



RESEARCH ARTICLE

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

Alexandre Mestre^{1,2} | Robert Poulin³ | Robert D. Holt⁴ | Michael Barfield⁴ | John C. Clamp^{5*} | Gregorio Fernandez-Leborans⁶ | Francesc Mesquita-Joanes¹

¹Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain; ²Department of Biology, Concordia University, Montreal, Quebec, Canada; ³Department of Zoology, University of Otago, Dunedin, New Zealand; ⁴Department of Biology, University of Florida, Gainesville, Florida; ⁵Department of Biological and Biomedical Sciences, North Carolina Central University, Durham, North Carolina and ⁶Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid (UCM), Madrid, Spain

Correspondence

Alexandre Mestre

Email: alexandre.mestre@uv.es

Funding information

Universitat de València, Grant/Award Number: UVPOST 2015; Generalitat Valenciana and European Union, Grant/Award Number: APOSTD 2017; Ministerio de Ciencia e Innovación, Grant/Award Number: CGL2008-01296/BOS; University of Florida Foundation

Handling Editor: Bethany Hoye

Abstract

1. The role of biotic interactions in shaping the distribution and abundance of species should be particularly pronounced in symbionts. Indeed, symbionts have a dual niche composed of traits of their individual hosts and the abiotic environment external to the host, and often combine active dispersal at finer scales with host-mediated dispersal at broader scales. The biotic complexity in the determinants of species distribution and abundance should be even more pronounced for hypersymbionts (symbionts of other symbionts).
2. We use a chain of symbiosis to explore the relative influence of nested biotic interactions and the abiotic environment on occupancy and abundance of a hypersymbiont.
3. Our empirical system is the epibiont ciliate *Lagenophrys discoidea*, which attaches to an ostracod that is itself ectosymbiotic on crayfish (the basal host). We applied multimodel selection and variance partitioning for GLMM to assess the relative importance of (a) traits of symbiotic hosts (ostracod sex and abundance), (b) traits of basal hosts (crayfish body weight, abundance and intermoult stage), (c) the abiotic environment (water chemistry and climate) and (d) geospatial autocorrelation patterns (capturing potential effects of crayfish dispersal among localities).
4. Our models explained about half of the variation in prevalence and abundance of the hypersymbiont. Variation in prevalence was partly explained, in decreasing order of importance (18%–4%) by shared effects of symbiotic host traits and the abiotic environment, pure fixed effects of symbiotic hosts, abiotic environment and geospatial patterns (traits of basal hosts were not relevant). Hypersymbiont abundance was most strongly explained by random effects of host traits (mainly the symbiotic host), in addition to weaker fixed effects (mostly abiotic environment).
5. Our results highlight the major role of the interplay between abundance of symbiotic hosts and water physico-chemistry in regulating populations of a

*Deceased.

hypersymbiotic ciliate, which is likely critical for dispersal dynamics, availability of attachment resources and suitability of on-host living conditions for the ciliate. We also found moderate signal of regulation by the basal host, for which we propose three mechanisms: (a) modulation of microhabitat suitability (crayfish-created water currents); (b) concentration of symbiotic hosts within crayfish; and (c) dispersal mediated by crayfish.

KEYWORDS

abundance, dispersal, host traits, nested hosts, niche, occupancy, symbiotic host

1 | INTRODUCTION

The relevance of biotic interactions in determining species distributions is a long-standing and controversial question (Godsoe, Jankowski, Holt, & Gravel, 2017; Louthan, Doak, & Angert, 2015; Soberón, 2007; Wiens, 2011). Biotic interactions have been often considered as secondary players relative to abiotic conditions, especially at broader scales (Soberón, 2007). However, this is not necessarily the case, especially for organisms that are tightly linked to others (Stewart et al., 2015), such as symbionts (i.e., species which depend upon other species for a place to live). Symbionts have a dual niche composed of traits of the individual hosts which harbour them, and the abiotic environment external to the host, and often combine active dispersal at finer scales with host-mediated dispersal at broader scales (Campbell & Crist, 2016; Krasnov et al., 2015). Hypersymbionts (i.e., symbionts of other symbionts) in a sense have a threefold niche—the external environment, traits of the basal host sustaining the symbiotic host and traits of the symbiotic host itself. Living in association with two nested hosts thus adds an extra level of biotic complexity, with potential impacts on species distribution and abundance. Here, we investigate the roles of nested biotic

interactions and the abiotic environment in governing occupancy and abundance of a hypersymbiont—based on a ciliate, living on an ectosymbiotic ostracod, which in turn resides on crayfish.

Our study species is the hypersymbiotic ciliate *Lagenophrys discoidea* Kellicott, 1887. The ectosymbiotic ostracod *Ankylocythere sinuosa* (Rioja, 1942), which hosts *L. discoidea*, will be referred to as the 'symbiotic host' (i.e., direct host of the hypersymbiont), and the crayfish *Procambarus clarkii* (Girard, 1852), which hosts the ostracod, as the 'basal host' of the symbiotic chain (Figure 1). Both nested hosts were introduced to Europe from their native North American range (Mestre et al., 2013). By contrast, *Lagenophrys discoidea* has a wide Holarctic and Neotropical distribution and uses freshwater ostracods as hosts, including both free-living and ectosymbiotic ostracods (Mayén-Estrada & Clamp, 2016). Peritrich ciliates of the genus *Lagenophrys* Stein, 1852 are sessile suspension feeders that attach to crustacean hosts in an ectosymbiotic lifestyle called epibiosis (Mayén-Estrada & Clamp, 2016). Lagenophryids feed on microorganisms, phytoplankton and organic particles suspended in the water by capturing them with their peristomial cilia whilst attached to their hosts (Clamp, 1973, 1989; Felgenhauer, 1982). Crustacean hosts, such as ostracods in our system, provide multiple benefits

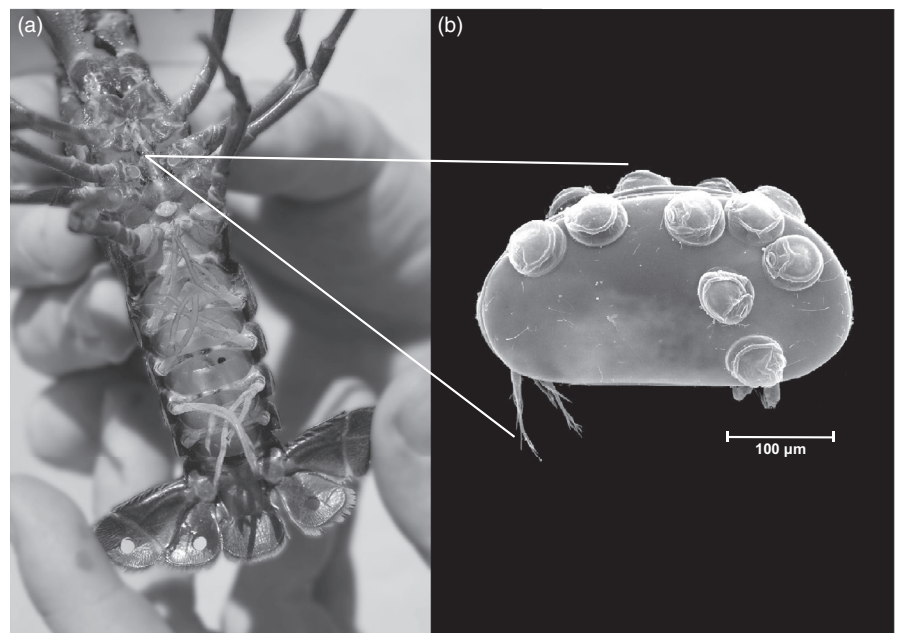


FIGURE 1 Chain of symbiosis comprised of the freshwater crayfish *Procambarus clarkii* (a), the symbiotic ostracod *Ankylocythere sinuosa* and the ciliate *Lagenophrys discoidea* (b). The ciliate acts as hypersymbiont with two nested hosts. The ostracod is the symbiotic host, and the crayfish is the basal host of the symbiotic chain (i.e., hosting the ostracod)

to their epibionts (Fernandez-Leborans, 2010): (a) an attachment surface; (b) increased efficiency in food acquisition from water currents provided by host respiration and feeding; (c) enhanced dispersal capability; and (d) avoidance of interspecific competition and predatory pressures. However, anti-fouling mechanisms of hosts, such as moulting or grooming, should hinder evolution towards obligate epibiosis. Whilst epibiosis has been documented as predominantly facultative (Wahl & Mark, 1999), the 62 known species of *Lagenophrys* are obligate epibionts specialized on a wide variety of crustacean hosts from many marine, brackish and freshwater habitats (Mayén-Estrada & Clamp, 2016). A large part of the success of *Lagenophrys* ciliates as obligate ectosymbionts of crustaceans is attributed to a special type of binary fission (called second-type division) just before host moulting, which prevents them from being lost (Clamp, 1991). Other adaptations to the ectosymbiotic lifestyle in *Lagenophrys* (Mayén-Estrada & Clamp, 2016) are as follows: (a) a mechanism for permanent attachment to the host; (b) a closure apparatus to protect the ciliate from drying when the host leaves water; and (c) a specialized free-living, dispersal stage.

