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Parasite species coexistence and limiting similarity: a multiscale look at phylogenetic, functional and reproductive distances

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Abstract The factors that control biodiversity have been the focus of numerous recent investigations; these include species interactions, speciation, environmental gradients and heterogeneity, all of these operating differently at each observation scale. We used a null model to examine the influence of two forces shaping the community structure of *Dactylogyrus* species parasitic on roach (*Rutilus rutilus*) gills: interspecific competition that might prevent the coexistence of the most similar species and environmental filters that might result in the most similar species coexisting together. The study was carried out on two sets of fish from two different localities in the Morava river basin (Czech Republic) to evaluate the consistency of the results across host populations, and at three different scales of observation to test for the scale dependence of assembly rules. In decreasing order, from largest to smallest, the three spatial scales investigated were: the individual fish, the individual gill arch and individual sections of each gill arch. The similarity between pairs of parasite species was

measured using three different criteria: (1) phylogeny, (2) quantitative functional traits consisting of parasite size and morphometric measurements of the attachment organ's sclerotized parts and (3) qualitative attributes of reproductive organs. First, our study reveals a strong conservatism of ecological characters for the nine *Dactylogyrus* parasite species, in particular regarding the attributes of their copulatory organs. Second, our study did not find any limitation of similarity among coexisting *Dactylogyrus* species due to interspecific competition, irrespective of the scale and the similarity measures considered. Conversely, our results support the niche filtering hypothesis, preventing the co-occurrence of species too dissimilar from one another. This process is particularly apparent at the scale of the individual fish and for functional traits associated with the hard parts of the parasites' attachment organs. In both localities, the *Dactylogyrus* species that occur on the same fish individuals tend to have similar values for the haptor dimensions. Our study supports previous studies on monogenean parasite communities indicating a weak influence of competition as a structuring force, but it goes a step further by identifying environmental filtering as a key process shaping these communities.

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Introduction

The recent success of macroecology and large-scale studies questions why ecologists are yet to understand community structure, which seems too complex to permit some general laws (Lawton 1999). Despite these controversies, Simberloff (2004) asks community ecologists “to move on” and argues that the study of community structure will provide crucial knowledge allowing advances in conservation and environmental management. A central question in community ecology

Table 1 Mean parasite length and mean standardized (^a) functional traits associated with the haptor's sclerotized parts (Fig. 2) for the nine *Dactylogyrus* species of *Rutilus rutilus*

| | <i>D. calballeroi</i> | <i>D. crucifier</i> | <i>D. fallax</i> | <i>D. nanus</i> | <i>D. rarissimus</i> | <i>D. rutili</i> | <i>D. similis</i> | <i>D. sphyrna</i> | <i>D. suecicus</i> |
|--|-----------------------|---------------------|------------------|-----------------|----------------------|------------------|-------------------|-------------------|--------------------|
| Parasitelength (mm) | 0.75 | 0.75 | 1.00 | 0.40 | 0.25 | 0.40 | 1.20 | 1.40 | 0.50 |
| Anchors ^a | -0.51 | -0.61 | -0.52 | 0.40 | 2.10 | 0.63 | -1.12 | -0.82 | 0.45 |
| Base of anchor ^a | -0.14 | -0.30 | -0.13 | 0.71 | 1.43 | 0.83 | -1.45 | -1.49 | 0.55 |
| Inner root of anchor ^a | -0.42 | -0.25 | 0.58 | -1.43 | 1.40 | -1.32 | 0.86 | 1.29 | -0.36 |
| Outer root of anchor ^a | -0.99 | -0.88 | 1.98 | -0.91 | -0.46 | -0.13 | 0.42 | 1.03 | -0.06 |
| Point ^a | 0.41 | 0.34 | 0.80 | -1.13 | 2.04 | -0.80 | -0.36 | -0.70 | -0.60 |
| Marginal hook ^a | -0.29 | -0.21 | -0.01 | 0.40 | 1.79 | 0.50 | -0.73 | -1.88 | 0.43 |
| Dorsal connective bar— length ^a | 0.15 | -0.04 | 2.08 | -1.28 | 0.70 | -0.70 | -0.89 | -0.33 | 0.33 |
| Dorsal connective bar— width ^a | -0.32 | -0.43 | 0.72 | 0.27 | 1.60 | 0.29 | -1.44 | -1.38 | 0.69 |
| Copulatory organ —length | L | M | L | S | S | S | M | M | S |
| Shape of vaginal armament | WT | WT | STLV | STSV | VA | STLV | VA | STSV | STSV |
| Shape of copulatory tube | BCBA | BCBA | SCC | TC | BC | TC | BC | SCC | TC |
| Shape of accessory piece | TTS | TTS | TTP | ANC | TTS | ANC | TTS | ASP | ANC |

