

**Formyl-tetrahydrofolate synthetase diversity in the gut  
of the wood-feeding cockroach *Cryptocercus punctulatus*  
and the omnivorous cockroach *Periplaneta americana***

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**Supplemental Materials**

**Supplemental Figure 1.** Phylogenetic analysis of roach and termite mitochondrial cytochrome oxidase II gene sequences.

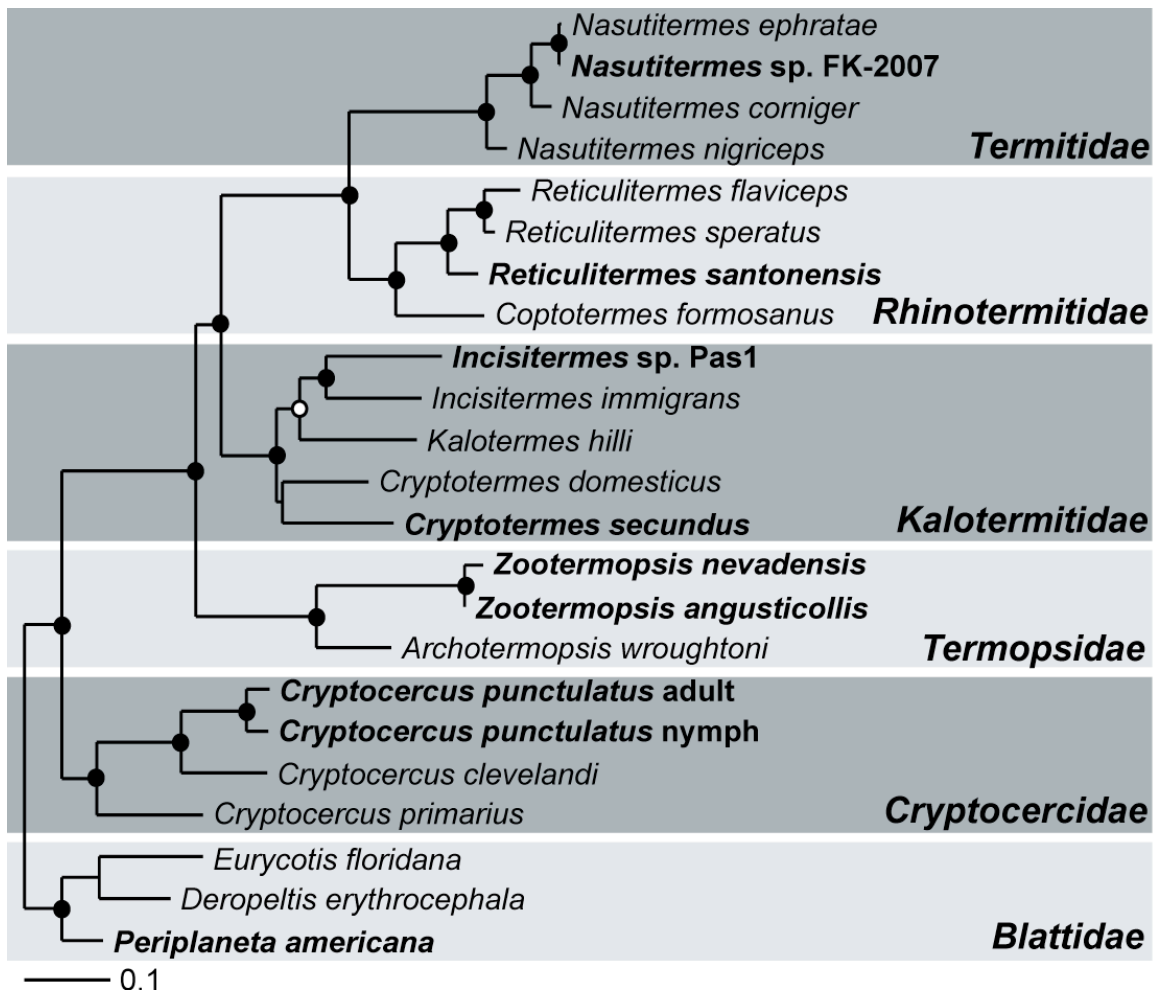
**Supplemental Figure 2.** Phylogenetic analysis of termite and roach FTHFS sequences.

