Host-parasite relationships in flatfish (Pleuronectiformes) – the relative importance of host biology, ecology and phylogeny

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SUMMARY

The extent to which host biology, ecology and phylogeny determine the diversity of macroparasite assemblages has been investigated in recent years in several taxa, including fish. However, consensus has not been reached probably as a result of data being collected from different sources, different temporal scales or host and parasite biogeography and phylogeny having greater influence than expected. The present study evaluates the relative importance of 27 biological, ecological and phylogenetic characteristics of 14 flatfish species on the diversity of their ecto- and endoparasite assemblages, comprising a total of 53 taxa. Redundancy analyses were applied to the mean abundance of each parasite taxa infecting each host and to the richness, taxonomic distinctness and variance in taxonomic distinctness calculated for each assemblage within each host. Only a few host characteristics contributed significantly to the observed patterns: host distribution was more important in determining the type and mean abundance of ectoparasites present in an assemblage, whereas diversity of these assemblages were mainly related to the host's maximum size. Endoparasite mean abundance and diversity were mostly influenced by the number of food items ingested and by the presence of Crustacea and Polychaeta in the diet. However, the sympatric occurrence of related hosts also played an important role in the diversity values found in macroparasite assemblages. Results showed that a host characteristic has different importance according to the host-parasite relationship being examined, suggesting an important role for host-parasite co-evolution on the diversity of extant macroparasite assemblages.

Key words: diversity, flatfish, host-characteristics, macroparasites, redundancy analyses, richness.

INTRODUCTION

Parasite communities of extant hosts are the result of repeated additions and losses of parasite species during evolutionary history and geological time. Developing over time, the ecological and biological characteristics of hosts and parasites determine host colonization, intra-host diversification and rates of parasite extinction, consequently influencing parasite community richness and diversity. The study of the factors influencing host-parasite relationships in vertebrates has been gaining the interest of ecological and evolutionary parasitologists in recent years, as illustrated in the increasing number of papers published on this subject in the last 2 decades (e.g. Poulin and Rohde, 1997; Krasnov *et al*. 2004; Korallo *et al*. 2007; Verneau *et al*. 2009).

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Host age, size, diet, habitat, behaviour, depth distribution and geographical range have all been recognized as variables influencing richness and diversity of parasite communities (e.g. Poulin and Rhode, 1997; Lile, 1998; Poulin and Morand, 2000; Luque *et al*. 2004; Aguirre-Macedo *et al*. 2007; Korallo *et al*. 2007; Luque and Poulin, 2008). However, there is no consensus about the relative importance of the host's traits in the evolution and diversification of the parasite fauna in general, suggesting that relationships between host features and parasite diversity are specific for each host-parasite association in a particular area (Aguirre-Macedo *et al*. 2007; Korallo *et al*. 2007; Luque and Poulin, 2008). Moreover, universal patterns in parasite community diversity have not yet been found (Poulin, 2007) and, for a given host group (e.g. marine fishes), the same host characteristic has shown a different influence, for instance, whereas Raibaut *et al*. (1998) and González and Poulin (2005) found no relationship between fish body size and parasite species richness, a positive relationship

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between these two variables was found by Timi and Poulin (2003).

These inconsistencies might be due to different sources of data or to data being collected and/or analysed by different people using different methods (Poulin and Rhode, 1997; Luque *et al*. 2004; Luque and Poulin, 2008). However, parasite species found within a host's distribution range are variable between geographical areas, limiting the number and type of species that can be acquired, regardless of host's characteristics, which is suggestive of an important role for host and parasite biogeography (Luque *et al*. 2004; Luque and Poulin, 2008). On the other hand, host and parasite phylogeny influence the composition of extant parasite communities (Poulin and Morand, 2000; Aguirre-Macedo *et al*. 2007; Poulin, 2007) since hosts might have inherited their parasites from their ancestors by 1 of the 4 main categories of evolutionary events (Verneau *et al*. 2009): co-speciation (synchronous divergence of host and parasite lineages over geological time); duplication (within host speciation of parasite lineages without the concomitant speciation of host lineages); host-switching (the parasite lineage colonizes a phylogenetically or ecologically close host species to its ancestral host); or extinction (following host speciation only 1 daughter species is parasitized by the ancestral parasite lineage – 'missing the boat' hypothesis – or both daughter species preserve the ancestral parasite lineages but it goes extinct in 1 of them – 'drowning on arrival' hypothesis). As such, and for a better understanding of the relative importance that host biology, ecology and phylogeny and environmental characteristics have on parasite richness and diversity patterns, these should be analysed in a group of related species inhabiting the same area (Luque *et al*. 2004; Korallo *et al*. 2007) and integrating both ecological and phylogenetic approaches (Poulin and Morand, 2000). Despite this recognition, most studies have focused solely on the effects of ecological factors (e.g. Krasnov *et al*. 2004; Korallo *et al*. 2007) and the few that take into account the importance of host phylogeny have usually considered it a confounding variable as closely related hosts are likely to harbour similar parasite communities (e.g. Poulin and Rhode, 1997; Luque and Poulin, 2008).

In the present study, the macroparasite fauna of 14 species of flatfish (Pleuronectiformes) inhabiting the Portuguese coast was investigated to assess the relative importance of several host features in the diversity of their macroparasite assemblages. Although monophyletic (Azevedo *et al*. 2008), this group of fishes comprises many different species, genera and families, displaying a wide variety of ecological and life-history patterns, including biological, environmental and dietetic requirements, and a relatively high species diversity within the Portuguese coast (25 species belonging to 13 genera and 5 different families). Therefore, Pleuronectiformes constitute a valuable group to evaluate the relative importance of host biology, ecology and phylogeny on the structure of host-parasite associations given the identical parasite species pool and environmental characteristics.

The richness (number of species), taxonomic distinctness (Δ^+) and the variance of taxonomic distinctness (Λ^+) (Clarke and Warwick, 1998, 1999; Warwick and Clarke, 2001) of each ecto- and endoparasite assemblage infecting a host species were calculated and the relative effect of 27 host characteristics on the patterns found were assessed by redundancy analyses in order to evaluate: (1) which host characteristic(s) are more important in the diversity found; (2) whether host phylogeny is, indeed, a good predictor of the structure of parasite communities, i.e. if closer hosts present more similar parasite assemblages; (3) whether the influence of a host characteristic varies according to the parasite taxa and guilds or if it is always identical independently of the type of parasites found; and (4) whether general patterns can be found at this local scale.