The copulatory organ attributes of these parasite species are coded as qualitative variables and are defined in Fig. 3: the copulatory organ length can be small (S), medium (M) or large (L), the shape of vaginal armament can be without vagina (VA), without tube (WT), with a sclerotized tube and short vagina (STSV) or with a sclerotized tube and long vagina (STLV), the shape of the copulatory tube can be with a broad copulatory tube (BC), with a broad

copulatory tube curved from insertion in the basal ampula (BCBA), with a thick copulatory tube at the insertion in the basal ampula (TC) or with spiral curved copulatory tube (SCC) and the shape of the accessory piece of the copulatory organ can be with a narrow central part (ANC), with two short but broad projections at the terminal part (TTS), with a prolonged projection at the terminal part (TTP) or with more short projections (ASP)

is whether communities are just random assemblages drawn from a general species pool. Null models are generally used to answer this question, with their rejection indicating structural forces or mechanisms shaping the non-random assembly of communities. There are traditionally two hypotheses explaining non-random patterns: interspecific competition and environmental filters. The former involves the competitive exclusion (Hardin 1960) and the limiting-similarity principles (MacArthur and Levins 1967): species are in competition and the magnitude of this competition is greater between those species that are most similar. The species eventually coexisting in a stable way are those that are dissimilar and thus functionally differentiated on at least one niche axis. The latter hypothesis assumes that coexisting species are more similar to one another than would be expected by chance, because environmental conditions (i.e., abiotic or biotic properties of the habitat) act as a filter allowing only a narrow spectrum of species to survive. The species that make it through the environmental filters are likely to share many characteristics. Both these hypotheses have been supported using field data (Franzen 2004; Stutzner et al. 2004; Stubbs and Wilson 2004); thus, the question is not which mechanism is operating but which mechanism has the strongest influence on community structure. To address this question, we definitely need more experiments and investigations of new types of communities; but first, we need to know on which kind of similarity the mechanism is acting and at which scale it operates.

The limitation of similarity leading to competitive exclusion is a very attractive and logical idea, but surprisingly very few studies have provided supportive evidence using field data (see Kingston et al. (2000) and Stubbs and Wilson (2004) for relevant examples using animal and plant data, respectively). Most studies seeking

this assembly rule failed to reject the null hypothesis of random assembly and failed to identify competition as a structuring force in communities (Franzen 2004; Peres-Neto 2004; Tofts and Silvertown 2000; Watkins and Wilson 2003). There are four main factors explaining this lack of evidence for non-randomness. First, some authors (Caswell 1976; Hubbell 2001) suggest that species are much more opportunistic than is generally believed and that species interaction might be neutral because species are ecologically equivalent. In this scenario, communities would be the random assemblages of species thrown together by chance and history, and competitive exclusion would not occur more frequently for similar species. Second, the metric used to estimate the similarity and thus the interactions among species influences the findings of a study. Usually, two species are supposed to compete when they apportion the same resource (Tokeshi 1999) and when they overlap in their functional niche (Rosenfeld 2002). Thus, it seems appropriate to use functional traits to quantify species similarity when seeking competition effects (Diaz and Cabido 2001; Dumay et al. 2004). Nevertheless, it remains difficult to measure the “right” traits, particularly those responsible for the strength of species competition. For instance, space and time are also fundamental resources for communities. Some dynamics related to reproductive life-history traits or related to resource allocation might explain species coexistence (Kelly and Bowler 2002). Thus, some traits based on reproductive characteristics should be included to estimate the similarity among species. From a phylogenetic point of view, the niche conservatism of many species traits in the evolution of a lineage often leads to a negative relationship between the phylogenetic distance between species and their net ecological similarity (Peterson et al. 1999; Webb et al. 2002). Thus, the limits in similarity, which we can expect for coexisting species, can be expressed by

using phylogenetic information as suggested by Tofts and Silvertown (2000). Third, the scale of observation clearly influences the processes detected in communities (Chase and Leibold 2002) and the influence of the limiting-similarity process may not be scale-invariant. Fourth, many other mechanisms can occur in the field and can weaken or prevent competitive exclusion. For instance, if an environment has changing abiotic factors, then surely dispersal and population dynamic variations may all realistically prevent the discovery of competitive exclusion (Kohler 1992; Litchman et al. 2004) even among species having identical responses to the environment (Snyder and Chesson 2004). More generally, all the mechanisms enhancing species coexistence (see review in Chesson 2000), such as the variability of pulsed environments (Chesson et al. 2004), are capable of preventing the occurrence of the limiting-similarity pattern.

In sharp contrast to the limiting-similarity process, the environmental filter process has received wide support irrespective of the use of any similarity measure or any of the scales of observation (Franzen 2004; Stutzner et al. 2004).

Parasite communities are relevant systems for tests of community structure and allow robust statistical testing because each host individual can harbour a replicate community (Holmes and Price 1986; Naeem and Hawkins 1994). Over the past decade, several studies have used parasite communities in the analyses of community structure and these communities are expected to be mostly unstructured because the total niche is always subjected to temporal variation, leading to the development of limited species interactions and a lack of time for resource-based competition. Indeed, several earlier studies have emphasized that most parasite communities appear non-saturated with species, with many empty niches and with interspecific competition not playing a major structuring role (Krasnov et al. 2005; Morand et al. 1999; Poulin 1996; Rohde 1991). For example, a recent study by Mouillot et al. (2003) found that replicates of fish parasite communities (i.e., infracommunities of metazoan parasites in individual hosts) fitted well within a random assortment model for species partitioning resource (Tokeshi 1999), suggesting a lack of interaction and an independent apportionment of resources between species in parasite communities. In addition, many investigators have used presence-absence data to test on the patterns of parasite species co-occurrence that depart from various null models (Gotelli and Rohde 2002; Guégan and Huguény 1994; Poulin 1996; Poulin and Valtonen 2001; Worthen and Rohde 1996). Taken as a whole, these investigations have revealed that departures from random species assembly do occur (competition intensity may increase with total biovolume of the parasite community (Mouillot et al. 2005)), but they are the exceptions rather than the norm: most patterns of species co-occurrence are not different from those predicted by null models. Nevertheless, to our knowledge, no one has considered functional and phylogenetic distances among

coexisting parasite species as a force shaping the assemblages of parasite species using null models.

