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Assembly rules of helminth parasite communities in grey mullets: combining components of diversity

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Note: Supplementary data associated with this article.

Abstract

Organisms associate themselves in ecological communities. It has been widely debated whether these associations are explained by deterministic or, in contrast, random processes. The answer may vary depending on the level of an organisational scale (α , β and γ) and the facet of diversity considered: Taxonomic (TD), Functional (FD) and Phylogenetic (PD). Diversity at the sampling unit (i.e. host individual) is the α diversity; β diversity represents the extent of dissimilarity in diversity among sampling units (within a level of an organisational scale, β_1 ; between levels of an organisational scale, β_2); and the total diversity of a system is γ diversity. Thus, the combination of facets and levels of scale may be useful to disentangle the mechanisms driving the composition of a parasite community. Using helminth parasite TD, FD, and a Proxy of PD (PPD) of three species of grey mullets (Teleostei: Mugilidae) from the Mediterranean Sea, we show that random and deterministic processes of different nature explain the assemblage of parasite communities. The parasite community at a host individual (α) was invariably a random subset of the total diversity in the community for the three facets of diversity. At β_1 level, TD was lower than expected by chance, whereas FD and PPD were random. At β_2 , diversity patterns suggested environmental filtering of the parasite assemblage: species, trait, and phylogenetic compositions of parasite communities seem to depend primarily on the species of host, but also on the locality and season. Our study shows that parasite communities are not totally understood if any of the components (i.e. facets and levels) of diversity is neglected.

Keywords

diversity; ecological communities; environmental filtering; helminths; limiting similarity; random processes

1. Introduction

Understanding the processes governing the distribution and assembly rules of biological diversity is one of the major challenges in biogeography and macroecology, and a prerequisite for successfully predicting global change impacts on biodiversity (Guisan and Rahbek, 2011). However, due to the extreme complexity of communities and ecosystems, we are still far from achieving this goal. The current state of affairs can be described as a schism between ecological and historical biogeography, which stems from the unresolved debate about the nature of the mechanisms governing community assembly. This divide, known as the Clements-Gleason controversy, has pervaded ecological thought for nearly a century. Whereas the Clementsian ecologists view ecological communities as tightly integrated entities consisting of interdependent species (the community-unit concept), the Gleasonians posit that species co-occur largely according to the individualistic response of each species to variable environmental conditions (the individualistic concept) (Liautaud et al., 2019). Accordingly, species would simply coexist because they tolerate the same habitat (Götzenberger et al., 2012; Liautaud et al., 2019). To a large degree, the latter view has prevailed in the last decades, based largely on empirical evidence of community composition along environmental gradients, and has eventually led to postulate the “disintegration of ecological community” (Ricklefs, 2008). Under this paradigm, species distributions would be mostly, if not exclusively, constrained by local environmental conditions, historical large-scale events, and dispersal capacity of the species. However, recent evidence suggests that community structure cannot be fully understood without taking into consideration deterministic processes, such as environmental filtering or limiting similarity (Pavoine and Bonsall, 2011).

Stochastic processes can affect diversity, influencing speciation, extinction, colonisation, or dispersal to a new community, and these can finally cause a random assemblage of diversity. On the other hand, deterministic processes can lead to either clustering (i.e. convergence) or overdispersion (i.e. divergence) of diversity (not to be confused with spatial clustering and overdispersion, Götzenberger et al., 2012). A clustering pattern of diversity assembly is interpreted as evidence of environmental filtering structuring the diversity of the

community, because abiotic factors select individuals with particular environmental tolerances, and it results in similar life strategies converging in a community (Pavoine and Bonsall, 2011). In contrast, the opposite pattern, i.e. overdispersion, would reflect a limiting similarity process, in which biotic forces tend to limit the coexistence of very similar life strategies (Pavoine and Bonsall, 2011; Götzenberger et al., 2012). Nonetheless, environmental filtering and limiting similarity are not the only potential deterministic processes acting on communities and alternative processes can lead to similar patterns of diversity dispersal. For instance, competition due to biotic interactions excluding the less competitive strategies can also result in diversity overdispersion or clustering depending on the species affected in each situation (Mayfield and Levine, 2010).

The understanding of the processes that drive biological communities can be examined for the multiple components of diversity to get a complete resolution of communities. Diversity encompasses multiple levels (e.g. α , β , γ) nested in organisational scales (e.g. spatial scales) and it has been argued that the importance of ecological processes is probably scale dependent. From the lowest to the highest of level of an organisational scale, three diversity levels are classically defined: α diversity or diversity at the sampling unit; β diversity or extent of dissimilarity in diversity among sampling units (within a level of an organisational scale, β_1 ; between levels of an organisational scale, β_2) and γ diversity or total diversity (Pavoine et al., 2016). Moreover, it includes multiple facets, such as the variety in species, traits, or evolutionary units (Pavoine and Bonsall, 2011). Regardless of the level considered, diversity has been studied under three facets: Taxonomic Diversity (TD, richness and abundance of taxonomic entities in a community), Functional Diversity (FD, richness and abundance of functional traits in a community) and Phylogenetic Diversity (PD, richness and abundance of genetically different entities in a community) (Pavoine and Bonsall, 2011). Traditionally, ecologists have considered TD as the single measure of diversity. This fact caused a continuous loss of ecological (FD) and evolutionary (PD) information, since TD considers that all the species in a community are equally similar and does not take into account the uniqueness of the functions or the phylogenetic distinctness of each species (Pavoine and Bonsall, 2011).

