Zoogeographical patterns of flatfish (Pleuronectiformes) parasites in the Northeast Atlantic and the importance of the Portuguese coast as a transitional area

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SUMMARY: Parasites are recognised as an excellent source of information on the distribution of their hosts. Here, the macroparasite fauna of 20 species of Pleuronectiformes belonging to five different families and inhabiting the Portuguese coast was investigated and compared with that known in four other areas (the North Sea, north Northeast Atlantic, Mediterranean Sea and Northwest African coast) in order to determine (1) their zoogeographical pattern and (2) the role of the Portuguese coast as an intermediate biogeographic province. Macroparasites infecting Pleuronectiformes sampled along the Portuguese coast were collected using standard parasitological techniques, whereas data on those in the other four areas were obtained from the literature, rendering a total of 73 macroparasite species. Both sets of data were then compiled in a presence/absence matrix. Hosts and macroparasites were placed into zoogeographical categories according to their known distribution, and patterns were evaluated using multidimensional scaling and cluster analysis. The zoogeography of hosts and parasites was not entirely concordant, although that of endoparasites was generally consistent with the patterns for marine free-living species. On the other hand, only specific ectoparasites truly mirrored the distribution of their hosts. These differences reflect the importance of host ecology and dispersal and environmental factors on the patterns revealed. The Portuguese coast seems to play a significant role in the distribution of Pleuronectiformes' parasites along the Northeast Atlantic and Mediterranean Sea, due to its transitional character and to the sympatric occurrence of related hosts, both promoting the acquisition of new parasite species or the maintenance of historical host-parasite relationships.

Keywords: biogeography, macroparasites, Pleuronectiformes, Northeast Atlantic, Mediterranean Sea, multivariate analyses.

RESUMEN: PATRONES ZOOGEOGRÁFICOS DE PARÁSITOS DE PLEURONECTIFORMES DE LA COSTA PORTUGUESA Y SU IMPORTANCIA COMO ÁREA DE TRANSICIÓN. – Los parásitos son reconocidos como una excelente fuente de información de la distribución de sus hospedadores. En este estudio, la fauna parasitaria de 20 especies de Pleuronectiformes, pertenecientes a cinco familias, que habitan la costa Portuguesa fue analizada y comparada con la conocida en otras cuatro áreas (Mar del Norte, norte del Atlántico Nordeste, Mediterráneo y costa del noroeste Africano) para determinar (1) sus patrones zoogeográficos y (2) el papel de la costa portuguesa como una provincia biogeográfica intermediaria. Los macroparásitos de los pleuronectiformes encontrados en la costa portuguesa fueron recogidos de acuerdo con las técnicas estándar en parasitología y los de las otras cuatro áreas fueron recopilados de la literatura, totalizando 73 especies. Ambos tipos de datos fueron posteriormente compilados en una matriz de presencia/ausencia. Hospedadores y macroparásitos fueron clasificados en categorías zoogeográficas, de acuerdo con su distribución y los patrones obtenidos mediante análisis multidimensional y de clúster. La zoogeografía de hospedadores y parásitos no fue totalmente concordante, aunque la de los endoparásitos fuera, en general, consistente con los patrones referidos para las especies marinas de vida libre. En cambio, sólo los ectoparásitos específicos reflejaron

verdaderamente la distribución de sus hospedadores. Estas diferencias reflejan la importancia de la ecología y dispersión de los hospedadores y de los factores ambientales en estos patrones. El carácter de transición de la costa portuguesa y la existencia simpátrica de hospedadores relacionados, contribuyen a que esta región desempeñe una función muy importante en la distribución de los macroparásitos de los pleuronectiformes del Atlántico nordeste y del Mediterráneo, ya que promueven la adquisición de nuevas especies de parásitos o el mantenimiento de las relaciones históricas hospedadores-parásitos.

Palabras clave: biogeografía, macroparásitos, pleuronectiformes, Atlántico nordeste, Mediterráneo, análisis multivariante.

INTRODUCTION

The last decade has seen increased interest in host-parasite relationships in ecological studies, with parasites providing important information about their hosts biology, ecology, phylogeny and population structure. Since it is highly improbable that parasite species are distributed randomly among their hosts, the geographical variation of the occurrence and abundance of parasite species is regarded as an excellent source of information on the geographic distribution and migration routes of marine fishes (Mosquera *et al.*, 2000; Poulin and Morand, 2000). However, though studies using parasites of marine fishes as indicators of extensive zoogeographical patterns are recognised as good models for studying biogeography (e.g. Carney and Dick, 2000; Poulin, 2003; Mejía-Madrid *et al.*, 2007; Waltari *et al.*, 2007; Pais *et al.*, 2008), they are remarkably scarce. Exceptions are some studies concerning the parasite fauna of economically important and well-studied fish species (e.g. Rohde and Hayward, 2000), although results have led to different conclusions. Blaylock *et al.* (1998) investigated the parasite fauna of adult *Hippoglossus stenolepis* (Schmidt, 1904) from 15 localities along the North American coast and defined three zoogeographic zones consistent with the ones previously established using the distribution of fishes and invertebrates. Furthermore, by examining the ectoparasite fauna of *Sebastes capensis* (Valenciennes, 1833) along its distribution range (Chilean, Argentinean and South African coasts), González and Moreno (2005) found that parasite communities did not follow the biogeographic pattern known for other marine species in the southeastern Pacific. Similar results were obtained by Byrnes and Rohde (1992) and Hayward (1997) for the ectoparasite fauna of four species of Sparidae of Australia and 26 species of Sillaginidae from the Indo-West Pacific, respectively. However, after examining the endoparasite fauna of *S. capensis*, González *et al.* (2006) concluded that its biogeographic pattern was concordant with that of free-living organisms.