Populations of *Lagenophrys* inhabiting free-living hosts are potentially affected by (a) physiological tolerances of physico-chemical and climatic conditions (Cabral, Utz, & Velho, 2017; Clamp, 1973, 1988; Sheehan, Lafferty, O'Brien, & Cebrian, 2011); (b) concentration of chlorophyll *a*, dissolved nutrients and percentage of organic matter in sediment, governing nutrient availability (Cabral et al., 2017; Clamp, 1973, 1989; Sartini et al., 2018); (c) host abundance and geospatial patterns that influence their dispersal (Cabral et al., 2017; Jones, Carrasco, Perissinotto, & Vosloo, 2016; Utz & Coats, 2005); and (d) host body size determining the total attachment surface available per host individual (Fernandez-Leborans, 2010; Poulin, 2007). In our study system, the body size of a symbiotic host depends on its sex. Adult females of the ostracod *A. sinuosa* are significantly larger than males, with little size variation within each sex (Aguilar-Alberola et al., 2012). In addition, crayfish with larger body size (which is highly variable among individuals) harbour larger numbers of symbiotic ostracods, whereas crayfish moulting temporarily decreases their abundance (Aguilar-Alberola et al., 2012; Mestre, Monrós, & Mesquita-Joanes, 2014). The ciliate *L. discoidea* has three

dispersal mechanisms acting at different spatial scales (Figure 2). First, free-swimming larval stages actively migrate among symbiotic ostracods within a single crayfish individual (Clamp, 1987). Second, sessile adults passively disperse among crayfish within a locality via dispersal of the symbiotic ostracods (Mestre, Castillo-Escrivà, Rueda, Monrós, & Mesquita-Joanes, 2015). Third, crayfish dispersing among localities (Hunt, Thomas, James, & Cable, 2018; Mestre et al., 2016) likely disseminate ciliates carried by symbiotic ostracods, a dispersal mechanism called hyperphoresy (Sabagh, Dias, Branco, & Rocha, 2011).

Here, we examined the relative importance of two nested hosts and the abiotic environment external to the hosts in regulating populations of the hypersymbiotic ciliate *L. discoidea*. A priori, the strong dependence on the chain of hosts for microhabitat suitability and dispersal should play a major role in driving population dynamics of the ciliate. The ciliate attaches specifically to ostracod valves, so that the bodies of symbiotic hosts should represent crucial attachment resources for the ciliate. Moreover, symbiotic hosts should play crucial roles for local-scale dispersal of the ciliate, including both active larval dispersal from one ostracod to another within a crayfish and passive transport among crayfish by the ostracod. On the other hand, basal hosts could affect populations of the ciliate in three ways. First, the crayfish could modulate microhabitat suitability, for example by creating water currents for food acquisition by the ciliate. Second, because ostracods are symbiotic, crayfish bodies act as containers that concentrate and regulate ostracod populations within and among their bodies, potentially influencing within-crayfish and local dispersal of the ciliate. Third, crayfish regional dispersal should leave a trace in occupancy and abundance of the ciliate due to dispersal limitations, demographic stochasticity or rescue effects experienced by the ciliate (Kanarek, Webb, Barfield, & Holt, 2015; Prenter, MacNeil, Dick, & Dunn, 2004; Torchin, Lafferty, Dobson, McKenzie, & Kuris, 2003). However, ectosymbionts by definition live on host exterior surfaces and therefore should also depend on external environmental conditions (DeWitt, Williams, Lu, Fard, & Gelder, 2013; Mestre et al., 2014), including food resource availability (e.g., for ectosymbionts that are suspension feeders; Sartini et al., 2018).

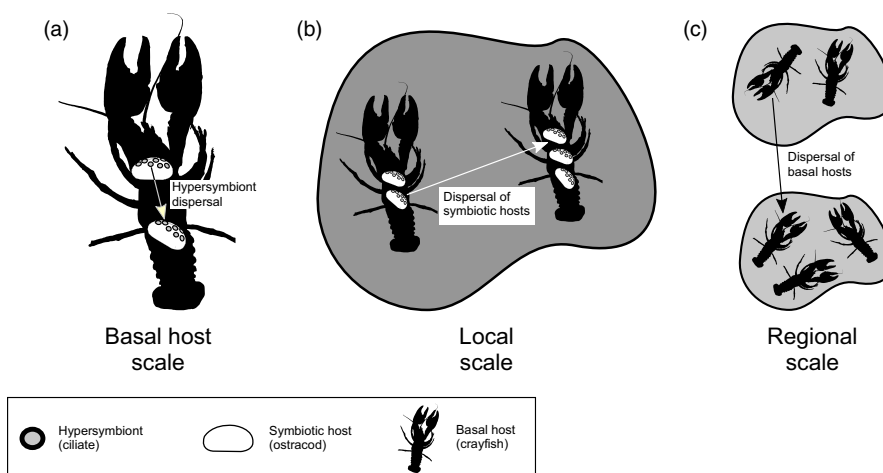


FIGURE 2 Dispersal mechanisms of the hypersymbiotic ciliate *Lagenophrys discoidea*: (a) at the basal host scale, free-swimming larval stages actively migrate among symbiotic ostracods within a single crayfish individual; (b) at the local scale, sessile adults passively disperse among crayfish within a locality via dispersal of symbiotic ostracods; and (c) at the regional scale, dispersing crayfish disseminate the ciliates carried by symbiotic ostracods among localities

TABLE 1 Description of explanatory variables and selection criteria used for modelling prevalence (Prev) and abundance (AbOH) of *Lagenophrys discoidea*

Class	Code	Description	Units/levels	Mean/ Median	SD/quartiles ^a
Response variables	Prev	Prevalence within a basal host	% of occupied symbiotic hosts	28.9	28.7
	Ab _{OH}	Abundance per occupied symbiotic host	Ind. within a symbiotic host	2	(1,4)
Symbiotic host	SexRatH1	Sex ratio of symbiotic hosts (used for Prev)	% of males	45.8	23.9
	SexH1	Sex of symbiotic host (used for Ab _{OH})	2 levels (♂ and ♀)		
	AbH1 ^b	Abundance of symbiotic hosts	Ind. within a basal host	13	(5,35)
	AbH1_Loc	Mean AbH1 in a locality	Av. ind. per basal host	23.1	16.3
	RE H1	Random effects of symbiotic host (nested within H2; used for Ab _{OH})	942 levels		
Basal host	WeightH2	Body weight of basal host	g	21.5	10.7
	MoultH2	Intermoult stage of basal host ^c	4 levels ^c		
	AbH2	Abundance of basal hosts	Av. ind. per trap	2.08	(1.25,3.91)
	RE H2	Random effects of basal hosts (nested within Loc; used for Ab _{OH})	143 levels		
Abiotic environment	Cond	Electric conductivity	mS/cm	1.26	1.13
	Chlor-a	Chlorophyll <i>a</i> concentration	µg/L	8.75	9.53
	Alk	(Bi-)carbonate alkalinity	meq/L	3.47	1.61
	O ₂	Oxygen concentration	mg/L	7.46	3.20
	NH ₄ ⁺	Ammonium concentration	mg/L	0.560	1.049
	NO ₃ ⁻	Nitrate concentration	mg/L	22.4	27.3
	PO ₄ ³⁻	Phosphate-P concentration	mg/L	0.116	0.158
	SO ₄ ²⁻	Sulphate concentration	mg/L	232.9	265.7
	RatAlkAn	Alkalinity/(chloride + sulphate)	(ratio)	1.32	1.81
	RatClS	Chloride/sulphate	(ratio)	2.10	3.25
	RatNO ₃ NH ₄	Nitrate/ammonium	(ratio)	99.5	124.7
	Sed _{OM}	% of organic matter in sediment	%	6.93	4.56
	T _{max}	Max. temperature of warmest month	°C	30.9	2.57
	T _{min}	Min. temperature of coldest month	°C	3.90	2.57
	T _{range}	Temperature annual range (T _{max} -T _{min})	°C	27.0	3.82
	Precip	Annual precipitation	mm	519.2	103.0
PrecipSeas	Seasonal precipitation (CV monthly precip.)	%	45.2	12.5	
Geographical space	MEM	Moran's eigenvector maps			
	RE Loc	Random effects of sampling locality	22 levels (Prev); 16 levels (Ab _{OH})		
Selection criteria	AICc	Second-order Akaike information criterion			
	Δ _i	AICc increase of the model 'i' in relation to the best model			
	ω _i	Relative likelihood of the model 'i' given the data and the set of best models			
	ER _i	Ratio of AICc weight of the best model to the model 'i' (ω _{best} /ω _i)			
	R _M ²	Marginal coefficient of determination (variance explained by fixed effects)			
	R _C ²	Conditional coefficient of determination (variance explained by both fixed and random effects)			

^aFor abundances, median and first and third quartiles are displayed. Mean and SD are given for other quantitative variables.

