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Genetic differentiation of Atlantic populations of the intertidal copepod *Tigriopus brevicornis*

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SUMMARY: The Harpacticoid copepod *Tigriopus brevicornis* belongs to the meiofauna of intertidal rock pools and is distributed widely along European coasts. Sixteen sites were sampled from the Irish Sea to the coasts of Spain. We used the ITS1 marker to analyse the relationship between the populations because it shows low intrapopulational variation (mean pairwise difference: 1.00 ± 0.8) and high interpopulational divergence (mean pairwise difference: 16.38 ± 7.39). A total of 57 bp out of 433 bp were recognised as informative nucleotides among the 61 individuals analysed. The analysis of the genetic relationships highlighted a north-south split in the distribution of the natural populations and showed a genetic break point around the Gironde estuary, which is probably due to differences in the geomorphologic characteristics of the coastal area on the two different sides of this estuary. Various populations were isolated and the ITS1 sequences indicated that there are specific genetic signatures in these populations. The northern set of populations, which was sampled along a large rocky coastline, had a metapopulation structure with genetic exchanges between geographically close populations and also between geographically far ones. The southern set of populations, which was sampled in small rocky pools on large sandy beaches, showed isolated populations as a consequence of the geomorphology of the area.

Keywords: biogeography, copepods, *Tigriopus*, genetic population structure.

RESUMEN: DIFERENCIACIÓN GENÉTICA DE POBLACIONES ATLÁNTICAS DE CHARCOS DE MAREA DEL COPÉPODO INTERMAREAL *TIGRIOPUS BREVICORNIS*. – Los copépodos harpacticoides *Tigriopus brevicornis* pertenecen a la meiofauna intermareal y están ampliamente distribuídos a lo largo de las costas europeas. Dieciséis lugares fueron muestreados desde el Mar de Irlanda hasta las costas de España. Nosotros usamos el marcador ITSI para analizar la relación entre poblaciones porque se mostró una variación interpoblacional baja (promedio diferencia de pares: 1.00 ± 0.8) y una divergencia interpoblacional alta (promedio diferencia de pares: 16.38 ± 7.39). Un total de 57 bp entre 433 bp fueron reconocidos como nucleótidos informativos entre los 61 individuos analizados. El análisis de relación genética resalta una partición Norte-Sur en la distribución de las poblaciones naturales y mostraba un punto de rotura genético alrededor del estuario de Gironde, probablemente debido a las diferencias en las características geomorfológicas de esta área costera en ambos lados del estuario. Algunas poblaciones fueron aisladas: las secuencias ITSI indicaban que había unas señales genéticas específicas en estas poblaciones. La población de la parte norte, que fue muestreada a lo largo de una línea rocosa de costa, evidencia la estructura de una metapoblación con intercambios genéticos entre poblaciones geográficamente próximas, pero también entre poblaciones geográficamente lejanas. La población de la parte sur, que fue muestreada en pequeños charcos de marea sobre vastas playas arenosas, mostraban poblaciones aisladas, destacando las consecuencias de la geomorfología del área.

Palabras clave: biogeografía, copépodos, *Tigriopu*s, estructura genética de la población.

INTRODUCTION

Although some physical factors, such as current patterns, may restrict or promote dispersal routes, oceans often appear to be free from gene flow barriers, and this limits our ability to understand the genetic exchange between the marine invertebrate populations. In the case of the marine invertebrates with a free-swimming stage (larvae and/or adult), the relationships between populations could be used to study the dispersal behaviour and the ability of species to recolonise highly variable environments.

The intertidal rock pools in which the copepod *Tigriopus* species live are described as a highly variable environment with large variations in the main physic-chemical parameters, in particular salinity and temperature (Davenport *et al.,* 1997) as well as pollutant concentrations (Crowe *et al.,* 2000), and in which there is evidence of extinction and recolonisation events (Dybdahl, 1994). If the species living in individual rock pools belong to a large metapopulation (Grimm *et al.,* 2003; Johnson, 2001), there is necessarily a flux of animals between the populations, which could explain the recolonisation events. Therefore, the spatial genetic structure could be a good tool for estimating the interdependence of the populations.

