

**UNIVERSITA' DEGLI STUDI DI
MILANO-BICOCCA**

Facoltà di Scienze Matematiche, Fisiche e Naturali

Dottorato di Ricerca in Biologia XXII Ciclo



**MODELLING THE ECOLOGICAL RELATIONSHIPS
BETWEEN FISH PARASITES AND THEIR HOSTS**

Tesi di Dottorato di:

Giovanni Strona

Coordinatore del Dottorato: **Prof. Paolo Tortora**

Tutor: **Dott. Paolo Galli**

Anno Accademico: 2009/2010

CONTENTS

CHAPTER 1 - Fish parasite and their hosts: an ecological scale model

1.1 – Overview	5
1.2 - Monogenoidea – Diversity	9
1.3 – Monogenoidea – Host specificity	12
1.4 – Monogenoidea – Taxonomy and classification	14

CHAPTER 2 - How host features affect parasite distribution in the compound community: a multivariate approach based on Ecological Niche Models.

2.1 – Introduction	20
2.1.1 - Host/Parasite Relationships - Overview	
2.1.2 - Host specificity, Ecology and Coevolution: the theoretical linkages	
2.1.3 - Application of ENMs to the host-parasite system	
2.2 – Methods	28
2.2.1 - From the geographical space to the compound community: general adjustments	
2.2.2 - An exemplicative case study: <i>Neobenedenia melleni</i> (MacCallum, 1927) Yamaguti, 1963	

2.3 - Results and discussion	34
CHAPTER 3 - Nestedness as a measure of intrinsic host specificity	
3.1 – Introduction	37
3.1.2 - The application of nestedness to the host/parasite complex	
3.2 – Methods	41
3.2.1 - Datasets and Nestedness analysis	
3.2.2. - How to compare nestedness of different matrices: principles and general procedure	
3.2.3 - Comparing nestedness of different parasite taxa to assess their “intrinsic” host specificity	
3.3 - Results and Discussion	50
CHAPTER 4 - Parasite host specificity within the component community: ecological and evolutionary implications.	
4.1 – Introduction	52
4.2 – Methods	56
4.3 Results and Discussion	58
CHAPTER 5 - Relationships between trophic level and host specificity	
5.1 – Introduction	62
5.2 - Material and Methods	65
5.2.1 - Host trophic level vs. parasite host range	

5.2.2 - Predation and host switch	
5.3 – Results and Discussion	70
5.3.1 - Host trophic level vs. parasite host range	
5.3.2 - Predation and host switch	
CHAPTER 7 – Overall discussion and conclusions	75
FIGURES	86
TABLES	110
REFERENCES	119

CHAPTER 1

Fish parasites and their hosts: an ecological scale model

1.1 - Overview

Many of the peculiarities between fish parasites and their hosts are very useful in the study of general ecological mechanisms. Treating a fish host as a locality is clearly a key point in this kind of analyses, as it make possible to apply at a small scale a wide range of the community and metacommunity ecological and biogeographical analyses. Comparing a host to a locality (or better, to an island) is intuitive but, at the same time, debatable. Prior to any further discussion, it is fundamental to define the level of organization at which hosts are regarded as islands. Kuris *et al.* (1980) discussed in detail many aspects related to the application of island biogeography assumptions to the host/parasite system. They individuated several difficulties associated to the theoretical steps necessary to assume the equivalence between hosts (considered at individual, population or species level) and islands. Comparing a host to a locality is

complicated by the fact that the host, as a living being, is capable of active responses against an harming external agent, through individual interaction and evolution of defensive strategies. However, most of the concerns they raised by Kuris *et al.* (1980) are related to the neutralism (species equivalence) implied by Island Theory (McArthur and Wilson, 1963). Differently, in a not-neutral approach, the use of host as locality is not biased by differences in host ecological and coevolutionary aspects. This statement will be supported throughout the various section of this thesis by several applicative examples (both original and from literature).

Biogeography offers many interesting hypothesis to be tested in the host/parasite system. Moreover, it provides the means to test such hypotheses. At this point it is important to distinguish between the biogeography of parasites *on* hosts, and the biogeography of parasites *and* hosts, since both of them do belong to a particular locality (meant both as an habitat and a biogeographical context). Nonetheless, it should be considered that geographical distribution of a parasite species at a certain moment is intrinsically related to biogeography of its host/s, being this relationship much stronger for high specific parasites than it is for low specific ones. The biogeography of parasites on their hosts, expecially for high specific

parasites does not exclude or weaken the study of the relationships between the symbiotic organisms and their geographical range. On the contrary, it offers new perspective of integration.

The biological definition of a parasitism has been argument of much debate among scientists. Some fundamental points distinguishing parasitic lifestyle from that of free living organisms are still unclear, even if the increasing efforts in parasitological studies and the improvements applied to techniques and equipments have enlightened many aspects of the matter. Recent studies demonstrated how the role of parasites in ecosystems has often been underestimated. Kuris *et al.* (2008) comparing the energetic implications of parasites to that of free living organisms in different ecosystems, found that parasites have substantial biomass, even exceeding that of top predators, while Lafferty *et al.* (2008) discussed the potential of parasites in altering food-web topology in terms of chain length, connectance and robustness.

The general tendency to exclude parasite from ecological studies is obviously due to the cryptic lifestyle characterizing most of parasite species, their reduced size and to the fact that their collection is subordinate to host accessibility. High prevalence and intensity (that are quite common among parasites) are helpful to the

investigator's job, but are often frustrated by aggregative behaviors (spatial and/or temporal) (Rhode, 1984; Shaw and Dobson (1995)). In vivo studies are not always performable, and require the controlled maintenance of the host prior to that of the parasite.

The practical outcome of these aspects is that many studies of fish parasite refer to species collected from hosts of commercial interest or eventually from hosts obtained by random samplings. Most of ecological studies on fish parasites and their hosts in the search for regular patterns (for example the search for nested subsets) have been conducted mostly at the infracommunity and component community level, while fewer studies have been performed in order to study the complexity of a parasite compound community integrating a quantitative and a qualitative point of view (Poulin, 2007). Moreover, although there are many available checklists reporting the fish parasite records in several biogeographical areas (see, for example, Hewitt and Hine 1972; Williams and Bunkley-Williams 1996; Holland and Kennedy 1997; Kohn and Cohen 1998; Kohn et al. 2006; Salgado-Maldonado 2006; Salgado-Maldonado 2008; Cohen and Kohn 2008; Strona et al. 2009), most of them group together data from hardly comparable sources, so that the provided information is very difficult to be

standardized for quantitative studies.

1.2 Monogenoidea – Diversity

Parasite can have complex life cycles, involving one or more intermediate hosts. Evolutionary radiation in parasite life cycles is so wide that is hard to individuate general rules in parasite development, reproduction, diffusion and transmission. Alternative behaviors are sometimes possible in a single species in response to different environmental stimuli (Schmidt and Roberts 1985). Virtually, each species is characterized by unique features that should be studied with a dedicated approach. The main difficulties in studying a species with a complex lifestyle are related to the collection of the different hosts (eventually comprising the paratenic ones) and to the morphological crypticity of most of larval stages (whose development in laboratory is usually hard to be achieved).

The study of direct developing parasites, such as Monogenoidea, is free from many of the described difficulties. In addition, apart from their direct development, there are a few other features that make Monogenoidea an optimal model to investigate host parasite relationships and get more insights into the ecological role of parasites in fish community. Monogenoidean parasites

usually are reported from very few host species (i.e. they have a small host range). Basing on the rough assumption of a 1:1 ratio between fish species and monogenoidean species, Whittington (1998) estimated the existence of almost 25000 monogenoidean species on Earth. The number of described species (less than 5000 according to Whittington) should be therefore considered as a large underestimation of the real biodiversity. On the other hand, Poulin (2002) observed that body size of monogenoidean species correlates negatively with the year of their first description, *i.e.* it decreases over time in a way that suggests that only some of the smallest species are left to be discovered.

It is nonetheless true, as discussed by Poulin and Mouillot (2005) that the width of host range influences the probability of a parasite species to be found and described (i.e. parasite host range is a significant predictor of the year of parasite species description). The narrow host ranges of monogenoidean species would therefore slow down the discovery and description processes, corroborating Whittington's hypothesis of the severe underestimation of the class biodiversity.

Moreover, the negative correlation found by Poulin (2002) between body size and year of description is likely to be biased by

host collections. Although Poulin randomly selected a significant number of records, the “universe” from which he chose its sample (published records since about 1800) has not been critically tested for statistical significance in terms of host size. As it is reasonable to assume that smaller parasite species are likely to be discovered before the bigger ones, it is nonetheless true that, at least in Monogenoidea, the relative range of variability of parasite size is much narrower than that of host size. Considering that a close correlation between the size of monogenoidean parasites and that of their fish host has not been put on evidence (Poulin 1996), the assumption of the reduction in size of described parasites over years as a prediction of monogenoidean biodiversity would be valid only if the pool of the considered hosts was representative of all the dimensional fish classes. This is hardly the case in Monogenoidean available studies, especially if we refer to the marine environment. Apart from some notable exceptions, most of the surveys on monogenoidean biodiversity are based on sampling procedures that are quite selective about fish size. For instance Strona *et al.* (2009) argued that the knowledge on Italian marine monogenoidean species is strongly affected by heterogeneity in the study effort, that has been primarily directed towards host species of commercial interest

(or eventually towards by-catches of commercial fisheries), with the result that the monogenoidean biodiversity is not only underestimated from a quantitative point of view (almost 80%, if we consider Whittington's host-specificity rule), but also from an ecological one: more than the 75% of the listed hosts belong to averagely big host fishes (>25 cm in total size) at the highest trophic levels. In Fig. 1 the frequency distribution of the (log-transformed) size of the known hosts for monogenoidean parasites in Italian marine (Strona *et al.* 2009) water is compared to that of the whole Italian marine fishfauna (according to Froese and Pauly 2009).

Usually the knowledge of parasite biodiversity in freshwater environment is less affected by similar bias, as a consequence of the closeness of the system, of the smaller number of available species and of several environmental features that make sampling in freshwater easier than in marine environment. This and the potential accessibility to the whole fish fauna assure the possibility of collecting a "real" random sample of the host species in the area of study.

1.3 – Monogenoidea – Host specificity

In the previous paragraph we discussed the (usually) restricted host

range of monogenoidean parasites. However we did not mean to associate it with the host specificity issue. From a theoretical point of view, the concepts of host specificity and host range do have to be distinguished, since host range results from the combination of parasite (intrinsic) host specificity and availability of compatible host species (Combes 1991)(Fig. 2).

The averagely narrow host range of monogenoidean parasites is considered as an obvious measure of their high (intrinsic) host specificity. This is hardly debatable and, as a consequence of the self-evidence of this fact, host specificity in Monogenoidea has been more often assumed than discussed. In some cases it has even been used to help host classification (Lambert and El Gharbi 1995). Although similar studies do not line up with the common approaches to the taxonomic investigation of Monogenoidea, yet they are indicative of a tendency of monogenoidean investigators to consider *where* a parasite is found as a constraining hint of *who* the parasite is.

A few considerations are therefore necessary to understand why a deeper, rigorous analysis of monogenoidean host specificity is necessary and how it could help understanding host specificity. First of all, several monogenoidean species have been collected on a quite wide range of host species. *Neobenedenia melleni*

(MacCallum, 1927) Yamaguti, 1963, which has been reported from more than 100 different host species, is probably the most remarkable example (Whittington and Horton 1996), but is not a unique case of (apparently) low specificity in Monogenoidea (see Chapter 2 for a more detailed discussion on *N. melleni* and its apparent low specificity). In Table 1 host range of several species of monogenoidean parasites from different areas and environments is reported to substantiate this assessment. Individuating the processes able to widen the host range is a reasonable way towards a better understanding of determinants of host specificity.

1.4 – Monogenoidea – Taxonomy and classification

Present classification of Monogenoidea is mostly based on morphometrical description of specimens (and particularly of their sclerified attachment hard part). Often the morphometrical discrimination of closely related specimens is tricky and relies on fine differences in intricate and small (<100µm) structures such as those of haptorial and copulatory sclerites (Fig. 3). The latter, in particular, are subjected to great morphological variability potentially related to the development of reproductive barriers in parapatric speciation events (Jarkovsky *et al.* 2004, Strona *et al.*,

2005). Morphometrical measurements of such structures can be deeply biased by manipulation and fixation of specimens. Monogenoidean sclerites are usually examined under optical microscopes and morphologically described using 2-dimensional drawings. Measurements are usually determined directly from specimens using a microscope equipped with an ocular or a micrometer, from drawings, or less frequently, using a digitizing system on photomicrographs (Ergens, 1969; Chisholm et al., 2001; Davidova et al., 2005).

The problem of these methods is that the sclerites of monogenoids normally do not lie within the visual plane of the microscope, thus requiring specimens to be moderately to heavily compressed on the microscope slide to orient structures to the optical plane of the microscope (see methods introduced by Malmberg 1957; Ergens 1969; Kritsky *et al.* 1978). Compression often results in the specimen being damaged, inevitably producing both morphological artifacts and metrical error. Moreover, such manipulations irreversibly compromise the natural relative and absolute positions of sclerites in the body. Galli *et al.* (2006, 2007) provided a non-destructive protocol of three-dimensional morphometry of monogenoidean sclerites using Laser Scanning

Confocal able to avoid over-estimation due to deformation and to reduce errors associated with different spatial orientations, permitting also the observation of morphological details not detectable in 2-D representations.

Molecular analysis could be a potential solution to the above described problems, but there are many limitations in collecting and preparing monogenoidean parasites for both morphological and molecular purposes. As already said, manipulating specimens usually requires the use of optical instruments and proper methodologies of preservation, staining and fixation to the study of inner organs and structures. Moreover, separating parasite specimens from their hosts in field is not always possible, and this makes the collection of the entire fish, or eventually of its gill baskets necessary to a further lab examination. The selected preservative medium, besides conserving parasites properly, is therefore required to maintain hosts in such a condition to allow an easy recover of monogenoideans. These requisitions are fully satisfied by hot (60 °C) 5% formalin, but it should be noticed that, normally, molecular analysis cannot be performed on formalin fixed specimens.

Actually, the most common protocols for genetic analysis of

monogenoids recommend to fix parasite specimens in ethanol at concentrations of 95% or more (Littlewood *et al.* 1998; Desdevises *et al.* 2002). As the collection of monogeneans from gills preserved in high concentrated ethanol is very difficult, and considering that specimens collected from ethanol-preserved gills appear dehydrated and breakable after a few time of storage, the general tendency of specialists is to work mainly on fresh fish, collecting parasite specimens from unfixed gills and then transferring them one by one in absolute ethanol. Obviously the possibility to perform a similar procedure is strongly limited by the time necessary to carry fish samples from their collecting site to laboratory. Moreover, there are many technical problems connected to field application of physical preservative techniques such as cryogenic conservation or drying.

Strona *et al.* 2008 demonstrating the suitability of using Dimethyl sulfoxide (DMSO) - sodium chloride (NaCl) solution (20% DMSO, 0.25 M disodium-EDTA, and NaCl to saturation, pH 7.5) as an alternative to formalin and ethanol in monogenoidean preservation for both field and laboratory analysis, encouraging studies involving large samplings in wide geographical areas, such as phylogeographic and biogeographic surveys, besides traditional systematic characterization of Monogenoidea (Fig. 4).

However, not many molecular studies have been performed on monogenoidean parasites, and most of them aim to describe evolutionary patterns more than to specific identification. Nonetheless there are just a few coevolutionary studies (which could eventually be much informative for the host specificity matter) for monogenoidean parasites and their hosts (see for example Desdevises et al. 2002).

On the other hand, monogenoidean ecology (and the ecological relationships between parasites and their hosts) has been quite extensively studied, both in natural environment and laboratory . Yet, the scale of most of these analyses refers to well known systems, i.e. the small natural ones or artificial (laboratory) ones (see for example Littlewood et al. 1997; Simkova et al. 2000, 2001a, 2001b; King and Cable 2007; King *et al.* 2009). Still, extensive analyses on general ecological processes IN Monogenoidea are still not common. This cannot be considered as a consequence of the unavailability of data of the class. A lot of information regarding species patterns in Monogenoidea is at scientists' disposal (see, for example, Kohn and Cohen 1998; Kohn *et al.* 2006; Salgado-Maldonado 2006; Salgado-Maldonado 2008; Cohen and Kohn 2008; Strona *et al.* 2009), even if it do not describe

the whole diversity of the class (see the above discussion regarding underestimation of monogenoidean diversity).

Poulin (2002) affirmed that if it is possible to detect a signal through the noise, it can not be dismissed because the coverage of the data set does not extend to all living species. Agreeing with this statement, we made use of large available datasets on monogenoidean parasites in different biogeographical areas and environments to investigate some major processes ruling the ecological relationships with their hosts.

CHAPTER 2

How host features affect parasite distribution in the compound community: a multivariate approach based on Ecological Niche Models.

2.1 – INTRODUCTION

2.1.1 - Host/Parasite Relationships - Overview

Many relationships between parasites and their hosts are closely related to host specificity. Host specificity is an intricate and complex concept, playing a fundamental structural role within the framework of ecological and evolutionary patterns and processes involved in the host-parasite symbiosis. Host specificity is obviously an intrinsic characteristic of the parasite but, at the same time, it is an adaptive answer to host evolution and ecology.

Coevolutionary scenarios have often been suggested as the most likely outcomes of parallel speciation processes in

host/parasite systems, as stated by Fahrenholz's Rule (parasite phylogeny mirrors host phylogeny) and Szidat's Rule (primitive hosts harbour primitive parasites) (Fahrenholz 1913; Szidat 1940; Eichler 1942, 1948) (Fig. 6). The development of molecular ecology provided new powerful tools to test the consistence of Fahrenzhold's prediction in well studied host parasite systems. Since it moved its first steps, the study of host parasite coevolution has arisen great interest among evolutionary scientists, offering a unique biological model to test theoretical assumptions of speciation mechanisms. The knowledge of host phylogeny and speciation events (i.e. the likely establishment of biological barriers potentially able to promote parasite speciation) provides an optimal background to study vicariance processes. Yet, most studies in which host and parasite phylogenies were found to be congruent refer to very particular groups with biological peculiarities that make host-switching events highly improbable (see Barker 1994). Coevolutionary scenarios appear to be less common than expected and modes of stenoxene speciation other than co-speciation also play an important role (Rozsa, 1991).

It should be noticed that most of the traditional methods to investigate coevolution in the host-parasite system are based on

different evolutionary assumptions and can therefore produce different results. Moreover, they are ideally designed for the one host-one parasite case, while the real situation is much more intricate (Legendre et al. 2002), considering also that cospeciation is not the only possible event related to simultaneous evolution of hosts and parasites: independent parasite speciation, disappearance of a parasite lineage on a host lineage, and host switching may eventually occur (Ronquist 1997; Charleston 1998; Page and Charleston 1998), making hard to reconcile host and parasite phylogeneses.

