



Allozyme variation and genetic divergence in populations of *Talitrus saltator* (Crustacea: Amphipoda) around the Atlantic coast, the Azores and the Canary Islands

Hans-Peter BULNHEIM* and Dorothea E. SCHWENZER

Biologische Anstalt Helgoland im Alfred-Wegener-Institut für Polar-und Meeresforschung, Notkestr. 31, D-22607 Hamburg, Germany

* Corresponding address: Dörpfeldstr. 53, D-22609 Hamburg, Germany

Abstract: Individuals from 15 supralittoral populations of the marine amphipod *Talitrus saltator* collected in a range from northern Portugal to south-west Spain, as well as from the Azores, and the Canary Islands, were electrophoretically analysed and compared with specimens from two NW-Atlantic populations. Patterns of significant differences in gene frequencies and geographic divergence were examined, using starch-gel electrophoresis. Out of ten enzymes loci investigated, 4 (glutamate-oxalacetate-transaminase-2, *Got-2*, mannose-phosphate-isomerase, *Mpi*, phosphoglucosomerase, *Pgi*, phosphoglucosomutase, *Pgm*,) were highly polymorphic. A clinal variation in allele frequency was obvious, and especially at two loci (*Pgi*, *Pgm*). Estimates of N_m (effective number of migrants per generation between populations) revealed reduced gene flow which is related to the restricted dispersal capacity of *T. saltator*. UPGMA cluster analysis, based upon polymorphic loci, shows two different groups of populations: (1) those from Portugal, including the populations of the Azores, and (2) those from the gulf of Cadiz which extends with modifications of allele frequencies to the Gibraltar region and to the Canary Islands. Genetic differentiation is proposed to be promoted by random genetic drift and natural selection.

Résumé : Variabilité et différenciation génétiques des populations de *Talitrus saltator* (Crustacea: Amphipoda) du littoral atlantique, des Açores et des îles Canaries. Les individus de quinze populations supralittorales de l'Amphipode marin *Talitrus saltator*, récoltés sur différentes plages localisées entre le nord du Portugal et le sud-ouest de l'Espagne, ainsi que ceux des Açores et des îles Canaries, ont été analysés au moyen de la technique d'électrophorèse sur gel d'amidon. Ces populations ont été comparées à deux populations du nord-ouest Atlantique. Des différences significatives dans la fréquence des gènes ont été mises en évidence. Dix locus enzymatiques ont été étudiés dont quatre (glutamate-oxalo-acétate-transaminase-2, *Got-2*, mannosephosphate-isomérase, *Mpi*, phosphogluco-isomérase, *Pgi*, phosphoglucomutase, *Pgm*) sont très polymorphes. Pour deux locus en particulier, une variation clinale de la fréquence électromorphe a été mise en évidence (*Pgi*, *Pgm*). L'estimation de N_m (nombre effectif de migrants entre populations, par génération) indique une limitation spatiale des échanges génétiques, en corrélation avec la capacité restreinte de dispersion de *Talitrus saltator*. Une analyse de regroupement par la méthode UPGMA montre que les populations sont scindées en deux groupes : (1) le groupe atlantique du Portugal, et les populations des Açores et (2) le groupe du Golfe de Cadix, s'étendant (avec modifications de fréquence d'allèles) à la région de Gibraltar et aux îles Canaries. La différenciation génétique semble être principalement due à la dérive génétique et à la sélection naturelle.

Keywords : *Talitrus saltator*, gene frequencies, geographic divergence, clinal variation, polymorphic loci, populations.

Introduction

Among the amphipods, members of the family Talitridae occur in many intertidal and supralittoral aquatic habitats, including sandy and stony marine beaches, rocky shores, salt marshes as well as estuarine and freshwater littoral zones. Among these, *Talitrus saltator* (Montagu, 1808), the common sandhopper, colonizes sandy shores above the high tideline and represents an important component of the sandy beach fauna. This amphipod, locally common in the marine narrow zone along the high tideline, has a geographical range from SW-Norway and the middle Baltic Sea to the Azores, Canary Islands, Morocco and nearly the whole Mediterranean (cf. Dahl, 1946; Bellan-Santini, 1993).

With regard to biogeography, the occurrence of *T. saltator* on the island of Tenerife is doubtful and, as confirmed from our own observations, populations of *Talitrus* in Gran Canaria, which were observed in various localities in the past, have ceased to exist due to anthropogenic pressure starting in the mid 20th century. *T. saltator* was also recorded from Lanzarote, Fuerteventura and La Graciosa (Purpurarias). However, this amphipod appears to be rare, especially on Lanzarote, as a result of human activities.

The taxonomic diagnosis shows that this crustacean species occupies quite an isolated position in the family Talitridae. Therefore, it has been considered as a monotypic genus (Bousfield, 1982). In the North-West coastal region of the European continent, Lincoln (1979) measured the maximal size of *T. saltator* male up to about 25 mm. On the other hand, in the semiterrestrial environment of the Mediterranean, two varieties of *T. saltator* were described (Bellan-Santini, 1993): a *mediterranean* form, somewhat smaller and slender than Atlantic specimens, and a *briani* form, limited to the Adriatic Sea. This morph was previously reported by Ruffo (1936) from the size of the male which measures only up to 13 mm.

The supralittoral talitrid under consideration exhibits out of placed life habit and specific ecological requirements. Hidden in temporary burrows during the day, it jumps on the shore at dusk or at night, foraging on plant material cast ashore, thus contributing to its decomposition. The adults and juveniles are poor swimmers and are generally rarely immersed in sea water. On many shores, they display a patchy distribution since they are strictly confined to sandy biotopes.

Like many other peracarid crustaceans, females carry the eggs and newly hatched young in a brood pouch, without a larval stage. Released juveniles, like adults, remain on sandy beaches amongst debris and decaying algae, close to the high water mark. For that reason, *T. saltator* is known to display limited dispersal abilities.

In view of these limitations to dispersal and habitat

colonization, there is evidence suggesting that gene flow between local populations is less important than it is for other marine crustaceans with planktonic larval stages. Previous studies along this line, based on enzyme electrophoresis, have been performed on the talitrids *Orchestia traskiana* Simpson (Busath, 1980), *Megalorchestia californiana* Brandt and *Platorchestia platensis* (Krøyer) (McDonald, 1985, 1987, 1989).