Tigriopus brevicornis belongs to the harpacticoid taxon, which has been widely used in studies of population genetics and structure (Burton *et al.,* 1981; Burton *et al.,* 1999; Johnson, 2001), physiology (McAllen and Block, 1997; Willett and Burton, 2002; Willett and Burton, 2003) and ecotoxicology (Pavillon *et al.,* 2002; Forget *et al.,* 2003; Kwok and Leung, 2005; Lee *et al.,* 2005). It is currently accepted that *T. brevicornis* is present all along the Atlantic coast from Iceland to Portugal. Distribution analyses of copepod populations have shown various degrees of differentiation over short distances (less than 1 km) (Burton and Feldmann, 1983; Burton, 1990; 1998; Burton and Lee, 1994) and also over long distances (hundred of kilometres) (Burton, 1998; Schizas *et al.,* 1999). In the latter case, the results suggest a distinct geographic pattern with breaks in the genetic composition. To analyse the interpopulation structure of *T. brevicornis* along the Atlantic coast we selected the nuclear marker, the ITS1 sequence, which has been widely used for intraspecific studies because of its high sequence variation (Hillis and Dixon, 1991; Schlötterer *et al.,* 1994; Miller *et al.,* 1996; Fabry *et al.,* 1999). The divergence observed in the spacer regions was appropriate for detecting differences between conspecific organisms and provided a useful marker for studying the relationships between populations (Vogler and DeSalle, 1994).

The objectives of this study were threefold: First, we defined an interpopulation variable sequence that could be used as a genetic marker for identifying *T. brevicornis* populations. Second, we investigated the gene variations between 16 populations of *T. brevicornis* and thus evaluated the connectivity between local or widely separated populations. We studied the geographic pattern of these variations at different spatial scales: a meso-geographic scale (10 - 400 km) and a macro-scale (around 100 km). Finally, we investigated the possible position of a potential genetic break point along the European Atlantic coast.

MATERIAL AND METHODS

Sixteen natural locations of *T. brevicornis* were explored (Table 1). The first sampling region was situated in the Irish Sea (1 place). The second one included 3 locations from the north coast of Brittany along the English Channel. The third region was along the European Atlantic coast and included 12 locations (Fig. 1). The animals were collected in one rock pool from each location in the high intertidal area, which is covered by seawater when the tide reaches a coefficient of 80. Except for the copepods from the Irish Sea, all the individuals were kept alive for one to three days until they were individually caught and their sexes determined. During this

TABLE 1. – Latitude position of each sampled locality along the west European coastline.

Names	Symbols	Latitude positions
Kearney	I	54.384
Dinard	II	48.133
Ile-Grande	III	48.800
Roscoff	IV	48.052
Logonna-Daoulas	V	48.017
Concarneau	VI	47.034
Pointe de la jument	VII	47.833
Erdeven	VIII	47.135
Locmariaquer	IX	47.067
Le Croisic	X	47.284
Pornic	XI	47.103
Sables d'Olonne	XII	46.150
Royan	XIII	45.117
Castro Urdiales	XIV	43.036
Ortigueira	XV	43.700
Porto do Son	XVI	42.051