Pleuronectiformes (flatfish) are a convenient group for studying biogeography and host-parasite interactions since they are monophyletic in origin (Berendzen and Dimmick, 2002; Pardo *et al.*, 2005; Kartavtsev *et al.*, 2007) but have evolved different ecological strategies and life-history patterns. This diversity and their cosmopolitan occurrence offer an excellent opportunity to investigate whether the biogeography of Pleuronectiformes' parasites follows that of their hosts or is highly dependent on the parasite itself, i.e. whether some kind of predictability of host-parasite associations (at the species or family level) can be depicted by evaluating parasite assemblages in several different areas. Comparisons of the parasite species infecting different flatfish species within the same area and infecting the same host species in different areas can be instruments for this investigation.

In a previous study comprising all flatfish species inhabiting the Atlantic Ocean, Marques *et al.* (2005) suggested that the parasitological relationships between the widely distributed families Scophthalmidae, Pleuronectidae and Soleidae, were influenced by the overlapping distribution of species of these families along the Portuguese coast. Indeed, given its physical and biological characteristics, a "Lusitanian marine province" has been suggested for the Portuguese coast (e.g. Dana, 1853). However, according to the current classification, it constitutes an important border area between the two major zoogeographic regions defined for the North Atlantic: the cool temperate and warm temperate (Briggs, 1974; Gubbay, 1995).

In the present study, the parasite fauna of Pleuronectiformes inhabiting the Portuguese coast was investigated and compared with that known in four other areas (the North Sea, north Northeast Atlantic, Mediterranean Sea and Northwest African coast) in order to 1) determine the biogeography of parasite taxa and 2) the importance of the Portuguese coast in the establishment of the patterns found.

MATERIALS AND METHODS

Sampling and data collection

Twenty species of Pleuronectiformes belonging to five different families (Table 1) were obtained

TABLE 1. – Macroparasites recorded from Pleuronectiformes on the Portuguese coast, their site of infection (Site), biogeographic distribution (Biog) and total number of host species (TNHsp). Citharidae (C): CL, *Citharus linguatula* (Linnaeus, 1758). Bothidae (B): AL, *Arnoglossus laterna* (Walbaum, 1792); AI, *Arnoglossus imperialis* Rafinesque, 1814. Scophthalmidae (S): LB, *Lepidorhombus boscii* (Risso, 1810); LW, *Lepidorhombus whiffiagonis* (Walbaum, 1792); SR, *Scophthalmus rhombus* (Linnaeus, 1758); SM, *Scophthalmus maximus* (Linnaeus, 1758); PR, *Phrynorhombus regius* (Bonnaterre, 1788); ZP, *Zeugopterus punctatus* (Bloch, 1787). Pleuronectidae (P): PF, *Platichthys flesus* (Linnaeus, 1758). Soleidae (L): DC, *Dicologlossa cuneata* (Moreau, 1881); MA, *Microchirus azevia* (Capello, 1868); MV, *Microchirus variegatus* Desoutter, 1990; MB, *Microchirus boscanion* (Chabanaud, 1926); MH, *Monochirus hispidus* Rafinesque, 1814; SK, *Solea kleinii* (Bonaparte, 1832); SL, *Solea lascaris* (Risso, 1810); SN, *Solea senegalensis* Kaup, 1858; SS, *Solea solea* (Linnaeus, 1758); SY, *Synaptura lusitanica* Capello 1868. TNPsp, total number of macroparasite species. Site: S, skin; G, gills; D, digestive tract; V, visceral cavity; B, branchial arches; M, mesenteries. (*) indicates parasites which have only been found infecting flatfishes. Abbreviations for biogeographic distribution are given in the legend to Figure 1. Med, Mediterranean Sea; Indo, Indopacific region.