Talitrus saltator and *Talorchestia deshayesii* (Audouin), also being semiterrestrial amphipods, may often co-occur as they have similar habitat preferences. They have been subjected to an intensive analysis of allozyme variation at 17 loci (Bulnheim & Scholl, 1986). Variations at the loci *Pgi* and *Pgm*, both highly polymorphic, were studied in order to characterize the microevolutionary process of genetic differentiation in geographically separated populations, both on micro- and macrogeographic scales. The surveyed area included samples from the western shores of the Baltic Sea, the Wadden Sea area of the North Sea, extending from Denmark to the Netherlands, as well from the Channel area and the western French Atlantic coast (overall genetic distance from *Pgi* and *Pgm* $D=0.051$ over a coastal transect of approximately 3500 km). Although the genetic distance was not very high, three geographic subgroups of *T. saltator* were distinguishable, in which a rather low genetic variation was detected: the eastern North Sea as well as Baltic populations, the northern French populations and the Atlantic populations.

De Matthaeis et al. (1994) examined the Mediterranean *T. saltator* populations of the Ligurian and Thyrrenian Sea coasts, and confronted them with those of the Adriatic coast. A remarkable genetic distance of about $D=0.4$ was found between western and eastern populations of the Italian peninsula. In addition to this, De Matthaeis et al. (1995) investigated electrophoretically Aegean populations of *T. saltator* at 23 loci; the authors showed that a great genetic differentiation exists between (1) Adriatic, (2) Aegean, and western Mediterranean including Atlantic populations. The second and the third were more related to each other ($D=0.34$) and more divergent from the first one ($D=0.45$), indicating the genetic isolation of the Mediterranean sandhopper populations. In addition, populations of *T. saltator* on islands surrounding the Aegean, were studied at 20 loci. Low levels of genetic variability were detected for these latter populations with an average value of $H_o=0.026$ (De Matthaeis et al., 1998).

The purpose of this study is to extend, by use of the same electrophoretic technique, the analysis of the gene pool structure of *T. saltator*. Investigations on populations of the Atlantic coast of Portugal and Spain (Iberian peninsula), and the insular populations of the Azores and the Canary Islands were achieved in order to estimate the amount of genetic differentiation between them. Furthermore, the biogeographic

divergence and the biochemical genetic information obtained, allow us to establish the status of the populations of *T. saltator*.

Material and methods

I - Sampling area

The analysis was conducted on 15 samples of *T. saltator* collected by hand from several sites of the Atlantic coast and Macronesia (Fig. 1), including the Azores and the Canaries. Madeira and Cape Verde Islands form part of the Macronesia and represent a group of volcanic islands, where the sandhopper failed to appear (Dahl, 1967).

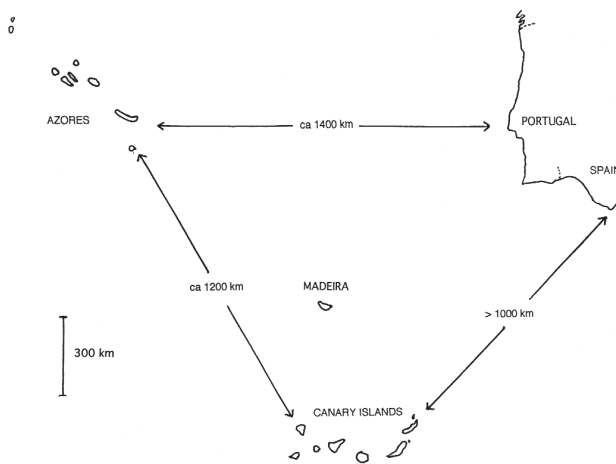


Figure 1. The regions sampled include Iberian peninsula (Portugal and Spain), Azores and Canary Islands.

Figure 1. Les régions échantillonnées comprennent la péninsule Ibérique (Portugal et Espagne), les Açores et les Iles Canaries.

The Azores are an archipelago of nine islands from which the samples sites are Lagoa=L (São Miguel, eastern group), Praia da Vitória=V (Terceira, central group) and Praia do Almoxarife (Faial, sample discarded). On the Atlantic coast of Portugal and Spain, several sites (Minho=M, Torrera=T, Guincho=G, Troia=Tr, Odeceixe=O, Lagos=La, Galé=Ga, Higuera=H, Tarifa=Ta, Sotogrande=So, in western Mediterranean) were also sampled. Canary Islands comprise eleven islands, and correspond to: La Graciosa=Gr (Graciosa), Playa Famara=F (Lanzarote) and Corralejo=C (Fuerteventura) (Fig.2). The island São Miguel is about 150 km away of the island Terceira and the island Fuerteventura is 115 km away of Morocco. Two additional localities of the north-western European continent (List/Sylt=S, Ambleteuse=A) were also sampled and correspond to known references (Bulnheim & Scholl, 1986). Samples varied from 10 to 85 specimens.

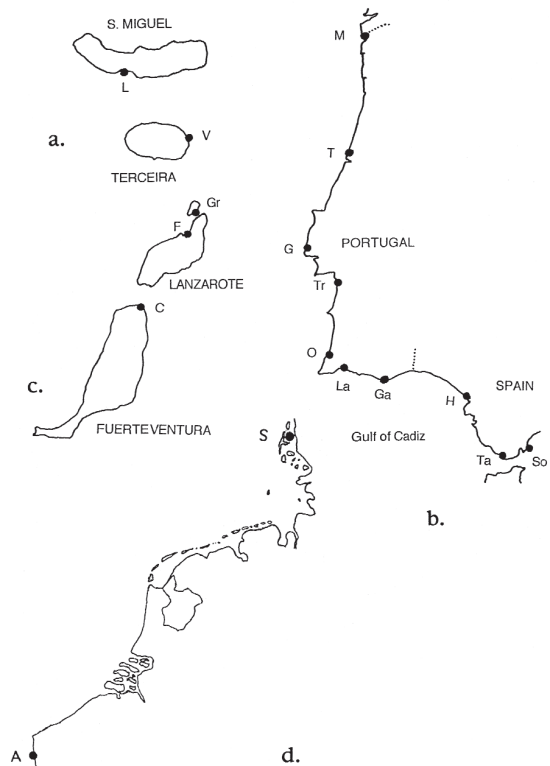


Figure 2. Sampling localities of *Talitrus saltator* (a) to (c) from the Atlantic Ocean. (a) Azores, L: Lagoa, V: Vitória. (b) Portugal and south-western Spain, M: Minho, T: Torrera, G: Guincho, Tr: Troia, O: Odeceixe, La: Lagos, Ga: Galé, H: Higuera, Ta: Tarifa, So: Sotogrande. (c) Canary, Gr: La Graciosa, F: Famara, C: Corralejo. (d) Coast of the Strait of Dover and North Sea, for comparative studies: A: Ambleteuse, S: Sylt.