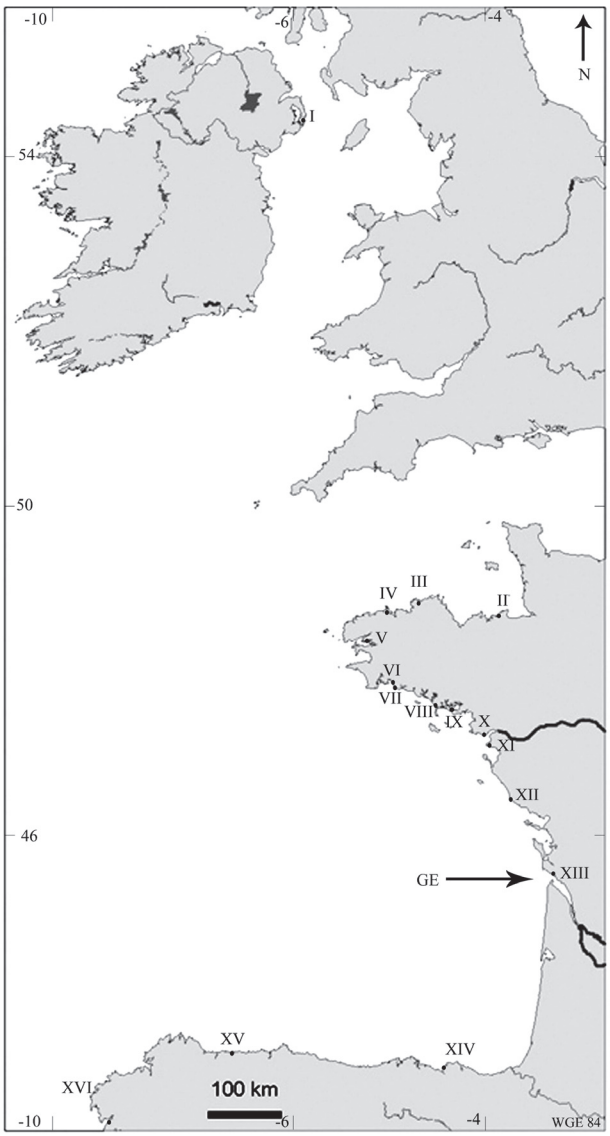

FIG. 1. – Geographic situation of the studied populations (Scale bar: 100 km). Sampling was carried out in 3 regions: Region 1 (1 population) = North Ireland (I: Kearney); Region 2 (3 populations) = Channel coast (II: Dinard, III: Ile-Grande, IV: Roscoff); Region 3 (12 populations) = Atlantic coast (V: Logonna-Daoulas, VI.: Concarneau, VII: Pointe de la Jument, VIII.: Erdeven, IX.: Locmariaquer, X.: Le Croisic, XI: Pornic, XII: Les Sables d'Olonnes, XIII: Royan, XIV: Castro-Urdiales, XV: Ortiguera, XVI: Porto do Son); G.E., Gironde Estuary: a potential phylogeographic limit.

period, no mortality was observed. They were used directly for DNA extractions. The copepods from the Irish Sea were kept in a 70% alcohol solution for one month until the DNA was extracted.

PCR reactions

The DNA extractions were carried out on individual adult males or non-ovigerous females in order to stay clear of the DNA from eggs. The total genomic DNA was extracted from the individual copepods using a standard proteinase K extraction with a 2% CTAB solution (Winnepenninckx *et al.,* 1993).

Conserved stretches of DNA in the coding rDNA adjacent to the ITS1 were used to design primers for amplifying the highly variable regions. The sequences of these primers were (5'-3'): ITS1a: CACACCGCCCGTCGCTACTA and ITS1r: TCGACSCACGAGCCRAGTGATC. The primer ITS1a begins at the nucleotide n° 5516 of the *Tigriopus californicus* sequence (GenBank: AY599492) including the 18S ribosomal RNA gene; the reverse primer ITS1r begins at the n° 6182 nucleotide of the same sequence in the 5.8S ribosomal RNA gene.

The standard PCR reactions were carried out with a PureTaq Amersham Ready-To-Go PCR Kit on a thermocycler (Appligene Oncor) with PCR conditions: 40 cycles of 1 min at 94°C as denaturing temperature/ 1 min at 52°C as annealing temperature/ 2 min at 72°C as extension temperature. PCR products were run on a 1% agarose gel with 0.001% ethidium bromide to separate the different sizes. The bands were purified using the QIAquick gel Extraction Kit (QIAGEN) according to the manufacturer's instructions.

Sequencing reaction

Amplified DNA fragments were sequenced directly to evaluate the degree of polymorphism between the sequences. The sequences were obtained with the forward primer. The sequencing reactions were carried out on the ABI PRISM® 310 Genetic Analyzer with the Big Die Terminator v3.1 Cycle Sequencing kit. After analyses, all sequences were double checked. The legibility of the sequence was high for all the PCR products, and no equivocal sites were observed. The sequences were edited and aligned together using CLUSTAL W (Thompson *et al.,* 1994).