| Parasite	Site	Biog	C	B	B	S	S	S	S	S	S	P	L	L	L	L	L	L	L	L	L	L	TNHsp
			CL	AL	AI	LB	LW	SR	SM	PR	ZP	PF	DC	MA	MV	MB	MH	SK	SL	SN	SS	SY	
Monogenea																							
Entobdella solea	S	SAA, NTA											x							x			2
Gyrodactylus sp.	G	-																x					1
Digenea																							
Hemipera sp.	D	-												x	x					x	x		4
Derogenes varicus	D	Cosmopolitan						x				x	x					x	x	x			6
Lecithochirium rufoviride	D	SAA, NTA, NTP	x	x		x		x	x			x							x				7
*Homalometron galaicus**	D	NTA											x	x	x					x			4
Macvicaria soleae	D	SAA, NTA										x	x	x	x				x	x	x		7
Prosorhynchus crucibulum	B	SA - T										x							x	x		x	4
Helicometra fasciata	D	NT - T				x																	1
*Lomasoma stephanskii**	D	NTA													x								1
Proctoeces maculatus	D	SA - T										x											1
Zoogonus rubellus	D	SA, NTA, STA, STP										x	x							x			3
Otodistomum sp.	V	-																x					1
Cestoda																							
*Didymobothrium rudolphii**	D	NTA																	x	x			2
*Bothriocephalus andresi**	D	NTA	x																				1
Bothriocephalus barbatus	D	NTA					x																1
*Bothriocephalus clavibothrium**	D	NTA			x																		1
Bothriocephalus gregarius	D	SAA, NTA							x														1
Bothriocephalus scorpii	D	SA, NTA, STA, STP					x					x	x							x			4
Diphyllobothrium sp.	D	-																			x		1
Grillotia sp.	D	-													x								1
Progrillotia dasyatidis	D	NTA					x						x	x	x				x	x			6
Nybelinia lingualis	M	SAA, NTA, NSA,T	x			x	x		x				x						x	x	x		8
Scolex pleuronectis	D	all but AR										x	x	x						x	x		5
Acanthocephala																							
Acanthocephaloides propinquus	D	NTA				x		x				x	x	x		x				x	x		8
*Acanthocephaloides geneticus**	D	NTA (Med)				x													x	x			3
*Acanthocephalus incrassatus**	D	NTA																x					1
Echinorhynchus gadi	D	all but AT				x																	1
Radinorhynchus sp.	D	-	x												x				x				3
Nematoda																							
Anisakis simplex s.s.	D	all but AR and AT	x	x	x	x	x			x		x						x	x				9
Anisakis pegreffii	D	NTA	x		x	x																	3
Anisakis typica	D	NTA - SSA										x											1
*Cucullanus campanae**	D	NTA										x	x	x				x	x	x			6
Cucullanus heterochrous	D	SAA, NTA, NTP												x									1
Dichelyne minutus	D	SAA, NTA										x											1
Hysterothylacium reliquens	D	NTA, T (Indop)												x	x	x							3
Hysterothylacium aduncum	D	all but AT										x			x				x				3
Hysterothylacium sp.	D	-													x				x				2
Capillaridae	D	-	x									x	x										3
Pycnogonida	S	-																	x				1
Copepoda																							
*Bomolochus soleae**	G	SAA, NTA											x					x	x	x	x		5
*Acanthochondria cornuta**	B	SAA, NTA										x											1
*Acanthochondria soleae**	B	NTA																	x		x		2
Caligus elongatus	S	Cosmopolitan													x						x		2
*Caligus brevicaudatus**	S	NT																	x	x	x		3
*Lepeophtheirus europaensis**	S	NTA (Med)						x				x											2
*Lepeophtheirus pectoralis**	S	AR, SAA, NTA										x								x			2
Lernaeocera sp.	G	-													x								1
Ergasilus sp.	G	-										x											1
Isopoda																							
Gnathia sp.	S	-								x	x		x						x		x		5
Rocinella sp.	S	-		x	x			x	x				x						x	x		x	8
Aega sp.	S	-		x																			1
Nerocilla orbignyi	S	NT - ST															x						1
Hirudinea																							
*Hemibdella soleae**	S	SAA, NTA											x	x				x	x	x	x	x	7
Caliobdella sp.	S	-																	x				1
Pentastomida	D	-											x					x					2
TNPsp			9	4	2	10	1	7	3	2	1	15	19	17	9	1	2	6	21	22	11	4	
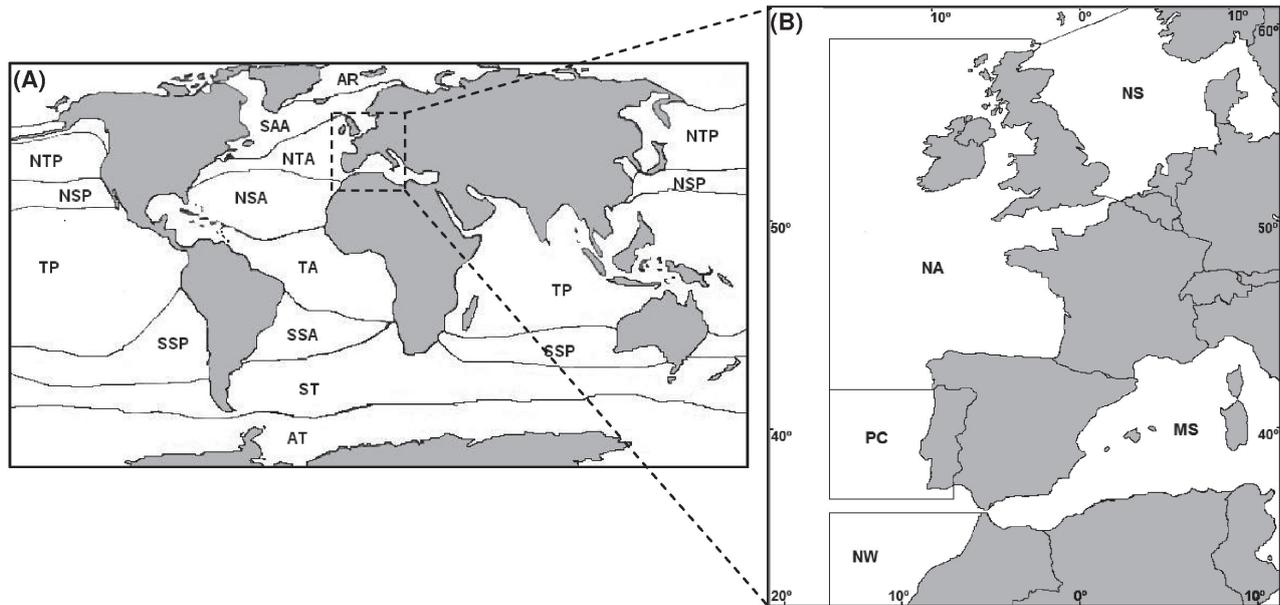
FIG. 1. – Zoogeographic regions used in the classification of macroparasites (A) and the five areas considered in the present study (B). AR, Arctic; SAA, Subarctic Atlantic; NTA, North temperate Atlantic; NTP, North temperate Pacific; NSA, North subtropical Atlantic; NSP, North subtropical Pacific; TA, tropical Atlantic; TP, tropical Pacific; SSA, South subtropical Atlantic; SSP, South subtropical Pacific; ST, South temperate; AT, Antarctic (Modified from Moyle and Cech, 1996). NS, North Sea; NA, north Northeast Atlantic; PC, Portuguese Coast; MS, Mediterranean Sea; NW, Northwest African coast.