The aim of our study is to search for assembly rules in parasite communities driven by phylogenetic relatedness or similarities in either reproductive attributes or functional traits. By using ectoparasitic species (Monogenea), the nine *Dactylogyru*s species found on the gills of roach (*Rutilus rutilus*), we examine the influence of two opposing forces on monogenean community structure: interspecific competition that might prevent the coexistence of the most similar species, and environmental filters that might result in the most similar species coexisting together. The present study was carried out on two sets of fish from two different localities to evaluate the consistency of the results across host populations, and at three different scales of observation to test for the scale dependence of assembly rules. After describing the null model which had been used to test these different hypotheses, we present the results for each locality and

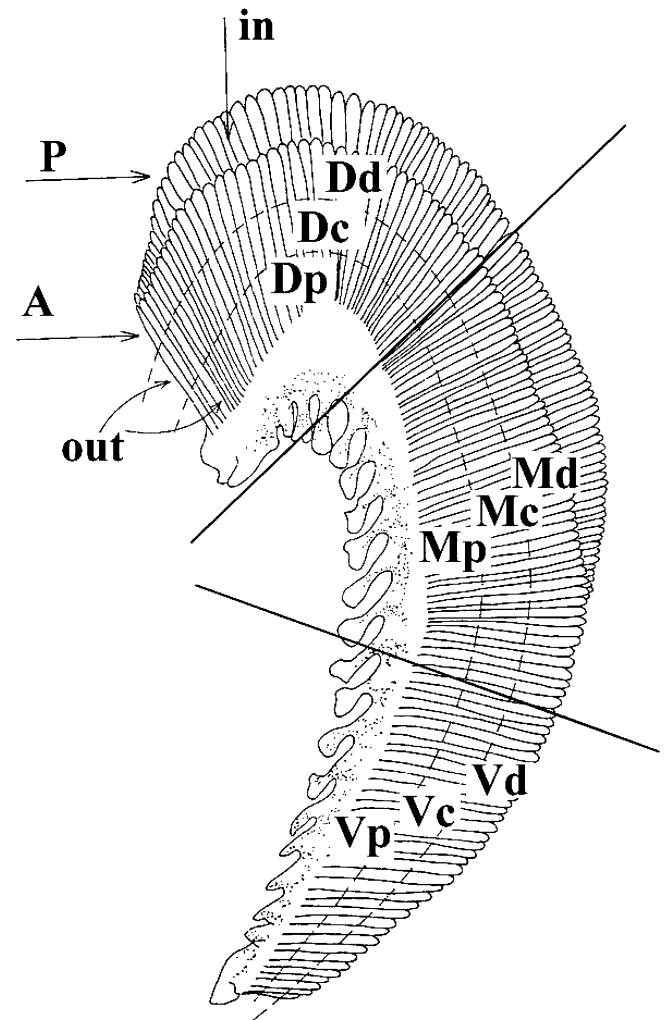


Fig. 1 Delimitation of spatial distribution of parasites on fish gills. Each gill arch is divided into gill segments (*D* dorsal, *M* medial, *V* ventral), gill areas (*d* distal, *c* central, *p* proximal), gill surfaces (*in* inner, *out* outer) and gill hemibranch (*A* anterior, *P* posterior)

for each scale of observation and discuss their ecological meanings and implications.

Materials and methods

Parasite sampling

A total of 328 individual roach *R. rutilus* (Linnaeus 1758) were caught by electrofishing between 1997 and 1998 in the Morava river basin (Czech Republic, Central Europe). These individuals come from two distinct localities with 194 and 134 individuals, respectively, from Moravska Nova Ves (hereafter Moravska) and Rohlik. All fish were immediately placed in aquaria and were killed within 24 h after capture.

The gills on the left-side of each fish were examined for *Dactylogyрус* species (class Monogenea, phylum Platyhelminthes) and a total of 939 individuals were identified on the 195 infected fish individuals (overall prevalence of infection was 63%). *Dactylogyрус* species belong to nine congeneric species: *D. caballeroi* Prost 1960, *D. crucifer* Wagener 1857, *D. fallax* Wagener 1857, *D. nanus* Dogiel & Bychowski 1934, *D. rarissimus* Gusev 1960, *D. rutili* Glaser 1965, *D. similis* Wagener 1910, *D. sphyrna* Linstow 1878 and *D. suecicus* Nybelin 1937. These *Dactylogyрус* species were identified using the sclerotized parts of the parasite haptor and the reproductive organs, according to Gusev (1985). More details are given in Šimková et al. (2000).

The co-occurrence of the nine parasite species was investigated at three scales of observation. The largest scale is the fish individual; i.e., we examined species co-occurrence using the pooled presence data from the four gill arches on each fish. The gill arch represents the

intermediate scale of coexistence; i.e., we examined the co-occurrence of species on the same gill arch. Finally, sections of each gill arch represent the smallest scale of observation. Each arch was divided into three gill segments (D: dorsal; M: medial; V: ventral) and into three gill areas (p: proximal; c: central; d: distal), according to Gelnar et al. (1990). Thus, for the smallest scale of observation, sections of the gill arch can be defined in two distinct ways, as seen in Fig. 1.