Figure 2. Les lieux de récolte de *Talitrus saltator* (a) à (c) sur les côtes de l'Océan Atlantique : (a) aux Açores, L : Lagoa, V : Vitória. (b) Au Portugal et au sud-ouest de l'Espagne, M : Minho, T : Torrera, G : Guincho, Tr : Troia, O : Odeceixe, La : Lagos, Ga : Galé, H : Higuera, Ta : Tarifa, So : Sotogrande. (c) aux Iles Canaries, Gr : La Graciosa, F : Famara, C : Corralejo. (d) Sur les côtes du Pas-de-Calais et de la Mer du Nord, pour des études comparatives : A : Ambleteuse, S : Sylt.

II - Electrophoresis, enzyme assays, and data analysis

Following the collection of living individuals, most samples were deep-frozen (at -70°C), or stored in liquid nitrogen until use in vertical electrophoresis. Samples were screened on 13% starch gels (Buchler instruments), using the four electrophoretic buffers as described formerly (Bulnheim & Scholl, 1986): Tris-citrate buffer (TC), Tris-borate buffer (TBE), phosphate buffer (PO_4), and histidine-citrate buffer (HC).

Electrophoresis was performed in a cold room at a constant temperature (4°C) for 16-17 hours. The staining procedure of the slices took place after electrophoresis. Gels

were stained according to protocols slightly modified from Harris & Hopkinson (1976). Agar overlays, in combination with the specific staining solution, were employed to locate the enzymes MPI, PGI and PGM.

Due to the poor resolution of zymograms in sporadic cases, the number of loci was lower than that assayed in our previous study. Electrophoretic phenotypes were interpretable from 9 enzymes encoded by a total of 10 loci. They included the following enzyme systems: APK (arginine phosphokinase, EC 2.7.3.3), FUM (fumarase, EC 4.2.1.2), GOT (glutamate-oxalacetate-transaminase, EC 2.6.1.1, two loci), HK (hexokinase, EC 2.7.1.1), LAP

(Leucineaminopeptidase, EC 3.4.11.1), ME (malic enzyme, EC 1.1.1.40), MPI (mannose phosphate isomerase, EC 5.3.1.8), PGI (phosphoglucose isomerase, EC 5.3.1.9), and PGM (phosphoglucomutase, EC 2.7.5.1). In contrast to the procedure employed in our former investigation (Bulnheim & Scholl, 1986), the enzymes APK and FUM were detected using the Tris-citrate gel buffer system. PGI and PGM were treated with the HC-, TC- or PO₄-buffer, MPI was detected with the TBE-buffer, and GOT (without treatment with agar-detector gels), was determined with the TC-buffer.

As a rule, the enzymes migrated in the anodal region. Allozyme mobilities were assessed by including individuals

Table 1. Distribution of allele frequencies at four polymorphic sites (*Got-2*, *Mpi*, *Pgi*, *Pgm*) in 17 populations of *Talitrus saltator*. N = sample size, Ho = observed heterozygosity, He = expected heterozygosity *= significant departure from Hardy-Weinberg equilibrium (p<0.05). For populations sites (S to C) see Figure 2.

Tableau 1. Distribution des fréquences alléliques au niveau de quatre locus polymorphes (*Got-2*, *Mpi*, *Pgi*, *Pgm*) dans 17 populations de *Talitrus saltator*. N = taille de l'échantillon, Ho = hétérozygotie observée, He = hétérozygotie attendue, *= écart significatif à l'équilibre de Hardy-Weinberg (p<0,05). Pour les localités des populations (S à C) voir Figure 2.

