Supplementary Figures

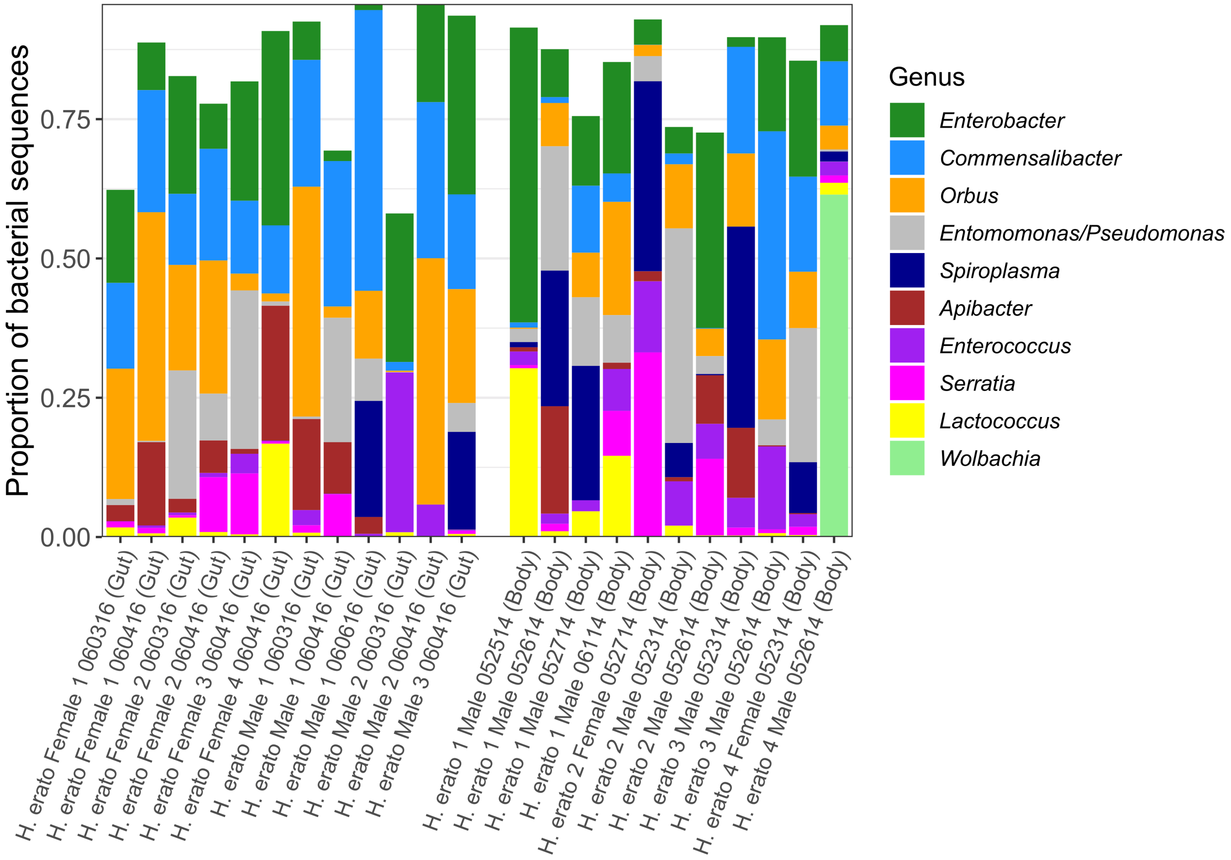

Figure S1. Whole-body bacterial communities are consistent among individuals and predominantly represent gut-associated taxa. Shown are the relative abundances of the top 10 bacterial genera (ranked by mean abundance) across *H. erato demophoon* collected from Gamboa, Panama. Remaining white space represents sequences belonging to other genera; these made up a median 16% of sequence libraries across individuals. Samples on the left are isolated guts from individuals collected in 2016, while samples on the right are whole-body homogenates from individuals collected in 2014. Note that *Enterobacter* here includes sequences originally assigned as *Klebsiella* and some other closely related Enterobacteriaceae genera (see Methods).