**Supplemental Table 1.** Operational taxonomic unit grouping of FTHFS sequences identified in this study

**Supplemental Table 2.** Sequences used in FTHFS phylogenetic analysis

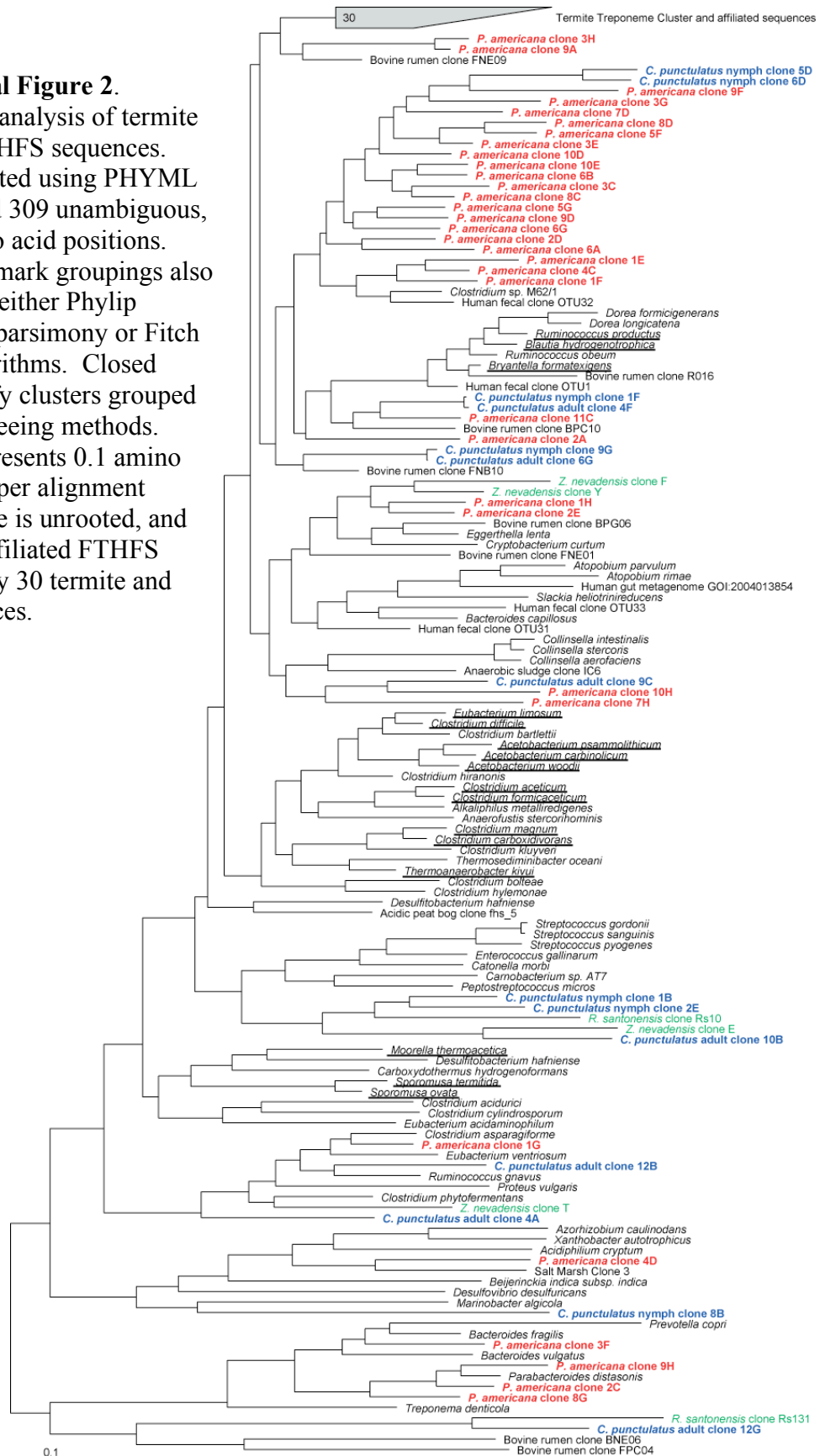
**Supplemental Table 3.** Sequences used in COII phylogenetic analysis