Statistical analyses

Direct sequencing of PCR products yielded different numbers of nucleotides between 572 and 603 from the transcribed region. Only the variable ITS1 region was conserved for further analyses. The common dataset of the ITS 1 region consisted of fragments with a length between 402 and 433 bp, and these fragments were used for the total alignment of the sequenced ITS1 region. The haplotypes were determined using

TABLE 2. – Interpopulation divergences estimated with the uncorrected average pairwise differences between populations. The symbols used for the populations are the same as those in Figure 1.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
I	-	5.50	5.70	5.20	8.17	6.30	5.17	5.50	5.50	8.90	8.75	8.50	43.33	33.25	46.00	26.67
II		-	10.00	4.80	13.67	3.80	1.67	6.00	6.00	9.60	9.25	9.00	43.33	33.00	45.00	25.67
III			-	8.76	5.66	10.20	10.47	9.00	9.00	6.40	6.25	6.00	41.13	37.60	43.80	30.47
IV				-	11.07	5.00	4.87	3.40	3.40	7.00	6.65	6.40	42.33	32.60	45.00	25.67
V					-	11.47	13.33	8.67	8.67	6.07	5.92	5.67	42.00	39.66	44.66	31.33
VI						-	3.47	3.80	3.80	7.40	7.05	6.80	42.73	33.40	44.40	25.47
VII							-	5.67	5.67	9.26	8.92	8.67	43.00	32.67	44.67	25.33
VIII								-	0.00	3.10	3.00	3.00	38.67	31.00	42.00	20.67
IX									-	3.60	3.25	3.00	39.33	31.00	42.00	22.67
X										-	0.85	0.60	36.93	34.60	39.60	26.27
XI											-	0.25	36.58	34.25	39.25	25.92
XII												-	36.33	34.00	39.00	25.67
XIII													-	30.33	9.66	27.33
XIV														-	23.00	39.00
XV															-	24.33
XVI																-

MEGA 4 (Tamura *et al.,* 2007). Standard diversity and molecular indices (length of the ITS1 sequence, number of haplotypes, nucleotide diversity) were performed using ARLEQUIN v 3.01 (Schneider *et al.,* 2000): the nucleotide diversities and divergences were based on the observed number of differences, and the interpopulation divergences were estimated with the uncorrected average pairwise differences. The evolution model was determined using Modelgenerator (Keane *et al.,* 2006). The molecular distances were then estimated with the Kimura 2 P model. The maximum likelihood (ML) tree was calculated with models. The parameters determined with Modelgenerator. Statistical support for internodes were tested by bootstrap percentages with 10000 replicates (Felsenstein, 1985). A Molecular Variance analysis (AMOVA) between the groups was carried out. A Mantel test was performed to evaluate the correlation between the genetic and the geographic distances in order to test the hypothesis of isolation by distance (Slatkin, 1987; 1993). The median-joining method (Bandelt *et al.,* 1999) was used to build a phylogenetic network in order to illustrate the relationships between populations (www.fluxus-engeneering.com). The gaps were treated as a fifth character and the ε parameter was set to 0.

RESULTS

Haplotypes and genetic diversity

Using the defined primers, a total of 61 individual *T. brevicornis* were analysed. The sequences were put in the EMBL/GenBank sequence database under the accession numbers: AM083335 (population from Concarneau - VI), AM083336 (population from Le Croisic - IX.), AM083337 (population from Roscoff - IV) AM083338 (population from Sables d'Olonne - XII), AM083339 (population from Castro Urdiales - XIV), AM083340 (population from Kearney - I), AM083341 (population from Ortiguera - XV), AM083342 (population from Royan - XIII).

The ITS1 sequence alignment showed 32 different haplotypes defined by a total of 67 polymorphic sites. Among them 90% were parsimoniously informative including 33 substitutions (15 transitions and 21 transversions) and 44 insertions/deletions. The total nucleotide diversity, π, was 0.0372 ± 0.0186. The uncorrected averages of pairwise interpopulation divergences are indicated in Table 2 with a mean number of 16.38 ± 7.39. A large part of the insertions/deletions consisted of more or less large gaps in relationship to a microsatellite sequence. The sequences were homogeneous in each population with regards to the length of this microsatellite sequence. This region, which was highly conserved at the intrapopulation level, appeared to be variable at the interpopulation level, and therefore can be considered to be a genetic marker of populations for each geographic area.

The genetic variation indices of the populations are indicated in Table 3. At the intrapopulation level, the nucleotide divergence index, π, ranged from 0.8 in the population of Ile Grande (III) to 0 for six populations (Fig. 2). Most of the haplotypes were private, and only 4 were found in different populations.