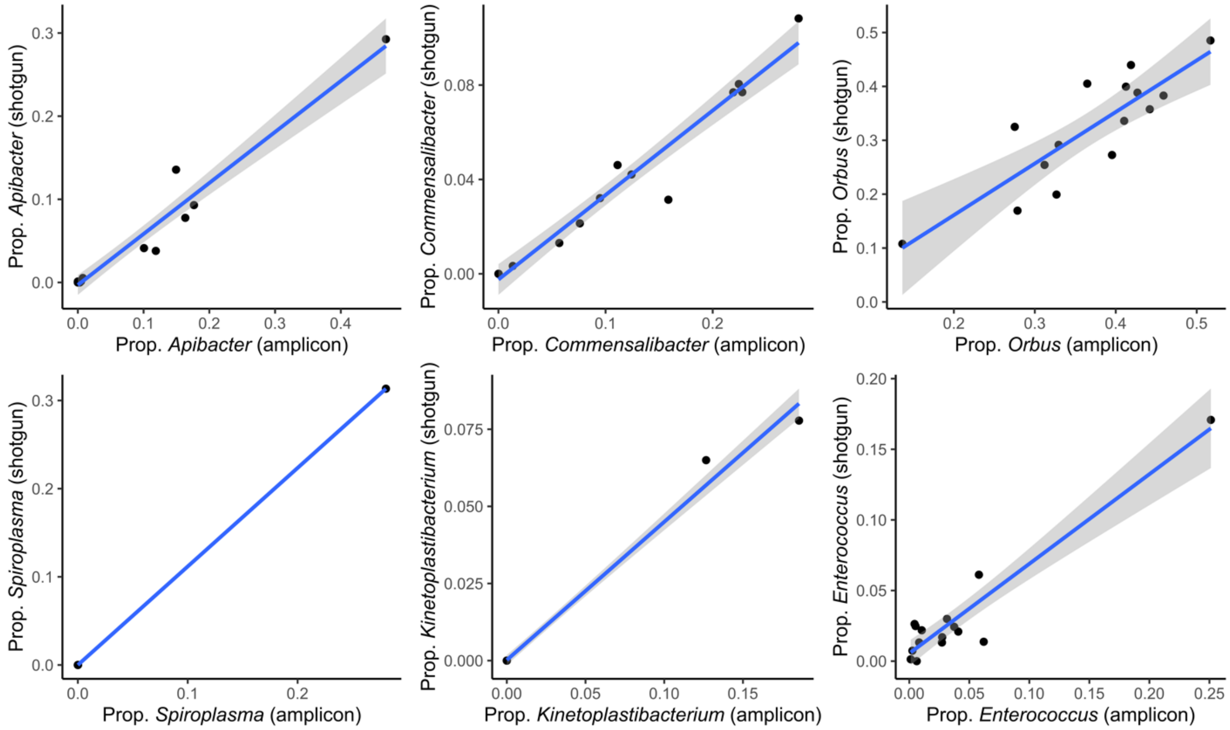

Figure S2. Bacterial genus-level relative abundances are highly correlated between the amplicon sequence libraries and metagenomes. Shown are relative abundances of six of the most abundant genera across the 15 butterfly gut samples for which we obtained both amplicon and shotgun metagenomic sequence data.