MATERIALS AND METHODS

Sampling procedures

Fourteen flatfish species, belonging to 5 different families (Table 1), were collected seasonally between January 2003 and June 2005 from commercial fishing vessels operating with gill nets and bottom trawls along the Portuguese coast. Each fish was measured (total length) and sexed prior to examination of its skin, fins, gills, nostrils and mouth cavity for the detection of ectoparasites. Digestive tract, liver, heart, spleen, gonads and mesenteries were carefully inspected under a stereomicroscope to search for endoparasites. Collected parasite specimens were then identified to the lowest possible taxonomical level, depending on their maturation stage and on the number of individuals available. Parasite collection and identification were always performed by the same researcher, reducing the variance in data associated with different sources of information and different methodologies.

Food items, gonads and otoliths were also collected from the same individual hosts and the data on feeding ecology, reproduction and growth were used as the hosts' ecological and biological characteristics (Table 2).

Data analyses

Mean abundance of each macroparasite taxa within each host species was calculated according to the method of Bush *et al*. (1997), i.e. the mean number of parasites of a given taxon per host. It has been shown that mean abundance values of macroparasite taxa

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infecting Pleuronectiformes are unaffected by a sample size larger than 40 individuals (Marques and Cabral, 2007). Thus, no corrections for sample sizes were performed despite the wide range of host individuals surveyed within each species. To evaluate the diversity of macroparasite assemblages, the total number of taxa (richness), taxonomic distinctness (Δ^+) and the variance of taxonomic distinctness (Λ^+) were calculated considering ectoparasites and endoparasites separately as they have different transmission pathways and life cycles (MacKenzie, 2002). Cases where only 1 parasite individual infected a single host individual (*Gyrodactylus* sp., *Anisakis typical* (Diesing, 1860), *Cucullanus heterochrous* Rudolphi, 1802, *Lernaeocera* sp.) were not included in the calculation of diversity indices, as they might be accidental infections.

For 2 host species presenting identical macroparasite richness some features may promote the acquisition of a broader taxonomic range of parasites whereas others may contribute to a narrower range. Hence, these assemblages may be different and, therefore, the present study considers measures of diversity that take species relatedness into account, i.e. Δ^+ and Λ^+. Moreover, whereas richness is highly dependent on sample size, 'taxonomic' measures are not (Clarke and Warwick, 1998). Taxonomic distinctness (Δ^+) is the expected path length between any 2 species in a sample and is calculated as the number of steps taken to reach a common taxon (Clarke and Warwick, 1998, 1999; Warwick and Clarke, 2001): 0 for the same species, 1 for different species of the same genus, 2 for different genera within a single family. The variance in taxonomic distinctness (Λ^+) reflects the representativeness of each taxon in the assemblage (Warwick and Clarke, 1995). Λ^+ can only be computed when at least 3 parasite taxa are found in a host sample (it always equals zero with 2 species); however, it is only reliable for assemblages with at least 4 parasite taxa (Luque *et al*. 2004). Macroparasite taxa were placed within the Linnaean taxonomic classification according to their species, genus, family, order, class, phylum and kingdom as indicated in the Natural History Museum host-parasite database (Gibson *et al*. 2008) and the European register of marine species database (MarBEF Data System, 2008) and Δ^+ and Λ^+ were calculated using PRIMER 5.2.8 (Clarke and Gorley, 2001). The higher the values of Δ^+ the lower the relatedness between the macroparasite species found within a host. The higher the values of Λ^+, the lower the evenness within macroparasite assemblages.

A total of 27 phylogenetic, biological and ecological host characteristics expected to have some degree of influence on the composition of macroparasite assemblages and infection levels of each macroparasite taxa within each host species were used (Table 2). Given that mean abundance and diversity values were not expected to be influenced by differences in sample size, as these were all larger than 40 individuals (Marques and Cabral, 2007), sample size was not considered as a variable in our study. The total number of genera and species known worldwide for each host family, as well as host family itself, were considered as phylogenetic characteristics. Reproductive season, zoogeographical classification and the proportional length of the gut of each host species were transformed from nominal to ordinal variables in the analysed data matrix. The minimum latitude reported for each species was transformed into negative values for southern latitudes in order to allow the calculation of host latitudinal range. The size range of the fishes examined in this study, in relation to the size range reported for each species, was also included as a variable given that body size has been shown to influence macroparasite communities (e.g. Luque *et al*. 2004; Muñoz *et al*. 2006) and very large individual hosts were only obtained for some of the host species inhabiting the Portuguese coast. Differences in the amount of epidermal mucus segregated by the 14 species were also detected when examining them for ectoparasites and, therefore, it was considered as a variable in ectoparasites' analyses. Although all Pleuronectiformes included in the present study are in the same trophic level (values between 3 and 4 according to Froese and Pauly (2009)), their different depth distribution and maximum size might result in different predation pressures exerted on them by other species (e.g. marine mammals) that, in turn, will influence the probability of endoparasite species completing their life cycle. Therefore, predation pressure can affect macroparasite assemblage composition and infection levels and was also included as a variable. Because endoparasites are differently distributed along the gut, or found in particular regions within it (e.g. pyloric caeca), the length of the gut in relation to host total length and the morphological differentiation of the gut were considered as variables in the analyses of endoparasite assemblages. The number of different prey ingested and the inclusion or absence of certain groups of taxa in the diet were also used in endoparasite analyses.

Redundancy analysis (RDA) was applied to investigate the relationships between host characteristics and the observed patterns of mean abundance, richness, Δ^+ and Λ^+. RDA is generally used to visualize linear correlations between explanatory variables, response variables and samples and to determine the relative importance of each explanatory variable and its significance in the variation observed in a data set. This multivariate technique was used to assess the relative importance of the phylogenetic, biological and ecological characteristics of hosts in (1) the taxa found infecting them and the mean abundance of each taxon and (2) the richness and diversity of their macroparasite assemblages. Analyses were performed in CANOCO 4.5

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Table 1. Mean abundance of each taxon infecting each host

(Codes used in figures and site of infection are shown. Host families (above host species) and host sample size (below host species, *n*) are also indicated. B, branchial arches; G, gills; L, gut lumen; M, mesenteries; S, skin; V, visceral cavity; W, gut wall.)

