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CLADOGENETIC EVENTS IN TWO RELICT SPECIES OF STENASELLIDAE (CRUSTACEA, ISOPODA) FROM SARDINIA ISLAND (TYRRHENIAN SEA) : A BIOCHEMICAL APPROACH

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GENETIC DIVERGENCE
MOLECULAR CLOCK
STYGOBITIC FAUNA
PALAEOGEOGRAPHY
STENASELLUS

SUMMARY. – Allozymic variation was studied in seven populations of exclusively stygobitic relict isopods of the genus *Stenasellus*. Two of the seven populations belong to different species (*S. nuragicus* and *S. assorgiai*) and were sampled in their type localities. With the exception of a population from the Su Mannau cave, the remaining populations, of uncertain systematic position, were sampled in wells from southern Sardinia. Levels of genetic divergence and the pattern of inferred phylogenetic relationships allow us to confirm that *S. nuragicus* and *S. assorgiai* are valid species. The populations of uncertain systematic position are phylogenetically related to these two species. Phylogenetic relationships are discussed in relation to the divergence times deducible from genetic distance data in order to assess the congruence between evolutionary events and Sardinian palaeogeographical dynamics.

DIVERGENCE GÉNÉTIQUE
HORLOGE MOLÉCULAIRE
FAUNE STYGOBIE
PALÉOGÉOGRAPHIE
STENASELLUS

RESUMÉ. – La variation des allozymes de sept populations d'Isopodes stygobies du genre *Stenasellusa* été étudiée. Deux des sept populations appartiennent à deux espèces différentes (*S. nuragicus* et *S. assorgiai*) et ont été récoltées à la station type. A l'exception de celle de la grotte de Su Mannau, les autres populations dont la position systématique est incertaine, ont été récoltées dans des puits du sud de la Sardaigne. Le degré de divergence génétique et les patterns de relations phylogénétiques obtenus, nous permettent de confirmer que *S. nuragicus* et *S. assorgiai* sont des espèces valides. Les populations dont la position systématique est incertaine sont phylogénétiquement proches de ces dernières. Les relations phylogénétiques sont discutées par rapport au temps de divergence que l'on peut obtenir à partir des distances génétiques pour évaluer la congruence entre phénomènes évolutifs et dynamique paléogéographique de la Sardaigne.

INTRODUCTION

The present position of the island of Sardinia in the centre of the Tyrrhenian Sea is the result of tectonic events leading to the separation of the Sardinia-Corsica microplate from the Iberian peninsula and to its subsequent rotation (Alvarez 1972). Various authors have correlated the high rate of endemicity that characterises the island's current fauna with these events, pointing to the phylogenetic relationships with Pyrenean elements (e.g. Baccetti 1983).

Despite the great difficulty in finding them, the Stenasellidae are certainly among the most interesting of the palaeogeographical indicator organisms (Magniez 1983 ; Messana *et al.* 1995). They are relict forms, decidedly stenoecious, have a limited capacity for dispersal, and are highly archaic. The last characteristic is indicated by the present chorology, the high degree of stygobitic specialisation, the lack of relationships with surface elements, and the primitiveness of some morphological characters (Magniez 1981).

For some years, we have been attempting to use the Sardinian Stenasellidae as reference material for a reconstruction of the dynamics of the colonisation of Sardinia, with particular respect to its initial phases (Argano *et al.* 1998). However, to achieve such a reconstruction, and to compare the Sardinian Stenasellidae with continental species, it is necessary to resolve the problems concerning the currently known species of the island.

We have already shown the high degree of genetic divergence between a Sardinian form of *Stenasellus,* discovered in the hyporheic systems of the Rio di Quirra basin of south-eastern Sardinia (previously only a single female was known), and *S. racovitzai*, an endemic species of some underground systems of Tuscany (Messana *et al.* 1995). In the past, the two entities were considered conspecific. The formal description of the newly identified Sardinian species is in preparation. According to Magniez (1974), *S. racovitzai* has affinities with the central European *virei* group.