**Supplemental References.**



**Supplemental Figure 1.** Phylogenetic analysis of roach and termite mitochondrial cytochrome oxidase II gene sequences. Tree constructed using PHYML algorithm and 394 unambiguous, aligned nucleic acid positions. Open circles mark groupings also supported by either Phylip DNAPARS parsimony or Fitch distance algorithms. Closed circles identify clusters grouped by all three treeing methods. Scale bar represents 0.1 amino acid changes per alignment position.

**Supplemental Figure 2.** Phylogenetic analysis of termite and roach FTHFS sequences. Tree constructed using PHYML algorithm and 309 unambiguous, aligned amino acid positions. Open circles mark groupings also supported by either Phylip PROTPARS parsimony or Fitch distance algorithms. Closed circles identify clusters grouped by all three treeing methods. Scale bar represents 0.1 amino acid changes per alignment position. Tree is unrooted, and treponeme-affiliated FTHFS represented by 30 termite and roach sequences.



**SUPPLEMENTAL TABLE 1.** Operational Taxonomic Unit Grouping of FTHFS sequences identified in this study

<b>Group</b>	<b>Phylotype</b>	<b>Abundance (%)<sup>a</sup></b>	<b>Genotypes<sup>b</sup></b>
<b><i>P. americana</i></b>			
Clone F Group	2E	25.6	<b>2E, 2F, 11F</b>
	1H	5.8	<b>1H, 10F</b>
<i>Clostridium</i> sp. M62/1	4C	4.7	<b>4C</b>
	5G	3.5	<b>5G</b>
	1F	2.3	<b>1F</b>
	3C	2.3	<b>3C</b>
	5F	2.3	<b>5F</b>
	6G	2.3	<b>6G</b>
	8D	2.3	<b>8D, 9C</b>
	10D	1.2	<b>10D</b>
	10E	1.2	<b>10E</b>
	1E	1.2	<b>1E</b>
	2D	1.2	<b>2D</b>
	3E	1.2	<b>3E</b>
	3G	1.2	<b>3G</b>
	6A	1.2	<b>6A</b>
	6B	1.2	<b>6B</b>
	7D	1.2	<b>7D</b>
	8C	1.2	<b>8C</b>
	9D	1.2	<b>9D</b>
	9F	1.2	<b>9F</b>
	Other	7B	5.8
	1A	5.8	<b>1A</b>
	3H	4.7	<b>3H</b>
	2B	3.5	<b>2B</b>
	1G	2.3	<b>1G</b>
	3F	2.3	<b>3F</b>
	10H	1.2	<b>10H</b>
	11C	1.2	<b>11C</b>
	2A	1.2	<b>2A</b>
	2C	1.2	<b>2C</b>
	4D	1.2	<b>4D</b>
	7H	1.2	<b>7H</b>
	8G	1.2	<b>8G</b>
	9A	1.2	<b>9A</b>
	9H	1.2	<b>9H</b>
<b><i>C. punctulatus</i> adult</b>			
<i>Treponeme</i> affiliated	1A	45	<b>1A, 1B, 1C, 3B, 3D, 3G, 4B, 5D, 10E</b>
	1F	20	<b>1F, 1G, 6E</b>
	7H	8.3	<b>7H, 4E</b>
	2H	6.7	<b>2H</b>