^bAbH1 was log-transformed because it showed a highly skewed distribution.

^c0: body, including chelae, completely and extremely soft; 1: sides of cephalothorax soft and very elastic; 2: sides of cephalothorax hard but slightly elastic; 3: sides of cephalothorax hard and totally rigid.

Moreover, the abiotic environment can affect local dispersal among hosts for horizontally transmitted symbionts with free-living dispersal stages (Studer & Poulin, 2013). Therefore, we expect a significant influence of abiotic conditions on occupancy and abundance of the ciliate, because ciliate individuals are directly exposed to the external aquatic environment when attached to symbiotic hosts, as well as during larval dispersal (Cabral et al., 2017; Clamp, 1973, 1988; Sheehan et al., 2011). We tested our hypotheses with multimodel selection and variance partitioning for GLMM, through which we assessed the relative importance of the following sources of variation in prevalence and abundance of the ciliate: (a) traits of symbiotic hosts (ostracod sex and abundance); (b) traits of basal hosts (crayfish body weight, abundance and intermoult stage); (c) the abiotic environment (water chemistry and climate); and (d) geospatial autocorrelation patterns (capturing potential effects of crayfish dispersal among localities).

2 | MATERIALS AND METHODS

2.1 | Sources of data

Two response variables describing hypersymbiont populations were modelled in this study. First, prevalence is defined as the proportion of symbiotic hosts occupied by the hypersymbiont within a basal host. Second, abundance is the number of hypersymbionts inhabiting an occupied symbiotic host (Table 1). The data used to model both variables were collected in the field and are available in the Dryad Digital Repository (Mestre et al., 2019).

The data were obtained from 22 localities in the Iberian Peninsula and Balearic Islands (SW Europe; Figure 3 and Table S1) with sampling concentrated in spring–summer (April to July) of two consecutive years (2010 and 2011; single sampling time per location). Individuals of *P. clarkii* were collected using 10–20 baited traps (40 cm × 40 cm × 80 cm) laid overnight, regularly distributed along the littoral of the waterbody (10 m distance between traps; up to 2 m depth). The next day, traps were retrieved and crayfish abundance (individuals per trap) was recorded. A maximum of 16 crayfish per location (depending on the local availability; see Table S1) were sampled for symbionts and hypersymbionts. Only crayfish with post-orbital cephalothorax length >10 mm were retained in the samples (and used to estimate crayfish abundance), as juvenile crayfish generally lack ostracod symbionts (Castillo-Escrivà, Mestre, Monrós, & Mesquita-Joanes, 2013). After applying the protocol for symbiont removal described below, crayfish hosts were preserved in 96% ethanol. In the laboratory, crayfish were sexed and weighed, and their intermoult stage estimated using hardness of the cephalothorax (following Castillo-Escrivà et al., 2013).

Individuals of the ostracod *A. sinuosa* (species identification from Mestre et al., 2014, based on Hart & Hart, 1974) were removed from each sampled crayfish individual in situ by immersing it in a container filled with carbonated water for 15 min (Mestre, Monrós, & Mesquita-Joanes, 2011). Afterwards, the water was filtered through a 63- μ m-mesh sieve to concentrate ostracods and their ciliates from each crayfish individual into separate 50-ml containers filled

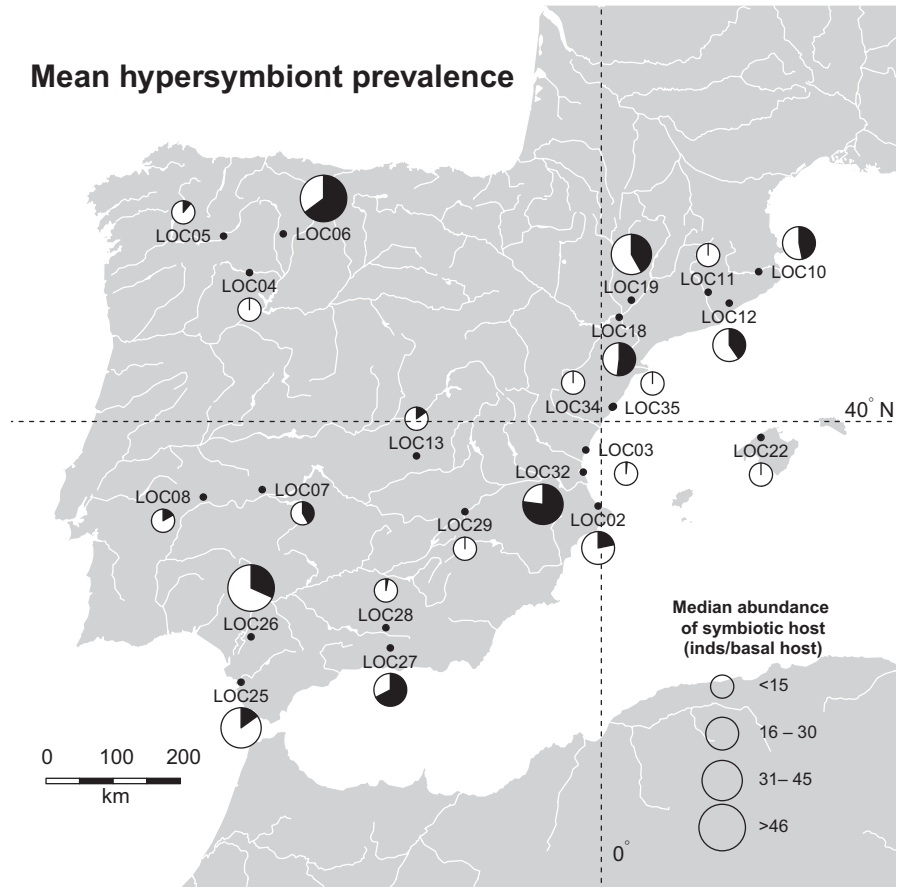
with 96% ethanol. The adult ciliates of *Lagenophrys* are sessile, living permanently attached to their hosts, and the ciliate–ostracod associations remain unaltered and preserved after the protocol of ostracod isolation. In the laboratory, all adult ostracods of *A. sinuosa* from each crayfish individual were counted under a stereomicroscope to obtain estimates of their abundance. Each adult ostracod was sexed, and the external surfaces of both valves were examined for the presence of hypersymbiotic ciliates. From each crayfish, a maximum of 10 ostracod individuals were randomly selected from the pool of adult ostracods harbouring *L. discoidea*. The selected ostracods were individually checked for hypersymbiont abundance (i.e., the total number of individuals attached to the valves). When a crayfish individual had fewer than 10 adult ostracods with ciliates, all these ostracods were checked for hypersymbionts. For species identification, representative samples of ciliates from each locality were mounted on a microscope slide, stained with safranin and suspended in a solution of glycerine plus distilled water (1:6). Species identification of ciliates relied on descriptions by Clamp (1990, 1991). All the ciliates proved to be *Lagenophrys discoidea*.

Oxygen concentration, pH, temperature and conductivity of the sampled waterbodies were measured in situ with portable probes. Water and sediment samples were collected from each locality and analysed in the laboratory. For water samples, concentrations of anions (alkalinity, Cl^- and SO_4^{2-}) and nutrients (NH_4^+ , NO_3^- and PO_4^{3-}) were determined using standard methods (APHA-AWWA-WEF, 2012); chlorophyll *a* concentration was estimated following Jeffrey and Humphrey (1975). From sediment samples, the percentage of organic matter was obtained by the loss-on-ignition method (Heiri, Lotter, & Lemcke, 2001). The following climatic data at 0.5 arcmin resolution were taken from WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005): maximum temperature of the warmest month, minimum temperature of the coldest month, temperature range, annual precipitation and precipitation seasonality. Moran's eigenvector maps (MEMs) were obtained from geographic coordinates of sampling locations, using SPDEP v. 0.5.88 (Bivand & Piras, 2015). The MEMs are spatial predictors that characterize a wide range of autocorrelation structures based on the distances between sampling sites (Dray, Legendre, & Peres-Neto, 2006). We used these geospatial variables representing patterns of spatial autocorrelation among localities to capture potential effects of crayfish dispersal among localities. Autocorrelated MEMs were identified through a 99-time permutation procedure implemented by SPACEMAKER v. 0.0.5 (Dray, 2013) and removed from the analyses. A total of 15 uncorrelated geospatial variables (MEM1–MEM15) were obtained from this procedure, representing a gradient of spatial autocorrelation patterns from broader (MEM1) to finer (MEM15) scales.

