Field-collected *Triatoma sordida* from central Brazil display high microbiota diversity that varies with regard to developmental stage and intestinal segmentation

Short title: ***Triatoma sordida* intestinal microbiota**

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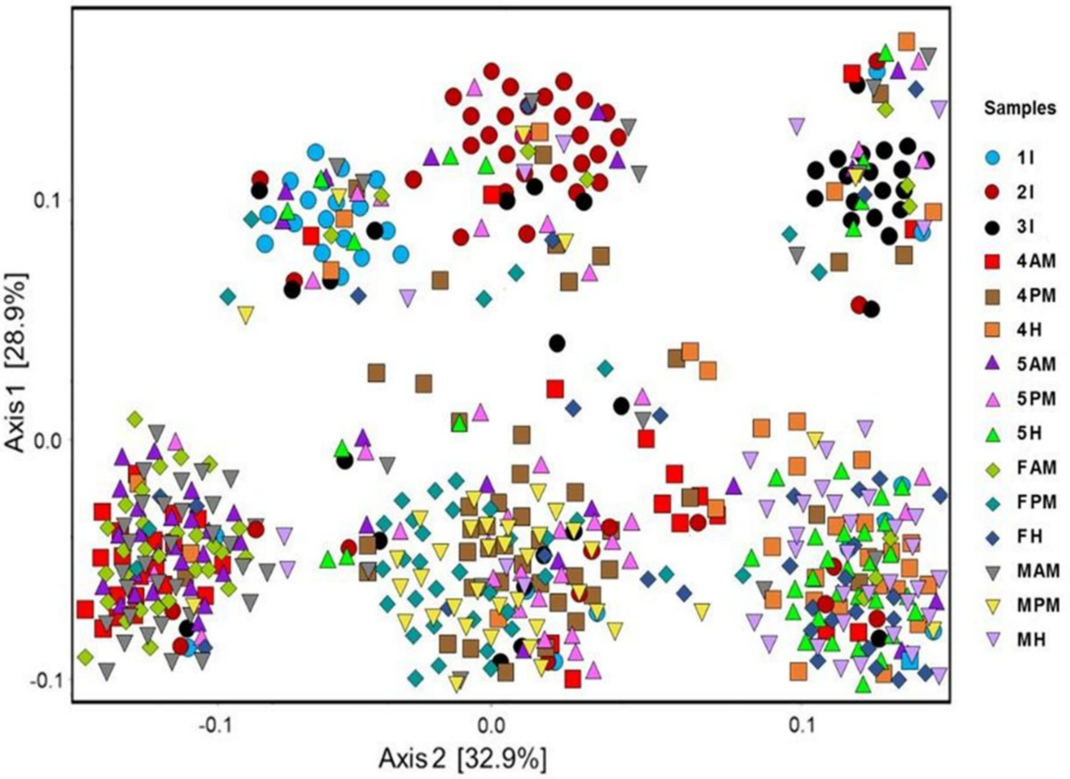
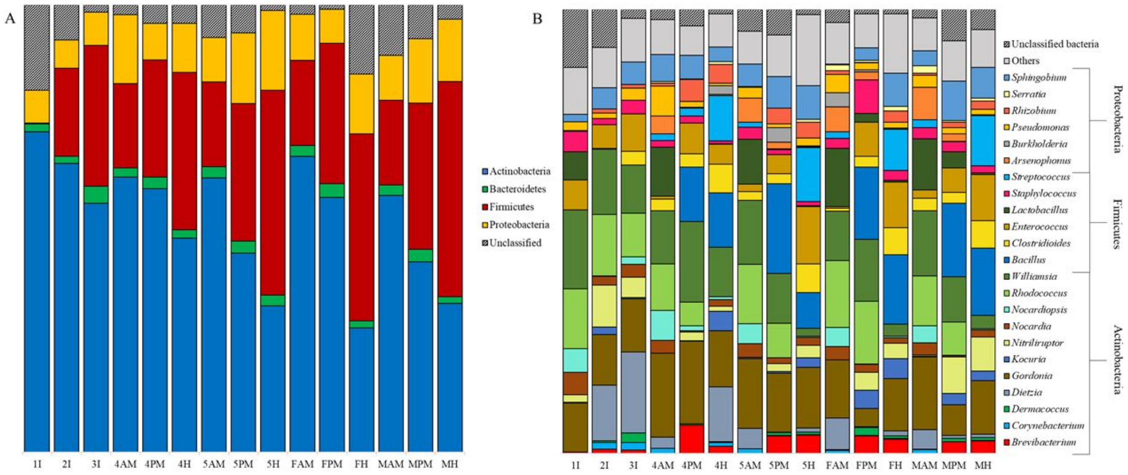
S1 Table. Analysis of similarity (ANOSIM) of the bacterial community of *T. sordida* intestine. Corrected P-values (<0.01) were calculated using the Bonferroni method. Significant comparisons are shown in italic. Significant and meaningful comparisons are shown both in italic and bold. Samples analyzed: entire intestine of 1st stage nymphs (1I); entire intestine of 2nd stage nymphs (2I); entire intestine of 3rd stage nymphs (3I); anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).