Similarity between species

The similarity between pairs of parasite species was measured using three different criteria. First, a phylogeny of the nine *Dactylogyрус* species based on morphological characters was obtained from Šimková et al. (2000). Homologous series of morphological characters and their polarization were described in Šimková et al. (2000). The topology of the phylogenetic tree based on morphological data was supported by information obtained from molecular analyses of large numbers of *Dactylogyрус* species (Šimková et al. 2004). However, as the available molecular phylogeny of *Dactylogyрус* species parasitizing central European cyprinid fish species includes only eight of our nine species of *Dactylogyрус* and as no sequence data are available for *D. suecicus*, we could not use the distances obtained on the basis of molecular phylogeny. Therefore, in the present study we use patristic phylogenetic distances obtained from the phylogenetic analysis of morphological characters as measures of phylogenetic distances among species.

Second, the functional distance between pairs of parasite species was estimated using nine quantitative functional traits, consisting of parasite body length and

Fig. 2 Morphometric measurements of haptor-sclerotized parts (applied following Gusev 1985). (a) Anchor: 1 total length of anchor, 2 length of base, 3 length of inner root, 4 length of outer root, 5 length of point; (b) marginal hook: 6 total length; (c) dorsal connective bar: 7 total length and 8 width

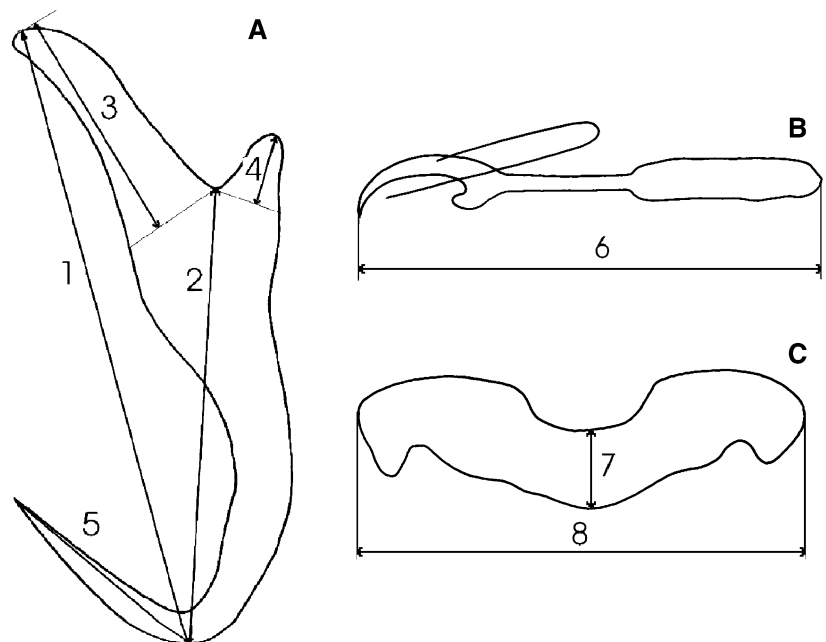
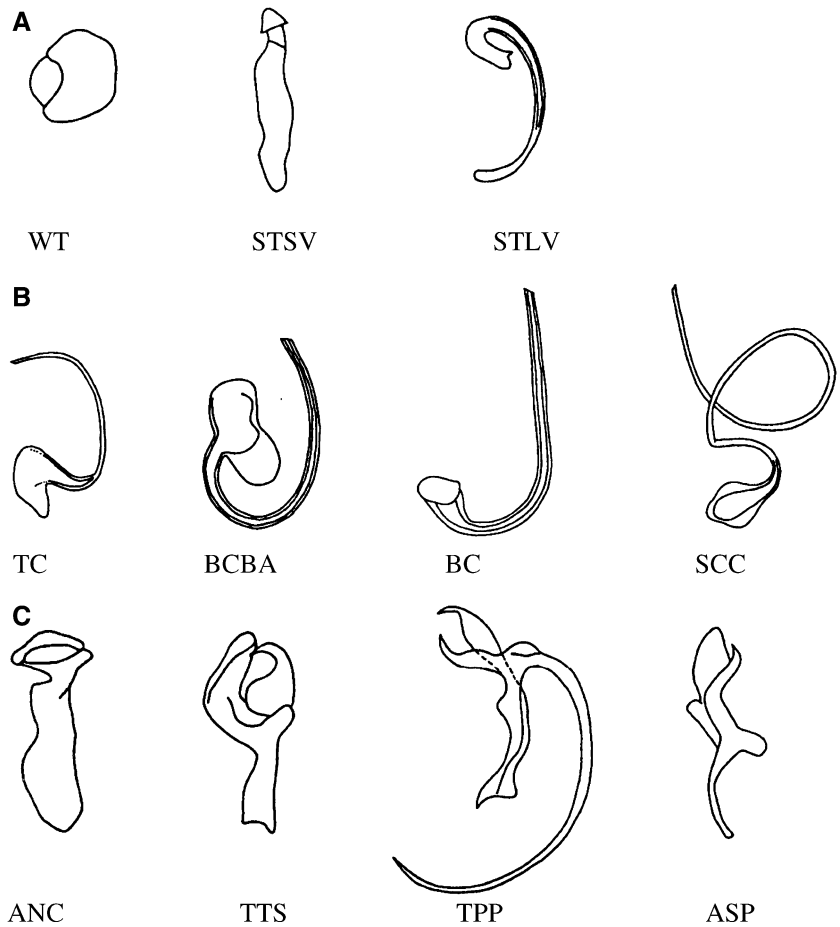


