

SUPPLEMENTARY INFORMATION

**The cockroach origin of the termite gut microbiota:
patterns in bacterial community structure reflect major
evolutionary events**

Carsten Dietrich, Tim Köhler & Andreas Brune

Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology,
Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

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Figures

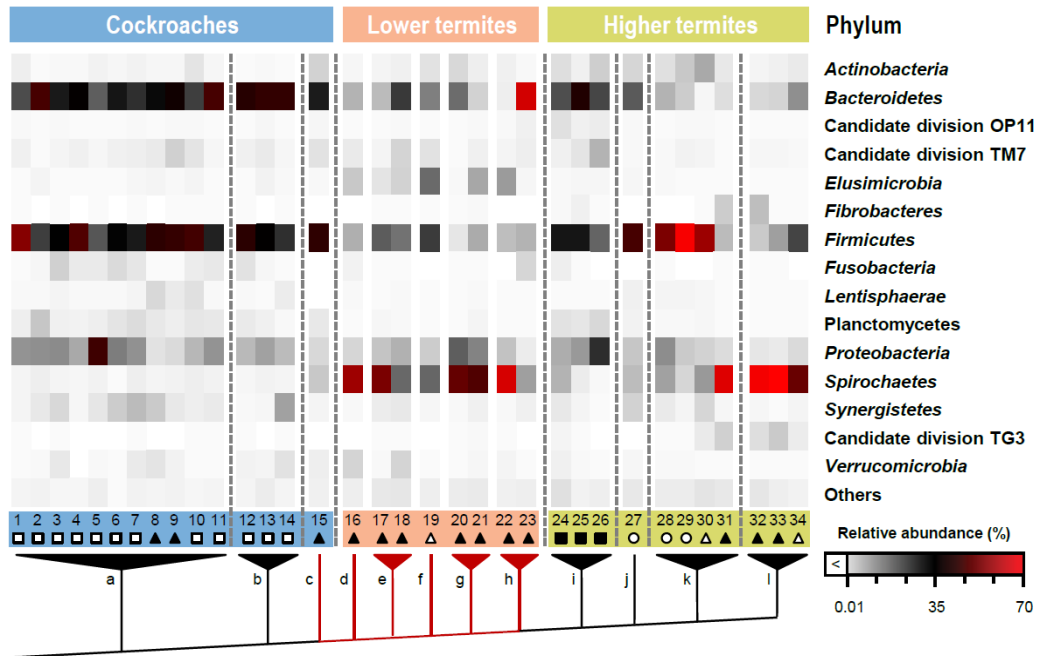


FIG S1 Relative abundance of sequence reads assigned to the major bacterial phyla in the gut microbiota of cockroaches, lower termites, and higher termites. The color scale is linear. Numbers indicate host species (see Table 1 or Table S2). Symbols indicate lifestyle: (□), wood feeding (▲), grass feeding (Δ), soil/humus feeding (○), and fungus cultivating (■). The schematic tree shows a simplified host phylogeny of the major host lineages (a, other cockroaches; b, *Blattidae*; c, *Cryptocercidae*; d, *Mastotermitidae*; e, *Termopsidae*; f, *Hodotermitidae*; g, *Kalotermitidae*; h, *Rhinotermitidae*; i, *Macrotermitinae*; j, *Apicotermitinae*; k, *Termitinae*; l, *Nasutitermitinae*). The red branches mark the presence of cellulose-digesting flagellates.

