

1 **Cicada endosymbionts have tRNAs that are correctly processed despite having genomes**
2 **that do not encode all of the tRNA processing machinery**

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26 **SUPPLEMENTARY MATERIAL**

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28 **Supplementary material S1.** Trinotate annotations for all transcripts assembled using Trinity.

29 Available at <https://doi.org/10.6084/m9.figshare.6687089.v1>.

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31 **Supplementary material S2.** Expression level of gene-level transcripts that were differentially

32 expressed between bacteriome and other cicada tissues. Shown as log₂ fold-change. Available at

33 <https://doi.org/10.6084/m9.figshare.6687089.v1>.

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35 **Supplementary material S3.** Trinotate annotations for transcripts there are expressed higher in

36 cicada bacteriome tissues than in other cicada tissues. Available at

37 <https://doi.org/10.6084/m9.figshare.6687089.v1>.

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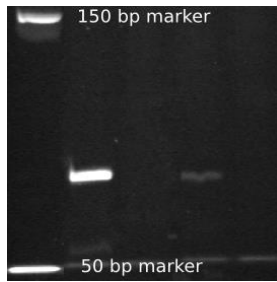
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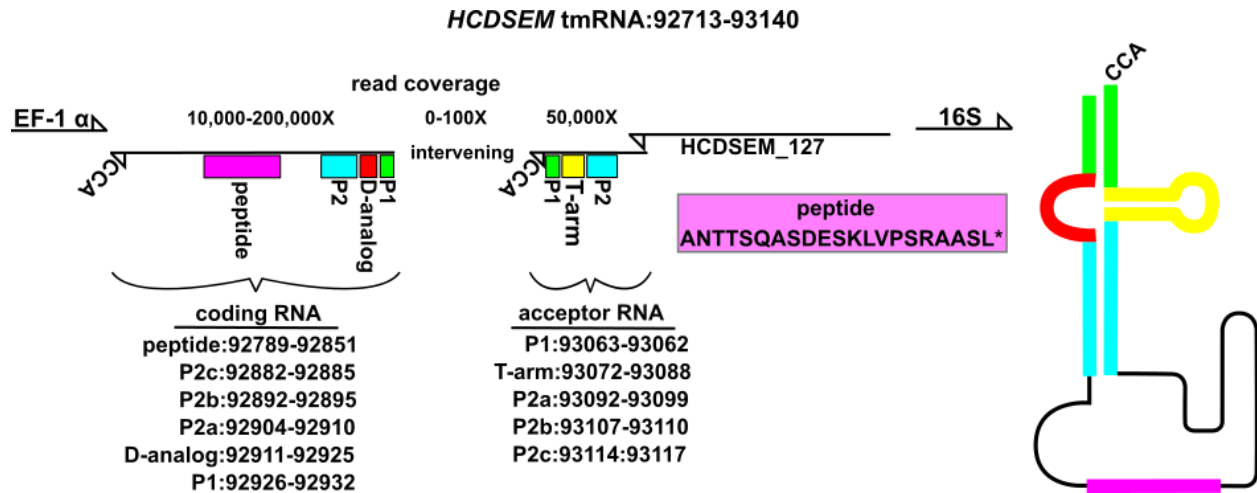
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62 **FIG S1.** To show a difference between highly expressed and lowly expressed transcripts, RT-
63 PCR was done on total bacteriome RNA using primers specific for *Hodgkinia* tRNA^{Ala} and
64 *Hodgkinia* tRNA^{Cys}. Lanes 1-5: DNA marker, tRNA^{Ala} primers, tRNA^{Ala} primers no RT control,
65 tRNA^{Cys} primers, tRNA^{Cys} primers no RT control.