As regarding for highly host specific parasite such as Monogenoidea, the theoretical likelihood of Fahrenholz's and Szidat's Rules is hardly debatable (Jovelin and Justine 2002; Noble *et al.* 1989; Kearn 1994). However, coevolution between monogenoidean parasites and their hosts was considered (and eventually tested) by few studies, most of which supporting the commonness of host switching events (Boeger and Kritsky 1997). Desdevises *et al.* (2000; 2002) put on evidence the absence of coevolution between mediterranean sparids and their *Lamellodiscus* spp. Monogenoids, claiming the importance of ecological factors in the determination of the host-parasite associations (Fig. 6).

Assessing the relative importance of co-speciation and ecological factors/niche constraints in the qualitative determination of parasite host range is a key point in the host specificity issue. As already stated, the matter has been approached nearly exclusively from a coevolutionary perspective, with the application of molecular techniques and tools aimed to obtain and reconcile host/parasite phylogenies. According to this approach, the presence of a coevolutionary scenery is enough to exclude a major role of ecological factors in determining the qualitative distribution of the parasite fauna on the potential hosts. By contrast, ecological factors are usually considered the leading players in case of absence of consistent coevolution. Nonetheless, in the latter case (such as in Desdevises *et al.* 2000 and 2002), no further ecological study is usually performed. Assuming a co-speciation scenario as a theoretical null model to be compared with the available data can be helpful in searching for the ecological features potentially involved in the divergence from host-parasite coevolution. In the following chapters we will provide the theoretical background supporting this approach and the methodological tools for its performance.

2.1.2 - Host specificity, ecology and coevolution: the theoretical

linkages

Host-specificity, coevolution and ecology are intrinsically related. A host-parasite coevolutionary scenery can be considered as a sort of null model characterized by high (eventually exclusive) host specificity, whose linearity can eventually be altered by ecological factors into a more complex framework.

The involved processes can be simplified in a two steps model, as described in Fig. 7. (1) Speciation in a single host species (A --> B, C) possibly results in the extinction of the parasite species in the newborn host species or, eventually, in its coevolutionary speciation ($P \rightarrow P1, P2$). (2) At this point, if speciation occurred, a new range of host switch possibilities is therefore set, each of them able to lower the average host specificity of the system. Host switch events could eventually involve the two newborn sister species or other not phylogenetically related host species. Parasite species extinction is another fundamental aspect to be considered, as it could produce an apparently incoherent non-coevolutionary scenery with a high level of host specificity.

Looking at the matter from an arbitrary perspective, namely that of the newborn sister species, it is possible to model out how ecological factors and phylogeny meet on a temporal scale, by

tracking the probability of the newborn parasite species to colonize the sister host species (Fig. 8). Basically, this is the combination of the probability of the parasite to cross the barrier, or of the barrier to become ineffective (the “barrier” is the ecological/biological factor responsible of host speciation), and the probability of parasite host switch, which is related to phylogenetic distance of available hosts. If we suppose the event of crossing the barrier as random, we could assume that the probability for it to happen increases linearly during time (line A in Fig. 8). On the other hand, the probability of parasite host switch is expected to be highest among the newborn sister species and then to progressively decrease together with the increase of the phylogenetic distance (i.e. with evolutionary time) of the candidate hosts, until it assesses to a value comparable to the “random” probability of colonization of each fish hosts of the community (i.e. the “average” phylogenetic distance of the potential host assemblage) (curve B in Fig. 8). The intersection point of the two lines indicates the time when the leading role in driving host switch and colonization processes shifts from coevolutionary to ecological factors. The first part of the graph (the green one, in Fig. 8) is virtually effective for all parasite *taxa*, independently from their intrinsic host specificity (that is the eventual specificity related

to phylogeny), while the second part regards the generalist parasite *taxa* only. The chances of host switching for a parasite with high intrinsic host specificity will decrease while getting nearer to the intersection point, as a host switch event happening after that point would be contrasting to the phylogenetical definition of intrinsic host specificity. The direct logical consequence of this is that only parasite *taxa* with high intrinsic host specificity are likely to maintain the host/parasite coevolutionary pattern over time.

The reality is obviously much more complex and usually tends to diverge quite significantly from these patterns, as the dichotomic classification of parasite into generalists and specialists is just an artificial simplification of a wide continuous range of specificity. Therefore, although parasite *taxa* characterized by high intrinsic host specificity are likely to produce coherent coevolutionary scenarios, deviations can (and are likely to) occur.

2.1.3 - Application of ENMs to the host-parasite system

The ecological relationships between monogenoidean species and their hosts have not been object of much study effort, especially from a qualitative point of view (see for example Guégan and Kennedy 1993; 1996). In general, most of the study investigating

fish parasite species assemblages focus on the potential factors responsible for species richness more than to those involved in the determination of species composition (Poulin, 1997). The application of Ecological Niche Models (ENMs) to the host parasite system could help reduce the gap.

ENMs are GIS applications developed to create maps of potential species distribution. ENMs relate point occurrence data of a species to the ecological and environmental characteristics of a landscape to model the dimensions of the species' niche. The obtained niche model can then be used to identify geographic regions "fitting" the species' niche, producing a hypothesis of a potential geographic distribution for the species (Anderson *et al.* 2002a; 2002b; 2003). A set of locality points of presence of the considered species and a stack of environmental GIS-based georeferenced layers describing the habitat of the area of study are requested as input. ENMs softwares are able to execute a multivariate analysis (according to different algorithms, usually selected by the user) on the environmental features at each locality where the considered species has been recorded, in order to build up a multidimensional niche model for the species. Then the softwares individuates all the other locations within the area of study

satisfying the criteria of the niche model. These locations represent the potential distribution of the species under examination. Genetic Algorithm for Rule-set Prediction (GARP) (Stockwell and Peters 1999) and Open Modeller (Muñoz et al. 2009) are the main ENM software applications.

The logical key points supporting the use of ENMs in the host/parasite system rely on the identification of the host to a locality (see Chapter 1). This approach is aimed to model the niche of a parasite species on its host species, and successively compare it with the niche of other species (eventually those inhabiting the biogeographic area/region of interest) in order to estimate the ecological probability of a fish species in the system to be colonized by the parasite species under study.

2.2. – Methods

2.2.1 - From the geographical space to the compound community: general adjustments

ENM softwares require the input files to be formatted according to particular standards of georeferenciation, as the spatial coherence of

occurrence points and environmental layers is the fundamental premise to draw projections of species distribution. Host-parasite data require therefore to be virtually georeferenced prior to be processed by the softwares. This can be achieved by attributing to each host an arbitrary pair of x/y coordinates (ideally corresponding to latitudinal and longitudinal values). Basically this implies to order the species in a grid, or, more simply, in a stack, to univocally codify rows and columns, and then to take note of the position of each species in the grid (or stack). Environmental layers containing information about the host species under study must be ordered coherently with the host positioning on the grid.

2.2.2 - An exemplificative case study: *Neobenedenia melleni*

(MacCallum, 1927) Yamaguti, 1963

To a clearer explanation of the ENM approach, a case study on the low host specific monogenoidean parasite *Neobenedenia melleni* is reported. Having been recorded from more than 100 fish host species (Whittington and Horton 1996), *N. melleni* is a remarkable exception in the general context of high specificity characterizing the Monogenoidea. According to recent molecular evidences (Whittington *et al.* 2006) the long time studied *N. melleni* is more

likely to be a complex of species than a single one. However, even if we assume that the results of these preliminary studies delineate the taxonomic position of *N. melleni* more realistically than the previous knowledge did, this does not affect the core question we are addressing to. The eventual fine molecular distinctions between the different species ascribable to *N. melleni* complex do not weaken the remarkable contrast (from a coevolutionary perspective) between host range width and phylogenetic closeness of the species of the complex. On the contrary, the phylogenetic peculiarities of *N. melleni* make it an optimal model to study how ecological factors can affect host range. Considering the diagram in Fig. 8, one could state that *N. melleni* has definitely crossed the ideal time border separating coevolutionary and ecological factors. A similar assumption is not unlikely, being indirectly supported by both ecological and evolutionary hints (large host range and phylogenetic variability respectively).

As already stated, the ecological factors considered by the described approach refer to features of the potential and effective host species in a certain area of study (the environmental ecological features of the area itself are therefore only indirectly considered). The analysis was performed using the dataset provided by Kohn *et*

al. (2006), which reports the host records for monogenoidean parasite in central America. Although *N. melleni* has been collected also from brackish and freshwater species, only the marine records were used.

The overall aims of the study were a) determine the niche boundaries of the “ideal” host for *N. melleni* by using a subset of the fish species known to host the parasite in the area; b) use the obtained niche model to produce a hypothesis of distribution of the *N. melleni* on a set of potential host species (other than those used to build up the model) in the area of study; c) attest the likelihood of the model by comparing its prediction to the known records of *N. melleni* on the projection species set (not necessarily referred to the area of study).

In particular we chose carangid species inhabiting the area of study as the projection set. From the records of *N. melleni* in Kohn *et al.* (2006) we therefore excluded those from carangid species to build up the model (in order to avoid circularity in the procedure). We used Open Modeller Software to create the models (our choice was influenced by the completeness of the software in terms of implemented algorithms). The list of species of both the training set (the species used to calculate niche boundaries) and the projection

set are reported in Table 2 and Table 3 respectively, together with the associated ecological and biological features used to build up and test the model. Considering the absence of any assumption or pre-knowledge regarding the ecological processes shaping parasite distribution on the host set, the choice of the host features to be included in the model was orientated towards a few macroscopical biological and ecological aspects such as standard length (cm), resilience (K), natural mortality, life span, trophic level and food consumption (as defined and calculated by Froese and Pauly 2009). As regarding for the arbitrary georeferenciation of the considered fish species, for a simpler observation and interpretation of the output produced by the software, we chose to arrange the host species in two columns, one containing the species of the training set, and the other one those of the projection set (i.e. central American carangid species).

The choice of the algorithms obviously affects deeply the resulting model. Another key point strongly influencing the outcome of the model is the choice of the ecological layers to be used in the analysis. However, the aim of our study was to create a model to be tested against a real situation and to test the relevance of the selected ecological parameters in the distribution of the parasite

species under analysis on the host set.

Using three different algorithms we created three different models that we compared to put on evidence the most coherent patterns. As a consequence of having no premises regarding the mechanism ruling relationships between the ecological layers we selected three of the less assumptive algorithms among those provided by Open Modeller Software:

- GARP: Genetic algorithm that creates ecological niche models that describe environmental conditions under which the species should be able to maintain populations;
- BIOCLIM: Uses mean and standard deviation for each environmental variable separately to calculate bioclimatic envelopes;
- ENVIRONMENTAL DISTANCE: Generic algorithm based on environmental dissimilarity metrics.

More detailed information for each algorithm is available in the software online help function. Then we checked out the projections obtained from the different models searching for coherence in the depicted probability patterns.

2.3 - Results and discussion

The projection maps of the different models and the overall probability map, obtained from the comparison of the latters, are shown in Fig. 9. Each square (corresponding to a fish species and virtually georeferenced) is associated to a value of probability of that species to host *Neobenedenia melleni*. The model integrates a self evaluation measure of its accuracy based on the expected/unexpected ratio of the species occurrence points used to build the model, calculated according to the probability values associated to each square. Although this mechanism could seem biased by circularity, it is not, since the measure of accuracy provided by the software should be regard just as a measure of coherence (or better, homogeneity) of the “environmental” parameters associated to the occurrences of the modeled species. It is to say that the model as it stands is not a provisional model. Or better, the projection map produced by the software is not meant to individuate the locations where a species is going to be found. Actually, the model just measures how much each of the considered locality fits with the “typical” habitat of the species under analysis, where “typical” refers just to the parameters taken into consideration

by the experimenter (i.e. the choice of the environmental layers is the most important point in the analysis). Considering the application of ENMs to the host/parasite system, the obtained results (providing probabilistic model of *N.melleni* host range) have to be critically considered according to the concept described by Fig. 2. The models calculated by the software give information on the compatibility of the considered parasite/s towards potential host species. The other key factor involved in the determination of parasite host range (i.e. the actual encounter between the parasite and its compatible host/s) obviously can not be integrated in the model. Another fundamental aspect to be discussed is the choice of the algorithm to be used to calculate parasite niche. Some of the algorithms provided by Open Modeller Desktop Software were originally developed for very specific applications and therefore are not suitable to be used in cases other than those. On the contrary the application of not assumptive algorithms (such as the ones we actually used) is virtually free from specific bias.

However, most of the problems connected to the choice of environmental layers and of the algorithm to combine them are limited by the possibility of performing multiple analyses, producing not a single model, but a set of models to be compared in

the search for coherent patterns.

From the three model we produced, it was possible to individuate a few species coherently suggested to be potential hosts for *Neobenedenia melleni*. It is interesting to notice the fact that the fish species indicated by the three model to have the highest overall compatibility with *N. melleni* is *Trachinotus goodei*, already known from literature (Salgado-Maldonado 2006) to actually host *N. melleni*. This should not be considered as a proof of the provisional power of the model, but a demonstration of the relevance of the considered ecological factors in the determination of *N. melleni* host range.

From a general point of view, testing the relevance of ecological factors in the qualitative determination of parasite host range is, as already stated in the introductory paragraph of this chapter, a fundamental point within the host specificity issue. On the other hand, there are many other more “practical” application of the described approach, such as the individuation of potential unrecorded host species, or the creation of “potential host maps” for the parasite within a biogeographical context, in order to assess risk related to the introduction of species.

CHAPTER 3

Nestedness as a measure of intrinsic host specificity

3.1 - Introduction

3.1.2 - *Nestedness*

The ecological approach described in the precedent chapter provides a set of statistical tools to test the relevance of host ecological features in shaping a compound community. As already stated, weighting the influence of ecology in the host parasite system is necessary to a complete comprehension of patterns and processes involved in the host specificity matter. However, ecological niche models applied to the host parasite system are more useful to the creation of null models to be compared with available data, than to the analyses of those data. Desdevises *et al.* (2002) stated the absence of coevolution between mediterranean sparids and their monogenoidean parasites *Lamellodiscus* spp (Fig. 6). Nonetheless,

considering what stated in paragraph 2.1.2, the intricate scenario of Fig. 6 does not necessarily imply the exclusive role of ecological features in determining the distribution of parasites on their hosts or, more important, the absence of intrinsic host specificity. Here we describe an approach based on nestedness analysis, proposing it as an instrument to measure the intrinsic host specificity of different parasite taxa without considering neither the host nor the parasite phylogeny.

Nestedness is a common natural pattern which occurs when the species composition within each community in an area is a proper subset of the species composition within the immediately richer community. In a nested metacommunity, the most common species is present in most of the communities, while the most rare one occur only in the richest community (Hultén 1937; Darlington 1957; Daubenmire 1975). Atmar and Patterson (1993) suggested the use of entropy (“temperature”) of a given species/area matrix as a measure of its nestedness. According to Atmar and Patterson's definition, the more nested a matrix is, the more nearer to 0° its temperature is. The protocol to assess nestedness of a matrix basically consists of three subsequent steps. The first one is ordering the matrix according to row and column sums, in order to draw an

isocline. This makes possible to individuate the “unexpected” presences or absences (in respect to the condition of perfect nestedness). The actual degree of nestedness is therefore calculated by measuring the distance of the unexpected presences/absences from the isocline (temperature) or, alternatively, by counting them. The third step is the validation of nestedness measure through the use of a set of random matrices produced according to certain rules/null models (Patterson and Atmar 2000).

Atmar and Patterson (1995) developed a software dedicated to the measure of nestedness (*NTC - Nestedness Temperature Calculator*) that has been used for a wide range of applications. Yet, much criticisms was arisen by their choice of temperature as a measure of nestedness and by a few other bias related to the poorly informative output produced by the software. *Nestedness* software (Ulrich 2006), as well as *BitMatNest* (Rodríguez-Gironés and Santamaría 2006) overcame most of these problems providing scientists with much more freedom in the choice of nestedness indexes and null model. A critical review on these subjects and a “consumer's guide” to nestedness are provided by Ulrich and Gotelli (2007) and Ulrich et al. (2009) respectively.

3.1.2 - The application of nestedness to the host/parasite complex

Although nestedness paradigm has been mainly used in medium scale metacommunities studies within a biogeographical context, it nonetheless revealed great theoretical potentialities when applied to different fields of research through various biological and ecological approaches. There are several studies regarding the application of nestedness to host-parasite systems (see, for example, Poulin 1996; Poulin and Valtonen 2001; Fellis et al. 2003; Nieberding et al. 2005; González and Oliva 2009). Most of them refer to the component community level (that is considering all parasite species in all the individual of a single host population), where significative nestedness is measured when species with high prevalence occur in all kinds of infracommunity and low prevalent (rare) parasite species occur in species rich infracommunities only. The results of the available studies are contrasting and sometimes apparently biased by the parameters applied to nestedness analyses.

However, not many studies have been performed at the compound community level, where nestedness is detected if each component community is a subset of the immediately species richer component community, i.e. when generalist parasite species occur in all kinds of component communities, while specialist parasite

species only occur in species rich component communities. This, in a natural condition of non-perfect nestedness, would suggest that generalist species host range is randomly assembled. On the contrary, a non-nested or an anti-nested pattern would suggest that host range is not randomly assembled even in generalist parasite species (Poulin 1997). According to these principles, we propose to use nestedness of fish parasites on their host species at the component community level as an indirect measure of intrinsic host specificity. A case study follows.

3.2 – Methods

3.2.1 - Datasets and Nestedness analysis

To test the approach described in the previous paragraphs of this Chapter, we measured and compared nestedness of different parasite taxa among a fish host set within a biogeographical region. Since as there are many problems in comparing nestedness from different matrices, we developed a procedure of standardization that will be extensively explained in the next paragraph.

Analyses aimed to compare nestedness of different parasite

taxa within a component community were performed on several parasite-taxa/fish-host matrices compiled using the checklists from Salgado-Maldonado (2006) (freshwater) and Williams and Bunkley-Williams (1996). In particular we considered the following parasite taxa: Acantocephala, Cestoda, Digenea and, obviously, Monogenoidea.

3.2.2 - How to compare nestedness of different matrices: principles and general procedure

Nestedness analysis was performed on those matrices using the software Nestedness, by Ulrich (2006). Nestedness was assessed using Z-values. For this purposes a large number of random matrices is generated according to certain rules (null-models), for each matrix the nestedness statistic is calculated and these nestedness values are averaged. Then, the difference between the observed value and the mean value of simulated matrices, divided by their standard deviation, is computed as Z-value. Because the distribution of Z-values follows that of a standardised Gaussian distribution, Z-values < -2 indicate a significant nestedness. So,

$$Z = [Ta - Tr] / SDr$$

where T_a is the actual temperature of the matrix under study, T_r is the average temperature of a set of matrices obtained from randomization of the matrix, and SD_r is the standard deviation of temperatures of the random matrices. No constraint null model was used to randomization.

As already stated negative Z values point to nestedness. Yet, it is quite common to obtain Z values far smaller than the significance limit. Although the use of Z s is appropriate to assess whether a given matrix is significantly nested or not, it does not help comparing matrices of different size to establish if a given matrix is more or less nested than another one. In other words, Z -values are not directly comparable.

To compare nestedness of different matrices, some researchers use the values of the observed statistics. For example, using the matrix temperature as a measure of nestedness, a matrix with a lower temperature is considered more nested than one with higher temperature (Patterson and Atmar 2000).

