Supporting Information for

Coevolution alters predator life history traits, behavior and morphology in experimental microbial communities

Johannes Cairns, Felix Moerman, Emanuel A. Fronhofer, Florian Altermatt, Teppo Hiltunen

Correspondence to: florian.altermatt@eawag.ch / teppo.hiltunen@helsinki.fi

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Supplementary methods:

R-code used in video analysis step for protist density measurements

Tables S1 to S6

Figures S1 to S10

Supplementary methods

R-code (including used parameter values) used in video analysis step for protist density measurements

```
#############################################################################
# R script for analysing video files with BEMOVI (www.bemovi.info)
#
# Emanuel A. Fronhofer
#
# October 2016
#############################################################################
rm(list=ls())

# load package
#library(devtools)
#install_github("efronhofer/bemovi", ref="experimental")
library(bemovi)

#############################################################################
# VIDEO PARAMETERS

# video frame rate (in frames per second)
fps <- 25
# length of video (in frames)
total_frames <- 500

# measured volume (in microliter)
measured_volume <- 34.4 # for Leica M205 C with 1.6 fold magnification, sample height 0.5 mm and Hamamatsu Orca Flash 4

# size of a pixel (in micrometer)
pixel_to_scale <- 4.05 # for Leica M205 C with 1.6 fold magnification, sample height 0.5 mm and Hamamatsu Orca Flash 4
```

```
# setup
difference.lag <- 10
thresholds <- c(10,255) # don't change the second value

########################################################################
# FILTERING PARAMETERS
#(optimized for Leica M205 C with 1.6 fold magnification, sample height 0.5 mm and Hamamatsu Orca Flash 4)
# tested species: Tet, Col, Pau, Eug, Chi, Ble, Ceph, Lox, Spi

# min and max size: area in pixels
particle_min_size <- 5
particle_max_size <- 1000

# number of adjacent frames to be considered for linking particles
trajectory_link_range <- 3
# maximum distance a particle can move between two frames
trajectory_displacement <- 16

# these values are in the units defined by the parameters above: fps (seconds), measured_volume (microliters) and
pixel_to_scale (micometers)
filter_min_net_disp <- 25
filter_min_duration <- 1
filter_detection_freq <- 0.1
filter_median_step_length <- 3
########################################################################
# MORE PARAMETERS (USUALLY NOT CHANGED)

# UNIX
# set paths to ImageJ and particle linker standalone
IJ.path <- "/home/felix/bin/ImageJ"
to.particlelinker <- "/home/felix/bin/ParticleLinker"

# directories and file names
to.data <- paste(getwd(),"/",sep="")
```

```
video.description.folder <- "0_video_description/"
video.description.file <- "video_description.txt"
raw.video.folder <- "1_raw/"
particle.data.folder <- "2_particle_data/"
trajectory.data.folder <- "3_trajectory_data/"
temp.overlay.folder <- "4a_temp_overlays/"
overlay.folder <- "4_overlays/"
merged.data.folder <- "5_merged_data/"
ijmacs.folder <- "ijmacs/"

# RAM allocation
memory.alloc <- c(60000) # hp machine

# RAM per particle linker instance
memory.alloc.perLinker <- c(10000)
#############################################################################

#############################################################################
# VIDEO ANALYSIS

# identify particles
locate_and_measure_particles(to.data, raw.video.folder, particle.data.folder, difference.lag, thresholds, min_size
= particle_min_size, max_size = particle_max_size, IJ.path, memory.alloc)

# link the particles
link_particles(to.data, particle.data.folder, trajectory.data.folder, linkrange = trajectory_link_range, disp =
trajectory_displacement, start_vid = 1, memory = memory.alloc, memory_per_linkerProcess =
memory.alloc.perLinker)

# merge info from description file and data
merge_data(to.data, particle.data.folder, trajectory.data.folder, video.description.folder, video.description.file,
merged.data.folder)

# load the merged data
```

```
load(paste0(to.data, merged.data.folder, "Master.RData"))

# filter data: minimum net displacement, their duration, the detection frequency and the median step length
trajectory.data.filtered <- filter_data(trajectory.data, filter_min_net_disp, filter_min_duration, filter_detection_freq, filter_median_step_length)

# summarize trajectory data to individual-based data
morph_mvt <- summarize_trajectories(trajectory.data.filtered, calculate.median=F, write = T, to.data, merged.data.folder)

# get sample level info
summarize_populations(trajectory.data.filtered, morph_mvt, write=T, to.data, merged.data.folder, video.description.folder, video.description.file, total_frames)

# create overlays for validation
create_overlays(trajectory.data.filtered, to.data, merged.data.folder, raw.video.folder, temp.overlay.folder, overlay.folder, 2048, 2048, difference.lag, type = "label", predict_spec = F, IJ.path, contrast.enhancement = 1, memory = memory.alloc)
```

Table S1 ANOVA table for linear model on log-transformed intrinsic growth rate (r_0) of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Prey evolution	1	1.81	1.82	29.8	**< 0.001**
Predator evolution	1	0.10	0.10	1.61	0.21
Prey species	6	6.26	1.04	17.1	**< 0.001**
Prey evolution × predator evolution	1	0.23	0.23	3.72	0.058
Prey evolution × prey species	6	3.31	0.55	9.03	**< 0.001**
Residuals	78	4.76	0.06		

