Supporting Information

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SI Materials and Methods

Genome Sequencing. The following amount of data were generated for each sequencing technology: 136,081,956 pairs of 100 × 2 short insert Illumina HiSeq reads for about 27 Gb total; 50,884,070 pairs of 100 × 2 large insert HiSeq reads for about 10 Gb total; and 259,593 reads averaging 1600 nt for about 421 Mb total of PacBio data.

Genome Annotation. Annotation of *Hodgkinia* DNA was done using the phmmer module of HMMER v3.1b1 (1). All ORFs beginning with a start codon that overlapped a phmmer hit were searched against a database of all *Hodgkinia* genes using BLASTX 2.2.28+. MAGTRE *Hodgkinia* genes were considered full length and presumably functional if they did not contain an internal stop codon and were at least 75% of the length of the functional homologs in the other cicada species. Ribosomal and transfer RNAs were annotated using RNAmmer v1.2 (2) and Aragorn v1.2.36 (3), respectively. To identify any potential genes in MAGTRE *Hodgkinia* that were not present in other cicada species, the 27 circles were searched against all nr using BLASTX v2.2.28+.

Microscopy. Potential regions for genome-targeted DNA probes were identified by using a sliding window of 1,000 nt and performing a BLASTN search on the full assembly to find unique genomic regions. PCR primers were designed to amplify the selected probe regions (MAGTRE001: AGGAGAAACTTAAAGTTCATTGATCC and ATTACAATCCTAGATGTCTACCC; MAGTRE0012: AGAAACAACAACATAATAAACAAAGC and AATTATCGAAACATTAACAACACAGC; MAGTRE005: ACACCTAAGCATAGCGTTCC and ATTTATCCAAGTTCATGTAAACCC; and MAGTRE006: AGTGGGTTTTGAATTTAATGTAGG and ATCCGAACTTAACCTTTGAAAACC). PCR products were A-tailed using Taq DNA polymerase (New England Biolabs M0267), cloned into pGEM-T-Easy vectors and transformed into *Escherichia coli* JM109 High Efficiency Competent Cells. Transformed cells were grown in 3 mL of LB broth at 37 °C overnight, and plasmids were purified with E.Z.N.A. plasmid DNA mini kit I. The purified plasmids were used as PCR templates to for further amplification of the probe region. The amplified probes were subject to nick translation (>175 ng/μL DNA, 1× nick-translation buffer, 0.25 mM unlabeled dNTPs, 50 μM labeled dNTPs, 2.3 U/μL DNA polymerase I, 9 mU/μL Dnase), using either Cy3 (MAGTRE006 and MAGTRE005), or Cy5 (MAGTRE001 and MAGTRE012), and size selected for sizes in the range of 100–500 bp using Ampure XP beads. Probes with at least seven incorporated labeled dNTPs per 1,000 nucleotides as determined by spectroscopy were used for hybridization.

PCR, Cloning, and Sequencing *Hodgkinia* 16S. Genomic DNA was extracted from bacteriomes of ethanol-preserved specimens using the Genomic DNA from tissue kit (Macherey-Nagel). PCR conditions for amplification of *Hodgkinia* 16S rDNA were 95 °C for 1 min followed by 30 cycles of 95 °C for 30 s, 62 °C for 30 s, 72 °C for 2 min, followed by a single step of 72 °C for 10 min, using the primers 10F_Hodg (AGYTTGATCCTGGCTCAGAACG) and 1507R_Hodg+Sulc (TACCTTGTTACGACTTMRCC) and TaKaRa Ex Taq (Takara Bio Inc). Amplicons were run on 1.5% (wt/vol) agarose gel and bands were cut and processed with the PCR clean-up Gel Extraction kit (Macherey-Nagel). Purified products were into cloned using Invitrogen's TOPO TA pCR 4 kit with One Shot Top 10 competent cells. Colonies were picked and directly amplified using M13F and M13R primers. The conditions for the PCR were 94 °C for 10 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1.5 min, with a final 10 min at 72 °C. Cloned products were cleaned with ExoSAP-IT (Affymetrix) and sequenced with BigDye 3.1 (ABI) and BDX64 (MCLAB) on an ABI 3130xl. Sequences were edited and aligned by eye using GeneiousR6. Neighbor Net networks (4) were produced using SplitsTree (5) using the GTR model.

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Table S1. *Hodgkinia* 16S clone distribution from various *Magicicada* species