from commercial fishing vessels seasonally along the Portuguese coast between January 2003 and July 2005. Because all fish of each species were adults of a similar size range and sampled at an identical depth and habitat, they were pooled into one sample. Samples collected in different areas (north, centre and south) and seasons were also pooled so that parasite assemblages of each host species could be representative of the entire area and comprise all species, including those with marked seasonal patterns. All sampled fish (480 individuals of each species) were then examined for macroparasites and these were collected, counted and identified to the lowest taxonomic level possible.

The current distribution of macroparasites was obtained from the host-parasite database of the Natural History Museum (UK), the European register of marine species (ERMS - MarBef), the ocean biogeographic information system (OBIS) and peer reviewed published papers, and classified according to the biogeographic regions defined by Briggs (1974) (Fig. 1A): Arctic, North temperate, North subtropical, Tropical, South subtropical, South temperate and Antarctic. In order to investigate macroparasite distribution patterns in the distribution range of the flatfish species considered in the present study (obtained from Froese and Pauly, 2006), five areas were considered (Fig. 1B): the North Sea, north Northeast

Atlantic (from northern Scotland – 57º59'N, 4º57'W to the northwest coast of Spain – 41º21'N, 8º53'W), Portuguese coast, Northwest African coast (from Senegal to Morocco) and Mediterranean Sea. Data on flatfish macroparasite infections in these areas were obtained from the literature (Nicoll, 1915; Williams, 1959; MacKenzie and Gibson, 1970; Gibson, 1972; Rodrigues *et al.,* 1975a,b; Papoutsoglou and Papaparaskeva-Papoutsoglou, 1977; van den Broek, 1979a,b; Carvalho-Varela and Cunha-Ferreira, 1987; Petter and Radujkovic, 1989; Renaud and Gabrion, 1988; Zeddam *et al.,* 1988; de Meeus *et al.,* 1992; Belghyti *et al.,* 1993; Cordero del Campillo *et al.,* 1994; Lile *et al.,* 1994; Petter and Cabaret, 1995; Van Damme and Ollevier, 1996; El-Darsh and Whitfield, 1999; Palm *et al.,* 1999; Álvarez *et al.,* 2002; Bartoli *et al.,* 2005) and compiled in a presence/absence matrix together with the data resulting from the parasitological examination of individuals collected along the Portuguese coast.

Data analyses

As in most biogeography studies (e.g. Carney and Dick, 2000; McDowall, 2000; Rohde, 2002), the present one compiles data from different sources. In order to diminish bias due to methodological differences, only those studies using standard parasi-

tological methods, similar and large sample sizes and formally described macroparasites identified to the species level were considered. The validity of species' abundance and its evolution over time could not be determined from the consulted studies, so presence/absence data was selected as the best measure of diversity. Although presented in Table 1, infection data from host species presenting sample sizes lower than 30 individuals along the Portuguese coast, such as *Lepidorhombus whiffiagonis* (Walbaum, 1792), *Phrynorhombus regius* (Bonnaterre, 1788), *Zeugopterus punctatus* (Bloch, 1787) and *Monochirus hispidus* Rafinesque, 1814, were discarded from the comparative analyses, since it has been shown that sample size is a major factor influencing the detection of parasitosis (Poulin, 1998; Marques and Cabral, 2007). Hosts infected by fewer than two macroparasite species were also discarded from the comparative analyses, as they proved to be differentiated from all other samples by not being infected.

Presence/absence data of ectoparasites and endoparasites were analysed separately, as these are influenced by different factors (MacKenzie and Abaunza, 1998) and show considerable differences in their life-cycles – whereas that of ectoparasites is direct, endoparasites require at least one intermediary host. Multivariate analyses were performed at three different scales (host species, host family and area) using the Bray-Curtis dissimilarity since this index accounts for joint presences. A multidimensional scaling (MDS) analysis, using the software PRIMER version 5 (PRIMER-E Ltd., 2001), was applied to host and macroparasite data, in order to reveal the similarity between areas in a two-dimensional space. Similarity between host species and host families was investigated by cluster analysis using the complete linkage agglomeration method, and their significance was tested by bootstrap analysis (1000 re-sampling steps) in Clustering Calculator version 1.0 (University of Alberta, 2002) in order to reveal the association at the area or family level.