TABLE 3. – Genetic variation of each population. Pop., populations from north to south (the symbols used for the populations are the same as those in Figure 1); Nb_{seq}, number of sequences in each population; Nb_{hap}, number of haplotypes in each population; length, length of the ITS1 sequence in each population; π, nucleotide diversity as a percentage of the total number of nucleotides; Nb_{TTG}, frequency of repetition of the triplet TTG in the microsatellite.

Pop.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
Nb_{seq}	4	4	5	5	3	5	3	3	3	5	4	5	3	3	3	3
Nb_{hap}	2	1	5	4	2	4	2	1	1	3	2	1	2	2	1	3
Length	418	421	421	421	421	421	421	421	421	424	424	424	419	402	419	433
π	0.24	0.0	0.80	0.57	0.16	0.28	0.16	0.0	0.0	0.24	0.12	0	0.32	0	0	0.92
Nb_{TTG}	4	5	5	5	5	5	5	5	5	6	6	6	6	5	6	5

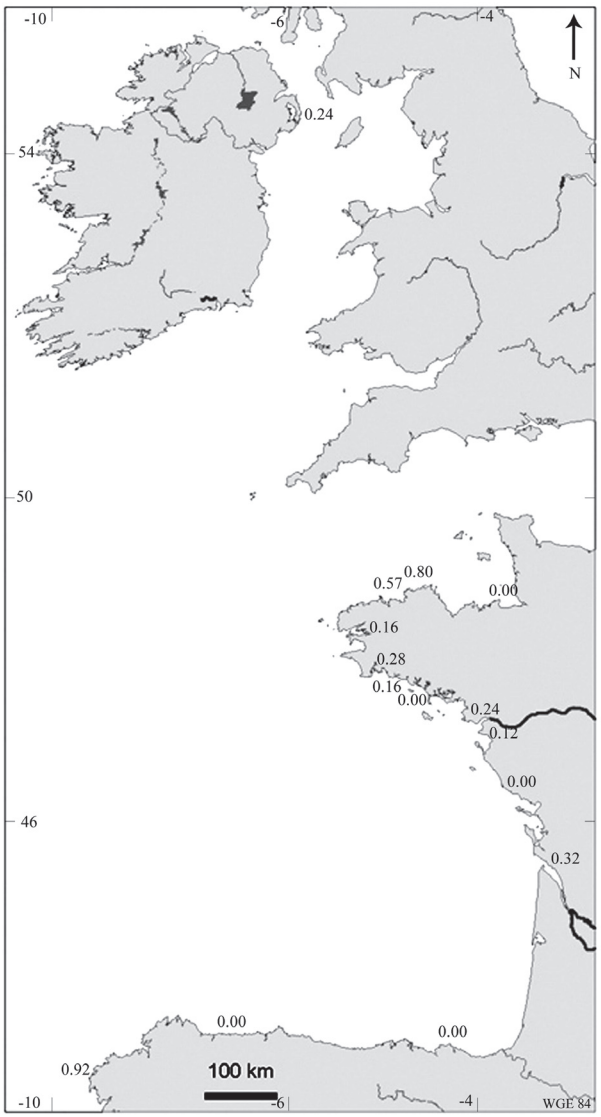

FIG. 2. – Map of haplotype diversity indices π estimated as a percentage of the total number of nucleotides. The symbols used for the populations are the same as those in Figure 1.