2.2 | Sources of variation in occupancy and abundance of the hypersymbiont

We used a model selection approach and variance partitioning to test the relative importance of traits of symbiotic hosts, traits of

FIGURE 3 Distribution of the 22 sampling localities across the Iberian Peninsula and Balearic Islands. Shaded parts of pie charts show local mean prevalences of the hypersymbiotic ciliate (averaged over all crayfish occupied by symbiotic ostracods). The diameters of pie charts indicate local median abundances of the symbiotic host (i.e., median number of symbiotic ostracods per individual crayfish)



basal hosts, the abiotic environment and geographical space, in explaining variation in prevalence and abundance of the hypersymbiont. Traits of symbiotic hosts included ostracod sex and abundance. Traits of basal hosts were crayfish body weight (as a measure of body size), intermoult stage and abundance. The set of variables describing the abiotic environment included the water chemistry and the climate variables listed above. Geographical space was represented by geospatial autocorrelation patterns (MEMs), used here to capture effects of crayfish dispersal among localities (see Table 1).

2.2.1 | Model selection

The general structure applied to our models was based on the nested nature of the study system and the overdispersion of ciliate abundance. The hierarchical structure was incorporated by using generalized linear mixed models (GLMM) with sampling locality (for prevalence and abundance) and basal host (for abundance) as nested random effects (i.e., basal host nested within locality). The GLMM were implemented with *lme4* v. 1.1.19 (Bates, Mächler, Bolker, & Walker, 2015). The model families were binomial for prevalence (with 'logit' link function) and lognormal-Poisson for abundance (with 'log' link function). The lognormal-Poisson was derived from a Poisson model by including individual of symbiotic host as a third random effect (nested within basal host), which allows us to control for overdispersion of abundance (Ozgul, Oli, Bolker, & Perez-Heydrich, 2009). We limited the initial set of geospatial variables

(15 MEMs) through backward selection. The preliminary backward selection pre-selected two geospatial variables for prevalence (i.e., MEM12 and MEM14) and nine for abundance (i.e., MEM3, MEM5, MEM6, MEM9-MEM11, MEM13-MEM15). All continuous explanatory variables were standardized. All pairs of explanatory variables (including pre-selected MEMs) had $|r| < 0.75$.

An automated model selection process was performed with *GLMULTI* v. 1.0.7 (Calcagno & de Mazancourt, 2010). It consisted of an exhaustive screening of a set of candidate models, using the AICc as selection criterion, and considering all combinations of explanatory variables subject to complexity limits. That is to say, to prevent overfitting and facilitate computation tasks, we discarded models with more than 8 explanatory variables (including random effects). Thus, the maximum number of fixed effects was set to seven for prevalence models (plus 1 random effect; 726,206 candidate models) and five for abundance models (plus 3 random effects; 242,825 candidate models). With that, we obtained a ratio of ca. 30 crayfish per fixed effect for both model variants. Moreover, interactions between explanatory variables were also discarded. The set of best models was composed of those with: $\Delta_i \leq 2$, where i is the model index and $\Delta_i = AICc_i - AICc_{best}$; $AICc_{best}$ is the model with the minimum AICc (Burnham & Anderson, 2002). When this criterion led to fewer than four models, we selected the four best models. Multimodel inference was performed on the set of best models to obtain model-averaged estimates of the parameters of fixed effects (Lukacs, Burnham, & Anderson, 2010).

2.2.2 | Percentages of explained variance

The relative influence of the different sources of variation in hypersymbiont populations was analysed through variance partitioning based on the marginal coefficient of determination for GLMM (R_M^2 ; Nakagawa & Schielzeth, 2013). R_M^2 represents the proportional contribution of fixed effects to the variance explained by the model. The analysis was made on a consensus model with all the explanatory variables with a relative importance (RVI) higher than 0.1. The RVI of an explanatory variable is the sum of the AICc weights across the best models containing the variable. The AICc weight of a best model represents its relative likelihood given the data and the full set of best models. We verified that consensus models were not biased by sampling month or sampling year (Figure S1).

3 | RESULTS

The dataset for the prevalence models was obtained from 212 crayfish (143 with hypersymbiotic ciliates on *A. sinuosa*) from 22 localities (16 with ciliates). A total of 4,901 ostracods were sampled for prevalence (2,577 females and 2,324 males), of which 2,045 harboured ciliates (913 females and 1,132 males). Thus, the global percentage of occupied ostracods was 41.7%. The mean prevalence per crayfish was 28.9% ($SD = 28.7\%$; SD is the standard deviation). The data for abundance models were obtained from 143 crayfish from 16 localities and 942 ostracods (422 females and 520 males). The median abundance per occupied host was 2 (IQR = 3; max. = 17; IQR is the interquartile range). General descriptive statistics of model variables are given in Table 1.

A single model was clearly superior for prevalence (with an AICc that was over 5 units below the second best model) and, therefore, we only selected the best four models including this one. The five best models for abundance were selected based on having an AICc within 2 units from the best one (Table 2). In all the models, both fixed and random effects explained around 40%–50% of the variation in both prevalence and abundance of the hypersymbiont. However, prevalence and abundance models differed in the relative contribution of fixed versus random effects. Whilst fixed effects explained most of the variation in prevalence, abundance was mostly explained by random effects. About 85% of the variance attributed to random effects in abundance models corresponded to symbiotic hosts, and 15% was attributed to basal hosts. The proportion of random-effects variance explained by locality was less than 0.1%.

The most important fixed effects for prevalence, which appeared in all the best models (i.e., $RVI = 1$), were (Table 2): (a) negative effects of a fine-scale geospatial autocorrelation pattern (MEM12; Figure S2), and (b) positive effects of average abundance of symbiotic hosts in a locality, sex ratio of symbiotic hosts (% of males) and phosphate concentration. Other important variables for prevalence ($0.85 < RVI < 1$) were (bi-)carbonate alkalinity (negative effects), maximum temperature of warmest month (positive effects) and precipitation seasonality (negative effects), all of which were present in the best model. The only explanatory variable that appeared in all the

abundance models was water conductivity, with negative effects. It was also the only variable showing significant effects on abundance according to confidence intervals of β estimates averaged across the best models.

The consensus model used for variance partitioning of prevalence had seven predictors and corresponded to Model 1 in Table 2. The variance partitioning for prevalence (Figure 4) showed that 17.8% was explained by the shared effects of symbiotic host traits and the abiotic environment. Pure fixed effects of symbiotic host traits explained 9% of the variance. The unique effects of the abiotic environment explained 6.1% and geospatial patterns 3.6% (no traits of basal hosts were present in the best models for prevalence). The consensus model for abundance had ten predictors (Table 2; AICc = 4,072.5; $R_M^2 = 0.100$; $R_C^2 = 0.486$). Random effects accounted for 39% of the variance explained by the consensus model for abundance, of which 88% was attributed to traits of the symbiotic host, and the other 12% to the basal host (locality-level random variation was zero). The fixed effects explained only 10% of the variance (Figure S3). The most important source of fixed effects explaining variation in abundance was the abiotic environment, with 4.7% of variance explained by pure effects, and 2.6% by shared effects with geographical space. Pure effects of host traits (considering both host types together) contributed with just 1.8%. Some factors thus have much more explanatory power than do others, and there is considerable residual unexplained variation.

4 | DISCUSSION

Populations of symbionts are spatially structured as aggregates among and within hosts, and their life cycles are adapted to both within-host living conditions and transmission among hosts. Thus, strong biotic interactions necessarily associated with a symbiotic lifestyle potentially affect both niche and dispersal processes (Borges, 2017; Buser, Newcomb, Gaskett, & Goddard, 2014; Lymbery, 2015). Using data from a chain of symbiosis, we investigated the relative influence of nested biotic interactions and the abiotic environment on occupancy and abundance of the hypersymbiotic ciliate *L. discoidea*. The most important source of variation in both occupancy and abundance of the ciliate was the symbiotic host. But we also found significant influence of water physico-chemistry and a moderate signal of regulation by basal hosts. We provide evidence suggesting that the interplay between two nested hosts and the abiotic environment external to the hosts regulates populations of *L. discoidea*, through their combined role in governing microhabitat suitability and dispersal dynamics of the ciliate.

