

**Supplemental Table 1** Overview of primers and probes used in this study

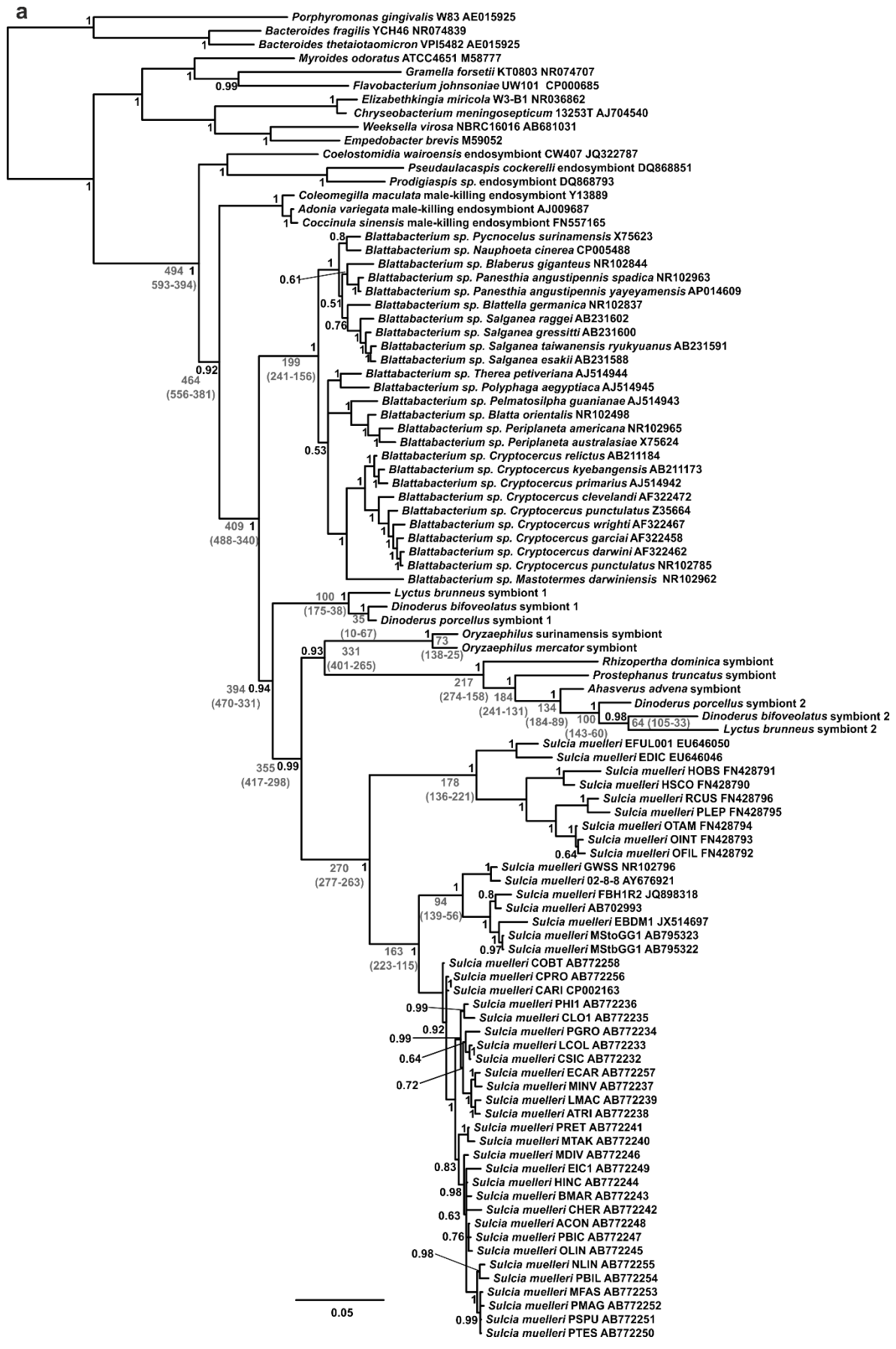
	Sequence	Specificity RDB	Specificity within beetle symbionts	Use	Reference
fd1 (fwd)	AGAGTTTGATCCTGGCTCAG	NA	NA	bacterial 16S PCR	(Weisburg et al., 1991)
rP2 (fwd)	ACGGCTACCTTGTTACGACTT	NA	NA	bacterial 16S PCR	(Weisburg et al., 1991)
M13_fwd	TGTAACGACGCGCCAGT	NA	NA	cloning PCR	StrataClone
M13_rev	GGAAACAGCTATGACCATG	NA	NA	cloning PCR	StrataClone
OsurSym_fwd2	GGCAACTCTGAACTAGCTACGC	0/3070243	<i>Oryzaephilus sp.</i> symbiont	diagnostic PCR, qPCR	
OsurSym_rev2	AGTCCCCAGCCAAACTGATG	40/3070243	<i>Oryzaephilus sp.</i> symbiont	diagnostic PCR	
RdomSym_fwd1	TATGAACGACAGTAGATAAGC	0/3070243	<i>R. dominica</i> symbiont	diagnostic PCR	
RdomSym_rev1	GCTTATAGTTACCTACTCGC	0/3070243	<i>R. dominica</i> symbiont	diagnostic PCR	
PtruSym_fwd1	ACCCCCACATTGGTACTGGA	0/3070243	<i>P. truncatus</i> & <i>A. advena</i> symbiont	diagnostic PCR	
PtruSym_rev1	GCCTTCATCCTACACGCGAA	3/3070243	<i>P. truncatus</i> symbiont	diagnostic PCR	