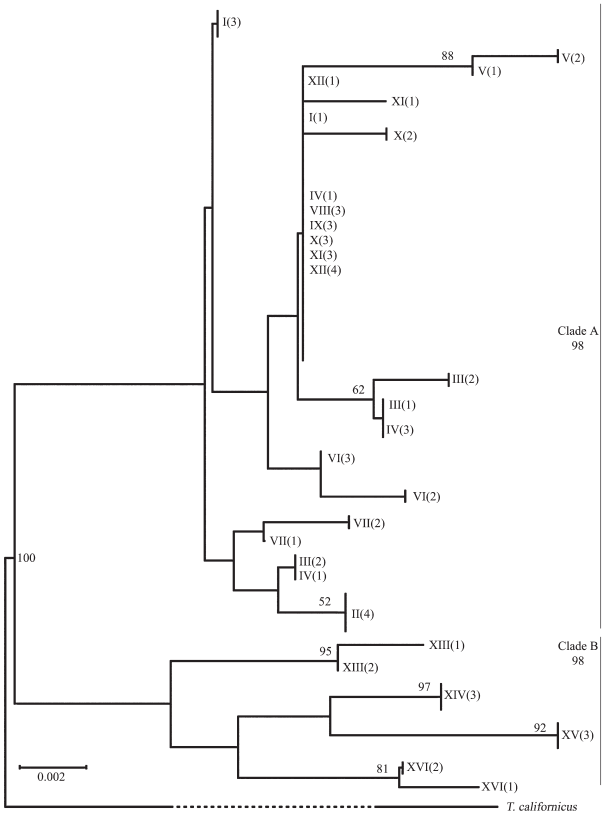

FIG. 3. – Phylogenetic relationships among the ITS 1 haplotypes illustrated with a Maximum Likelihood tree based on the Kimura 2 P model. The symbols used for the populations are the same as those in Figure 1. The numbers in brackets indicate the number of sequences of each population at the node. The tree root used the homologous sequence of *T. californicus*. Branch lengths are proportional to the nucleic divergences. The bootstrap support values for the two clades are provided in bold at the right near the name of the clades. Only the intra-clade bootstrap values higher the 50% are given above the branches.

Phylogeographic analysis

The 61 sequences from individual *T. brevicornis* were used to construct the ML tree (Fig. 3), which was estimated with the best-fit model (K80 + G), (-lnL= 1551.57) based on 433 bp segments of ITS1 sequences. The out-group was the ITS1 sequence determined from the ribosomal DNA complex sequence of *T. californicus* (gb:AY599492). The phylogenetic tree grouped all sequences of *T brevicornis* into a monophyletic cluster (supported by BP = 100%), which included two divergent clades (Fig. 3). Clade A (supported by BP = 98%) enclosed the haplotypes from the 12 northern populations sampled between

TABLE 4. – AMOVA based on genetic distances between ITS1 sequences for 61 *T. brevicornis* sampled from 16 populations with and without regional structure.

Source of variation	df	sum of squares	variances components	percentage of variation	P value
No regional group					
Among populations	15	459.75	7.92	93.47	0
Within populations	45	24.92	0.55	6.58	0
Total	60	484.67	8.84		
Two groups: North/South					
Among groups	1	221.66	10.74	68.95	0
Among populations within groups	14	238.09	4.28	27.50	0
Within populations	45	24.97	0.55	3.55	0
Total	60	484.67	15.58		

the locations Kearney (I) and Sables d'Olonne (XII) (1245 km) with an average nucleotide diversity index π of 0.0022 ± 0.0024. In this group, no subclade was statistically identified (BP<50). Clade B was formed by the 4 southern populations sampled all along 2338 km of the Atlantic coast from Royan (XIII) to Porto do Son (XVI) with an average nucleotide divergence index π of 0.31 ± 0.37. The Mantel test showed that the correlation between genetic and geographic distances was low (r=0.314; p=0.005). Less than 10% of the genetic distances were geographically explained. Likewise, no correlation was detected between the indices π and the latitude position of the sampled locations. According to the AMOVA (Table 4), the major part of the total genetic variance was expressed between the north and the south clades (69%), whereas 3% was estimated among the populations. Only 27% of the total variation was expressed among the populations within the clusters. The AMOVA analysis confirmed the significant genetic heterogeneity between the two clusters (F_{CT} = 0.689 p<0.001). Inside the northern clade, the north-south organisation was generally respected as it was shown in the minimum spanning tree (Fig. 4). Nevertheless, the relationship between the genetic distance vs. geographic distance was weak, especially due to the deviations associated with the east-west axis in relation to indentations in the coast (Channel, Bay of Brest), which could be responsible for the isolation phenomena. Moreover, some populations that were geographically close were not genetically closer than geographically distant populations (genetic differences VI-VII= 3.4; genetic differences II-VII= 1.67). Clade B showed a higher genetic interpopulation diversity. The populations were clearly defined and separated. Neither the common haplotype nor the cluster was observed among these populations. Neither the north/south organisation nor the relationships between the geographic and genetic distances was respected.