4.1 | Main role of symbiotic hosts

In symbiotic species, host traits (e.g., body size or abundance) govern host susceptibility to symbiont acquisition, maintenance and transmission and thus can be key drivers of symbiont dynamics (Poulin,

TABLE 2 Results of selection of the best models built to evaluate the relative importance of traits of symbiotic host, traits of basal host, abiotic environment and geospatial autocorrelation patterns in explaining prevalence (Prev) and abundance (Ab_{OH}) of *Lagenophrys discoidea*

Type	Model	CLASS					Symbiotic host			Basal host			Abiotic environment				Geographical space ^a		
		AICc	Δ_i	ω_i	ER_i	R_M^2	R_C^2	PREDICTOR	ABH1_Loc	SexRatH1 ^b	PO ₄ ³⁻	Alk	T _{max}	PrecipSeas	MEM12	MEM9	MEM10		
Prev	1	909.78	0	0.87	1	0.417	0.421	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	2	915.09	5.32	0.06	14.3	0.396	0.414	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	3	916.04	6.27	0.04	23.0	0.490	0.500	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	4	916.80	7.02	0.03	33.5	0.449	0.458	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
		β^c																	
								0.799	0.504	0.504	0.771	-0.491	0.415	-0.362	-3.779				
								± 0.227	± 0.122	± 0.122	± 0.189	± 0.290	± 0.337	± 0.290	± 1.224				
								1	1	1	1	0.936	0.875	0.875	1				
								$\log_{10}(AbH1)$	SexH1 ^a	WeightH2	Cond	PO ₄ ³⁻	RatAlkAn	O ₂	RatCIS	MEM9	MEM10		
Ab_{OH}	1	4,077.0	0	0.29	1.00	0.080	0.484	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	2	4,077.3	0.30	0.25	1.16	0.084	0.486	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	3	4,077.8	0.86	0.19	1.54	0.080	0.485	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	4	4,078.4	1.40	0.14	2.00	0.080	0.484	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
	5	4,078.6	1.67	0.13	2.30	0.080	0.483	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
		β																	
								0.045	0.055	-0.021	-0.142	0.091	-0.065	-0.096	-0.050	0.273	-0.030		
								± 0.093	± 0.132	± 0.079	± 0.080	± 0.170	± 0.130	± 0.219	± 0.120	± 0.526	± 0.183		
								0.583	0.479	0.250	1.000	0.562	0.562	0.438	0.438	0.562	0.126		

Notes: Codes and abbreviations of model variables and selection criteria are defined in Table 1. Only predictors with RVI > 0.1 were presented (i.e., those predictors that were selected to build the consensus models for variance partitioning).

^aThe three selected geospatial variables are shown in Figure S2.

^bThe index used for SexRatH1 was % of males, and β of SexH1 represents the effects of the male gender.

^c β , model-averaged estimates of fixed effects.

^dCI, 95% confidence intervals of β .

^eRVI, relative importance of a variable.

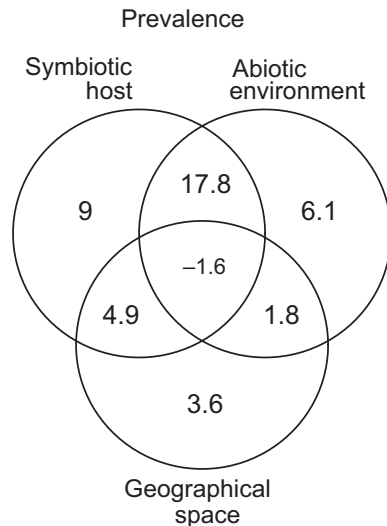


FIGURE 4 Percentages of contribution of pure and shared effects of traits of symbiotic hosts, the abiotic environment external to the hosts and geospatial autocorrelation patterns to the explained variation in prevalence of the hypersymbiotic ciliate *Lagenophrys discoidea*. No variable representing traits of basal hosts was included in the best models for prevalence. Small negative values in shared fields appear in cases of suppression/enhancement between explanatory variables and can be interpreted as zeros

2007). The main source of variation in prevalence of the hypersymbiont *L. discoidea* corresponded to traits of the symbiotic host (i.e., ostracod abundance and sex). We found positive effects of local-scale average abundance of the symbiotic ostracods on prevalence of the ciliate. Host abundance is expected to be an important factor affecting prevalence and abundance of horizontally transmitted symbionts (Arneberg, Skorping, Grenfell, & Read, 1998; Stanko, Krasnov, & Morand, 2006). World-wide, the ciliate *L. discoidea* uses both free-living and symbiotic ostracods as attachment surfaces (Mayén-Estrada & Clamp, 2016). However, there are no other published records of *L. discoidea* in the Iberian Peninsula (Mayén-Estrada & Clamp, 2016), and findings of peritrich ciliates in samples of free-living ostracods from the study region are rare (authors' pers. obs.). Our results strongly support the symbiotic ostracod as being a key host for *L. discoidea* in the study region. Very high prevalences and high transmissibility observed for *A. sinuosa* suggest highly efficient dispersal of the symbiotic ostracod among crayfish (Aguilar-Alberola et al., 2012; Mestre et al., 2015, 2014). Thus, local-scale dispersal of symbiotic ostracods among crayfish (Figure 2b) should be crucial for transmission dynamics of the ciliate. The lack of significance of crayfish abundance in our analyses adds support to the main role of ostracod dispersal (instead of ciliate active dispersal) in governing among-crayfish transmission. In addition, the lower numbers of ciliates observed in ostracod females are explained by the precopulatory behaviour of the ostracod, whereby females are guarded by males. Valves of guarded females are partially covered by the male's body (Aguilar-Alberola et al., 2012), thereby impeding colonization of females by ciliates, or dislodging them when the precopulation

position is established. Thus, ostracod sex (and sexual behaviour) likely influences the availability of attachment surfaces for the ciliate.

The observed large proportion of variance in ciliate abundance explained by random effects of symbiotic hosts was expected, in part, from the colonization–extinction dynamics at the scale of symbiotic hosts. Symbiotic ostracods have seven juvenile instars separated by moult events (Aguilar-Alberola et al., 2012), and adults live less than one year (Young, 1971). The highly ephemeral nature of ostracod valves exacerbates the necessity for frequent movement among symbiotic hosts by second-type division to avoid being lost during host moult or after host death (Clamp, 1991). The resulting highly transient colonization–extinction dynamics should generate high levels of stochasticity in ciliate abundance. However, other host traits that we did not consider here could conceivably account for a portion of this random unexplained variation as well (e.g., time from last moult).

4.2 | Significant influence of the abiotic environment

Our analysis of populations of a hypersymbiotic ciliate showed a strong signal of regulation by water chemistry and climate. The abiotic environment external to the hosts may influence populations of ectosymbionts both directly, modulating on-host living conditions and/or mortality during dispersal, and indirectly through its effect on host attributes (e.g., abundance or quality). The selected abiotic variables (i.e., those present in the best models; see Table 2) are likely to affect physiological tolerances (alkalinity, temperature and precipitation seasonality) and nutrient availability (phosphate) of the ciliate (Cabral et al., 2017; Clamp, 1973, 1988, 1989; Sartini et al., 2018; Sheehan et al., 2011). Phosphate is likely a proxy for food sources of *L. discoidea* (i.e., micro-organisms and organic particles suspended in the water). Adult ciliates live outside their hosts, attached to their external surface, where they are exposed directly to the external environment from which they obtain their food. Thus, the on-host microhabitat suitability for the ciliate should also depend on the abiotic environment. Moreover, larval stages of *L. discoidea* move actively through the water environment during dispersal. Both microhabitat suitability and dispersal-related factors should make the ciliate more sensitive to the abiotic environment, compared to, say, endosymbionts with direct host-to-host transmission which are more isolated from the external environment. Moreover, because crustacean hosts such as freshwater crayfish and ostracods are aquatic ectotherms, thermal conditions of their bodies should closely match the environment. Thus, they likely do not offer a thermally regulated microenvironment for ectosymbionts (though habitat selection by crayfish, for example choosing water depth, could indirectly influence thermal conditions). Accordingly, we found an effect of ambient temperature on prevalence of *L. discoidea*. The amount of shared effects of the abiotic environment with traits of the symbiotic host can be explained by the already documented effects of water chemistry on abundances of the ostracod *A. sinuosa* (Mestre et al., 2014). Our study system represents a clear example of indirect effects of the abiotic environment through modulation of host traits (i.e., host abundance).