RESULTS

Host-parasite relationships

Although the 25 species of flatfish inhabiting the Portuguese coast were examined, only 20 of them

were infected, harbouring 23652 macroparasite individuals belonging to 56 species (Table 1): 2 Monogenea, 11 Digenea, 11 Cestoda, 5 Acanthocephala, 10 Nematoda, 1 Pycnogonida, 9 Copepoda, 4 Isopoda, 2 Hirudinea and 1 Pentastomida. Most species were endoparasites in the digestive tract and a total of 34 species infected more than one host, generally two or three closely related species, i.e. within the same family. The highest number of macroparasite species was collected from Soleidae: *Solea senegalensis* Kaup, 1858, *Solea lascaris* (Risso, 1810), *Dicologlossa cuneata* (Moreau, 1881) and *Microchirus azevia* (Capello, 1868) harboured 22, 21, 19 and 17 species, respectively. However, most of the hosts were infected by fewer than 10 macroparasite species, with three of them (*L. whiffiagonis*, *Z. punctatus* and *Microchirus boscanion* (Chabanaud, 1926)) harbouring only one species (Table 1).

According to the currently known distribution of the 40 macroparasites identified to the species level, only the digenean *Derogenes varicus* (Müller, 1784) and the copepod *Caligus elongatus* von Nordmann, 1832 were truly cosmopolitan. On the other hand, the acanthocephalan *Acanthocephaloides geneticus* (Buron, Renaud and Euzet, 1985) and the copepod *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut and Gabrion, 1988 had only been reported from flatfishes inhabiting the Mediterranean Sea. The majority of macroparasite species had, nevertheless, a broad distribution in the North temperate Atlantic region or in the Subarctic and North temperate regions of the Atlantic and Pacific (Table 1).

The information collected for the macroparasite species infecting flatfish in the five areas considered in this study showed that most species were reported in more than one area and infecting more than one host. With a few exceptions, macroparasite species found in Pleuronectiformes were also found in other diverse and unrelated marine fish families (Appendix 1, Electronic Supplementary Material). However, within each host, most macroparasite species were found in only one area and, for the more widespread species, one of the areas of distribution was usually the Portuguese coast (Appendix 1, Electronic Supplementary Material).

Zoogeographic patterns

The multidimensional scaling (MDS) analysis carried out using the presence/absence data of hosts within each area revealed the North Sea (NS)
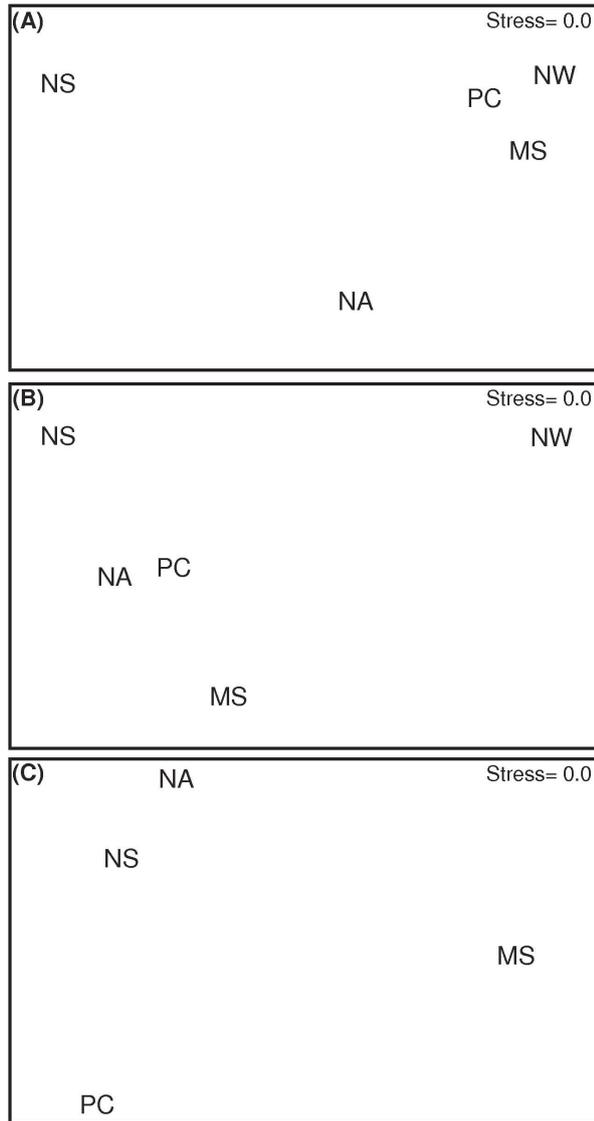
FIG. 2. – Multidimensional scaling analysis of the five areas, based on the presence/absence data of (A) Pleuronectiformes species, (B) endoparasites and (C) ectoparasites. Abbreviations for areas are given in the legend of Figure 1.

to be the most differentiated area and the Portuguese coast (PC) and the Mediterranean Sea (MS) to be the most similar ones (Fig. 2A). However, the MDS analysis performed on macroparasite data revealed a different pattern: the PC was very similar to the north Northeast Atlantic (NA) with regard to endoparasite data (Fig. 2B), and differentiated from all other areas (widely separated from all other areas in the two-dimensional plot) with regard to ectoparasite data (Fig. 2C). The cluster analysis performed on the presence/absence data of endoparasites within each flatfish family (Fig. 3) showed an overall dissimilarity between families, although phylogenetically closer families (Soleidae and Pleuronectidae; Bothidae and Citharidae) had more similar assemblages, with all clusters being supported by high bootstrap values. The removal of hosts with only one ectoparasite species led to the exclusion of three of the families and the cluster analysis using ectoparasite data was therefore not performed.