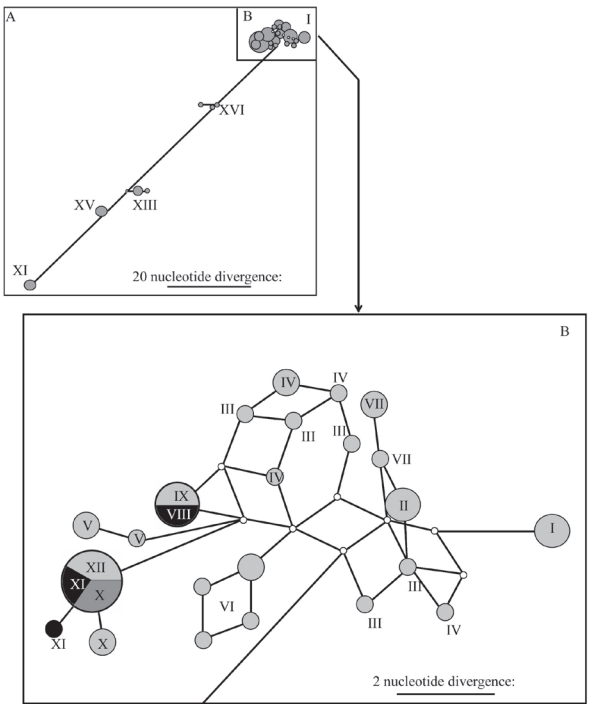

FIG. 4. – Haplotypic networks of the 16 *T. brevicornis* populations. The symbols used for the populations are the same as those in Figure 1. The symbol size is in agreement with the number of identical sequences. A, general network which showed the north-south axis structure (Scale bar: 20 nucleotide divergence); B, specific northern group network which showed the knit network structure (Scale bar: 2 nucleotide divergence).

DISCUSSION

The results showed that ITS1 sequences can be used as a suitable population marker of *T. brevicornis* and their usefulness as phylogenetic markers

at low taxonomic levels was demonstrated. These sequences were characterised by low intrapopulation variation and high interpopulation diversity. Among the *T. brevicornis* sequences analysed, the variation of the length was population specific and partially due to the presence of indels in a microsatellite sequence. Variation in the length between non-rDNA genes in the rDNA complex is well documented in other species, and is associated with the variation in the number of various sub-repeats within the region. Inter-specific comparison was impossible due to the high variability of the ITS1 region, which emphasised that the ITS1 region is an intra-specific marker.

The studied *T brevicornis* populations were genetically structured and were characterised by low inner variation. As rock pools are stochastic environments (Igarashi, 1959), the *Tigriopus* populations can often disappear and the rock pools recolonised with a small number of individuals after high tides or big storms. Therefore, the low intra-population variations could reflect the recent and variable formation of the populations, as expected in an ephemeral habitat.

The informative characteristic of the ITS1 sequences was due, in part, to some alleles restricted to single populations, which indicates that these populations had locality accuracy. As regular bottleneck processes limit the number of haplotypes in the populations, stochastic environmental events can reduce populations to such a small size that drift may occur and produce genetic divergences among neighbouring populations. Thus, because the populations were restricted to small pools, the genetic drift may be large. This level of divergence suggests that the dispersal rates were not high enough to erode genetic diversity. According to Burton (1997), gene flow could be absent between *Tigriopus* sp. populations located less than 1 km apart. The consequences were that, in the north part of the studied area, some geographically close ponds were not more genetically similar than distant ones, which Boileau and Taylor (1994) also observed.

Nevertheless, the gene flow seems to be greater in the case of *T brevicornis* than *T. californicus* (Burton, 1997) as the north cluster showed some genetic exchanges which may be due to local currents (between VIII and XII populations in south Brittany) or to very strong ebb and flow tides (between II and IV populations in north Brittany) and which could explain the structure of a metapopulation in this geographic area. Although there are no studies that mention *T. brevicornis* as planktonic fauna or as passive drifting adults or larvae, there must be restrictions for pelagic periods as this copepod has free-swimming stages. The hypothesis that pieces of algae are used as means of transport cannot be rejected. Such a means of passive migration in a neustonic habitat may affect the colonisation rate, especially for species with low active migratory potential, as shown with the isopod *Idotea metallica* (Gutow and Franke, 2003). Moreover, *Tigriopus* sp. is restricted to linear habitats of coastal environments where the prevailing current directions may accentuate clinal biogeographic patterns (Edmands, 2001).