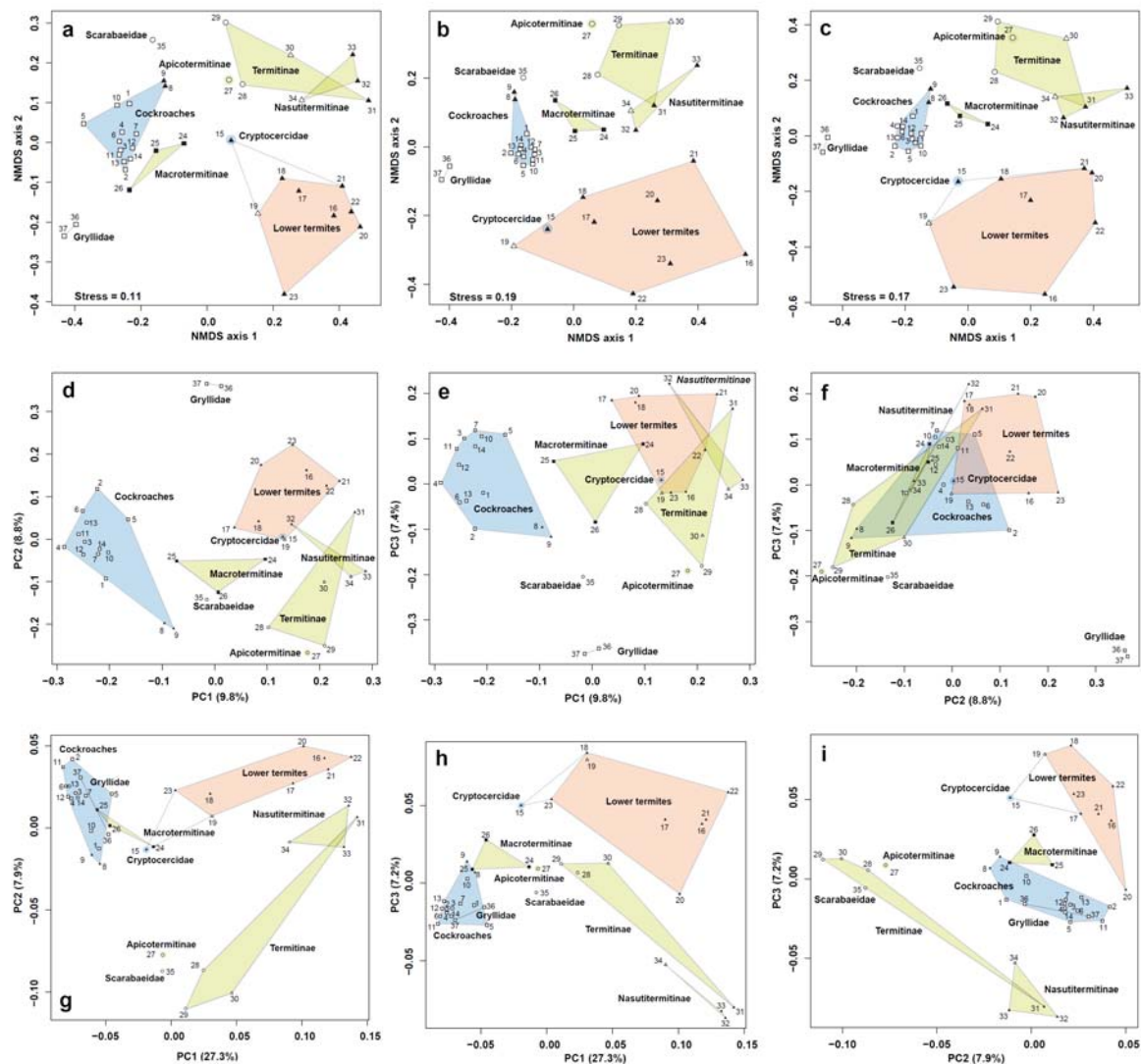


FIG S2 Comparison of bacterial community structure in cockroaches and termites using various ordination techniques and taxon-independent (OTU-based) approaches. Non-metric multidimensional scaling (NMDS) of a pairwise Bray-Curtis dissimilarity distance matrix based on genus-level taxa (**a**) (relative abundance of 16S rRNA genes) or on the distribution of operational taxonomic units (OTUs) with 3% (**b**) and 5% sequence divergence (**c**). Principal coordinate analysis of the unweighted (**d-f**) and weighted (**g-i**) UniFrac distance matrix (normalized dataset); the variance explained by the three principal coordinates (PC) is indicated on the respective axes. Numbers indicate host species (see Table 1 or Table S2). Clustering of cockroaches, lower termites and different subfamilies of higher termites was significant ($p < 0.001$).

Tables

TABLE S1 Excel table containing information with the classification results for all samples (relative abundance of reads at different phylogenetic levels). **Please download Table_S1.xlsx for interactive spreadsheet.**

TABLE S2 Classification success at different taxonomic levels for all insects included in this study.

