

SUPPLEMENTARY INFORMATION FOR

Mating changes the genital microbiome in both sexes of the common bedbug *Cimex lectularius* across populations

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Separate Excel files:

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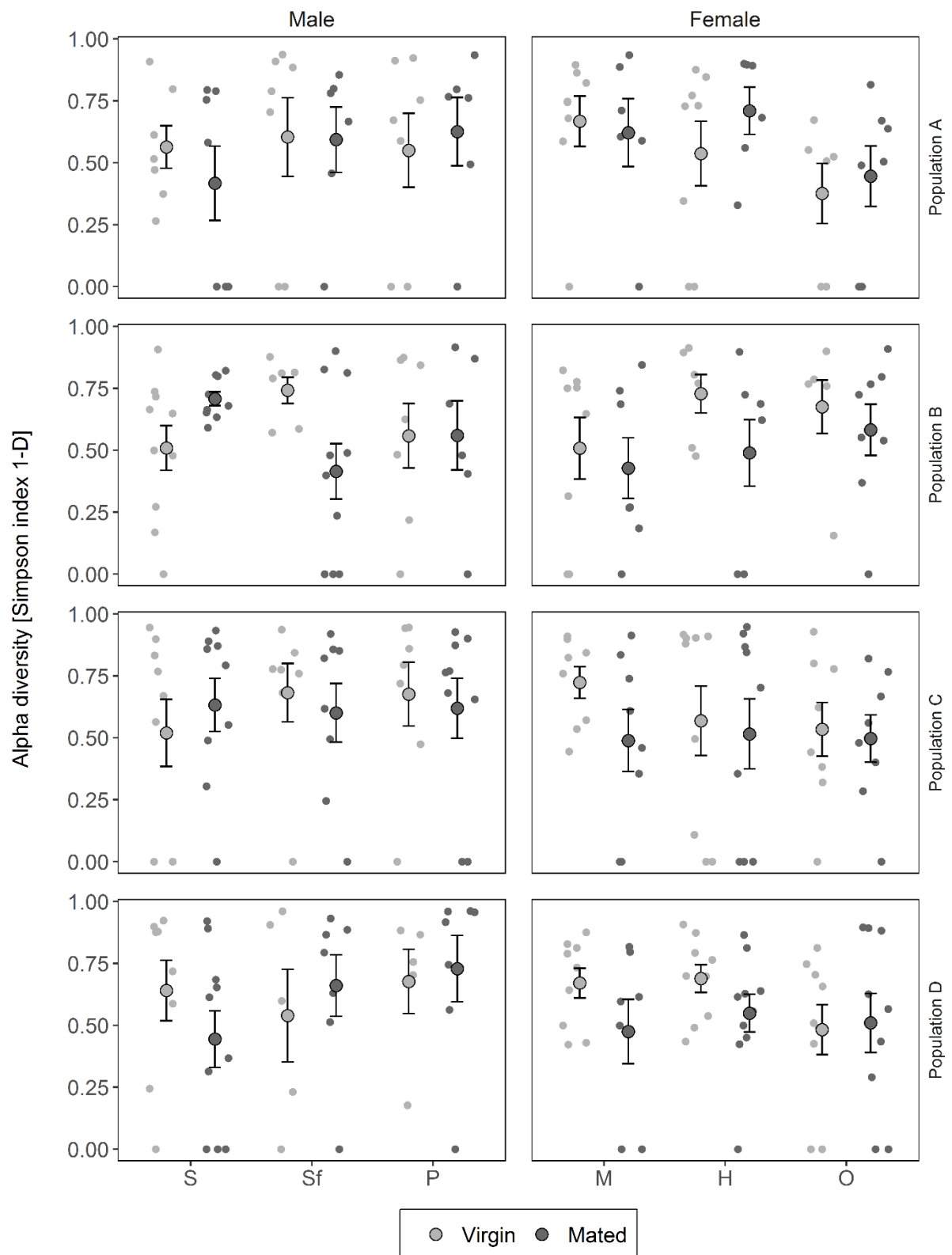


Figure S1 Alpha diversity of each sample in the sperm vesicle (S), the seminal fluid vesicle (Sf), on the paramere (P), in the mesosperma (M), the haemolymph (H) and the ovary (O). Depicted are means, standard errors of the mean, and all individual data points.

