Supporting Information

Title

The interplay of nested biotic interactions and the abiotic environment regulates populations of a hypersymbiont

Authors

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Summary of Supporting Information that can be found in this file:

Appendix 1. Occupancy-abundance and variance-to-mean-abundance at the scale of basal hosts.

As a complement to the main analysis, two patterns involving occupancy and abundance expected for symbionts (e.g. Matthee & Krasnov, 2009) were tested in the ciliate at the scale of basal hosts. First, 'occupancy-abundance' is the relationship between prevalence and ln(mean abundance per occupied symbiotic host). Second, 'variance-to-mean-abundance' describes the relationship between ln(variance of abundance) and ln(mean abundance). In the variance-to-mean-abundance relationship, unlike our other abundance analyses, the abundance measure was obtained from all the symbiotic ostracods, including those free of ciliates. The 'occupancy-abundance' pattern could emerge from the effects of concentration of symbiotic ostracods within crayfish bodies on hypersymbiont transmission dynamics. We expect a positive correlation between occupancy and abundance when colonisations of empty ostracods by active dispersal of the ciliate are favoured within crayfish that harbour more ciliates. On the other hand, the slope (β coefficient) of the variance-to-mean relationship informs about the degree of aggregation of ciliates among symbiotic hosts. A $\beta > 1$ indicates aggregation; symbionts with higher β are more aggregated. We expect a limit to the degree of aggregation in symbionts on hosts with relatively small body sizes (i.e. due to limited within-host carrying capacity). Both occupancy-abundance and variance-to-mean-abundance relationships were analysed through GLMM (binomial and Gaussian families, respectively), with locality as random effects factor. Crayfish without ciliates were removed from these analyses.

Both occupancy-abundance and variance-to-mean-abundance patterns were tested using data from 16 localities (140 and 143 crayfish, respectively). The occupancy-abundance pattern (Fig. S4) was significant, with a positive effect of mean abundances on prevalences at the scale of basal hosts (β = 0.55; *95%* CI = 0.37 – 0.73; *Marginal* R^2 = 0.017; *Conditional* R^2 = 0.152). The variance-to-mean-abundance (Fig. S5) had a slope somewhat greater than 1, indicating a moderate degree of aggregation of hypersymbionts among symbiotic hosts (β = 1.32; *95%* CI = 1.23 – 1.42; *Marginal* R^2 = 0.89; *Conditional* R^2 = 0.90).