DspSym1_fwd2	CGTACGTAATCTACCTTTTGC	0/3070243	ancestral symbiont of <i>Dinoderus sp.</i> , <i>L. brunneus</i>	diagnostic PCR	
DspSym1_rev2	CTCCGTCGAAGAATAAATT	0/3070243	ancestral symbiont of <i>Dinoderus sp.</i>	diagnostic PCR	
DspSym2_fwd1	GATAAACGCTAGCGATTAGC	0/3070243	derived bostrichid clade symbionts	diagnostic PCR	
DspSym2_rev1	CCTTCCTCTAAACTTACGTTAG	0/3070243	derived symbiont of <i>Dinoderus sp.</i>	diagnostic PCR	
mod. CFB563_rev	GACCCCTTTAAACCAAT	NA	all except <i>L. brunneus</i> symbionts & derived symbiont of <i>D. bifoveolatus</i>	qPCR, FISH	(Weller et al., 2000)
28Srr	GGGACCCGTCTTGAACAC	NA	NA	qPCR	(Levkanicova, 2009)
Osur_28Sr	TCTTCCTTAGTTTACGCAAGG	NA	NA	qPCR	
Eub338	GCTGCCTCCCGTAGGAGT	NA	NA	FISH	(Amann et al., 1990)
OsurSym_16S	AGCAGCCCATGGACTAACC	0/3070243	<i>Oryzaephilus sp.</i> symbiont	FISH	
Bostrichidae_Sym1	TACTCGATGGCAATTAACAAC	0/3070243	ancestral symbiont of <i>Dinoderus sp.</i> , <i>L. brunneus</i>	FISH	
Bostrichidae_Sym2	CTTCCTACACGCGAAATAG	0/3070243	derived bostrichid clade symbionts except <i>L. brunneus</i>	FISH	

