

Genetic differentiation and evolutionary process of speciation in the *Idotea chelipes* complex (Crustacea, Isopoda)

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Abstract – Genetic differentiation and evolutionary relationships among 12 populations, representing three subspecies of the polytypic *Idotea chelipes* were studied by examining geographic variation at 13 loci assayed using polyacrylamide gel electrophoresis. Interbreeding tests and isozyme variations were investigated to compare these three subspecies and *Idotea balthica* and to see whether morphological differences coincided with genetic differentiation. A dendrogram using Nei's genetic distance values and a pattern of speciation are reported. The taxonomic status, both specific and subspecific, were confirmed. © Inra/Elsevier, Paris

Idotea / cross breed / genetic distance / speciation

Résumé – Différenciation génétique et processus évolutif de la spéciation d'*Idotea chelipes* (Crustacé Isopode). *Idotea chelipes* a été définie, sur la base des critères morphologiques et de l'hémocyanine, comme espèce polytypique qui groupe trois sous-espèces : *I. c. bocqueti*, *I. c. mediterranea* et *I. c. chelipes*. Le statut taxinomique de ces trois sous-espèces est réexaminé en utilisant les données du polymorphisme enzymatique et les résultats des tests d'interfertilité par comparaison avec une autre espèce, *Idotea balthica*. Douze populations d'*I. chelipes* ont été analysées par électrophorèse sur gel de polyacrylamide mettant en évidence 13 loci. À partir des fréquences alléliques, les distances génétiques de Nei ont été estimées. Celles-ci sont

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faibles entre les populations mais augmentent entre les sous-espèces et deviennent importantes entre les espèces. Un dendrogramme est construit et un modèle de spéciation est proposé. Les statuts taxinomiques, spécifique et subs spécifique sont confirmés. © Inra/Elsevier, Paris

***Idotea* / hybridation / distance génétique / spéciation**

1. INTRODUCTION

Idotea chelipes [22] was determined as a polytypic species including three subspecies, *bocqueti*, *mediterranea* and *chelipes*, on the basis of morphological resemblance and study of hemocyanin when compared with *Idotea balthica basteri* [1]. These subspecies or geographical races occupy, respectively, the eastern Mediterranean basin (eastern Tunisia coasts), the western Mediterranean basin (north Tunisia and south French lagoons) and the Atlantic coasts (Morocco, Spain, France), North Sea and Baltic.

The majority of the *I. chelipes* subspecies features, particularly those concerning the secondary sexual male characteristics are identical. However, they are very different from *I. b. basteri* ones [8].

Similar observations were reported that concern the hemocyanin electrophoretic mobility and its molecular weight. Hemocyanin, used as a crustacean specific marker, showed the same relative electrophoretic mobility and the similar molecular weight within *I. chelipes* subspecies. Thus, we observed clear differences between *I. chelipes* and *I. b. basteri* [4, 9].

The former results, when combined with some minor morphological characteristics (coxal plates and pleotelson shape) and with biochemical markers [7], allowed the following:

- to confer a subspecific level to *I. bocqueti*, the endemic species of the eastern Tunisia coasts, which was described as a new species [26];
- to separate the western Mediterranean and the Atlantic *I. chelipes* populations into two subspecies, *I. c. mediterranea* [4] and *I. c. chelipes* [22].

The purpose of the present paper is to verify this viewpoint. Two approaches are used:

- breeding tests to determine whether genetic divergence is high enough for the populations or the subspecies to be considered as separate species;
- enzymatic polymorphism to estimate the intraspecific and the interspecific genetic divergence.

2. MATERIALS AND METHODS

Laboratory cross-breeding tests were tested: intrasubspecific, intersubspecific and interspecific crosses were made.