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108 **FIG S2.** Proposed tmRNA gene in *Hodgkinia* lies between genes for EF-1 alpha and 16S rRNA.
 109 The direction of transcription is indicated by an arrow. EF-1 alpha and 16S are encoded on the
 110 sense strand. The tmRNA and *Hodgkinia_127* are encoded on the anti-sense strand. Two small
 111 RNA transcripts with high coverage were identified as shown by separate arrows. Read depth
 112 across the tmRNA gene varies from 0-50,000X. Coordinates of tmRNA features are shown for
 113 the coding and acceptor RNAs.
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147 **Table S1.** tRNA and aaRS genes in the smallest bacterial genomes. Shaded cells indicate that the
 148 aaRS gene in the left column is missing. ¹aaRS is heteromeric. ²probable pseudogenes.
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	DICSEM	TETULN	TETUND1	TETUND2	MAGTRE	TPPAVE	TPPLON	TPPCIT
valS		UAC		UAC		GAC	GAC(2)	² CAC
ileS	GAU	GAU		GAU	GAU	CAU,GAU	CAU	GAU
proS	UGG	UGG	UGG	UGG	UGG	UGG		
hisS	GUG	GUG		GUG		GUG	GUG	² GUG
trpS	UCA	UCA	UCA	UCA	UCA	CCA		CCA
metG	CAU(3)	CAU(3)	CAU(2)	CAU(2)	CAU	CAU(2)	CAU	CAU
gltX	UUC	UUC		UUG		UUC	UUC	
pheS ¹	GAA	GAA	GAA	GAA		GAA	GAA	GAA
pheT ¹								
alaS	UGC	UGC	² GGC,UGC(2)	UGC		UGC	ACG,CCU	UGC
glyS ¹	UCC,GCC(2)	UCC,GCC	UCC	UCC,GCC	UCC,GCC	GCC,UCC	GCC,UCC	
glyQ ¹								
serS						CGA,GCU,GGA, UGA	ACU	UGA
asnS						GUU	GUU	
tryrS						GUA		
glnS	UUG	UUG	UUG	UUG	UUG	UUG		UUC
lysS	UUU	UUU		UUU	UUU	CUU,UUU	CUU(2)	CUU(3)
leuS						CAA,UAG	CAG	
argS						ACG,UCU		ACG
aspS		GUC	GUC	GUC		GUC	GUC	
thrS		UGU,GGU	UGU	UGU,GGU		CGU,GGU,UGU		
cysS	GCA	GCA	GCA	GCA	GCA	GCA	GCA	

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162 **Table S2.** Number of reads in the small RNA dataset.

	Index1 (TAP)	Index2 (untreated)	Index3 (untreated)	Index4 (untreated)
Raw	77,189,680	19,096,461	47,564,122	82,862,668
Quality/length filtered	60,627,486	12,657,156	38,406,203	33,486,002
18-90nts	48,521,525	10,914,315	28,597,483	24,618,283
Mapped to <i>Hodgkinia</i>	7,484,021	1,565,097	7,677,511	5,903,119
Mapped to <i>Sulcia</i>	21,424,135	5,094,798	9,313,149	5,360,814
Mapped to mitochondria	261,693	83,062	57,994	77,055
Mapped to <i>Hodgkinia</i> tRNAs	2,545,941	635,453	101,749	386,447
Mapped to <i>Sulcia</i> tRNAs	15,582,990	3,489,686	1,732,283	2,646,973
Mapped to mitochondrial tRNAs	338,590	104,637	60,532	120,499
48-90nts	13,855,233	3,713,578	21,300,447	13,163,499
Mapped to <i>Hodgkinia</i>	3,706,879	24,261	7,151,011	4,812,614
Mapped to <i>Sulcia</i>	4,127,670	1,067,020	6,835,654	2,087,571
Mapped to mitochondria	81,003	24,261	47,331	38,985
Mapped to <i>Hodgkinia</i> tRNAs	13,254	4,277	17,712	39,408
Mapped to <i>Sulcia</i> tRNAs	885,324	229,957	691,271	714,197
Mapped to mitochondrial tRNAs	57,593	18,308	47,960	51,929
70-100nts	17,917,568	3,862,380	23,160,979	14,622,761
Mapped to <i>Hodgkinia</i>	3,263,929	554,659	6,775,227	4,046,344
Mapped to <i>Sulcia</i>	9,572,979	1,591,156	10,075,795	6,042,490
Mapped to mitochondria	26,122	4,996	6,320	3,718
Mapped to <i>Hodgkinia</i> tRNAs	5,644	1,767	8,209	8,723
Mapped to <i>Sulcia</i> tRNAs	520,386	144,229	617,538	659,736
Mapped to mitochondrial tRNAs	674	128	321	558