Population S	A	L	V	M	T	G	Tr	O	La	Ga	H	Ta	So	Gr	F	C	
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
GOT-2																	
N	21	11	10	17	50	50	42	75	35	78	50	36	29	34	14	12	12
82	.000	.000	.000	.000	.000	.000	.000	.000	.000	.032	.000	.194	.000	.990	1.000	1.000	1.000
90	.000	.000	.000	.059	.000	.000	.024	.343	.414	.874	.890	.737	.931	.010	.000	.000	.000
100	1.000	1.000	1.000	.941	1.000	1.000	.976	.657	.586	.103	.110	.069	.069	.000	.000	.000	.000
Ho	0	0	0	.12	0	0	.05	.36	.43	.27	.18	.36	.03	.03	0	0	0
He	0	0	0	.12	0	0	.05	.47*	.50	.22	.20	.42	.13	.02	0	0	0
MPI																	
N	15	10	41	36	40	20	42	63	36	46	42	30	36	43	38	41	47
94	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.014	.035	.000	.000	.000
97	.000	.000	.000	.014	.050	.000	.000	.040	.000	.011	.036	.033	.028	.174	.000	.000	.000
100	1.000	1.000	.900	.986	.925	.900	1.000	.952	.986	.967	.952	.933	.833	.802	.724	.744	.436
104	.000	.000	.100	.000	.025	.090	.000	.008	.014	.022	.012	.033	.125	.000	.276	.256	.564
Ho	0	0	.19	.02	.15	.23	0	.04	.03	.07	.04	.13	.24	.34	.24	.28	.40
He	0	0	.21	.02	.14	.18	0	.10	.04	.06	.09	.13	.30	.33	.41*	.38	.50
PGI																	
N	39	82	42	44	38	50	43	69	37	77	52	28	39	43	37	38	48
84	.000	.000	.000	.000	.000	.010	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
91	.000	.000	.000	.000	.065	.060	.000	.043	.000	.058	.077	.143	.051	.000	.176	.092	.000
96	.000	.000	.000	.091	.051	.130	.209	.239	.351	.812	.750	.679	.885	.942	.824	.908	.948
100	.987	.701	.667	.864	.764	.570	.640	.638	.622	.117	.173	.161	.064	.058	.000	.000	.052
101	.000	.000	.000	.000	.020	.030	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
103	.013	.299	.333	.045	.100	.200	.151	.072	.027	.013	.000	.018	.000	.000	.000	.000	.000
105	.000	.000	.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.000	.000	.000	.000	.000
Ho	.03	.46	.38	.26	.42	.60	.49	.43	.41	.32	.31	.46	.24	.09	.32	.17	.10
He	.02	.42	.44	.24	.40	.63	.51	.52	.49	.33	.40	.49	.21	.13	.30	.17	.10
PGM																	
N	26	85	42	22	33	30	35	62	35	53	51	30	38	43	38	36	43
89	.000	.000	.000	.000	.000	.017	.000	.008	.014	.009	.000	.000	.013	.023	.000	.000	.000
92	.000	.000	.000	.227	.012	.000	.071	.073	.029	.066	.029	.117	.097	.093	.000	.000	.000
95	.019	.082	.738	.250	.348	.300	.286	.556	.514	.726	.765	.667	.711	.826	.397	.431	.279
97	.019	.118	.083	.023	.045	.017	.224	.032	.086	.113	.078	.167	.145	.047	.456	.403	.547
100	.942	.782	.179	.477	.561	.617	.371	.306	.386	.085	.127	.033	.053	.000	.132	.139	.093
103	.019	.018	.000	.023	.000	.050	.057	.016	.000	.000	.000	.017	.000	.000	.015	.028	.081
105	.000	.000	.000	.000	.015	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Ho	.12	.42	.38	.45	.62	.52	.66	.56	.63	.34	.37	.50	.54	.31	.68	.79	.64
He	.12	.37	.41	.66*	.55	.53	.73	.58	.60	.44	.39	.51	.46	.30	.59*	.64*	.61

from reference samples (List/Sylt or Ambleteuse) from which electrophoretic mobilities are well documented. Alleles were labelled according to the relative differences between their electrophoretic mobility. They were referred to the most common allele at each locus of the reference samples for which a relative mobility of 100 was given.

Electrophoretic data were analysed using the computer program BIOSYS-1 (Release 1.7) (Swofford & Selander, 1981). For each population, observed and expected heterozygosities and polymorphisms ($P_{95\%}$), as well as deviations from Hardy-Weinberg equilibrium were calculated. Nei's (1972) genetic distance (D) and identity (I) were estimated among all pairs of population for each locus, and averaged across loci. Cluster analysis was performed to draw the dendrogram using the unweighed pair group method (UPGMA) of Sneath & Sokal (1973).

F-statistics (estimated from Nei's G_{ST}) were calculated according to Wright (1978). His formula $F_{ST} = 1/(1+4Nm)$ ("island model") was used to estimate flow patterns from the standardized variances of allele frequencies between populations (N being the effective population size, and m the effective migration rate). This equation can be transformed to an estimation of the number of migrants per generation: $Nm = 1/4(1 - F_{ST})$.

Results

As reported from the French coast of the Atlantic ocean, the loci *Apk*, *Fum*, *Hk*, *Lap* and *Me* of *Talitrus saltator* were monomorphic (Bulnheim & Scholl, 1986). Anyhow, the molecular genetic data (for example *Got-1* and *Fum*) vary from the western Mediterranean by different allozymic patterns (Bulnheim & Scholl, unpublished results).

In addition, the *Gpt* locus, which is monomorphic from Denmark to Troia (southern Portugal), displays an additional rare allele (heterozygote) at Odeceixe (96/100). Allele 100 tends to become less and less frequent until Sotogrande and is replaced by allele 90 in the western Mediterranean for which the *Gpt* locus is fixed. Unfortunately, the enzyme GPT was not consistently detectable in all the populations, and therefore has been omitted in this study.

Electromorph frequencies of the four polymorphic enzymes encoded by the loci *Got-2*, *Mpi*, *Pgi* and *Pgm* are summarized in Table 1, together with observed (H_o) and expected (H_e) heterozygosities. Nearly all of them followed the expectations of Hardy-Weinberg equilibrium. MPI and PGM are monomeric, GOT-2 and PGI are dimeric enzymes. Three alleles were detected at the *Got-2* locus, four at the *Mpi* locus, and seven alleles were observed at the *Pgi* and *Pgm* loci. Figure 3 depicts the large geographic pattern of the relative allele frequencies in 15 populations along the coast and islands investigated.