Parasite communities offer excellent models to study the assembly rules of the facets of diversity at different levels of organisation. The sampling unit (i.e. host individual) can be precisely defined (as required by Götzenberger et al., 2012) and it is often relatively easy to sample a large number of communities to eventually get strong statistical conclusions (Poulin and Valtonen, 2001). Although pioneering studies exist (e.g. Mouillot et al., 2005; Krasnov et al., 2014, 2015, 2016), up to now little is known about the mechanisms driving the different facets of diversity at different organisational scales of an entire parasite community. In studies of free-living organisms, assembly mechanisms of diversity can hierarchically be assessed at different spatial levels (e.g. local communities, regions, continents) or even temporal levels (e.g. years, decades, centuries) (Pavoine et al., 2009a). The study of parasite communities provides an additional organisational scale, the host, which also shapes parasite diversity. For example, parasite diversity can be examined at host taxonomic levels (i.e. species, genus, family, and so on).

Here, we will investigate the assembly mechanisms (deterministic vs stochastic processes) of the helminth adult parasite communities of grey mullets (Teleostei: Mugilidae) from the Western Mediterranean Sea under the influence of host and environmental factors. This model is appropriate because, first, these parasite communities include parasite species from distant phylogenetic origins and are functionally disparate. Second, it comes from three out of the six sympatric grey mullet species that coexist in this area of the Mediterranean (Blasco-Costa, 2009. Taxonomy of the Haploporinae Nicoll, 1914 and Bunocotylinea Dollfus, 1950 (Digenea) from Mediterranean mullets (Teleostei): morphological and molecular approaches. Doctoral dissertation, University of Valencia, Valencia, Spain) and from localities that vary in their environmental parameters. This allows us to test whether the different facets of helminth diversity provide congruent results and whether host factors (phylogenetic proximity and similarity in life strategies) and/or environmental factors (geographical location and habitat conditions) select for different parasitic life strategies. Particularly, two of the host species, *Chelon auratus* and *Chelon ramada*, are phylogenetically closer to each other than to *Mugil cephalus* (Durand et al., 2012), whereas *M. cephalus* and *C. ramada* show greater similarities in

their life strategies between them than with *C. auratus* (Cardona, 2001; Cardona, 2006). Additionally, our samples are from three coastal localities that differ in their habitat conditions (two marine: Ebro Delta, Sea - EDS and Santa Pola, Sea - SPS; and one brackish lagoon: Santa Pola, Lagoon - SPL) and in their geographical proximity (SPS and SPL are very close, ~10 km apart; whereas EDS is more distant from the other two, ~290 km). Finally, our nested sampling design allows us to measure and compare diversity at the sampling unit (α diversity or parasite diversity at the host individual) and among sampling units within and between levels of a factor (i.e. parasite diversity within (β_1) or between (β_2) host individuals of a host species or locality).

We asked whether diversity patterns differ between the facets of parasite diversity and across two hierarchical scales (i.e. locality and host), and which are the factors (i.e. host phylogeny vs life strategies; habitat conditions vs geographic proximity) related to such variation. Based on evidence from free-living organisms (Cavender-Bares et al., 2006; Kraft and Ackerly, 2010), and previous studies of our host-parasite system (Blasco-Costa, 2009. Taxonomy of the Haploporinae Nicoll, 1914 and Bunocotylineae Dollfus, 1950 (Digenea) from Mediterranean mullets (Teleostei): morphological and molecular approaches. Doctoral dissertation, University of Valencia, Valencia, Spain; Blasco-Costa et al., 2012; Míguez-Lozano et al., 2012; Sarabeev et al., 2013), we hypothesise that both host phylogeny and host life strategy will influence the parasite community, whereas habitat conditions will be a stronger determinant of the parasite communities than the geographic distance. Parasite communities will be overdispersed at low organisational levels (within a locality or a host species) since similar life strategies will be limited (i.e. not able) to coexist. In contrast, at higher levels, we expect that clustering will be the driver of the parasite community, because the environment filters (i.e. selects for) certain life strategies.

2. Material and methods

2.1. Data

Fish were obtained from local harbour markets and surveyed for parasites as described in Blasco-Costa (2009. Taxonomy of the Haploporinae Nicoll, 1914 and Bunocotylineae Dollfus,

1950 (Digenea) from Mediterranean mullets (Teleostei): morphological and molecular approaches. Doctoral dissertation, University of Valencia, Valencia, Spain). Adults of helminth parasites were identified following Yamaguti (1958), Gaevskaya and Dmitrieva (1992) and Blasco-Costa (2009. Taxonomy of the Haploporinae Nicoll, 1914 and Bunocotylinea Dollfus, 1950 (Digenea) from Mediterranean mullets (Teleostei): morphological and molecular approaches. Doctoral dissertation, University of Valencia, Valencia, Spain) for 18 trematode species; Paperna (1964), Euzet and Combes (1969) and Sarabeev et al. (2013) for 9 monogenean species; Orecchia and Paggi (1987) for one nematode species; and Orecchia et al. (1988) and Tkach et al. (2014) for two acanthocephalan species. The dataset includes 272 host individuals and 30 parasite species from three seasons (two autumns and one spring) of two years (2004 and 2005) (Table 1). The Supplementary Material Table S1 contains the prevalence of the parasite species in each host species, locality, and season. Data analysis was entirely performed in R (R Core Team, 2019. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria).