Electrophoretic study used specimens collected from natural populations (*figure 1*). Descendants and hybrids obtained from laboratory intra or interspecific cross-breeding tests were used to determine the genetic control of the different allozymes. Thirteen populations were submitted to enzymatic analysis:

- Arcachon basin (A) and Pouliguen salt-marsh (P) from Atlantic coasts;
- Leucate (L), Canet (C), Gruissan Salin (GS), Ichkeul (I), Bizerte (B), Ghar-el-Melh (GM) and Tunis (T) from western Mediterranean lagoons;

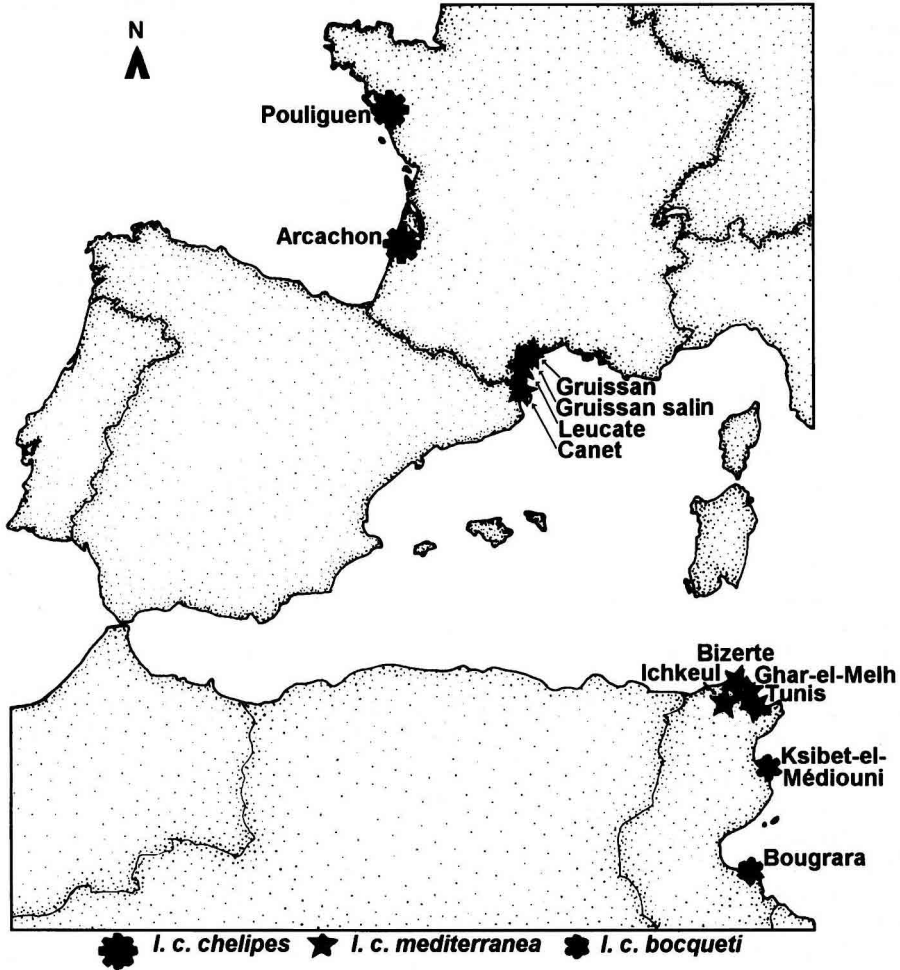


Figure 1. Sampling areas of *Idotea chelipes*: *I. c. chelipes* (Atlantic coasts); *I. c. mediterranea* (western Mediterranean basin); *I. c. bocqueti* (eastern Mediterranean basin).

- Ksibet-el-Médiouni (K), Bougrara (BG) from eastern Tunisia coasts;
- *I. b. basteri* population of Bizerta Lake was used as an outgroup to compare the three geographical *I. chelipes* subspecies described above with this *I. balthica basteri*.

The enzymatic polymorphism was studied on polyacrylamide gels. Specimens at stage C of the moult cycle interecdysis [12] were homogenized in a migration buffer (Tris-glycin pH 8,6) and saccharose 40 % in the same proportions.