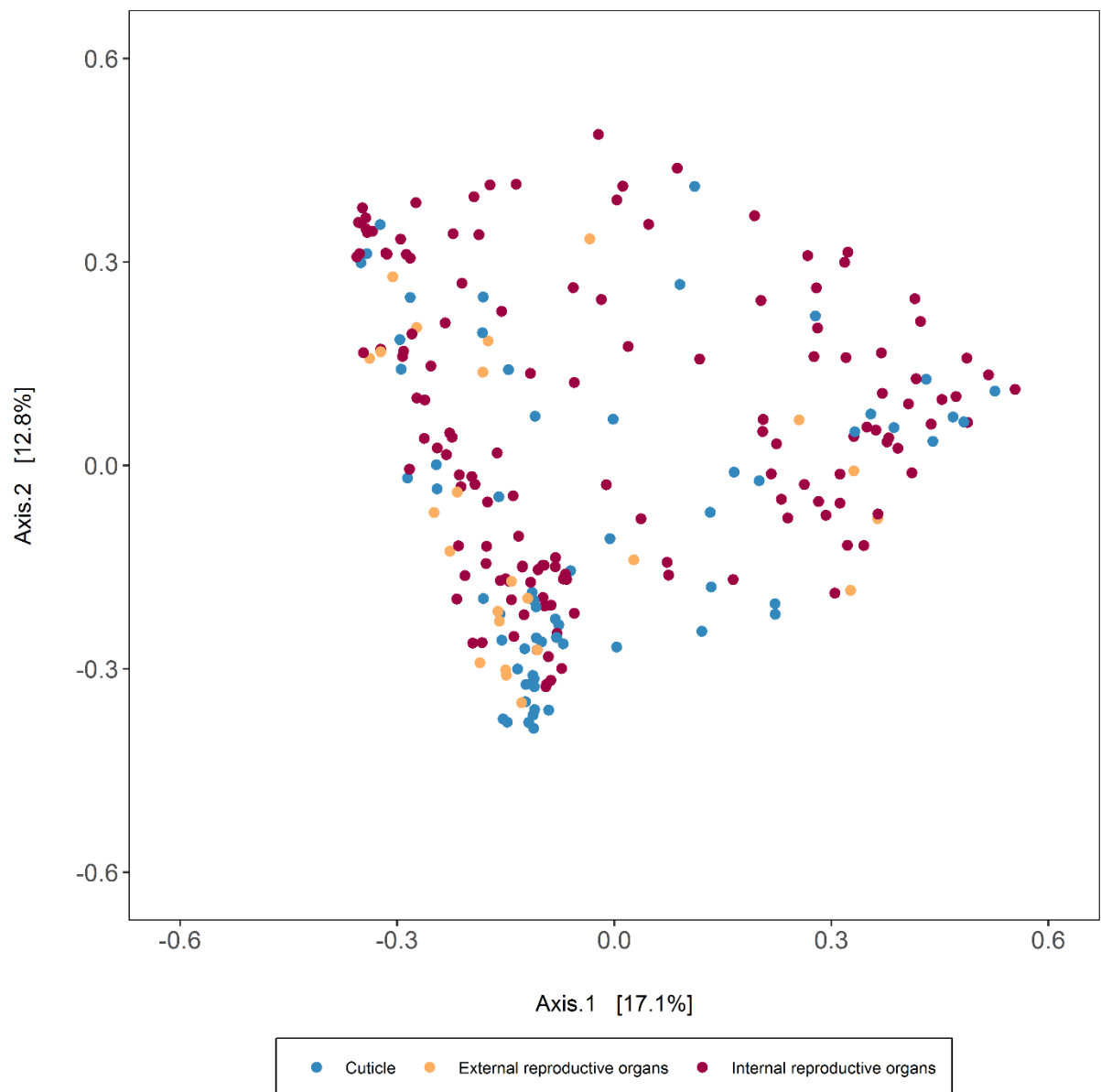


Figure S2 PCoA of microbiomes from cuticle in comparison to the external reproductive organ of males) and internal reproductive organs of both sexes based on Bray-Curtis dissimilarities.