4.3 | Moderate signal of regulation by the basal host

Random effects of hosts were the main sources of abundance variation identified by our models. The basal host accounted for about 15% of the variance in abundance explained by random effects of hosts. The amount of random variation explained by basal hosts suggests that crayfish bodies play a moderate role in regulating the ciliate abundances at the within-crayfish scale. Crayfish bodies may modulate ciliate abundances through their effects on microhabitat suitability or within-crayfish dispersal. At a local scale, because crayfish abundance affects the abundance of their symbiotic ostracods (Mestre et al., 2014), crayfish populations should indirectly regulate ciliate populations through modulation of ostracod abundances. At a regional scale, the significance of geospatial autocorrelation patterns could be a signal of the impact of crayfish dispersal on ciliate distribution.

Water currents from hosts are considered critical factors of microhabitat suitability and selection in epibiont ciliates of the genus *Lagenophrys* (Clamp, 1973, 1988, 1989, 1990, 1993, 2006; Felgenhauer, 1982; Roberts & Chubb, 1998). A potential advantage of using ostracods living on crayfish as substrate for attachment could be related to feeding benefits from the water current around the ciliates created by the respiratory and locomotory activities of the crayfish. Because the symbiotic ostracods are tiny and not filter feeders, they have a limited capacity to generate water currents that the ciliate could use for feeding. Moreover, *L. discoidea* selects preferentially the laterodorsal margin of the ostracod valves (Figure 1b), which is not the location where the ostracod-generated water currents should be stronger (i.e., the ventral area near the mouth). Furthermore, symbiotic ostracods often select the branchial cavity or ventral areas of the crayfish as their preferred microhabitat (Hart & Hart, 1974; Hobbs & Peters, 1977). Hence, individual variation in intensity of crayfish-created currents could generate variation in microhabitat conditions among crayfish bodies that potentially explain the significance of crayfish random effects in our abundance models. In addition, abundances of the symbiotic ostracod are dependent on crayfish body size and intermoult stage (Aguilar-Alberola et al., 2012; Mestre et al., 2014), suggesting that body size and moulting of crayfish individuals could regulate ciliate populations indirectly through their effects on the abundances of the symbiotic host.

Variation in crayfish individual traits influencing within-host dispersal could produce random variation at the level of basal hosts as well. For instance, variation among crayfish individuals in the intensity of their water currents could generate variation in the ability of ciliate larvae to find and colonize new ostracods. We found a positive relationship between occupancy and abundance of the ciliate at the scale of basal hosts, after controlling for locality-scale effects (see Appendix 1 and Figure S4). In a given locality, crayfish with higher abundances of the ciliate also have higher prevalences, thus supporting the relevance of within-crayfish dispersal dynamics for the ciliate. Otherwise, the relationship would have been diluted by dispersal at higher scales (i.e., among hosts). Previous studies proposed that active transmission by larval stages in epibiont ciliates may be favoured by host aggregation

(Sartini et al., 2018), as in other symbionts (Patterson & Ruckstuhl, 2013). The microscopic size and short life span of free-living larvae (Reynoldson, 1951) suggest that their active dispersal is limited in range. Symbiotic ostracods are among the smallest hosts used by lagenophryids (Mayén-Estrada & Clamp, 2016). The variance-to-mean abundance relationship (see Appendix 1 and Figure S5) indicates a moderate degree of aggregation in *L. discoidea* within symbiotic hosts ($\beta = 1.32$), compared to other symbionts (Matthee & Krasnov, 2009). The spatial limitation on intra-host population size imposed by the small size of the symbiotic ostracod (adult length 360–420 μm ; see Figure 1b) constrains the degree of aggregation of *L. discoidea* (max. abundance within a symbiotic host = 17). In contrast, the symbiotic ostracod has a higher degree of aggregation within crayfish ($\beta = 1.67$; Mestre et al., 2014; max. abundance within a basal host = 145). Hence, the basal host is most likely to be relevant in aggregating and favouring active dispersal of the hypersymbiotic ciliate.

Geospatial autocorrelation patterns served to test for the relevance of potential footprints of dispersal processes on occupancy and abundance patterns of the ciliates. When attached to their hosts, adult ciliates of the genus *Lagenophrys* have the ability to seal their bodies hermetically within their lorica to avoid desiccation when they leave water (Clamp, 1991). The amphibious behaviour of crayfish offers opportunities for their aquatic symbionts to disperse among isolated ponds, or different riverine systems (Hunt et al., 2018). Moreover, the introduction and spread of the crayfish *P. clarkii* in the Iberian Peninsula was driven by human-mediated translocations (Mestre et al., 2016). The full chain of symbiosis is widespread across the study region (Figure 3). Thus, crayfish dispersal (either active or passive) among nearby locations is a potential driver of the fine-scale geospatial autocorrelation patterns observed in occupancy and abundance of the Iberian populations of *Lagenophrys discoidea* (Figure S2). For example, crayfish carrying ostracods free of ciliates could colonize localities that are environmentally optimal for the ciliate. Multiple causes can explain the lack of ciliates in the crayfish colonizers (Prenter et al., 2004; Torchin et al., 2003): (a) environmental unsuitability (either abiotic or biotic) in the origin, implying that the colonizing ostracods were originally ciliate-free; (b) loss of ciliates during the overland movement, for example because they did not tolerate well the dry overland conditions; or (c) simply absence by chance (or dispersal limitation) in the subset of colonizers from the origin. Moreover, ciliates could become locally extinct after colonization due to demographic or environmental stochasticity. On the other hand, crayfish carrying large numbers of ostracods and ciliates could colonize a pond environmentally suboptimal for the ciliate, leading to prevalences or abundances of ciliates that would be unexpectedly high based on the environmental conditions (either abiotic or biotic) of the colonized locality (Kanarek et al., 2015).

4.4 | Future perspectives

A symbiotic lifestyle alters organism–environment relationships because it partitions the niche space into two main components:

host individual traits and the abiotic environment (Campbell & Crist, 2016; Krasnov et al., 2015). One potential advantage of symbiosis is the function of host bodies as 'environmental stabilizers', buffering the effects of the off-host environment on symbiont fitness (Hairston & Bohonak, 1998; Mestre et al., 2014). If so, within-host populations of the symbiont should acquire a degree of insensitivity to external environmental conditions. That is, the abiotic environment would become a less important component of the symbiont's niche, especially when combined with transmission by host-to-host contact. In contrast to our case study, we predict that populations of parasites and mutualists that obtain food resources directly from their hosts will be more independent from external food resources (Mestre et al., 2014). In addition, we expect less influence of the abiotic environment in endosymbionts, and in ectosymbionts of endothermic hosts, which a priori should be more protected from the external environment by host bodies. The use of macroecological approaches to search for patterns across symbiont-host systems (Stephens et al., 2016), including assessing the relative sensitivity of symbiont populations to variation in host traits and the abiotic environment, is a promising approach to test and refine these hypotheses. Finally, we propose that future research might focus on three potential benefits of hypersymbiosis: (a) improvement of microhabitat suitability (crayfish currents in our example); (b) facilitation of active hypersymbiont dispersal mediated by aggregation of symbiotic hosts; and (c) increased dispersal capacity by hyperphoresy.

ACKNOWLEDGEMENTS

This research was funded by the Spanish Ministry of Science and Innovation Project ECOINVADER (CGL2008-01296/BOS), the University of Valencia and the Spanish regional government of Generalitat Valenciana (UVPOST 2015 and APOSTD 2017 postdoctoral grants to A.M.). We thank the regional Spanish governments of Castilla-La Mancha, Castilla-León, Extremadura, Aragón, Andalucía, Navarra, Illes Balears, Catalunya and C. Valenciana for permission to sample in specific localities. R.D.H. and M.B. also thank the University of Florida Foundation for support. We are grateful for the constructive and insightful reviews provided by two anonymous reviewers, as well as the valuable feedback provided by the associate editor.

AUTHOR'S CONTRIBUTIONS

A.M. and F.M.-J. designed the study. A.M. collected the data, performed the analyses and wrote the MS. J.C. was involved in the initially submitted draft. The rest of the authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data used in this work are available from Dryad Digital Repository: <https://doi.org/10.5061/dryad.bs8313h> (Mestre et al., 2019).