2.1.1. Data accessibility

Parasite abundance, functional traits and phylogenetic-like data analysed in this study and a step-by-step manual of the code, functions and packages used to analyse data and display figures are stored in DOI ##### (<https://github.com/crisLB/diversity>). Thus, we expect to favour the reproducibility of our analyses and the expansion studies of parasite communities and to make future studies comparable under this framework.

2.2. The multiple facets of diversity

2.2.1. Functional trait information

The number of functional traits (i.e. features measurable at individual level which impacts the fitness of individuals and reflects their performance in ecosystems) that can potentially be assessed in any organism is large, but our ability to measure functional traits of parasites is often limited. This is mainly due to technical impediments or lack of knowledge about the

relationship between life-history information of the parasites and the components of fitness. Functional traits considered in this study conform to the definition of functional trait currently accepted in ecology (see above) and are susceptible to affect parasite fitness depending on environmental factors (see Table 2 in Llopis-Belenguer et al., 2019).

Here, we used five functional traits based on the framework and core list developed by Llopis-Belenguer et al. (2019): attachment organ, type of life cycle, body mass, egg size and number of eggs. We extracted information of categorial functional traits (i.e. attachment organ and type of life cycle) from direct observations, whereas, we obtained information of continuous functional traits (i.e. body mass, egg size and number of eggs) from species descriptions or as the mean value measured from a varying number of individuals of each species. To estimate individual body mass, we resorted to indirect methods “Area by Depth by Density” (for flatworms: trematodes and monogeneans) and “Volume of Revolution by Density” (for organisms with subcircular transversal section along their bodies: acanthocephalans and nematodes) (Llopis-Belenguer et al., 2018). Mass of a parasite species was computed as the mean mass of a range of individuals (mean number of individuals per species 9; range 2-12). Egg size was estimated as the mean maximum egg length and mean width from species descriptions when these measures were available. Otherwise, we measured these features from 3 to 20 eggs (mean 10) from a varying number of individuals from each species. Then egg volume (μm^3) was estimated assuming an ellipsoid shape (i.e. depth equal to width). For the number of eggs of monogeneans and trematodes, we counted eggs from 1 to 20 (mean 9) individuals per species mounted on slides. Since acanthocephalans and nematodes possessed too many eggs to be counted directly, the number of eggs was estimated as follows: 10 females of each species were dissected individually, and their eggs diluted in 1 ml of saline solution each. Then the total number of eggs was estimated from aliquots of 0.1 ml from each specimen. Regardless of the method used, we performed the procedure twice to obtain the mean number of eggs for each individual. Finally, we log-transformed data of continuous traits.

We built a functional trait (columns) by parasite species (rows) matrix with the `dist.ktab` function from package `ade4` (Thioulouse et al., 2018) and calculated the Gower’s distance

(Gower, 1971) between species. This distance allows combining several types of traits (continuous and categorical as described above) and incorporating observations with missing data (4% in our dataset). Then, we transformed the Gower matrix of pairwise distances into Euclidean pairwise distances (function `lingoes` in `ade4`) and divided the resulting matrix by its maximum to bound values between 0 and 1 (Pavoine et al., 2009b).

2.2.2. *Proxy of phylogenetic diversity*

Since we did not have a complete phylogeny of the parasite species in the community, we used a Proxy of Phylogenetic Diversity (PPD) to estimate the phylogenetic pairwise distance between parasite species. The PPD can be seen as a measure of the length of the path connecting two species traced through a Linnaean classification of the full set of species in the community (Clarke and Warwick, 1998). We created a Euclidean pairwise distance matrix between parasite species by means of the `taxa2dist` function from `vegan` (Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.S., Szoecs, E., Wagner, H., 2019. `vegan`: Community Ecology Package. R package version 2.5-5) applied to a classification table with parasite species at rows and taxa of such species at higher levels (genus, subfamily, family, suborder, order, class, phylum, in our case) at columns. We divided the resulting distance matrix by its maximum to bound the cell values between 0 and 1.

2.2.3. *Correlation between functional trait and taxon-level distances*

We assessed the correlation between the matrix of pairwise functional distances and the matrix of pairwise phylogenetic distances to study the relationship between the evolutionary history and ecological processes behind community assembly (Pavoine and Bonsall, 2011). We tested such correlation by means of the Mantel test (`mantel` function in `vegan`).