**Supplemental Table 2** Infection frequencies in Silvanid and Bostrychid grain pest beetles based on diagnostic PCR and FISH

<b>Species</b>	<b>N</b>	<b>Ancestral symbiont present</b>	<b>Derived symbiont present</b>	<b>Any symbiont present</b>
<i>A. advena</i>	10	n.a.	30%	30%
<i>O. surinamensis</i>	100	n.a.	99%	99%
<i>O. mercator</i>	21	n.a.	91%	91%
<i>R. dominica</i>	25	n.a.	76%	76%
<i>P. truncatus</i>	21	n.a.	91%	91%
<i>D. bifoveolatus</i>	21	100%	90%	100%
<i>D. porcellus</i>	21	86%	71%	95%
<i>L. brunneus</i>	25	96%	68%	100%

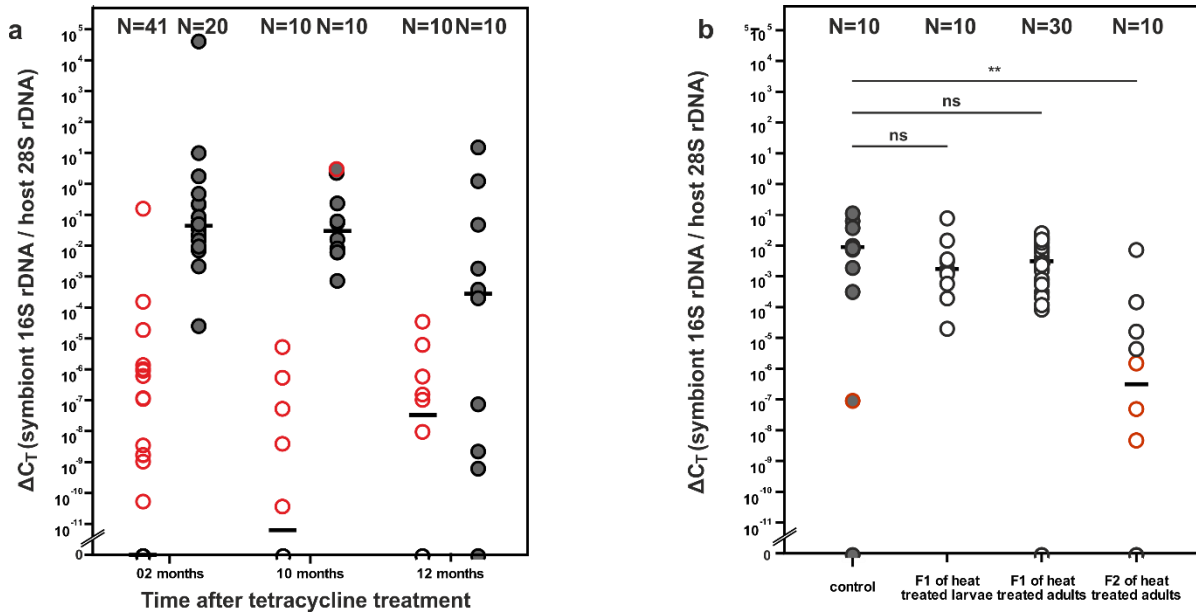
**Supplemental Table 3** Model parameters and results of the phylogenetic dating analyses using BEAUti and BEAST

	substitution model	base frequencies	clock model	age <i>Blattabacterium</i>	age <i>Sulcia</i>	ESS values	Log marginal likelihood using		age of all endosymbionts (Mya; mean and 95% HSD)	age of male killing endosymbionts & mutualists (Mya, mean and 95% HSD)	age of <i>Blattabacteria</i> , beetle symbionts, <i>Sulcia</i> (Mya mean and 95% HSD)
							path sampling	stepping stone sampling			
TE_51	HKY	empirical	strict	normal: 220 ± 25	normal: 270 ± 3.5	good	-20671	-20670	432 (392-470)	411 (377-450)	384 (323-418)
TE_52	HKY	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-20498	-20498	503 (404-613)	462 (375-563)	410 (341-484)
TE_53	GTR	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-20387	-20387	504 (406-606)	465 (373-554)	413 (340-488)
TE_54	TN93	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-20475	-20474	503 (415-612)	462 (384-550)	409 (341-483)
TE_55	HKY + G	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18728	-18728	489 (384-607)	457 (369-568)	404 (333-490)
TE_56	HKY +G +I	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18695	-18695	498 (392-626)	465 (355-574)	412 (328-505)
TE_57	GTR + G	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18721	-18721	502 (388-627)	470 (374-586)	414 (335-508)
TE_58	GTR +G +I	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18687	-18688	494 (394-593)	464 (381-557)	409 (340-488)
TE_59	TN93 +G	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18729	-18729	498 (384-613)	463 (362-567)	409 (334-501)
TE_60	TN93 +G +I	estimated	relaxed; lognormal	normal: 220 ± 25	normal: 270 ± 3.5	good	-18694	-18694	494 (386-613)	460 (360-569)	408 (328-498)
TE_61	GTR +G +I	estimated	strict	normal: 220 ± 25	normal: 270 ± 3.5	good	-18841	-18842	423 (381-464)	410 (373-450)	392 (359-432)
TE_62	GTR +G +I	estimated	relaxed; lognormal		normal: 270 ± 3.5	good	-18692	-18693	475 (372-567)	446 (356-538)	396 (320-473)
TE_63	GTR +G +I	estimated	relaxed; lognormal	normal: 220 ± 25		good	-18689	-18690	593 (364-872)	557 (340-797)	495 (310-722)



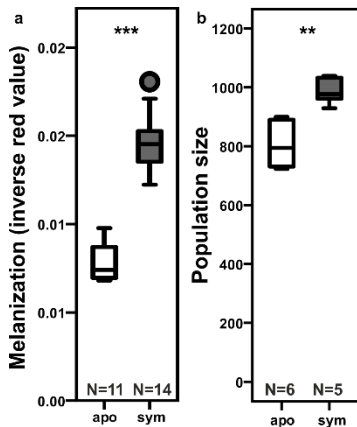


1 **Supplemental Figure 1** Detailed phylogenetic analysis of Bacteroidetes symbionts in grain pest beetles,  
2 cockroaches and cicadas with (a) Bayesian inference, (b) maximum likelihood analysis. Black node values  
3 are Bayesian posteriors and bootstrap values of 10,000 replicates, respectively. Grey values indicate  
4 mean node age and in brackets 95% HSD intervals in Mya.