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180 **Table S3.** Primer sequences to amplify tRNAs from total RNA, genomic DNA, and finished
 181 library preparations.
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	Forward primer 5' to 3'	Reverse primer 5' to 3'
<i>Ala_129_Hodgkinia</i>	GGGGCTGTAGCTCAATTGG	TGGAGCTAAGCGGACTCG
<i>Cys_041_Hodgkinia</i>	GGCTTCGTGGTATAGGGGT	GGCTTCGCTCAGACTCG
<i>Thr_Sulcia_flanking</i>	CCTGGACAATCTACATGAGCA	GGTAGAGCATCAGCCTTCCA
Split_tRNA_1	AGAGTTGCCGGAGGGGTTAAC	TGGAGAATATCGGATTTGAACCG
Split_tRNA_2	TATGGCAATAACCAAG	TGGAGAATATCGGATTTGAACCG
Split_tRNA_3	GGTGGAGCAGTTGGTAGC	AGCTAAGCGGACTCGAACCGC
Split_tRNA_4	GGTGAACGTAGCTCAATTGG	TGGAGCTAAGCGGACTCG
Split_tRNA_5	GGATGTAGCGTAGGTTGG	CGGTACCGGGAATCGAACC
Split_tRNA_6	CGCGGGGTGGAGCAGTTGG	CAACGGGGGCAGGAGTCG

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199 **Table S4.** Number of reads mapping to each tRNA gene (plus 15bp flanking sequence) using
 200 bowtie. The 18-90 SAM file was parsed for reads that map to the tRNA with nearly the perfect
 201 length and ending in CCA.
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	18-90	48-90	70-100	tRNA count
SMDSEM_264_Arg	171822	5010	4216	3522
SMDSEM_216_Gln	569993	13458	12207	291
SMDSEM_212_Glu	2345816	170866	163305	120854
SMDSEM_189_Met	390387	33319	29288	12978
SMDSEM_187_Leu	80814	1747	1746	542
SMDSEM_170_Met	110296	16461	15727	8919
SMDSEM_164_Leu	499915	54916	679	219
SMDSEM_163_Leu	142900	14619	14306	2513
SMDSEM_152_Ser	602352	96386	156066	35534
SMDSEM_151_Pro	1192993	2460	1995	1459
SMDSEM_150_Arg	4004740	56468	54731	36894
SMDSEM_138_Ser	232144	122460	4854	2366
SMDSEM_126_Lys	220987	16320	14855	6793
SMDSEM_125_Asp	557210	56272	51479	40892
SMDSEM_115_Val	92904	52027	50574	30918
SMDSEM_091_Leu	127084	28779	22692	17190
SMDSEM_090_Gly	50342	32083	29976	4065
SMDSEM_081_Ala	3605265	263269	122850	91939
SMDSEM_080_Ile	645895	159599	147330	95551
SMDSEM_070_Thr	1204147	175998	132236	68762
SMDSEM_069_Tyr	297423	21774	4172	1938
SMDSEM_068_Gly	249994	24795	22514	17837
SMDSEM_066_Trp	226724	38543	36839	30700
SMDSEM_057_His	272166	11285	9114	7518
SMDSEM_053_Phe	374054	2042	1844	1203
SMDSEM_030_Cys	219277	97275	96060	70828
SMDSEM_021_Asn	4102498	540031	335843	178660
SMDSEM_018_Met	861790	412487	404391	305158
HCDSEM_189_Met	306977	2522	1118	831
HCDSEM_187_His	220501	1654	373	250
HCDSEM_164_Ile	10009	65	8	3
HCDSEM_163_Gln	15973	103	48	30
HCDSEM_143_Pro	52512	6800	5805	9
HCDSEM_142_Glu	141333	37552	981	946
HCDSEM_132_Met	498517	330	108	78
HCDSEM_129_Ala	2254846	11053	7459	5591
HCDSEM_114_Lys	20565	112	58	13
HCDSEM_108_Gly	1859	60	3	0
HCDSEM_103_Phe	3561	156	4	3
HCDSEM_099_Gly	379	171	138	8
HCDSEM_096_Met	11795	1107	83	74
HCDSEM_062_Trp	51683	11390	7899	6754
HCDSEM_061_Gly	75091	388	1	0
HCDSEM_041_Cys	3989	1188	257	168