No.	Host species	Classified (%)				
		Phylum	Class	Order	Family	Genus
Cockroaches						
1	<i>Ergaula capucina</i>	99.3	96.1	93.1	88.0	61.6
2	<i>Symploce macroptera</i>	99.5	98.7	90.1	82.5	70.8
3	<i>Rhyarobia maderae</i>	99.4	97.2	94.2	83.5	67.4
4	<i>Elliptorhina chopardi</i>	99.6	97.2	95.3	87.3	65.1
5	<i>Panchlora</i> sp.	99.1	97.2	92.7	86.5	73.5
6	<i>Diploptera punctata</i>	99.8	97.9	94.4	88.1	74.1
7	<i>Opisthoplatia orientalis</i>	99.0	95.8	89.6	83.9	63.3
8	<i>Panesthia angustipennis</i>	99.3	96.1	94.2	86.0	61.0
9	<i>Salganea esakii</i>	99.6	92.4	90.7	85.7	63.5
10	<i>Eublaberus posticus</i>	99.2	94.0	90.1	82.3	68.5
11	<i>Schultesia lampyridiformis</i>	99.5	97.8	94.3	89.2	73.7
12	<i>Eurycotis floridana</i>	99.4	96.4	94.0	90.1	66.2
13	<i>Shelfordella lateralis</i>	99.4	95.3	93.5	90.1	74.6
14	<i>Blatta orientalis</i>	99.3	97.7	96.3	93.4	66.6
15	<i>Cryptocercus punctulatus</i>	99.0	93.4	90.2	82.8	67.7
Lower termites						
16	<i>Mastotermes darwiniensis</i>	98.6	94.8	91.9	84.1	78.5
17	<i>Zootermopsis nevadensis</i>	97.9	90.1	88.3	76.0	70.0
18	<i>Hodotermopsis sjoestedti</i>	98.5	96.4	95.3	92.9	86.3
19	<i>Hodotermes mossambicus</i>	98.8	93.9	90.5	86.8	72.4
20	<i>Incisitermes marginipennis</i>	99.2	98.3	97.3	94.9	91.4
21	<i>Neotermes jouteli</i>	99.3	95.7	92.2	86.8	81.8
22	<i>Reticulitermes santonensis</i>	99.2	96.2	95.6	94.5	93.3
23	<i>Coptotermes niger</i>	98.2	94.2	93.8	92.7	91.0

No.	Host species	Classified (%)				
		Phylum	Class	Order	Family	Genus
Higher termites						
24	<i>Odontotermes</i> sp.	99.0	93.2	89.9	83.9	76.3
25	<i>Macrotermes</i> sp.	99.2	90.7	87.4	83.7	76.7
26	<i>Macrotermes subhyalinus</i>	99.9	86.4	81.0	72.6	66.1
27	<i>Alyscotermes trestus</i>	99.7	95.7	93.0	87.1	68.0
28	<i>Cubitermes ugandensis</i>	98.7	97.4	94.9	83.3	69.4
29	<i>Ophiotermes</i> sp.	99.8	97.4	95.2	90.6	73.7
30	<i>Amitermes meridionalis</i>	97.8	93.3	89.6	81.6	68.7
31	<i>Microcerotermes</i> sp.	99.2	98.3	96.8	93.2	87.7
32	<i>Nasutitermes corniger</i>	98.9	97.4	96.5	95.3	91.0
33	<i>Nasutitermes takasagoensis</i>	98.8	97.7	97.0	94.9	88.9
34	<i>Trinervitermes</i> sp.	99.8	98.9	98.2	95.7	87.3
Others						
35	<i>Pachnoda ehippiata</i>	99.8	98.6	95.3	85.9	69.1
36	<i>Acheta domesticus</i>	99.7	99.4	98.0	96.0	79.8
37	<i>Gryllus assimilis</i>	99.9	99.7	97.0	94.9	75.4

TABLE S3 Contribution of the 100 most important genus-level taxa to the separation of cockroaches, lower termites, and higher termites in the ordination analyses. The taxa are ranked in order of their contribution, and their cumulative abundance among the total reads of all samples and their representation among the three major host lineages are given. Taxa represented by > 70% of the species in a host group were considered core lineages (marked in bold). Taxa with core status for all hosts groups that were included in the community similarity analysis (Fig. 5) are indicated with grey shading.