Fig. 3 Morphological characters of reproductive organs. **(a)** Shape of vaginal armament: vaginal armament without tube (*WT*), sclerotized tube and short vagina (*STSV*), sclerotized tube and long vagina (*STLV*); **(b)** shape of copulatory tube: broad copulatory tube (*BC*), broad copulatory tube, curved from insertion in the basal ampulla (*BCBA*), thick copulatory tube at the insertion in the basal ampulla (*TC*), spiral curved copulatory tube (*SCC*); **(c)** shape of accessory piece of copulatory organ: narrow central part and broadened points of insertion to basal ampulla (*ANC*), terminal part with two short and broad projections (*TTS*), terminal part with prolonged projection (*TPP*), terminal part with prolonged short projections (*ASP*)



eight morphometric measurements of the haptor's sclerotized parts, as shown in Fig. 2. The haptor is the anchoring device that monogeneans use to attach to their host surfaces and/or gill apparatus. The dimensions of the haptor's sclerotized parts are positively correlated with the parasite size, and thus we standardized all quantitative measures (eight functional traits) using the ratio $y = \text{Ln}(x)/\text{Ln}(l)$, where x is the functional trait, l the parasite length and y the functional trait standardized for body size. However, our eight variables still have differences in their values distributions and need to be standardized because no one has a higher "biological importance" than the others. Thus, in order to give the same mean and the same variance to each variable, we applied the following transformation $z = (y - m_y)/s_y$, where m_y and s_y are, respectively, the mean and the standard error of the size-standardized functional trait y , and z is the final variable used in the analyses. Thus, each functional trait represents an axis in the functional space (Rosenfeld 2002), and the Euclidean distance between pairs of species was calculated to obtain the final triangular distance matrix.

Third, we estimated a distance between each pair of parasite species using four qualitative attributes of their reproductive organs: the relative length of the copulatory organ (S: small; M: medium; L: large), the shape of the vaginal armament (Fig. 3a), the shape of the

copulatory tube (Fig. 3b) and the shape of the accessory piece of the copulatory organ (Fig. 3c). We calculated a binary distance matrix based on the species \times qualitative reproductive attributes matrix using the S-plus software (Copyright 1988, 2002 Insightful Corp.). The binary distance is the proportion of non-zeros that two vectors do not have in common in a binary-coded matrix (the number of occurrences of a zero and a one, or a one and a zero divided by the number of times that at least one vector has a one).

The first question concerns the niche conservatism for the nine *Dactylogyrus* species, and we therefore investigated the relationship between matrices of distances based on phylogeny, functional traits related to the haptor, and copulatory organ attributes. To this end, we implemented a Mantel test between each pair of distance matrix among pairs of parasite species using the S-plus software, the null hypothesis being the independence of any two distance matrices, i.e., that affinities among species are not consistent between any two matrices of distance.

Null model

To test which of the limiting-similarity or the environmental filter hypotheses best explains the observed pat-

terns, we used a null model assuming that coexisting parasite species are randomly associated, i.e., that they represent assemblages independent from phylogenetic, functional or reproductive affinities. In each test, we have four observed matrices, one for observed co-occurrences of species and three corresponding to the three distance matrices considered in this study. The test is based on the simulation of null occurrence matrices of randomly assembled species. As suggested by previous studies (Connor and Simberloff 1979; Gotelli 2000; Gotelli and Entsminger 2001), we used a “fixed–fixed” algorithm fixing the sums of rows and columns in order to constantly hold both the number of species in each replicate and the total number of times a species was observed. Thus, we maintained the observed community structure within all the randomized communities in order to get enough parasite species per sample to calculate our statistics. Following Gotelli (2000) and Gotelli and Ellison (2002), we randomly swapped checkerboard units throughout the presence/absence matrix for each scale of observation to generate 9,999 new matrices from which we calculated 9,999 simulated statistics. The checkerboard units are four-cells’ submatrices of the main matrix corresponding to two species and two samples where the two species do not coexist and where each sample has only one of the two species. In a competitively structured community, the number of checkerboard units between similar species tends to be larger than expected by chance. Conversely, if environmental filters constrain coexisting species to share some similar attributes, the number of checkerboard units between similar species tends to be lower than expected by chance.

The statistics were chosen to challenge the two hypotheses we wanted to test. We used the minimum nearest-neighbour distance (minNND) to test for absolute limits to similarity (Stubbs and Wilson 2004; Weither et al. 1998), i.e., a limit to phylogenetic, functional or reproductive closeness among coexisting parasite species within replicates. Conversely, to test for absolute limits to dissimilarity, i.e., an upper limit to phylogenetic, functional or reproductive distance among coexisting parasite species within replicates due to environmental filters, we used the maximum distance (maxD) among pairs of coexisting species within the community.