Table S2 ANOVA table for linear model on log-transformed competitive ability (α) of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Prey evolution	1	1.59	1.59	15.1	**< 0.001**
Predator evolution	1	0.12	0.12	1.12	0.29
Prey species	6	9.23	1.54	14.6	**< 0.001**
Prey evolution × predator evolution	1	0.26	0.26	2.51	0.12
Prey evolution × prey species	6	3.46	0.58	5.48	**< 0.001**
Residuals	78	8.20	0.11		

Table S3 ANOVA table for linear model on log-transformed equilibrium density (K) of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Prey evolution	1	0.01	0.01	0.33	0.57
Prey species	6	1.56	0.26	11.2	**< 0.001**
Prey evolution × prey species	6	1.91	0.32	13.7	**< 0.001**
Residuals	80	1.86	0.02		

Table S4 ANOVA table for linear model on cell size of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Prey evolution	1	313	313	2.97	0.085
Predator evolution	1	695	695	6.59	**0.010**
Log prey population size	1	2964	2964	28.1	**< 0.001**
Log predator population size	1	6072	6072	57.6	**< 0.001**
Log prey population size × predator evolution	1	509	509	4.82	**0.028**
Log predator population size × predator evolution	1	266	266	2.52	0.11
Log predator population size × log prey population size	1	894	894	8.47	**0.004**
Residuals	767	80886	106		

Table S5 ANOVA table for linear model on gross speed of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Prey evolution	1	19715	19715	2.94	0.087
Predator evolution	1	20961	20961	3.13	0.077
Prey species	6	345734	57622	8.60	**< 0.001**
Log predator population size	1	635913	635913	94.9	**< 0.001**
Log predator population size × prey evolution	1	36601	36601	5.46	**0.020**
Log predator population size × predator evolution	1	26668	26668	3.98	**0.046**
Residuals	763	5113281	6702		

Table S6 ANOVA table for linear model on cell turning angle distribution of ciliate.

Model terms	*d.f.*	*SS*	*MS*	*F*	*p*
Predator evolution	1	0.40	0.40	7.33	**0.007**
Prey species	6	3.72	0.62	11.4	**< 0.001**
Log prey population size	1	0.45	0.45	8.24	**0.004**
Log predator population size	1	8.24	8.24	152	**< 0.001**
Log predator population size × predator evolution	1	0.58	0.58	10.7	**0.001**
Log predator population size × prey species	1	3.34	0.56	10.3	**< 0.001**
Residuals	758	41.0	0.05		