DICSEMmt_Val_c(13936..14031)	51028	6575	2	295
DICSEMmt_Tyr_c(1625..1720)	3553	107	10	5
DICSEMmt_Trp_1509..1602	6858	75	4	28
DICSEMmt_Thr_9849..9944	17606	692	51	9
DICSEMmt_Ser_6308..6404	142889	114076	108	75887
DICSEMmt_Ser_11602..11697	6822	23	41	1
DICSEMmt_Pro_c(9915..10007)	37229	6479	117	55
DICSEMmt_Phe_c(6433..6528)	2035	8	0	1
DICSEMmt_Met_405..500	29118	1469	3	160
DICSEMmt_Lys_3969..4068	80942	3324	129	83
DICSEMmt_Leu_c(12647..12745)	23661	4271	788	645
DICSEMmt_Leu_3225..3319	15019	421	8	75
DICSEMmt_Ile_266..359	36577	2388	4	162
DICSEMmt_Ile_119..211	476	14	0	0
DICSEMmt_His_c(8193..8286)	1948	13	0	4
DICSEMmt_Gly_5701..5793	8489	3501	85	351
DICSEMmt_Glu_6373..6465	50668	17779	7	61
DICSEMmt_Gln_c(340..433)	34924	2491	323	479
DICSEMmt_Cys_c(1565..1655)	25463	3374	0	59
DICSEMmt_Asp_4039..4130	20488	1068	0	32
DICSEMmt_Asn_6244..6338	17953	3333	0	1370
DICSEMmt_Ala_6114..6207	10512	4309	1	396

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229 **Table S5.** No difference found between TAP treated and untreated libraries by Spearman's rank
 230 correlation and ANOVA, indicating that the 5' of *Hodgkinia* and *Sulcia* tRNAs are properly
 231 processed. Spearman's rank shows significant correlation between tRNA expression of TAP
 232 treated and untreated samples. ANOVA shows no significant difference between tRNA relative
 233 abundance between treated and untreated samples. Relative abundance is the number of reads
 234 corresponding to each tRNA over the total number of reads assigned to all tRNAs in the sample.
 235 Categories for "Organism" include *Sulcia*, *Hodgkinia*, mitochondrial (DICSEM), and other
 236 (unidentified). Significant F values for the ANOVA are F(0.01) = 3.14, F(0.05) = 4.95.
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Spearman's Rank

	<i>Sulcia</i> n (p=0.005 critical value)	<i>Hodgkinia</i> n (p=0.005 critical value)	Rho value <i>Sulcia</i>	Rho value <i>Hodgkinia</i>
20-100nts	28 (0.496)	16 (0.666)	0.932	0.988
70-100nts	28 (0.496)	16 (0.666)	0.943	0.962

ANOVA

	Df	Sum of squares	Mean of squares	F value	Pr (>F)
Organism	4	8.17	2.04	0.4586	0.7658
Amino acid	45	193.69	4.30	0.9665	0.5423
Anticodon	16	59.13	3.70	0.8298	0.6482
Organism:Amino acid	3	4.51	1.50	0.3374	0.7984
Organism:Anticodon	35	208.40	5.95	1.3371	0.1529
Anticodon:Amino acid	3	5.61	1.87	0.4197	0.7394

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257 **Table S6.** tRNA modifications sorted by site. Those shown are at 2% or greater in frequency. The
 258 number of reads matching each of the four nucleotides is shown. The genome sequence at that
 259 position is greyed. ^aEdit occurs on mismatched base-pair in stem region, ^btRNA secondary
 260 structure suggests that the gene is pseudogenized, ^ctRNA with high nucleotide similarity exists in
 261 nuclear genome. 48-90 nucleotide reads used in mapping.
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		A	T	G	C
N1	<i>Hodgkinia_164</i>	18 ^a	0	10	0
	Mito_Ala_6114-6207	137	2	174	0
N2	<i>Hodgkinia_103</i>	0	0	0	8 ^a
N3	<i>Hodgkinia_099</i>	0	15	0	112
N4	<i>Hodgkinia_061</i>	19	0	2	0
	Mito_Cys_1565-1655	0	67	0	48 ^a
N6	Mito_Ile_266-359	281	0	90	2
	Mito_Met_405-500	39	0	279	1
N7	<i>Sulcia_151</i>	64	4	2072	5
N9	<i>Hodgkinia_132</i>	3	16	105	3
	<i>Hodgkinia_142</i>	7	12	1023	15
	Mito_Ile_266-359	7	12	571	16
	Mito_Gln_340-433	210	77	38	0
	Mito_Met_405-500	323	11	3	1
	Mito_Cys_1565-1655	96	23	14	0
	Mito_Lys_3969-4068	4	26	108	20
	Mito_Asp_4039-4130	55	12	18	0
	Mito_Gly_5701-5793	656	190	144	1
	Mito_Ala_6114-6207	515	147	29	0
	Mito_Asn_6244-6338	618	622	1560	28
	Mito_Glu_6373_6465 ^c	310	60	72	0
	Mito_Leu_12647-12745	610	268	113	4
	Mito_Val_13936-14031 ^c	314	179	263	0
N15	<i>Hodgkinia_061</i>	22	0	6	0
	<i>Hodgkinia_142</i>	139	0	1122	0
N16	<i>Hodgkinia_164</i>	20	0	27	0
	Mito_Asn_6244-6338	0	1698	1445	0
N18	<i>Hodgkinia_096</i>	3	0	104	0
N20	<i>Hodgkinia_061</i>	0	10	0	28
	<i>Hodgkinia_062</i>	108	7813	4	43
	<i>Hodgkinia_096</i>	11	67	0	177
	<i>Hodgkinia_103</i>	0	3	0	51
	<i>Hodgkinia_108</i>	0	18	0	4
	<i>Hodgkinia_114</i>	1	7	0	50
	<i>Hodgkinia_132</i>	7	85	1	57
	<i>Hodgkinia_142</i>	97	1258	3	70