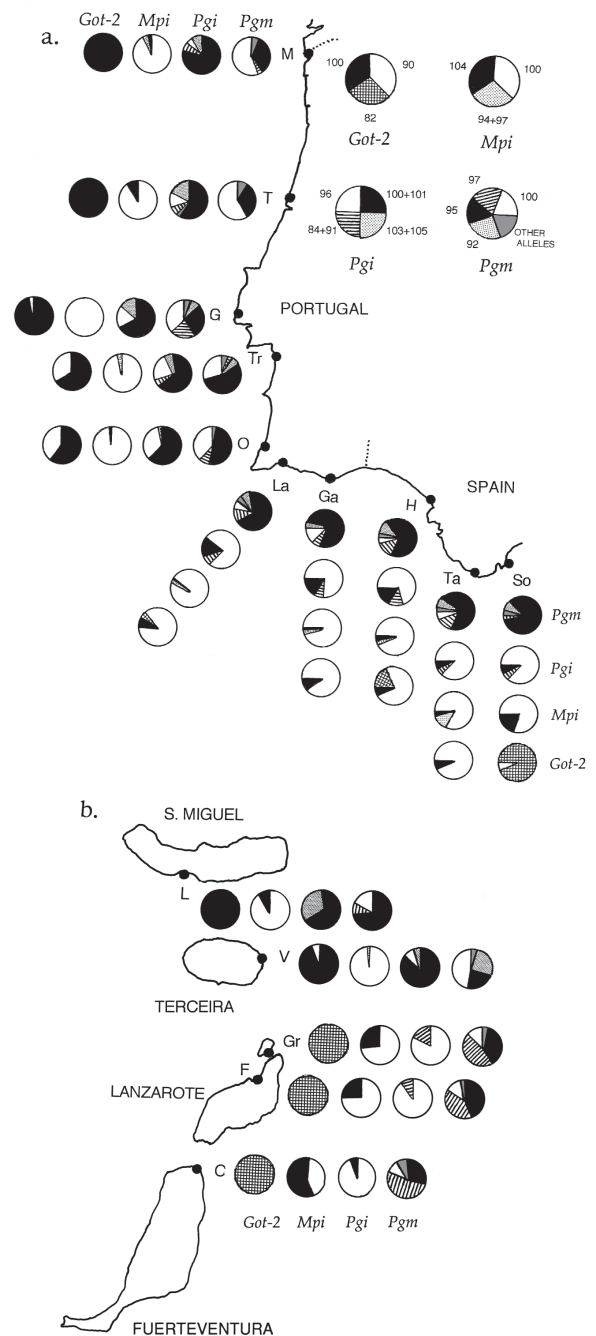


Figure 3. Geographical patterns of relative allele frequencies of four polymorphic loci (*Got-2*, *Mpi*, *Pgi*, *Pgm*) in the populations of *Talitrus saltator* studied, (a) along the continental coast of Portugal and Spain, (b) in two sites in the Azores and in three sites in the Canary Islands. For the different sites, see legend of Figure 2

Figure 3. Distribution géographique des fréquences relatives des allèles de quatre locus polymorphes (*Got-2*, *Mpi*, *Pgi*, *Pgm*) dans les populations de *Talitrus saltator* étudiées, (a) le long des côtes du Portugal et de l'Espagne, (b) sur deux sites des Açores et sur trois sites des Iles Canaries. Pour les différents sites, voir la légende de la Figure 2.

Table 2. Mean genetic identity (I, above diagonal) and mean genetic distance (D, below diagonal) between *Talitrus saltator* populations. Calculations (according to Nei, 1972) are based on four polymorphic loci (*Got-2*, *Mpi*, *Pgi*, *Pgm*).

Tableau 2. Identité génétique moyenne (I, au dessus de la diagonale) et distance génétique moyenne (D, au dessous de la diagonale) entre des populations de *Talitrus saltator*. Les calculs (selon Nei, 1972) sont basés sur quatre locus polymorphes (*Got-2*, *Mpi*, *Pgi*, *Pgm*).

Populations		S	A	L	V	M	T	G	Tr	O	La	Ga	H	Ta	So	Gr	F	C
Sylt	S	-	0.975	0.812	0.956	0.959	0.949	0.919	0.845	0.835	0.381	0.408	0.393	0.315	0.250	0.245	0.271	0.180
Ambleteuse	A	0.025	-	0.868	0.956	0.970	0.978	0.953	0.863	0.848	0.423	0.442	0.435	0.356	0.286	0.286	0.312	0.211
Lagoa	L	0.208	0.141	-	0.911	0.938	0.923	0.936	0.922	0.881	0.550	0.571	0.571	0.490	0.447	0.347	0.372	0.261
Vitoria	V	0.045	0.045	0.093	-	0.984	0.965	0.975	0.933	0.912	0.517	0.536	0.537	0.453	0.377	0.316	0.348	0.240
Minho	M	0.042	0.031	0.064	0.016	-	0.988	0.977	0.929	0.905	0.498	0.523	0.515	0.434	0.376	0.324	0.351	0.240
Torrera	T	0.052	0.022	0.080	0.036	0.012	-	0.973	0.911	0.892	0.509	0.529	0.518	0.450	0.383	0.354	0.380	0.276
Guincho	G	0.084	0.048	0.066	0.025	0.023	0.027	-	0.936	0.920	0.570	0.581	0.588	0.511	0.438	0.402	0.434	0.328
Troia	Tr	0.168	0.147	0.081	0.069	0.073	0.093	0.067	-	0.992	0.766	0.786	0.777	0.712	0.549	0.441	0.479	0.345
Odeceixe	O	0.180	0.165	0.127	0.093	0.100	0.114	0.084	0.008	-	0.809	0.826	0.813	0.760	0.573	0.480	0.521	0.393
Lagos	La	0.964	0.861	0.597	0.660	0.696	0.674	0.562	0.267	0.212	-	0.997	0.988	0.992	0.716	0.607	0.650	0.529
Galé	Ga	0.897	0.815	0.561	0.623	0.649	0.638	0.542	0.241	0.191	0.003	-	0.984	0.987	0.695	0.576	0.619	0.493
Higuera	H	0.935	0.833	0.561	0.622	0.664	0.658	0.531	0.253	0.207	0.012	0.016	-	0.974	0.772	0.681	0.717	0.592
Tarifa	Ta	1.154	1.033	0.713	0.791	0.836	0.798	0.671	0.340	0.275	0.008	0.013	0.026	-	0.694	0.605	0.641	0.549
Sotogrande	So	1.388	1.253	0.806	0.975	0.977	0.958	0.826	0.600	0.557	0.334	0.364	0.259	0.365	-	0.899	0.932	0.828
La Graciosa	Gr	1.406	1.253	1.059	1.152	1.127	1.039	0.911	0.820	0.734	0.500	0.552	0.385	0.503	0.106	-	0.993	0.970
Famara	F	1.305	1.164	0.988	1.055	1.047	0.967	0.835	0.736	0.651	0.431	0.480	0.333	0.444	0.071	0.007	-	0.954
Corralejo	C	1.714	1.557	1.343	1.427	1.427	1.288	1.114	1.065	0.934	0.637	0.707	0.524	0.600	0.188	0.031	0.047	-