The whole algorithm of the null model combining the occurrence matrix and the distance matrices (based on phylogeny, functional or reproductive data) is summarized as follows:

1. Using the observed occurrence matrix and the observed distance matrix, we calculated the observed statistic for each sample and then the mean observed statistic I_o (Step 1).
2. We randomized the observed occurrence matrix using 1,000 successful swaps to obtain a null occurrence matrix with the same sums for both rows and columns (Step 2).
3. Using the null occurrence matrix and the observed distance matrix, we calculated the randomized statistic for each simulated sample and then the mean statistic for the randomization r : I_r (Step 3).
4. We repeated Steps 2 and 3 9,999 times to obtain a null distribution for the mean statistic I (Step 4).
5. We compared the mean observed statistic I_o with the distribution in order to obtain the P value which is the type I error when we reject the null hypothesis (Step 5).

For each of the two statistics, we used a one-tailed test to reject the null hypothesis because we are only interested in results that are greater or lower than the observed statistic for, respectively, minNND and maxD (Manly 1998; Weither et al. 1998). Thus the alternative hypothesis of limiting similarity is accepted if $p_{LM} < 5\%$ where $p_{LM} = ((\text{the number of randomized minNND} > \text{the observed minNND}) + 1) / (\text{the number of randomizations} + 1)$. Conversely, the environmental filter, alternative hypothesis, is accepted if $p_{EF} < 5\%$ where $p_{EF} = ((\text{the number of randomized maxD} < \text{the observed maxD}) + 1) / (\text{the number of randomizations} + 1)$.

This procedure was repeated 48 times, one time for each scale of observation (four, including the two ways in which a gill arch can be sectioned), for each locality (two), for each distance matrix considered (three) and for two statistics. We used only samples or replicates with at least two parasite species present, because our study focuses on coexistence and species interactions. For the two versions of our smallest study scale, more than half of the samples had to be deleted because they contained only one or no *Dactylogyrus* species.

Results

The mean values for the functional traits of the nine *Dactylogyrus* parasite species of *R. rutilus* are reported in Table 1. The mean parasite length varies from 0.25 mm for *D. rarissimus* to 1.40 mm for *D. sphyrna*. For the functional traits related to the haptor system, the values are given in a standardized way to avoid the correlation with parasite body length and differences in mean and variance. The same table presents the qualitative attributes related to the copulatory organs.

First of all, we tested the relationships between each pair of distance matrices among parasite species. Each distance matrix is significantly and positively related to the two other distance matrices. The standardized Mantel statistic (r) is 0.415 for the phylogenetic–functional relationship ($P=0.018$), 0.610 for the phylogenetic–reproductive relationship ($P=0.001$) and 0.375 for the functional–reproductive relationship ($P=0.016$).

The results for the tests of the null model assuming random assemblages of coexisting species are presented in Table 2. Whatever the measure of similarity among species that we used, the scale of observation, and the locality considered, we found no supporting evidence for

Table 2 Type I error (P -value) results from a one-tailed test of the null hypothesis assuming that coexisting species are random assemblages independent of phylogenetic affinities, functional traits or reproductive attributes

| Distance base | Scale | Locality | P_{LM} (minNND) | P_{EF} (maxD) |
|---------------|----------|----------|-------------------|-----------------|
| Phylogeny | Fish | Moravska | 0.977 | 0.005 |
| | | Rohlik | 0.781 | 0.032 |
| | Arch | Moravska | 0.971 | 0.007 |
| | | Rohlik | 0.913 | 0.078 |
| | Segment | Moravska | 0.654 | 0.212 |
| | | Rohlik | 0.899 | 0.074 |
| Functional | Area | Moravska | 0.812 | 0.164 |
| | | Rohlik | 0.751 | 0.209 |
| | Fish | Moravska | 0.928 | 0.004 |
| | | Rohlik | 0.890 | 0.007 |
| | Arch | Moravska | 0.996 | 0.002 |
| | | Rohlik | 0.814 | 0.122 |
| Reproductive | Segment | Moravska | 0.743 | 0.101 |
| | | Rohlik | 0.834 | 0.059 |
| | Area | Moravska | 0.989 | 0.100 |
| | | Rohlik | 0.833 | 0.116 |
| | Fish | Moravska | 0.801 | 0.112 |
| | | Rohlik | 0.758 | 0.048 |
| Arch | Moravska | Moravska | 0.956 | 0.010 |
| | | Rohlik | 0.894 | 0.340 |
| | Segment | Moravska | 0.695 | 0.124 |
| | | Rohlik | 0.887 | 0.142 |
| Area | Moravska | 0.748 | 0.073 | |
| | Rohlik | 0.813 | 0.097 | |

minNND is the minimum nearest neighbour distance statistic to test for the lower limit in the species similarity observed in parasite communities and its associated P -value is $P_{LM} = ((\text{the number of randomized minNND} > \text{the observed minNND}) + 1) / (\text{the number of randomizations} + 1)$. maxD is the maximum distance observed between parasite species in a community and is related to the environmental filter which prevents coexisting species from being

too dissimilar. Its associated P -value is $p_{EF} = ((\text{the number of randomized maxD} < \text{the observed maxD}) + 1) / (\text{the number of randomizations} + 1)$. We reject the null hypothesis when p_{LM} or p_{EF} is lower than 0.05 (results in bold). Two localities and three scales of observation were considered: the fish, the arch and a portion of the arch, which can be a segment or an area, as detailed in Fig. 1

the limiting similarity and the competitive exclusion hypothesis. All the minNND P -values were higher than 0.5, and 6 out of 24 were higher than 0.9, i.e., 90% of the randomized minNND values under the null model were higher than the observed minNND. This P -value needed to be lower than 0.05 to reject the null hypothesis and to accept the limiting similarity process as a structuring force.