Only one or two enzymes were scored per specimen. The hemocyanic fractions and the following enzymes were analysed: amylases (AMY, EC 3.2.1.1.),

phosphoglucose isomerase (PGI, EC 5.3.1.9.), alkaline phosphatase (ALP, EC 3.1.3.1.), aldehyde oxidases (AO, EC 1.2.3.1), malate dehydrogenases (MDH, EC 1.1.1.37), glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1.), lactate dehydrogenase (LDH, EC 1.1.1.27.), esterases (EST, EC 3.1.1.1.). The technical details were described by Sims [27], Harris and Hopkinson [13], Legrand-Hamelin et al. [17], Laulier [14], Pasteur et al. [24] and Charfi-Cheikhrouha [5]. According to the population, the number of specimens varied from 14 to 184.

Genetic interpretation of the gels was based upon the nomenclature of Pasteur et al. [24]: the most common allele at each locus was named 100. For the other alleles, numerical values were obtained by adding or subtracting migration distances from 100.

The Biosys-1 program of Swofford and Selander [29] was used:

- to calculate the coefficients [20, 21] of genetic identity (I) and genetic distance (D);

- to construct the phylogenetic tree using the unweighted pair group method (UPGMA) and the single linkage (SL) or nearest-neighbour method [28].

This last method offers the advantage of requiring no assumptions about equal rates of evolution.

3. RESULTS

3.1. Experimental cross-breedings

Owing to the cannibalism of males against females during ecdysis, only some cross-breedings were realized in the laboratory. The interspecies cross-breedings (*I. balthica basteri* *I. c. mediterranea* and *I. c. bocqueti*) were unsuccessful. The intersubspecies cross-breedings (*I. c. mediterranea* and *I. c. bocqueti*) yielded viable and fertile individuals. The intrasubspecies cross-breedings (*I. c. mediterranea*, *I. c. bocqueti* and *I. c. chelipes*) were always successful. The progeny numbers produced by the intra and the inter cross-breedings (table I) were compared statistically. The *F* test application indicates no statistical difference between the matings ($F = 0.1383$, $P < 0.05$).

3.2. Electrophoretic analysis

3.2.1. Allozyme variation

Genetic variation was examined at 13 loci in 12 populations of *I. chelipes*. Seven loci were found monomorphic in all populations: AO-1, MDH-1, MDH-2, GOT, LDH, EST-1 and hemocyanin (HCY). Six loci were polymorphic: AMY-1, AMY-2, PGI, ALP, AO-2, EST-2. The number of alleles varied from 2 to 6. Enzyme structure, deduced from pedigree analysis, is monomeric (AMY, AO-2) or dimeric (ALP, EST-2).

Table II indicates allelic frequencies for the different *I. chelipes* populations. At the esterase locus (EST-2), diagnostic alleles were identified: each subspecies is characterized by its own allele. According to this table, we can observe that the alleles with the highest frequencies are the same in each subspecies.

Table I. Intra and intersubspecific cross-breeding results.

Cross-breeding type	<i>I. c. mediterranea</i>	<i>I. c. bocqueti</i>	Male <i>c. mediterranea</i> × female <i>c. bocqueti</i>	Male <i>c. bocqueti</i> × female <i>c. mediterranea</i>
Cross-breeding Number	13	10	12	6
descendents	14.15	13.60	12.50	13.80
Mean variance	38.81	46.93	41.73	57.30

For some genes, frequencies differ notably between the three subspecies. Such results are observed when *I. c. bocqueti* is compared with the other *I. chelipes* subspecies (AMY-2). The same results are also observed within *I. chelipes mediterranea* when the northern lagoons of Tunisia are compared with the ponds of Roussillon (PGI).

The similarity of hemocyanin fractions was observed. The homology of the fraction, considered as the constitutive monomer, was demonstrated. These observations support the hypothesis of the hemocyanic allele identity.

