

Supplementary Tables

Table S1. Gene content and gene order of the chloroplast genomes of three *Macrosolen*

Species	Regions	Positions	species				Length (bp)	
			T(U) (%)	C (%)	A (%)	G (%)		
<i>Macrosolen cochinchinensis</i>	Total		31.9	19.0	30.8	18.3	129,570	
	IRa		29.0	22.0	28.8	20.3	25,397	
	IRb		28.8	20.3	29.0	22.0	25,397	
	LSC		33.4	18.0	31.8	16.9	73,052	
	SSC		40.6	12.5	35.3	11.6	5,724	
	CDS		31.3	17.3	31.4	19.9	64,743	
		1st position		23.8	18.5	31.4	26.2	21,581
		2nd position		32.3	19.6	30.7	17.4	21,581
		3rd position		37.9	13.9	32.1	16.1	21,581
<i>Macrosolen tricolor</i>	Total		31.8	19.2	30.5	18.5	126,621	
	IRa		29.0	22.1	28.6	20.3	24,703	
	IRb		28.6	20.3	29.0	22.1	24,703	
	LSC		33.3	18.2	31.5	17.0	71,895	
	SSC		40.3	13.4	33.8	12.4	5,320	
	CDS		31.3	17.4	31.3	20.0	64,794	
		1st position		23.8	18.6	31.3	26.2	21,598
		2nd position		32.3	19.6	30.6	17.5	21,598
		3rd position		37.7	13.9	32.1	16.2	21,598
<i>Macrosolen bibracteolatus</i>	Total		31.7	19.3	30.5	18.6	127,169	
	IRa		29.0	22.1	28.7	20.3	25,445	
	IRb		28.7	20.3	29.0	22.1	25,445	
	LSC		33.1	18.4	31.4	17.1	70,692	
	SSC		39.8	13.4	34.0	12.7	5,587	
	CDS		31.2	17.4	31.3	20.0	64,560	
		1st position		23.8	18.8	31.3	26.2	21,520
		2nd position		32.3	19.6	30.6	17.5	21,520
		3rd position		37.6	13.9	32.1	16.4	21,520

1. CDS: protein-coding regions; 2. 1st position: 1st base of codons; 3. 2nd position: 2nd base of codons; 4. 3rd position: 3rd base of codons.

Table S2. Genes content introns of three *Macrosolen* species

Species	Gene	Location	Exon1(bp)	Intron1(bp)	Exon2(bp)	Intron2(bp)	Exon3(bp)
<i>M. cochinchinensis</i>	<i>atpF</i>	LSC	150	750	405		
	<i>clpP</i>	LSC	71	838	292	617	228
	<i>petB</i>	LSC	6	755	642		
	<i>petD</i>	LSC	5	538	478		

<i>M. tricolor</i>	<i>rpl2</i>	LSC, IR	395	655	433		
	<i>rpl16</i>	LSC	9	990	399		
	<i>rpoC1</i>	LSC	432	749	1,614		
	<i>rps12</i>	LSC, IR	114	--	232	520	26
	<i>trnA-UGC</i>	IR	38	815	35		
	<i>trnI-GAU</i>	IR	35	912	37		
	<i>trnK-UUU</i>	LSC	37	2,473	35		
	<i>trnL-UAA</i>	LSC	37	291	50		
	<i>ycf3</i>	LSC	125	726	229	698	153
	<i>atpF</i>	LSC	150	745	405		
	<i>clpP</i>	LSC	71	828	292	594	228
	<i>petB</i>	LSC	6	758	642		
	<i>petD</i>	LSC	5	556	478		
	<i>rpl2</i>	LSC, IR	395	656	433		
	<i>M. bibracteolatus</i>	<i>rpl16</i>	LSC	9	1,003	399	
<i>rpoC1</i>		LSC	432	721	1,614		
<i>rps12</i>		LSC, IR	114	--	232	531	26
<i>trnA-UGC</i>		IR	38	815	35		
<i>trnI-GAU</i>		IR	35	909	37		
<i>trnK-UUU</i>		LSC	37	2,497	35		
<i>trnL-UAA</i>		LSC	37	283	50		
<i>ycf3</i>		LSC	125	728	229	680	153
<i>atpF</i>		LSC	150	744	405		
<i>clpP</i>		LSC	71	790	292	598	228
<i>petB</i>		LSC	6	752	642		
<i>petD</i>		LSC	5	532	478		
<i>rpl2</i>		LSC, IR	395	656	433		
<i>rpl16</i>		LSC	9	990	399		
<i>rpoC1</i>		LSC	432	721	1,614		
<i>rps12</i>	LSC, IR	114	--	232	531	26	
<i>trnA-UGC</i>	IR	38	814	35			
<i>trnI-GAU</i>	IR	35	909	37			
<i>trnK-UUU</i>	LSC	37	2,490	35			
<i>trnL-UAA</i>	LSC	37	278	50			
<i>ycf3</i>	LSC	125	758	229	689	153	