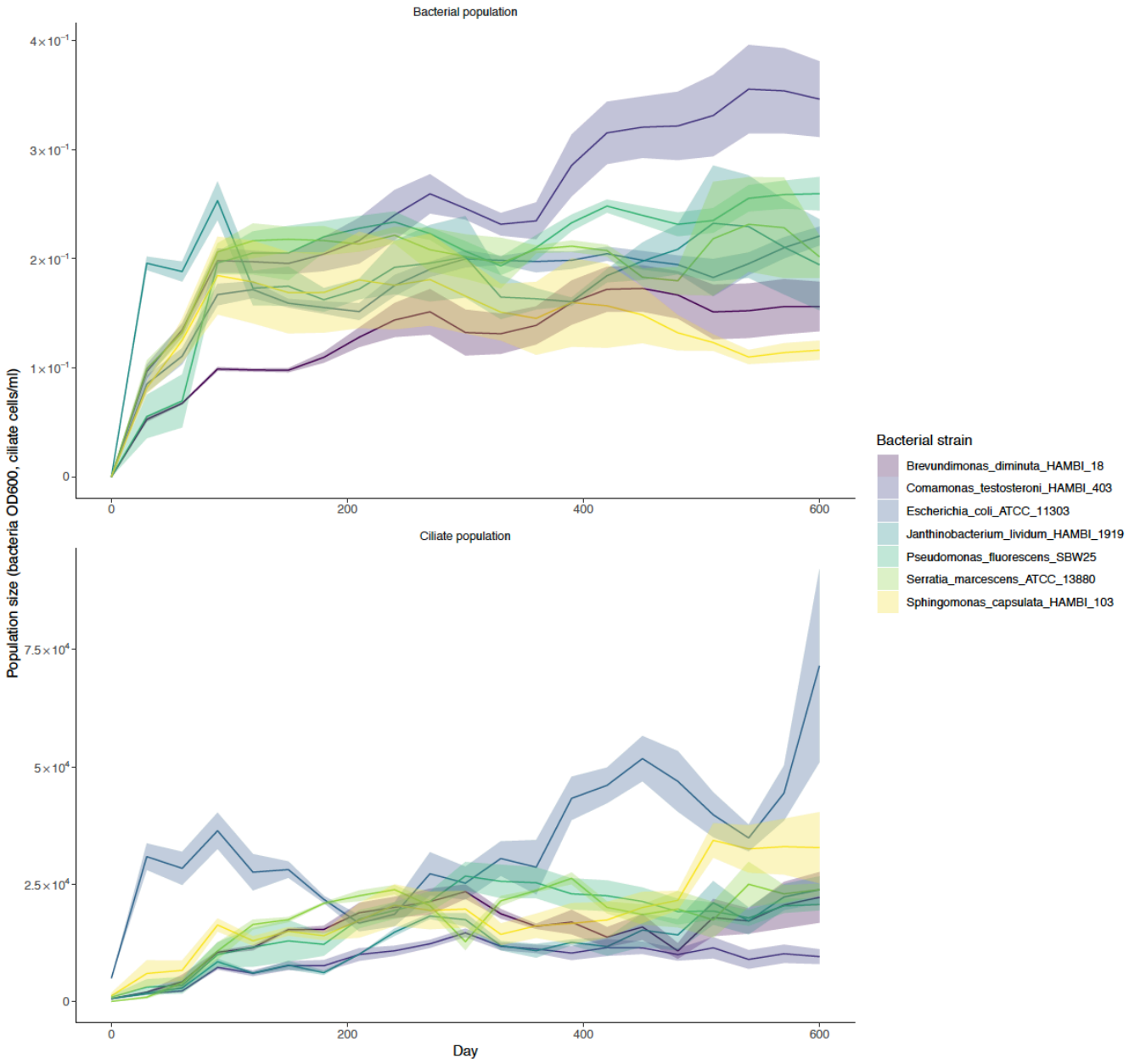

Figure S1 Bacterial and ciliate population size during first 20 months in long-term predator-prey coevolutionary experiment (mean ± s.e.m. smoothed over 90 day sliding window). The figure shows different population sizes for different bacterial species and an increasing trend over time.

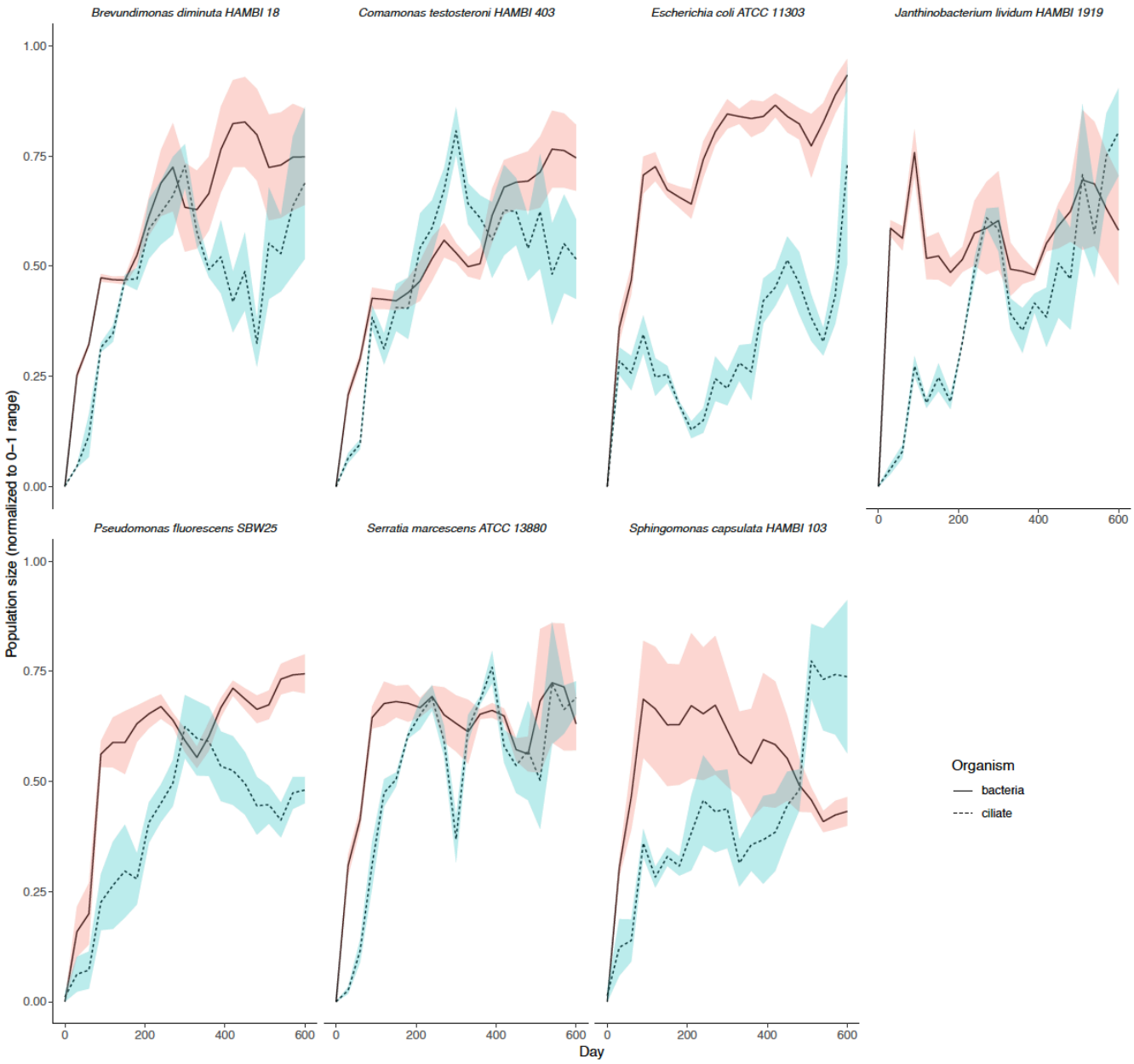

Figure S2 Bacteria-ciliate community dynamics during first 20 months in long-term predator-prey coevolutionary experiment (mean ± s.e.m. standardized to 0-1 range and smoothed over 90 day sliding window). The figure shows a negative association between bacterial and ciliate population size as well as differences in community dynamics depending on the prey (bacterial) species.