	<i>Hodgkinia</i> _163	2	51	0	18
	<i>Hodgkinia</i> _187	18	254	1	514
N23	<i>Hodgkinia</i> _189	478 ^a	8	1824	0
N26	<i>Hodgkinia</i> _062	190	3	1	8394
	<i>Hodgkinia</i> _103	86	0	38	0
	<i>Sulcia</i> _080	102	2873	147868	29
	<i>Sulcia</i> _091	22	471	17805	6
	<i>Sulcia</i> _138	2	125	4770	1
	Mito_Gly_5701-5793	1257	0	2172	0
N27	<i>Hodgkinia</i> _062	12	224	7915	3
	<i>Hodgkinia</i> _099	0	27	0	116
N34	<i>Sulcia</i> _126	4	15802	7	407
	<i>Sulcia</i> _264	4546	2	380	0
N37	<i>Hodgkinia</i> _189	8	14	1403	981
	<i>Hodgkinia</i> _132	2	21	287	5
	<i>Hodgkinia</i> _143	63	243	6143	122
	<i>Sulcia</i> _151	38	89	2137	91
	<i>Sulcia</i> _187	4	15	1290	12
N43	<i>Hodgkinia</i> _041	0	442	0	371 ^a
	<i>Hodgkinia</i> _187	28	1	1569	1
N45	<i>Hodgkinia</i> _061	91	1	264	0
	<i>Hodgkinia</i> _189	470	12	1902	1
	Mito_Asp_4039-4130	237	0	644	0
N46	<i>Hodgkinia</i> _187	27	1	1571	0
N49	<i>Hodgkinia</i> _189	12	468	0	1899
N57	<i>Hodgkinia</i> _132	2563	3	3922	0
	Mito_Gln_340-433	1488	0	246	0
N58	<i>Hodgkinia</i> _041	424	148	179	0
	<i>Hodgkinia</i> _061	188	26	123	1
	<i>Hodgkinia</i> _103	103	8	19	0
	<i>Hodgkinia</i> _108	33	1	5	0
	<i>Hodgkinia</i> _114	77	1	10	0
	<i>Hodgkinia</i> _163	40	26	30	0
	<i>Hodgkinia</i> _187	1501	22	29	0
	<i>Hodgkinia</i> _189	2158	42	17	0
	<i>Sulcia</i> _189	29461	671	17	2
N61	Mito_Asn_6244-6338	1811	1	1362	0
N62	<i>Hodgkinia</i> _189 ^b	21 ^a	0	115	0
	<i>Hodgkinia</i> _108	36	0	2	0
N67	Mito_Gln_340-433	0	222	0	1479
	Mito_Leu_12647-12745	1772	2085	2	2
N68	<i>Hodgkinia</i> _062	2	842	1	7800 ^a
T-loop	Mito_Met_405-500	580	570	0	0
	Mito_Cys_1565-1655	0	267	0	1305

	Mito_Cys_1565-1655	1303	266	2	0
	Mito_Asp_4039-4130	643	0	0	242
D-loop	Mito_Val_13936-14031 ^c	0	5108	1	588