This approach, however, has been questioned, because nestedness metrics, and in particular matrix temperature, are influenced by matrix size . Although it is well known that

temperature tends to increase in larger matrices, there is no clear relationship (e.g. a linear dependence) between temperature and matrix size. So, it is difficult to standardise temperature values by matrix size (Gonzalez and Poulin 2005).

This problem is particularly compelling when the original matrix is partitioned into submatrices. When analysing a nested matrix, researchers can be interested in finding if some sectors of the matrix are more nested than others. For example, in biogeographical analyses, one can ask if a certain group of areas in the whole matrix is more or less nested than other area groups.

In spite of the interest in this topic, the problem of comparing nestedness of submatrices has been so far largely unanswered. An approach to circumvent this problem is here presented and applied to the host/parasite matrices under analysis. To make comparable the Z-values of submatrices of different size we elaborated a method based on the construction of a regression line. This curve (which is typical for each matrix) is constructed to model variation of Z-values in response to matrix size using randomly extracted submatrices of different size. Thus, the curve represents the expected trend and its confidence intervals can be used to assess if Z-value of a particular submatrix deviates significantly from that predicted by

the curve for a submatrix of the same size extracted at random from the complete matrix.

To develop this approach we therefore proceeded in the following way. First, we noticed that the negativeness of Z-scores depends on the mean of the values of the nestedness metrics calculated from randomized matrices. Z-scores are defined as

$$(1) Z = (x - \mu) / SD$$

where x is the nestedness value observed for the examined matrix, μ is the mean of the nestedness values calculated for the simulated matrices, and SD is the respective standard deviation.

Now, for a given matrix, the larger μ , more negative Z . It is expected that μ tends to increase with matrix size. To make less abstract the reasoning, we will refer to the behaviour of the temperature (T) as a metric of nestedness. Although temperature is not appropriate in all circumstances, it is a widely used nestedness metric and it can be easily interpreted with reference to the concept of entropy. We expect that the values of T of the simulated matrices (i.e. the expected T_E values under the null-model), and their mean value $T_{E\mu}$ (corresponding to μ in the general formula of (1)) increase with matrix size. This is because these values of T are calculated on random matrices, which become more and more ‘disordered’ as

their size increases. Larger matrices offer more opportunities of re-allocating species presences in a random way. In fact, in addition to matrix size, also matrix fill should be considered, and we empirically tested our predictions using three parameters that are expected to influence T_E , i.e. matrix size (number of columns x number of rows), matrix fill and number of occurrences. Obviously, these three measures are not completely independent, because if fill is fixed (i.e. for equally filled matrices), the number of occurrences is proportional to matrix size. However, we anticipate here that, using matrices of different size, fill and number of occurrences, we found tight correlations between these parameters and $T_{E\mu}$ (see next paragraph). The same does not hold for the observed temperature, T_0 . While T_E values are calculated on random matrices, T_0 is the value actually observed for a given matrix. We expect that, as a result of a random distribution of presences in the randomised matrices, T_E (and hence their mean $T_{E\mu}$) tends to increase with matrix size, while T_0 is not expected to be strictly dependent on matrix size. We can find a small and highly ordered (nested) matrix, a small but highly disordered matrix, a large and highly disordered matrix, and a large and highly ordered matrix. On the other hand, in the real world, we can presume that at increasing size of the matrix,

even under conditions that should determine highly nested patterns, there will be random factors that will introduce a certain degree of disorder. For example, at increasing matrix size, absences due to lack of research, or occurrences due to unpredictable and rare events, become more likely, thus introducing a certain degree of ‘additional’ disorder, to which smaller matrices are less exposed. Thus, although T_0 are not expected to be strictly influenced by matrix size as the randomly generated T_E , a certain influence of matrix size is expected.

As a result of the different behaviour of T_0 and T_E (and hence $T_{E\mu}$), we expect to find: (1) a tight positive correlation between $T_{E\mu}$ and matrix size, (2) an irregular pattern of variation of T_0 in response of matrix size (with T_0 becoming however typically high in larger matrices), and, finally, (3) a dependence of Z-values on matrix size. We then partitioned a given matrix into several submatrices of different sizes. These submatrices are constructed by a extraction of a given number of columns (sites) from the original, complete matrix. Because columns and rows are taken at random, we can construct several sub-matrices of a given size, by changing the number of rows and/or columns to be retained. For each set of submatrices we calculated Z values, each given by the formula:

$$(2) Z_i = (T_s - T_r) / SD$$

where T_s is the temperature observed for the i -th matrix obtained from the original matrix, and T_r is the mean of the temperatures obtained from the matrices constructed by randomising the i -th matrix.

Then we regressed each one of these Z -values against a parameter that indicates how large is the corresponding i -th matrix (e.g. matrix size, fill or number of occurrences). This regression line expresses the increase in the magnitude of Z (i.e. its increasing negativity) with the size of submatrices obtained by random sampling different numbers of rows or columns and can therefore be used to calculate the expected Z -value for a submatrix of any size under the assumption that sites have been extracted at random. Moreover, the observed Z -value for a selected submatrix of a given size can be compared to the expected Z -value for a theoretical submatrix of that size constructed by random sampling the columns of the original matrix, as expressed by the regression line. Significant deviations between expected and observed Z -values suggest that the observed sub-assemblage of sites has a nestedness significantly different from that expected for a random sub-assemblage of the same size, quantifying such difference using the

confidence intervals of the regression line. Moreover, the distance of observed Z-values from the regression line may be used to compare Z-values, which are per se not comparable.

3.2.3 - Comparing nestedness of different parasite taxa to assess their “intrinsic” host specificity

We compiled a host/parasite presence absence matrix for each one of the considered checklists (Salgado-Maldonado 2006; Williams and Bunkley-Williams 1996), reporting the known host records of several taxa of metazoan fish parasites in Mexican freshwater fishfauna and Puerto Rico marine fishfauna, respectively. From each of these matrices we then extracted four submatrices reporting host records for Acantocephala, Cestoda, Digenea and Monogenoidea respectively. The complete matrices were processed according to the statistical protocol described in the previous paragraph. A set of one hundred submatrices was created for each complete matrix by extracting a random number of random rows and columns from the two complete matrices. Z values were calculated for each submatrix (no constraints - equiprobable rows and columns - null model for randomization; 100 iterations to compute standard deviation of the null model; 0.5 set as minimum distance to the borderline; matrix

packed according to richness). A regression analysis was performed between the Z values of each submatrix and the corresponding matrix occupancies (number of presences). The so obtained regression lines were used to estimate the expected Z values for a set of random matrices with a number of occupancies respectively equal to that of the parasite-taxa submatrices. Actual Z values of the parasite-taxa submatrices were then compared to the expected ones.

3.3 - Results and Discussion

All the matrices compiled to be processed for nestedness analysis are provided as supplementary material (S1), as well as the script (in Python language) for row and column extraction (S2).

The graphs plotting Z values of the random submatrices against their cell occupancy number are reported in Fig. 10 and Fig. 11 together with the equations of the regression lines and the R^2 values assessing their accuracy. Z values resulting from Nestedness analyses of the submatrices for both Mexican freshwater and Puerto Rican marine waters are reported in Table 4, together with some basic information regarding the matrices themselves (row and column number, fill). The expected Z values (calculated using the equations of the regression lines), and the difference between them

and the actual Z values of the submatrices are reported as well.

In both the considered parasite assemblages (hosted respectively by Mexican freshwater fish fauna and Puerto Rican marine fishfauna, among the considered parasite taxa, monogenoidean parasite species showed the largest negative difference between the expected and the calculated Z values. This is coherent with the ideal high intrinsic host specificity of Monogenoidea, since it means that the considered monogenoidean assemblage is less nested than expected. Considering what already stated in the introductory paragraph of this Chapter, this means that in the host range of monogenoidean “generalist” species (i.e. those species present in more than one host species) is not randomly assembled as it appears to be for the generalist species of other metazoan fish parasite taxa. In other words, monogenoidean generalists are not species with no particular preference for one host instead of another, but are just specialists whom specificity is directed towards a quite wide range of host. Their intrinsic host specificity is therefore higher than that of other fish parasites taxa. Although this could appear quite obvious considering the Monogenoidea, yet this approach offer a new way to easily ponder host specificity from an ecological perspective, overcoming most of

the difficulties related to the coevolutionary approach.

CHAPTER 4

Parasite host specificity within the component community: ecological and evolutionary implications.

4.1 - Introduction

Although host specificity is a property of parasites, it should be considered that the main evolutionary pressures affecting it derive from their interactions with the hosts (both effective and potential) and, eventually, by interspecific interactions of parasites within a host (considered either as an individual or as a species). The necessary premise for a parasite to colonize a new host species is the presence of an available niche on the new host. The niches on a single host species are supposed to be quite limited in number and size. However, given the repetitiveness of biological and ecological features among a wide range of host *taxa* (or, even better, guilds), the number of available niches increases together with the number of available potential host species (at least from an evolutionary perspective) (Rhode, 1981). The parasite species distribution within

a compound community is therefore to be considered as the result of:

- niche availability on the (potential) hosts;
- a potential of host colonization intrinsic of the parasite (and closely related to the niche/s to be colonized);
- host peculiarities determining its chances to be colonized by a certain parasite species: the presence of a suitable niche on a host does not necessarily imply the presence of a parasite species potentially able to colonize that niche; there are a few other necessary premises to be satisfied for the colonization to be successful, such as the contactability of the host by the parasite and the ability of the parasite species to evade host defenses (immunitary system and eventual strategies to avoid infections).

Another important aspect to be considered is that of the interspecific interactions (such as competition) within the host species, as they can play a significant role in shaping the qualitative composition of the component community. Among the various potential consequences of competition among parasite species within a single host species, the most likely two are the exclusion of

the weakest competitor/s or, eventually, the niche splitting. Niche splitting usually implies an increment in parasite specialization, that should almost necessarily be paired to an increment in parasite host specificity (Bush *et al.* 2001).

Even for the most studied host/parasite complex it is very difficult to individuate the ecological and evolutionary key factors determining and derived from (eventual) niche splitting. Another, more feasible approach is a general comparison between the overall, potential competitive pressure (simply measured as number of parasite species in a component community) and the eventual adaptive response (i.e. the average host specificity of the parasite species of each component community). Yet, most of the ecological studies conducted on the host/parasite system attempted to individuate the determinants of parasite species richness within a host species (see for example, as regarding for the freshwater environment, Guégan *et al.* 1992; Guégan and Kennedy, 1993; Kennedy and Guégan, 1994). On the contrary, little is known about the qualitative composition of parasite assemblage (i.e. about what types of parasites are present in an assemblage in respect to the species richness of the assemblage). The studies by Kennedy and Bush (1994) and by Poulin (1997) are an exception to this tendency.

Both works show how a distinction between generalists and specialists is useful to describe the structure of parasite assemblages: rich parasite assemblages (on a host species) usually consist of both generalist and specialist parasites, whereas poor assemblages usually include only generalists. This means that the parasite species assemblage is nested across the host species set. The two above-cited papers focus on temperate freshwater fish (from Canada and Great Britain). Only Poulin (1997) approached the matter using nestedness, and he encountered some difficulties in detecting nested patterns, due to significant differences in study effort of the available data (that he finally had to standardize).

However, the assumption of parasite nestedness among the host range implies: an high degree of interspecific interaction among the considered parasites (from an evolutionary perspective, as we will discuss later); a parasite species assemblage rich enough to put on evidence the significant distributional patterns of parasite species within the considered host assemblage. Considering that diversity in the temperate areas is much lower than that in the tropics (Pimm and Borwn 2004), and the crucial differences between freshwater and marine environment (see Chapter 1), it is self-evident how the issue of the (eventual) relationships between

parasite specificity and parasite species richness within a fish assemblage still needs to be extensively tested. Here we provided new results on this matter by examining different host/parasite assemblages from marine and freshwater tropical and temperate environment.

4.2 - Methods

Host parasite datasets for different biogeographical regions and environment were compiled using the checklists by: Williams and Bunkley-Williams 1996 (Puerto Rico and Western Atlantic, marine); Holland and Kennedy 1997 (Ireland, freshwater); Kohn and Cohen 1998 (Amazon river, Monogenoidea only); Kohn *et al.* 2006 (Central America, marine and freshwater, Monogenoidea only); Salgado-Maldonado 2006 (Mexico, freshwater); Cohen and Kohn 2008 (Amazon river, Monogenoidea only).

For each area of study a host/parasite presence/absence matrix was compiled basing on the host/parasite records provided by the checklist. Taking into account the biological, ecological and evolutionary differences between ecto- and endoparasites (Noble et al. 1989), we created two distinct sets of matrices (one for the endoparasites grouped together – Acantocephala, Cestoda, Digenea,

Nematoda –, and the other for the Monogenoidea only). Total number of host records (host range) for each parasite species was used as a measure of its host range (according to Poulin (1997)). For each fish species within the matrix the average host range of the harbored parasite species was calculated and plotted against the corresponding species richness of the known parasite assemblage within that host. A set of random compound communities of different sizes were created as null models to be compared with the observed patterns (the script, in R language, used to produce the random matrices is provided as supplementary material to this thesis (S3))

A nestedness analysis was then performed on the same matrices using the software Nestedness (Ulrich 2006) set according the following parameters:

- no constraints (equiprobable rows and columns) null model for randomization;
- 100 iterations to compute standard deviation of the null model;
- 0.5 set as minimum distance to the borderline;
- matrix packed according to richness.

Nestedness was assessed as Z values (see Chapter 3 for a deeper discussion on nestedness theoretical aspects and methods).

4.3 - Results and Discussion

Host/parasite presence absence matrices are reported as supplementary material to this thesis (S4). Three of them (referring to the following biogeographical areas/environments: Ireland – freshwater; Mexico – freshwater; Puerto Rico and Western Atlantic – marine) reported data from both freshwater and marine compound communities of different metazoan low specific fish endo-parasite taxa (comprising acantocephalans, digenetic trematodes, cestodes and nematodes), while the others (referring to the following biogeographical areas/environments: Amazon River – freshwater; Central America – freshwater; Atlantic Central America - marine) include monogenoidean species only.

Results of Nestedness analysis are reported in Table 5. The matrices containing different parasite taxa (Ireland – freshwater; Mexico – freshwater; Puerto Rico and Western Atlantic – marine) were significantly nested, as expected considering the results obtained by Poulin (1997). On the contrary, the three matrices reporting monogenoidean occurrences only showed an antinested pattern, confirming what already stated in the previous Chapter.

Graphs illustrated in Fig. 12 plot the average host range of parasite species of each host species versus the corresponding number of parasite species recorded on that host. A common general pattern repeats itself throughout the graphs, characterized by a heteroscedastic distribution. Variability in average host range is much higher in poor parasite assemblages than in rich ones. Considering that the number of rich component community in each of the studied areas tends to decrease together with the corresponding number of hosted species, we can not *a priori* exclude the eventual effect of random sampling: a wide number of high and low values are apparently likely to assess average host range of parasite species in a large component community to a medium value; on the contrary the average host range in a poor component community is much more affected by the presence of parasite species with very high or very low specificity. However, the distributions produced by hypothetical random compound communities (Fig. 13) appear to be very different from the real ones, pointing to a non-randomness of the observed patterns (and therefore supporting the underlying assumptions. Additional random matrices and their resulting outputs are provided as supplementary material (S5).

Even if the observed patterns reflect exactly what we expected to detect, still it is noteworthy the fact that the parasite species with lowest and highest specificity are present in the poorest component community, while specificity tends to assess itself to relatively low values in richer component communities.

It is reasonable to hypothesize that the processes determining the size of the compound community are involved in the described patterns. Fish species bearing a low number of parasite species can be assumed to be difficult to be infested by parasite species. One of the most important features involved in infestation processes is the contactability of the host by the parasite, that is (especially for parasite with direct life cycle such as Monogenoidea) partially determined by the degree of interspecific interactions of the considered host species (both in terms of duration and number of contacts). A host that interacts weakly with other species has few chances of being infested, and he would probably be much more susceptible to be colonized by very low specific parasites (called them “opportunistic”). On the other hand, if the contact with the “parasite donor” is episodic or restricted in terms of time duration, given the stable establishment of the symbiotic relationship, the new colonized host will provide enough isolation to the parasite species

from its original pool to acquire more and more specialization on the host, taking advantage of the low number of potential competitors. A similar evolutionary scenario appear quite likely at least to explain the fact that very highly specific parasites are present mainly in species poor component community.

CHAPTER 5

Relationships between trophic level and host specificity

5.1 - Introduction

Monogenoidea have a single-host, direct life cycle. However certain marine genera (*Pricea* and *Gotocotyla*) have been observed to infect various fish where they do not develop beyond a certain stage, and to achieve maturation only after ingestion by a “final” host (usually a large Cybiidae mackerel) (Rohde 1993). Circumstantial evidences of this behavior have been provided by Bychowsky and Nagibina (1967), even if still not supported by experimental proofs.

The (presumable) *Pricea* and *Gotocotyla* are clearly two exceptions to the monoxeny of Monogenoidea, still this singular adaptation is related to evolutionary pressures eventually responsible for alteration in parasite host specificity and indirectly connected to host trophic ecology.

Actually, host trophic level could be assumed to affect the specificity of the parasites of its component community, both at an

ecological and evolutionary scale. A simplified vision of the ecology of the two guilds at the opposite extreme of the trophic level range in fish (i.e. piscivorous and prey fish) is useful to understand the seeds of these assumption. Ecology of predators is obviously very different from that of prey. Among the several self-evident peculiarities distinguishing the two groups, the one most likely affecting host specificity are once again related to host contactability. Predator populations are smaller than the prey ones, so that the density of individual of a certain predator species in an area is much lower than that of the preys it feeds on. The tendency for predators to live in small groups or even isolated is obviously closed related to this aspect, as well as to other ones of behavioral ecology induced by competitive pressures. On the contrary the common behavior for the prey is that of sticking together as a defensive strategy (and obviously as a direct consequence of the high population density). In other words, a predator is much less prone to intraspecific interactions than a prey is (though it would eventually interact with a wide range of prey species). From the perspective of the potential parasite species, this makes the intraspecific transmission of direct life cycle parasites unlikely to happen (unless the prey intraspecific isolation is somehow reduced,

as it happens for example in case of reproductive aggregations).

Such difficulties should result in a low host specificity of the parasites harbored by a predator fish, as specialization on an habitat difficult to be reached would constitute a clear evolutionary paradox: although specialization on a niche difficult to be reached could reveal worth evolving, it should have to be driven by some common vector events. For parasite with complex life cycle this happens quite often. The predation behavior of a certain species on a restricted range of host species is able to determine the specialization of some parasite species (generalist on the prey set) on the predator species as the final host. For monoxenous parasite species predation obviously can not be considered as a potential vector to drive the parasites towards the host, as it would negate the monoxeny. It is therefore noteworthy that a similar phenomenon apparently evolved even in an exclusively monoxenous group such as Monogenoidea.

Assuming this as an (eventual) exception, the described theoretical premises suggest, from an ecological point of view, a potential correlation between the trophic level of a species and the relative specialization of its monoxenous parasites. This would eventually led to a high species richness in the component

communities of fish species at upper trophic levels.