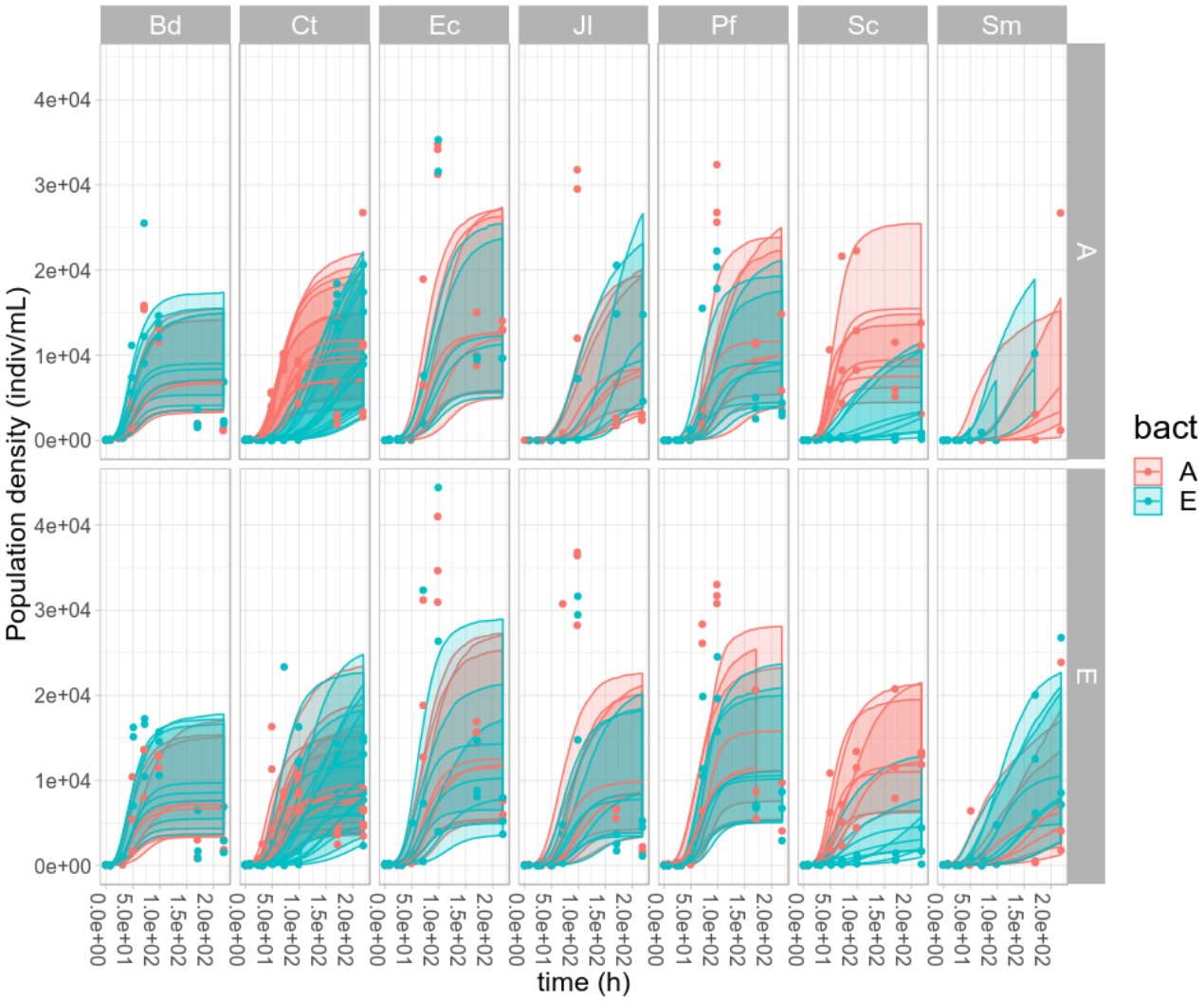

Figure S3 Beverton-Holt continuous-time growth models for ciliate population density (mean ± 95 % confidence intervals). A = ancestral ciliate; E = evolved ciliate; Bd = *Brevundimonas diminuta* HAMBI 18; Ct = *Comamonas testosteroni* HAMBI 403; Ec = *Escherichia coli* ATCC 11303; Jl = *Janthinobacterium lividum* HAMBI 1919; Pf = *Pseudomonas fluorescens* SBW25; Sc = *Sphingomonas capsulata* HAMBI 103; Sm = *Serratia marcescens* ATCC 13880.