When host species were clustered based on their endoparasite fauna (Fig. 4A), most samples were highly differentiated (76.2 bootstrap value associated with the dissimilarity of the seven major clusters), although some species of the same family inhabiting the same area showed small dissimilarities supported by high bootstrap values. The cluster of host species based on their ectoparasite fauna (Fig. 4B) revealed a significantly high similarity between all samples of *Platichthys flesus* (Linnaeus 1758), regardless of the area where the host was collected, and between species of *Solea* from the Portuguese coast, the clustering of *S. lascaris* and *S. solea* being supported by a moderately high bootstrap value (66.7).
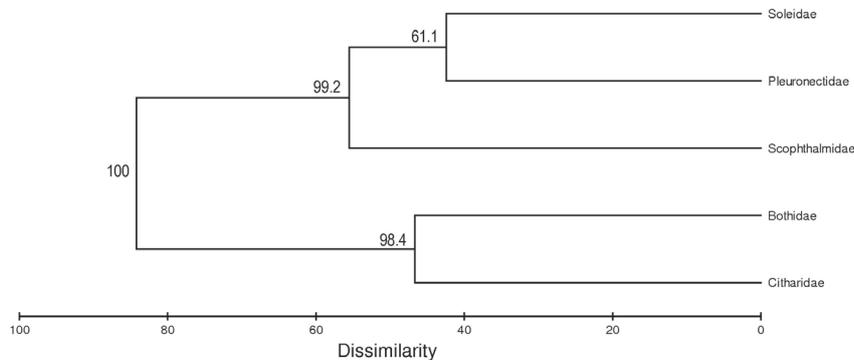
FIG. 3. – Cluster analysis of the Pleuronectiformes families based on the presence/absence data of endoparasite species using the Bray-Curtis similarity index and the complete linkage algorithm. Numbers in cluster nodes indicate bootstrap values (1000 re-sampling steps).
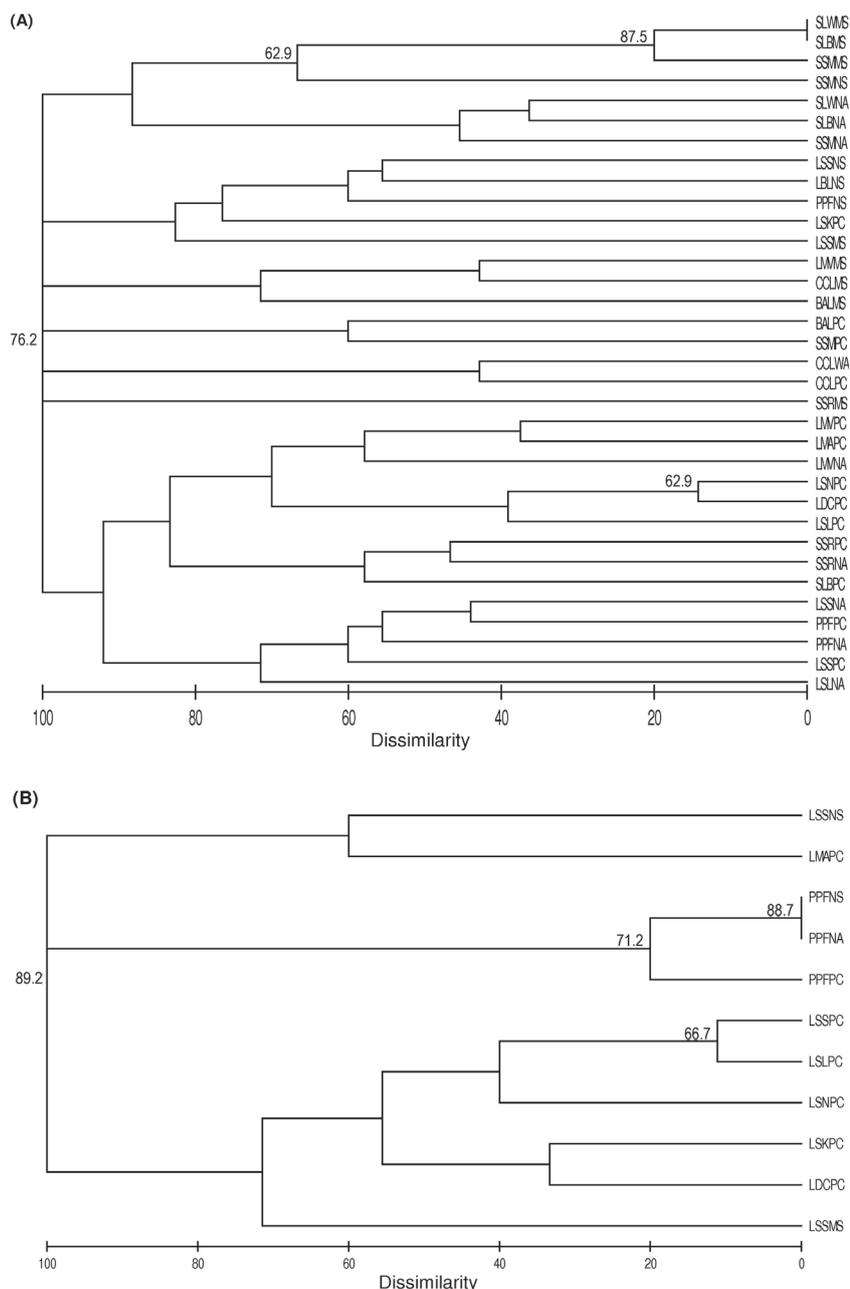
FIG. 4. – Cluster analyses of the Pleuronectiformes species within each area based on the presence/absence data of macroparasite species using the Bray-Curtis dissimilarity index and the complete linkage algorithm: (A), Endoparasites; (B), Ectoparasites. Acronyms are defined by five characters, with the first corresponding to the family, the following two to the species and the last two to the area. Family: C, Citharidae; B, Bothidae; S, Scophthalmidae; P, Pleuronectidae; L, Soleidae. Abbreviations for species are given in Table 1 and for areas in the legend of Figure 1. Numbers in cluster nodes indicate bootstrap values (1000 re-sampling steps).