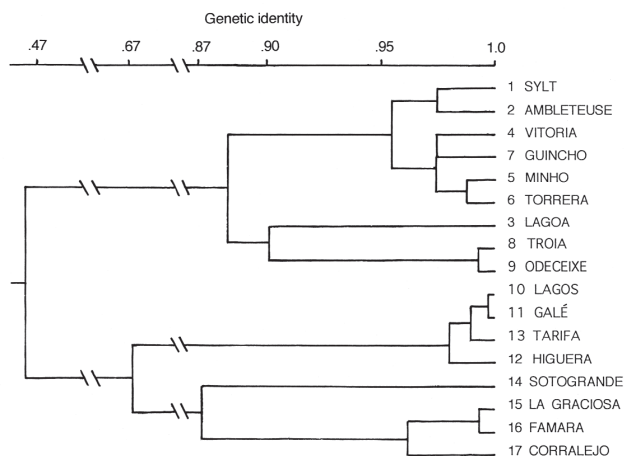


Figure 4. UPGMA-Dendrogram obtained from genetic identity (I) of pairwise combinations between 17 different *Talitrus saltator* populations: 15 populations examined in this study and 2 reference populations from North Sea and Strait of Dover (from Bulnheim & Scholl, 1986).

Figure 4. Dendrogramme UPGMA obtenu à partir des indices de similitude génétique (I) par paires de populations, entre 17 populations différentes de *Talitrus saltator* : 15 populations examinées dans cette étude et 2 populations de référence de la Mer du Nord et du Pas de Calais (voir Bulnheim & Scholl, 1986).

Considering the allele frequency distribution at the four polymorphic loci, there is a marked geographic variation in allele frequencies of *T. saltator*, with genetic distances up to 1.72 (Table 2). From the UPGMA dendrogram, two main groups can be distinguished, the Atlantic group and the Gulf of Cadiz group (Fig. 4). Within the Atlantic group, there is

Table 3. Values (F_{st} Nm, number of alleles) of *Talitrus saltator* (A) from De Mattheaie et al. (1995) for mediterranean populations including two Atlantic regions and (B) from this paper.

Tableau 3. Valeurs (F_{st} Nm, nombre d'allèles) de *Talitrus saltator* (A) d'après De Mattheaie et al. (1995) pour des populations méditerranéennes incluant deux régions atlantiques, et (B) d'après ce travail.

Enzymes	A			B		
	F_{st}	Nm	Alleles	F_{st}	Nm	Alleles
Got-2	1.000	0.000	2	0.799	0.063	3
Mpi	0.402	0.372	4	0.217	0.904	4
Pgi	0.542	0.211	5	0.434	0.326	7
Pgm	0.417	0.350	6	0.242	0.782	7
Averages	0.590	0.233	4.25	0.423	0.519	5.25

little differentiation from Grenen (North Sea, Denmark) to Guincho (middle Portugal). As a striking fact, Vitoria from the Azores forms part of these Atlantic localities (Minho, Torrera, Guincho). As a result, the *Talitrus* populations from the Azores seem to be more or less related to the western coast of Portugal, in spite of the huge geographic distance of ca. 1400 km. An additional finding from the gene-pool structure of *T. saltator* is that the Azores population undoubtedly greatly differ from those of the Canaries (see also Fig. 5), with genetic distances of $D \geq I$.

The second main group is made of Lagos (southern Portugal) and populations from the Gulf of Cadiz. The geographical distance between Tarifa and Sotogrande is more than 40 km; a mountain-range separates the two localities. With $D=0.36$, there is a pronounced and

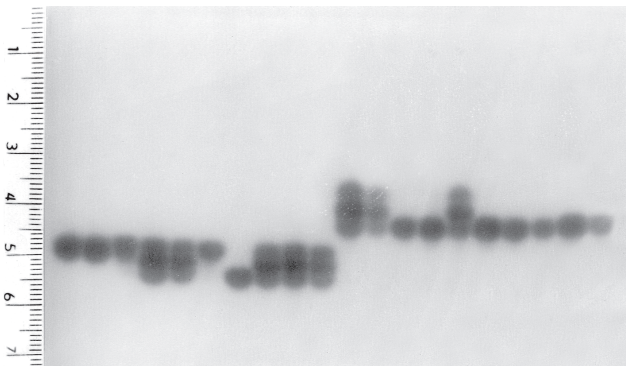


Figure 5. Zymogram of phosphoglucose isomerase obtained from different *Talitrus saltator* populations: Graciosa (Canary Islands, 10 individuals), Lagoa (Azores, 7 individuals), Praia da Vitória (Azores, 3 individuals).

Figure 5. Zymogramme de la phosphoglucose isomérase obtenu à partir de différentes populations de *Talitrus saltator* : Graciosa (Iles Canaries, 10 individus), Lagoa (Açores, 7 individus), Praia da Vitória (Açores, 3 individus).

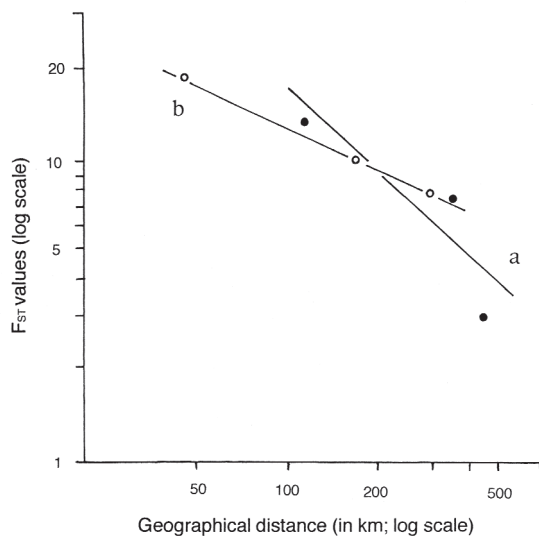


Figure 6. F_{st} values versus geographical distance (km) in *Talitrus saltator*. (a) For the Atlantic group, pairwise combinations from Minho to Troia. The locality Odeceixe omitted. Regression coefficient $r = -0.85$ (<0.87 , $ddf = 3$, $\alpha = 0.05$). r is not significant. (b) for the Gulf of Cadiz group, pairwise combinations from Lagos to Tarifa. $r = -0.90$ (>0.87 , $ddf = 3$, $\alpha = 0.05$). r is significant.

