

Table S1. Housekeeping genes for codon usage measurement

Gene ID	Abbreviation	Protein
PF00281.14	<i>rplE</i>	Ribosomal protein L5
PF00297.17	<i>rplC</i>	Ribosomal protein L3
PF00347.18	<i>rplF</i>	Ribosomal protein L6
PF00416.17	<i>rpsM</i>	Ribosomal protein S13/S18
PF00573.17	<i>rplD</i>	Ribosomal protein L4/L1 family
TIGR00116	<i>tsf</i>	translation elongation factor Ts
TIGR00158	<i>rplK</i>	ribosomal protein L9
TIGR00855	<i>rplL</i>	ribosomal protein L7/L12
TIGR01009	<i>rpsC</i>	ribosomal protein S3
TIGR01011	<i>rpsB</i>	ribosomal protein S2
TIGR01021	<i>rpsE</i>	ribosomal protein S5
PF00380.14	<i>rpsI</i>	Ribosomal protein S9/S16
TIGR01067	<i>rplN</i>	ribosomal protein L14
TIGR01164	<i>rplP</i>	ribosomal protein L16
TIGR01169	<i>rplA</i>	ribosomal protein L1
TIGR01171	<i>rplB</i>	ribosomal protein L2
TIGR01632	<i>rplK</i>	ribosomal protein L11
TIGR01953	<i>nusA</i>	transcription termination factor NusA

Table S2. The close relatives of “*Ca. Spiroplasma holothuricola*” and symbionts for comparison

Species	Accession
<i>Spiroplasma chrysopicola</i> DF 1	CP005077
<i>Spiroplasma apis</i> B31	CP006682
<i>Spiroplasma diminutum</i> CUAS 1	CP005076
<i>Spiroplasma syrphidicola</i> EA 1	NC 021284
<i>Spiroplasma taiwanense</i> CT 1	CP005074
<i>Mycoplasma suis</i> KI3806	NC_015153
<i>Mycoplasma</i> sp. Bg1	PRJNA309720
<i>Mycoplasma</i> sp. Bg2	PRJNA309720
<i>Buchnera aphidicola</i>	NC 002528
" <i>Candidatus</i> Moranella endobia"	NC 01573
" <i>Candidatus</i> Nasuia deltocephalinicola"	CP013211;CP013212
<i>Ureaplasma urealyticum</i> serovar 10 ATCC 33699	NC_002162
" <i>Candidatus</i> Spiroplasma holothuricola"	CP022928;CP022929

Table S3. tRNA genes in “*Ca. Spiroplasma holothuricola*” genome

ID	chromosome	start	end	AA type	anticodon
2_trna3	2	115516	115592	Elongator Met	CAT
1_trna3	1	120679	120756	Initiator Met	CAT
1_trna8	1	108012	107937	Ala	TGC
1_trna27	1	83108	83033	Arg	TCT
1_trna12	1	210086	210012	Arg	CCT
2_trna2	2	111918	111994	Arg	TCG
1_trna15	1	246627	246553	Asn	GTT
2_trna4	2	118414	118488	Asp	GTC
1_trna2	1	108146	108069	Cys	GCA
1_trna12	1	223027	223101	Gln	TTG
1_trna6	1	176362	176436	Gln	CTG
1_trna18	1	246527	246452	Glu	TTC
1_trna17	1	246424	246349	Glu	CTC
1_trna9	1	213976	214049	Gly	TCC
1_trna26	1	105173	105098	His	GTG
1_trna22	1	120859	120934	Ile	GAT
1_trna23	1	209920	209994	Ile	TAT
1_trna5	1	176238	176325	Leu	GAG
1_trna7	1	183226	183311	Leu	TAG
1_trna14	1	224541	224627	Leu	TAA
1_trna16	1	230619	230705	Leu	CAA
1_trna13	1	224465	224539	Lys	TTT
2_trna1	2	66201	66276	Lys	CTT
2_trna5	2	118490	118564	Phe	GAA
1_trna19	1	107930	107852	Pro	TGG
1_trna4	1	120952	121042	Ser	TGA
1_trna20	1	246765	246859	Ser	GCT
1_trna11	1	222851	222928	Thr	TGT
1_trna1	1	117	190	Trp	TCA
1_trna21	1	252493	252566	Trp	CCA
1_trna24	1	222930	223014	Tyr	GTA
1_trna10	1	222770	222845	Val	TAC

Table S4. tRNAs in transcriptomic data as identified by Blastn

frag ment	subject ID	tRNA Type	anti- codon	% identity	aligned region(bp)	query start	query end	subject start	subject end	e-value
1	1_trna10	Val	TAC	100	43	1	43	53	11	1E-21
2	1_trna11	Thr	TGT	100	47	1	47	75	29	6.00E-24
3	1_trna16	Leu	CAA	100	36	2	37	83	48	2.00E-17
4	1_trna26	His	GTG	100	43	1	43	59	17	1E-21
5	1_trna26	His	GTG	100	59	2	60	75	17	8.00E-29
6	1_trna26	His	GTG	100	39	1	39	51	13	9.00E-18
7	1_trna27	Arg	TCT	100	37	1	37	40	76	1.00E-16
8	1_trna27	Arg	TCT	100	31	26	56	76	46	3.00E-13
9	1_trna27	Arg	TCT	100	29	23	51	76	48	3.00E-12
10	1_trna4	Ser	TGA	100	41	1	41	61	21	2.00E-20
11	1_trna5	Leu	GAG	100	53	1	53	69	17	1.00E-25
12	1_trna5	Leu	GAG	100	63	1	63	9	71	5.00E-31
13	1_trna7	Leu	TAG	100	58	1	58	10	67	3.00E-28
14	1_trna7	Leu	TAG	100	57	1	57	69	13	1.00E-27
15	1_trna9	Gly	TCC	100	47	1	47	70	24	6.00E-24
16	1_trna9	Gly	TCC	100	59	23	81	74	16	1.00E-28
17	2_trna2	Arg	TCG	100	49	4	52	77	29	2.00E-23
18	2_trna2	Arg	TCG	100	49	4	52	77	29	2.00E-23
19	2_trna3	Met	CAT	100	39	1	39	39	1	3.00E-19
20	2_trna3	Met	CAT	100	39	1	39	39	1	3.00E-19

The tRNA IDs were described in Table S1. The query and subject of the Blastn search were the Illumina cDNA fragments and tRNA genes, respectively.