Conversely, we obtained some significant results for the maxD statistic related to the power of the environmental filter. When the P -value is lower than 0.05 it means that no more than 5% of the randomized maxD values were lower than the observed maxD. All the P -values are lower than 0.5 for maxD, suggesting that globally the coexisting species are more similar than expected at random due to environmental constraints acting as filters for *Dactylogyrus* species on *R. rutilus*. More precisely, the environmental filter seems to be strongest on functional traits associated with the length of the parasites and their haptor's sclerotized parts at the scale of the individual fish; this result is consistent for the two localities ($P = 0.004$ and 0.007 for Moravska and Rohlik, respectively). The other consistent result concerns the convergence in similarity for parasites occupying the same arch at the Moravska locality, irrespective of the measure of similarity we use between species. Finally, not a single significant result was found at the smallest scales of observation (either segment or

area of the gill arch), i.e., we cannot reject the possibility that, at that small scale, *Dactylogyrus* parasite species are assembled independently from phylogenetic, functional or reproductive affinities.

Discussion

The first result we obtained concerns about the positive and significant relationships between phylogenetic, functional and reproductive attributes of the nine *Dactylogyrus* parasite species. This result has to be placed within the context of niche conservatism. It is widely assumed that the conservatism of many species traits (embracing morphological, life-history and functional traits) in the evolution of a lineage leads to a negative relationship between the phylogenetic distance between species and their net ecological similarity (Peterson et al. 1999; Webb et al. 2002). For example, von Euler and Svensson (2001) reported that functional and taxonomic diversity were positively correlated in bird communities, and Chazdon et al. (2003) observed that patterns of trait distribution across forest types are closely linked with patterns of floristic composition at the genus and family levels. Nevertheless, this relationship is far from universal: Bohning-Gaese et al. (2003), using passerine birds, and Losos et al. (2003), using lizards, demonstrated that phylogeny and ecological

niches were only weakly associated. This last result was explained by competition between species that promotes evolutionary diversification through ecological character displacement. Therefore, distantly related species can be ecologically similar, and ecological similarity and phylogenetic relatedness need not be linked (Day and Young 2004; Schluter 1994). For instance, Schluter (2000) highlighted that closely related species show exaggerated differences in morphological traits influencing resource use. In some cases evolutionary divergence overcomes niche conservatism, and we must thus examine the relationship for each community under study. As observed in most studies on animal and plant species (see synthesis in Webb et al. 2002), our study reveals a strong conservatism of ecological characters for nine *Dactylogyrus* parasite species, in particular regarding the attributes of their copulatory organs ($P=0.001$). This result partly explains the consistency which we obtain in Table 2, irrespective of the similarity measure used between species.

Our results provide unambiguous evidence that hypotheses based on species interaction such as limiting similarity (MacArthur and Levins 1967) or competitive exclusion (Grime 1973; Hardin 1960) are not likely to apply to our system. The minNND statistic, which was used to reveal the limits in similarity that coexisting species can endure, is far from being significantly higher than expected under the null model of randomly assorted species (Table 2). Moreover, this result is highly consistent between the two localities and among scales. Our study is in accordance with previous studies showing weak species interaction in parasite communities, especially communities of ectoparasites on fish. Several earlier workers using mainly data on presence/absence and also relative numerical abundance have emphasized that most parasite communities appear non-saturated with species, with many empty niches and random apportionment of resources among species, and finally with interspecific competition not playing a major structuring role (Gotelli and Rohde 2002; Mouillot et al. 2003; Poulin 1996; Poulin et al. 2003; Rohde 2001). More generally, the very appealing and logical idea that several similar species cannot easily coexist has seldom been supported when considering the whole community (see Kingston et al. (2000) and Stubbs and Wilson (2004) for relevant examples using animal and plant data, respectively). In the introduction section, four explanations were provided for a failure to identify competition as a structuring force in communities. We tried to overcome the second and third explanations by considering three measures of similarity among species and by considering three different scales of observation. We showed that these two variables do not affect the results and cannot be retained as an explanation for the lack of evidence for non-randomness. In addition, the neutral theory and particularly its main assumptions can generally be rejected for parasite species. Poulin (2004) and Šimková et al. (2000) have highlighted some fundamental differences among the nine *Dactylogyrus*

parasite species studies here. The fourth potential reason, as to why competition may not appear to be a force shaping the communities, is that particular dynamics or mechanisms may prevent competitive exclusion to occur. For instance, short-term disturbances, such as daily variations in irradiance for phytoplankton (Litchman et al. 2004) or flow regimes for stream invertebrates (Kohler 1992), may slow rates of competitive exclusion in communities. We cannot rule out this possibility in our case, i.e., that resource or environmental fluctuations allow highly similar parasite to coexist, but cannot find any evidence for it either. Our failure to find a limitation in the similarity of coexisting parasite species could be due to changes in individual allometry which can promote species coexistence without any niche separation (Rastetter and Agren 2002). Unfortunately, we are not able to test this hypothesis using our dataset. We are also unable to test whether limiting similarity among parasites occur preferably during free living host stages or during settlement.