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307 **Table S7.** List of transcripts that are involved in tRNA maturation and are up regulated in cicada
 308 bacteriocytes. Genes identified by edgeR ($P < 0.05$) and those highly expressed in bacteriocytes
 309 ($P = NS$) are listed. Gene expression values are given in CPM.
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Trinity name	Trinotate name	1b	1o	2b	2o	3b	3o	4b	4o	pvalue
DN38936_c1_g1	Arginine--tRNA ligase, cytoplasmic	129.9	5.5	6.2	1.3	34.3	3.4	20.8	5.0	NS
DN38565_c0_g1	Cysteine--tRNA ligase, cytoplasmic	9.1	1.7	3.9	0.0	1.8	1.2	4.0	0.6	NS
DN46824_c3_g1	Cysteine--tRNA ligase, mitochondrial	51.7	0.9	3.6	0.7	9.0	1.7	8.2	2.1	NS
DN51624_c0_g1	Serine--tRNA ligase, cytoplasmic	54.9	3.7	1.2	0.3	2.1	2.7	2.7	2.7	NS
DN36332_c0_g2	Putative tRNA pseudouridine synthase Pus10	68.4	3.2	4.4	3.3	10.8	7.1	17.5	4.9	NS
DN38950_c0_g1	tRNA 2'-phosphotransferase 1	118.9	13.3	5.9	0.3	19.9	7.6	20.5	6.1	NS
DN40306_c0_g1	tRNA (guanine(10)-N2)- methyltransferase homolog	15.1	1.1	0.6	2.6	2.3	1.4	6.2	1.8	NS
DN43098_c0_g1	Methionyl-tRNA formyltransferase, mitochondrial	15.5	0.2	0.9	0.0	1.5	0.1	1.4	0.1	NS
DN47148_c0_g2	Queuine tRNA-ribosyltransferase subunit QTRTD1 homolog	60.7	3.2	1.8	0.3	4.4	0.6	11.3	1.7	NS
DN34313_c0_g1	D-tyrosyl-tRNA(Tyr) deacylase 1	22.4	0.0	3.6	0.0	28.8	0.0	19.5	0.0	0.001
DN41591_c0_g1	tRNA modification GTPase MnmE	3.3	0.0	119.8	0.0	0.7	0.1	6.3	0.0	0.012
DN45267_c0_g1	D-tyrosyl-tRNA(Tyr) deacylase 1	28.6	0.0	17.8	1.0	121.0	1.4	21.8	0.5	0.043
DN45267_c2_g1	D-tyrosyl-tRNA(Tyr) deacylase 1	20.7	0.0	4.2	0.0	48.3	0.0	8.7	0.0	0.002
DN45267_c2_g2	D-tyrosyl-tRNA(Tyr) deacylase 1	9.7	0.0	0.0	0.0	3.2	0.0	3.9	0.0	0.043
DN47407_c0_g1	tRNA (uracil(54)-C(5))- methyltransferase homolog-B	160.2	0.3	4.4	0.3	1.6	0.5	21.7	0.8	0.031
DN48812_c5_g1 4	D-tyrosyl-tRNA(Tyr) deacylase	31.0	0.0	0.6	0.0	13.6	0.0	4.2	0.0	0.006
DN48899_c1_g2	Aminoacyl tRNA synthase complex- interacting multifunctional protein 1	10.2	0.0	0.0	0.0	2.9	0.0	2.8	0.0	0.049
DN52199_c1_g2	Aminoacyl tRNA synthase complex- interacting multifunctional protein 1	93.4	0.1	1.2	0.0	13.0	0.2	12.6	0.0	0.011

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330 **Table S8** Horizontally transferred genes identified in the cicada transcriptome. Gene expression
 331 values are given in CPM.
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Trinity	Gene	Product	1b	1o	2b	2o	3b	3o	4b	4o
DN89433_c0_g1	N/A	AAA-ATPase	0.2	0	0	0	0	0	0.1	0
DN22517_c1_g1	<i>pel</i>	Pectin lyase	0	0	0	0	0	1.4	0	1.4
DN65002_c0_g1	<i>yebC-1</i>	Transcription regulator	9.9	0	0.4	0	9.9	0	2.6	0
DN39720_c1_g1	<i>yebC-2</i>		12	0	0.4	0	1.5	0	5.4	0
DN56768_c0_g1	<i>frr</i>	Ribosome recycling factor	0	0	0	0	3.6	4.1	0	0

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370 **Table S9.** Number of differentially expressed tRNA genes encoded on the *Hodgkinia*, *Sulcia*, and
 371 the cicada mitochondria genomes by edgeR analysis (66 total genes). The analysis was
 372 performed for all four small RNA samples and for three read size ranges. Index1: TAP treated
 373 2010 sample, index2: 2010 sample, index3: 2012 sample, index4: 2012 sample.
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		70-100 nt			
index1	0				
index2	0	0			
index3	9	10	0		
index4	8	5	3	0	
		48-90 nt			
index1	0				
index2	0	0			
index3	18	16	0		
index4	11	9	4	0	
		18-90 nt			
index1	0				
index2	0	0			
index3	20	20	0		
index4	11	11	3	0	

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