2.3. *Diversity analyses*

Our samples were organised by three key factors: host species, locality, and season (Table 1). Since *C. auratus* does not naturally occur in SPL, we evaluated the effect of the host species and the locality by splitting the diversity analyses into two case studies. In Case 1, we assessed the influence of host species on the three facets of diversity. To that end, we analysed and compared TD, FD and PPD of the helminth parasite communities from the three host species, at SPS in two autumn surveys (2004 and 2005). In Case 2, we evaluated the effect of locality and season survey on the parasite communities. To that end, we analysed and compared TD, FD and PPD of the helminth parasite communities from *M. cephalus* at the three localities surveyed in spring and autumn 2005.

2.3.1. *Influence of one factor on diversity*

In both case studies, we measured diversity in two different ways. First, we used the Double Principal Coordinate Analysis (DPCoA) (Pavoine et al., 2004), that is a combined version of the Rao index of diversity (Rao, 1982) and the Weighted Principal Coordinate Analysis (Gower and Legendre, 1986). DPCoA allows comparing the partitioning of diversity at different levels of an organisational scale and the different facets of diversity. It is based on the matrix of pairwise distances (functional or phylogenetic) between species in a sample and an abundance matrix of such species. When the scope of the analysis is TD, all cells of the distance matrix are defined as 1. Hence, all species are considered equally and maximally distant, and Rao's index becomes equal to the Simpson's index of diversity, i.e. equals to the probability that any two individuals randomly drawn from a community are of different species (Pavoine et al., 2004). Furthermore, the ordination of species according to the previous two matrices (i.e. pairwise distances and abundance) assembles species in a multivariate space related with the decomposition of diversity in organisational levels (Pavoine et al., 2004). Since the DPCoA only allows studying one factor at a time, we analysed the databases by season survey to avoid crossed factors.

Under the DPCoA framework we measured α diversity with function `dpcoa` in `ade4`. In order to examine the relationship between α diversity (i.e. parasite community in a host individual) and the factors host species (Case 1) or locality (Case 2), we used function `lm.rpp`

of package RRPP (Collyer and Adams, 2018) that performs a linear model by residual randomisation and provides empirical sampling distributions for further ANOVAs. Following Collyer and Adams (2018), univariate α values were log-transformed. Then, we performed ANOVAs (type I of sums of squares) using random distributions of the F-statistics (Collyer and Adams, 2018) for TD, FD and PPD, independently. When differences between samples from different host species or localities were significant, we ran posteriori pairwise comparisons of α TD, FD and PPD between host species or localities using function pairwise in RRPP.

We calculated β diversity at two different organisational levels under the context of the Rao index of diversity. β_1 represents dissimilarity in parasite diversity among sampling units (hosts) within the same host species or locality. To calculate β_2 , one must group together parasite communities of hosts of the same species or from the same locality and then, calculate dissimilarities in the parasite communities between different host species or localities. In both cases, we calculated β diversities under the equivalent number approach (Ricotta and Szeidl, 2009) using the third proposition of the Rao index of diversity in Pavoine et al. (2016) (EqRao function in adiv (Pavoine, S., 2018. adiv: Analysis of diversity. R package version 1.2)). Indeed, this proposition is specifically developed for unbalanced samplings. Furthermore, we compared each of the TD, FD and PPD β_1 and β_2 diversities with 999 randomly simulated β_1 and β_2 values (rtestEqRao function in adiv) in order to establish whether the observed values significantly differ from those randomly simulated ($p < 0.05$). When significant, we compared observed and simulated results to determine whether the observed β_1 or β_2 were greater or lower than expected at random. This allows determining whether parasite communities from fish of the same species or locality (β_1) or of different fish species or locality (β_2) are more similar (the observed value is lower than simulated values) or more dissimilar (the observed value is greater than simulated values) to each other than expected by chance. Finally, we used the standardised β_1 and β_2 given by EqRao function and defined as follows: (observed β – mean of randomly simulated β s)/standard deviation of randomly simulated β s. This standardisation enables us to infer if the parasite species, traits or the phylogenetic proxy are overdispersed

(negative standardised β) or clustered (positive standardised β) (Head et al., 2018) within a level of a factor (β_1) or between levels of a factor (β_2).

2.3.2. *Influence of two crossed factors on diversity*

To evaluate and disentangle the effect of crossed factors on diversity, we used crossed-DPCoA (Pavoine et al., 2013a). In both case studies, we analysed the effect of two crossed factors simultaneously: host species (Case 1) or locality (Case 2), and season. The crossed-DPCoA is grounded on the DPCoA. It analyses the effect of two crossed factors at the same time. Thus, it distinguishes the proportional contribution of the sampling unit, each factor individually and the effect of the interaction of both factors on the diversity of the community.

The crossed-DPCoA consists of three consecutive analyses. Following Pavoine et al.'s (2013a) terminology, each parasite community is associated with a component of the factor A (hosts species or locality) and a component of a factor B (season). The main version of the crossed-DPCoA plots the parasite species, the sampling units, and the variables of the main factor A, without taking into account seasonal differences (factor B), in a DPCoA space. Then, the first version of the crossed-DPCoA removes the amount of diversity among sampling units due to the sole effect of factor B, but retains combined effects of factors A and B (i.e. the interaction between factors A and B). Finally, the second version of the crossed-DPCoA eliminates any influence of the factor B on the factor A (including the interaction term). Thus, it provides diversity exclusively under the light of the main crossed-factor, factor A (host species or locality). We carried out the main, the first and the second versions of the crossed-DPCoA with functions `crossdpcoa_maineffect`, `crossdpcoa_version1` and `crossdpcoa_version2` in `adiv`, respectively.