Our most surprising result is certainly the niche-filtering process occurring in these parasite communities, i.e., coexisting species tend to be more similar than would be expected from chance alone. The maxD statistic is significantly smaller than it should be under the null model (Table 2) for eight occasions. This process is particularly active at the scale of the fish individual and for functional traits associated with the hard parts of the haptor. The *Dactylogyrus* species that occur on the same fish individuals tend to have the same values for the haptor dimensions, in both localities. This process has received broad support in previous studies (Franzen 2004; Stätzner et al. 2004), but we provide here the first example by using parasite data and considering morphological characters as functional traits on such small scales. Different monogenean species with similar haptors are presumably adapted to anchor to similar substrates. The non-random assortment of *Dactylogyrus* species among fish individuals with respect to haptor dimensions would seem to suggest that differences existing among the gills of different fish individuals may be due to age, damage or intra-species phenotypic plasticity. Moreover, fish individuals were caught at different time periods of the year during 2 years (Šimková et al. 2000) and this time variable could influence parasite community composition allowing more similar species to co-occur at the same period.

After discussing general patterns, it is now relevant to turn to particular species associations. To this end, we consider two different pairs of species: one is the association of two very dissimilar species (*D. rutili* and *D. sphyrna*), whereas the other is the association of two similar species (*D. rutili* and *D. nanus*). *D. rutili* and *D. sphyrna* have the greatest phylogenetic distance of all the species pairs, have no common features for their copulatory organs (Table 1) and have very different functional traits. *D. sphyrna* is the largest species with, on its haptor, a relatively large inner root of the anchor, whereas *D. rutili* is the second smallest species with a

relatively small inner anchor root (Table 1). These species have thus some functional dissimilarities, and interspecific competition between them is expected to be weak compared to interspecific competition between similar species. As a consequence, if the filtering process is acting due to some environmental constraints, these opposite species are not expected to be present in the same samples. We tested this hypothesis for the scales and the localities where the filtering process is occurring, i.e., $P_{EF} < 0.05$ (Table 2). For the Moravska locality the presences and absences of *D. rutili* and *D. sphyrna* are statistically independent ($\chi^2 = 0.94$, $df = 1$ and $P = 0.33$), while for the Rohlik locality the presences and absences of *D. rutili* and *D. sphyrna* are statistically dependent ($\chi^2 = 5.15$, $df = 1$, $P = 0.02$ and $\chi^2 = 6.96$, $df = 1$, $P = 0.01$ for, respectively, the fish and the gill arch scale of observation). The latter result means that co-occurrence and co-absence of these two species are less than expected if the presences and absences of *D. rutili* and *D. sphyrna* were independent. In other words, these two contrasting species do not occur together in parasite communities in the Rohlik locality at the fish and gill arch scales. This two-species pattern can explain the rejection of the null hypothesis in these two tests.

Both *D. rutili* and *D. nanus* are very close species: the phylogenetic distance between them is the smallest among the species in our study; they have the same modal value for three variables out of four for their reproductive organs, and they are functionally similar because they are small species with a relatively small inner root of the anchor on their haptor (Table 1). If the limiting similarity and the competitive exclusion processes are operating, these species would not be expected to co-occur. Using presences and absences of *D. rutili* and *D. nanus*, we showed their independence ($P > 0.05$ for all χ^2 tests), irrespective of any scale of study or the locality considered. This result suggests that the supposed competition between *D. rutili* and *D. nanus* does not influence their occurrences.

Using phylogenetic, reproductive and functional attributes of nine *Dactylogyrus* species, we were not able to demonstrate the presence of current or recent competitive forces shaping the parasite community structure, as observed in other studies seeking such assembly rules (e.g., Franzen 2004; Peres-Neto 2004; Tofts and Silvertown 2000; Watkins and Wilson 2003). Conversely, we found an intensive niche-filtering process at the fish individual level preventing species too dissimilar from one another from co-occurring on the same fish, and therefore limiting the biodiversity in each community sub-unit. Earlier studies on monogenean parasite communities, based solely on presence/absence data, have generally concluded that competition was not a dominant structuring force in these communities. (e.g., Worthen and Rohde (1996) and Gotelli and Rohde (2002)). Our results are not in accordance with the hypotheses proposing that the coexistence of congeneric ectoparasites with morphologically similar attachment organs should be reinforced by reproductive barriers (i.e., differences in size

or type of reproductive organs) to prevent their hybridization (Rohde 1991). For instance, Rohde et al. (1994) found that congeneric monogenean parasites of *Lethrinus miniatus* living in the same microhabitat have very different copulatory organs and that species with similar copulatory organs live in a different microhabitat. In contrast, our results indicate that co-occurring *Dactylogyrus* species, whatever the observation scale, are more similar in all respects than what one would expect from chance alone. Using the same data set, Šimková et al. (2002) suggested that species coexistence should be facilitated by specialist adaptations, and also by reproductive isolation based on the morphology of the copulatory organs. This was based on the fact that similar niches were occupied by parasite species differing in the size of copulatory organs but not based on random models testing whether these differences were higher than expected by chance. Jarkovsky et al. (2004) used random models to test for dissimilarities within infracommunities among copulatory organs of 52 *Dactylogyrus* species parasitizing 17 species of cyprinid fishes. They found different trends for specialist and generalist species. Some are in accordance with our study (coexisting specialist species possess more similar attachment apparatus); some are random patterns but none of them significantly contradicts our results. In conclusion, our study suggests that haptor attributes or copulatory organs, as we measured them, are not the main factors affecting the reinforcement of reproductive barriers, if any. Owing to the lack of any knowledge of how these organs function, further work is needed to evaluate whether differences in copulatory organs are sufficient to guarantee reproductive segregation.

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