Only nine loci were scored in the *I. balthica basteri* population. Three of them were polymorphic (AMY-1, PGI, LDH) and six were monomorphic within-population (AMY-2, GOT, MDH-2, MDH-1, EST-1, HCY). The monomorphic loci are as important as the polymorphic ones. The three last loci were considered as biochemical markers. They separate *I. b. basteri* from *I. chelipes* samples. Allele frequencies are compiled in *table II*.

3.2.2. Genetic divergence

The genetic similarity (I) and distance (D) values were calculated among *I. chelipes* subspecies on the basis of the allelic frequency data (*table III*). (I) varies from 0.776 to 0.999 and (D) varies from 0.001 to 0.254 according to Nei [20]. Nei's unbiased [21] estimates of similarity and genetic distance varied, respectively, from 0.777 to 1 and from 0 to 0.252.

Genetic distance values (*table III*) are low ($0.001 < D < 0.051$) when comparing populations of the same subspecies. They notably increase when populations of the different subspecies are considered. The average values are equal to 0.109 (*I. c. chelipes* and *I. c. mediterranea*), to 0.118 (*I. c. bocqueti* and *I. c. mediterranea*) and to 0.211 (*I. c. chelipes* and *I. c. bocqueti*).

The interspecific genetic distances (*table IV*), based on nine loci are high ($d = 0.898$) when comparing the two species, *I. b. basteri* and *I. chelipes*. The average values are, respectively, equal to 0.996, 0.955 and 0.7435 between *I. b. basteri* and *I. c. chelipes* populations, *I. c. mediterranea* and *I. c. bocqueti* ones.

Table II. Allele frequencies at 13 loci in the 3 subspecies of *Idotea chelipes* and at 9 loci in *Idotea balthica* (last line).

Taxa	Populations	Loci		N		AMY1		AMY2		N		MDH1		MDH2	
		Alleles													
<i>I. chelipes chelipes</i>	Pouliguen	97	100	103	106	100	103	100	103	62	100	98	100	100	100
	Arcachon	36	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	62	1.00	0.00	1.00	1.00
<i>Idotea chelipes mediterranea</i>	Gruissan salin	65	0.01	0.98	0.01	0.00	0.99	0.01	0.01	17	1.00	0.00	1.00	1.00	1.00
	Gruissan	33	0.05	0.95	0.00	0.00	0.97	0.03	0.03	33	1.00	0.00	1.00	1.00	1.00
	Leucate	30	0.02	0.98	0.00	0.00	0.97	0.03	0.03	22	1.00	0.00	1.00	1.00	1.00
	Canet	68	0.00	1.00	0.00	0.00	0.96	0.04	0.04	48	1.00	0.00	1.00	1.00	1.00
	Bizerte	46	0.00	1.00	0.00	0.00	1.00	0.00	0.00	28	1.00	0.00	1.00	1.00	1.00
	Ichkeul	184	0.00	1.00	0.00	0.00	0.98	0.02	0.02	126	1.00	0.00	1.00	1.00	1.00
	Ghar-el-Melh	180	0.00	1.00	0.00	0.00	0.98	0.02	0.02	140	1.00	0.00	1.00	1.00	1.00
	Tunis	139	0.01	0.99	0.00	0.00	0.96	0.04	0.04	114	1.00	0.00	1.00	1.00	1.00
	Ksibet-el-Médiouni	152	0.00	1.00	0.00	0.00	0.93	0.07	0.07	141	1.00	0.00	1.00	1.00	1.00
	Bougrara	150	0.00	1.00	0.00	0.00	0.03	0.97	0.97	136	1.00	0.00	1.00	1.00	1.00
<i>I. chelipes bocqueti</i>	Bougrara	57	0.00	1.00	0.00	0.00	0.00	1.00	1.00	48	1.00	0.00	1.00	1.00	1.00
	Bizerte	65	0.00	0.00	0.85	0.15	0.00	1.00	1.00	14	1.00	1.00	0.00	1.00	1.00
<i>I. balthica</i>	Bizerte	65	0.00	0.00	0.85	0.15	0.00	1.00	1.00	14	1.00	1.00	0.00	1.00	1.00

Table II. (continued).