3. Results

Parasite functional and phylogenetic-like pairwise distance matrices were highly correlated (Mantel test, $F = 0.79$, $p < 0.001$).

3.1. Case 1: host species is a significant predictor for TD, FD and PPD of parasite communities of *Chelon auratus*, *C. ramada* and *Mugil cephalus* from Santa Pola Sea (SPS) (autumn 2004 and 2005)

At α level and in the first survey (autumn 2004), we found non-significant differences in terms of TD and FD of parasites hosted by the different fish species (Table 2). However, the analysis of the PPD showed significant differences in α diversity among host species because the parasite community of *C. ramada* was less diverse than that of *M. cephalus* (Fig. 1: autumn 2004; Table 2; Supplementary Table S2 A). In the second survey (autumn 2005), α diversity of the parasite community of *C. ramada* was significantly lower in terms of parasite species (lowest TD) and functional traits (lowest FD) than the parasite communities hosted by other two fish species (Fig. 1: autumn 2005, Supplementary Table S2 B and C). As for PPD, the three host species displayed significantly different α diversity values with *C. ramada* having the lowest diversity and *M. cephalus* the highest (Fig. 1: autumn 2005; Table 2; Supplementary Table S2 D).

At β_1 level, we found that the parasite communities of fish from the same sample (within host species) tended to be more similar than expected by chance only in “TD – autumn 2004” (Table 3, Supplementary Fig. S1 A-C and Fig. S2 A-C). The negative values of the standardised β_1 in both autumn surveys indicated that parasite species, traits and phylogeny tended to be overdispersed within host species (Table 3). At β_2 level, the parasite communities from fish from different host species always differed significantly from randomness (Table 3, Supplementary Fig. S1 D-F and Fig. S2 D-F). The positive values of the standardised β_2 s indicated that the composition in parasite species, traits and phylogenies differed between host species in both autumn surveys (Table 3).

When taking crossed-effects into account, diversity within each host individual constituted the highest proportion of parasite diversity in the whole community (50%, 50% and 56% for TD, FD and PPD respectively), followed by host species, the crossed effect of host species and seasons, and season (Table 4). The graphical representations of the second version of the crossed-DPCoA considering TD, FD and PPD (Fig. 2) were congruent. In all three cases,

the first axis separated the parasite communities of the two coastal-related grey mullets (*C. ramada* and *M. cephalus*) from the marine-related *C. auratus*, although those of *C. ramada* were always in an intermediate position. The parasite communities of the three host species showed an overlap in the parasite functional trait and phylogenetic-like spaces. However, host species was still a significant predictor for trait and phylogenetic diversity of the parasite communities (Fig. 2).

3.2. *Case 2: parasite communities of Mugil cephalus from spring and autumn (2005) overlapped in TD, FD and PPD of marine localities (Ebro Delta Sea, Santa Pola Sea) but were separated from those of the lagoon (Santa Pola Lagoon)*

The statistical analyses of α TD, FD and PPD did not show congruent results. In spring, localities did not differ in the composition of the parasite species (TD in Table 2) but did in FD and PPD. This was due to SPL being significantly less diverse than the other two localities in terms of FD and than EDS in terms of PPD (Table 2; Supplementary Table S3 A and B; Fig. 3: spring 2005). In the autumn survey (Fig. 3: autumn 2005), SPL had significantly lower TD than the other two localities (Supplementary Table S3 C). As for FD, the three localities differed significantly (Supplementary Table S3 D). For PPD, SPS had significantly higher diversity than the other two localities (Supplementary Table S3 E).

At β_1 level, the dissimilarity in diversity of the parasite communities from hosts within the same locality tended to be lower than expected by chance only for TD in both season surveys (Table 5, Supplementary Fig. S3 A-C and Fig. S4 A-C). The standardised observations with negative values indicated that parasite species, traits and the phylogenetic proxy were overdispersed within hosts from the same locality, except for “PPD - autumn” (Table 5). At β_2 level, differences in parasite diversity between localities always differed significantly from randomness (Table 5) and were always greater than expected (Supplementary Fig. S3 D-F and Fig. S4 D-F). The positive values of the standardised β_2 reflected that parasite species, traits and phylogeny were clustered at this level of the organisational scale.

When we considered the crossed factors simultaneously, diversity within each host individual constituted the highest proportion of the parasite diversity in the whole community (58% for TD, 54% for FD and 52% for PPD), followed by the crossed effect of locality and season, the locality and the season, for the three facets of diversity (Table 4). The graphical representation of the second version of the crossed-DPCoA displayed similar patterns for TD, FD and PPD (Fig. 4). The first axis slightly separated the diversity of parasite communities of the two marine localities (SPS and EDS) from the lagoon locality (SPL). Furthermore, both marine localities (EDS and SPS) overlapped substantially in the species (TD), trait (FD) and phylogenetic-like (PPD) spaces (Fig. 4).