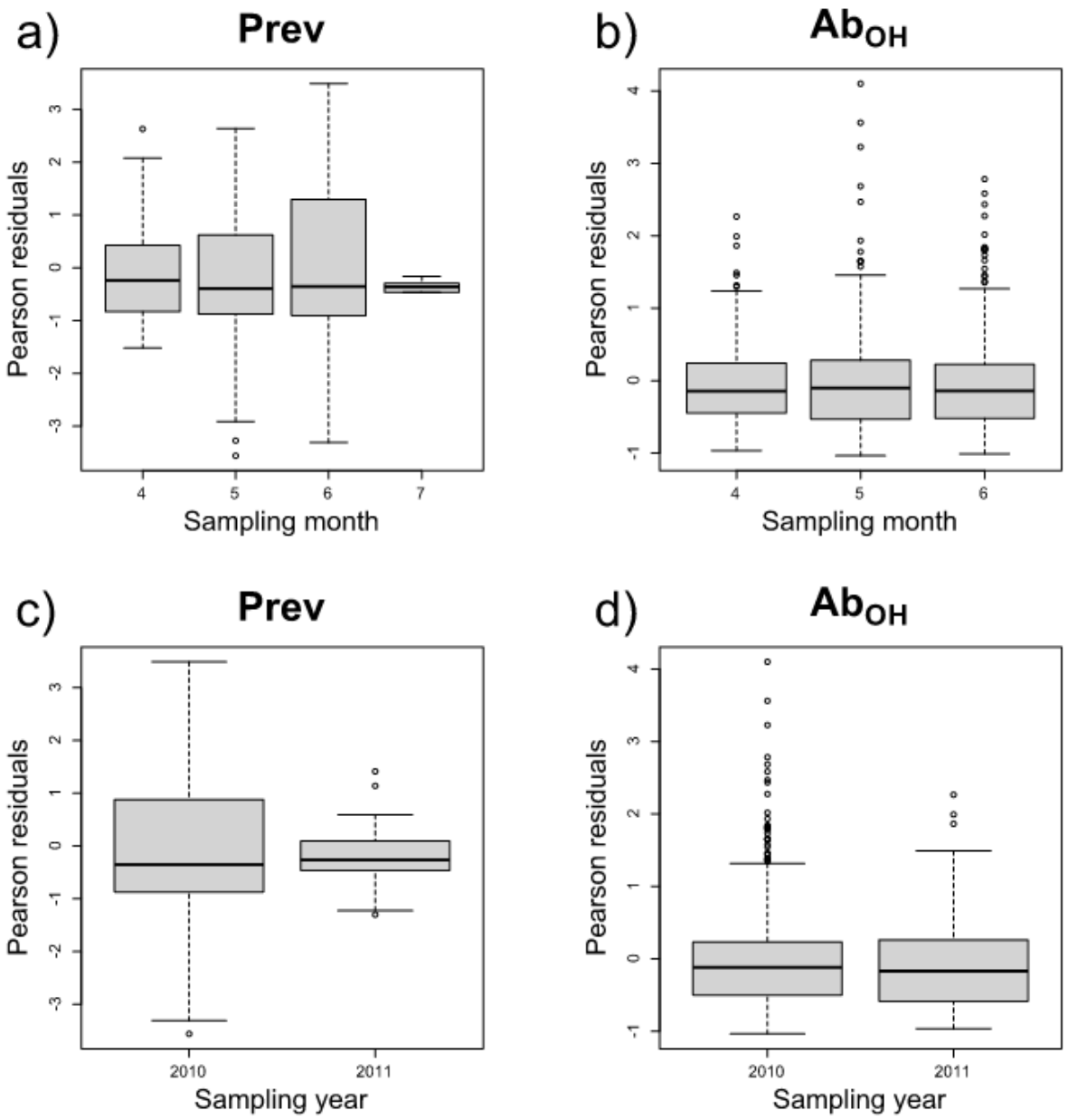

Figure S1. Temporal patterns in Pearson residuals of consensus models for prevalence (Prev) and abundance per occupied host (Ab_{OH}) of the hypersymbiotic ciliate *Lagenophrys discoidea*. Codes 4 to 7 in labels of x-axis refer to months April to July (the seasonal range of field sampling in this study).