			Citharidae	Bothidae	Scophthalmidae			Pleuronectidae
Taxon	Code	Site	*Citharus linguatula* (*n* = 486)	*Arnoglossus laterna* (*n* = 159)	*Lepidorhombus boscii* (*n* = 439)	*Scophthalmus rhombus* (*n* = 223)	*Scophthalmus maximus* (*n* = 68)	*Platichthys flesus* (*n* = 261)
Monogenea								
Entobdella solea	*Esol*	S						
Gyrodactylus sp.	—	G						
Digenea								
Derogenes varicus	*Dvar*	L				0·063		0·192
Helicometra fasciata	*Hfas*	L			0·021			
Hemipera sp.	*Hemi*	L						
Homalometron galaicus	*Hgal*	L						
Lecithochirium rufoviride	*Lruf*	L	0·144	0·006	0·073	2·040	1·132	0·011
Lomasoma stephanskii	*Lste*	L						
Macvicaria soleae	*Msol*	L						0·854
Otodistomum sp.	*Otod*	V						
Proctoeces maculatus	*Pmac*	L						0·119
Prosorhynchus crucibulum	*Pcru*	B						
Zoogonus rubellus	*Zrub*	L						0·433
Cestoda								
Bothriocephalus andresi	*Band*	L	0·280					
Bothriocephalus barbatus	*Bbar*	L				0·184		
Bothriocephalus clavibothrium	*Bcla*	L		0·057				
Bothriocephalus gregarius	*Bgre*	L					1·529	
Bothriocephalus scorpii	*Bsco*	L			0·011			0·015
Didymobothrium rudolphii	*Drud*	L						
Diphyllobothrium sp.	*Diph*	L						
Grillotia sp.	*Gril*	W						
Nybelinia lingualis	*Nlin*	M	0·043		0·011	0·090		
Progrillotia dasyatidis	*Pdas*	L			1·287			
Scolex pleuronectis	*Sple*	L						0·015

Acanthocephala								
Acanthocephaloides geneticus	*Agen*	L			0·027			
Acanthocephaloides propinquus	*Apro*	L			0·137	0·332		
Acanthocephalus incrassatus	*Ainc*	L						
Echinorhynchus gadi	*Egad*	L			0·036			
Radinorhynchus sp.	*Radi*	L	0·002					
Nematoda								
Anisakis simplex	*Asim*	W	0·885	0·057	0·232			
Anisakis typica	—	W						0·004
Capillaria sp.	Capi	L	0·019					0·188
Cucullanus campanae	*Ccam*	L						0·004
Cucullanus heterochrous	—	L						
Cucullanus sp.	*Cucc*	L		0·013				
Dichelyne minutus	*Dmin*	L						1·494
Hysterothylacium aduncum	*Hadu*	L						0·027
Hysterothylacium reliquens	*Hrel*	L						
Hysterothylacium sp.	*Hyst*	L						
Copepoda								
Acanthochondria cornuta	*Acor*	B						6·184
Acanthochondria soleae	*Asol*	B						
Bomolochus soleae	*Bsol*	G						
Caligus brevicaudatus	*Cbre*	S						
Caligus elongatus	*Celo*	S						
Ergasilus sp.	*Erga*	G						
Lepeophtheirus europaensis	*Leur*	S				0·004		
Lepeophtheirus pectoralis	*Lpec*	S						4·418
Lernaeocera sp.	—	G						
Isopoda								
Gnathia sp.	*Gnat*	S			0·002			0·008
Rocinella sp.	*Roci*	S	0·002	0·006		0·022	0·029	
Pentastomida	Pent	L						
Hirudinea								
Caliobdella sp.	*Cali*	S						
Hemibdella soleae	*Hsol*	S						

Table 1 (*Cont.*)

			Soleidae							
Taxon	Code	Site	*Dicologlossa cuneata* (*n* = 490)	*Microchirus azevia* (*n* = 329)	*Microchirus variegates* (*n* = 327)	*Solea kleinii* (*n* = 47)	*Solea lascaris* (*n* = 480)	*Solea senegalensis* (*n* = 479)	*Solea solea* (*n* = 424)	*Synaptura lusitanica* (*n* = 270)
Monogenea										
Entobdella solea	*Esol*	S	0·002					0·117		
Gyrodactylus sp.	—	G					0·002			
Digenea										
Derogenes varicus	*Dvar*	L	0·031			0·043	2·358	0·067		
Helicometra fasciata	*Hfas*	L								
Hemipera sp.	*Hemi*	L		0·006	0·006		0·010	0·006		
Homalometron galaicus	*Hgal*	L	0·794	0·878	0·483			0·006		
Lecithochirium rufoviride	*Lruf*	L		0·003						
Lomasoma stephanskii	*Lste*	L			0·654					
Macvicaria soleae	*Msol*	L	0·061	0·261	0·003		0·538	0·132	0·519	
Otodistomum sp.	*Otod*	V				31·468				
Proctoeces maculatus	*Pmac*	L								
Prosorhynchus crucibulum	*Pcru*	B	0·008				0·840	0·010		0·007
Zoogonus rubellus	*Zrub*	L	0·061					0·015		
Cestoda										
Bothriocephalus andresi	*Band*	L								
Bothriocephalus barbatus	*Bbar*	L								
Bothriocephalus clavibothrium	*Bcla*	L								
Bothriocephalus gregarius	*Bgre*	L								
Bothriocephalus scorpii	*Bsco*	L	0·004					0·002		
Didymobothrium rudolphii	*Drud*	L					2·925	0·006		
Diphyllobothrium sp.	*Diph*	L								0·007
Grillotia sp.	*Gril*	W			0·009					
Nybelinia lingualis	*Nlin*	M	0·020				1·150	0·004	0·017	
Progrillotia dasyatidis	*Pdas*	L	0·124	0·021	0·021		0·035	0·040		
Scolex pleuronectis	*Sple*	L	0·208	0·027				0·718	0·455	

Acanthocephala										
Acanthocephaloides geneticus	*Agen*	L					0·473	0·002		
Acanthocephaloides propinquus	*Apro*	L	0·406	0·024	0·006			14·407	0·005	
Acanthocephalus incrassatus	*Ainc*	L					1·131			
Echinorhynchus gadi	*Egad*	L								
Radinorhynchus sp.	*Radi*	L		0·003			0·002			
Nematoda										
Anisakis simplex	*Asim*	W	0·010			0·277	0·002			
Anisakis typica	—	W								
Capillaria sp.	Capi	L	0·006							0·122
Cucullanus campanae	*Ccam*	L	0·727	0·043			0·023	0·557	0·238	
Cucullanus heterochrous	—	L		0·003						
Cucullanus sp.	*Cucc*	L								
Dichelyne minutus	*Dmin*	L								
Hysterothylacium aduncum	*Hadu*	L			0·009		0·002			
Hysterothylacium reliquens	*Hrel*	L		0·015	0·040					
Hysterothylacium sp.	*Hyst*	L		0·009			0·035			
Copepoda										
Acanthochondria cornuta	*Acor*	B								
Acanthochondria soleae	*Asol*	B					0·004		0·005	
Bomolochus soleae	*Bsol*	G	0·016			0·128	0·198	1·299	0·302	
Caligus brevicaudatus	*Cbre*	S					0·027	0·127	0·007	
Caligus elongatus	*Celo*	S		0·003					0·002	
Ergasilus sp.	*Erga*	G	0·022							
Lepeophtheirus europaensis	*Leur*	S	0·035							
Lepeophtheirus pectoralis	*Lpec*	S						0·008		
Lernaeocera sp.	—	G		0·003						
Isopoda										
Gnathia sp.	*Gnat*	S		0·003			0·010		0·009	
Rocinella sp.	*Roci*	S		0·012			0·008	0·013		0·007
Pentastomida	*Pent*	L	0·045			0·021				
Hirudinea										
Caliobdella sp.	*Cali*	S						0·013		
Hemibdella soleae	*Hsol*	S	0·006	0·006		0·021	0·123	0·225	0·019	0·011