<b>Group</b>	<b>Phylotype</b>	<b>Abundance (%)<sup>a</sup></b>	<b>Genotypes<sup>b</sup></b>
	7C	3.3	<b>7C</b>
	5B	1.7	<b>5B</b>
	6D	1.7	<b>6D</b>
	10F	1.7	<b>10F</b>
Clone E Group	10B	1.7	<b>10B</b>
Other	4A	1.7	<b>4A</b>
	4F	1.7	<b>4F</b>
	6G	1.7	<b>6G</b>
	9C	1.7	<b>9C</b>
	12B	1.7	<b>12B</b>
	12G	1.7	<b>12G</b>
<b><i>C. puntulatus</i> nymph</b>			
<i>Treponeme</i> affiliated	1G	16	<b>1G, 1E, 11G</b>
	2B	15	<b>2B, 1C, 3G, 10H</b>
	3H	6.8	<b>3H, 7F</b>
	1A	3.4	<b>1A, 12G</b>
	2H	3.4	<b>2H, 1D, 9D</b>
	6F	2.3	<b>6F, 6E</b>
	7A	2.3	<b>7A, 9C</b>
	6B	1.1	<b>6B</b>
<i>Clostridium</i> sp. M62/1	5D	1.1	<b>5D</b>
	6D	1.1	<b>6D</b>
Clone E Group	1B	40	<b>1B, 3A, 3C</b>
	2E	1.1	<b>2E</b>
Other	1F	3.4	<b>1F, 6A</b>
	8B	2.3	<b>8B</b>
	9G	1.1	<b>9G</b>
<b><i>Incisitermes</i> sp. Pas1</b>			
<i>Treponeme</i> affiliated	1B	40	<b>1B, 11F</b>
	2A	34	<b>2A, 3C, 3F, 3G, 8B</b>
	3D	12	<b>3D, 4B, 11B</b>
	1F	4.4	<b>1F</b>
	1E	2.2	<b>1E</b>
	3A	2.2	<b>3A</b>
	7D	2.2	<b>7D</b>
	11C	2.2	<b>11C, 11G</b>

<sup>a</sup>Defined as percent of full-length clones

<sup>b</sup>Sequenced RFLP type clones. Group representative marked in bold.

**SUPPLEMENTAL TABLE 2.** Sequences used in FTHFS phylogenetic analysis

Source / Sequence Type	Accession	Reference
<i>Acetobacterium carbinolicum</i>	DQ152908	(16)
<i>Acetobacterium psammolithicum</i>	AJ494824	(5)
<i>Acetobacterium woodii</i>	AF295701	(6)
Acidic Peat Bog Clone fhs_5	EF173429	
<i>Acidiphilium cryptum</i> JF-5	ZP_01145990	
Anaerobic sludge clone IC6	EU009524	
	199190..200863	
<i>Anaerofustis stercorihominis</i>	NZ_ABIL02000006	
<i>Alkaliphilus metalliredigenes</i> QYMF	CP000724	
<i>Atopobium parvulum</i>	YP_003179642	(27)
	58616..60289	
<i>Atopobium rimae</i>	NZ_ACFE01000001	
<i>Azorhizobium caulinodans</i>	YP_001523813	(7)
<i>Bacteroides capillosus</i>	53815..55617	
	NZ_AAAG02000001	
<i>Bacteroides fragilis</i>	2570198..2571865 NC_006347	(4)
<i>Bacteroides vulgatus</i>	3818795..3820462 NC_009614	(29)
<i>Beijerinckia indica subsp. indica</i> ATCC 9	1870025..1871692 NC_010581	
<i>Blautia hydrogenotrophica</i> DSM 10507	EEG47205	
<i>Bryantella formatexigens</i>	ZP_05346660	
Bovine Rumen Clone BNE06	AB085284	
Bovine Rumen Clone BPC10	AB085350	
Bovine Rumen clone BPG06	AB085389	
Bovine Rumen Clone FNB10	AB085422	
Bovine Rumen Clone FNE01	AB085446	
Bovine Rumen Clone FNE09	AB085454	
Bovine Rumen Clone FPC04	AB085514	
Bovine Rumen clone R016	AB282707	(10)
<i>C. secundus</i> Gut Clone Cs18	DQ278253	(17)
<i>C. secundus</i> Gut Clone Cs27	DQ278254	(17)
<i>C. secundus</i> Gut Clone Cs3	DQ278251	(17)
<i>C. secundus</i> Gut Clone Cs56	DQ278258	(17)
<i>Catonella morbi</i>	ZP_04449844	
<i>Carboxydotherrmus hydrogenoformans</i> Z-2901	ABB16038	(28)
<i>Carnobacterium sp. AT7</i>	52555..54231 ABHH01000002	
<i>Clostridium aceticum</i>	AF295705	(6)
<i>Clostridium acidurici</i>	P13419	(26)
<i>Clostridium asparagiforme</i> DSM 15981	EEG52107	
<i>Clostridium bartlettii</i>	171762..173447	
	NZ_ABEZ02000016	
<i>Clostridium bolteae</i>	21178..22857	