From an evolutionary point of view, apart from the weakly documented deviations from monoxeny, it is reasonable thinking at the eventuality of predation-driven host switch events in response of repetitive predation on a restricted range of prey species, particularly if we consider contactability of the host by parasite species as a key point in the establishment of the symbiotic relationship.

5.2 - Material and Methods

5.2.1 - Host trophic level vs. parasite host range

Host/parasite data were obtained from the checklists by Cohen and Kohn (1998) (South America, freshwater) and by Kohn *et al.* (2006) (Central America Marine). Host were grouped into families. Trophic level values for the hosts were obtained from fishbase (Froese and Pauly 2009). Among the various families, we selected for our analyses the most homogeneous in terms of trophic level of their species members: Characidae, Cichlidae, Loricaridae and Serrasalminidae as regarding for freshwater environment; Lutjanidae, Carangidae and reef prey fish as regarding for marine environment.

It is self evident that “reef prey fish” is not a family; nonetheless, the similarity in the trophic level of the species included in this category justifies its use. It should be noticed that the choice of using families to test our assumptions is not driven by any phylogenetic implications, since we are testing the evolutionary effect of a process (predation) that is purely ecological. We used families simply as a shortcut to group easily fish species with similar diets. Standard deviation from the average value of the trophic level of the considered families was calculated and used as a confidence measure of this assumption.

Host range for each parasite species was calculated as total number of hosts known to be infested by the parasite species in the area of study. A regression analysis was then performed between the average trophic level of the fish species in the area of study belonging to the same family and the overall average host range of the monogenean species harbored by the members of the family.

5.2.2 - Predation and host switch

The impossibility of a direct test of the past occurrence of host switch events driven by predation is self evident, as in any other analysis of patterns produced by processes over evolutionary time.

Yet, there are a few analyses useful to an indirect test of the hypothesis.

Among these an interesting possibility is that of testing the eventual overlapping in the specific distribution of parasite species in each possible host species pair, in order to test how often the parasitofauna of a predator is significantly shared by a prey (in a biogeographic area, i.e., potentially, in the same food web). The fundamental premise to this approach is the likely assumption that the commonness in host switch events due to predation would produce an overlapping pattern of parasite occurrence in predator and prey communities in a biogeographical context.

A straightforward method to test the eventual overlapping of parasite species between host species pairs is co-occurrence analysis (Gotelli 2000; Gotelli and McCabe 2002). Here we used a host/parasite matrix (*not a parasite/host one*) instead of the species/locality meant for classical cooccurrence analysis, in order to test how many times the monogenoidean species assemblage infesting a particular host species was significantly shared by another host species.

We obtained host/monogenoid records from Kohn *et al.* (2006) (Central America, marine and freshwater). To test the

described approach we needed a set of host containing fish species easy to be distinguished into preys and predators. We therefore selected from the fish host reported in the checklist those belonging to the Carangidae and those we already referred in the previous paragraph as "reef prey fish". We have already discussed how the similarity in the trophic level of the species included in this category justifies its use. Trophic levels for the host species were obtained from Fishbase (Froese and Pauly 2009). Standard deviation from the average value of the trophic level of the considered families was calculated to test the homogeneity of trophic level within the considered host assemblages.

Three presence absence matrices of monogenoidean parasite species on predators (Carangidae) and preys (reef fishes) were compiled: one reporting the monogenoidean species parasite recorded from predator fish, one reporting those recorded from prey fish, and a third reporting both.

To determine which parasite species pairs have a co-occurrence frequency different from that expected by chance, we processed the three host/parasite matrices with Ecosim (Gotelli and Entsminger 2004), setting C-score as the co-occurrence index, with fixed sum rows and columns constraints, the Sequential Swap

Randomization algorithm, and 5000 interactions. The 5000 simulated “null” matrices were analyzed with Cooc software (Sfenthourakis et al. 2004), which estimates the null frequency distribution of each species pair, and individuates the species pairs in the original matrix with significant (χ^2 test, $P < 0.05$) higher or lower occurrence.

Finally, we measured the fraction of significant overlaps between parasite assemblages of predators and preys in respect to the total number of possible interactions (given by $n!/[(n-2)!2]$, where n is the total number of host species).

In addition we performed some more cooccurrence analyses in order to test the eventual overall influence of competition (from an evolutionary point of view, as it will be extensively discussed in the next paragraph) in the qualitative composition of monogenoidean communities in large fish assemblages in different biogeographical and environmental contexts. To this purpose, we tested (with the same procedure described above) the overall number of significantly cooccurring and competing monogenoidean species pairs in various wide fish species assemblages (namely, all the host/monogenoidean parasite records from Cohen and Kohn (1998) (Amazon river, freshwater) and by Kohn *et al.* (2006)

(Central America, freshwater and marine).

5.3 – Results and Discussion

5.3.1 - Host trophic level vs. parasite host range

The descriptive statistics of the trophic level for the considered host-assemblages are reported in Table 6. A nearly perfect correlation was found between the average trophic level of the considered fish assemblages and the average host range of their monogenoidean parasites (R^2 : 0.95). Regression line is shown in Fig. 14. This is a strong evidence suggesting that trophic ecology plays a major role in the determination of the qualitative composition of parasite assemblages. A few considerations regarding the way we approached this issue should be added. We have already discussed the choice of using families to group together species similar in their trophic ecology, as well as the exception represented by the category “reef prey fish” (see previous paragraphs). One drawback of our study could be individuated in the use of average values of parasite host range and host trophic level instead of individual ones. Yet, our approach allows to reduce some potential errors related to sampling

effort (as regarding for the determination of parasite species host range) and estimation of host trophic level. The correlation we detected on averaged value enlightens the presence of a clear overall pattern, that was exactly the aim of our approach, limiting the confounding effect of the eventual outliers and, in general, of the background noise related to individual statistical behavior of single species.

5.3.2 - Predation and host switch

Presence/absence parasite matrices used in the cooccurrence analyses are provided as supplementary material to this thesis in S6 (comprising monogenoidean parasite records on fish species of different trophic levels within a biogeographical area - Atlantic Central America - ideally categorizable into predators and prey according to the fish consumption information provided by Froese and Pauly 2009) and S7 (comprising all the available monogenoidean records in different biogeographical and environmental contexts: Amazon river basin, Atlantic Central American marine waters, Central American freshwaters). Table 7 report the number and percentage of significantly cooccurring (and competing) parasite species pairs (i.e. those species pairs found to

share more (or less) hosts than expected by chance, with $P < 0.05$) within the fish assemblage as reported in S6. In Table 8 we report the number and percentage of significantly cooccurring and competing species pairs for the monogenoidean species assemblage of matrices S7 (comprising all the available monogenoidean records in different biogeographical and environmental contexts: Amazon river basin, Atlantic Central American marine waters, Central American freshwaters).

In general, the number of species pairs detected to cooccur more or less frequently than expected by chance was a small percentage of the potential number of pair interactions. In particular, competition appears to be very rare in all the examined assemblage.

At this point it is fundamental to make clear that we use the term “competition” to indicate that two species share less host species than expected by chance, without any ecological time scale implication. This should emerge quite clearly from the fact the our analyses are based on all the available monogenoidean records within an area of study, independently from the time of collection (i.e. we do not assume cooccurring parasites to actually share the resources of a host species, be it an individual or a population).

Nonetheless, since the use of cooccurrence analysis to detect

phenomena of competitive exclusion has been object of much debate (Hastings (1987) stated that using species co—occurrence data to look for competition is likely not to detect even strong competition), there is no harm in making it clear that our aim was to detect an eventual pattern of monogenoidean niche/host splitting related to their evolutionary history and not to their ecological ongoing processes.

However, as already stated, a very small number of monogenoidean species pairs resulted to share less host species than expected by chance (less than 0.15%). Cooccurring patterns resulted more common, even if the number of significantly cooccurring pairs was (in all analyses) quite small in respect to the total number of possible pair interactions (less than 5%). The apparently absence of evidences reflecting the effect of interspecific interactions in the examined host/parasite assemblages was somehow expected (at least from a statistical point of view), considering the generally small host range of Monogenoidea. Yet, it is interesting to take under examination the results from the cooccurrence analysis performed on the prey/predator matrix (S6). The total percentage of significantly cooccurring species pair in the prey/predator matrix is almost 6 times higher than that calculated for the “complete”

host/monogenoidean parasite assemblage for the Atlantic Central American marine waters (see Table 8). This is obviously a consequence of having selected two groups respectively homogeneous (at least from an ecological point of view, letting alone the potential coevolutionary effect in the monogenoidean assemblages of the Carangidae). For the same reason it is not surprising that cooccurrence within the two trophic assemblages (intra-groups) is higher than that among the assemblages (inter-groups). The higher fraction of cooccurring species pairs detected on the Carangidae (in respect to reef prey fish) is related to the average species richness of their parasite assemblages: each carangid species hosts an average of 2.7 monogenoidean species, while the considered prey fish species host an average of 1.9 monogenoidean species (see paragraph 5.1).

On the contrary it is very interesting to notice how our hypotheses regarding an eventual role of predation in promoting host switch events (and therefore qualitatively affecting the parasite assemblage) is supported by the fact that also the percentage of inter-groups cooccurring species alone is larger than the overall percentage for the area (1.31% Vs. 0.73%), putting on evidence a significant species overlap between monogenoids of prey and

monogenoids of predators (in respect to the general pattern of the region).

CHAPTER 7

Overall discussion and conclusions

We examined the relationships between fish parasites and their hosts from an ecological perspective. We assumed host specificity one of the major issues related to the host parasite system. To test our assumption we proposed an alternative ecological approach other than those based on the coevolutionary paradigm. We did not assume available data on parasite distribution as a quantitative measure of host specificity. Instead we used the known patterns to test different ecological and evolutionary processes strongly related to host specificity (in terms of both cause and effect), with the aim to define and quantify “intrinsic” specificity (opposed to the one measured from available records).

The concept of intrinsic host specificity has not been much debated (especially at higher taxonomic levels), being at most implicitly identified with phylogenetic host specificity. However, coevolutionary studies do not allow to compare measures of phylogenetic coherence between different host/parasite systems. In

addition, most of the time, the absence of strong coherence in the pattern is considered enough to state the exclusive relevance of ecological factors in determining the distribution of parasite species on the considered hosts (and vice-versa). We handled the problem from the ecological perspective, proposing two approaches (an *a priori* and an *a posteriori* one) integrated by a theoretical framework.

Looking for a formal coherent integration of coevolution and ecology as determinants of the qualitative composition of host/parasite systems, we theorized a probabilistic temporal model integrating phylogenetic host specificity (i.e. that related to host/parasite coevolution) and ecological factors (potentially responsible for the alteration of coevolutionary scenarios). The model aims to reduce the complexity of coevolutionary mechanisms by assuming host/parasite co-speciation as a keystone and then diagramming the probability of a parasite species to colonize the newborn host species. It also individuates an evolutionary temporal border separating the major role of coevolution and ecology in determining the distribution of parasite species on closely related host species. Intrinsic host specificity is the balancing force which determines the strength of the border. The higher is intrinsic host

specificity of a parasite group, the more difficult is for its member (and its evolutionary lineages) to pass across the border. The absence of strong coevolutionary patterns even in intrinsic host specific parasites is therefore coherent with the model. Ecological factors are likely to be the major responsible for deviation from coevolutionary scenarios. Yet, how to address them is debatable.

According to these premises, we provided a new method based on multivariate statistics and ecological niche modeling to evaluate the effective influence of ecological features (primarily referred to the host) in the qualitative distribution of parasite species in a considered fish assemblage. In particular, we produced a set of exemplifying models for the low specific monogenoidean *Neobenedenia mellenii* in Atlantic Central American marine waters. We evaluated the consistency of the model by comparing the predicted host range composition with that known from literary data. At least as regarding for *N. mellenii*, the ecological features included in the models demonstrated to be significant in the qualitative specific determination of host range.

Then we proposed nestedness analysis at the compound community level as an indirect measure of intrinsic host specificity. Nestedness paradigm has already been applied to the host parasite

system, but mostly at the component community level (with a nested pattern implying that species with high prevalence occur in all kinds of infracommunity, while low prevalent parasite species only occur in species rich infracommunities). At the compound community level a nested pattern is verified when each component community is a subset of the immediately species richer component community, *i.e.* when generalist parasite species occur in all kinds of component communities, while specialist parasite species occur in species rich component communities only. An indirect consequence of the occurrence of generalists in all component communities is the random assemblage of their host range (where “random” means not phylogenetically). We then developed a new methodology of nestedness validation based on the concept of “relative” nestedness of submatrices selected from a larger, more comprehensive nested matrix (aimed to overcome the problems related to the comparison of nestedness of presence/absence matrices of different size and fill). We used such method to assess and compare nestedness of various fish metazoan endo- and ectoparasite taxa.

The relative degree of nestedness in Monogenoidea hosted by various fish assemblages in different biogeographic areas and environments resulted to be lower than that of the other considered

parasite taxa. This pattern suggests that host range in Monogenoidea (at least in the investigated fish assemblages) is *not* randomly assembled, i.e. intrinsic host specificity of Monogenoidea is apparently higher than that of the other parasite taxa. Although this result is far from being surprising, it is nonetheless relevant since it provides an ecological support to an assumption that, as far as now, has only been indirectly tested through coevolutionary studies often leading to ambiguous results. Yet, even if the described approach, as well as that based on ENMs, are clearly different from the coevolutionary one, they are far from being unrelated to the cospeciation issue, since they all aim to the answer the same basic questions.

As already stated, considering the general short span of free living stages of fish parasites, infection, colonization and host switch processes require the hosts to get in close contact. Colonization and host switch are obviously related to interactions among host belonging to different species. The number of parasite species found on a single host species can therefore be considered as an indirect measure of interspecific interactions.

We therefore compared the number of parasite species found on a single host species (i.e. the indirect measure of host

interspecific interactions) with the host specificity of the parasite species recorded on that host. We used different datasets, referring to different habitats and parasite taxa (not only the Monogenoidea). We chose to perform this analysis on not-monoxenous parasite species too, because we cannot state that the basic assumption of the component community richness as a measure of inter specific interactions is valid for them too.

Plotting the number of parasite species recorded on a single host species against the average host range of the parasite species recorded on that host in the area of study produced comparable results for all the considered datasets. An heteroscedastic distribution is the common pattern that emerged from our analyses (for both monoxenous and not-monoxenous parasite taxa). Most of the variability in host specificity (from very specialist to very generalist parasite species) is restricted to poor parasite assemblages, while richer assemblages host mainly medium/high specific parasite species.

Successively, we chose to study in depth how and how much trophic level of fish host affects specificity of monogenoidean parasite. Among the various ecological host features considered in the previous analyses, we decided to concentrate on trophic level

because it is the much closely related to one of the most important ecological key-facts ruling parasite species distribution, that is interaction in host species. At the extreme levels of the trophic web, the rate of inter- and intraspecific interactions reverses. Piscivorous fish are much more prone to interspecific interactions (with the prey species) than prey fish (that usually stick together in wide intraspecific aggregations), also as a consequence of the differences in population size and density.

Transmission and infection processes in parasitic species with direct life cycle are enhanced by high densities in host populations or, more generally, by repetitive interactions between potential host individuals. If such repeated interactions involve different species, host switch events may occur. Thus, we assumed high host specificity to be a not stable evolutionary adaptation for monoxenous parasites of fish species belonging to higher trophic levels (and with populations characterized by low densities and weak intraspecific interactions), since finding the “right” host may be problematic.

According to this premise, we compared the trophic level of a large sample of host species belonging to different habitats and ecological guilds, to the host specificity of their monogenoidean

parasites. The strong linear correlation that emerged from the regression analyses performed on the considered datasets corroborated the assumptions and induced us to perform one more analysis on the effect of intra/inter specific interactions of host species on specificity of parasite species.

Another aspect related to host trophic ecology is that of the potential relevance of predation in promotion of host switch events in monoxenous parasites such as monogenoids. The most likely outcome of the eventual commonness of predation-driven host switch events is the absence of a strong qualitative divergence in the species composition of parasite assemblages of predators in respect to those of preys. We therefore compared monogenoidean fauna of two sets of predators and preys within a fish assemblage in a biogeographical area (namely, Carangidae Vs. herbivorous reef fish in Atlantic Central American marine waters) through cooccurrence analysis. Several of the parasite species under examination resulted to be shared by predator and prey species more often than expected by chance. In other words, there is a significant overlapping between the monogenoidean fauna of prey and that of predators, corroborating the starting hypothesis.

The overall patterns emerging from the analyses described in

this thesis are all related to a theoretical aspect that appears to be fundamental in the host specificity issue, both from an ecological and an evolutionary perspectives. Throughout the text we often referred to it with the term “contactability” (of a fish host by a parasite). Yet, we deliberately avoided to give it a straightforward definition. Its literal meaning (namely, the reciprocal potentiality of a host and a parasite to come in contact) is much limiting, since it appears to be much more related to the ecological aspects of the symbiosis than to the evolutionary ones.

In a more refined definition, contactability could be indicated as the actual factor responsible for the theoretical incongruence between *actual* parasite host range (the fish species where the parasite species has been found) and *potential* host range (the fish species virtually compatible with the considered parasite species). This distinction is much more intricate than it seems. First of all, a part from the evidences of compatibility eventually provided by laboratory experiments, there is no way to determine which hosts are compatible with a parasite species and which are not.

If we give host range of time and space boundaries (host range of a parasite in a geographical context, or over a certain period), one could identify the suitable hosts in those from which

the parasite has eventually already been recorded in another area of period. However, it is much more common to refer to host range in general, without limiting it into space and/or time. In this case, the “compatible” host species of the last definition do correspond to parasite host range. The ecological niche modeling approach we proposed is the first attempt to provide a better, ecology-based definition of host compatibility.

From a evolutionary point of view, the concept of contactability becomes even more complex, becoming deeply involved also in the other factor affecting host range (i.e. the compatibility issue).

Host range is often identified with host specificity. However, although it certainly gives a measure of a parasite specificity, it does not coincide with it. As a matter of fact, the intrinsic host specificity of a parasite is responsible (together with contactability) for the parasite host range. In fact, parasite *intrinsic* host specificity and host compatibility, though not totally equivalent, are closely related.

Our work was aimed to create a logical framework for the integration of intrinsic host specificity and host range. The obtained results provide new, statistically supported insights on the host specificity matter. The fundamental ecological and evolutionary role

played contactability emerging from our analyses suggests the necessity of reconsider the traditional relationship stating that host range results from the intersection of the compatible host species set and the encountered host species one, since all the three factors appear more likely to be contemporaneously (and reciprocally) cause and effect one of another.