The other two known Sardinian species, *S. nuragicus* and *S. assorgiai*, were discovered in two different caves of the Iglesias area, in the south-western part of the island (Argano 1968). Magniez (1981) claimed an affinity of these species with the group of Balkan species belonging to the genus *Balkanostenasellus*.

The two species are extremely similar to each other morphologically (the only differentiating diagnostic trait is the form of the second female pleopod). The discovery of some specimens in wells in the intermediate band of the Piana del Campidano which cannot be reliably attributed on morphological grounds (because of a certain variability in the morphology of the 2nd female pleopod) to one or the other species of the Iglesias area, prompted the hypothesis that there is a single species distributed throughout the southern part of the island (Manicastri *et al.* 1983, Messana *et al.* 1995).

During research in the Rio di Quirra basin, we identified a population attributable on morphological grounds to *S. nuragicus-assorgiai*. The level of genetic divergence between the Quirra population and the type-population of *S. nuragicus* has been assessed (Messana *et al.* 1995). However, to confirm the affiliation of this population with one or the other species, it remained to define the validity of *S. assorgiai.* To shed light on this entangled taxonomic situation, we assessed levels of genetic divergence among the type-populations of *S. nuragicus* and *S. assorgiai* and some specimens from the Piana del Campidano and Rio di Quirra basin, collected in 1996-97 during several surveys. In addition to confirming the validity of the two species, the data allow us to formulate an hypothesis about the times and mode of their mutual isolation.

MATERIALS AND METHODS

We examined seven populations of *Stenasellus* attributed on morphological grounds to the *nuragicus-assorgiai* group. The sampling sites, illustrated in Fig. 1, are listed below (a 3 capital letter code identifies the study populations).

The material comes from the type localities of *S. nuragicus* (S. Pietro Cave, PIE) and *S. assorgiai* (Pitzu' e Crobisi Cave, PIT), both in the Iglesias massif. A control sample comes from the Su Mannau Cave, MAN, which is part of the same karstic system as the S. Pietro Cave. The rest of the samples are from artesian wells, the specimens being captured with small baited fish-pots. Of the tens of wells tested, only 4 furnished samples suitable for electrophoretic examination. Two of them were on the slopes of the Iglesias massif (Musei, MUS, and Vallermosa, VER), one on the southeastern extremity of the island (Villasimius, VIL) and another in the Rio di Quirra basin (Tertenia, TER).

12 % starch gel electrophoresis was used to test variation at 11 enzymatic proteins presumably encoded by 15 structural loci : *Acph, Aph, Est-1, Est-2, Est-3, Idh-1, Idh-2, Ldh, Mdh, No-Dh, Pep-1, Pep-2, Pgm, Phi, To.* For details on study enzymes, the buffer systems and staining techniques, see Messana *et al.* (1995).

The levels of genetic differentiation among the study populations were quantified on the basis of the allele frequencies by means of the index of genetic distance D (Nei, 1978). An UPGMA dendrogram was constructed on the basis of the matrix of D values (Sneath & Sokal 1973).

The relationships among populations were also investigated by Principal Components Analysis. We obtained the relative pattern of ordering by extracting the first two Factors and considering the populations as variables characterised by a genetic order determined by the allele frequencies at the single loci. The statistical analyses were carried out using Biosys-1 (Swofford & Selander 1981) and Statistica for Windows.

RESULTS

Table I reports the allele frequencies at each locus for all the populations; of the 15 loci studied, 3 are monomorphic in all the study populations (*Est 3, No-Dh, To*) while the remaining 12 are polymorphic in at least one population.

The values of genetic distance D (Nei 1978) are reported in Table II : D ranges from 0.022 (MAN vs. PIE) to 0.662 (MAN vs. PIT). The level of genetic differentiation between the type populations of *S. nuragicus* (PIE) and *S. assorgiai* (PIT) is D = 0.587. The UPGMA dendrogram of Fig. 2 summarises the genetic affinities between the groups of populations and species studied. It reveals the existence of two groups of populations (PIT-VER-MUS and TER-VIL-MAN-PIE), that are distinct from each other and relatively homogeneous internally.