SUPPLEMENTARY TABLES

Table S1 Sample sizes and number of bacterial communities that were successfully sequenced and endured quality filtering for each group of samples. Sampled organs were: cuticle (C), sperm vesicle (S), seminal fluid vesicle (Sf), paramere (P), mesospermae (M), haemolymph (H) and ovary (O).

Population	Sex	Organ	Mating status	N total	N sequenced
A	Male	C	Virgin	10	7
A	Male	C	Mated	10	6
A	Male	S	Virgin	10	7
A	Male	S	Mated	11	7
A	Male	Sf	Virgin	10	7
A	Male	Sf	Mated	10	6
A	Male	P	Virgin	10	7
A	Male	P	Mated	10	6
A	Female	C	Virgin	11	7
A	Female	C	Mated	11	8
A	Female	M	Virgin	10	8
A	Female	M	Mated	10	6
A	Female	H	Virgin	11	8
A	Female	H	Mated	10	6
A	Female	O	Virgin	10	6
A	Female	O	Mated	9	7
B	Male	C	Virgin	10	9
B	Male	C	Mated	10	8
B	Male	S	Virgin	10	10
B	Male	S	Mated	10	9
B	Male	Sf	Virgin	10	6
B	Male	Sf	Mated	10	10
B	Male	P	Virgin	12	7
B	Male	P	Mated	8	6
B	Female	C	Virgin	10	10
B	Female	C	Mated	10	8
B	Female	M	Virgin	10	8
B	Female	M	Mated	10	7
B	Female	H	Virgin	10	6
B	Female	H	Mated	10	8
B	Female	O	Virgin	10	6
B	Female	O	Mated	10	8
C	Male	C	Virgin	10	9
C	Male	C	Mated	10	7
C	Male	S	Virgin	10	9
C	Male	S	Mated	10	9
C	Male	Sf	Virgin	10	7
C	Male	Sf	Mated	10	8
C	Male	P	Virgin	10	7
C	Male	P	Mated	10	9
C	Female	C	Virgin	10	9

C	Female	C	Mated	10	8
C	Female	M	Virgin	10	8
C	Female	M	Mated	10	8
C	Female	H	Virgin	10	9
C	Female	H	Mated	10	9
C	Female	O	Virgin	10	8
C	Female	O	Mated	10	8
D	Male	C	Virgin	10	8
D	Male	C	Mated	10	8
D	Male	S	Virgin	10	8
D	Male	S	Mated	10	10
D	Male	Sf	Virgin	10	5
D	Male	Sf	Mated	10	7
D	Male	P	Virgin	10	5
D	Male	P	Mated	10	7
D	Female	C	Virgin	10	8
D	Female	C	Mated	10	10
D	Female	M	Virgin	10	9
D	Female	M	Mated	10	7
D	Female	H	Virgin	10	9
D	Female	H	Mated	10	10
D	Female	O	Virgin	10	9
D	Female	O	Mated	10	9

Table S2 Contaminants found with the *decontam* package (Davis et al. 2018) based on prevalences in controls. Given are the contaminants and their prevalences within each type of control, i.e. control for dissection, control for DNA isolation, control for target PCR, and control for indexing PCR.