Taxa	Populations	Loci	N	PGI			N	AO1	AO2	N	LDH					
				98	100	103					104	106	108	100	105	
<i>I. chelipes chelipes</i>	Pouliguen	Alleles	42	0.00	0.56	0.33	0.00	0.11	0.00	22	1.00	0.84	0.16	28	1.00	0.00
	Arcachon		22	0.00	0.55	0.29	0.00	0.16	0.00	17	1.00	0.82	0.18	20	1.00	0.00
<i>Idotea chelipes mediterranea</i>	Gruissan salin		21	0.00	0.33	0.50	0.00	0.17	0.00	16	1.00	0.81	0.19	20	1.00	0.00
	Gruissan		27	0.00	0.22	0.70	0.00	0.08	0.00	34	1.00	0.60	0.40	21	1.00	0.00
	Leucate		61	0.00	0.37	0.40	0.01	0.20	0.02	31	1.00	0.53	0.47	38	1.00	0.00
	Canet		28	0.00	0.28	0.60	0.00	0.10	0.02	36	1.00	0.30	0.70	36	1.00	0.00
	Bizerte		57	0.00	0.26	0.44	0.01	0.29	0.00	25	1.00	0.68	0.32	44	1.00	0.00
	Ichkeul		49	0.00	0.41	0.32	0.00	0.27	0.00	49	1.00	0.69	0.31	66	1.00	0.00
	Ghar-el-Melh		36	0.00	0.44	0.38	0.00	0.18	0.00	51	1.00	0.81	0.19	107	1.00	0.00
Tunis		49	0.00	0.42	0.22	0.00	0.33	0.03	79	1.00	0.95	0.05	105	1.00	0.00	
<i>I. chelipes bocqueti</i>	Ksibet-el-Médiouni		51	0.06	0.68	0.16	0.00	0.10	0.00	42	1.00	0.83	0.17	120	1.00	0.00
	Bougrara		48	0.08	0.67	0.19	0.02	0.04	0.00	37	1.00	0.90	0.10	21	0.78	0.22
<i>I. balthica</i>	Bizerte		40	0.00	0.02	0.68	0.00	0.04	0.26					132		

Table II. (continued).

Taxa	Populations	Loci		N		ALP		N		EST1		EST2		HCY	
		Alleles													
<i>I. chelipes chelipes</i>	Pouliguen		31	98	100	0.18	0.58	0.24	14	1.00	104	100	0.00	1.00	104
	Arcachon		17	0.35	0.53	0.12	0.12	14	1.00	0.00	0.00	0.00	0.00	1.00	0.00
<i>Idotea chelipes mediterranea</i>	Gruissan salin		15	0.43	0.27	0.30	0.27	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Gruissan		35	0.33	0.46	0.21	0.21	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Leucate		30	0.52	0.33	0.15	0.15	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Canet		27	0.45	0.33	0.22	0.22	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Bizerte		47	0.29	0.35	0.36	0.36	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Ichkeul		42	0.21	0.35	0.44	0.44	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	Ghar-el-Melh		50	0.25	0.39	0.36	0.36	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00
Tunis		49	0.21	0.42	0.37	0.37	14	1.00	0.00	0.00	1.00	0.00	1.00	0.00	
<i>I. chelipes bocqueti</i>	Ksibet-el-Médiouni		37	0.19	0.66	0.15	0.15	14	1.00	0.00	0.00	0.00	0.00	1.00	0.00
	Bougrara		31	0.19	0.63	0.18	0.18	14	1.00	0.00	0.00	0.00	0.00	1.00	0.00
<i>I. balthica</i>	Bizerte							28	0.00	1.00				0.00	1.00

Table III. Matrix of genetic distance and similarity coefficients (Nei [20]) between 12 populations of *Idotea chelipes* estimated on the basis of 13 loci. Genetic distance (below diagonal) and genetic similarity (above diagonal).