	1I	2I	3I	4AM	4PM	4H	5AM	5PM	5H	FAM	FPM	FH	MAM	MPM	MH
1I	-	0.0682	0.0127	**0.0042**	**0.0040**	**0.0099**	**0.0011**	**0.0011**	**0.0011**	0.0504	0.0509	**0.0009**	0.0505	0.0505	**0.0007**
2I		-	0.0122	**0.0013**	**0.0053**	**0.0011**	0.0405	**0.0051**	**0.0045**	**0.0042**	**0.0051**	**0.0008**	0.0452	**0.0050**	**0.0008**
3I			-	**0.0011**	0.0100	**0.0008**	0.0421	0.0102	**0.0045**	0.0412	**0.0040**	**0.0008**	**0.0010**	0.0110	**0.0009**
4AM				-	**0.0001**	**0.0052**	0.0826	**0.0008**	**0.0051**	0.0155	**0.0015**	**0.0023**	0.0457	**0.0013**	**0.0009**
4PM					-	0.0008	0.3495	0.2010	**0.0012**	**0.0011**	0.0729	0.2733	0.0827	0.0802	**0.0022**
4H						-	**0.0094**	0.0280	0.0495	**0.0007**	0.2395	**0.0010**	**0.0008**	0.0999	**0.0098**
5AM							-	0.0290	**0.0039**	0.0222	**0.0052**	**0.0010**	**0.0012**	0.0119	**0.0010**
5PM								-	**0.0013**	0.0224	0.0829	**0.0042**	0.0822	0.0830	0.5115
5H									-	**0.0053**	0.3341	0.0110	0.1000	0.2113	0.0612
FAM										-	**0.0015**	0.0110	**0.0015**	**0.0010**	0.1002
FPM											-	**0.0083**	0.0322	0.0210	**0.0011**
FH												-	0.0112	0.3924	0.1001
MAM													-	0.0100	0.1021
MPM														-	**0.0010**
MH															-