Contaminant	Dissection (N=1)	DNA isolation (N=1)	Target PCR (N=16)	Indexing PCR (N=13)
Unclassified Enterobacteriaceae	1	0	0.81	0.62
<i>Wolbachia</i> sp.	0	0	0.75	0.77
<i>Bradyrhizobium</i> sp.	0	0	0.44	0.08
<i>Stenotrophomonas</i> sp.	0	0	0.25	0.08
<i>Myroides</i> sp.	0	0	0.13	0.00
<i>Methylobacterium</i> sp.	0	0	0.38	0.08
<i>Pseudomonas</i> sp.	0	0	0.13	0.00
<i>Methylobacterium</i> sp.	0	0	0.19	0.08
<i>Enterococcus</i> sp.	0	0	0.13	0.00
<i>Sphingomonas</i> sp.	0	0	0.25	0.00
Unclassified Streptophyta	0	0	0.13	0.00
<i>Curvibacter</i> sp.	0	0	0.25	0.08
<i>Rickettsia</i> sp.	0	0	0.06	0.08
<i>Sphingomonas</i> sp.	0	0	0.19	0.00
<i>Curvibacter</i> sp.	0	0	0.19	0.00
Unclassified Streptophyta	0	0	0.06	0.00
Unclassified Erythrobacteraceae	0	0	0.13	0.08
<i>Janthinobacterium</i> sp.	0	0	0.13	0.00
Unclassified Erythrobacteraceae	0	0	0.13	0.00
Unclassified Lactobacillales	0	0	0.06	0.00
<i>Janthinobacterium</i> sp.	0	0	0.13	0.00
<i>Pseudomonas</i> sp.	0	0	0.06	0.08
Unclassified Lactobacillales	0	0	0.06	0.00
<i>Sphingomonas</i> sp.	0	0	0.13	0.00
<i>Methylobacterium</i> sp.	0	0	0.06	0.00
<i>Methylobacterium</i> sp.	0	0	0.06	0.00
<i>Novosphingobium</i> sp.	0	0	0.13	0.08
<i>Paracoccus</i> sp.	0	0	0.13	0.00
<i>Renibacterium</i> sp.	0	0	0.13	0.00
Unclassified Pseudomonadaceae	0	0	0.06	0.00
Unclassified Rhodospirillaceae	0	0	0.06	0.00
<i>Bacillus thermoalkalophilus</i>	0	0	0.06	0.00
<i>Prevotella</i> sp.	0	0	0.13	0.00
<i>Pseudoxanthomonas taiwanensis</i>	0	0	0.13	0.00
Unclassified Rhodospirillaceae	0	0	0.06	0.00
<i>Rickettsia</i> sp.	0	0	0.06	0.08
Unclassified Pseudomonadaceae	0	0	0.06	0.00
<i>Thermomonas</i> sp.	0	0	0.06	0.00
Unclassified Erythrobacteraceae	0	0	0.06	0.00
Unclassified Rhodospirillaceae	0	0	0.06	0.00
<i>Bacillus thermoalkalophilus</i>	0	0	0.06	0.00
<i>Thermus</i> sp.	0	0	0.06	0.00

<i>Rubrivivax</i> sp.	0	0	0.06	0.00
<i>Bosea</i> genosp.	0	0	0.06	0.00
Unclassified Pseudomonadaceae	0	0	0.06	0.00
Unclassified Phyllobacteriaceae	0	0	0.06	0.00
<i>Sphingobacterium</i> sp.	0	0	0.06	0.00
Unclassified Comamonadaceae	0	0	0.06	0.00
<i>Marinobacter</i> sp.	0	0	0.06	0.00
<i>Bosea</i> genosp.	0	0	0.06	0.00
<i>Marinobacter</i> sp.	0	0	0.06	0.00
<i>Nevskia</i> sp.	0	0	0.06	0.00

Table S6 SVs potentially transmitted from males to females. Given are prevalence and relative abundance of all SVs that were found in mated females and in virgin males but not in virgin females.