Subspecies	<i>I. chelipes chelipes</i>		<i>Idotea chelipes mediterranea</i>					<i>I. chelipes bocqueti</i>				
	Populations		Gruissan	Gruissan	Leucate	Canet	Bizerte	Ichkeul	Ghar-el-Melh	Tunis	Ksibet-el-Médioni	Bougrara
<i>I. chelipes chelipes</i>	Pouliguen											
	Arcachon	0.002	0.998	0.901	0.895	0.899	0.876	0.901	0.904	0.909	0.906	0.830
<i>I. chelipes mediterranea</i>	Gruissan Salin	0.104	0.102		0.991	0.991	0.976	0.996	0.993	0.996	0.990	0.815
	Gruissan	0.111	0.111	0.009	0.991	0.991	0.990	0.993	0.986	0.987	0.973	0.807
	Leucate	0.113	0.106	0.009	0.009	0.009	0.982	0.993	0.989	0.989	0.976	0.811
	Canet	0.133	0.129	0.024	0.010	0.008	0.983	0.977	0.983	0.977	0.950	0.784
	Bizerte	0.104	0.105	0.004	0.007	0.007	0.017	0.998	0.998	0.996	0.990	0.812
	Ichkeul	0.101	0.103	0.007	0.014	0.011	0.024	0.002	0.998	0.998	0.993	0.818
	Ghar-el-Melh	0.095	0.097	0.004	0.013	0.013	0.030	0.004	0.002	0.996	0.996	0.827
	Tunis	0.098	0.100	0.010	0.027	0.024	0.051	0.010	0.007	0.004	0.004	0.832
	Ksibet	0.186	0.187	0.205	0.214	0.209	0.243	0.208	0.200	0.189	0.184	0.999
	Bougrara	0.191	0.192	0.208	0.220	0.217	0.254	0.214	0.207	0.193	0.187	0.001

Table IV. Matrix of genetic similarity and distance coefficients (Nei [20]) between 13 populations of *Idotea chelipes* and *Idotea balthica* estimated on the basis of nine loci. Genetic distance (below diagonal) and genetic similarity (above diagonal).

Subspecies	<i>I. chelipes</i>		<i>Idotea chelipes mediterranea</i>							<i>I. chelipes bocqueti</i>		<i>I. balthica</i>	
	Populations	Pouliguen Arcachon	Gruissan	Gruissan Salin	Gruissan Leucate	Canet Bizerte	Ichkeul Ghar-el-Melh	Tunis Ksibet-el-Médiouni	Bougrara Bizerte	Bougrara Bizerte			
<i>I. chelipes chelipes</i>	Pouliguen	1.000	0.995	0.985	0.997	0.991	0.992	0.997	0.999	0.995	0.886	0.879	0.369
	Arcachon	0.000	0.995	0.983	0.997	0.990	0.993	0.998	0.999	0.997	0.887	0.880	0.370
<i>I. chelipes mediterranea</i>	Gruissan Salin	0.005	0.005	0.996	0.999	0.999	0.998	0.997	0.998	0.993	0.879	0.874	0.391
	Gruissan	0.015	0.017	0.004	0.992	0.999	0.993	0.987	0.990	0.980	0.866	0.862	0.403
	Leucate	0.003	0.003	0.001	0.008	0.996	0.999	0.999	1.000	0.997	0.886	0.879	0.384
	Canet	0.009	0.010	0.001	0.004	0.996	0.993	0.993	0.995	0.987	0.867	0.862	0.391
	Bizerte	0.008	0.007	0.002	0.001	0.004	0.998	0.998	0.997	0.995	0.875	0.868	0.383
	Ichkeul	0.003	0.002	0.003	0.001	0.007	0.002	0.999	0.999	0.999	0.884	0.877	0.373
	Ghar-el-Melh	0.001	0.001	0.002	0.000	0.005	0.003	0.001	0.999	0.997	0.890	0.883	0.381
Tunis	0.005	0.003	0.007	0.020	0.013	0.005	0.001	0.003	0.003	0.895	0.887	0.375	
<i>I. chelipes bocqueti</i>	Ksibet	0.121	0.129	0.144	0.121	0.142	0.134	0.123	0.117	0.111	1.000	1.000	0.473
	Bougrara	0.129	0.128	0.135	0.128	0.148	0.142	0.131	0.124	0.120	0.000	0.000	0.478
<i>I. balthica</i>	Bizerte	0.997	0.995	0.939	0.910	0.958	0.959	0.985	0.965	0.980	0.748	0.739	