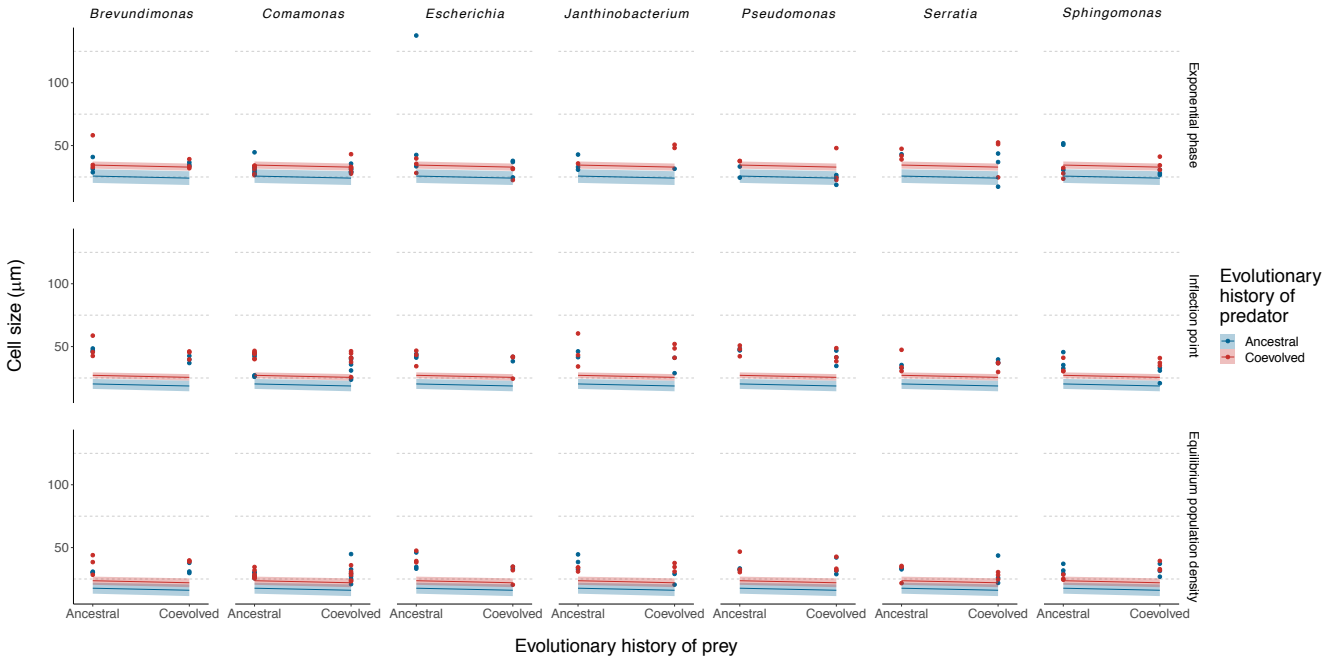

Figure S4. Reaction norms showing effect of predator-prey coevolution on cell size of predator at low (5 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

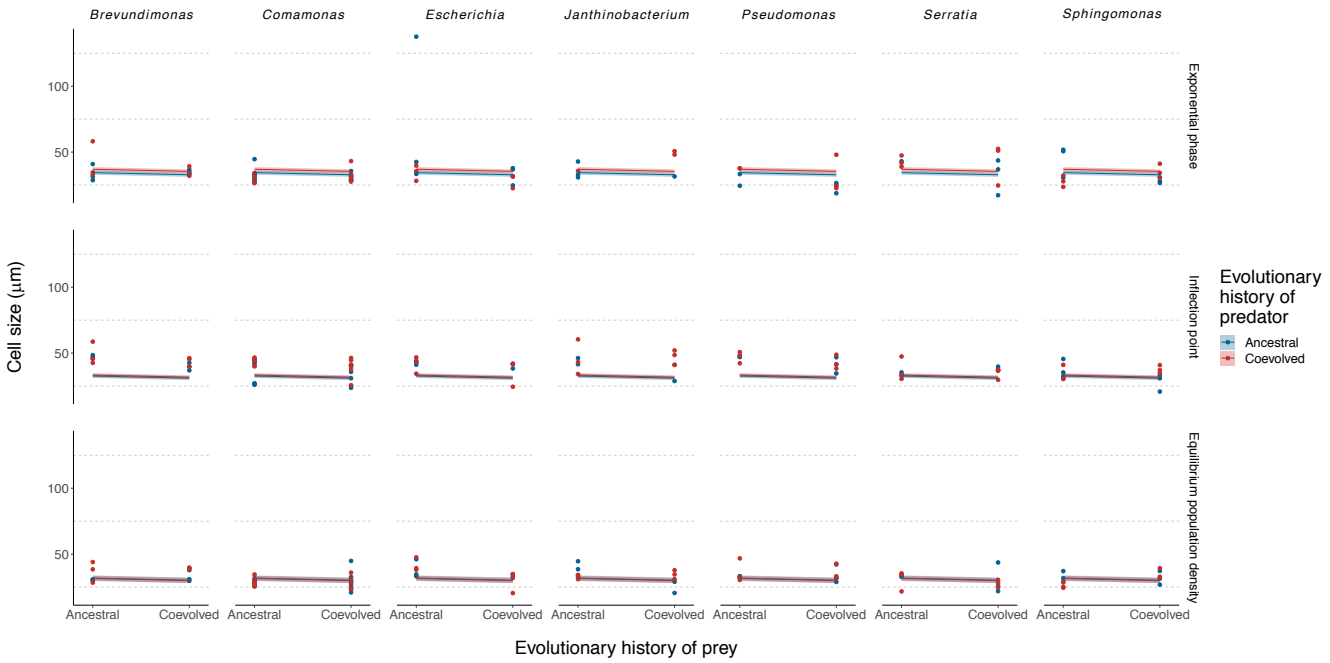

Figure S5. Reaction norms showing effect of predator-prey coevolution on cell size of predator at medium (50 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