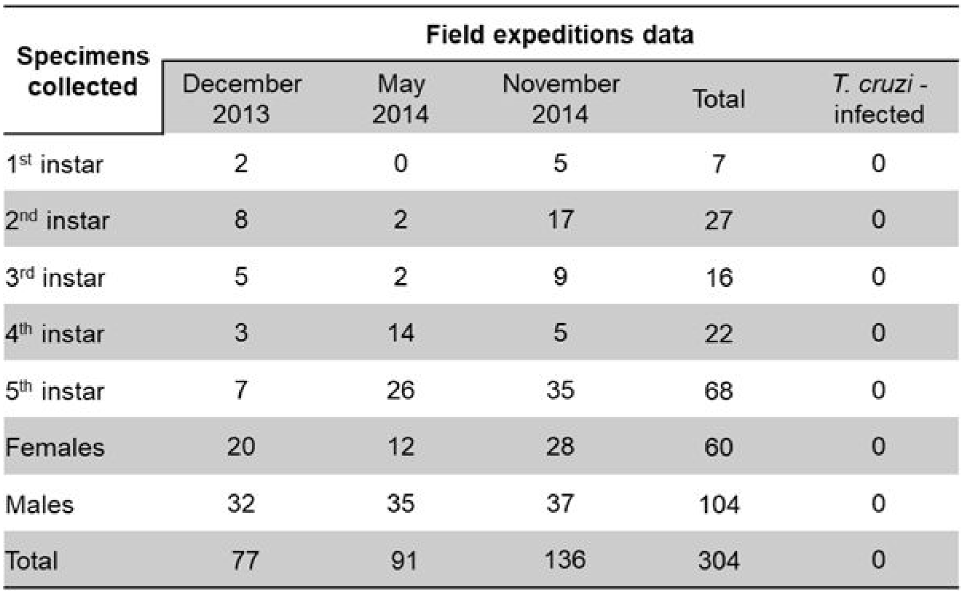


S1 Appendix. Principal coordinate analysis (PCoA) of weighted-UniFrac distances comparing the bacterial communities of *T. sordida* intestine. Weighted-UniFrac distances matrices were calculated for each sample using one representative sequence for each OTU (3%). Axes represent the greatest proportion of variances in the communities for each analysis. Samples analyzed: entire intestine of 1st stage nymphs (1I); entire intestine of 2nd stage nymphs (2I); entire intestine of 3rd stage nymphs (3I); anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).



S2 Appendix. Relative abundance of bacterial phyla (A) and genera (B) found in *T. sordida* intestinal microbiota. Samples: entire intestine of 1st stage nymphs (1I); entire intestine of 2nd stage nymphs (2I); entire intestine of 3rd stage nymphs (3I); anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).

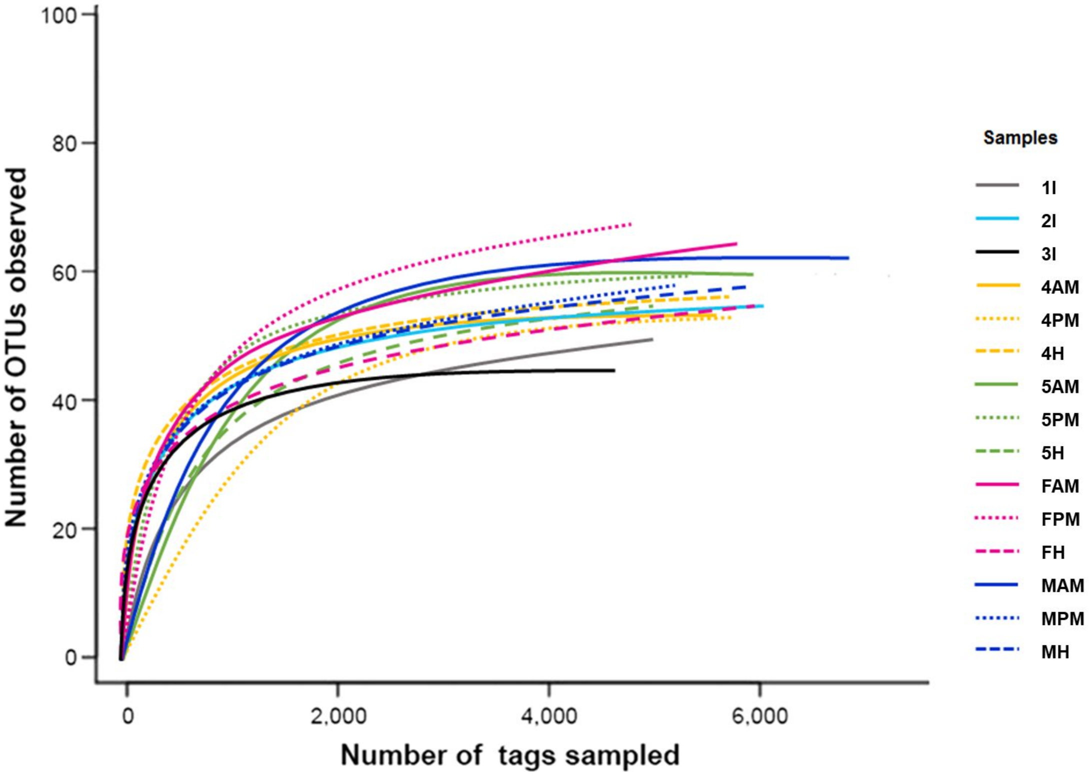
S2 Table. Field expedition data. Number of collected specimens of *T. sordida* in three expeditions and positivity to *T. cruzi*.