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abundance (%) ^b	Representation in hosts (%) ^c		
					Individual	Cumulative		Cockroaches	Lower termites	Higher termites
1	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i> Ia	3907	5.5	5.5	9.6	87	100	100
2	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i> Ic	901	3.6	9.1	3.6	47	63	100
3	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	<i>Alistipes</i> 2	969	3.5	12.6	7.4	100	100	100
4	<i>Bacteroidetes</i>	Cluster V	Subcluster Va	9	3.5	16.1	1.8	13	13	0
5	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i> Id	42	2.2	18.3	1.2	0	50	18
6	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 24	326	2.0	20.3	1.4	87	63	100
7	<i>Elusimicrobia</i>	<i>Endomicrobia</i>	Endomicrobium	306	1.9	22.3	2.4	100	100	91
8	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i> If	981	1.8	24.1	1.9	40	63	100
9	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	Uncultured 65	1268	1.8	25.9	3.9	100	88	100
10	<i>Proteobacteria</i>	<i>Campylobacteraceae</i>	<i>Arcobacter</i>	226	1.6	27.5	1.1	53	50	55

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
11	<i>Bacteroidetes</i>	<i>Blattabacteriaceae</i>	<i>Blattabacterium</i>	665	1.5	29.0	2.2	100	75	64
12	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Treponema</i> II	185	1.5	30.6	1.2	47	75	55
13	<i>Bacteroidetes</i>	<i>Bacteroidaceae</i>	<i>Bacteroides</i>	286	1.3	31.9	1.5	100	63	73
14	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	<i>Alistipes</i> 1	422	1.3	33.2	1.7	93	75	73
15	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Parabacteroides</i>	439	1.2	34.4	2.0	100	100	64
16	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Dysgonomonas</i>	628	1.2	35.5	2.1	100	75	91
17	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	<i>Cand. Arthromitus</i>	1647	1.0	36.5	2.1	100	88	100
18	<i>Bacteroidetes</i>	Insect cluster I	Insect Cluster	2	1.0	37.5	0.7	53	38	27
19	<i>Bacteroidetes</i>	Cluster V	Subcluster Ve	1	1.0	38.4	1.5	100	50	73
20	<i>Proteobacteria</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i> 3	769	1.0	39.4	2.1	100	88	100
21	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Insect Cluster 1	84	0.9	40.3	1.9	100	63	91
22	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Paludibacter</i>	98	0.8	41.1	1.8	100	100	100
23	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	Gut Cluster 2	1728	0.8	41.9	1.8	100	100	100

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
24	<i>Actinobacteria</i>	<i>Corynebacteriaceae</i>	<i>Corynebacterium</i>	8	0.8	42.7	0.5	53	25	18
25	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Gut Cluster 1	61	0.8	43.5	1.7	100	88	100
26	TG3	Termite cluster	Termite Cluster I	286	0.8	44.3	0.6	47	38	82
27	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 23	407	0.7	45.0	0.6	67	50	82
28	<i>Synergistetes</i>	<i>Synergistaceae</i>	Uncultured 6	297	0.7	45.7	1.2	100	100	100
29	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	<i>Anaerotruncus</i>	110	0.7	46.3	0.8	100	75	100
30	<i>Proteobacteria</i>	<i>Rhodocyclaceae</i> 3	Termite cluster	157	0.6	47.0	0.4	40	75	55
31	<i>Firmicutes</i>	Family XIII	Uncultured 5	269	0.6	47.6	1.2	100	100	100
32	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	<i>Robinsoniella</i>	1	0.6	48.2	0.4	20	25	64
33	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 12	529	0.6	48.8	1.3	93	100	100
34	<i>Firmicutes</i>	<i>Streptococcaceae</i>	<i>Lactococcus</i> 1	10	0.6	49.3	0.5	73	50	64
35	<i>Bacteroidetes</i>	<i>Flavobacteriaceae</i> 2	Uncultured a	113	0.5	49.9	0.4	40	50	18
36	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 30	762	0.5	50.4	0.9	93	88	100

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
37	<i>Bacteroidetes</i>	Cluster V	Subcluster Vb	7	0.5	50.9	0.5	7	13	91
38	<i>Fibrobacteres</i>	Termite cluster	Termite cluster II	181	0.5	51.4	0.3	40	50	73
39	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 36	632	0.5	51.8	0.9	100	50	100
40	<i>Proteobacteria</i>	<i>Acetobacteraceae</i>	<i>Acidisoma</i>	4	0.5	52.3	0.2	20	0	9
41	<i>Fusobacteria</i>	<i>Leptotrichiaceae</i>	<i>Sebaldella</i>	103	0.4	52.7	0.5	80	63	55
42	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	<i>Alistipes</i>	42	0.4	53.2	0.4	73	25	55
43	<i>Bacteroidetes</i>	<i>Marinilabiaceae</i>	Uncultured 1	74	0.4	53.6	0.5	93	38	27
44	<i>Firmicutes</i>	<i>Lactobacillaceae</i>	<i>Lactobacillus</i> 5	43	0.4	54.0	0.3	60	38	0
45	<i>Proteobacteria</i>	<i>Neisseriaceae</i> 1	Uncultured a	1	0.4	54.4	0.2	7	0	0
46	<i>Firmicutes</i>	<i>Veillonellaceae</i>	Uncultured 7	155	0.4	54.7	0.8	100	75	73
47	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	<i>Spirochaeta</i> 1	73	0.4	55.1	0.4	73	50	55
48	<i>Firmicutes</i>	<i>Enterococcaceae</i>	<i>Enterococcus</i> 2	24	0.4	55.5	0.4	80	50	9
49	<i>Firmicutes</i>	<i>Veillonellaceae</i>	<i>Phascolarctobacterium</i>	30	0.4	55.9	0.4	67	38	18