4. Discussion

We examined the mechanisms driving the helminth parasite assemblages of grey mullets. Four main findings can be extracted from our results. First, the three facets of diversity (i.e. TD, FD and PPD) did not always show congruent results among them, which is unsurprising and follows the general trend reported for diverse groups of free-living organisms (e.g. Devictor et al., 2010; Hevia et al., 2016). However, these results have been hardly reported for parasite communities. Thus, conclusions of a parasite community study might be biased if any of the facets of diversity is overlooked (Jarzyna and Jetz, 2016). Second, the diversity of the parasite communities shows at least two opposed patterns (i.e. overdispersion and clustering). These patterns are found at different levels (β_1 and β_2 , respectively) of the two organisational scales (i.e. host species or locality). Third, the diversity of the two organisational scales is influenced by several variables. Fourth, the phylogenetic-like signal on functional traits suggested that functional traits are, at least, constrained by phylogeny. However, these two facets of diversity did not always display the same results, which suggests that the FD of this parasite community is not a surrogate of PPD (see Pavoine et al., 2013b).

At the lowest organisational level (α diversity), the most notable case of incongruences among the three facets of diversity was the spring survey at SPL (Fig. 3), which was the richest in terms of number of species (highest TD) mainly because of trematodes. However, SPL

displayed the lowest mean for α FD and α PPD probably because trematode species are redundant (sensu Carmona et al., 2016) in terms of FD and PPD. At the α level as well, we found that host individual explains for most of its the three facets (~50% of the total diversity, crossed-DPCoA results), regardless of the case study considered. This strongly suggests that the parasite community found at each host individual (α TD) is a random subset of a larger pool of parasite species within a host species or locality (Poulin, 1996). However, it also highlights that the parasite community found at this level is to a large extent a random subset of a larger pool of parasite ecological (FD) and evolutionary (PPD) strategies. This can be explained as α being product of random events (Poulin, 1996) or as result of several forces acting simultaneously in different directions in the development of parasite communities at α level (Poulin, 2005). However, it has been recently shown that α TD of parasite communities is determined by host age-class like an ecological succession of communities of free-living organisms. Thus, if hosts of different age-classes were analysed together it could mask an actual deterministic pattern of parasite community assemblage (Espínola-Novelo et al., 2020). Nonetheless, we consider that this is not a likely scenario in this study since parasite communities were mainly sampled from fish of two consecutive age-classes out of nine (classes 3 and 4 according to those described for *M. cephalus* in Espínola-Novelo et al., 2020).

In the case of β 1 diversity (within a host species or locality), differences among host individuals of the same species were less important than differences between host individuals of the same locality to determine the variation of the parasite communities (Table 4). In other words, host species is more determinant of the parasite community than locality, at least for TD since the parasite communities from different host species (Fig. 2) and localities (Fig. 3) are overlapped in FD and PPD spaces. In fact, the parasite community of grey mullets, especially monogeneans of genus *Ligophorus* (Dactylogyridae), tend to be host-species specific while they are not so geographically constrained within the Mediterranean Sea (e.g. Sarabeev et al., 2013).

In addition, the negative standardised value of β 1 TD indicated that diversity of parasite species were overdispersed within hosts from the same species, which may indicate that parasite species sharing the same resources are limited to coexist (e.g. Krasnov et al., 2005a).

Nonetheless, parasite FD and PPD were randomly distributed among host individuals (Table 3 and 4). A similar result was found by Krasnov et al. (2005b). These authors proposed that when the number of parasite species infecting a host species becomes saturated, a random assemblage in PPD suggests that all the parasite species are ecologically interchangeable and contribute equally to the saturation. Here we show that FD and PPD are random assemblages. This would indicate that parasite species are functional and phylogenetic interchangeable entities. Furthermore, the span of the boxes (Fig. 1 and 3) and the size of the ellipses (Fig. 2 and 4) reflect the heterogeneity in infection among host individuals from the same species or locality. This could result from the almost universal observation of parasite aggregation among host individuals (Poulin, 2007a). Unfortunately, it is difficult to interpret the causes of parasite aggregation in host individuals in our system because we do not have enough data about inequality in environmental pressures among host individuals (Thieltges and Reise, 2007), host genetic background (Poulin, 2007a), host traits (Timi and Poulin, 2003) or parasite characteristics (such as dispersal ability) (Poulin, 2007b).