The dendrogram reported in *figure 2* summarizes genetic relationships among all populations. It shows three levels of genetic differentiation corresponding to three main clusters:

- a first subdivision which separates the two species, *I. b. basteri* and *I. chelipes*;
- a second subdivision which isolates *I. c. bocqueti* populations from *I. c. chelipes* and *I. c. mediterranea* ones;
- a third subdivision which separates *I. c. mediterranea* and *I. c. chelipes* populations.

The groupings of the various populations of *I. c. mediterranea*, using the UPGMA and the SL methods, are different. These populations are represented on the dendrogram at the same level.

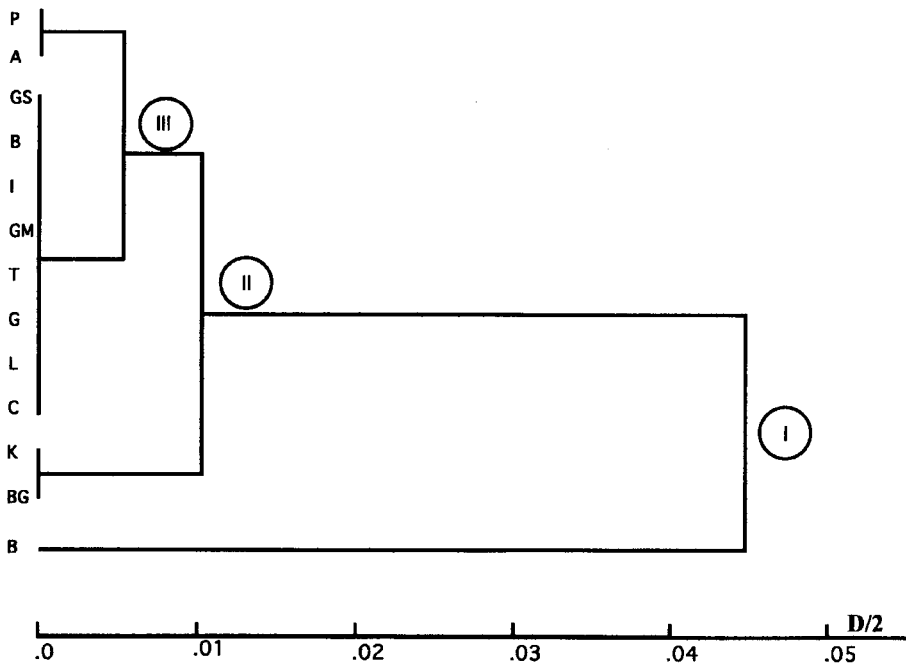


Figure 2. Dendrogram of *Idotea chelipes* populations based on UPGMA clustering of the genetic distance (D) data reported in *table III*. Data reported in *table IV* for the specific cladogenetic event (Level I).

4. DISCUSSION

Experimental studies showed the similarity of interbreedings between and within *I. chelipes* subspecies. Negative results and unsuccessful matings were observed when *I. b. basteri* was involved. According to the biological species concept: the mating of *I. bocqueti* sensu Rezig with other *I. chelipes* populations

produces hybrid offspring that interbreed with both parents and with one another [6]. We conclude that *I. bocqueti* is not a 'good' species like *I. b. basteri* but a race or a subspecies which belongs to the same species *I. chelipes*. However, these conclusions would be confirmed by further experiments based on the possibility of the choice of partners and the investigation of sympatric areas such as the Siculo Tunisia strait.