FIGURES

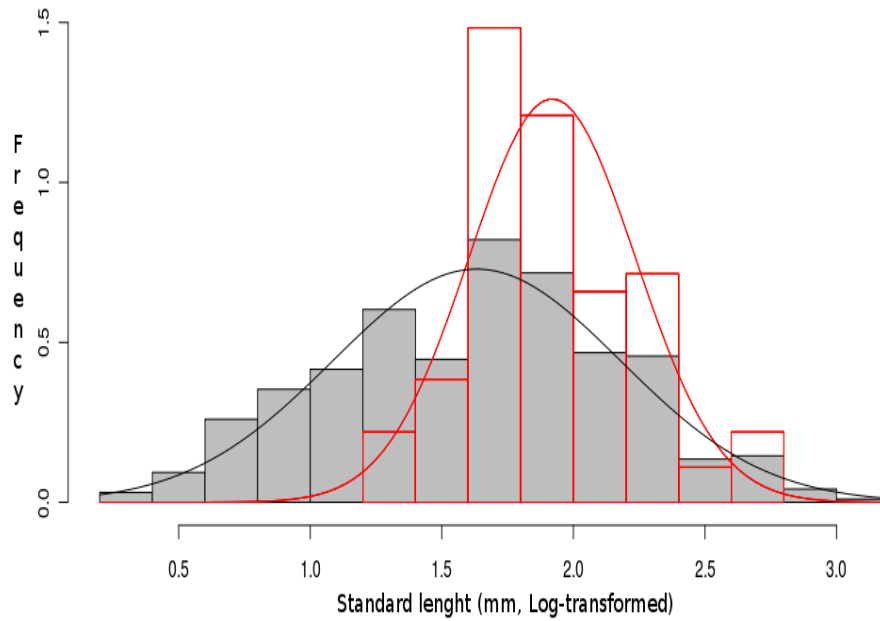


Figure 1 – Grey histogram, black line: frequency distribution of the Log-transformed standard length (mm) of Italian marine fish species (according to Froese and Pauly 2009). Red histogram and line: frequency distribution of the Log-transformed standard length (mm) of the Italian marine fish species included in the checklist by Strona *et al.* (2009).

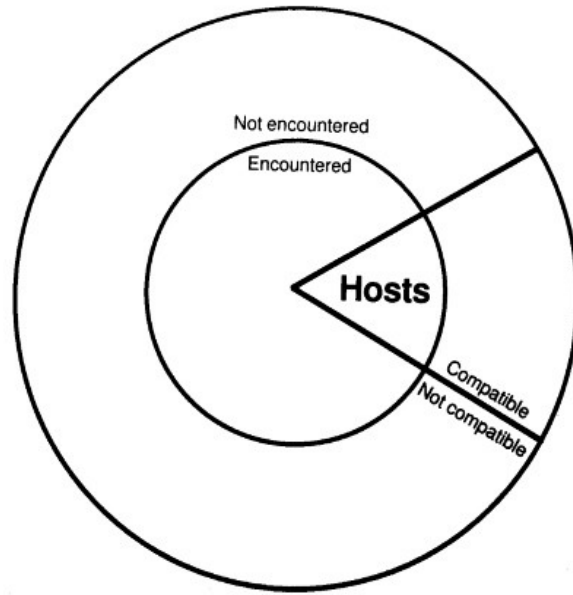


Figure 2 – Schematic representation of how a parasite species host range is determined: parasite are able to infect only a subset of the potential hosts (the compatible ones, i.e. those where the parasite can develop); not all the potentially compatible host species are encountered by the parasite species (some of them are just “virtual” potential hosts); host range of a parasite species is therefore composed by the compatible host species it actually comes in contact with (Poulin 1998).

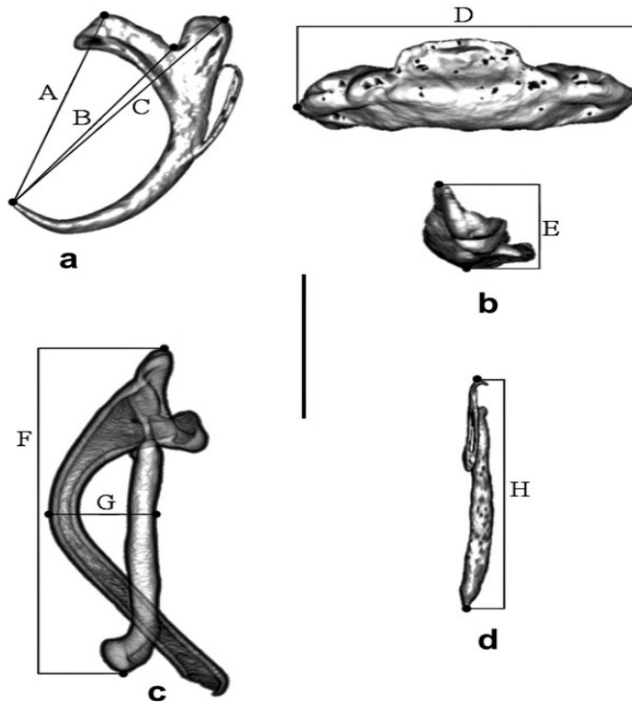


Figure 3 – Images of haptoral and copulatory sclerites of *Dactylogyrus extensus* Mueller and Van Cleave 1932 with indication of the morphometrical measurements necessary to taxonomical identification. (a) Anchor (A, superficial root edge to point tip; B, shaft edge to point tip; C, deep root edge to point tip); (b) Bar (D, bar length; E, bar width); (c) Male copulatory organ with accessory piece (F, length; G, width); (d) Hook (H, hook length). Scale bar: a, c: 30 μ m; b, d: 15 μ m (From Galli *et al.* 2007).

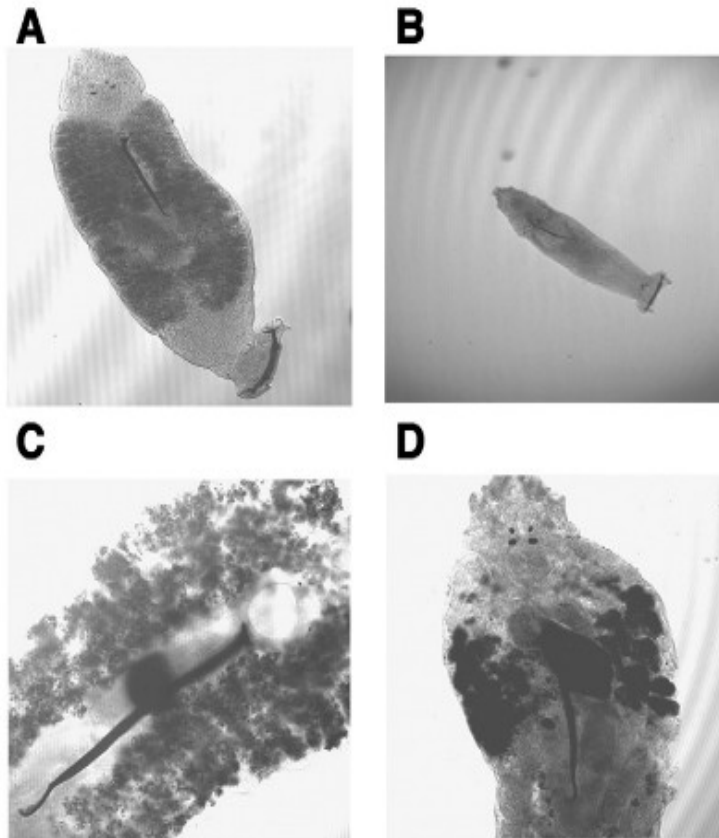


Figure 4 - Micrographs showing overall structural preservation of 6 different specimens of *Diplectanum aequans* (Wagener 1857) Diesing 1858 after 8 weeks of storage. (A, C): specimens preserved in DMSO; (B, D): specimens preserved in 70% EtOH. (A, B): entire body; (C, D): copulatory complex. Scale bar: (A, B): 1 mm; (C, D): 120 μ m (Modified from Strona et al. 2009).

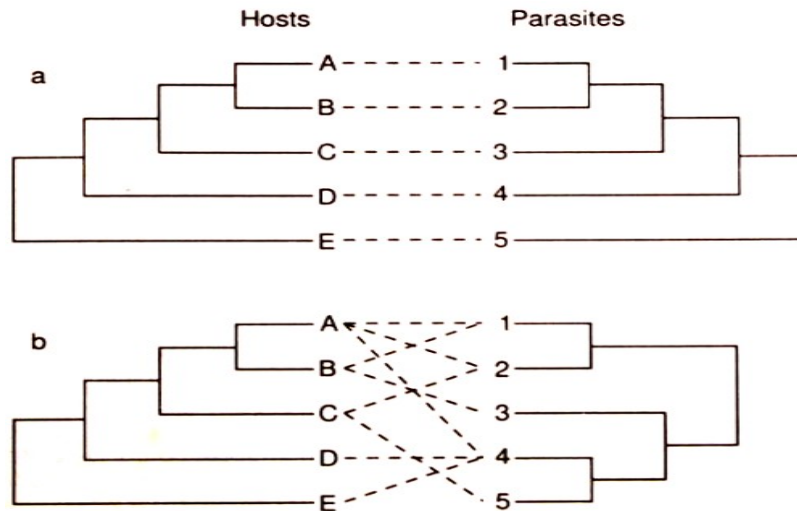


Figure 5 – Phylogenetic trees of an hypothetical host/parasite assemblage (broken lines indicate host/parasite association). (a) Host/parasite phylogenies are congruent as predicted by Fahrenholz's Rule; host specificity is high (ideally each species parasitize a single host species); ecological factors and evolutionary processes other than cospeciation do not affect the distribution of parasite species on host species. (b) Host and parasite phylogenies are incongruent; host specificity varies among the host parasite assemblage; ecological factors and processes such as parasite host switch or parasite (local) extinction are fundamental in the qualitative determination of the host/parasite assemblage (Modified from Poulin 1998).

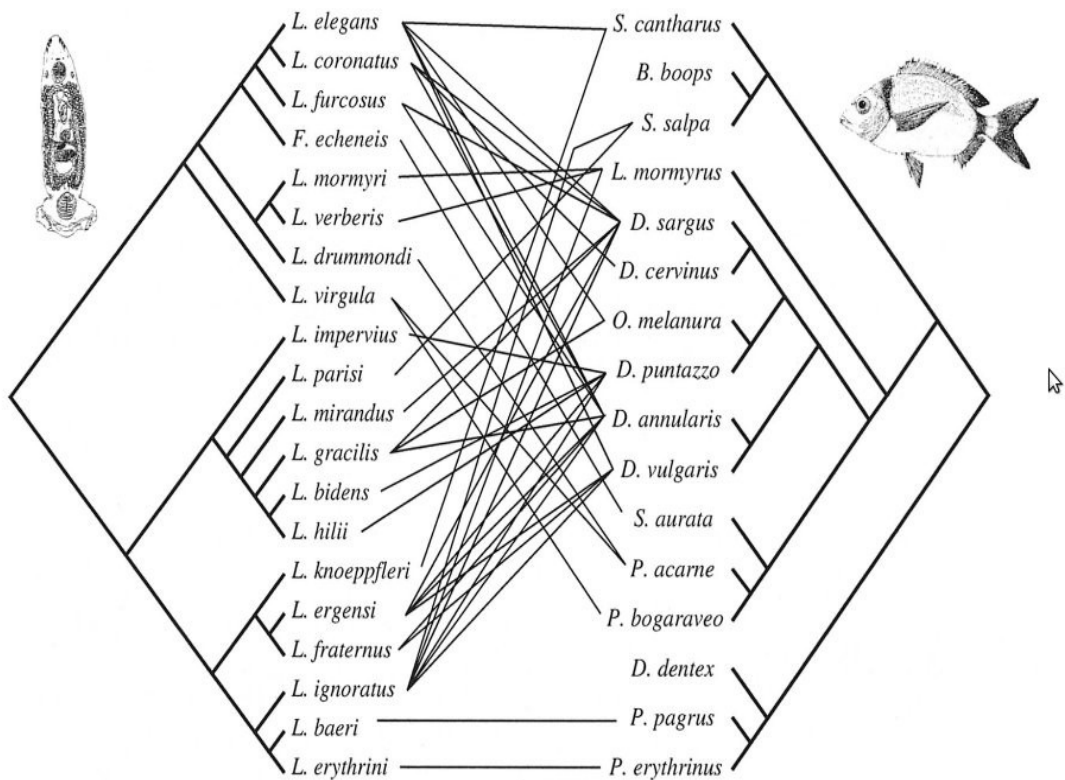


Figure 6 - Pattern of associations estimated for the host/parasite system Sparidae/*Lamellodiscus* spp. Lines depict the observed host-parasite associations. No widespread cospeciation processes can be observed (Modified from Desdevises *et al.* 2002).

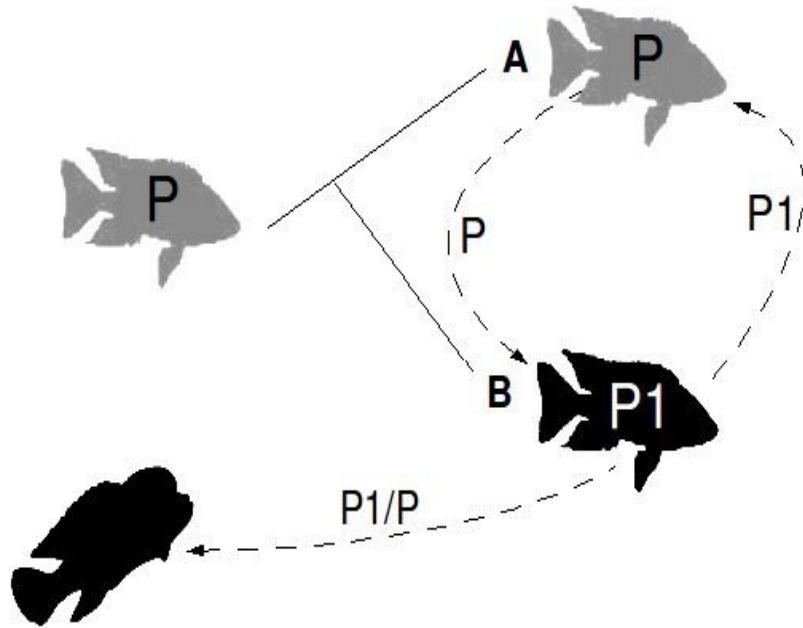


Figure 7 - Processes involved in the alteration of a coherent host-parasite coevolutionary scenery. Speciation in a single host species (A --> B, C) possibly results in the extinction of the parasite species in the newborn host species or, eventually, in its coevolutionary speciation ($P \rightarrow P1, P2$). At this point, if speciation occurred, a new range of host switch possibilities is therefore set, each of them able to lower the average host specificity of the system. Host switch events could eventually involve the two newborn sister species or other not phylogenetically related host species.

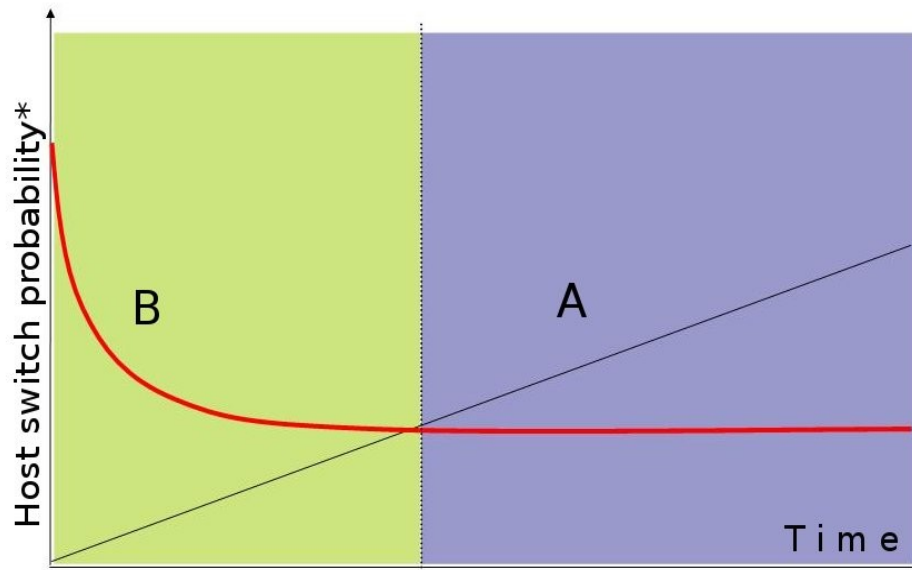


Figure 8 – *Probability of the newborn parasite species to colonize the sister host species (i.e. probability of P1 of moving from host species A to host species B in **Fig. 7**). (A - red curve): probability of the parasite to cross the barrier, or of the barrier to become ineffective (the “barrier” is the ecological/biological factor responsible of host speciation); (B – black line): probability of parasite host switch related to phylogenetic distance of available hosts. The intersection point of the two lines indicates the time when the leading role in driving host switch and colonization processes shifts from coevolutionary to ecological factors.

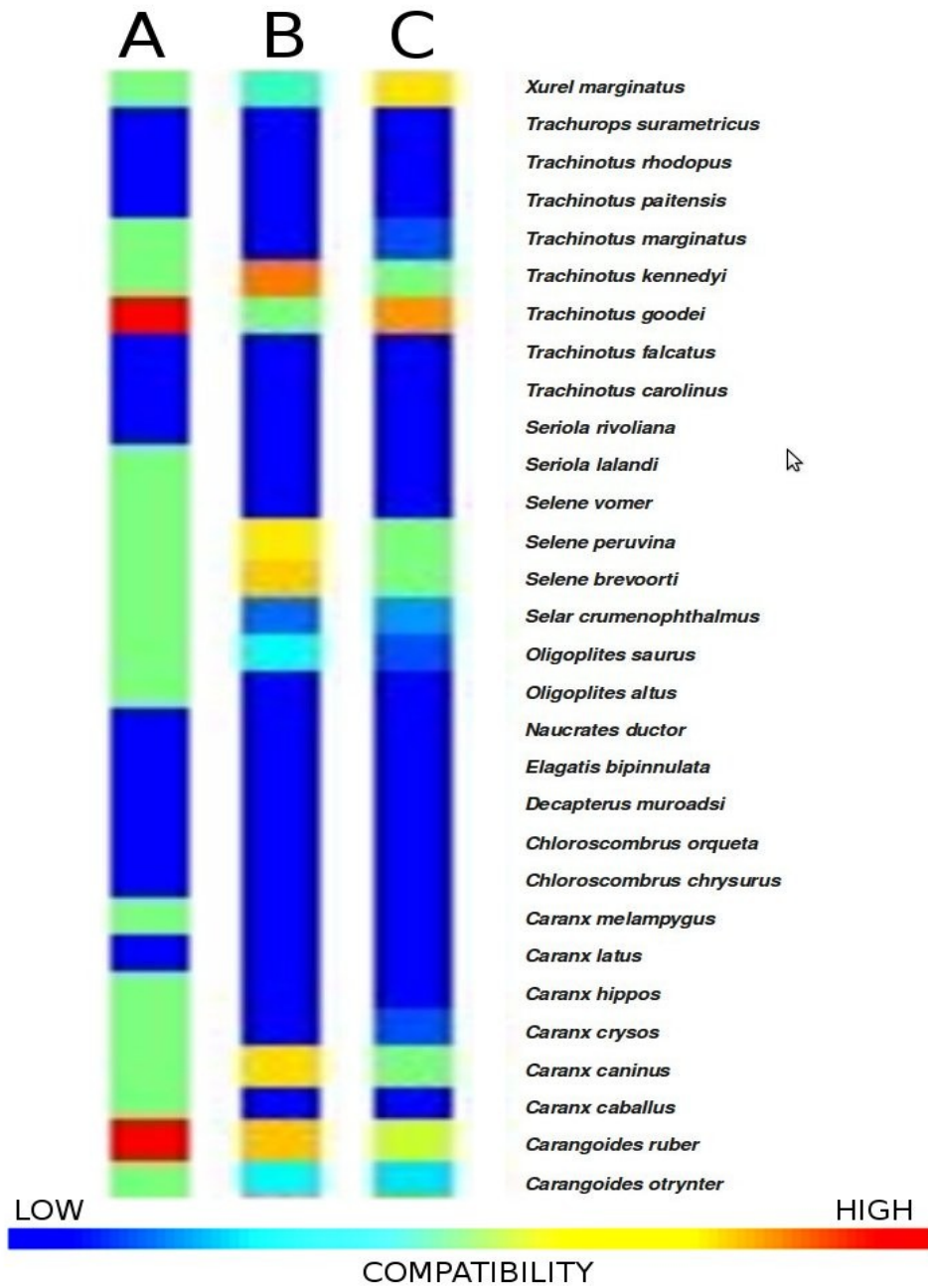


Figure 9 – Projections of the three models produced by Open Modeller Desktop, expressing the degree of compatibility of the consider carangid species towards the monogenoidean parasite *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963. Algorithms used to produce the models: (A) Bioclim; (B) Environmental Distance; (C) Garp. See Muñoz *et al.* (2009) for further details.