At β_2 level (between host species or localities), the positive standardised observations indicated that species, traits and the proxy of the phylogeny were clustered, i.e. the species, trait, and phylogenetic compositions of parasite communities depend on the species host and the locality. Both environmental filtering and competition can be responsible for these patterns (Cadotte and Tucker, 2017). Moreover, intrahost speciation has also been pointed out as a cause of clustering in parasites (Krasnov et al., 2014). Parasite species competition is difficult to demonstrate (Mideo, 2009). However, the filtering of parasite species has received much more attention, particularly after the framework developed by Combes (2001). Due to the strong association between parasite and host species (Fig. 2), we consider that environmental (host) filtering is an important driver in these communities. As for intrahost speciation, it has been suggested for some *Ligophorus* spp. in our system (Blasco-Costa et al., 2012). Consequently, intrahost speciation could be an additional mechanism accounting for the clustering of such species. As for geographical differences in parasite assemblages, the overlap in the three spaces of diversity of the two marine localities was considerable (Fig. 4). This suggests that habitat

condition (marine vs lagoon) is perhaps a stronger driver than geographical distance of the parasite communities. The environmental characteristics of the lagoon together with the high site fidelity of *M. cephalus* to lagoons (Chang et al., 2004) might eventually determine the slightly differentiated parasite community from SPL (Fig. 4). These results are indicative of environmental filtering in the parasite facets of diversity, which conforms with previous evidence only reported for TD of parasite communities of fish populations (e.g. Levy et al., 2019).

Finally, the season survey had a moderate effect on the parasite diversity of the host species and localities (crossed-DPCoA results). However, the capacity to differentiate parasite communities from different localities might be seasonal-dependent. Since freshwater effluents widely vary in EDS seasonally, and, thus, the parasite diversity in EDS and SPL localities could be more similar in some seasons than in others (Míguez-Lozano et al., 2012).

To sum up, our study supports the idea that the assembly rules driving parasite communities depend on the level of the analysis and the facet of diversity considered. Particularly, communities at host individual level appear to represent a random subset of parasite species of a larger pool of species, traits, and phylogeny of parasites. At intermediate levels, similar parasite species are limited to coexist, whereas at higher levels, the environment filters the parasite diversity, since a joint influence of host phylogenetic origin (*Chelon* vs *Mugil*) and environmental preferences (marine-related vs coastal-related) might drive the parasite TD, FD and PPD. Although this was less clear for the last two facets of diversity. Finally, the habitat conditions of the locality seem to be more determinant not only of the TD, but also of FD and PPD than geographic distance. The fact that diversity in these parasitic communities are subjected to random and deterministic processes simultaneously, but at different organisational levels, brings together Clements and Gleason ideas. These can be seen as two polar cases along a single deterministic-stochastic continuum of community organisation outcomes (Götzenberger et al., 2012; Liautaud et al., 2019). Clearly, parasitologists should pay attention to patterns of diversity at different facets and organisational levels.

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638

639 **Fig. 1. Parasite α diversity in terms of taxonomic (TD), functional (FD) and a proxy of the**
640 **phylogenetic (PPD) diversity for each host individual of each fish species (Case 1).**

641 Different lowercase letters indicate significant differences between host species.

642

Fig. 2. Position of each host individual (dots) in the (A) parasite species space, (B) parasite trait space, and (C) parasite phylogenetic-like space (Case 1). Fish are grouped by host species. Abbreviations: Mc, Cr and Ca represent the centroid of each fish species, and stand for *Mugil cephalus*, *Chelon ramada* and *C. auratus*, respectively. These analyses were carried out with the second version of the crossed-DPCoA. The width and height of the ellipses are given by the variance of the coordinates of the individuals, and the covariance between the coordinates on the two axes gives the slope of the ellipse. “d” (top-right) indicates the length of the side of the grey squares of the background grid.

Fig. 3. Parasite α diversity in terms of taxonomic (TD), functional (FD) and a proxy of the phylogenetic (PPD) diversity for each locality (Case 2). Different lowercase letters indicate significant differences between localities. Abbreviations: EDS, Ebro Delta Sea; SPS, Santa Pola Sea; SPL, Santa Pola Lagoon.

659

660 **Fig. 4. Position of each host individual (dots) in the (A) parasite species space, (B) parasite**
661 **trait space and (C) parasite phylogenetic-like space (Case 2).** Fish were grouped by
662 localities. Abbreviations: EDS, SPL and SPS represent the centroid of each locality, and stand

663 for Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea, respectively. These analyses were
664 carried out with the second version of the crossed-DPCoA. The width and height of the ellipses
665 are given by the variance of the coordinates of the individuals, and the covariance between the
666 coordinates on the two axes gives the slope of the ellipse. “d” (top-right) indicates the length of
667 the side of the grey squares of the background grid.

Supplementary Figure S1. Observed and simulated β diversity values (Case 1: autumn

2004). (A, B, C) β_1 diversity or extent of dissimilarity in the diversity of parasite communities

among host individuals within each host species (*Chelon auratus*, *Mugil cephalus* and *Chelon*

ramada). (D, E, F) β_2 diversity or extent of dissimilarity in the diversity of parasite

communities between host species. Diversity was measured in terms of (A, D) Taxonomic

Diversity (TD), (B, E) Functional Diversity (FD) and (C, F) the Proxy of Phylogenetic Diversity (PPD). Samples are from Santa Pola Sea and autumn 2004 (Case 1). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

Supplementary Figure S2. Observed and simulated β diversity values (Case 1: autumn 2005). (A, B, C) β_1 diversity or extent of dissimilarity in the diversity of parasite communities

among host individuals within each host species (*Chelon auratus*, *Mugil cephalus* and *Chelon ramada*). (D, E, F) β^2 diversity or extent of dissimilarity in the diversity of parasite communities between host species. Diversity was measured in terms of (A, D) Taxonomic Diversity (TD), (B, E) Functional Diversity (FD) and (C, F) the Proxy of Phylogenetic Diversity (PPD). Samples are from Santa Pola Sea and autumn 2005 (Case 1). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

Supplementary Fig S3. Observed and simulated β diversity values (Case 2: spring 2004).