Table 2. Host characteristics of the flatfish species included in the analyses

(Clin, *Citharus linguatula*; Alat, *Arnoglossus laterna*; Lbos, *Lepidorhombus boscii*; Srho, *Scophthalmus rhombus*; Smax, *Scophthalmus maximus*; Pfle, *Platichthys flesus*; Dcun, *Dicologlossa cuneata*; Maze, *Microchirus azevia*; Mvar, *Microchirus variegatus*; Skle, *Solea kleinii*; Slas, *Solea lascaris*; Ssen, *Solea senegalensis*; Ssol, *Solea solea*; Slus, *Synaptura lusitanica*. Reproductive season: AuWt, autumn/winter; WtSp, winter/spring; SpSm, spring/summer; SmAu, summer/autumn. Zoogeographical classification: PL, polar; ST, subtropical; TM, temperate; TP, tropical. Mucus secretion was classified as 1 (low) or 2 (high), depending on the thickness of the mucus layer covering host's body; Predation pressure was classified from 1 (low) to 4 (high) based on host size and its habitat (coastal or deep); Proportional length of the gut was classified as 1 (smaller than fish total length), 2 (identical to fish total length), 3 (longer than fish) or 4 (much longer than fish). Binary variables (indicated with '?') are coded as '0' – no, '1' – yes. Italicized variables were only used in endoparasite assemblages' analyses; 'Mucus secretion' was only used in ectoparasites assemblages' analyses.)

Host characteristics	Clin	Alat	Lbos	Srho	Smax	Pfle	Dcun	Maze	Mvar	Skle	Slas	Ssen	Ssol	Slus
Number of genera registered worldwide[2]	4	20	4	4	4	21	29	29	29	29	29	29	29	29
Number of species registered worldwide[2]	7	145	9	9	9	60	139	139	139	139	139	139	139	139
Life span (years)[3,4,6,7]	8	8	10	6	26	15	–	10	15	–	15	20	28	8
Growth coefficient[3,4,6,7]	0·19	0·80	0·20	0·40	0·28	0·11	0·47	0·40	0·37	0·30	0·82	0·17	0·23	0·27
Age at first maturation (years)[3,4,6,7]	2	3	2	5	4	3	–	3	3	–	3	3	9	4
Reproductive season[3,4,6,7]	SmAu	WtSp	AuWt	SpSm	SpSm	AuWt	WtSp	SpSm	WtSp	WtSp	WtSp	AuWt	AuWt	SpSm
Reproductive season length (months)[3,4,6,7]	4	5	4	6	5	4	4	4	4	4	3	4	3	4
Maximum size reported (mm)[1,3,5,6,7]	300	260	400	750	1000	600	300	420	370	451	400	620	700	480
Proportion of size range examined (%)*[,1]	83	100	89	60	24	68	88	71	36	100	82	71	41	100
Maximum latitude reported (degrees)[3]	44·0	59·5	62·2	58·9	71·2	68·4	57·0	40·0	57·0	43·7	50·1	44·8	58·9	39·0
Minimum latitude reported (degrees)[+,3]	−8·7	21·0	35·5	−8·1	30·5	−33·0	−22·9	13·0	16·0	−34·0	−5·8	5·2	13·2	−5·8
Latitude range (degrees)[1]	52·7	38·5	26·7	67·0	40·7	101·4	80·3	27·0	40·9	77·7	55·9	39·5	45·7	44·8
Zoogeographical classification[3]	ST	ST	TM	TM	TM	PL	ST	TM	ST	ST	ST	ST	ST	TP
Maximum depth reported (m)[3]	200	200	800	50	70	100	460	250	400	460	350	65	150	60
Adults inhabit brackish waters?[1,3,4,5,6,7]	0	0	0	0	1	1	0	0	0	1	0	1	1	1
Present in rocky bottoms?[1,3,4,5,6,7]	0	1	0	1	1	0	0	0	0	0	0	0	0	0
Mucus secretion[1]	1	1	1	2	2	2	2	2	2	2	2	2	2	2
Predation pressure[1]	3	3	4	1	1	1	4	2	3	2	3	1	1	1
Differentiated gut?[1]	1	1	1	1	1	1	0	0	0	0	0	0	0	0
Proportional length of the gut[1]	1	2	1	1	1	1	3	3	4	2	2	2	2	2
Number of different prey[1,3,4,5,6,7]	40	39	14	21	19	16	48	17	4	6	49	50	63	12
Polychaeta?[1,5,6,7]	0	0	1	0	0	1	1	1	1	1	1	1	1	1
Crustacea?[1,5,6,7]	1	1	1	1	1	1	1	1	1	0	1	1	1	0
Mollusca?[1,5,6,7]	1	0	1	0	0	1	1	1	0	1	1	1	1	0
Echinodermata?[1,5,6,7]	1	0	1	0	0	1	0	1	0	0	1	1	1	0
Cephalochordata?[1,5,6,7]	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Teleostei?[1,5,6,7]	1	0	1	1	1	1	0	1	0	0	0	1	1	0

Sources: [1] present study; [2] Munroe (2005); [3] Froese and Pauly (2009); [4] Deniel (1981); [5] Cabral *et al*. (2003); [6] Teixeira *et al*. (2009); [7] Teixeira *et al*. (2010); [8] Teixeira and Cabral (2010).

* Proportion of the size range of fish examined in the present study considering the size at first maturity as the minimum and the maximum size reported as the maximum.

[+] Degrees south are indicated by (–).