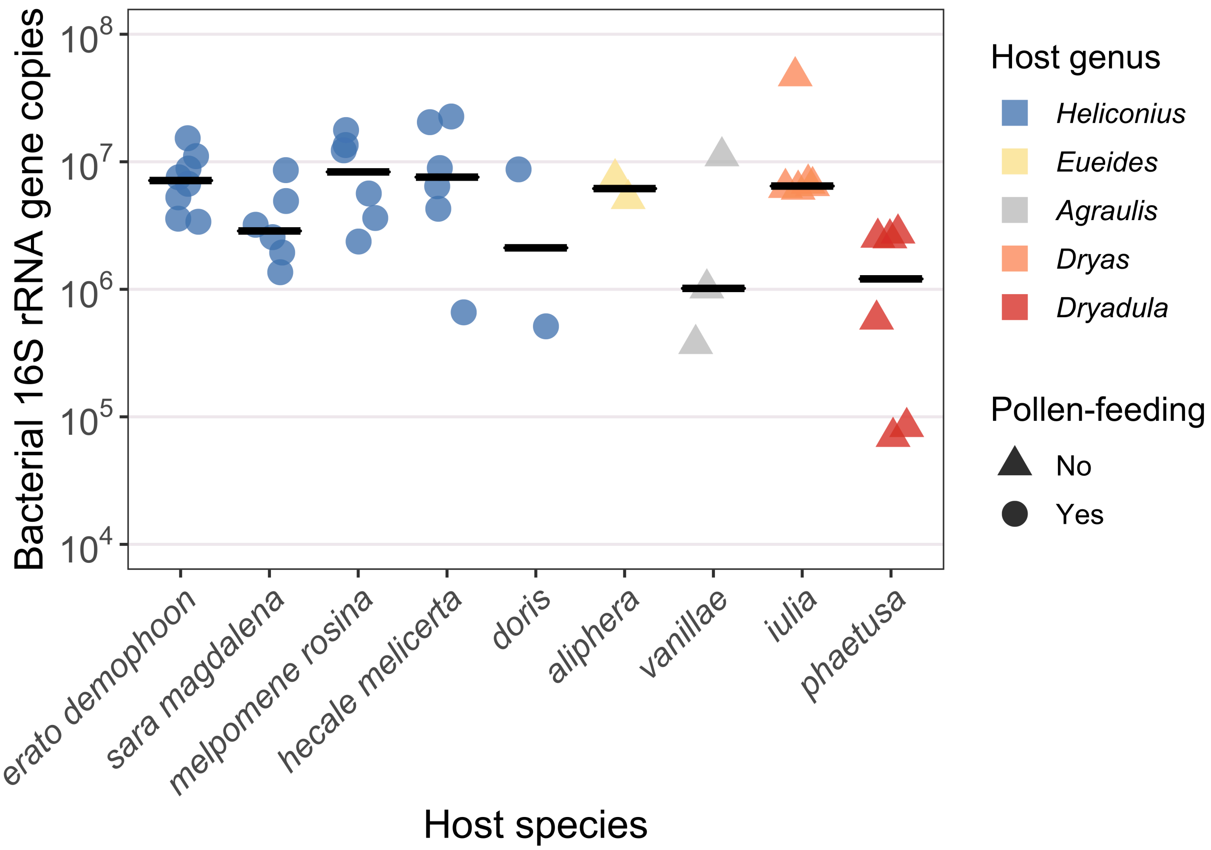

Figure S3. Absolute abundances of bacteria in the head and thorax of adult heliconiine butterflies, derived from qPCR. Shown are the number of bacterial 16S rRNA gene copies per subsample (approx. 50 mg) of homogenized, combined head and thorax tissue. These individuals were collected from Gamboa, Panama in 2016 (N = 28 *Heliconius*, 16 other Heliconiini).

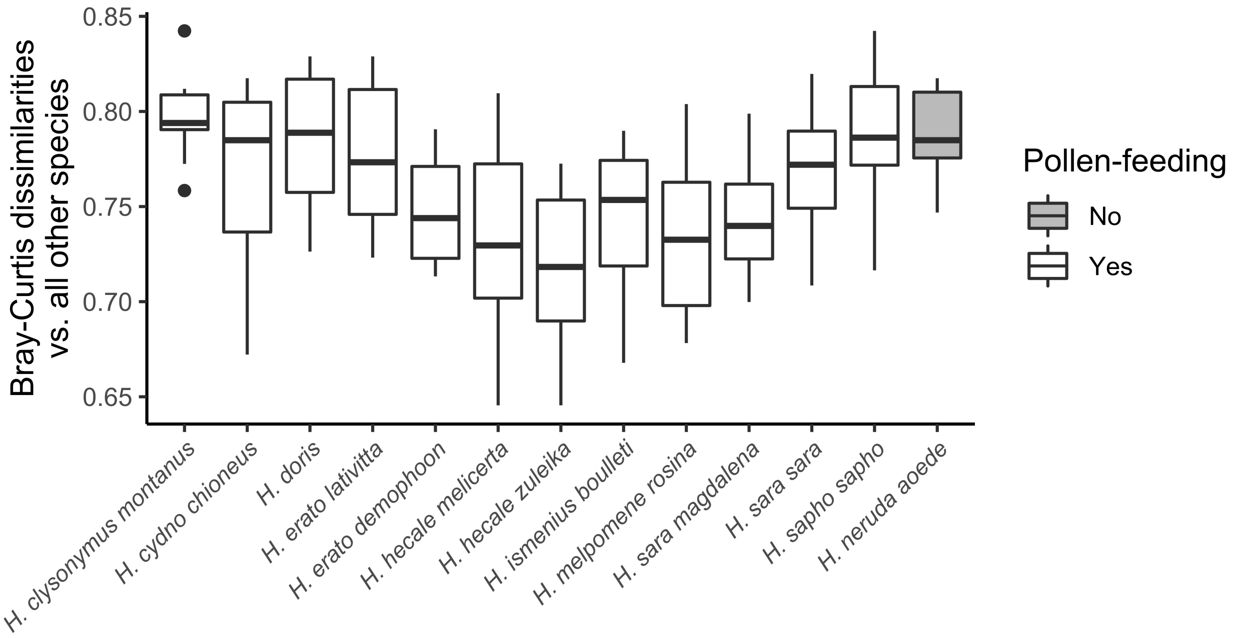

Figure S4. The non-pollen-feeding species *Heliconius aoede* is not uniquely distinct from pollen-feeding *Heliconius* species. Whole-body microbiomes from each focal species (x axis) are compared to all other species using Bray-Curtis dissimilarities. For a given comparison of two species' microbiomes, intraspecific replication was handled by averaging dissimilarities among all pairs of individuals.