Lineage 1	Lineage 2	Lineage 3A	Lineage 3B	Lineage 4	Lineage 5	Total Clones Seq.	Species	Brood*	County, state	Year	Location
4	8	0	0	2	4	18	*M. tredecim*	XIX.S.a	Pope Co., IL	2011	Dixon Springs Agricultural Center
1	4	0	0	1	2	8	*M. septendecim*	X.M.a	Franklin Co., OH	2004	Southwest of Columbus
0	4	0	3	1	0	8	*M. septendecim*	X.M.b	Franklin Co., OH	2004	Southwest of Columbus
1	6	0	0	1	2	10†	*M. tredecim*	XIX.S.b	King William Co., VA	2011	Dabney Mill Rd. and Etna Mills Rd.
3	5	1	2	4	1	16	*M. neotredecim*	XIX.W	Boone Co., MO	2011	Rt. K, 1 mile east of McBain, MO
0	5	4	1	7	3	20	*M. tredecassini*	XIX.W	Pope Co., IL	2011	Dixon Springs Agricultural Center
0	6	0	1	0	0	7	*M. cassini*	VIII.E.a	Westmoreland Co., PA	2002	Keystone State Park
0	1	0	6	0	0	7	*M. cassini*	VIII.E.b	Westmoreland Co., PA	2002	Keystone State Park
3	3	1	3	3	7	20	*M. septendecula*	VI.E.a	Burke Co., NC	2000	Piedmont Road
0	3	2	1	1	1	8	*M. septendecula*	VI.E.b	Burke Co., NC	2000	Piedmont Road
2	2	0	0	0	3	7	*M. septendecula*	VI.E.c	Burke Co., NC	2000	Piedmont Road
2	9	1	1	0	4	17	*M. tredecula*	XXII.M	Adams Co., MS	2014	Natchez State Park Campground

Each row represents clones sequenced from a single individual cicada.
**Magicicada* brood (Roman numeral) followed by mt-DNA–defined *Magicicada* geographic lineage (East, Middle, West, South), and individual cicada identifier (a,b,c) if more than one individual sampled from a single locality.
†These 10 16S versions were obtained from genome sequencing.

Table S2. Relative d_S values for several *Hodgkinia* orthologs

Ortholog	DICSEM	TETULN	TETUND1	TETUND2
Pairwise d_S values for *Hodgkinia*				
TETULN	3.6819			
TETUND1	4.3319	0.5422		
TETUND2	3.2595	0.4839	0.2078	
MAGTRE	NC	NC	NC	NC
Pairwise d_S values for *Sulcia*				
TETULN	0.0309			
TETUND	0.0317	0.0115		
MAGTRE	0.0258	0.0378	0.0419	0.0419
Ratio of *Hodgkinia* to *Sulcia* d_S values				
TETULN	119			
TETUND1	137	47		
TETUND2	100	53	17	
MAGTRE	NC	NC	NC	NC

Protein and DNA sequences for all possible orthologs in each comparison were aligned using MACSE and d_S values were calculated using PAML (NSites = 0, model = 1, and runmode = 0). We used the model of Nei and Gojobori (1) to estimate d_S, which is known to underestimate this term for large values. As such, the values reported here are conservative. The d_S value for MAGTRE comparisons was not calculable (NC) for *Hodgkinia* by any method because of the extreme dissimilarity of the sequences.

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Table S3. Cicada species with known life cycle lengths

Species	Location	Median total life cycle length, y	Range of life cycle length, y	Source
Amphipsalta zealandica	New Zealand	4	3–4	(1)
Chremistica ribhoi	Ri Bhoi District, Meghalaya, India	4	no variation	(2, 3)
Cicadetta calliope	United States plains states	4	?	(4)
Cryptotympana facialis	Japan	4	3–5	(5)
Cyclochila australasiae	Australia	6–7	?	(6)
Cystosoma saundersi	Australia	4	4–5	M. S. Moulds, Australian Museum, Sydney
Diceroprocta apache	SW United States	3–4	2–5	(7)
Graptopsaltria nigrofuscata	Japan	3	2–5	(5, 8)
Hyalessa maculaticollis	Japan	3	2–5	(5)
Kikihia muta	New Zealand	3	3 (grass) to 4 (flax)	(9, 10)
Kikihia ochrina	New Zealand	3	3–4	(11)
Magicicada cassini	Northeastern and central United States	17	13–21	(12)
Magicicada septendecim	Northeastern + central United States	17	13–21	(12)
Magicicada septendecula	Northeastern + central United States	17	13–21	(12)
Magicicada tredecassini	Southeastern + central United States	13	9–17	(12)
Magiciada neotredecim	Southeastern + central United States	13	9–17	(13)
Magicicada tredecim	Southeastern + central United States	13	9–17	(14)
Magicicada tredecula	Southeastern + central United States	13	9–17	(14)
Meimuna kuroiwae	Japan	2	2–4	(5)
Meimuna opalifera	Japan	2	2–5	(5)
Meimuna oshimensis	Japan	3	2–4	(5)
Mogannia minuta	Japan (sugar cane and grass)	2 (sugarcane), 3 (grass)	1–4 (sugarcane), 2–5 (grass)	(14, 15)
Mogannis hebes	Taiwan	2–3	2–3	(16)
Munza (Platypleura) kuroiwae	Japan	4	?	(8)
Myopsalta crucifera	Australia (sugar cane)	1	?	(6, 17) Misidentified as *Melampsalta puer* in ref. 17
Okanagana rimosa	Eastern United States and Canada	9	?	(18)
Okanagana synodica	Alberta, Canada	19?	17 to 19?	(19)
Oncotympana coreana	Japan	7	?	(20)
Parnkalla muelleri	Australia	1	?	(6)
Platypleura kaempferi	Japan	4	?	(8, 21)
Platypleura kaempferi	Japan	2	2–5	(5)
Raiateana knowlesi	Fiji, Serua and Navosa	8	No variation	(22, 23)
Tettigades "chilensis"	Central Chile	19	?	(24)
Yanga guttulata	Madagascar (sugar cane)	2	1–3	(25)

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