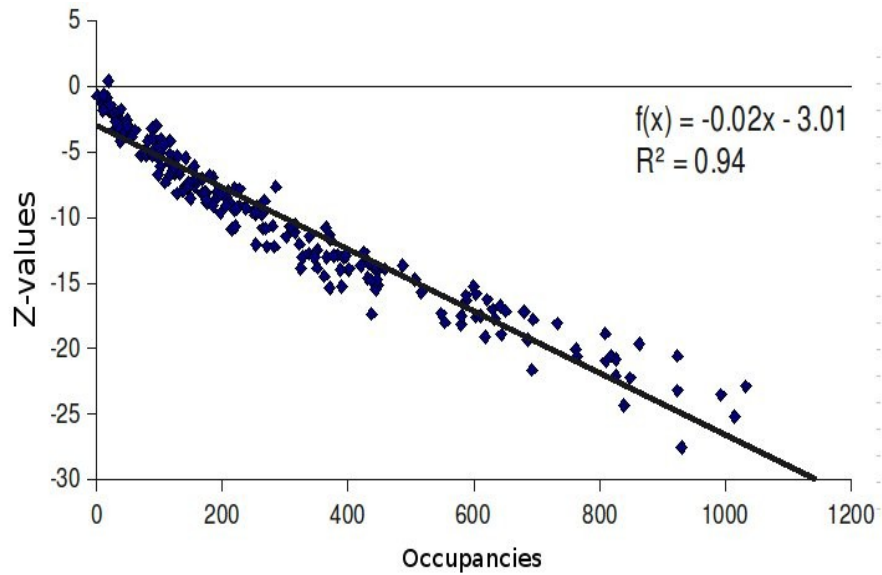


Figure 10 – Regression line expressing the relationship between the Z values and numbers of presences (occupied cells) of the random submatrices obtained from the complete host parasite matrix of Mexican freshwater fish, based on the checklist by Salgado-Maldonado 2006.

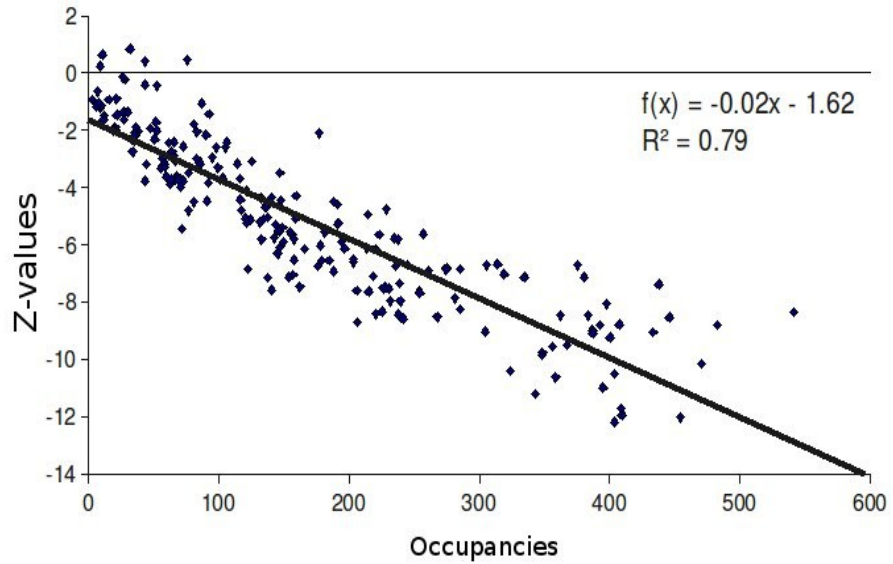


Figure 11 – Regression line expressing the relationship between the Z values and numbers of presences (occupied cells) of the random submatrices obtained from the complete host parasite matrix of Puerto Rico marine fish, based on the checklist by Williams and Bunkley-Williams 1996.

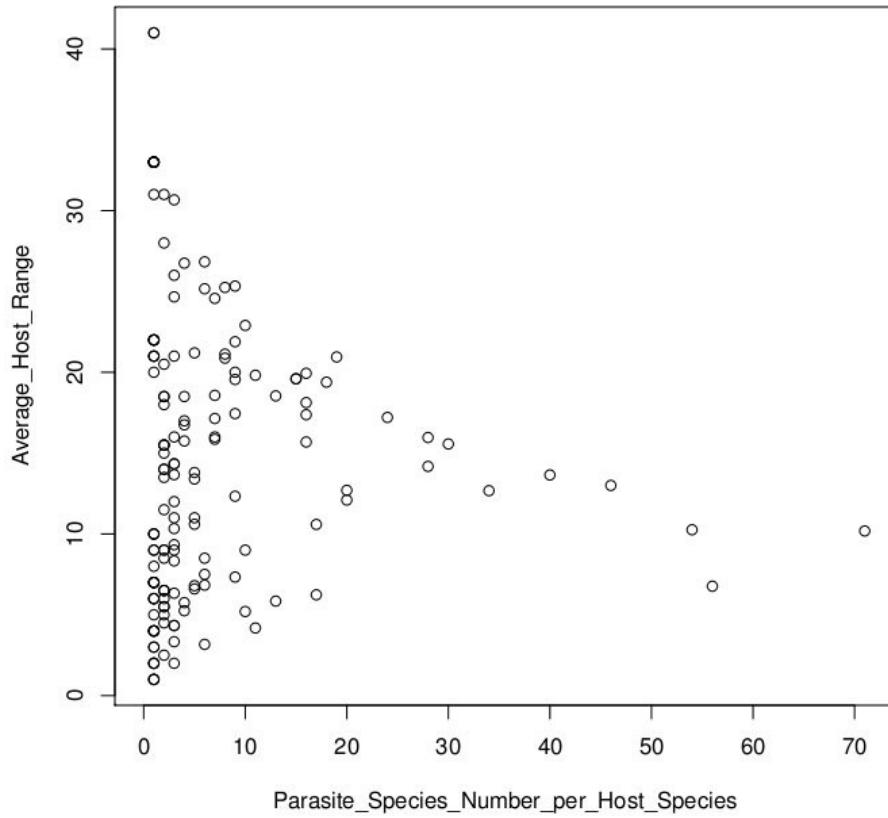


Fig. 12 - A

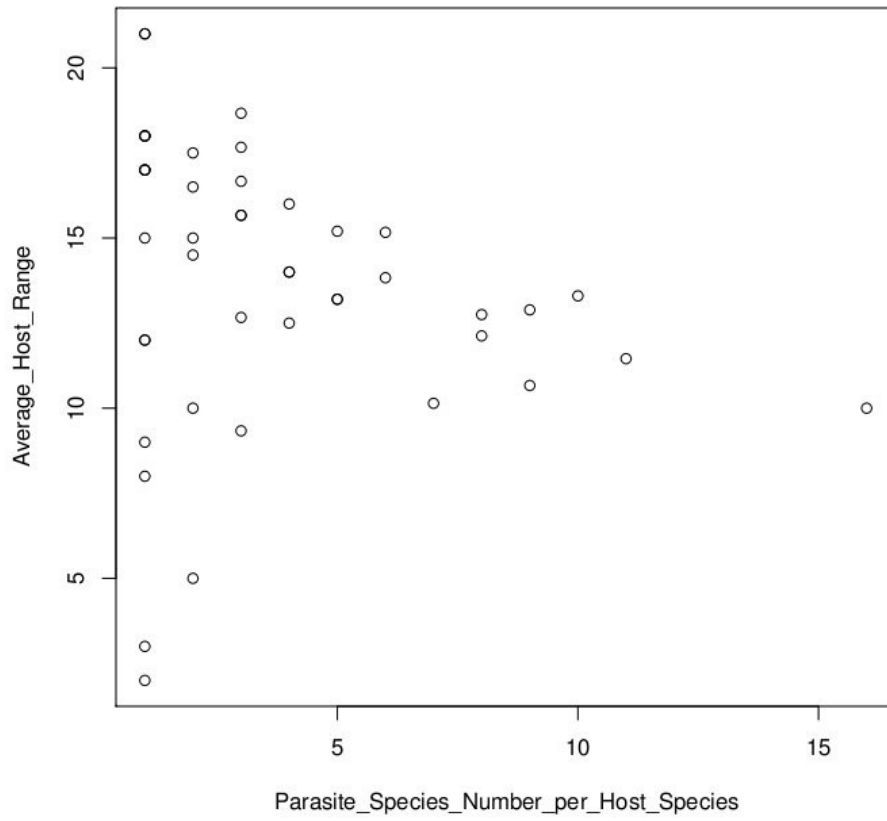


Fig. 12 - B

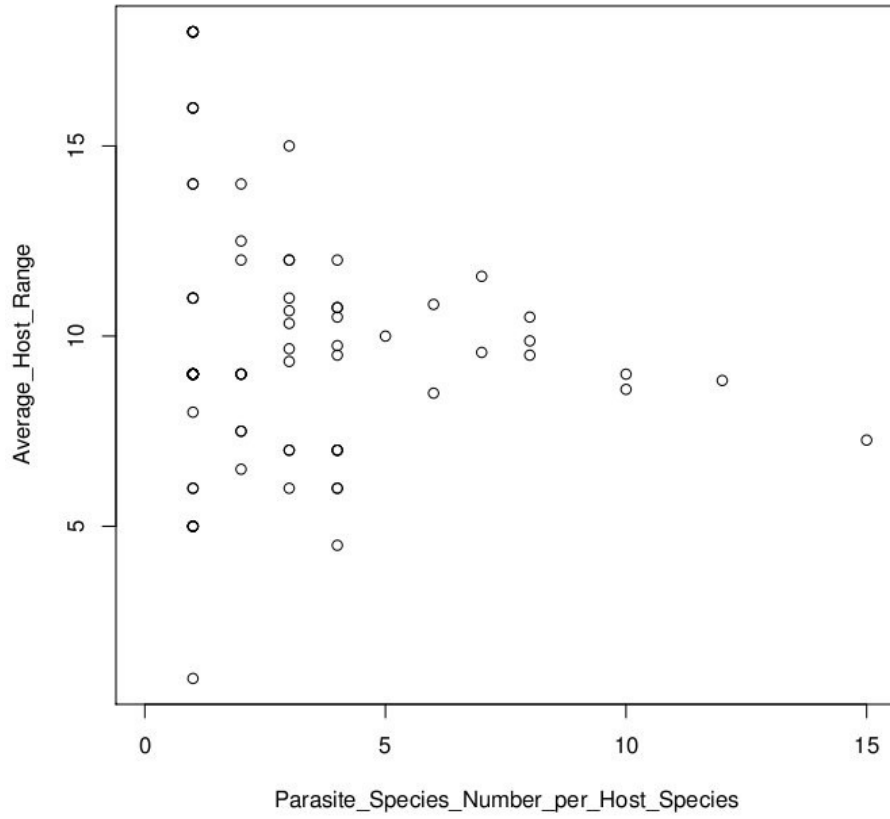


Fig. 12 - C

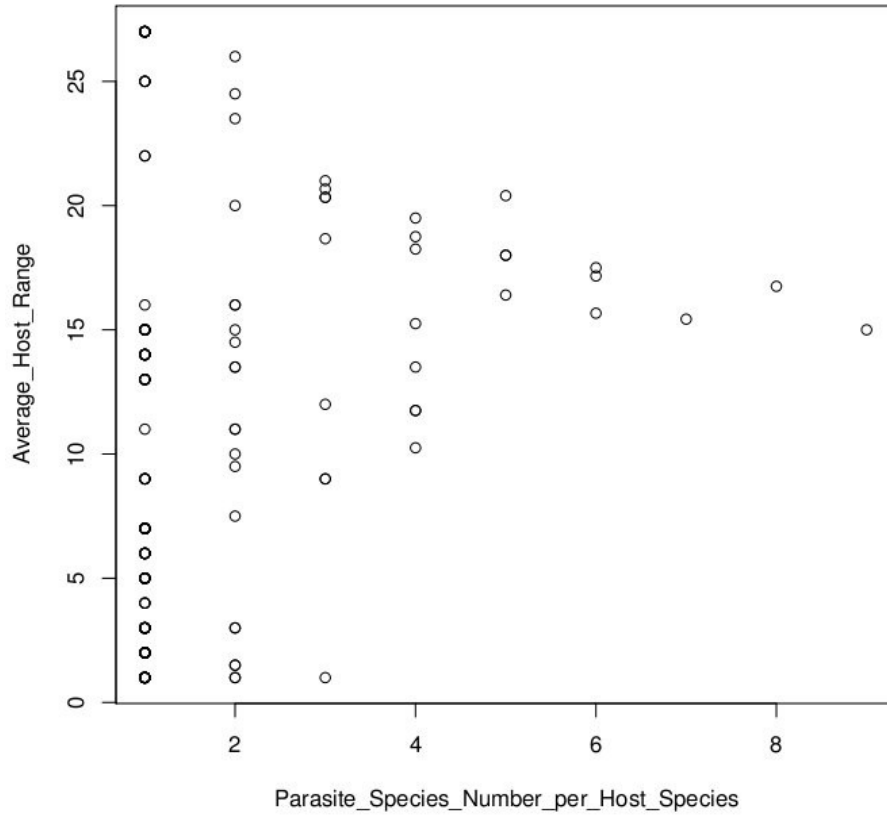


Fig. 12 - D

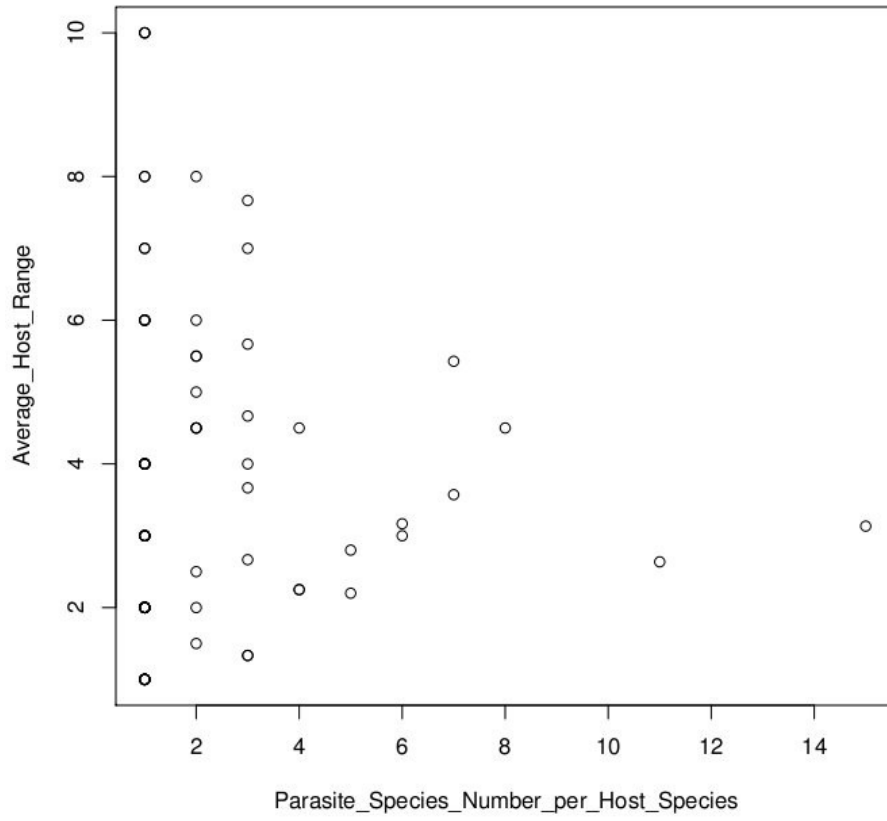


Fig. 12 - E

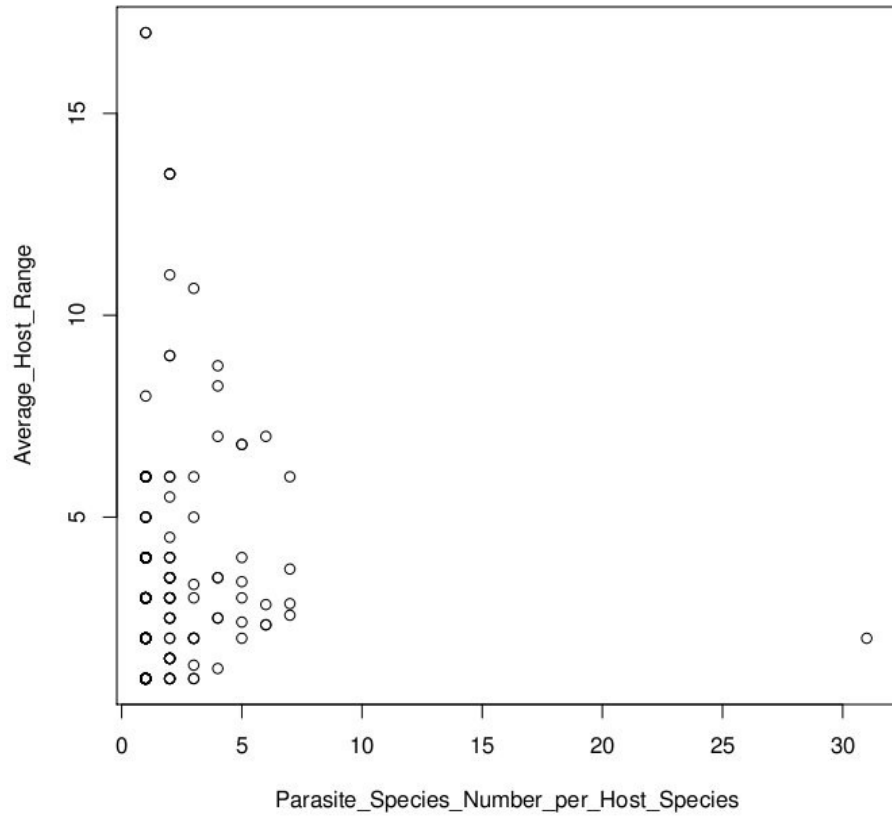


Fig. 12 - F

Figure 12 - Average host range of parasite species of each host species versus the corresponding number of parasite species recorded on that host. A common general pattern repeats itself throughout the graphs, characterized by a heteroscedastic distribution. Variability in average host range is much higher in poor parasite assemblages than in rich ones. A: Mexico (freshwaters); B: Ireland (freshwaters); C: Puerto Rico (marine waters); D: Amazon River Basin (freshwaters, Monogenoidea only); E: Central America (freshwaters, Monogenoidea only); F: Atlantic Central America (marine waters, Monogenoidea only).