Figure 6. Valeurs de F_{st} obtenues par paire de populations versus la distance géographique (km) chez *Talitrus saltator*. (a) Pour le groupe Atlantique, de Minho à Troia. La localité Odeceixe est omise. Coefficient de regression $r = -0.85$ (<0.87 , $ddl = 3$, $\alpha = 0.05$). r n'est pas significatif. (b) Pour le groupe du Golfe de Cadix, combinaisons couplées de Lagos à Tarifa. $r = -0.90$ (>0.87 , $ddl = 3$, $\alpha = 0.05$). r est significatif.

unexpected level of genetic divergence between these populations. The population of Sotogrande displays the highest similarity to populations of the Canary islands ($D=0.07-0.19$), these latter exhibiting a very distinct genetic structure from that of the continental ones ($D=0.34-1.7$).

These relationships can be illustrated by values of F_{st} which reflect the proportion of the observed genetic variation that can be interpreted by partitioning between populations to estimate patterns of gene flow among populations (Slatkin, 1981). Hence, with $F_{st} = 0.8$, *Got-2* is extremely variable and could be taken as a diagnostic locus, separating regional groups (Atlantic, Gulf of Cadiz, Canaries incl. Sotogrande), while F_{st} values obtained from the locus *Mpi* ($F_{st} = 0.2$) and to a lesser extent from the locus *Pgm* are lower but indicate a barrier to gene flow between the populations at a global scale.

The comprehensive data from 15 populations indicate that substantial barriers to gene flow, a major component of population structure, occur over small geographical distances. Based on estimates over all the investigated loci, F_{st} equals 0.42; the number of migrants exchanged per generation is found as $Nm=0.52$ (Table 3), which is low between conspecific populations.

Isolation by distance seems to be one of the most likely mechanisms whereby *T. saltator* is speciating. In a subdivided population, random genetic drift may result in genetic divergence among such subpopulations. In this connection an attempt is made to test this process by estimating the Nm values between pairwise combinations from F_{st} values against geographical distance. Two groups, the Atlantic and the Gulf of Cadiz group, were analysed in logarithmic transformations. The populations along the continental coast and those of the archipelagos were submitted to a regression analysis, in order to see if isolation by distance exists. The regression coefficients estimated from all populations of the continental coast, which expands over a distance of ca. 800 km, are illustrated in Figure 6. In this context, the allele 96 and 103 frequencies of the *Pgi* locus considerably increased with decreasing latitudes from Denmark to northern France, comprising a distance of about 1000 km (Bulnheim & Scholl, 1986). In contrast to this, the allozymic patterns between the archipelagos and the continental coast, namely Azores versus Atlantic coast and Canary versus Gulf of Cadiz are not too different despite great geographical distances.

Discussion

Measures of genetic identity (I) and genetic distance (D) may be used to estimate the level of genetic divergence between taxa. In general, for the taxonomic classifications, values of genetic identity are reported as 1-0.95 for

populations, 0.98-0.84 (0.85) for subspecies, and 0.90-0.13 for species (Nei, 1976; Thorpe, 1983).

The present study reveals that the different forms of *T. saltator* do not merit a full specific status on the basis of the morphology, whereas high values of genetic distance (up to $D=0.596$) reported by De Matthaeis et al. (1995) (Mediterranean populations: overall similarity $I=0.792$) suggests that *T. saltator* represents a species' complex. Scapini & Fasinella (1990) obtained reciprocal crosses between Ligurian-Thyrrhenian and Adriatic populations. However, in F2 generation, offspring is only made of a few young indicating that some disruptions may occur in reproduction. This demonstrates that geographical races when getting into secondary contact either produce a more or less infertile offspring or do not hybridize.

In the high-water mark of sandy beaches, the two species *Talitrus saltator* and *Talorchestia deshayesii* were repeatedly found together. *T. deshayesii*, however, does not show any pattern of genetic differentiation or geographic isolation between northern European, Atlantic and Mediterranean populations (Bulnheim & Scholl, 1986; De Matthaeis et al., 1994). Moreover, the taxonomic separation between *T. saltator* and *T. deshayesii* is well documented with a value of $D=0.88$ (De Matthaeis et al., 1998).

The absence of dispersal phase could be one of the factors producing such a genetic structure in *T. saltator*. So far, gene flow between populations of this species is low and restricted (De Matthaeis et al., 1995). The Pleistocene climatic fluctuations, comprising glacial periods, could have played an important role in the isolation of Adriatic and Aegean populations in the Mediterranean, which is a relatively young sea (ca. 6 million years ago). During that period, marginal populations became gradually separated from the central Mediterranean and began to diverge genetically. By comparison, it is worth noting that populations from the southern part of Portugal or the Gulf of Cadiz, respectively, may have created another subspecies or race with some allelic alterations.

A number of genetic differences were obviously found between Atlantic and Mediterranean invertebrates. A pronounced level of genetic differentiation exists in *T. saltator* between the nearby populations of Tarifa (Atlantic ocean) and Sotogrande (western Mediterranean). As outlined by Pannaculli et al. (1997) for the barnacles *Chthamalus montagui* Southward and *C. stellatus* (Poli) and by Sanjuan et al. (1997) for the mussel *Mytilus galloprovincialis* Lmk., differences in allele frequency between Atlantic and Mediterranean localities were documented. This is due to the Almerian-Oran front, located east of Gibraltar region, which act as the major barrier to gene flow between these Atlantic and Mediterranean species.

As indicated in the above investigation, both the western Portugal and the southern Spain populations receive a steep environmental gradient in that area. Otherwise, the assumption reveals that the gradual fixation of enzyme alleles which could reflect clinal patterns of gene frequencies, particularly at the *Pgi* and *Pgm* loci, in possible response to climatic conditions, is included as evidence to infer natural selection on allele frequencies. These clines in allele frequency are situated along various geographical distances.