(A, B, C) β_1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each locality (Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea). (D, E, F) β_2 diversity or extent of dissimilarity in the diversity of parasite communities between localities. Diversity was measured in terms of (A, D) Taxonomic Diversity (TD), (B, E) Functional Diversity (FD) and (C, F) the Proxy of Phylogenetic Diversity (PPD). Samples are of

the host species *Mugil cephalus* and from spring 2005 (Case 2). Observed β values (black diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β values (grey bars).

Supplementary Fig S4. Observed and simulated β diversity values (Case 2: autumn 2005).

(A, B, C) β_1 diversity or extent of dissimilarity in the diversity of parasite communities among host individuals within each locality (Ebro Delta Sea, Santa Pola Lagoon and Santa Pola Sea). (D, E, F) β_2 diversity or extent of dissimilarity in the diversity of parasite communities between localities. Diversity was measured in terms of (A, D) Taxonomic Diversity (TD), (B, E)

709 Functional Diversity (FD) and (C, F) the Proxy of Phylogenetic Diversity (PPD). Samples are of
710 the host species *Mugil cephalus* and from autumn 2005 (Case 2). Observed β values (black
711 diamond on the top of the black vertical line) and distribution of the simulated (x-axis: sim) β
712 values (grey bars).

713 **Tables**

714 **Table 1. Sample summary.** Fish (host individuals) sample sizes by host species, seasons, and
715 localities.

	Santa Pola Sea			Santa Pola Lagoon			Ebro Delta Sea		
	Autumn 2004	Spring 2005	Autumn 2005	Autumn 2004	Spring 2005	Autumn 2005	Autumn 2004	Spring 2005	Autumn 2005
<i>Chelon auratus</i>	12		30						
<i>Mugil cephalus</i>	20	22	30		25	31		28	29
<i>Chelon ramada</i>	30		15						

716

717 **Table 2. Results of Type I ANOVAs of α Taxonomic (TD), Functional (FD) and Proxy of**
 718 **Phylogenetic (PPD) diversity.** *P* values are given (significance level $P < 0.05$).

	Case 1		Case 2	
	Autumn 2004	Autumn 2005	Spring 2005	Autumn 2005
TD	0.447	0.001	0.16	0.001
FD	0.693	0.002	0.001	0.001
PPD	0.023	0.001	0.03	0.001

719

Table 3. Partition of diversity at two organisational levels of the host species factor (Case 1). Statistical results of the partitioning of Taxonomic (TD), Functional (FD) and the Proxy of Phylogenetic (PPD) Diversity at two organisational levels (β_1 [among host individuals within host species] and β_2 [between host species]) in comparison to a distribution of 999 random replicates. *P* values are given (significance level $P < 0.05$). Standardised observed values are given in parenthesis. Standardised β is negative when the community structure is overdispersed, and positive when the community structure is clustered.

	β_1			β_2		
	TD	FD	PPD	TD	FD	PPD
autumn 2004	0.007 (-2.6)	0.07 (-1.8)	0.17 (-1.4)	0.001 (14.6)	0.001 (13)	0.001 (13.3)
autumn 2005	0.85 (-0.3)	0.61 (-0.7)	0.42 (-0.8)	0.001 (13.8)	0.001 (10.1)	0.001 (11.2)

Table 4. Percentage of diversity associated with each factor. Taxonomic (TD), Functional (FD) and Proxy of Phylogenetic (PPD) Diversity of parasite communities (A) of three host species and autumn 2004 and 2005 (Case 1); (B) and from three localities and spring and autumn 2005 (Case 2).

A	TD	FD	PPD
Host individual	50.3	50.2	56.3
Host species	31.3	28	20.6
Season	1.6	2.1	1.5
Host species $\times$ season	16.8	19.7	21.6
B			
Host individual	57.9	53.7	52.1
Locality	5.8	12.4	12.1
Season	1.3	0.6	1
Locality $\times$ Season	35	33.3	34.8

Table 5. Partition of diversity at two organisational levels of the locality factor (Case 2).

Statistical results of the partitioning of Taxonomic (TD), Functional (FD) and Proxy of Phylogenetic (PPD) Diversity at two organisational levels (β_1 [among host individuals within locality] and β_2 [between localities]) in comparison to a distribution of 999 random replicates. P values are given (significance level $P < 0.05$). Standardised observed values are given in parenthesis. A standardised β is negative when the community structure is overdispersed, and positive when the community structure is clustered.

	β_1			β_2		
	TD	FD	PPD	TD	FD	PPD
spring 2005	0.01 (-2.9)	0.25 (-1.2)	0.57 (-0.6)	0.001 (4.3)	0.001 (10.2)	0.001 (6.7)
autumn 2005	0.01 (-3.2)	0.2 (-1.2)	0.91 (0.1)	0.01 (3)	0.001 (7)	0.001 (5.2)