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
50	<i>Proteobacteria</i>	<i>Desulfovibrionaceae</i>	<i>Desulfovibrio</i> TC I	78	0.4	56.3	0.4	47	100	36
51	<i>Fibrobacteres</i>	<i>Termite cluster</i>	Termite cluster I	166	0.4	56.6	0.3	13	25	73
52	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	Cockroach cluster	1	0.4	57.0	0.4	80	38	27
53	<i>Proteobacteria</i>	Cluster 1	Insect cluster	1	0.4	57.3	0.6	100	38	55
54	<i>Firmicutes</i>	<i>Lactobacillaceae</i>	<i>Lactobacillus</i> 2	12	0.3	57.7	0.3	53	25	55
55	<i>Verrucomicrobia</i>	<i>Verrucomicrobiaceae</i>	<i>Akkermansia</i>	19	0.3	58.0	0.4	60	25	18
56	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	Insect cluster	2	0.3	58.3	0.4	100	100	73
57	<i>Synergistetes</i>	<i>Synergistaceae</i>	Uncultured 2	14	0.3	58.7	0.2	47	13	0
58	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Odoribacter</i>	26	0.3	59.0	0.2	40	25	27
59	<i>Firmicutes</i>	<i>Lactobacillaceae</i>	<i>Lactobacillus</i> 4	17	0.3	59.3	0.4	67	25	9
60	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	Gut cluster 1	61	0.3	59.6	0.5	100	63	55
61	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	<i>Papillibacter</i>	143	0.3	59.8	0.5	87	75	91
62	<i>Actinobacteria</i>	<i>Coriobacteriaceae</i>	Uncultured 10	170	0.3	60.1	0.6	93	100	100

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
63	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	Higher termite cluster	2	0.3	60.4	0.2	13	0	55
64	TG3	Termite cluster	Termite cluster II	152	0.3	60.6	0.2	7	13	73
65	<i>Proteobacteria</i>	<i>Campylobacteraceae</i>	<i>Sulfurospirillum</i>	125	0.3	60.9	0.3	53	50	55
66	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Tannerella</i>	209	0.2	61.1	0.4	93	63	100
67	<i>Bacteroidetes</i>	<i>Porphyromonadaceae</i>	<i>Bacteroidales</i> Cluster Vg	23	0.2	61.4	0.5	100	88	82
68	<i>Firmicutes</i>	<i>Enterococcaceae</i>	<i>Enterococcus</i> 7	2	0.2	61.6	0.1	20	0	0
69	<i>Firmicutes</i>	<i>Peptococcaceae</i>	Uncultured 2	10	0.2	61.9	0.4	93	50	82
70	<i>Proteobacteria</i>	<i>Enterobacteriaceae</i>	<i>Pragia</i>	8	0.2	62.1	0.1	7	13	0
71	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 35	380	0.2	62.3	0.4	87	88	100
72	<i>Proteobacteria</i>	<i>Desulfobulbaceae</i>	<i>Desulfobulbus</i>	80	0.2	62.5	0.4	93	38	82
73	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Incertae Sedis 1	1	0.2	62.7	0.2	60	88	100
74	<i>Firmicutes</i>	<i>Erysipelotrichaceae</i>	<i>Turicibacter</i>	59	0.2	62.9	0.3	0	13	91
75	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 28	97	0.2	63.1	0.4	87	50	73