Specimens collected	Field expeditions data				
	December 2013	May 2014	November 2014	Total	*T. cruzi* - infected
1st instar	2	0	5	7	0
2nd instar	8	2	17	27	0
3rd instar	5	2	9	16	0
4th instar	3	14	5	22	0
5th instar	7	26	35	68	0
Females	20	12	28	60	0
Males	32	35	37	104	0
Total	77	91	136	304	0

S3 Table. Coverage indices of *T. sordida* intestinal samples. Good's coverage of 454 pyrosequencing for each sample ($p < 0.05$) calculated with the Mothur program. Samples analyzed: entire intestine of 1st stage nymphs (1I); entire intestine of 2nd stage nymphs (2I); entire intestine of 3rd stage nymphs (3I); anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).

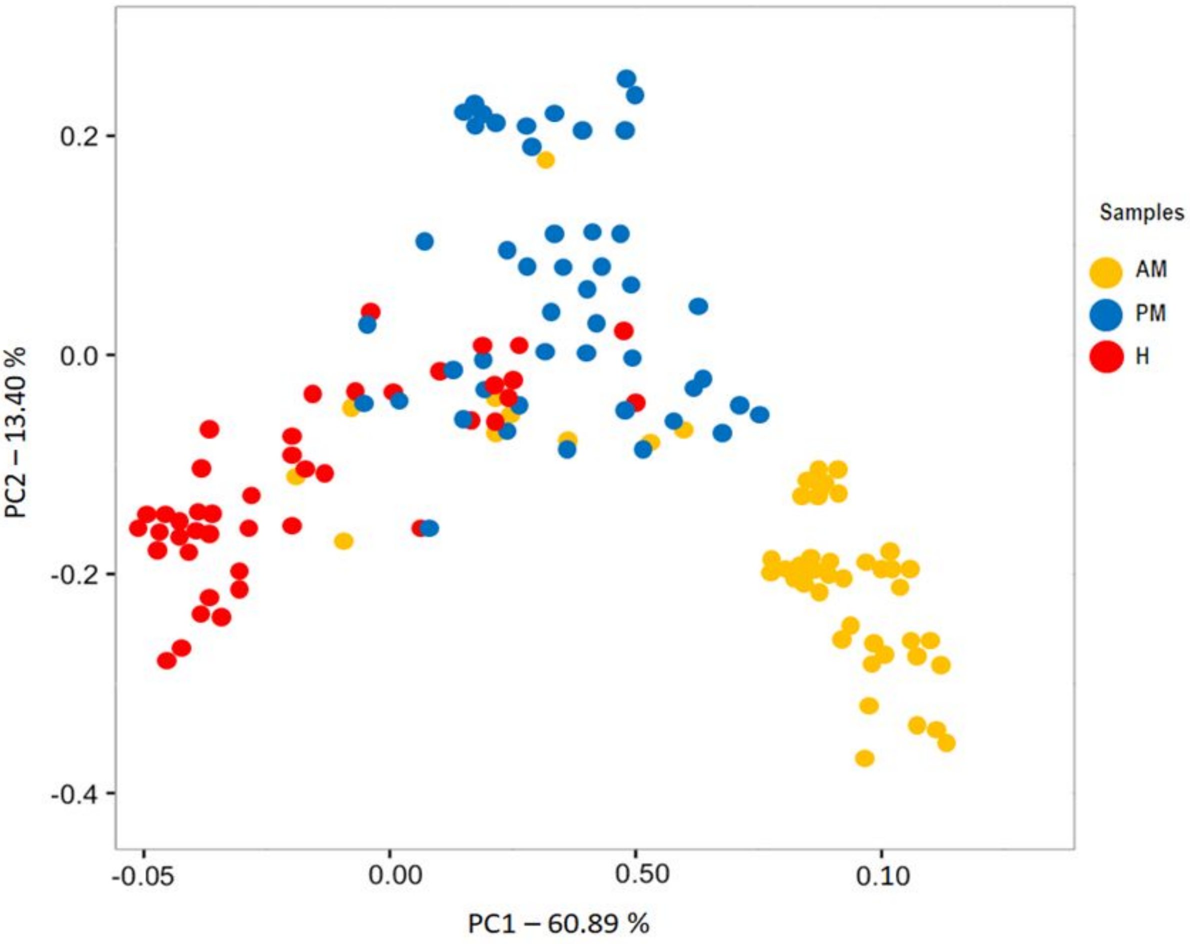
Samples	**Good's coverage (%)**
1I	93.76
2I	96.13
3I	96.84
4AM	97.02
4PM	97.14
4H	96.03
5AM	97.20
5PM	97.20
5H	97.32
FAM	96.44
FPM	95.49
FH	97.23
MAM	97.35
MPM	97.16
MH	97.41