Table 3. Diversity of parasite assemblages within each host species

(Clin, *Citharus linguatula*; Alat, *Arnoglossu laterna*; Lbos, *Lepidorhombus boscii*; Srho, *Scophthalmus rhombus*; Smax, *Scophthalmus maximus*; Pfle, *Platichthys flesus*; Dcun, *Dicologlossa cuneata*; Maze, *Microchirus azevia*; Mvar, *Microchirus variegatus*; Skle, *Solea kleinii*; Slas, *Solea lascaris*, Ssen, *Solea senegalensis*; Ssol, *Solea solea*; Slus, *Synaptura lusitanica*. Only parasite species represented by more than 1 individual were considered in taxonomic indices. Taxonomic indices were only computed for hosts harbouring at least 4 ecto- or endoparasite taxa.)

	Clin	Alat	Lbos	Srho	Smax	Pfle	Dcun	Maze	Mvar	Skle	Slas	Ssen	Ssol	Slus
Ectoparasites														
Richness	1	1	1	2	1	3	5	5	0	2	7	7	6	2
Taxonomic distinctness (Δ^+)	—	—	—	—	—	—	75·7	73·8	—	—	70·5	75·5	66·7	—
Variance of taxonomic distinctness (Λ^+)	—	—	—	—	—	—	246·9	232·4	—	—	203·2	313·8	371·9	—
Endoparasites														
Richness	6	4	9	5	2	12	14	12	9	4	14	14	5	3
Taxonomic distinctness (Δ^+)	81·0	76·2	75·4	72·9	—	73·0	76·0	75·8	73·8	78·6	75·0	71·1	80·0	—
Variance of taxonomic distinctness (Λ^+)	72·6	249·4	290·4	181·6	—	249·9	174·0	266·2	289·1	255·1	339·1	228·7	89·8	—

(ter Braack and Smilauer, 2002) and the significance of each host characteristic to the identified patterns evaluated by permutation tests considering $\alpha = 0{\cdot}05$.

RESULTS

Of the 4482 flatfish examined, 53 macroparasite taxa (23 518 individuals) were found (Table 1). Although Digenea, Cestoda and Nematoda were the most frequent and abundant groups infecting almost every host, Monogenea, Acanthocephala, Copepoda, Isopoda, Pentastomida and Hirudinea were also present. Whereas the majority of the taxa infected several hosts, others were more specific and found in only 1 or 2 flatfish species; nevertheless, and even for the less specific macroparasites, a considerably higher mean abundance was generally observed in one of the host species (Table 1). The largest values of mean abundance found for ectoparasites were registered in *Acanthochondria cornuta* (Müller, 1776) and *Lepeophtheirus pectoralis* (Müller, 1776), both infecting *Platichthys flesus* (Linnaeus, 1758). *Otodistomum* sp. infecting *Solea kleinii* (Bonaparte, 1832) and *Acanthocephaloides propinquus* (Dujardin, 1845) infecting *Solea senegalensis* Kaup, 1858 presented the highest mean abundance values obtained for endoparasites.

Richness of ectoparasite species ranged between zero, in *Microchirus variegatus* Desoutter, 1990, and 7, in *S. senegalensis* and *Solea lascaris* (Risso, 1810) (Tables 1 and 3). Values of taxonomic distinctness (Δ^+) and its variance (Λ^+), only computed for hosts presenting at least 4 ectoparasite taxa represented by more than 1 individual, were higher in *Dicologlossa cuneata* (Moreau, 1881) and *Solea solea* (Linnaeus, 1758), respectively (Table 3). The ectoparasite species infecting *D. cuneata* were, therefore, the least related and comprised of Monogenea, Copepoda and Hirudinea (Table 1) whereas the ectoparasite assemblage of *S. solea* was the less taxonomically even, with most of its ectoparasites being Copepoda (Table 1). This proportion of Copepoda also contributed to *S. solea* presenting the lowest value of Δ^+, i.e. the assemblage comprising the most related ectoparasite taxa (Table 3). The lowest Λ^+ was found in *S. lascaris* (Table 3) indicating that this host harboured the most taxonomically even ectoparasite assemblage.

Endoparasite assemblages presented the highest richness in *D. cuneata*, *S. lascaris* and *S. senegalensis*, all infected by 14 taxa, although 3 of the parasite taxa found in *S. lascaris* were only represented by 1 individual and can, therefore, be considered accidental infections. The lowest endoparasite richness was found in *Scophthalmus maximus* (Linnaeus, 1758), infected by only 2 species (Tables 1 and 3). *Citharus linguatula* (Linnaeus, 1758) presented the highest Δ^+ and *S. lascaris* the highest Λ^+ (Table 3). Although presenting only 6 taxa, the endoparasite

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assemblage of *C. linguatula* comprised at least 1 species of each group, thus it was the most distinct. On the contrary, the endoparasite assemblage of *S. lascaris* was comprised of many taxa of the same genus or family (Table 1). *S. senegalensis* presented the lowest value of Δ^+, related to the many species within the Digenea and Cestoda infecting this host, and *C. linguatula* the lowest Λ^+, in agreement with its low number of Digenea, Cestoda, Acanthocephala and Nematoda parasites (Table 1).

The redundancy analysis (RDA) performed on ectoparasite taxa (Fig. 1A) evidenced the zoogeographical classification of *S. senegalensis*, *S. solea*, *S. lascaris* and *S. kleinii* (subtropical species, Table 2) as the main characteristic influencing their higher mean abundance of *Bomolochus soleae* (Claus, 1864), *Caligus brevicaudatus* Scott, 1901, *Entobdella solea* van Beneden and Hesse, 1864 and *Hemibdella soleae* van Beneden and Hesse, 1863 (all in the left half of the RDA diagram). A larger latitudinal range was directly related to the higher mean abundance of *A. cornuta* and *L. pectoralis* found in *P. flesus* (right half of the diagram). Although the first (horizontal) axis explained 90% of all variation in host characteristics-ectoparasite taxa relationships, the second (vertical) axis accounted for another 10% of all variation, suggesting that the ectoparasite assemblages of these 5 hosts are influenced by adults inhabiting brackish environments (except *S. lascaris*) and by spawning in colder seasons (autumn-winter or winter-spring) (Table 2). The size and direction of the vectors 'Life span', 'Number of genera' and 'Number of species' was also indicative of the importance of these variables in the type and mean abundance of ectoparasite taxa found in *Solea* spp. (Fig. 1A). However, the permutations test performed (1000 permutations) revealed that only zoogeography and latitudinal range of hosts had a significant effect ($P=0{\cdot}010$ and $P=0{\cdot}017$, respectively). When the RDA was performed on the diversity of the ectoparasite assemblages (Fig. 1B) only the 5 host species infected with at least 4 ectoparasite taxa could be considered. Entering brackish waters, reproducing in autumn-winter and attaining a larger size than other analysed species were the most important characteristics determining the richness and variance in taxonomic distinctness of *S. solea* and *S. senegalensis* (right half of the diagram) in comparison to the other 3 host species. Because the first RDA axis explained 99% of the variation in host characteristics-ectoparasite taxa, all the other host-characteristics represented in the diagram contributed little to the attained diversity values, despite the size of the vectors displayed in the diagram. The permutations test also revealed that only the maximum size reported was significantly important for the observed pattern ($P=0{\cdot}036$).