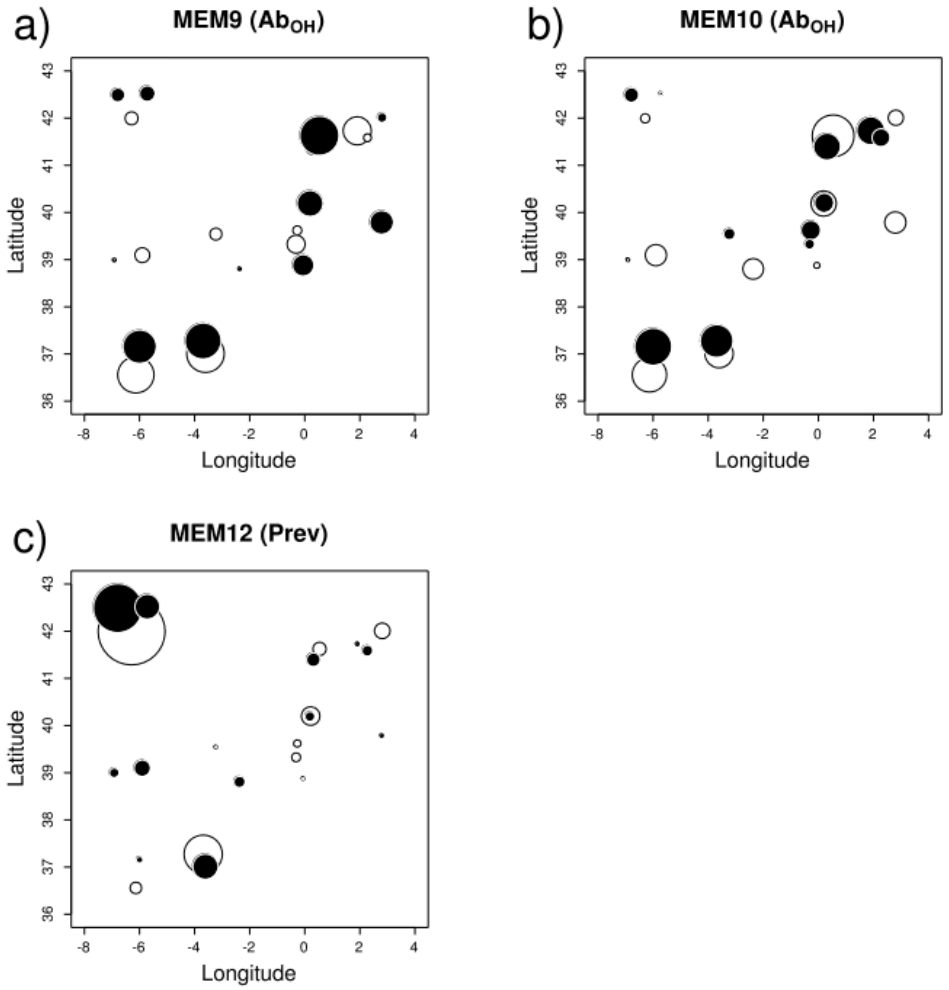

Figure S2. Moran's Eigenvector Maps (MEMs) selected as explanatory variables of the models for explaining prevalence (Prev) and abundance per occupied host (Ab_{OH}) of the hypersymbiotic ciliate *Lagenophrys discoidea*. (a) MEM9 and (b) MEM10 were selected for Ab_{OH}; (c) MEM12 was selected for Prev. White circles are positive values, whereas black circles are negative values. Circles of similar size and same colour indicate populations with similar scores (large black and white circles describe opposite extremes on the MEM axes).

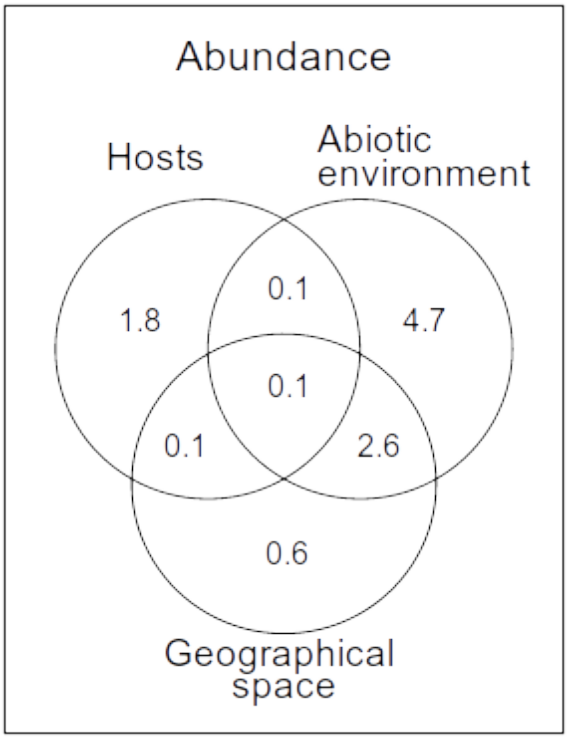

Figure S3. Percentages of contribution of pure and shared effects of host traits (including those of both symbiotic and basal hosts), the abiotic environment external to the hosts, and geospatial autocorrelation patterns to the explained variation in abundance of the hypersymbiotic ciliate *Lagenophrys discoidea*.