ORCID

Alexandre Mestre  <https://orcid.org/0000-0003-1764-2248>
 Robert Poulin  <https://orcid.org/0000-0003-1390-1206>
 Robert D. Holt  <https://orcid.org/0000-0002-6685-547X>
 John C. Clamp  <https://orcid.org/0000-0003-4513-0514>
 Francesc Mesquita-Joanes  <https://orcid.org/0000-0001-7168-1980>

REFERENCES

- Aguilar-Alberola, J. A., Mesquita-Joanes, F., López, S., Mestre, A., Casanova, J. C., Rueda, J., & Ribas, A. (2012). An invaded invader: High prevalence of entocytherid ostracods on the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in the Eastern Iberian Peninsula. *Hydrobiologia*, 688, 63–73. <https://doi.org/10.1007/s10750-011-0660-1>
- APHA-AWWA-WEF. (2012). *Standard methods for the examination of water and watershed*, 22nd ed. Washington, DC: American Public Health Association.
- Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 1283–1289. <https://doi.org/10.1098/rspb.1998.0431>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bivand, R., & Piras, G. (2015). Comparing implementations of estimation methods for spatial econometrics. *Journal of Statistical Software*, 63, 1–36. <https://doi.org/10.18637/jss.v063.i18>
- Borges, R. M. (2017). Co-niche construction between hosts and symbionts: Ideas and evidence. *Journal of Genetics*, 96, 483–489. <https://doi.org/10.1007%2Fs12041-017-0792-9>
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*, 2nd ed. New York, NY: Springer-Verlag.
- Buser, C. C., Newcomb, R. D., Gaskett, A. C., & Goddard, M. R. (2014). Niche construction initiates the evolution of mutualistic interactions. *Ecology Letters*, 17, 1257–1264. <https://doi.org/10.1111/ele.12331>
- Cabral, A. F., Utz, L. R. P., & Velho, L. F. M. (2017). Structure and distribution of ciliate epibiont communities in a tropical floodplain. *Hydrobiologia*, 787, 167–180. <https://doi.org/10.1007/s10750-016-2955-8>
- Calcagno, V., & de Mazancourt, C. (2010). glmulti: An R package for easy automated model selection with (generalized) linear models. *Journal of Statistical Software*, 34, 1–29. <https://doi.org/10.18637/jss.v034.i12>
- Campbell, K. U., & Crist, T. O. (2016). Species traits and environmental characteristics together regulate ant-associated biodiversity. *Ecology and Evolution*, 6, 6397–6408. <https://doi.org/10.1002/ece3.2276>
- Castillo-Escrivà, A., Mestre, A., Monrós, J. S., & Mesquita-Joanes, F. (2013). Population dynamics of an epibiont Ostracoda on the invasive red swamp crayfish *Procambarus clarkii* in a western Mediterranean wetland. *Hydrobiologia*, 714, 217–228. <https://doi.org/10.1007/s10750-013-1542-5>
- Clamp, J. C. (1973). Observations on the host-symbiont relationships of *Lagenophrys lunatus* Imamura. *The Journal of Protozoology*, 20, 558–561. <https://doi.org/10.1111/j.1550-7408.1973.tb03572.x>
- Clamp, J. C. (1987). Five new species of *Lagenophrys* (Ciliophora, Peritricha, Lagenophryidae) from the United States with observations on their developmental stages. *The Journal of Protozoology*, 34, 382–392. <https://doi.org/10.1111/j.1550-7408.1987.tb03197.x>

- Clamp, J. C. (1988). The occurrence of *Lagenophrys aselli* (Ciliophora: Peritricha: Lagenophryidae) in North America and a description of environmentally-induced morphological variation in the species. *Transactions of the American Microscopical Society*, 107, 17–27. <https://doi.org/10.2307/3226402>
- Clamp, J. C. (1989). Redescription of *Lagenophrys eupagurus* Kellicott (Ciliophora, Peritricha, Lagenophryidae) and a comparison of it with three similar species. *The Journal of Protozoology*, 36, 596–607. <https://doi.org/10.1111/j.1550-7408.1989.tb01102.x>
- Clamp, J. C. (1990). Redescription of three species of *Lagenophrys* (Ciliophora: Peritricha: Lagenophryidae) and a new North American species of *Lagenophrys* from hypogean amphipods. *Transactions of the American Microscopical Society*, 109, 1–31. <https://doi.org/10.2307/3226589>
- Clamp, J. C. (1991). Revision of the family Lagenophryidae Bütschli, 1889 and description of the family Usconophryidae n. fam. (Ciliophora, Peritricha). *The Journal of Protozoology*, 38, 355–377. <https://doi.org/10.1111/j.1550-7408.1991.tb01373.x>
- Clamp, J. C. (1993). A new species of *Lagenophrys* (Ciliophora: Peritrichia) symbiotic on marine amphipods. *Transactions of the American Microscopical Society*, 112, 62–68. <https://doi.org/10.2307/3226782>
- Clamp, J. C. (2006). Redescription of *Lagenophrys cochinesis* Santhakumari & Gopalan, 1980 (Ciliophora, Peritrichia, Lagenophryidae), an ectosymbiont of marine isopods, including new information on morphology, geographic distribution, and intraspecific variation. *Journal of Eukaryotic Microbiology*, 53, 58–64. <https://doi.org/10.1111/j.1550-7408.2005.00074.x>
- DeWitt, P. D., Williams, B. W., Lu, Z.-Q., Fard, A. N., & Gelder, S. R. (2013). Effects of environmental and host physical characteristics on an aquatic symbiont. *Limnologia*, 43, 151–156. <https://doi.org/10.1016/j.limno.2012.07.009>
- Dray, S. (2013). spacemaker: spatial modelling. R package version 0.0-5/r113.
- Dray, S., Legendre, P., & Peres-Neto, P. R. (2006). Spatial modelling: A comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*, 196, 483–493. <https://doi.org/10.1016/j.ecolmodel.2006.02.015>
- Felgenhauer, B. E. (1982). A new species of *Lagenophrys* (Ciliophora: Peritrichida) from the fresh-water shrimp *Palaemonetes kadiakensis*. *Transactions of the American Microscopical Society*, 101, 142–150. <https://doi.org/10.2307/3225767>
- Fernandez-Leborans, G. (2010). Epibiosis in Crustacea: An overview. *Crustaceana*, 83, 549–640. <https://doi.org/10.1163/001121610X532648>
- Godsoe, W., Jankowski, J., Holt, R. D., & Gravel, D. (2017). Integrating biogeography with contemporary niche theory. *Trends in Ecology & Evolution*, 32, 488–499. <https://doi.org/10.1016/j.tree.2017.03.008>
- Hairston, N. G. J., & Bohonak, A. J. (1998). Copepod reproductive strategies: Life-history theory, phylogenetic pattern and invasion of inland waters. *Journal of Marine Systems*, 15, 23–34. [https://doi.org/10.1016/S0924-7963\(97\)00046-8](https://doi.org/10.1016/S0924-7963(97)00046-8)
- Hart, D. G., & Hart, C. W. Jr (1974). *The ostracod family Entocytheridae*, 1st ed. Lancaster, UK: Fulton Press Inc.
- Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology*, 25, 101–110. <https://doi.org/10.1023%2FA%3A1008119611481>
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978. <https://doi.org/10.1002/joc.1276>
- Hobbs, H. H. Jr, & Peters, D. J. (1977). The entocytherid ostracods of North Carolina. *Smithsonian Contributions to Zoology*, 247, 1–73. <https://doi.org/10.5479/si.00810282.247>
- Hunt, R., Thomas, J. R., James, J., & Cable, J. (2018). Transmission and terrestrial dispersal of non-native ectosymbionts on invasive crayfish. *Hydrobiologia*, 820, 135–144. <https://doi.org/10.1007/s10750-018-3647-3>
- Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. *Biochemie Und Physiologie Der Pflanzen*, 167, 191–194. [https://doi.org/10.1016/S0015-3796\(17\)30778-3](https://doi.org/10.1016/S0015-3796(17)30778-3)
- Jones, S., Carrasco, N. K., Perissinotto, R., & Vosloo, A. (2016). Association of the epibiont *Epistylis* sp. with a calanoid copepod in the St Lucia Estuary, South Africa. *Journal of Plankton Research*, 38, 1404–1411. <https://doi.org/10.1093/plankt/fbw069>
- Kanarek, A. R., Webb, C. T., Barfield, M., & Holt, R. D. (2015). Overcoming Allee effects through evolutionary, genetic, and demographic rescue. *Journal of Biological Dynamics*, 9, 15–33. <https://doi.org/10.1080/17513758.2014.978399>
- Krasnov, B. R., Shenbrot, G. I., Khokhlova, I. S., Stanko, M., Morand, S., & Mouillot, D. (2015). Assembly rules of ectoparasite communities across scales: Combining patterns of abiotic factors, host composition, geographic space, phylogeny and traits. *Ecography*, 38, 184–197. <https://doi.org/10.1111/ecog.00915>
- Louthan, A. M., Doak, D. F., & Angert, A. L. (2015). Where and when do species interactions set range limits? *Trends in Ecology and Evolution*, 30, 780–792. <https://doi.org/10.1016/j.tree.2015.09.011>
- Lukacs, P., Burnham, K., & Anderson, D. (2010). Model selection bias and Freedman's paradox. *Annals of the Institute of Statistical Mathematics*, 62, 117–125. <https://doi.org/10.1007/s10463-009-0234-4>
- Lymbery, A. J. (2015). Niche construction: Evolutionary implications for parasites and hosts. *Trends in Parasitology*, 31, 134–141. <https://doi.org/10.1016/j.pt.2015.01.003>
- Matthee, S., & Krasnov, B. R. (2009). Searching for generality in the patterns of parasite abundance and distribution: Ectoparasites of a South African rodent, *Rhabdomys pumilio*. *International Journal for Parasitology*, 39, 781–788. <https://doi.org/10.1016/j.ijpara.2008.12.003>
- Mayén-Estrada, R., & Clamp, J. C. (2016). An annotated checklist of species in the family Lagenophryidae (Ciliophora, Oligohymenophorea, Peritrichia), with a brief review of their taxonomy, morphology, and biogeography. *Zootaxa*, 4132, 451–492. <https://doi.org/10.11646/zootaxa.4132.4.1>
- Mestre, A., Aguilar-Alberola, J. A., Baldry, D., Balkis, H., Ellis, A., Gil-Delgado, J. A., ... Mesquita-Joanes, F. (2013). Invasion biology in non-free-living species: Interactions between abiotic (climatic) and biotic (host availability) factors in geographical space in crayfish commensals (Ostracoda, Entocytheridae). *Ecology and Evolution*, 3, 5237–5253. <https://doi.org/10.1002/ece3.897>
- Mestre, A., Butlin, R. K., Kelso, W. E., Romaine, R., Bonvillain, C. P., Monrós, J. S., & Mesquita-Joanes, F. (2016). Contrasting patterns of genetic diversity and spatial structure in an invasive symbiont-host association. *Biological Invasions*, 18, 3175–3191. <https://doi.org/10.1007/s10530-016-1207-1>
- Mestre, A., Castillo-Escrivà, A., Rueda, J., Monrós, J. S., & Mesquita-Joanes, F. (2015). Experimental spillover of an exotic ectosymbiont on an European native crayfish: The importance of having a chance. *Hydrobiologia*, 755, 225–237. <https://doi.org/10.1007/s10750-015-2236-y>
- Mestre, A., Monrós, J. S., & Mesquita-Joanes, F. (2011). Comparison of two chemicals for removing an entocytherid (Ostracoda: Crustacea) species from its host crayfish (Cambaridae: Crustacea). *International Review of Hydrobiology*, 96, 347–355. <https://doi.org/10.1002/iroh.201111343>
- Mestre, A., Monrós, J. S., & Mesquita-Joanes, F. (2014). The influence of environmental factors on abundance and prevalence of a commensal ostracod hosted by an invasive crayfish: Are 'parasite rules' relevant to non-parasitic symbionts? *Freshwater Biology*, 59, 2107–2121. <https://doi.org/10.1111/fwb.12412>