The pattern of ordering obtained from the Principal Components Analysis (Fig. 3) agrees with the relationships expressed by the UPGMA dendrogram; the first two axes explain 86.9 % of the total variance.

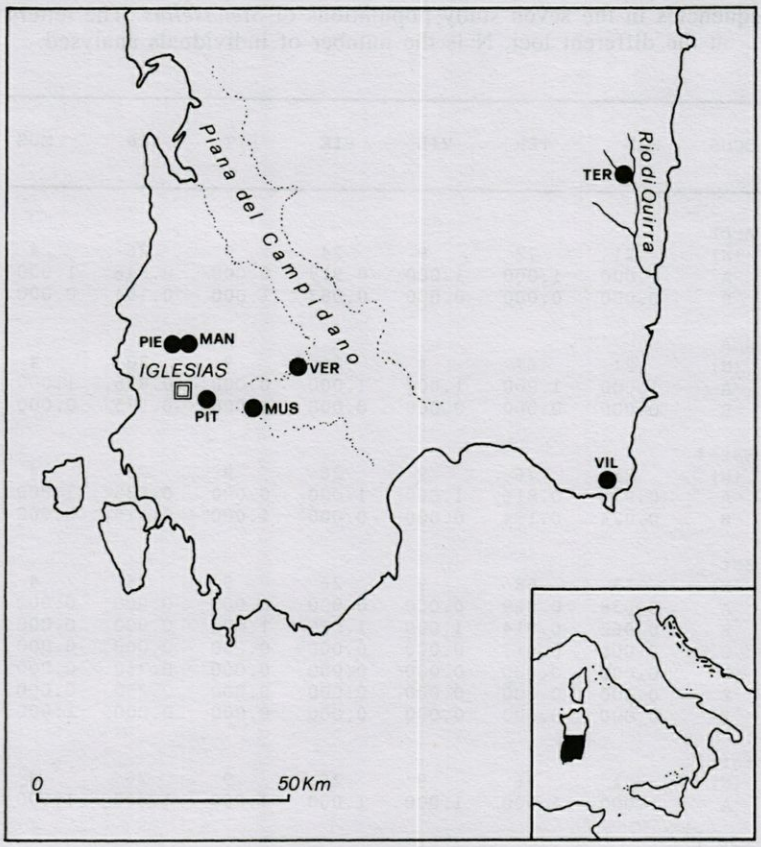

Fig. 1. – Sampling locations of the study populations of *Stenasellus*. For the abbreviations see Materials and Methods.

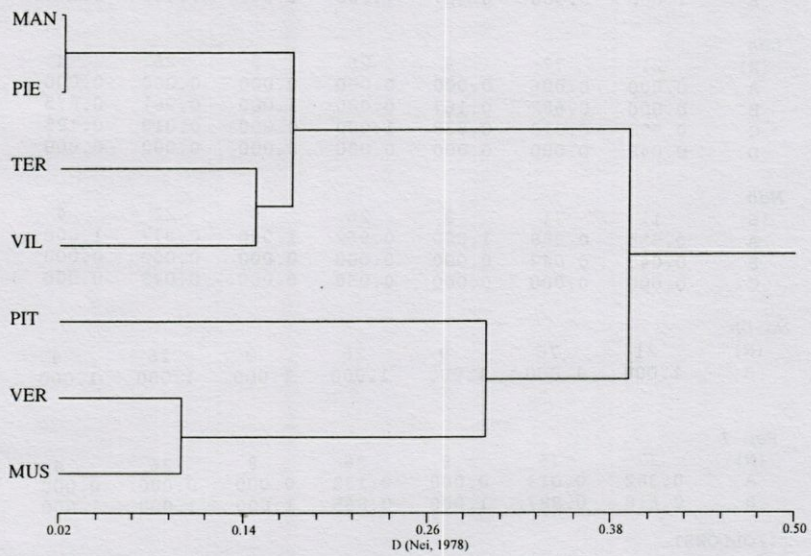

Fig. 2. – UPGMA dendrogram constructed from the values of genetic distance D (Nei, 1978) reported in Table I.