DISCUSSION

Host-parasite relationships

Parasite assemblages have successfully been used in the study of their hosts distribution and relationships, but few studies have used parasites as indicators of larger-scale zoogeographical patterns (e.g. Oliva and González, 2005; Mejía-Madrid *et al.,* 2007; Vinarski *et al.,* 2007; Waltari *et al.,* 2007; Pais *et al.*, 2008). Focusing on widely distributed flatfishes, and covering most of their range, the present study identified some patterns in the host-parasite associations for this group of marine fishes.

According to the 'first law of geography', similarity between two observations decays as their

geographic distance increases due to a decrease in similarity of their environmental conditions, the existence of geographical barriers between them, or both (Nekola and White, 1999). Since most endoparasite species infecting flatfish are acquired by ingesting invertebrate intermediate hosts whose dispersal is ultimately influenced by oceanic current patterns, the results obtained for the endoparasite fauna might be the reflection of similar environmental conditions between the Portuguese coast and the north Northeast Atlantic, which are the most geographically closest areas and have no geographical barriers between them. A decay of similarity in parasite communities over distance has been reported for other host-parasite assemblages (Poulin, 2003; Krasnov *et al.,* 2005; Oliva and González, 2005). However, because parasites are influenced by the physical environment and the 'host environment', factors such as the host ecology and host species community composition must also play a role in the associations found. In the present study, most endoparasite species were recovered from the generalist Soleidae (Link *et al.,* 2005), which feed on the most abundant Crustacea and Mollusca. Similar environmental characteristics between the north Northeast Atlantic and Portuguese coast might therefore lead to similar feeding ecology of Soleidae in both areas, also contributing to the similarity found.

On the other hand, the distribution of marine ectoparasites, which have direct life-cycles, is mainly determined by the characteristics of the water mass during their brief free-living stage, and by the host's dispersal ability when they are adults (Mackenzie and Abaunza, 1998; Bush *et al.,* 2001). This gives ectoparasites an advantage in biogeographic studies as their distribution is not confounded by factors related to intermediate host abundance (González and Moreno, 2005), and it was expected that areas with similar host assemblages would also have similar ectoparasite assemblages. However, similarly to reports from other studies using marine fish ectoparasites (e.g. Byrnes and Rohde, 1992; Hayward, 1997; González and Moreno, 2005), no congruence was found between the distribution of flatfish and that of their ectoparasites, with the Portuguese coast being highly differentiated from all other areas. Host distribution, abundance and behaviour, alone or in conjunction, might have contributed to this differentiation.

Host-parasite evolutionary relationships have been the subject of several studies (e.g. Mas-Coma,

1992; Sasal *et al.,* 1998; Rohde, 2002; Stireman, 2005) and a positive relationship has been found between the diversity of hosts and the diversity of parasites within one area and between parasite and host phylogenies. The present study is in agreement with these findings, since the most diverse family within the Portuguese coast, the Soleidae, showed a higher number of parasite species than the least represented families, such as the Citharidae or the Bothidae. Results also indicate that more closely related families, such as the Soleidae and the Pleuronectidae, have more similar macroparasite faunas, as evidenced by the cluster analysis performed on families of hosts. However, the high variability of the macroparasite faunas among hosts inhabiting different areas, evidenced by the low similarities obtained in the cluster analyses of both endoparasites and ectoparasites, suggests that host-parasite associations result mostly from environmental-driven evolution. Under this scenario, no predictability of host-parasite associations can be made, except for specific parasites, such as *Acanthochondria cornuta* (Müller, 1776) and *Lepeophtheirus pectoralis* (Müller, 1776), whose distribution actually mirrors that of their host, *Platichthys flesus* (Linnaeus, 1758), as revealed by the significant similarity between *P. flesus* ectoparasite assemblages regardless of the area where the fish were sampled.