Table S3. Codon usage of three *Macrosolen* species

Amino acid	Codon	No.			RSCU			Amino acid	Codon	No.			RSCU		
		①	②	③	①	②	③			①	②	③	①	②	③
Phe	TTT	874	876	860	1.35	1.36	1.34	Tyr	TAT	631	629	626	1.62	1.63	1.62
Phe	TTC	421	414	422	0.65	0.64	0.66	Tyr	TAC	149	143	145	0.38	0.37	0.38
Leu	TTA	697	700	686	1.89	1.89	1.86	Stop	TAA	33	33	32	1.46	1.46	1.41
Leu	TTG	481	486	489	1.30	1.31	1.32	Stop	TAG	17	16	18	0.75	0.71	0.79
Leu	CTT	466	458	459	1.26	1.24	1.24	Stop	TGA	18	19	18	0.79	0.84	0.79

Leu	CTC	146	150	151	0.40	0.40	0.41	Trp	TGG	368	372	370	1.00	1.00	1.00
Leu	CTA	279	282	282	0.76	0.76	0.76	Gln	CAA	603	605	611	1.51	1.52	1.52
Leu	CTG	143	147	150	0.39	0.40	0.41	Gln	CAG	198	189	192	0.49	0.48	0.48
Ile	ATT	894	889	887	1.46	1.46	1.46	Asn	AAT	827	823	807	1.54	1.53	1.53
Ile	ATC	350	348	354	0.57	0.57	0.58	Asn	AAC	246	250	249	0.46	0.47	0.47
Ile	ATA	592	593	577	0.97	0.97	0.95	Lys	AAA	1,005	1,010	1,006	1.53	1.54	1.54
Met	ATG	477	475	479	1.00	1.00	1.00	Lys	AAG	311	304	303	0.47	0.46	0.46
Val	GTT	411	411	407	1.44	1.43	1.42	Asp	GAT	737	745	732	1.62	1.63	1.64
Val	GTC	141	148	144	0.49	0.52	0.50	Asp	GAC	173	170	163	0.38	0.37	0.36
Val	GTA	427	420	427	1.49	1.46	1.49	Glu	GAA	824	820	823	1.46	1.46	1.46
Val	GTG	166	170	168	0.58	0.59	0.59	Glu	GAG	302	305	306	0.54	0.54	0.54
Ser	TCT	441	433	433	1.62	1.58	1.59	Pro	CCT	301	295	295	1.42	1.38	1.38
Ser	TCC	267	270	268	0.98	0.99	0.99	Pro	CCC	199	204	200	0.94	0.95	0.94
Ser	TCA	334	342	339	1.23	1.25	1.25	Pro	CCA	247	248	246	1.17	1.16	1.15
Ser	TCG	164	171	169	0.60	0.62	0.62	Pro	CCG	100	109	111	0.47	0.51	0.52
Ser	AGT	344	342	341	1.26	1.25	1.25	Arg	CGT	283	285	288	1.29	1.29	1.30
Ser	AGC	82	84	82	0.30	0.31	0.30	Arg	CGC	66	70	70	0.30	0.32	0.32
Cys	TGT	177	176	176	1.47	1.47	1.48	Arg	CGA	308	321	316	1.40	1.45	1.43
Cys	TGC	64	63	62	0.53	0.53	0.52	Arg	CGG	98	97	100	0.45	0.44	0.45
Thr	ACT	432	437	428	1.58	1.61	1.57	Arg	AGA	411	395	397	1.87	1.79	1.79
Thr	ACC	213	213	212	0.78	0.78	0.78	Arg	AGG	151	157	158	0.69	0.71	0.71
Thr	ACA	315	307	311	1.15	1.13	1.14	His	CAT	421	424	421	1.49	1.49	1.49
Thr	ACG	131	132	137	0.48	0.48	0.50	His	CAC	144	144	145	0.51	0.51	0.51
Ala	GCT	479	476	476	1.76	1.76	1.77	Gly	GGT	462	454	453	1.33	1.3	1.30
Ala	GCC	179	181	178	0.66	0.67	0.66	Gly	GGC	150	155	154	0.43	0.44	0.44
Ala	GCA	300	299	298	1.10	1.11	1.11	Gly	GGA	535	539	534	1.54	1.54	1.53
Ala	GCG	129	124	126	0.47	0.46	0.47	Gly	GGG	247	251	253	0.71	0.72	0.73

① *M. cochinchinensis*; ② *M. tricolor*; ③ *M. bibracteolatus*

Table S4. Potential RNA editing sites of three *Macrosolen* species

Gene	Nucleotide Position			Codon change	Amino acid change	Score
	①	②	③			
<i>accD</i>	-	-	-	-	-	-
<i>atpA</i>	773	773	773	TCA-TTA	S-L	1
	914	914	914	TCA-TTA	S-L	1
	1,148	1,148	1,148	TCA-TTA	S-L	1
<i>atpB</i>	-	-	-	-	-	-
<i>atpF</i>	92	92	92	CCA-CTA	P-L	0.86
<i>atpI</i>	23	23	23	ACC-ATC	T-I	1
	629	629	629	TCA-TTA	S-L	1
<i>ccsA</i>	137	137	137	ACA-ATA	T-I	1
<i>clpP</i>	559	559	559	CAT-TAT	H-Y	1
<i>matK</i>	322	322	322	CTT-TTT	L-F	0.86