The first 2 RDA axes explained only 64% of the variance in host characteristics – endoparasite mean

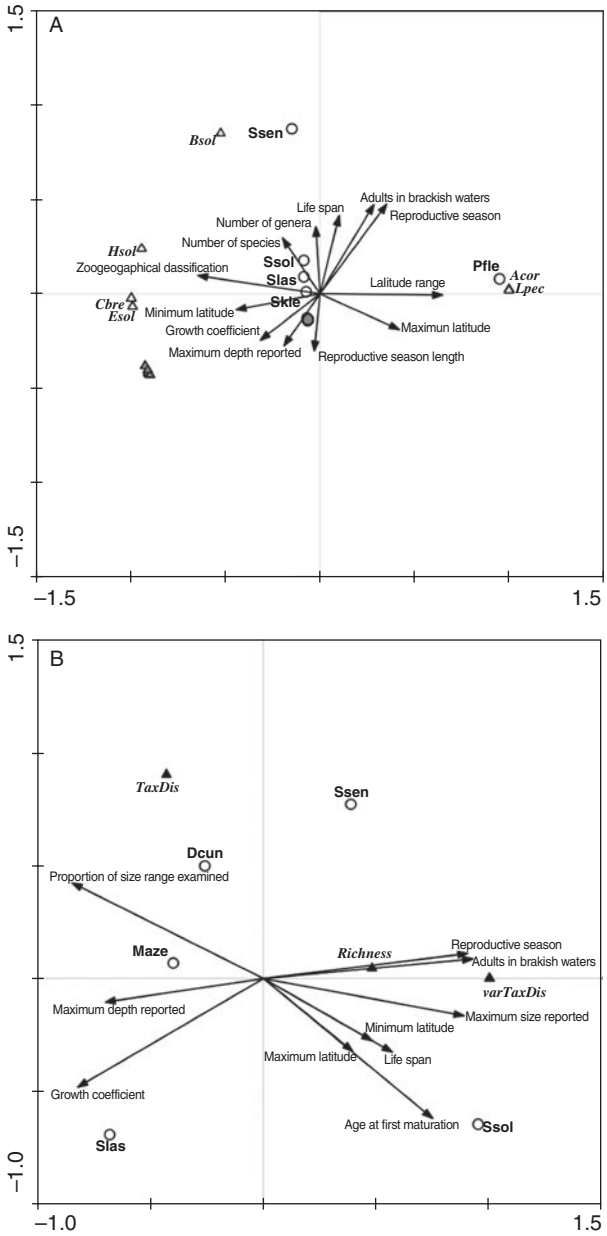

Fig. 1. Redundancy analysis (RDA) between host biological, ecological and phylogenetic characteristics as independent variables and the mean abundance of each ectoparasite taxa (A) or ectoparasite assemblage diversity measures (B) as dependent variables. Vectors represent host characteristics, circles represent host species, open triangles represent ectoparasite taxa (A) and filled triangles represent diversity measures (B). Acronyms for parasite and host taxa are given in Tables 1 and 2, respectively. Acronyms for diversity measures are: TaxDis, taxonomic distinctness; varTaxDis, variance in taxonomic distinctness. Grey symbols correspond to clumped hosts and ectoparasite taxa whose acronyms are not shown due to space constrictions.

abundance relationships, evidenced by the almost identical size of the vectors representing the more important variables, nevertheless *S. kleinii*, *S. senegalensis*, *S. lascaris* and *D. cuneata* appeared separated from the remaining species (Fig. 2A). Whereas the

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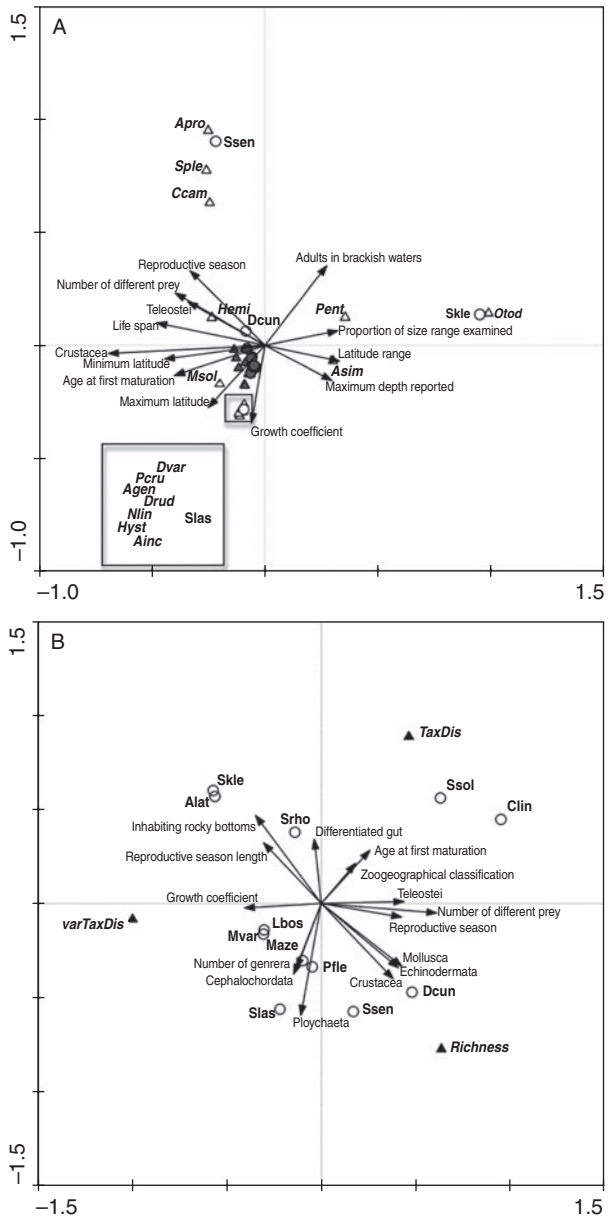

