeats	Mono-polymer	Total errors
<i>O. sativa</i>	Os1	215,000,114	0.5	24.63	4	1	5	0	2	8
	Os2	430,000,026	1	49.31	5	5	2	0	0	7
	Os3	860,000,052	2	99.26	5	0	0	0	1	1
	Os4	1,290,000,078	3	148.67	3	4	0	0	0	4
	Os5	1,720,000,104	4	198.48	3	3	6	0	0	9
	Os6	2,150,000,130	5	248.5	3	2	0	0	0	2
	Os7	4,300,000,260	10	496.03	4	4	0	0	1	5
	Os8	8,600,000,520	20	980.42	4	7	7	1	0	15
	Os9	25,800,001,560	50	2,534.87	6	2	1	0	1	4
	Os10	44,425,734,760	100	5,006.54	10	13	38	3	1	55
<i>P. ginseng</i>	Pg1	75,750,000	0.025	24.49	3	6	4	3	0	13
	Pg2	151,500,000	0.05	49.89	3	9	2	1	0	12
	Pg3	303,000,000	0.1	99.06	3	4	0	2	0	6
	Pg4	454,500,000	0.15	150.38	3	3	1	2	0	6
	Pg5	606,000,000	0.2	200.54	3	3	0	2	0	5
	Pg6	757,500,000	0.25	260.13	3	3	0	2	0	5
	Pg7	1,515,000,000	0.5	512.77	3	0	0	4	0	4
	Pg8	3,030,000,000	1	1,044.53	3	4	0	2	0	6
	Pg9	7,575,000,000	2	2,564.97	3	1	0	3	0	4
	Pg10	15,150,000,000	5	5,101.95	3	2	0	2	0	4
	Pg11	30,300,000,000	10	10,008.88	2	0	2	5	0	7

^a Coverage to genome was determined by calculation of ratio of total bases to genome size (430 Mb of *O. sativa* and 3.12 Gb of *P. ginseng*). ^b Coverage to cp genome and nR sequence were based on contents of cp and nR reads determined by mapping raw reads to reference cp genome and nR sequence. ^c Number of contigs representing the entire cp genome sequence.

Supplementary Table S4. Estimate of nR content based on FISH signal size in the rice genome

Chr. no.	Relative size (%) in the genome		Estimated DNA content (Mbp)		Estimated nR copy number
	Chromosome	nR	Chromosome	nR	
1	10.84		46.61		
2	10.03		43.12		
3	9.24		39.71		
4	9.10		39.13		
5	8.71		37.47		
6	7.78		33.46		
7	7.74		33.30		
8	7.05		30.31		
9	8.29	1.65	35.67	7.09 ^a	894 ^c
10	6.86		29.51		
11	8.01	0.56	34.46	2.40 ^b	7,409 ^d
12	6.33		27.24		
Total	100.00		429,88		

^a45S, ^b5S, ^cbased on one 45S unit of 7.928 kb, ^dbased on 324 bp Nipponbare 5S unit length. Note: 5S and 45S loci are based on previous studies.

Supplementary Table S5. Number of monopolymer tracts in cp genome sequences of rice (*O. sativa J. cv. Nipponbare*)

Monopolymer type	Number
(A) ₈₋₁₃	49
(T) ₈₋₁₇	40
(C) ₈₋₁₁	4
(G) ₈₋₁₀	2
Total	95

Supplementary Table S6. Primers used for validation of cp genome polymorphism

Primer name	Primer sequence (5' → 3')	Tm (°C)
Os07-F	TGGATGAATCAGTTCAAAGAATTTA	59.1
Os07-R	GTGCCGCCTGATCAATAAAA	61.0
Os17-F	TTCCGCTACACATAAAAATGGA	60.7
Os17-R	TCCCTTCGTAACCAGTCGTC	60.1
Os18-F	AACCATGGGTCGCTTCTAGT	58.7
Os18-R	GGGAGGAGTGGACTCTAGG	60.1

Supplementary Datasets

Supplementary Dataset S1.

The cp genome sequences of seven *Oryza* species. WGS sequences of these seven accessions were retrieved from GenBank (Table 1) and the cp genome sequence was obtained using the dnaLCW method. The header of each FASTA sequence indicates species name and accession number.

>O.rufipogon_AC111008369_cp

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Supplementary Dataset S2.

The 45S transcription unit sequences of seven *Oryza* species. WGS sequences of these seven accessions were retrieved from GenBank (Table 1) and the 45S sequence was obtained using the dnaLCW method. The header of each FASTA sequence indicates species name and accession number.

>O.rufipogon_AC11-1008369_45S

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Supplementary Dataset S3.

The 5S transcription unit sequences of seven *Oryza* species. WGS sequences of these seven accessions were retrieved from GenBank (Table 1) and the 5S sequence was obtained using the dnaLCW method. The header of each FASTA sequence indicates species name and accession number.

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