	646	646	646	CAT-TAT	H-Y	1
	1,384	1,384	1,384	CCG-TCG	P-S	0.86
	-	1433	-	TCG-TTG	S-L	1
<i>petB</i>	418	418	418	CGG-TGG	R-W	1
	611	611	611	TCA-TTA	S-L	1
<i>petD</i>	-	-	-	-	-	-
<i>petG</i>	-	-	-	-	-	-
<i>petL</i>	-	-	-	-	-	-
<i>psaB</i>	-	-	-	-	-	-
<i>psaI</i>	-	-	-	-	-	-
<i>psbB</i>	305	305	305	GCA-GTA	A-V	0.86
<i>psbE</i>	-	-	-	-	-	-
<i>psbF</i>	-	-	-	-	-	-
<i>psbL</i>	2	-	-	ACG-ATG	T-M	1
<i>rpl2</i>	-	-	-	-	-	-
<i>rpl20</i>	308	308	308	TCA-TTA	S-L	0.86
<i>rpl23</i>	-	-	-	CAT-TAT	H-Y	1
<i>rpoA</i>	368	368	368	TCG-TTG	S-L	1
	745	745	745	CTT-TTT	L-F	0.86
<i>rpoB</i>	338	338	338	TCT-TTT	S-F	1
	430	430	430	CCG-TCG	S-L	0.86
	-	473	473	TCA-TTA	S-L	0.86
	551	551	551	TCA-TTA	S-L	1
	566	566	566	TCG-TTG	S-L	1
	898	898	898	CTT-TTT	L-F	0.86
	2,342	-	2,342	ACA-ATA	T-I	1
<i>rpoC1</i>	-	41	41	TCG-TTG	S-L	1
	-	1,762	1,762	CAT-TAT	H-Y	0.86
<i>rpoC2</i>	815	815	815	GCC-GTC	A-V	0.86
	827	-	-	ACA-ATA	T-I	1
	1,256	1,256	1,256	GCG-GTG	A-V	0.86
	3,686	3,686	3,692	TCA-TTA	S-L	0.86
	4,064	4,076	4,082	TCT-TTT	S-F	0.8
<i>rps2</i>	248	248	248	TCA-TTA	S-L	1
<i>rps8</i>	92	92	92	ACC-ATC	T-I	1
	182	182	182	TCA-TTA	S-L	1.86
<i>rps14</i>	80	80	80	TCA-TTA	S-L	1
	149	149	149	CCA-CTA	P-L	1
<i>ycf3</i>	-	-	-	-	-	-

① *M. cochinchinensis*; ② *M. tricolor*; ③ *M. bibracteolatus*

Table S5. Primer sequences at the boundaries between SC and IR regions of three *Macrosolen* species

Species	Regions	Forward/Reverse	Primer sequence (5' to 3')	
<i>M. cochinchinensis</i>	LSC-IRa	F	TCCGCCTTTGTGTTAAGCTT	
		R	CGGTCTGAATGCTACATGAAGA	
	IRa-SSC	F	GCGCACTCAAAGGCGTAAAA	
		R	AAAACCTGCTGTCATGCCCT	
	SSC-IRb	F	AACCGTTGTGATGGGAAAGACA	
		R	AAAGGTTCCATGCGCACTCA	
	IRb-LSC	F	CGGTCTGAATGCTACATGAAGA	
		R	CCGTGCTAACCTTGGTATGG	
	<i>M. bibracteolatus</i>	LSC-IRa	F	GTAGATGCTCGGGACCAAGT
			R	ACATAAGCCAGATGACGGAAC
		IRa-SSC	F	TGATCAAGCAGAGGAGGTGG
			R	GGAGAAACCCCATGAAAGGA
SSC-IRb		F	ACCGTTGGGATGTGAAAGACA	
		R	GCGCACTCAAAGGCGTAAAA	
IRb-LSC		F	ACATAAGCCAGATGACGGAAC	
		R	TGGTTCACAGCCTTAGGTATCA	
<i>M. tricolor</i>		LSC-IRa	F	CCCCTTCCGTTATAGATCGAAA
			R	CCATGAGTGATTTGGCAGATTT
		IRa-SSC	F	CAAGCAGAGGAGGTGGCTTT
			R	CTGCTGTCATGCCCTTTTCTG
	SSC-IRb	F	AAACCGTTGTGATGGGAAAGACA	
		R	TGCGCACTCAAAGGCGTAAA	
	IRb-LSC	F	ACATAAGCCAGATGACGGAAC	
		R	CCGTGCTAACCTTGGTATGG	

The thermocycler conditions for the PCR:

94°C 5min;

94°C 30s, 56°C 30s, 72°C 45s, 40 cycles;

72°C 10min.