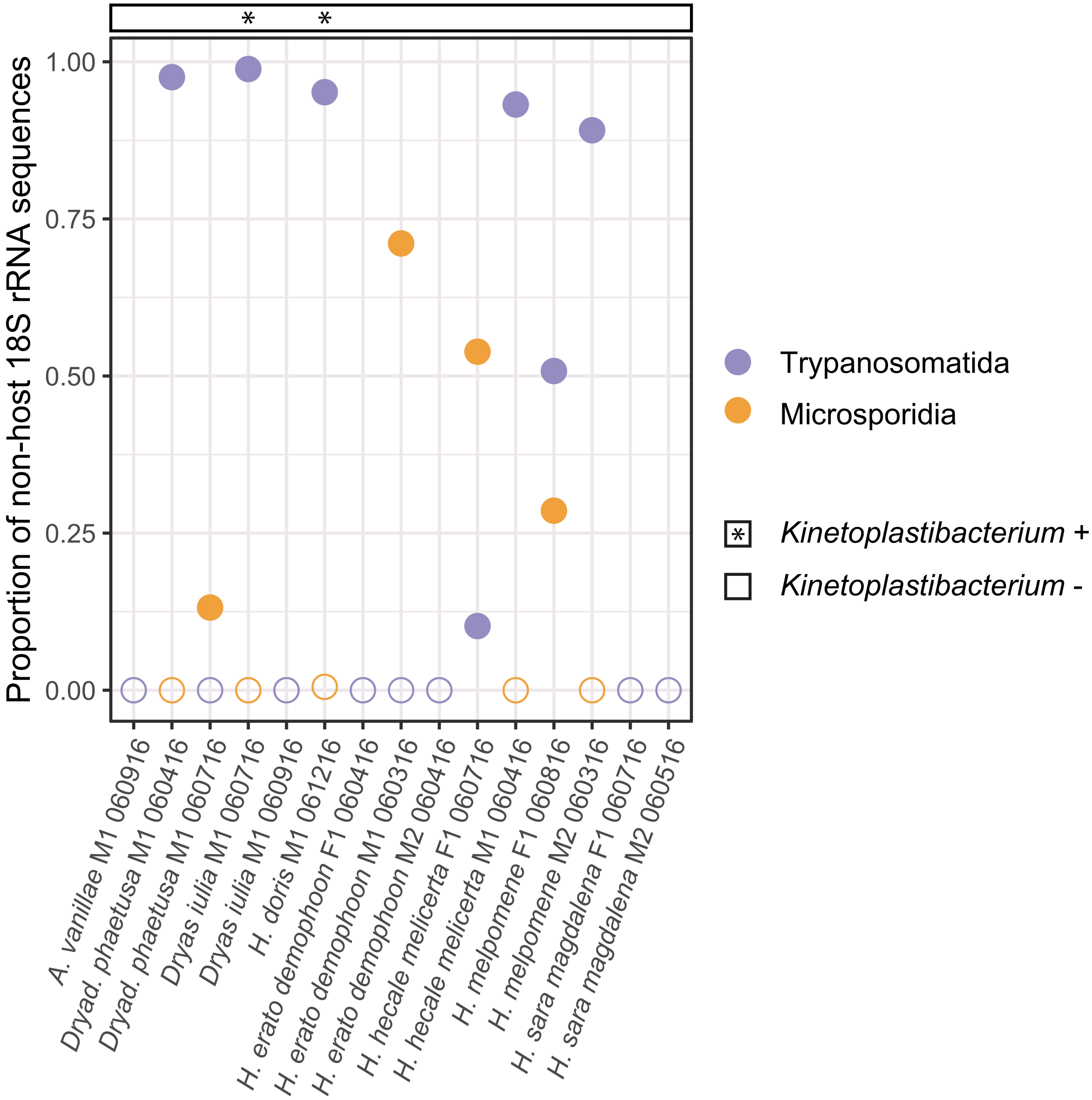

Figure S5. Microsporidia and Trypanosomatidae (related to *Crithidia*, *Leishmania*, and *Trypanosoma*) are prevalent in the 15 butterfly individuals with sequenced gut metagenomes. Points show the proportion of each microeukaryotic taxon out of all non-host 18S rRNA sequences identified by phyloFlash. Circles are filled if the proportion of microsporidia or trypanosomatids exceeded 0.01. Asterisks indicate samples in which *Kinetoplastibacterium*, a bacterial endosymbiont of trypanosomatids, was detected in metagenomes. Other microeukaryotes not shown here comprise a variety of very low-abundance taxa (i.e. ≤ 30 total reads per metagenome) classified mainly as coccidia, ascomycete fungi, acanthamoeba, and algae.