The sequence analysis distinguished 2 clades, which were constituted by the southern populations (from XIII to XVI) and the northern ones (from I to XII). The results showed that there was no genetic continuity between these 2 sets. Burton (1986) suggested that some genetic divergences may be observed between populations of *Tigriopus* sp. in relation to adaptive stress, which could explain directional selection. However, in our study, no environmental variation could justify a selective process. Therefore, a genetic barrier could exist that isolates the clades from one side of the Gironde estuary to the other. This could be due to the distribution of appropriate rock pools, as the *Tigriopus* populations were only observed on granite rock. The presence of large beaches along the south coast in the studied area might result in separated populations, particularly if currents are low. No geographic break point has been identified around the Gironde estuary for other species. Nevertheless, the structure of the *Tigriopus* populations, which are characteristic of intertidal and rocky habitats, was impacted by the differences in the physical characteristics of the coastal area on both sides of this estuary. The absence of concordance between the *Tigriopus sp.* phylogeographic break point and the biogeographic break point has already been noted (Burton and Lee, 1994; Burton, 1998).

Other differences between the population distributions of *Tigriopus* sp. and other species were also observed. Our study did not reveal any phylogeographic limit between the English Channel and Atlantic populations, as found by Jolly *et al.* (2005) and Jolly (2005) with the Polychaete *Pectinaria koreni*. This difference of genetic organisation was observed at the other break point between the Atlantic and the Mediterranean basin (Remerie *et al*., 2006). Among others, two hypotheses could explain these differen-

tiations: i) the populations were initially similar and diverged via selection or drift phenomena over a period long enough to allow the observed differences, or ii) the original colonists derived from already differentiated populations and the observed genetic differences reflect the original divergence.

As the genetic marker was a highly variable sequence and due to the short generation time of *T. brevicornis*, we can expect that the historical processes which reached the coastline a long time ago did not directly influence the structure of the relationship between the populations. These processes modified the geomorphologic characters of the coastline, which now can limit the gene flow and so increase the locality's separateness, particularly in the southern area of the study. However, we cannot completely exclude the impact of the phenomena which isolated and/or destroyed the populations long ago. The founder effect can produce initial genetic differentiation among neighbouring populations, especially if the number of colonists is small (Wade and McCauley, 1988). Previous genetic studies on populations of meiofaunal organisms in small isolated ponds demonstrated divergence among nearby populations (Crease *et al.* 1990, Boileau and Taylor, 1994). In the case of *T. brevicornis*, the size of the populations might be limited by the size of the rockpool in which each population developed.

Nonetheless, the analysis of the genetic structure showed that a north-south organisation existed. This suggests that the *Tigriopus* populations spread along the coast step by step. The major recolonisation event of the littoral by the copepods probably took place after a large glaciation event. Our results suggest that this event could have gone from the south area to the north area. In this case, the original population may have been located in the Bay of Biscay, which was genetically far from the north clade. This population could have survived until the post-glaciation age in a glacial refugia and could be the source population for all the Atlantic European populations. If we consider that the ITS1 sequences evolve about 2.5 times faster than expressed genes like COI (Navajas *et al.*, 1998) and apply the rate of mutation used by Knowlton and Weight (1998), we can evaluate the time which was necessary for the colonisation of the north-west Atlantic coast (from the Bay of Biscay to Ireland) by *T. brevicornis* around 2.5 MY. This evolution rate agrees with the one estimated for *Alpheus cylindricus* between the Caribbean and Pacific areas (unpublished data).

Nevertheless, other studies have shown that two refuge areas might have existed along the European coasts: one in the Mediterranean/Iberian area, which our hypothesis confirmed, and another along the west coasts of England and Scotland (Jolly 2005). As we only collected one population in this geographical area (Kearney: population I), we could not confirm this second unsupported northern glacial refuge.

The present study gave evidence of a marked subdivision between marine harpacticoid copepod *T. brevicornis* populations. The interpopulation genetic divergence suggested that there may be a gene flow along the European coast but it could be restricted to some areas depending on the geographic dispersion of the natural habitat of this species. The founder effect and genetic drift seemed to be the most important processes, which could explain the observed genetic structure. Nevertheless, the analysis of the genetic divergence showed that the populations had a general north-south structure, which points to a new genetic break point in accordance with the geomorphology of the area**.** Other markers and more sampling sites around the Gironde estuary are now necessary in order to analyse the role of this tableland as a contemporary barrier to gene flow.

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