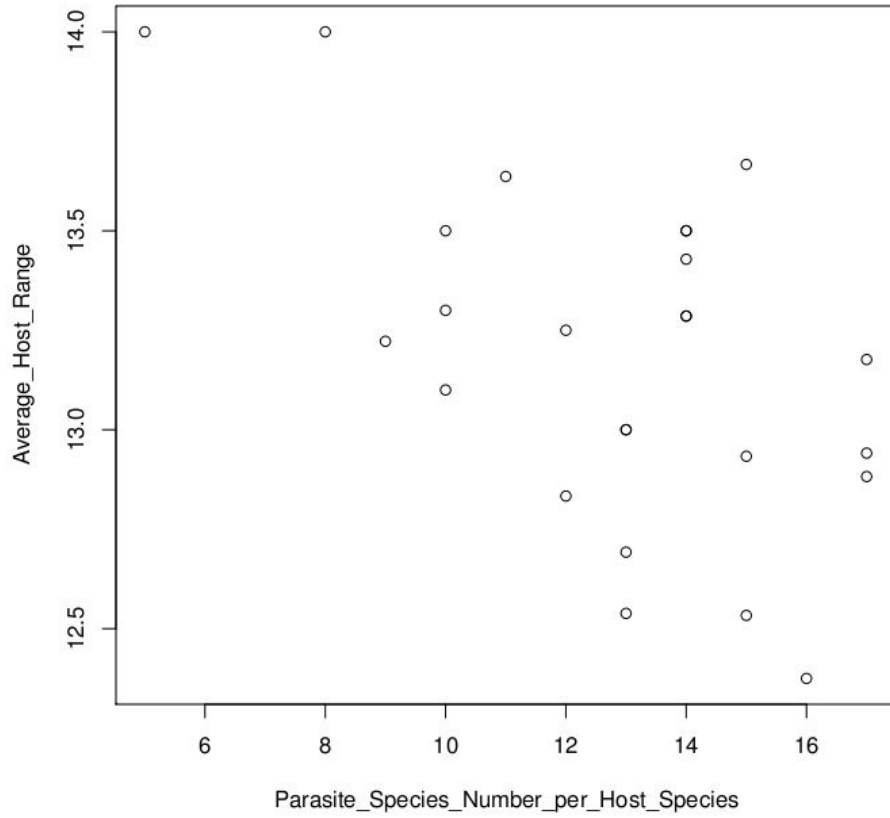


Fig. 13 - A

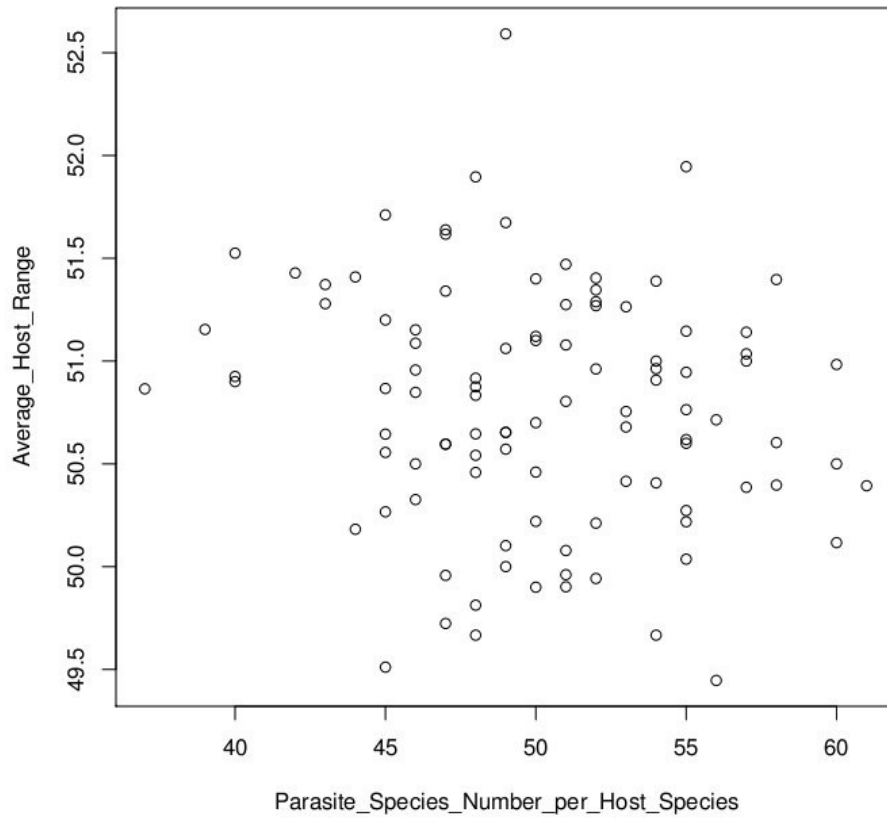


Fig. 13 - B

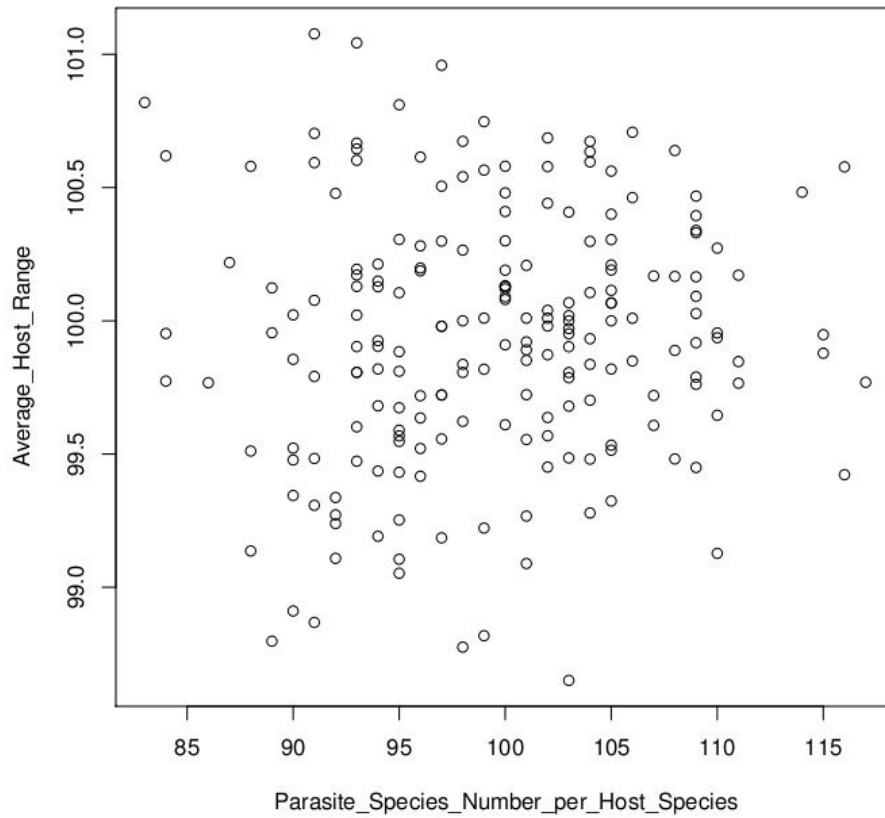


Fig. 13 - C

Figure 13 - Average host range of parasite species of each host species versus the corresponding number of parasite species recorded on that host in simulated host/parasite assemblages (i.e. matrices) of different size (rows X columns). A: 25 rows X 25 columns; B: 100 rows X 100 columns; C: 200 rows X 200 columns.

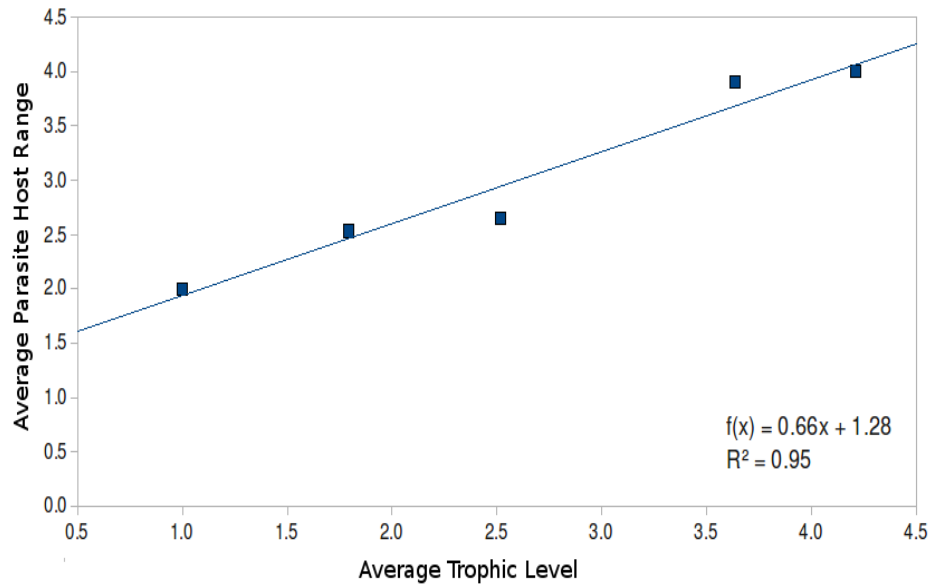


Figure 14 – Regression line expressing the relationship between average trophic level of different host taxa/categories and the respective average host range of their monogenoidean parasites. Correlation between the two variables is very tight, with a determination coefficient close to 1.

TABLES

Table 1 - Regional host range (number of host species found to be infested by the considered monogenoidean species in the biogeographical area of study) of various monogenoidean species from different localities and environments.

Monogenoidean Species	Host Range	Area/Environment	References
<i>Sciadicleithrum bravohollisae</i>	15	Central America (Freshwater)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Sciadicleithrum mexicanum</i>	11	Central America (Freshwater)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Amphithecium falcatum</i>	9	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Cichlidogyrus tilapiae</i>	8	Central America (Freshwater)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Enallothecium aegidatum</i>	8	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Alpua piscicola</i>	7	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Mexicotyle mexicana</i>	7	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Microcotyle incisa</i>	7	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Pseudomazocraes monsvaisae</i>	7	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Notothecium cyphophallum</i>	7	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Cichlidogyrus sclerosus</i>	6	Mexico (Freshwater)	Salgado-Maldonado 2006
<i>Amphithecium dicionophallum</i>	6	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Anacanthorus mesocondylus</i>	6	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Notothecium teinodendrium</i>	6	Amazon River basin	Kohn and Cohen 1998; Cohen and Kohn 2008
<i>Cynoscionicola srivastavai</i>	6	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Macrovalvitrema sinaloense</i>	6	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Protomicrocotyle mirabilis</i>	6	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Thoracocotyla crocea</i>	6	Central America (Marine)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Haplocladius furcatus</i>	6	Central America (Freshwater)	Kohn <i>et al.</i> 2006; Salgado-Maldonado 2008
<i>Lamnelodiscus ignoratus</i>	6	Italy (marine)	Strona <i>et al.</i> 2009

Table 2 - List of the fish host species of the training set (i.e. the species used to calculate niche boundaries), together with the associated ecological and biological features used to build up the model as described in Chapter 2.

Species	Standard length (cm)	Resilience (K)	Natural mortality	Life span	Trophic level	Food consumption
<i>Paracanthurus hepatus</i>	31	0.43	0.87	6.60	3.40	9.20
<i>Chaetodon capistratus</i>	8	2.57	3.85	1.10	3.00	11.20
<i>Chaetodon ocellatus</i>	20	1.04	1.86	2.70	3.20	9.60
<i>Chaetodon striatus</i>	16	1.27	2.28	2.20	3.20	10.80
<i>Coryphaena hippurus</i>	210	1.41	1.38	2.10	4.40	8.50
<i>Holocentrus adscensionis</i>	61	0.32	0.61	9.00	3.50	5.70
<i>Lutjanus apodus</i>	67	0.35	0.77	8.10	4.20	8.80
<i>Haemulon plumieri</i>	53	0.11	0.28	26.00	3.60	5.70
<i>Epinephelus guttatus</i>	76	0.12	0.31	24.00	3.90	2.80
<i>Epinephelus striatus</i>	122	0.12	0.29	23.90	4.10	5.50
<i>Mycteroperca rosacea</i>	86	0.13	0.28	22.10	4.50	4.80
<i>Calamus calamus</i>	56	0.15	0.29	19.00	3.30	3.50
<i>Balistes vetula</i>	60	0.30	0.63	9.50	3.40	6.30
<i>Haemulon sciurus</i>	46	0.30	0.66	9.50	3.40	7.00
<i>Haemulon album</i>	79	0.20	0.43	14.30	3.20	5.60
<i>Lutjanus synagris</i>	60	0.20	0.46	14.30	3.80	6.80
<i>Pomacanthus paru</i>	41	0.21	0.51	13.30	2.80	6.70
<i>Acanthurus coeruleus</i>	39	0.11	0.32	25.40	2.00	18.80
<i>Acanthurus chirurgus</i>	39	0.25	0.63	11.20	2.00	22.70
<i>Scarus perrico</i>	76	0.24	0.45	12.00	2.00	13.50
<i>Aluterus scriptus</i>	110	0.08	0.15	36.00	2.80	13.10
<i>Melichthys niger</i>	50	0.26	0.44	11.00	2.40	15.50
<i>Holacanthus ciliaris</i>	45	0.22	0.4	13.00	3.00	14.00
<i>Holacanthus tricolor</i>	35	0.28	0.61	10.20	3.00	23.10
<i>Pomacanthus arcuatus</i>	60	0.17	0.37	16.80	2.90	16.90
<i>Sphaeroides annulatus</i>	44	0.28	0.48	10.20	3.10	14.3

Table 3 - List of the fish host species of the projection set, together with the associated ecological and biological features used to test the model as described in Chapter 2.

Species	Standard length (cm)	Resilience (K)	Natural mortality	Life span	Trophic level	Food consumption
<i>Canangoides otrynier</i>	60	0.34	0.62	8.40	4.40	6.20
<i>Canangoides ruber</i>	59	0.14	0.33	20.00	4.40	10.60
<i>Canax caballus</i>	55	0.34	0.76	8.40	4.00	4.30
<i>Canax caninus</i>	101	0.19	0.76	15.20	3.90	3.00
<i>Canax crysos</i>	70	0.38	0.75	7.50	4.40	6.90
<i>Canax hippos</i>	124	0.17	0.33	17.00	3.50	6.70
<i>Canax latus</i>	101	0.19	0.76	15.20	4.40	2.20
<i>Canax melampygus</i>	117	0.23	0.42	12.40	4.50	4.30
<i>Chloroscombrus chrysurus</i>	65	0.56	1.09	5.10	3.20	13.80
<i>Chloroscombrus orqueta</i>	30	0.65	0.99	4.40	2.50	21.10
<i>Decapterus muroadsi</i>	50	0.37	0.76	7.70	3.40	6.90
<i>Elaagatis bipinnulata</i>	180	0.60	0.86	4.80	3.60	4.90
<i>Naucrates ductor</i>	70	2.57	2.70	1.10	4.00	5.20
<i>Oligoplites altus</i>	56	0.33	0.76	8.70	4.10	6.50
<i>Oligoplites saurus</i>	35	0.56	0.85	5.10	4.50	6.70
<i>Selar crumenophthalmus</i>	30	0.89	1.56	3.20	4.10	8.90
<i>Selene brevoortii</i>	38	0.47	0.76	6.10	3.80	8.20
<i>Selene peruvina</i>	40	0.50	0.76	5.70	4.20	5.20
<i>Selene vomer</i>	48	0.42	0.64	6.80	4.30	4.50
<i>Seriola lalandi</i>	250	0.14	0.22	21.20	4.10	2.30
<i>Seriola rivoliana</i>	180	0.13	0.76	22.20	4.50	2.10
<i>Trachinotus carolinus</i>	64	0.32	0.49	9.00	3.50	5.30
<i>Trachinotus falcatus</i>	122	0.39	0.64	7.40	3.20	11.20
<i>Trachinotus goodei</i>	50	0.40	0.61	7.20	4.30	4.60
<i>Trachinotus kennedyi</i>	90	0.23	0.42	12.50	3.80	4.90
<i>Trachinotus marginatus</i>	45	0.27	0.76	10.60	3.90	4.90
<i>Trachinotus paitensis</i>	51	0.40	0.61	7.20	3.70	4.50
<i>Trachinotus rhodopus</i>	61	0.34	0.51	8.40	4.00	4.00
<i>Trachurops surametricus</i>	50	0.40	0.61	7.20	4.00	4.60
<i>Xarel marginatus</i>	28	0.63	0.76	4.50	4.10	3.90

Table 4 - Z values resulting from Nestedness analyses of the parasite taxa - submatrices for both Mexican freshwater and Puerto Rican marine waters, together with some basic information regarding the matrices themselves (row and column number, fill). The expected Z values (calculated using the equations of the regression lines), and the difference between them and the actual Z values of the submatrices are reported as well.

Parasite Taxa	Row Number	Column Number	Fill	Cell occupancies	Z	Expected Z	Expected Z-Z
Mexico							
Acantocephala	8	186	0.05	74.4	-4.36	-4.77	-0.41
Cestoda	27	186	0.03	150.66	-5.28	-6.57	-1.29
Digenea	68	186	0.05	632.4	-17.77	-17.94	-0.17
Monogenea	35	186	0.01	65.1	-3.11	-4.55	-1.44
Puerto Rico							
Acantocephala	6	38	0.09	20.52	-0.78	-2.05	-1.27
Cestoda	25	38	0.09	85.5	-4.91	-3.4	1.51
Digenea	39	38	0.07	103.74	-4.4	-3.78	0.62
Monogenea	27	38	0.05	51.3	1.96	-2.69	-4.65

Table 5 – Results of nestedness analyses conducted on host/parasite matrices from different biogeographic areas/environments. The output was produced by Nestedness software (Ulrich 2006). For a detailed discussion on terminology, refer to Chapter 3.