ID	SV	Prevalence	Min. relative abundance	Max. relative abundance
<i>Population A</i>		(N=19)		
SV30	Unclassified <i>Aeromonas</i>	6	0.0009	0.0065
SV44	<i>Staphylococcus</i> sp.	2	0.0001	0.0003
SV51	<i>Bradyrhizobium</i> sp.	6	0.0006	0.0091
SV66	<i>Caulobacter</i> sp.	7	0.0001	0.0045
SV68	<i>Alcanivorax</i> sp.	4	0.0004	0.0024
SV69	<i>Brevibacterium</i> sp.	1	0.0004	0.0004
SV73	<i>Staphylococcus</i> sp.	2	0.0010	0.0018
SV77	<i>Alcanivorax</i> sp.	4	0.0003	0.0017
SV83	<i>Staphylococcus</i> sp.	1	0.0010	0.0010
SV86	Unclassified Comamonadaceae	3	0.0004	0.0020
SV94	<i>Bradyrhizobium</i> sp.	3	0.0003	0.0020
SV97	<i>Cutibacterium acnes</i>	5	0.0001	0.0029
SV98	<i>Acinetobacter</i> sp.	2	0.0003	0.0016
SV99	<i>Bradyrhizobium</i> sp.	4	0.0005	0.0009
SV100	Unclassified Gammaproteobacteria	1	0.0002	0.0002
SV106	<i>Corynebacterium</i> sp.	5	0.0002	0.0023
SV109	<i>Paracoccus</i> sp.	1	0.0014	0.0014
SV111	<i>Pseudomonas</i> sp.	3	0.0005	0.0008
SV114	<i>Cloacibacterium</i> sp.	2	0.0000	0.0007
SV115	Unclassified Alcaligenaceae	1	0.0003	0.0003
SV116	<i>Acinetobacter</i> sp.	5	0.0001	0.0018
SV117	Unclassified Lactobacillaceae	1	0.0004	0.0004
SV118	<i>Cloacibacterium</i> sp.	2	0.0002	0.0005
SV120	<i>Lactobacillus</i> sp.	1	0.0002	0.0002
SV122	Unclassified Gammaproteobacteria	2	0.0003	0.0018
SV124	<i>Pseudomonas</i> sp.	2	0.0006	0.0006
SV125	<i>Acinetobacter lwoffii</i>	4	0.0003	0.0021
SV126	<i>Comamonas</i> sp.	3	0.0004	0.0015
SV135	Unclassified Comamonadaceae	2	0.0004	0.0047
<i>Population B</i>		(N=22)		
SV135	Unclassified Comamonadaceae	1	0.0030	0.0030
<i>Population C</i>		(N=25)		
SV111	<i>Pseudomonas</i> sp.	4	0.0002	0.0063
SV124	<i>Pseudomonas</i> sp.	1	0.0004	0.0004
SV140	Unclassified Rhodobacteraceae	1	0.0001	0.0001
<i>Population D</i>		(N=26)		
SV33	<i>Stenotrophomonas</i> sp.	1	0.0003	0.0003
SV117	Unclassified Lactobacillaceae	3	0.0001	0.0023
SV118	<i>Cloacibacterium</i> sp.	2	0.0001	0.0009

SV138	<i>Corynebacterium</i> sp.	1	0.0003	0.0003
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Table S7 SVs potentially transmitted from females to males. Given are prevalence and relative abundance of all SVs that were found in mated males and in virgin females but not in virgin males.

ID	SV	Prevalence	Min. relative abundance	Max. relative abundance
<i>Population A</i>			<i>(N=19)</i>	
-	-	-	-	-
<i>Population B</i>			<i>(N=25)</i>	
SV90	<i>Bradyrhizobium</i> sp.	2	0.0011	0.0033
SV93	<i>Halomonas</i> sp.	2	0.0015	0.0024
SV122	Unclassified Gammaproteobacteria	2	0.0004	0.0008
SV134	Unclassified Gammaproteobacteria	2	0.0004	0.0009
<i>Population C</i>			<i>(N=26)</i>	
-	-	-	-	-
<i>Population D</i>			<i>(N=24)</i>	
SV44	<i>Staphylococcus</i> sp.	1	0.0016	0.0016
SV108	<i>Lactobacillus</i> sp.	1	0.0002	0.0002

SUPPLEMENTARY REFERENCES

Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. 2018 Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* **6**, 226. (doi:10.1186/s40168-018-0605-2)