Fig. 2. Redundancy analysis (RDA) between host biological, ecological and phylogenetic characteristics as independent variables and the mean abundance of each endoparasite taxa (A) or endoparasite assemblage diversity measures (B) as dependent variables. Vectors represent host characteristics, circles represent host species, open triangles represent endoparasite taxa (A) and filled triangles represent diversity measures (B). Acronyms for parasite and host taxa are given in Tables 1 and 2, respectively. Acronyms for diversity measures are: TaxDis, taxonomic distinctness; varTaxDis, variance in taxonomic distinctness. Grey symbols correspond to clumped hosts and endoparasite taxa whose acronyms are not shown due to space constrictions. The acronyms for *Solea lascaris* and its endoparasite taxa, whose symbols are contained in the small square in the left side of diagram A, are outlined in the larger square on the bottom left of the diagram.

higher mean abundance of *Otodistomum* sp. and *Anisakis simplex* (Rudolphi, 1809) found in *S. kleinii* were mainly related to the larger proportion of size range examined and larger latitudinal range of this Soleidae compared to the other 4 (Table 2), inhabiting brackish waters and spawning in autumn-winter were the 2 host characteristics most contributing to the higher mean abundance of *Scolex pleuronectis* Müller, 1788, *Acanthocephaloides propinquus* (Dujardin, 1845) and *Cucullanus campanae* Lebre & Petter, 1985 in *S. senegalensis* (Fig. 2A, Table 2). Nevertheless, feeding on Crustacea and ingesting a higher number of different prey also contributed to the mean abundance of the endoparasite taxa found in *S. senegalensis* and to the differentiation between *S. kleinii* and the other 4 Soleidae along the first RDA axis (Fig. 2A). These 2 characteristics were also the main ones influencing the higher mean abundance of *Derogenes varicus* (Müller, 1784), *Hemipera* sp., *Macvicaria soleae* (Dujardin, 1845), *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905, *Didymobothrium rudolphii* (Monticelli, 1890), Nybelin, 1922, *Nybelinia lingualis* Cuvier, 1817, *Acanthocephaloides geneticus* (Buron, Renaud and Euzet, 1985), *Acanthocephalus incrassatus* (Molin, 1858), *Hysterothylacium* sp. and Pentastomida found in *D. cuneata* and *S. lascaris*. The differentiation of *S. senegalensis*, *D. cuneata* and *S. lascaris* along the second RDA axis (Fig. 2A) was also related, to some extent, to the higher growth coefficient obtained for *S. lascaris* (Table 2). Still, ingesting Crustacea was the only variable with a significant effect on the patterns found ($P = 0{\cdot}032$). When the RDA was performed on endoparasite diversity, each index was mostly influenced by a type of host characteristics (Fig. 2B): whereas a higher number of different prey and ingesting Mollusca, Crustacea and Echinodermata influenced the higher endoparasite richness and taxonomic distinctness found in some hosts (right half of the diagram), inhabiting rocky bottoms and having a higher growth coefficient were important to the higher variance in taxonomic distinctness found in other hosts (left of the diagram). Although the first axis explained 57% of the variance found, the second contributed with 43%, suggesting that preying on Polychaeta and Cephalochordata and belonging to a family with a higher number of genera were also important characteristics influencing the higher values of richness and variance in taxonomic distinctness found in the majority of the Soleidae and in *P. flesus* (lower half of the diagram); gut differentiation also contributed to the values of taxonomic distinctness found in *C. linguatula* and *Arnoglossus laterna* (Walbaum, 1792) (upper half of the diagram). However, none of the host features presented an overall significant effect in the pattern found.

DISCUSSION

Results showed considerable differences in the macroparasite assemblages of the 14 analysed flatfish species. Since this study was based on data collected

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in a single area by the same researchers and all data were analysed using standard methods by the same research team, all these factors can be excluded as possible causes for the observed differences in mean abundance, richness, taxonomic distinctness and its variance between hosts (Luque *et al*. 2004). Therefore, these ought to be related to host and parasite phylogenetic, biological and ecological characteristics within the study area, which overall determined the likelihood of host colonization, parasite speciation and extinction and the survival of parasite lineages within new hosts.

Since this study was performed in a group of related hosts, a certain similarity in parasite assemblages was expected (e.g. Poulin and Rhode, 1997; Luque *et al*. 2004; Luque and Poulin, 2008), especially for those hosts with similar ecology (Muñoz *et al*. 2006). However, assemblages were not more similar between species within the same genus than between species of different genera or families (e.g. endoparasite assemblage of *S. solea* and its congeners *vs* endoparasite assemblage of *D. cuneata* and *S. senegalensis* or *D. cuneata* and *P. flesus*). In addition, species sharing life-history patterns and feeding ecology, such as *S. senegalensis* and *S. solea* (Teixeira and Cabral, 2010) also presented differentiated endoparasite and ectoparasite assemblages even if they had 8 species in common. Nevertheless, and with the exception of *C. campanae*, *B. soleae*, *C. brevicaudatus* and *H. soleae*, these were generalist parasites that also occur in non-flatfish hosts (Marques *et al*. 2009 and references therein). On the other hand, considering other closely related species presenting similar diets but different biological traits and ecological requirements (e.g. *S. lascaris* and *S. senegalensis*), ecto- and endoparasite assemblages were more alike than those of less-related hosts with similar biology and ecology (e.g. *D. cuneata* and *S. lascaris*). These results seem to agree to some extent with those of Lile (1998), that found a higher similarity in endoparasitic helminth communities of less-related hosts (*P. flesus* and *Hippoglossoides platessoides* (Fabricius, 1780)) than between more-related hosts (*P. flesus* and *Glyptocephalus cynoglossus* (Linnaeus, 1758)) in 4 Pleuronectidae from Norway, and to those of Muñoz *et al*. (2006), that found no consistent pattern in endoparasite communities of 5 Labridae from Lizard Island (Australia).