Biogeographic Area	Parasite Species n.	Host Species n.	Matrix Fill	Temperature	Simulated Temperature	Standard Deviation	Z
Mexico - Freshwater	194.00	186.00	0.03	3.08	12.76	0.39	-24.68
New Zealand - Marine	292.00	114.00	0.01	1.63	2.34	0.17	-4.31
Ireland - Freshwater	55.00	21.00	0.17	9.93	34.51	2.47	-9.96
Puerto Rico - Marine	205.00	38.00	0.07	8.89	20.54	0.98	-11.85
Central America - Freshwater	82.00	78.00	0.03	7.93	6.86	0.69	1.56
Central America - Marine	209.00	204.00	0.01	3.16	2.12	0.17	6.27
Central America - Brackish	34.00	22.00	0.05	26.20	7.31	1.81	10.46
Amazon River Basin	204.00	75.00	0.02	6.45	5.45	0.41	2.39

Table 6 – Descriptive statistics of the host range and trophic level of the host-assemblages considered in the regression analysis (see Chapter 5 and Fig. 13).

Host Assemblage	Average Host Range	Standard Deviation	Average trophic level	Standard Deviation	n
Lutjanidae	4.2	1.6	4.0	0.3	11
Carangidae	3.6	1.8	3.9	0.5	29
Reef fish	2.5	1.4	2.7	0.6	18
Characidae	1.8	0.7	2.5	0.5	21
Loricaridae	1.0	0.0	2.0	0.0	11

Table 7 – Number and percentage of significantly cooccurring (and competing) parasite species pairs (i.e. those species pairs found to share more or less hosts than expected by chance, with $P < 0.05$) within the fish assemblage (reported in S6) comprising monogenoidean parasite records from Atlantic Central America, ideally categorizable into predators and prey according to the fish consumption information provided by Froese and Pauly (2009). Percentages are calculated on the total number of possible interactions (1378).

	Intra-group	Carangidae	Intra-group	Reef	Prey	Inter groups	Tot
Cooccurrence							
n	27		18			13	58
%	1.96		1.31			0.94	4.21
Competition							
n	0		0			1	1
%	0		0			0	0

Table 8 – Number and percentage of significantly cooccurring and competing species pairs for the monogenoidean species assemblage of matrices S7 (comprising all the available monogenoidean records in different biogeographical and environmental contexts: Amazon river basin, Atlantic Central American marine waters, Central American freshwaters).

		Significant Competing Pairs	Significant Cooccurring Pairs	Number of potential couple interactions
Amazon River Basin				20706
	n	52	802	
	%	0.12	1.93	
Central America Marine				21736
	n	27	321	
	%	0.06	0.73	
Central America Freshwater				3321
	n	1	107	
	%	0.03	3.22	

REFERENCES

Anderson R.P., Laverde M., Peterson A.T. 2002a. Geographical distributions of spiny pocket mice in South America: Insights from predictive models. *Global Ecology and Biogeography* 11: 131-141.

Anderson R.P., Laverde M., Peterson A.T. 2002b. Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. *Oikos* 93: 3-16.

Anderson R.P., Lew D., Peterson A.T. 2003. Evaluating predictive models of species' distributions: Criteria for selecting optimal models. *Ecological Modelling* 162: 211-232.

Atmar W., Patterson B.D. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96: 373-382.

Atmar W., Patterson B.D. 1995. The nestedness temperature calculator: a Visual Basic program, including 294 presence-absence matrices. - AICS Research, Univ. Park, NM and Field Museum, Chicago.

Barker S.C. 1994. Phylogeny and classification, origins, and evolution of

host associations of lice. *International Journal for Parasitology* 24: 1285-1291.

Boeger W.A., Kritsky D.C. 1989. Phylogeny, coevolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). *International Journal for Parasitology* 19: 425–440.

Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83:575–583.

Bush A.O., Fernandez J.C., Esch G.W., Seed R. 2001. Parasitism. The diversity and ecology of animal parasites. Cambridge University Press. 566 pp.

Bychowsky B.E., Nagibina L.T. 1967. On “intermediate” hosts in monogeneans (Monogenoidea). *Parazitologiya* 1: 117-122 (in Russian).

Charleston M.A. 1998. Jungles: A new solution to the host/parasite phylogeny reconciliation problem. *Mathematical Biosciences* 149: 191–223.

Chisholm L., Whittington I.D., Kearn G.C. 2001. *Dendromonocotyle*

colorni sp.n. (Monogenea: Monocotyliidae) from the skin of *Himantura uarnak* (Dasyatididae) from Israel and a new host record for *D. octodiscus* from the Bahamas. *Folia Parasitologica* 48: 15-20.

Cohen S., Kohn A. 2008. South American Monogenea - list of species, hosts and geographical distribution from 1997 to 2008. *Zootaxa* 1924: 1-42.

Combes C. 1991. Evolution of parasite life cycles. In: Toft C.A., Aeschlimann A., Bolis L. "Parasite-Host associations: Coexistence or Conflict?", Oxford University Press, Oxford, pp. 62-82.

Combes C. 1991. Evolution of parasite life cycles. In: Toft C.A., Aeschlimann A., Bolis L. "Parasite-Host associations: Coexistence or Conflict?", Oxford University Press, Oxford, pp. 62-82.

Darlington P.J. 1957. *Zoogeography: the geographical distribution of animals.* - Wiley.

Daubenmire R. 1957. Floristic plant geography of eastern Washington and northern Idaho. - *Journal of Biogeography* 2:1-18.

Davidova M., Jarkovsky J., Matějusová I., Gelnar M. 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Zitnan 1964 (Monogenea, Gyrodactylidae), Parasitology Research 95: 398-405.

Desdevises Y., Jovelin R., Jousson O., Morand S. 2000. Comparison of ribosomal DNA sequences of *Lamellodiscus* spp, (Monogenea, Diplectanidae) parasitising Pagellus (Sparidae, Teleostei) in the north Mediterranean sea: species divergence and coevolutionary interactions. International Journal for Parasitology 30:741–746.

Desdevises Y., Morand S., Jousson O., Legendre P. 2002. Coevolution between *Lamellodiscus* (monogenea: Diplectanidae) and Sparidae (teleostei): the study of a complex host-parasite system. Evolution 56(12): 2459–71.

Eichler W. 1942. Die Entfaltungsregel und andere Gesetzmässigkeiten in den parasito-genetischen Beziehungen der Mallophagen und anderer ständer Parasiten zu ihren Wirten. Zoologischer Anzeiger 137: 77–83.

Eichler W. 1948. Evolutionsfragen der Wirtsspezifität. Biologisches Zentralblatt 67: 373–406.

Ergens R. 1969. The suitability of ammonium picrate-glycerin in

preparing slides of lower Monogenoidea. *Folia Parasitologica* 16: 320.

Fahrenholz H. 1913. Ectoparasiten und Abstammungslehre. *Zoologischer Anzeiger* 41: 371–374.

Fellis K.J., Negovetich N.J., Esch G.W., Horak I.G., Boomker J. 2003. Patterns of association, nestedness, and species co-occurrence of helminth parasites in the greater kudu, *Tragelaphus strepsiceros*, in the Kruger National Park, South Africa, and the Etosha National Park, Namibia. *Journal of Parasitology* 89(5):899-907.

Froese R. and Pauly D. (Eds) 2009. Fishbase, World Wide Web electronic publication. www.fishbase.org, version (10/2009).

Galli P., Strona G., Villa A.M., Benzoni F., Stefani F., Doglia S.M., Kritsky D.C. 2006. Three-dimensional imaging of monogenoidean sclerites by confocal laser scanning microscopy. *Journal of Parasitology*, 92(2): 395–9.

Gerasev P.I. 2009. Fauna of monogeneans (Monogenea, Platyhelminthes) of gudgeons (Gobioninae, Cyprininae). *Coevolution. Parazitologîâ* 43(1): 12-31.

Gonzalez M.T., Poulin R. 2005. Nested Patterns in parasite component communities of a marine fish along its latitudinal range on the Pacific coast of South America. *Parasitology* 131(4): 569-577.

González M.T., Oliva M.E. 2009. Is the nestedness of metazoan parasite assemblages of marine fishes from the southeastern Pacific coast a pattern associated with the geographical distributional range of the host? *Parasitology* 136(4):401-9.

Gotelli N.J. 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81(9): 2606-2621.

Gotelli N.J., McCabe D.J. 2002. Species Co-Occurrence: A Meta-Analysis of J. M. Diamond's Assembly Rules Model. *Ecology* 83(8): 2091-2096.

Guégan J.F., Lambert A., Lévêque C., Combes C., Euzet L. 1992. Can body size explain the parasite species richness in tropical freshwater fishes? *Oecologia* 90: 197-204.

Guégan J.F., Kennedy C.R. 1993. Maximum local helminth parasite community richness in British freshwater fish: a test of the colonization time hypothesis. *Parasitology* 106: 91-100.

Guégan J.F., Kennedy C.R. 1996. Parasite richness/Sampling effort/Host range: The Fancy Three-piece Jigsaw Puzzle. *Parasitology Today* 12(9):367-369.

Hafner M.S., Nadler S.A. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* 332: 258-259

Hastings A. 1987. Can Competition Be Detected Using Species Co-Occurrence Data? *Ecology*: Vol. 68, No. 1, pp. 117-123.

Hewitt G.C., Hine P.M. 1972. Checklist of Parasites of New Zealand fishes and of their hosts. *New Zealand Journal of Marine and Freshwater Research* 6: 69-114.

Holland C.V., Kennedy C.R. 1997. A checklist of parasitic helminth and crustacean species recorded in freshwater fish from Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* 3: 225 – 243.

Hultén E. 1937. Outline of the history of Arctic and Boreal biota during the Quaternary period. - Thule, Stockholm.

Jarkovsky J., Morand S., Šimkova A., Gelnar M. 2004. Reproductive barriers between congeneric monogenean parasites (*Dactylogyrus*: *Monogenea*): attachment apparatus morphology or copulatory organ incompatibility?. *Parasitology Research*, 92(2): 95-105.

Jovelin R., Justine J-L. 2001. Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *International Journal for Parasitology* 31: 393-401.

Kearn G.C. 1994. Evolutionary expansion of the *Monogenea*. *International Journal for Parasitology* 24:1227–1271.

Kennedy C.R., Guégan J.F. 1994. Regional versus local helminth parasite richness in British freshwater fish: saturated or unsaturated parasite communities? *Parasitology* 109: 175-185.

Kennedy C.R., Bush A.O. 1994. The relationship between pattern and scale in parasite communities: a stranger in a strange land. *Parasitology*

109: 187-196.

King T.A., Cable J. 2007. Experimental infections of the monogenean *Gyrodactylus turnbulli* indicate that it is not a strict specialist. *International Journal for Parasitology* 37(6): 663-672.

King T.A., van Oosterhout C., Cable J. 2009. Experimental infections with the tropical monogenean, *Gyrodactylus bullatarudis*: Potential invader or experimental fluke? *Parasitology International* 58(3): 249-254.

Kohn A., Cohen S. 1998. South American Monogenea - list of species, hosts and geographical distribution. *International Journal for Parasitology* 28: 1517 – 1554.

Kohn A., Cohen S., Salgado-Maldonado G. 2006. Checklist of Monogenea parasites of freshwater and marine fishes, amphibians and reptiles from Mexico, Central America and Caribbean. *Zootaxa* 1289: 114 pp.

Kritsky D.C., Leiby P.D., Kayton R.J. 1978. A rapid stain technique for the haptor bars of *Gyrodactylus* species (Monogenea). *Journal of Parasitology*, 64(1): 172-174.

Kuris, A.M., Blaustein, A.R., Aliò J.J. 1980. Hosts as islands. *American Naturalist* 116: 570-586.

Kuris A.M., Hechinger R.F., Shaw J.C., Whitney K.L., Aguirre-Macedo L., Boch C.A., Dobson A.P., Dunham E.J., Fredensborg B.L., Huspeni T.C., Lorda J., Mababa L., Mancini F.T., Mora A.B., Pickering M., Talhouk N.L., Torchin M.E., Lafferty K.D. 2008. Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454(7203):515-8.

Lafferty K.D., Allesina S., Arim M., Briggs C.J., De Leo G., Dobson A.P., Dunne J.A., Johnson P.T.J., Kuris A.M., Marcogliese D.J., Martinez N.D., Memmott J., Marquet P.A., McLaughlin J.P., Mordecai E.A., Pascual M., Poulin R., Thielctges D.W. 2008. Parasites in food webs: the ultimate missing links. *Ecology Letters* 11(6): 533–546.

Lambert A., El Gharbi S. 1995. Monogenean host specificity as a biological and taxonomic indicator for fish. *Biological Conservation* 72: 227-235.

Legendre P., Desdevises Y., Azin E.B. 2002. A Statistical Test for Host-

Parasite Coevolution. *Systematic Biology* 51(2):217–234.

Littlewood D.T.J., Rohde K., Clough K.A. 1997. Parasite speciation within or between host species?- phylogenetic evidence from site-specific polystome monogeneans. *International Journal for Parasitology* 27: 1289-1297.

Littlewood D.T.J., Rohde K., Clough K.A. 1998. The phylogenetic position of *Udonella* (Platyhelminthes). *International Journal for Parasitology* 28:1241–50.

Malmberg G. 1957. Om förekomsten av *Gyrodactylus* på svenska fiskar. Särtryck ur Skrifter utgivna av Södra Sveriges Fiskeriförening, Årsskrift 1956, 19-76.

Muñoz M.E.S., Giovanni R., Siqueira M.F., Sutton T., Brewer P., Pereira R.S., Canhos D.A.L., Canhos V.P. 2009 "OpenModeller: a generic approach to species' potential distribution modelling". *GeoInformatica*. DOI: 10.1007/s10707-009-0090-7.

Nieberding C., Göüy de Bellocq J., Morand S. 2005. The biogeography of host-parasite interactions: from nested assemblages to comparative phylogeography. *Mammal Study* 30: 87-93.

Noble E.R., Noble G.A., Schad G.A., MacInnes A.J. 1989. Parasitology. The biology of animal parasites. 6th ed. Lea and Febiger, Philadelphia.

Page R.D.M., Charleston M.A. 1998. Trees within trees-Phylogeny and historical associations. *Trends in Ecology and Evolution* 13:356–359.

Patterson B.D., Atmar W. 2000. Analyzing species composition in fragments. - *Bonner Zoological Monographs* 46.

Pimm S.L., Brown J.H. 2004. Domains of Diversity. *Science* 304 (5672): 831 – 833.

Poulin R. 1996. Richness, nestedness, and randomness in parasite infracommunity structure. *Oecologia* 105(4):1432-1939.

Poulin R. 1996. The evolution of body size in the Monogenea: the role of host size and latitude. *Canadian Journal of Zoology* 74(4): 726–732.

Poulin R. 1997. Parasite faunas of freshwater fish: the relationship between parasite richness and the specificity of parasites. *International Journal for Parasitology* 27(9): 1091-1098.

Poulin R., Valtonen E.T. 2001. Nested assemblages resulting from host size variation: the case of endoparasite communities in fish hosts. *International Journal for Parasitology* 31(11): 1194-1204.

Poulin R. 2002. The evolution of monogenean diversity. *International Journal for Parasitology* 32:245–254.

Poulin R., Mouillot D. 2005. Host specificity and the probability of discovering species of helminth parasites. *Parasitology* 130: 709–715.

Poulin R. 2007. Are there general laws in parasite ecology? *Parasitology* 134: 763–776.

Rodríguez-Gironés M. A., Santamaría L. 2006. A new algorithm to calculate the nestedness temperature of presence-absence matrices. *Journal of Biogeography*, 33: 924-935.

Rohde K. 1981. Niche width of parasites in species-rich and species-poor communities *Cellular and Molecular Life Sciences* 37(4): 359-361

Rohde K. 1993. *Ecology of Marine Parasites: An Introduction to Marine Parasitology*, 2nd Edition. CAB International, Wallingford, 298 pp.

Ronquist F. 1997. Phylogenetic approaches in coevolution and biogeography. *Zoologica Scripta* 26:313-322.

Rozsa L. 1991. Points in question: flamingo lice contravene Fahrenholz. *International Journal for Parasitology* 21(2): 151-152.

Salgado-Maldonado G. 2006. Checklist of helminth parasites of freshwater fishes from Mexico. *Zootaxa* 1324: 357 pp.

Salgado-Maldonado G. 2008. Helminth parasites of freshwater fish from Central America. *Zootaxa* 1915: 29–53.

Schmidt G.D., Roberts L.S. 1985. *Foundations of Parasitology*. Times Mirror-Mosby College, St. Louis, pp. 655-668.

Shaw D.J., Dobson, A.P. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* 111: 111–133.

Simkova A., Desdevises Y., Gelnar M., Morand S. 2000. Co-existence of nine gill ectoparasites (*Dactylogyrus*: Monogenea) parasitising the roach *Rutilus rutilus* (L.): history and present ecology. *International Journal for Parasitology* 30: 1077-1085.

Simkova A., Gelnar M., Morand S. 2001. Order and disorder in ectoparasite communities: the case of congeneric gill monogeneans (*Dactylogyrus* spp.). *International Journal for Parasitology* 31, 1205-1210.

Simkova A., Gelnar M., Sasal P. 2001. Aggregation of congeneric parasites (Monogenea: *Dactylogyrus*). *Parasitology* 123: 599-607.

Stockwell D.R.B., Peters D.P. 1999. The GARP modeling system: Problems and solutions to automated spatial prediction. *International Journal of Geographic Information Systems* 13: 143-158.

Strona G., Stefani F., Galli P. 2009. Field preservation of monogenean parasites for molecular and morphological analyses. *Parasitology International* 58: 51–54.

Strona G., Stefani F., Galli P. 2009. Monogenoidean parasites of Italian marine fish: an updated checklist. *Italian Journal of Zoology* (in press).

Szidat L. 1940. Beiträge zum Aufbau eines natürlichen Systems der Trematoden. I. Die Entwicklung von *Echinocercaria choanophila* U. Szidat zu *Cathaemasia hians* und die Ableitung der Fasciolidae von den Echinostomidae. Z. Parasitenk. 11:239–283.

Ulrich W., Gotelli N.J. 2007. Null model analysis of species nestedness patterns. Ecology 88: 1824-1831.

Ulrich W. 2006. Nestedness – a FORTRAN program for calculating ecological matrices temperatures. - www.uni.torun.pl/~ulrichw.

Ulrich W., Almeida-Neto M., Gotelli N.J. 2009. A consumer's guide to nestedness analysis. Oikos 118:3-17.

Whittington I.D., Horton M.A. 1996. A revision of *Neobenedenia* Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of *N. melleni* (MacCallum, 1927) Yamaguti, 1963. Journal of Natural History 30(8): 1113-1156.

Whittington I.D. 1998. Diversity ‘down under’: monogeneans in the Antipodes (Australia) with a prediction of monogenean biodiversity worldwide. International Journal for Parasitology 28: 1481–1493.

Whittington I.D., Perkins E.M., Chisholm L.A., Donnellan S.C. 2006. *Neobenedenia* 'melleni' (Monogenea: Capsalidae): is this enigmatic infamous pest of fishes worldwide a cryptic species complex? 11th International Congress of Parasitology (ICOPA XI), August 6-11 2006, Scottish Exhibition & Convention Centre, Glasgow, UK.

Williams E.H. Jr., Bunkley-Williams L. 1996. Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Puerto Rico Department of Natural and Environmental Resources, San Juan, PR, and the University of Puerto Rico, Mayaguez, PR, 382 p.