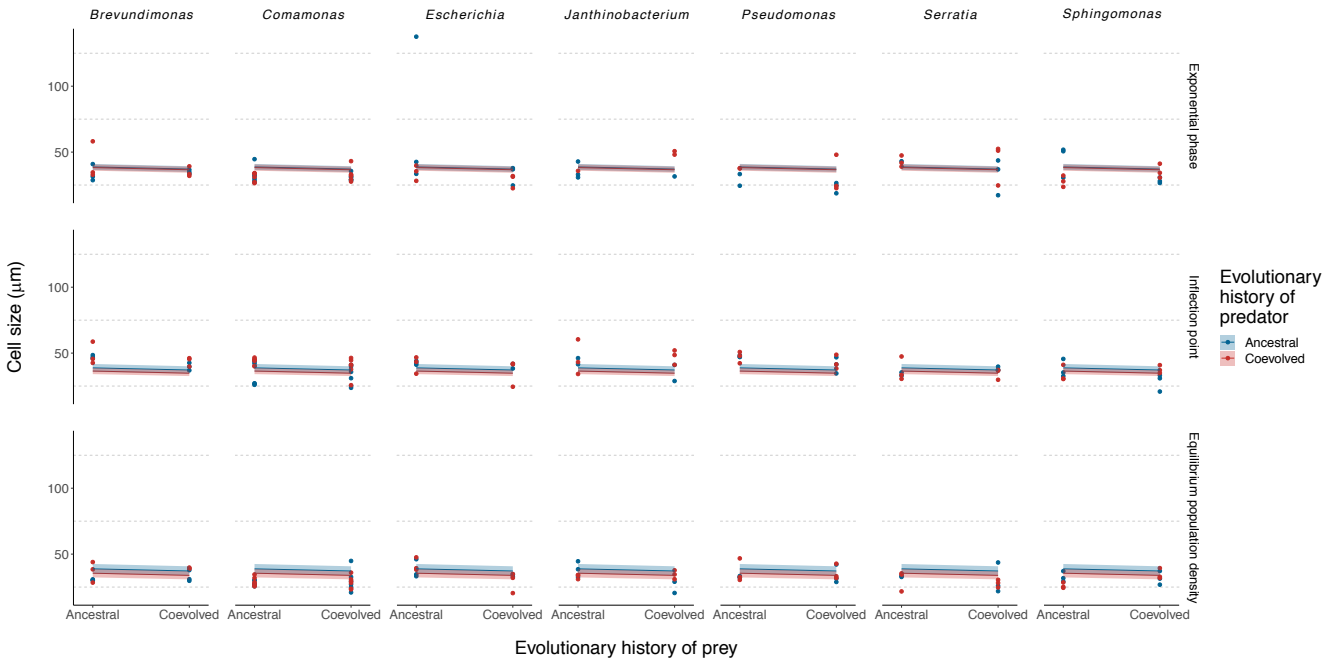

Figure S6. Reaction norms showing effect of predator-prey coevolution on cell size of predator at high (95 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

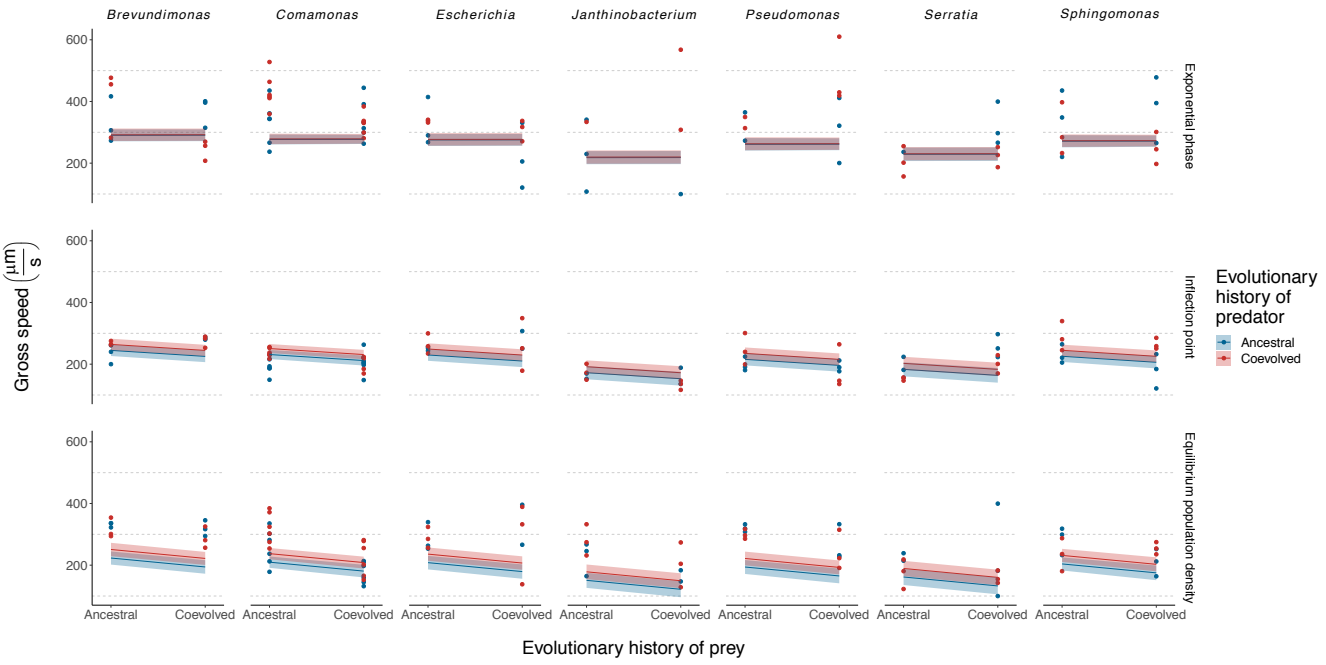

Figure S7. Reaction norms showing effect of predator-prey coevolution on gross speed of predator at medium (50 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Since the statistical analysis did not show an effect of prey density on gross speed of the predator, only one prey density is plotted for speed unlike for cell size and turning angle distribution. Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