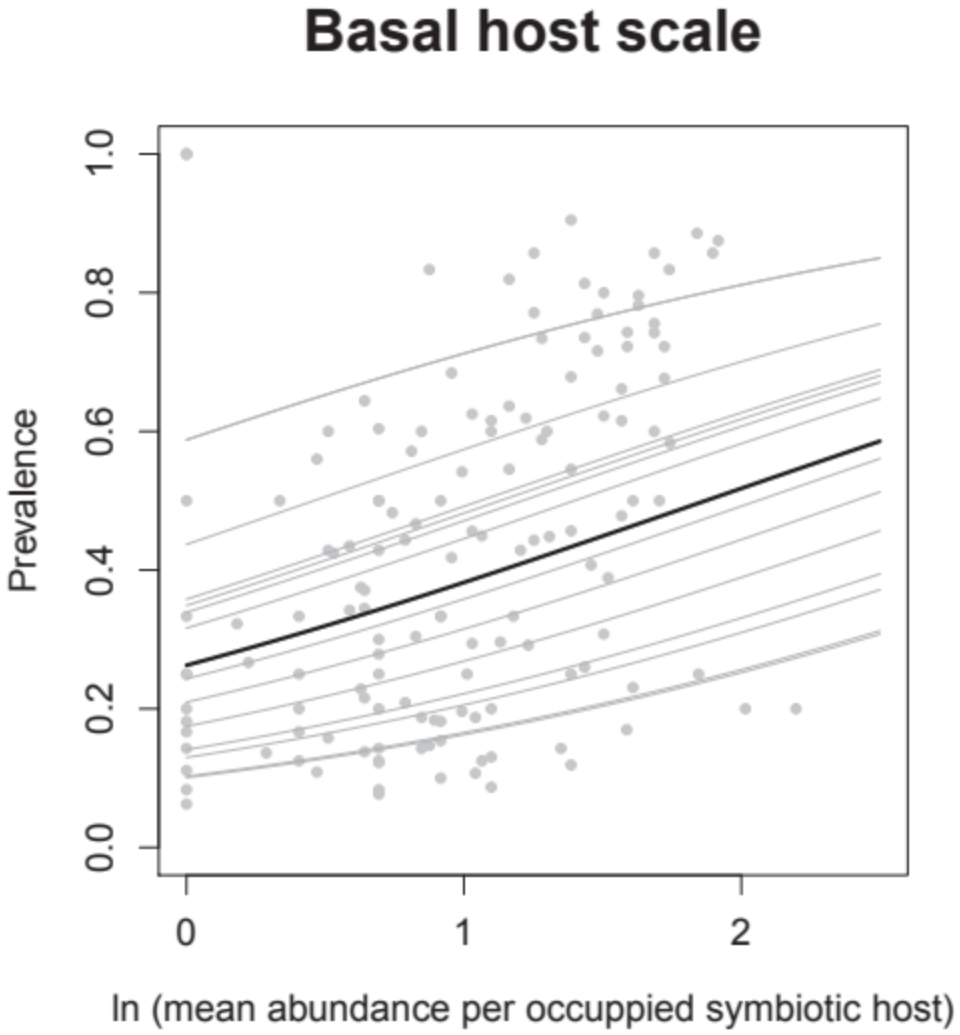

Figure S4. Occupancy-abundance relationship of the hypersymbiotic ciliate *Lagenophrys discoidea* at the scale of basal hosts (i.e. crayfish). The estimate of ciliate abundance is the average abundance per occupied ostracod within a crayfish. The estimate of occupancy is the prevalence, i.e. the percentage of occupied ostracods within a crayfish. The black line represents the fixed effects; grey lines represent the random effects of locality. A total of 143 crayfish (grey dots) and 16 sampling localities were included in this analysis; six sampling localities where the ciliate was absent were excluded.