S3 Appendix. Rarefaction curves of 16S rRNA sequences from intestinal segments of *T. sordida* samples. Samples analyzed: entire intestine of 1st stage nymphs (1I); entire intestine of 2nd stage nymphs (2I); entire intestine of 3rd stage nymphs (3I); anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).

S4 Table. Richness and diversity indices for *T. sordida* intestinal microbiota in different intestinal segments. OTUs were defined based on 97% identity cutoff. Samples analyzed: Anterior midgut (AM = 4_AM+5_AM+F_AM+M_AM); Posterior midgut (PM = 4_PM+5_PM+F_AM+M_AM); Hindgut (H = 4_H+5H+F_H+M_H).

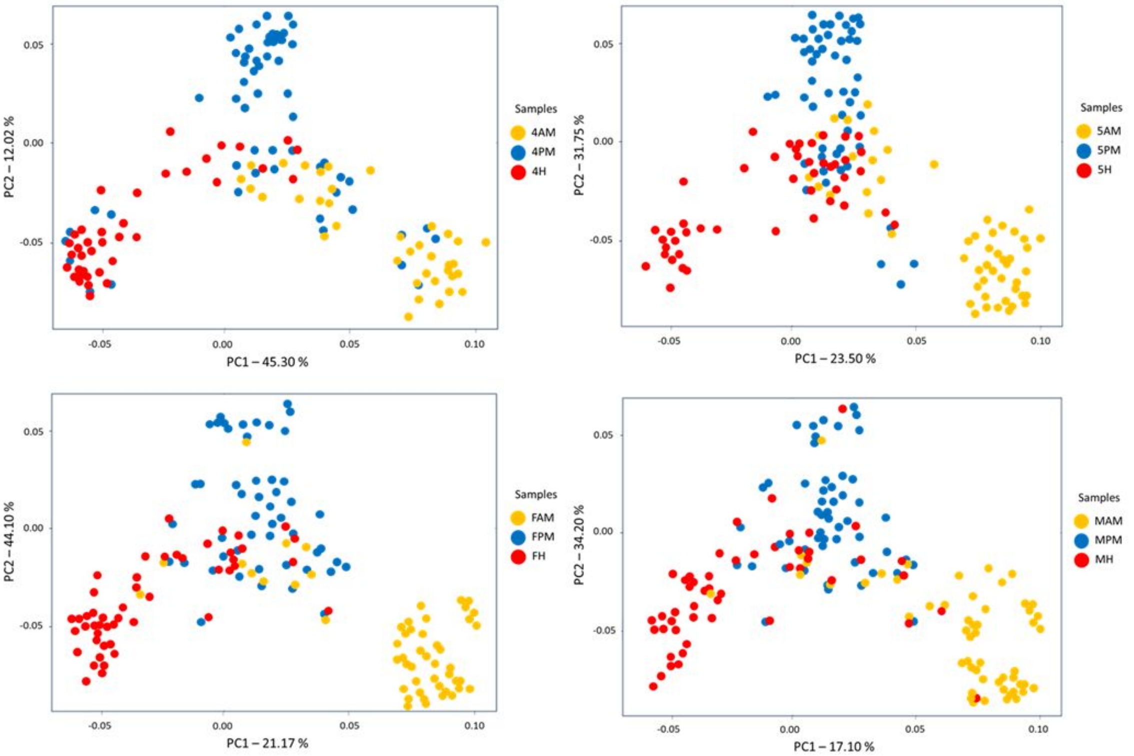
Samples	Nº of observed OTUs	Chao1	Shannon
AM	46	47.5 ± 0.70	3.50 ± 0.75
PM	35	36.5 ± 0.50	3.25 ± 0.25
H	33	38.5 ± 1.50	2.30 ± 0.30