Table I. – Allele frequencies in the seven study populations of *Stenasellus*. The letters indicate the alleles at the different loci, N is the number of individuals analysed.

LOCUS	MAN	TER	VIL	PIE	PIT	VER	MUS
Acph							
(N)	21	72	9	24	9	26	4
A	1.000	1.000	1.000	0.917	0.000	0.846	1.000
B	0.000	0.000	0.000	0.083	1.000	0.154	0.000
Aph							
(N)	21	67	4	26	9	20	4
A	1.000	1.000	1.000	1.000	0.000	0.825	1.000
B	0.000	0.000	0.000	0.000	1.000	0.175	0.000
Est-1							
(N)	21	76	9	26	9	26	4
A	0.976	0.816	1.000	1.000	0.000	0.885	1.000
B	0.024	0.184	0.000	0.000	1.000	0.115	0.000
Est-2							
(N)	13	58	9	26	9	26	4
A	0.038	0.069	0.000	0.000	0.000	0.000	0.000
B	0.962	0.914	1.000	1.000	1.000	0.000	0.000
C	0.000	0.017	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.750	0.000
E	0.000	0.000	0.000	0.000	0.000	0.250	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Est-3							
(N)	21	76	9	26	9	26	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1							
(N)	21	71	9	26	8	26	4
A	0.000	0.190	0.056	0.000	0.000	0.058	0.000
B	0.000	0.739	0.944	0.000	1.000	0.942	1.000
C	1.000	0.000	0.000	1.000	0.000	0.000	0.000
D	0.000	0.071	0.000	0.000	0.000	0.000	0.000
Idh-2							
(N)	21	76	8	26	9	26	4
A	0.000	0.020	0.063	0.000	0.000	0.000	0.125
B	1.000	0.980	0.937	1.000	1.000	1.000	0.875
Ldh							
(N)	21	77	9	26	9	26	4
A	0.000	0.006	0.000	0.000	0.000	0.000	0.000
B	0.000	0.682	0.167	0.000	1.000	0.981	0.875
C	0.952	0.312	0.833	1.000	0.000	0.019	0.125
D	0.048	0.000	0.000	0.000	0.000	0.000	0.000
Mdh							
(N)	11	71	9	26	9	22	4
A	0.955	0.958	1.000	0.962	1.000	0.977	1.000
B	0.045	0.042	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.038	0.000	0.023	0.000
No-Dh							
(N)	21	76	9	26	9	26	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pep-1							
(N)	17	76	9	26	8	26	4
A	0.382	0.013	0.000	0.135	0.000	0.000	0.000
B	0.618	0.987	1.000	0.865	1.000	1.000	1.000

(FOLLOWS)

Table I. – (*Continues*).

LOCUS	MAN	TER	VIL	PIE	PIT	VER	MUS
Pep-2							
(N)	18	58	9	26	9	24	4
A	0.194	0.336	1.000	0.481	0.000	0.104	0.000
B	0.806	0.664	0.000	0.519	1.000	0.271	1.000
C	0.000	0.000	0.000	0.000	0.000	0.625	0.000
Pgm							
(N)	21	85	9	26	9	26	4
A	0.000	0.476	0.000	0.000	0.000	0.058	0.000
B	1.000	0.436	1.000	1.000	0.000	0.000	0.000
C	0.000	0.088	0.000	0.000	1.000	0.942	1.000
Phi							
(N)	22	69	9	21	6	20	4
A	0.000	0.007	1.000	0.476	1.000	0.975	1.000
B	0.000	0.007	0.000	0.000	0.000	0.000	0.000
C	0.182	0.805	0.000	0.024	0.000	0.025	0.000
D	0.818	0.014	0.000	0.500	0.000	0.000	0.000
E	0.000	0.167	0.000	0.000	0.000	0.000	0.000
To							
(N)	21	76	9	26	9	26	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table II. – Matrix of genetic distance values (D) calculated according to Nei (1978). For the abbreviations, see text.