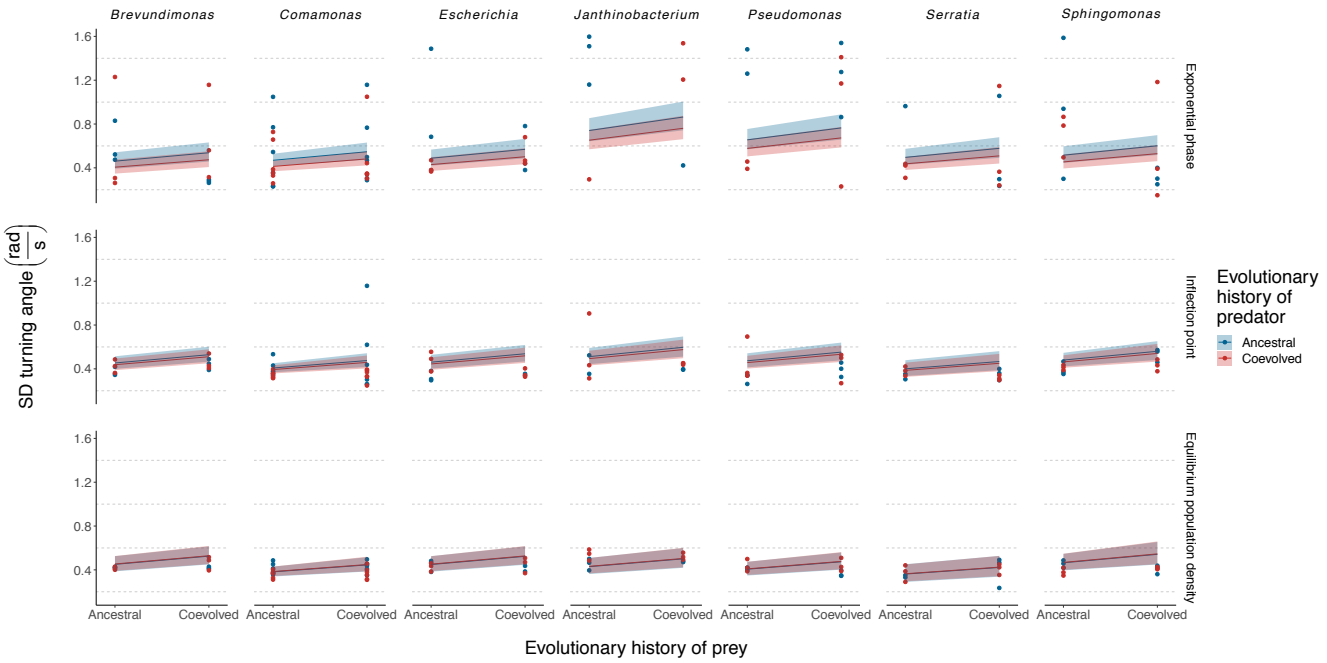

Figure S8. Reaction norms showing effect of predator-prey coevolution on turning angle distribution of predator at low (5 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Cell turning angle distribution (standard deviation, SD) is used as a proxy for directionality of cell movement which is higher at lower values. Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