Source / Sequence Type	Accession	Reference
	NZ_ABCC02000077	
<i>Clostridium carboxidivorans</i>	ZP_05391913	
<i>Clostridium cylindrosporium</i>	Q07064	(19)
<i>Clostridium difficile</i> QCD-32g58	ZP_01033134	
<i>Clostridium formicaceticum</i>	AF295702	(6)
<i>Clostridium hiranonis</i>	75293..76969 ABWP01000070	
<i>Clostridium hylemonae</i> DSM 15053	EEG73673	
<i>Clostridium kluyveri</i>	413480..415156 NC_009706	(21)
<i>Clostridium magnum</i>	AF295703	(6)
<i>Clostridium phytofermentans</i>	13457..15127	
	NZ_AAQT01000065	
<i>Clostridium</i> sp. M62/1	ZP_03733593	
<i>Collinsella aerofaciens</i>	144672..146339	
	NZ_AAVN02000005	
<i>Collinsella intestinalis</i>	43156..44823 ABXH01000019	
<i>Collinsella stercoris</i>	21057..22724 ABXJ01000142	
<i>Cryptobacterium curtum</i>	YP_003150638	(27)
<i>Desulfitobacterium hafniense</i> Y51	NC_007907	(13)
<i>Desulfovibrio desulfuricans</i>	AJ494753	(5)
<i>Dorea formicigenerans</i>	6079..7764 NZ_AAAXA02000014	
<i>Dorea longicatena</i>	6080..7762 NZ_AAXB02000001	
<i>Eggerthella lenta</i>	YP_003183199	(27)
<i>Enterococcus gallinarum</i>	ZP_05648325	
<i>Eubacterium acidaminophilum</i>	AAU84895	
<i>Eubacterium limosum</i>	AF295706	(6)
<i>Eubacterium ventriosum</i>	279152..280822	
	NZ_AAVL02000038	
Human fecal clone OTU1	AB291639	(14)
Human fecal clone OTU31	AB291669	(14)
Human Fecal Clone OTU32	AB291670	(14)
Human Fecal Clone OTU33	AB291671	(14)
Human gut metagenome	JGI GOI:2004013854	(1)
<i>Marinobacter algicola</i>	ZP_01892361	
<i>Moorella thermoacetica</i>	NC_007644	(18)
<i>Nasutitermes</i> sp. FK-2007 Contig40968	JGI GOI: 2004144560	(25)
<i>Parabacteroides distasonis</i>	1173123..1174790 NC_009615	(29)
<i>Peptostreptococcus micros</i>	NZ_ABEE02000017	
<i>Prevotella copri</i> DSM 18205	ZP_03615969	
<i>Proteus vulgaris</i>	AF295710	(6)
<i>R. santonensis</i> Gut Clone Rs10	DQ278259	(17)
<i>R. santonensis</i> Gut Clone Rs119	DQ278226	(17)
<i>R. santonensis</i> Gut Clone Rs129	DQ278222	(17)
<i>R. santonensis</i> Gut Clone Rs13	DQ278232	(17)
<i>R. santonensis</i> Gut Clone Rs131	DQ278221	(17)