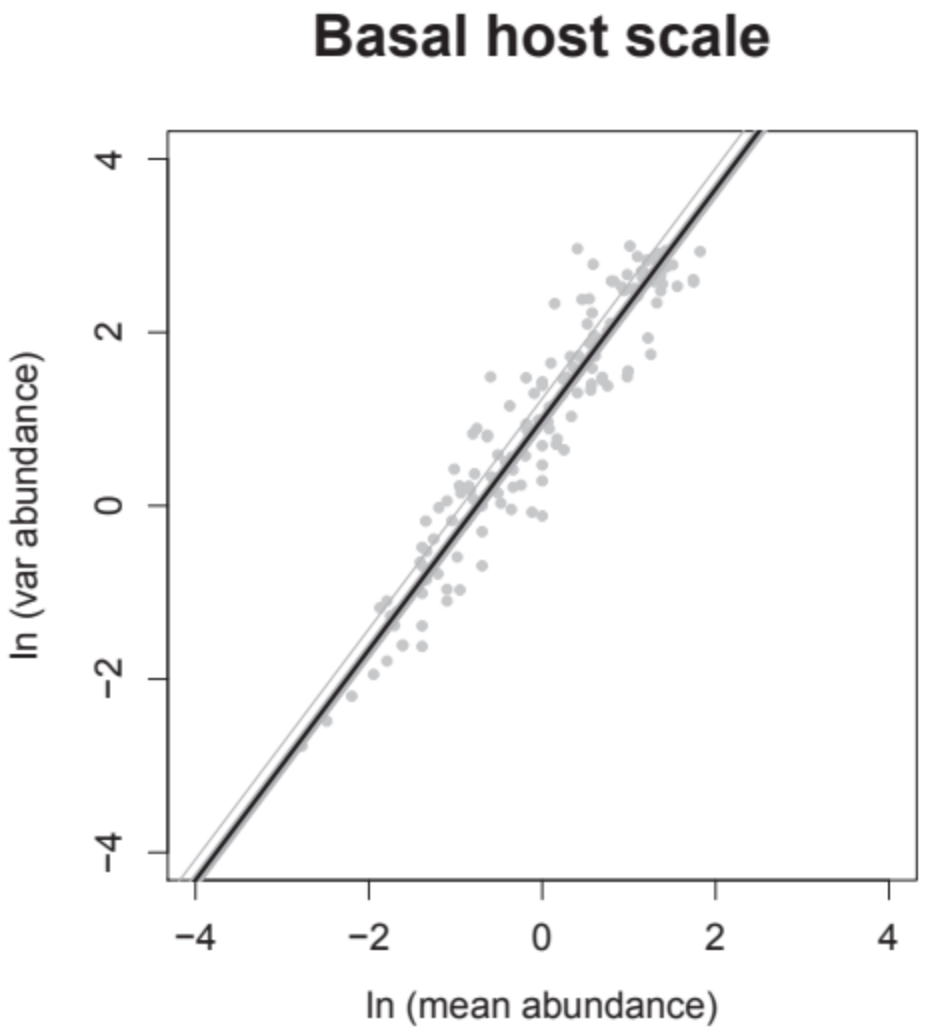

Figure S5. Variance-to-mean-abundance of the hypersymbiont *Lagenophrys discoidea* at the scale of basal hosts. The variance-to-mean abundance is the relationship between ln(variance of abundance) and ln(mean abundance) (where ln is the natural logarithm). In this analysis, the ciliate abundance was obtained from all the symbiotic ostracods, including those free of ciliates. Because we only measured the ciliate abundances from up to 10 ostracods with ciliates per crayfish, the numbers of ostracods without ciliates for mean abundance estimations were deduced from prevalences. For example, for a crayfish with a prevalence of 0.5 and 10 measured ostracods with ciliates, 10 additional data points were added with abundance equal 0 to estimate the mean abundance. Crayfish harbouring a single ostracod individual were disregarded here because the variance in ciliate abundance is not available at crayfish scale. The black line represents the fixed effects; grey lines represent the random effects of locality. A total of 140 crayfish (grey dots) and 16 sampling localities were included in this analysis; six sampling localities where the ciliate was absent (see Fig. 3) were excluded.

Table S1. Summary of the 22 sampling sites considered in this study focused on the freshwater hypersymbiotic ciliate *Lagenophrys discoidea* from the Iberian Peninsula (SW Europe), including the number of hosts sampled per locality.

Code	Locality name	Description	Coordinates (E-W°, N°)	Altitude (m)	Sampling date	n_{H1}	n_{H2}
LOC02	Oliva	Canal	-0.0555, 38.8775	0	28/04/10	278	11
LOC03	Puçol	Canal	-0.2715, 39.6207	0	30/04/10	60	13
LOC04	Valparaíso	Reservoir	-6.2882, 41.9952	853	06/05/10	28	5
LOC05	Carucedo	Lake	-6.7838, 42.4879	495	07/05/10	113	10
LOC06	Chozas de Arriba	Lagoon	-5.7139, 42.518	893	07/05/10	301	7
LOC07	Vivares	Canal	-5.8978, 39.0948	279	15/05/10	131	15
LOC08	Valdebótoa	Pond	-6.9160, 38.9958	183	15/05/10	15	7
LOC10	Sarrià de Ter	Stream	2.8126, 42.0077	55	20/05/10	489	15
LOC11	Navarcles	Stream	1.9040, 41.7309	262	20/05/10	31	6
LOC12	Granollers	River	2.2767, 41.5841	107	21/05/10	41	2
LOC13	Quero	River-stream	-3.2275, 39.5422	666	26/05/10	118	15
LOC18	Mequinenza	River	0.3170, 41.3938	61	02/06/10	325	12
LOC19	Alpicat	Reservoir	0.5354, 41.6246	203	03/06/10	518	14
LOC22	Sòller	Reservoir	2.7939, 39.7873	745	09/06/10	2	2
LOC25	Puerto Real	Canal	-6.1326, 36.5578	15	16/06/10	691	16
LOC26	Palacios	Canal	-5.9913, 37.1524	7	16/06/10	672	15
LOC27	Padul	Canal	-3.6052, 37.0075	720	18/06/10	391	12
LOC28	Cubillas	River	-3.6887, 37.2728	612	18/06/10	158	16
LOC29	Robledo	Canal	-2.3660, 38.8032	923	23/06/10	21	4
LOC32	El Palmar	Pond	-0.3147, 39.3268	2	20/04/11	509	10
LOC34	Torreblanca	Canal	0.2123, 40.2009	4	19/07/11	1	1
LOC35	Torreblanca	Canal	0.1914, 40.1899	0	19/07/11	8	4

n_{H1} is the number of symbiotic hosts (H1; i.e. adult ectocommensal ostracods of the sp. *Ankylocythere sinuosa*, acting as direct hosts of the hypersymbiont); n_{H2} is the number of basal hosts (H2; i.e. crayfish *Procambarus clarkii* acting as hosts of H1) that were sampled for symbiotic hosts.