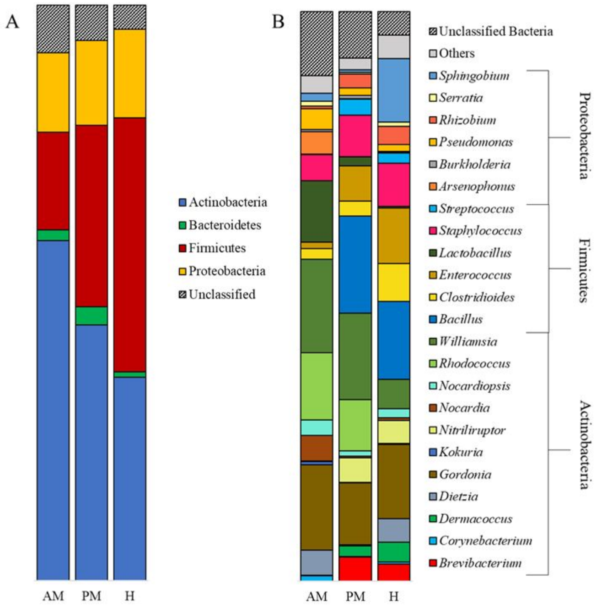
S4 Appendix. Principal coordinate analysis (PCoA) of weighted-UniFrac distances comparing the bacterial communities of *T. sordida* intestinal segments. Weighted-UniFrac distances matrices were calculated for each sample using one representative sequence for each OTU (3%). Axes represent the greatest proportion of variances in the communities for each analysis. Samples analyzed: Anterior midgut (AM = 4_AM+5_AM+F_AM+M_AM); Posterior midgut (PM = 4_PM+5_PM+F_AM+M_AM); Hindgut (H = 4_H+5H+F_H+M_H).

S5 Table. Analysis of similarity (ANOSIM) of the bacterial community of intestinal segments of *T. sordida*. Corrected P-values (<0.025) were calculated using the Bonferroni method. Significant comparisons are shown in bold. Samples analyzed: Anterior midgut (AM = 4_AM+5_AM+F_AM+M_AM); Posterior midgut (PM = 4_PM+5_PM+F_AM+M_AM); Hindgut (H = 4_H+5H+F_H+M_H).

	AM	**PM**	**H**
AM	-	0.0357	**0.0086**
PM		-	**0.0054**
H			-



S5 Appendix. Principal coordinate analysis (PCoA) of weighted-UniFrac distances comparing the bacterial communities of *T. sordida* intestine in different intestinal segments. Weighted-UniFrac distances matrices were calculated for each sample using one representative sequence for each UTO (3%). Axes represent the greatest proportion of variances in the communities for each analysis. Samples analyzed:Anterior midgut of 4th stage nymphs (4AM); posterior midgut of 4th stage nymphs (4PM); hindgut of 4th stage nymphs (4H); anterior midgut of 5th stage nymphs (5AM); posterior midgut of 5th stage nymphs (5PM); hindgut of 5th stage nymphs (5H); anterior midgut of females (FAM); posterior midgut of females (FPM); hindgut of females (FH); anterior midgut of males (MAM); posterior midgut of males (MPM); hindgut of males (MH).



S6 Appendix. Relative abundance of bacterial phyla (A) and genera (B) found in *T. sordida* intestinal microbiota. Samples: **a**nterior midgut of 4th instar, 5th instar, female and male (AM); posterior midgut of of 4th instar, 5th instar, female and male and hindgut of 4th instar, 5th instar, female and male (H). I = entire intestine. AM = anterior midgut, PM = posterior midgut, H = hindgut.