- Mestre, A., Poulin, R., Holt, R. D., Barfield, M., Clamp, J. C., Fernandez-Leborans, G., & Mesquita-Joanes, F. (2019). Data from: The interplay of nested biotic interactions and the abiotic environment regulates populations of a hypersymbiont. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.bs8313h>
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133–142. <https://doi.org/10.1111/j.2041-210x.2012.00261.x>
- Ozgul, A., Oli, M. K., Bolker, B. M., & Perez-Heydrich, C. (2009). Upper respiratory tract disease, force of infection, and effects on survival of gopher tortoises. *Ecological Applications*, 19, 786–798. <https://doi.org/10.1890/08-0219.1>
- Patterson, J. E. H., & Ruckstuhl, K. E. (2013). Parasite infection and host group size: A meta-analytical review. *Parasitology*, 140, 803–813. <https://doi.org/10.1017/S0031182012002259>
- Poulin, R. (2007). *Evolutionary ecology of parasites*, 2nd ed. Princeton, NJ: Princeton University Press.
- Prenter, J., MacNeil, C., Dick, J. T., & Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in Ecology & Evolution*, 19, 385–390. <https://doi.org/10.1016/j.tree.2004.05.002>
- Reynoldson, T. B. (1951). The dispersal of *Urceolaria mitra* (Peritricha) epizoic on flatworms. *Journal of Animal Ecology*, 20, 123–131. <https://doi.org/10.2307/1650>
- Roberts, G. N., & Chubb, J. C. (1998). The distribution and location of the symbiont *Lagenophrys aselli* on the freshwater isopod *Asellus aquaticus*. *Freshwater Biology*, 40, 671–677. <https://doi.org/10.1046/j.1365-2427.1998.00365.x>
- Sabagh, L. T., Dias, R. J. P., Branco, C. W. C., & Rocha, C. F. D. (2011). News records of phoresy and hyperphoresy among treefrogs, ostracods, and ciliates in bromeliad of Atlantic forest. *Biodiversity and Conservation*, 20, 1837–1841. <https://doi.org/10.1007/s10531-011-0050-z>
- Sartini, B., Marchesini, R., D'ávila, S., D'Agosto, M., & ... Dias, R. J. P. (2018). Diversity and distribution of peritrich ciliates on the snail *Physa acuta* Draparnaud, 1805 (Gastropoda: Physidae) in a eutrophic lotic system. *Zoological Studies*, 57, 42. <https://doi.org/10.6620/ZS.2018.57-42>
- Sheehan, K. L., Lafferty, K. D., O'Brien, J., & Cebrian, J. (2011). Parasite distribution, prevalence, and assemblages of the grass shrimp, *Palaemonetes pugio*, in Southwestern Alabama, U.S.A. *Comparative Parasitology*, 78, 245–256. <https://doi.org/10.1654/4427.1>
- Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, 10, 1115–1123. <https://doi.org/10.1111/j.1461-0248.2007.01107.x>
- Stanko, M., Krasnov, B. R., & Morand, S. (2006). Relationship between host abundance and parasite distribution: Inferring regulating mechanisms from census data. *Journal of Animal Ecology*, 75, 575–583. <https://doi.org/10.1111/j.1365-2656.2006.01080.x>
- Stephens, P. R., Altizer, S., Smith, K. F., Alonso Aguirre, A., Brown, J. H., Budischak, S. A., ... Poulin, R. (2016). The macroecology of infectious diseases: A new perspective on global-scale drivers of pathogen distributions and impacts. *Ecology Letters*, 19, 1159–1171. <https://doi.org/10.1111/ele.12644>
- Stewart, A. J. A., Bantock, T. M., Beckmann, B. C., Botham, M. S., Hubble, D., & Roy, D. B. (2015). The role of ecological interactions in determining species ranges and range changes. *Biological Journal of the Linnean Society of London*, 115, 647–663. <https://doi.org/10.1111/bij.12543>
- Studer, A., & Poulin, R. (2013). Cercarial survival in an intertidal trematode: A multifactorial experiment with temperature, salinity and ultraviolet radiation. *Parasitology Research*, 112, 243–249. <https://doi.org/10.1007/s00436-012-3131-3>
- Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J., & Kuris, A. M. (2003). Introduced species and their missing parasites. *Nature*, 421, 628–630. <https://doi.org/10.1038/nature01346>
- Utz, L. R. P., & Coats, D. W. (2005). Spatial and temporal patterns in the occurrence of peritrich ciliates as epibionts on calanoid copepods in the Chesapeake Bay, USA. *Journal of Eukaryotic Microbiology*, 52, 236–244. <https://doi.org/10.1111/j.1550-7408.2005.00025.x>
- Wahl, M., & Mark, O. (1999). The predominantly facultative nature of epibiosis: Experimental and observational evidence. *Marine Ecology Progress Series*, 187, 59–66. <https://doi.org/10.3354/meps187059>
- Wiens, J. J. (2011). The niche, biogeography and species interactions. *Philosophical Transactions of the Royal Society of London B*, 366, 2336–2350. <https://doi.org/10.1098/rstb.2011.0059>
- Young, W. (1971). Ecological studies of Entocytheridae (Ostracoda). *American Midland Naturalist*, 85, 399–409. <https://doi.org/10.2307/2423764>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Mestre A, Poulin R, Holt RD, et al. The interplay of nested biotic interactions and the abiotic environment regulates populations of a hypersymbiont. *J Anim Ecol*. 2019;88:1998–2010. <https://doi.org/10.1111/1365-2656.13091>