Supplementary Table

Host taxon	Phylum	Class	Order	Family	Genus	Mean prop. gut +/- SEM	Mean prop. head/thorax +/- SEM	p value
Heliconius	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Apibacter*	0.036 +/- 0.009	0.002 +/- 0.001	< 0.001
					Chishuiella	0.007 +/- 0.003	0.095 +/- 0.027	0.001
					Chryseobacterium	0.011 +/- 0.004	0.096 +/- 0.024	0.001
	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	0.031 +/- 0.008	0.021 +/- 0.006	0.117
				Streptococcaceae	*Lactococcus*	0.02 +/- 0.006	0.033 +/- 0.009	0.793
	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Asaia*	0.024 +/- 0.007	0.004 +/- 0.002	0.001
					Commensalibacter	0.12 +/- 0.02	0.007 +/- 0.003	< 0.001
					Swaminathania	0.013 +/- 0.01	0.016 +/- 0.014	0.745
		Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Variovorax*	0.01 +/- 0.004	0.021 +/- 0.006	0.017
			Enterobacteriales	Enterobacteriaceae	*Enterobacter*	0.233 +/- 0.034	0.031 +/- 0.007	< 0.001
					Serratia	0.037 +/- 0.01	0.027 +/- 0.017	0.013
			Orbales	Orbaceae	*Orbus*	0.163 +/- 0.024	0.021 +/- 0.01	< 0.001
			Pseudomonadales	Moraxellaceae	*Acinetobacter*	0.023 +/- 0.007	0.293 +/- 0.041	< 0.001
				Pseudomonadaceae	*Entomomonas/Pseudomonas*	0.086 +/- 0.017	0.024 +/- 0.01	0.291
	Tenericutes	Mollicutes	Entomoplasmatales	Spiroplasmataceae	*Spiroplasma*	0.012 +/- 0.007	0.003 +/- 0.002	0.927
Other Heliconiini	Bacteroidetes	Bacteroidia	Flavobacteriales	Weeksellaceae	*Apibacter*	0.139 +/- 0.045	0.008 +/- 0.004	0.083
					Chishuiella	0.009 +/- 0.003	0.138 +/- 0.029	0.016
					Chryseobacterium	0 +/- 0	0.015 +/- 0.012	0.808
	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	0.056 +/- 0.027	0.031 +/- 0.011	0.843
				Streptococcaceae	*Lactococcus*	0.036 +/- 0.026	0.012 +/- 0.011	0.098
	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Asaia*	0.153 +/- 0.039	0.004 +/- 0.001	0.01
					Commensalibacter	0.031 +/- 0.015	0.002 +/- 0.002	0.106
					Swaminathania	0.007 +/- 0.004	0 +/- 0	0.226
		Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	*Variovorax*	0.005 +/- 0.002	0.016 +/- 0.004	0.292
			Enterobacteriales	Enterobacteriaceae	*Enterobacter*	0.156 +/- 0.042	0.014 +/- 0.006	0.01
					Serratia	0.02 +/- 0.012	0.001 +/- 0	0.075
			Orbales	Orbaceae	*Orbus*	0.139 +/- 0.043	0.007 +/- 0.004	0.042
			Pseudomonadales	Moraxellaceae	*Acinetobacter*	0.046 +/- 0.018	0.45 +/- 0.054	< 0.001
				Pseudomonadaceae	*Entomomonas/Pseudomonas*	0.02 +/- 0.017	0.011 +/- 0.004	0.393
	Tenericutes	Mollicutes	Entomoplasmatales	Spiroplasmataceae	*Spiroplasma*	0.068 +/- 0.038	0.052 +/- 0.052	0.843

Table S1. Relative abundances and within-body distribution of the top 15 bacterial genera (ranked by mean abundance) across butterflies collected from Gamboa, Panama in 2016. These individuals were dissected to compare microbiomes between isolated gut tissue and the combined head and thorax. Abundances are shown for *Heliconius* (top) and species belonging to other heliconiine genera (bottom) separately. P values are from a nonparametric statistical test of proportions in guts versus head/thorax samples, after FDR correction (see Methods).