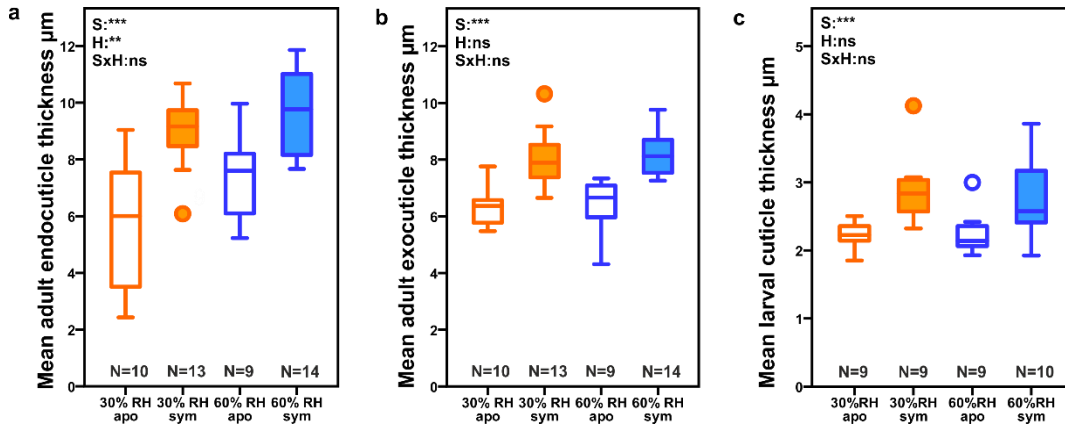
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7 **Supplemental Figure 2** Success of symbiont elimination in *O. surinamensis*, measured as relative abundance  
 8 of symbiont 16S rDNA in eggs. (a) Tetracycline treatment could completely and stably eliminate symbionts  
 9 in the offspring of treated larvae. (b) Heat treatment slightly, but non-significantly reduced relative  
 10 symbiont abundance in the F1 offspring of treated larvae and adults (Mann-Whitney-U test,  $p > 0.05$ ), but led  
 11 to a significant reduction in the F2 offspring of treated adults (Mann-Whitney-U test,  $p = 0.003$ ). Filled circles  
 12 are symbiotic controls, empty one symbiont depletion treatments. Red circles represent off-target  
 13 amplification during late qPCR cycles, identified by melting curve analysis. Horizontal lines show medians of  
 14 each treatment including off target amplifications. Sample sizes are given within the graphs.



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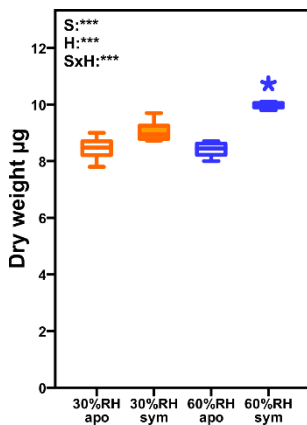
16 **Supplemental Figure 3** Melanization and population growth at standard culturing conditions. (a)  
 17 Melanization of two day old symbiotic and aposymbiotic *O. surinamensis* adults, and (b) growth of  
 18 populations with initially 50 symbiotic or aposymbiotic beetles over three months reared at 30°C and ~50-  
 19 60% RH. Melanization and population growth are significantly reduced in the absence of symbionts (Mann-  
 20 Whitney-U tests,  $p < 0.001$  and  $p = 0.004$ ). Boxplots show medians, quartiles, and minimum/maximum values.  
 21 Sample sizes are given underneath the boxes. Empty boxes represent aposymbiotic beetles, filled ones  
 22 symbiotic beetles.



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24 **Supplemental Figure 4** Thickness of adult endo- and exocuticle and larval cuticle. (a) Endocuticle thickness  
 25 of *O.surinamensis* adults was significantly influenced by symbiont presence (S; GLM,  $p < 0.001$ ) as well as  
 26 environmental humidity (H; GLM,  $p = 0.009$ ), whereas (b) adult exocuticle and (c) larval cuticle were only  
 27 influenced by symbiont presence (S; GLM,  $p < 0.001$ ). Boxplots show medians, quartiles, and  
 28 minimum/maximum values. Sample sizes are given under each box. Filled boxes represent symbiotic and  
 29 empty ones aposymbiotic beetles, orange boxes indicate rearing at 30% RH, blue ones at 60% RH

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32 **Supplemental Figure 5** Body mass of *O.surinamensis* adults. Dry weight was significantly influenced by  
 33 symbiont presence, environmental humidity as well as their interaction (S, H, S\*H; GLM,  $p < 0.001$ ,  $N = 8$ ).  
 34 Boxplots show medians, quartiles, and minimum/maximum values. Sample sizes are given under each box.  
 35 Filled boxes represent symbiotic and empty ones aposymbiotic beetles, orange boxes indicate rearing at  
 36 30% RH, blue ones at 60% RH