Apart from the morphological similarities, there are diagnostic alleles at the esterase 2 locus which constitute the best way to characterize hybrids in potential contact zones. At the locus EST-2 of the hybrids *I. c. bocqueti*-*I. c. mediterranea*, three bands at equal distances are evident. This result proves a diallelic locus and a dimeric structure of the EST-2.

Like other Crustaceans, the hemocyanic electrophoregram might be used as a specific taxonomic criterion. Manwell and Baker [19] and Maguire and Fielder [18] reported a similar hemocyanin pattern of various Crustacean species. Furthermore, in the case of *Sphaeroma*, the hemocyanin is both specific and subspecific [15]. In the Idotea genus, the comparison of hemocyanic fractions showed the identity of *I. c. chelipes*, *I. c. mediterranea* and *I. c. bocqueti*. This pattern differs from that of *I. b. basteri* [9]. These results are consistent with the hypothesis of genetic identity of the hemocyanin fraction inside closely related taxa.

An interesting observation should be made: the genetic distinction of the three subspecies is related to the geographic areas based on the enzymatic polymorphism and the allelic frequencies. Many geographically distinct populations of *Jaera*, *Talitrus saltator* were separated when allelic frequencies were used [3, 11].

The genetic distance values (*table IV*) might be directly compared with the taxonomic categories based on morphological criteria. The interspecific (*chelipes*-*balthica*) genetic distance as well as the subspecific one (*c. bocqueti*, *c. mediterranea* and *c. chelipes*) agree with estimates reported for other animal groups [2, 23]. The former values confirm that populations of the western Mediterranean and the Atlantic lagoons of *I. chelipes* would be considered as local populations of the two subspecies, *I. chelipes mediterranea* and *I. chelipes chelipes* and suggest that *I. bocqueti* cannot be isolated from the *I. chelipes* complex and elevated to the rank of species like *I. balthica basteri*. This example shows the excellent overall agreement between the genetic data and the taxonomic grouping.

The genetic distances within *I. chelipes* subspecies were used to draw the dendrogram (*figure 2*). The results suggest a relatively recent separation of the three subspecies populations. The first cladogenetic event would isolate the two species, *I. chelipes* and *I. balthica*. The second one would lead to the separation of *I. c. bocqueti* from *I. c. mediterranea*-*I. c. chelipes* and the third one would separate *I. c. mediterranea* and *I. c. chelipes*. It would be important to examine, with particular attention, the presumed contact zones considering that the process of geographic differentiation is reversible, whether there is an opportunity of gene exchange.

The clustering in the dendrogram agrees with the morphological affinities. The two species, *I. chelipes* and *I. balthica basteri*, are distinguishable by strong features notably the male sexual characteristics such as *appendix masculina* and pereopod's two seta. Only minor features corresponding to the pleotelson and

the first pereonite shape are observed to differ within *I. chelipes* subspecies. Thus, these characteristics are more pronounced in *I. c. bocqueti* than in the two other *I. chelipes* subspecies.

The results of the genetic analysis confirm our morphological observations and our tests of tentative hybridization and reinforce our hypothesis of the distinction of three subspecies, *I. c. bocqueti*, *I. c. mediterranea* and *I. c. chelipes* of a single polytypic species *I. chelipes*.

Contrary to some assertions [30, 31] for *Idotea balthica* [16] for *Sphaeroma*, the pattern of *I. chelipes* would suggest colonization from the western Mediterranean sea [10]. These apparently contradictory results must be reconsidered. The present Mediterranean Isopoda fauna might be related to a conquest by species which originated from the present oriental basin and which did not undergo drying during the salinity crisis as expounded by Charfi-Cheikhrouha and Zaghbib-Turki [10] and a reconquest by Atlantic species [25, 16]. To better solve this puzzle, the study must be enlarged to other species of the *Idotea* genus. The cladogram obtained must be compared with the corresponding geological events as has been carried out for *Jaera* genus on the basis of morphological characters [32].

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