RDA results revealed that the diversity of macroparasite assemblages of the 14 analysed flatfish species can be explained by only a few host features. Of the characteristics considered in each analysis, only a few were identified as important, as depicted by the size of the vectors in the RDA diagrams. Moreover, depending on considering parasite taxa mean abundance or the diversity of ecto- or endoparasites assemblages (a) different host characteristics have different influence – e.g. zoogeography and latitude range of the host were the most important characteristics determining ectoparasite infection levels but the diversity of ectoparasite assemblages were mostly influenced by habitat, reproductive season and maximum size of the host. (b) A given host characteristic has a different importance according to the macroparasites' guild analysed – e.g. the maximum size attained by the host was significantly important in the diversity of ectoparasite assemblages but not in the diversity of endoparasite assemblages in the same host species. (c) The same host characteristic may have different effects according to host and parasite taxa. For example, a wider latitude range in host distribution was important in the *P. flesus* – *A. cornuta*/*L. pectoralis* relationships, contributing to higher mean abundance of these parasites, but had the contrary effect on the relationships between *D. cuneata* or *S. kleinii* and their ectoparasites, as evidenced by the direction of the vector 'Latitude range' regarding these host-parasite pairs. Therefore, these results support the hypothesis that different diversity measures are related with different host characteristics, as suggested in previous studies (Poulin and Rhode, 1997; Raibaut *et al*. 1998; Luque *et al*. 2004; Aguirre-Macedo *et al*. 2007; Korallo *et al*. 2007; Luque and Poulin, 2008). However, these studies used data collected from different sources and areas, fewer host characters and only ecto- or endoparasite taxa, unlike the present study. In addition, as RDA was used, it was possible to assess the relative importance of each host characteristic to each diversity measure and determine why some flatfish species were more parasitized than others by the same macroparasite taxa. The latter, to our knowledge, has only been performed previously with the same objective in fish hosts by Aguirre-Macedo *et al*. (2007), on unrelated fish.

Hosts with subtropical distribution and latitudinal ranges around 50° presented ectoparasite assemblages clearly differentiated from those of hosts with polar (*P. flesus*) or temperate (*Scophthalmus* spp.) distributions and wider latitude ranges, suggesting an important link between host and macroparasite geographical distribution despite most of the analysed ectoparasite taxa being subarctic or northern temperate (Marques *et al*. 2009). On the other hand, feeding on a higher number of different prey and including Crustacea in the diet appeared to be key factors determining the endoparasite assemblages of *D. cuneata, S. lascaris* and *S. senegalensis*, even if Soleidae are not very selective and usually prey on the more abundant items (Link *et al*. 2005). In fact, the influence of diet preferences in macroparasite infection levels of 7 Soleidae along the Portuguese coast has been examined by Marques *et al*. (2006) and these authors found that much of the variation of prevalence and mean abundance was related to the type and quantity of food ingested as well as by habitat use. The present study corroborates the results found for those 7 Soleidae, and expands the importance of host

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diet to other Pleuronectiformes, revealing that even when other ecological, biological and phylogenetic factors are considered, diet is the most important and significant host feature determining the composition of endoparasite assemblages.

Taxonomic distinctness and richness of ectoparasite assemblages appeared to be mostly influenced by the maximum size and reproductive season of the host as well as by the host inhabiting brackish waters. Although the significant effect of the hosts' maximum reported size in ectoparasite diversity is in agreement with the positive correlations between this host characteristic and ectoparasite richness and diversity in some marine fish (e.g., Timi and Poulin, 2003) and related with the fact that larger fish have a larger surface for ectoparasite attachment (Luque *et al*. 2004), hosts presenting small body lengths, such as *D. cuneata* and *M. azevia*, harboured a more diverse ectoparasite assemblage than *S. maximus* and *S. rhombus*, which were the largest analysed flatfish species. Despite living in brackish and marine environments, which is suggested to contribute to higher ectoparasite richness and taxonomic distinctness as hosts can acquire parasite species in both environments, *P. flesus* presented low ectoparasite diversity. Thus, the importance of these 2 host characteristics has to be taken with care since the RDA was conducted on a small number of host species (since only 5 were infected with at least 4 different ectoparasites), therefore restricting the conclusions about the relative importance of this characteristic in the Pleuronectiformes inhabiting the Portuguese coast.

Results also showed that phylogeny must play a role, as Muñoz *et al*. (2006) and Aguirre-Macedo *et al*. (2007) pointed out in their studies. Although the hosts' phylogenetic variables were not significant, RDAs revealed some importance of the number of host species and genera. Moreover, the most diverse assemblages were found in one of the most speciose families within the Pleuronectiformes, i.e. the Soleidae. However, the host belonging to the family comprising the largest number of species, the Bothidae *A. laterna* was infected by few taxa. Thus, and similarly to that described for ectoparasites in Mediterranean fishes (Raibaut *et al*. 1998), small mammals (Krasnov *et al*. 2004) and bats (Bordes *et al*. 2008), the sympatric occurrence of closely related hosts on the Portuguese coast (13 species occurring in this area) might have contributed to the higher richness and taxonomic distinction of macroparasite assemblages, allowing the infection of closely related hosts sharing ecological and biological features by host-switch, increasing lateral transfer in ectoparasites and favouring parasite speciations (Verneau *et al*. 2009). Therefore, our data corroborate the suggestion that host proximate factors are more important than host species-wide characteristics in ectoparasite assemblage diversity (Patterson *et al*. 2008) suggesting a similar relationship for endoparasite assemblage diversity.

RDA results found for the diversity of endoparasite assemblages revealed that a more diverse diet (type and number of different prey ingested) contributes to a higher diversity of endoparasite assemblages. This has been referred for other marine fishes' endoparasite assemblages (Lile, 1998; Luque *et al*. 2004; Muñoz *et al*. 2006; Aguirre-Macedo *et al*. 2007; Luque and Poulin, 2008) as feeding on a more diverse array of taxa exposes hosts to a broader pool of Digenea, Cestoda and Nematoda macroparasites which use those taxa as intermediate hosts. Because Crustacea are the first or second intermediate hosts of these three macroparasite groups (Marcogliese, 2004) a diet including them must contribute to a higher richness of endoparasite assemblages. However, and since some parasites can only be acquired by ingesting Polychaeta (e.g. *Dichelyne minutus* (Rudolphi, 1819) and *C. heterochrous*, (Koie, 2001)), including this group of taxa in the diet was also a major factor in Pleuronectiformes' endoparasite assemblages diversity.

The wider taxonomic range obtained in the macroparasite assemblages of Soleidae when compared to all the other families may, therefore, be explained by some of their phylogenetic, biological and ecological characteristics, each contributing to the acquisition and/or diversification of a broad range of parasite species. The complexity of host-macroparasite relationships, the importance of phylogenetic and biological characteristics and the unpredictability of host-parasite associations due to host characteristics contributing differently to the observed patterns of diversity, highlight the importance of using multivariate analysis to unveil these relationships. Results also suggest that the observed diversity might be more related to host-parasite co-evolution than first thought, emphasizing the need to focus future research towards the historical processes shaping these relationships. One way to achieve this might be via the reconstruction of host and parasite phylogenies, dating the several evolutionary events leading to the extant relationships and integrating this with host ecological data and parasite life-cycle characteristics.

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