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
76	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Uncultured 25	115	0.2	63.4	0.4	80	75	100
77	<i>Proteobacteria</i>	<i>Enterobacteriaceae</i>	Enteric cluster	38	0.2	63.6	0.2	60	63	45
78	<i>Firmicutes</i>	<i>Streptococcaceae</i>	<i>Lactovum</i>	77	0.2	63.7	0.2	7	25	73
79	<i>Actinobacteria</i>	<i>Coriobacteriaceae</i>	Marine group	84	0.2	63.9	0.2	13	38	64
80	<i>Bacteroidetes</i>	Insect cluster II	Uncultured a	79	0.2	64.1	0.4	87	50	91
81	<i>Proteobacteria</i>	<i>Desulfobacteraceae</i>	<i>Desulfatiferula</i>	52	0.2	64.3	0.3	93	63	36
82	<i>Spirochaetes</i>	<i>Spirochaetaceae</i>	Higher termite cluster	40	0.2	64.5	0.2	47	75	91
83	<i>Firmicutes</i>	<i>Lachnospiraceae</i>	Incertae sedis 34	9	0.2	64.7	0.2	87	63	36
84	<i>Firmicutes</i>	<i>Clostridiaceae</i> 1	<i>Cand. Savagella</i> -related	56	0.2	64.9	0.2	67	38	82
85	<i>Proteobacteria</i>	<i>Rhodocyclaceae</i> 2	Uncultured	36	0.2	65.0	0.2	33	88	91
86	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Termite cluster	84	0.2	65.2	0.3	60	88	73
87	<i>Proteobacteria</i>	Cluster 1	Higher termite cluster	129	0.2	65.4	0.2	7	50	100
88	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	Insect Cluster	2	0.2	65.5	0.3	53	100	91

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
89	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	RC9	25	0.2	65.7	0.2	80	38	18
90	<i>Actinobacteria</i>	<i>Nakamurellaceae</i>	<i>Nakamurella</i>	2	0.2	65.9	0.1	0	0	9
91	<i>Firmicutes</i>	<i>Ruminococcaceae</i>	Incertae Sedis 4	1	0.2	66.0	0.4	93	88	91
92	<i>Planctomycetes</i>	<i>Planctomycetaceae</i>	Termite cluster	85	0.2	66.2	0.3	87	50	73
93	<i>Lentisphaerae</i>	<i>Victivallaceae</i>	<i>Victivallis</i>	70	0.2	66.4	0.3	87	38	36
94	<i>Firmicutes</i>	<i>Peptococcaceae</i>	<i>Desulfitibacter</i>	35	0.2	66.5	0.1	33	50	64
95	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	Gut cluster c	89	0.2	66.7	0.3	93	38	55
96	<i>Firmicutes</i>	<i>Leuconostocaceae</i>	<i>Weissella</i> 1	5	0.2	66.8	0.1	40	25	9
97	<i>Proteobacteria</i>	<i>Enterobacteriaceae</i>	<i>Morganella</i>	1	0.2	67.0	0.1	0	0	0
98	<i>Firmicutes</i>	<i>Leuconostocaceae</i>	<i>Leuconostoc</i>	2	0.2	67.2	0.1	20	13	0
99	<i>Bacteroidetes</i>	<i>Rikenellaceae</i>	Insect cluster 2	2	0.2	67.3	0.3	67	75	91
100	<i>Bacteroidetes</i>	Insect custer II	uncultured b	81	0.2	67.5	0.2	13	25	64
119	<i>Firmicutes</i>	<i>Enterococcaceae</i>	<i>Enterococcus</i> 4	2	0.1	70.0	0.1	47	25	9

Rank	Phylum	Family	Genus level	No. of OTUs (3%)	Contribution (%) ^a		Read abund- ance (%) ^b	Representation in hosts (%) ^c		
					Indivi- dual	Cumula- tive		Cock- roaches	Lower termites	Higher termites
320	<i>Acidobacteria</i>	<i>Acidobacteriaceae</i>	Uncultured 5	1	<0.1	80.0	0.0	53	100	55
598	<i>Proteobacteria</i>	<i>Comamonadaceae</i>	<i>Ottowia</i>	2	<0.1	90.0	0.0	47	75	91
884	<i>Proteobacteria</i>	<i>Desulfarculaceae</i>	<i>Desulfarculus</i>	21	<0.1	100.0	0.0	0	13	9

^a The contribution to the overall clustering in a PCA analysis, expressed as the fraction of the total variance explained by this particular genus.

^b Fraction of reads in all samples belonging to a particular taxon (normalized samples).

^c To increase sensitivity, datasets were not subsampled for the core analysis.