Source / Sequence Type	Accession	Reference
<i>R. santonensis</i> Gut Clone Rs144	DQ278223	(17)
<i>R. santonensis</i> Gut Clone Rs158	DQ278226	(17)
<i>R. santonensis</i> Gut Clone Rs23	DQ278210	(17)
<i>R. santonensis</i> Gut Clone Rs239	DQ278201	(17)
<i>R. santonensis</i> Gut Clone Rs280	DQ278207	(17)
<i>R. santonensis</i> Gut Clone Rs296	DQ278208	(17)
<i>R. santonensis</i> Gut Clone Rs44	DQ278211	(17)
<i>R. santonensis</i> Gut Clone Rs57	DQ278215	(17)
<i>Ruminococcus gnavus</i>	NZ_AAYG02000005	
<i>Ruminococcus obeum</i>	35465..37150	
	NZ_AAVO02000015	
<i>Ruminococcus productus</i>	AF295707	(6)
Salt Marsh Clone 3	AJ494817	(5)
<i>Slackia heliotrinireducens</i>	YP_003144459	(27)
<i>Sporomusa ovata</i>	AF295708	(6)
<i>Sporomusa termitida</i>	AF295709	(6)
<i>Streptococcus gordonii</i>	NC_009785	(24)
<i>Streptococcus pyogenes</i> SSI-1	BAC64868	(12)
<i>Streptococcus sanguinis</i>	NC_009009	(30)
<i>Thermoanaerobacter kivui</i>	AF295704	(6)
<i>Thermosediminibacter oceani</i> JW/IW-1228P	JGI GOI: 2500808311	
<i>Treponema azotonutricium</i> ZAS-9	AY162316	(20)
<i>Treponema denticola</i>	NC_002967	(22)
<i>Treponema primitia</i> ZAS-1	AY162313	(20)
<i>Treponema primitia</i> ZAS-2	AY162315	(20)
<i>Xanthobacter autotrophicus</i>	YP_001416288	
<i>Z. nevadensis</i> Gut Clone A	AY162294	(20)
<i>Z. nevadensis</i> Gut Clone E	AY162296	(20)
<i>Z. nevadensis</i> Gut Clone F	AY162298	(20)
<i>Z. nevadensis</i> Gut Clone H	AY162302	(20)
<i>Z. nevadensis</i> Gut Clone N	AY162306	(20)
<i>Z. nevadensis</i> Gut Clone P	AY162307	(20)
<i>Z. nevadensis</i> Gut Clone T	AY162309	(20)
<i>Z. nevadensis</i> Gut Clone Y	AY162311	(20)



**SUPPLEMENTAL TABLE 3.** Sequences used in COII phylogenetic analysis

<b>Source</b>	<b>Accession</b>	<b>Reference</b>
<i>Archotermopsis wroughtoni</i>	DQ442080	(3)
<i>Coptotermes formosanus</i>	AB109529	(15)
<i>Cryptocercus clevelandi</i>	DQ007626	(8)
<i>Cryptocercus primarius</i>	DQ007644	(8)
<i>Cryptotermes domesticus</i>	AF189086	(23)
<i>Cryptotermes secundus</i>	AF189093	(23)
<i>Deropeltis erythrocephala</i>	DQ874271	(2)
<i>Eurycotis floridana</i>	DQ874283	(2)
<i>Incisitermes immigrans</i>	AB109542	(15)
<i>Kaloterme hilli</i>	AF189101	(23)
<i>Nasutitermes corniger</i>	AB037327	(11)
<i>Nasutitermes ephratae</i>	AB037328	(11)
<i>Nasutitermes nigriceps</i>	AB037329	(11)
<i>Nasutitermes sp. FK-2007</i>	EU236539	(25)
<i>Reticulitermes flaviceps</i>	AB109532	(15)
<i>Reticulitermes santonensis</i>	AF291743	(9)
<i>Reticulitermes speratus</i>	AB109530	(15)
<i>Zootermopsis angusticollis</i>	DQ442267	(3)

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