Zoogeographic patterns

The endoparasite fauna of flatfishes inhabiting the Portuguese coast appears to be dominated by Subarctic and North temperate Atlantic species that also occur in other marine fish, their zoogeographical pattern being generally consistent with that of marine free-living species. The wide distribution of Monogenea and Digenea from marine fish and their considerable host specificity (Manter, 1966) led Lebedev (1969) to distinguish 10 zoogeographical regions differing in the composition of their Monogenea and Digenea faunas. In this zoogeographic model, the Portuguese coast was included in the Atlanto-Mediterranean region, which also comprised the Mediterranean and Black Seas and the Atlantic coast of Spain and Morocco. The results obtained in this study are therefore consistent with this model since most Digenea are common between the north Northeast Atlantic and the Portuguese coast or between these areas and the Mediterranean Sea, resulting in a similar endoparasite assemblage between

these areas, as revealed by the MDS analysis and, to some extent, by the cluster analysis performed on flatfish species.

Although flatfish assemblages from the more southern areas were quite similar (Fig. 2A), some of the species that have their northern distribution limit along the Portuguese coast (*Arnoglossus thori* Kyle, 1913*, Bothus podas* (Delaroche, 1809)*, Dicologlossa hexophthalma* (Bennett, 1831), *Microchirus ocellatus* (Linnaeus, 1758)) are not abundant in this area, resulting in low sampling sizes and their exclusion from the comparative analyses of macroparasite assemblages, and increasing the discrepancy between host and macroparasite infections results. Ecological host-switching (Poulin, 1998; Page, 2003), through the contagious transmission of generalist ectoparasites between similar host species, or between widespread host species and those living near the limit of their distribution range, might be promoted by the high overlap of host species along the Portuguese coast. However, the relatively sedentary behaviour of adult flatfishes might contribute to a minimal dispersion of these ectoparasite species, resulting in a higher richness in this area and its differentiation from the other four considered.

Despite all the ecological factors pointed out so far, historical reasons cannot be ruled out to explain the results found in the present study. The origin and distribution of the great majority of our present species probably took place in tropical centres during the Pliocene and Pleistocene (Briggs, 2006), and Atlantic and Mediterranean marine faunas are no exception. Results from a palaeontologic study conducted in the central region of the Portuguese coast (40ºN), indicates that the Pliocenic faunal association in this area had a typical Atlantic affinity, with no fossil species from the Mediterranean being found and two exclusively Atlantic fossil species being present (Nolf and Marques da Silva, 1997). After the mass extinction of Mediterranean fauna due to the Messinian salinity crisis, in the early Pliocene, this area was colonised by warm temperate Atlantic species that are found here, and on the western coast of Africa, their refuges during the Pleistocene glaciations, before they recolonised temperate eastern Atlantic waters when conditions were favourable (Almada *et al.,* 2001; Domingues *et al.,* 2008). These events offer a possible explanation for the similarity found between flatfish communities along the Portuguese coast, Mediterranean Sea and Northwest African coast, and also for the differences found between

macroparasite assemblages: some parasite lineages established in flatfishes might have been lost when hosts migrated southwards (warm-temperate species) during glaciation peaks, due to extreme differences in environmental conditions or the absence of the required intermediate hosts to complete their life-cycles; glacial advances and retreats during the Pleistocene and the recent (less than 10000 years, after the last ice-age) colonisation of the Portuguese coast by some subtropical species (e.g. *D. hexophthalma*, *M. ocellatus*, also found in the warmer waters of the Mediterranean and Northwest African coast) have not yet allowed the development of stable host-parasite relationships. This is supported by the fact that most macroparasite species found in the present study had Subartic and North temperate distributions and infected hosts with temperate affinities, suggesting that similarities found between parasite faunas from Pleuronectiformes inhabiting the north Northeast Atlantic and the Portuguese coast are the result of long-lasting interactions between parasites and their hosts.

Results of the present study point out the high importance of the Portuguese coast as a transition area in shaping macroparasite assemblages of Pleuronectiformes species occurring in the Northeast Atlantic and Mediterranean Sea and in the establishment of host-parasite relationships, as suggested by Marques *et al.* (2005). The sympatric occurrence of closely related host species along this area, allows the colonisation of new, but similar, hosts by macroparasites specific at the genus or family level through evolutionary events, but also the continuum of host-parasite relationships involving specific parasite lineages. However, phylogenies of both parasites and hosts are essential in the study of the biogeography of the extant biodiversity (Cowie and Holland, 2006), and knowledge of the macroparasite faunas infecting Pleuronectiformes in more remote regions and of the rare Pleuronectiformes species occurring along the Portuguese coast are needed in order to clarify the relationships found in the present study.

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SUPPLEMENTARY MATERIAL

The following appendix is available through the web page http://www.icm.csic.es/scimar/supplm/sm73n3461sm.pdf

APPENDIX 1. – Macroparasites recorded from the Pleuronectiformes species considered in the present study in the North Sea (NS), Northeast Atlantic (NA), Portuguese coast (PC), Mediterranean Sea (MS) and Northwest coast of Africa (NW).