The genetic distance used to estimate the average level of gene divergence among populations is, from northern Portugal (Minho-Guincho) up to the Gulf of Cadiz (Lagoa-Tarifa) equal to $D=0.67$ ($I=0.51$), whereas it reaches, when Sotogrande is included, $D=0.92$ ($I=0.40$). On the other hand, the genetic distance between Sotogrande and the Canary Islands (La Graciosa-Corralejo) is equal to $D=0.12$ ($I=0.89$) which indicates a close relationship between these *T. saltator* populations.

Considering the four polymorphic loci, De Matthaeis et al. (1995) found mean values of $N_m=0.23$ and $F_{st}=0.59$, respectively, for Mediterranean populations including two Atlantic ones (Archachon, western France and Rosa Coruna, northern Spain) (Table 3). When all six populations on islands around the Aegean were taken into consideration, low levels of gene flow ($N_m < 1$) were indicated using different methods estimating gene flow (De Matthaeis et al., 1998). Gene flow models demonstrate that values of $N_m < 0.5$ will result in populations being genetically unconnected with regard to neutral alleles (Slatkin & Burton, 1989). Genetic drift and bottlenecks will effect in local differentiation if N_m -values are less than 1.

Geographical barriers, such as mountains with steep coast, are sufficient to prevent gene flow between sandhopper populations. Allopatric speciation can therefore occur, when a geographical barrier splits a population living over a wide range of habitats into two or more subpopulations between which gene flow is strongly reduced. Under these conditions, each of the subpopulation evolves by drift toward an adaptive peak appropriate to its local environment. The speciation appears to result from genetic drift and foundations which are known to produce significant differences between allelic frequencies and the genetic isolation of populations. The cross-fertilization experiments between individuals from separated populations are necessary to test the hypothesis of allopatric speciation which, however, have largely failed to appear.

The extinction and recolonization of local populations is an important mechanism of gene flow. The evolutionary effect of indirect measures in the level and pattern of gene flow is greatly enhanced by a long-distance migration and by an extinction and recolonization by local *Talitrus* populations on the volcanic archipelagos, for which founder

effects are expected. It might be reasonable to suppose that the dispersal tendencies would indicate the potential for colonizing vacant sandy habitats. If there are low levels of gene flow, well-adapted combinations of genes can be fixed in one local population through the combined action of genetic drift and natural selection. In Wright's (1978) shifting balance theory both dispersal between established populations and patterns in gene frequencies of local populations are important for spreading new combinations of genes.

T. saltator has been considered as a typical component of the supralittoral level of sandy beaches. This crustacean is one of those species able to extend its range from marine areas up to the brackish-water, for instance of the Baltic, for which salinity falls within 7-6‰. It demonstrates a marked ability to regulate the haemolymph osmolarity and to maintain a high salt-concentration in its blood over a wide range of external osmolarities or environmental salinities (Moritt, 1988). However, like other euryhaline invertebrates, *T. saltator* populations of the western Baltic (salinity ca. 15‰) show only slight genetic differences when compared with North Sea populations (salinity 32‰).

Attempts have been made to relate interspecific variation to environmental factors. As outlined by Scapini (1997), Atlantic and Mediterranean talitrid populations show behavioural differences in orientation, zonation, and spontaneous migration on the beach, all of which being referred to variations in tidal amplitude. The observed differences seem to be due to an adaptive response to the presence or nearly absence of tides at places of sampling which may be also explained by genetic factors.

Dahl (1946) observed that the supralittoral amphipod species are poor swimmers and the possibility of their active dispersal in the sea water column must therefore be precluded. Thrown into the sea water, *T. saltator* are only able to move slowly forward, suspended by common blows of the tender pleopods and their shortened urosom. This type of climbing or rolling on the bottom, as a form of passive dispersal, occurs mainly during the summer period of temperate regions at which the amphipods reproduces below the high-water marks of the sandy beaches. In the boreal Atlantic, during winter, *T. saltator* burrows higher upshore at a greater depth (maximum: 50 cm), usually beyond the influence of tidal floodings. The amphipods experience an overwintering phase of quiescent, diurnal period of inactivity.

On the other hand, *T. saltator* juveniles were recorded in the lower area of the bottom zone, usually in association with fucoid wrack deposits. They occur within the algal-mass or are superficially burrowed in the sand below the algae (Moritt, 1987; Williams, 1995). The downshore movement of the juveniles would partly compensate the flooding of the burrow zones. Inactive amphipods which

remain in contact with the beach substrate or algal weed, would be possibly displaced into the water level. In this way, the juveniles might be transported over long distances relative to their origin. It is difficult to assess the efficiency of the dispersal when juveniles are swept along by sea water (wave action) but the amphi-atlantic distribution can hardly account for such an immigration process (Karlbrink, 1969). It is possible that the supralittoral amphipods had colonized new areas by being carried through bilge water or ballast tanks of the ships along the Atlantic passage. However, this mode of dispersal appears to be very seldom and it is almost impossible that *T. saltator* and some related talitrids can conquer new habitats along the huge geographical distance without the aid of external relief actions (e.g. floating wrack and driftwood).

Altogether, populations may be founded by a few colonists. Given burst of random drift with founder events, this could enhance genetic differentiation between local populations. Limited dispersal capabilities and small founder populations were thought to contribute for *T. saltator* to the observed patterns of distribution. Natural selection under different environmental regimes and random genetic drift are the likely basis of population differentiation (Barton, 1996).

In the past, the prevalent opinion was that during the late Tertiary, Macronesia was possibly connected to the continental Europe or Africa by one or more land bridges. However, from our present knowledge, the archipelago of the Azores - located in the middle North Atlantic region - Madeira and the Canary Islands are supposed to be totally oceanic, and to have a volcanic origin (Balletto et al., 1990). The more western islands of the Azores and the Canary Islands are assumed to be the youngest ones (about 1-2 Myr). The North Macronesian fauna suggests that it may have derived from species, originally coming from the European continent, which probably reached the islands in a stepping-stone process. This is in contrast to Madeira, and probably also to the Canaries, as these islands seem to have been colonized from the African continent. Nevertheless, on grounds of the extensive distance separating the Atlantic coast from the Azores, the occurrence of the supralittoral amphipod *T. saltator* cannot be considered as clarified, and casts doubt on the possibility of a passive dispersal from the western coast of Portugal toward the sandy beaches of these islands.

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