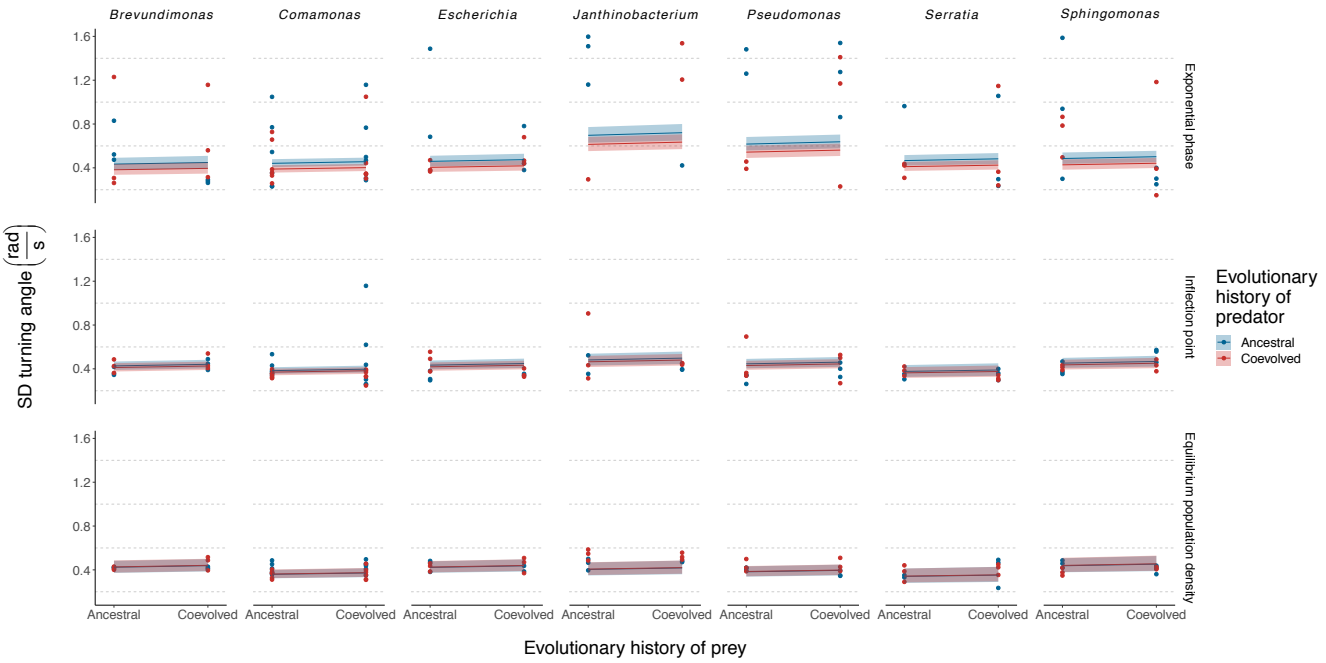

Figure S9. Reaction norms showing effect of predator-prey coevolution on turning angle distribution of predator at medium (50 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Cell turning angle distribution (standard deviation, SD) is used as a proxy for directionality of cell movement which is higher at lower values. Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.

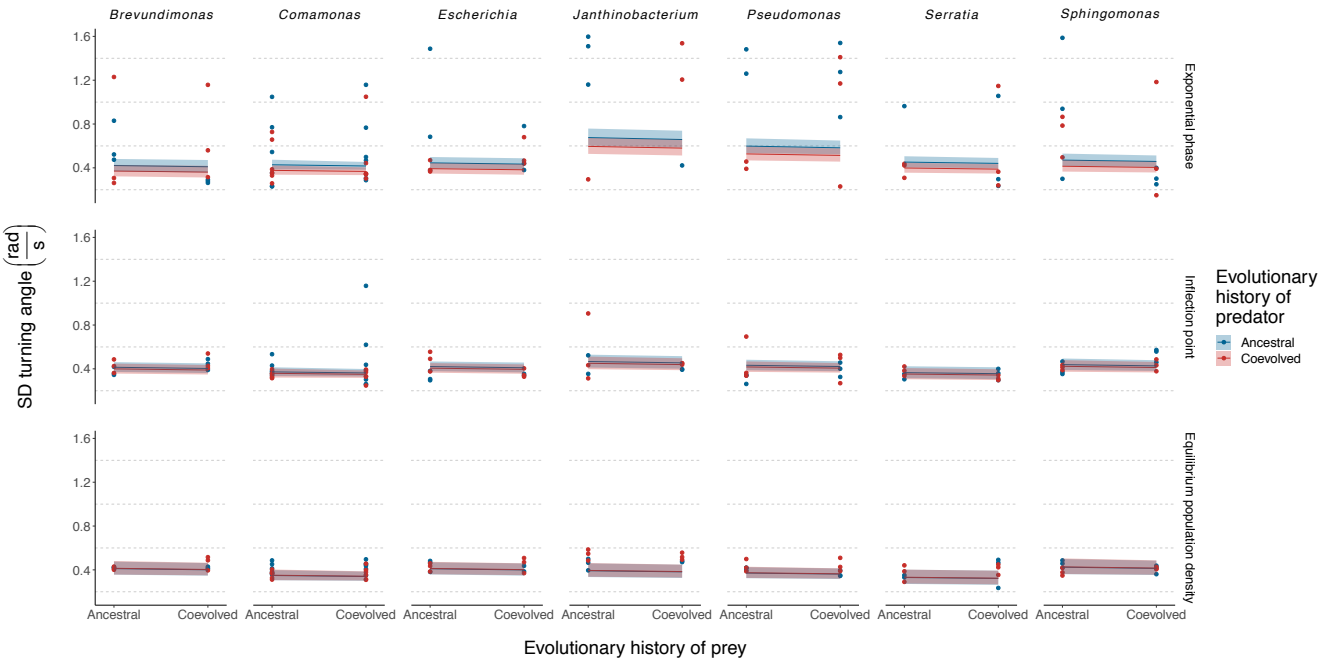

Figure S10. Reaction norms showing effect of predator-prey coevolution on turning angle distribution of predator at high (95 % quantile) prey density and three different predator densities (data points with linear model estimate ± 95 % confidence intervals.; N = 3 except 6 for *Comamonas*). Cell turning angle distribution (standard deviation, SD) is used as a proxy for directionality of cell movement which is higher at lower values. Predator densities have been taken from different growth phases estimated using Beverton-Holt population models. The reaction norms for predators (one strain of the ciliate *Tetrahymena thermophila*) feeding on ancestral or coevolved prey (seven bacterial strains indicated by genus name) are depicted separately for ancestral and coevolved predators (color coding). Predators coevolved with a particular prey taxon have always been coupled with ancestral or coevolved populations of the same taxon, while the ancestral predator is the same for all prey taxa.