POP.	MAN	TER	VIL	PIE	PIT	VER	MUS
MAN	*****						
TER	0.185	*****					
VIL	0.204	0.147	*****				
PIE	0.022	0.182	0.113	*****			
PIT	0.662	0.384	0.487	0.587	*****		
VER	0.463	0.223	0.254	0.387	0.280	*****	
MUS	0.406	0.211	0.273	0.362	0.316	0.100	*****

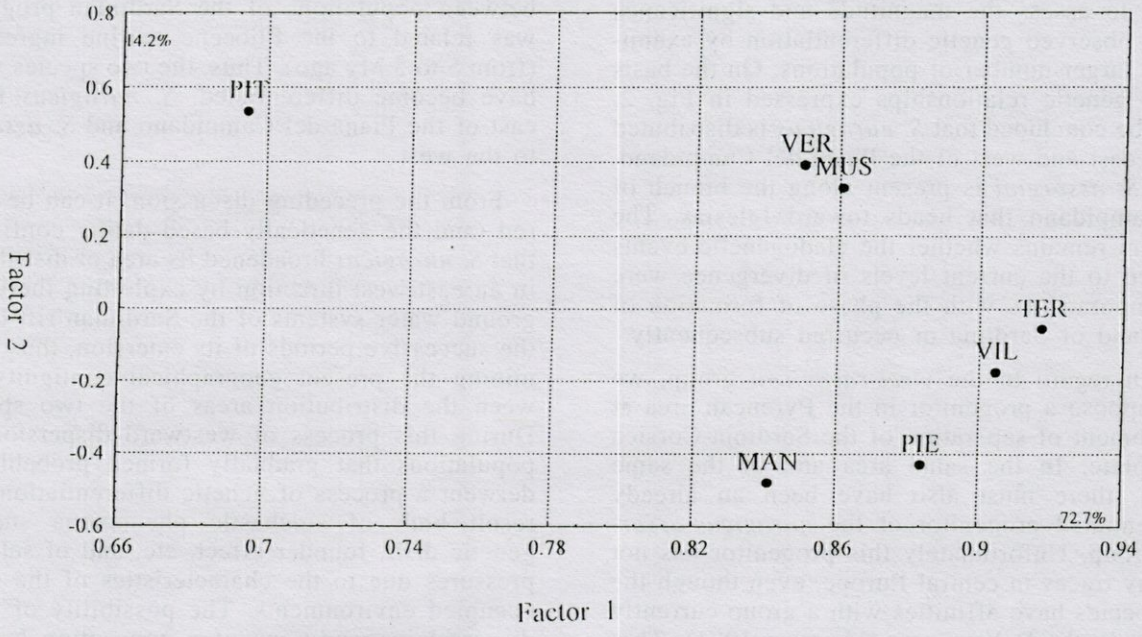

Fig. 3. – Ordering of the study populations by Principal Components Analysis. The first two factors are shown.

DISCUSSION

The PIE and PIT samples exhibit a genetic divergence of D = 0.587. This value falls within the range usually found in heterospecific comparisons, both in various groups of Crustacea and in other taxa (Hedgecock *et al.* 1982, Ayala 1983, Thorpe 1983, Stewart 1993). As these samples derive from populations of the type localities of the two study species (respectively, S. Pietro Cave and Pitzu' e Crobisi Cave), it follows that *S. nuragicus* and *S. assorgiai* can be considered valid species. Hence, the perplexities expressed by Messana *et al.* (1995) have been resolved.

The mean level of differentiation among the four samples PIE, MAN, TER and VIL (D mean = 0.142) is sufficiently low to consider the respective populations conspecific and referable to *S. nuragicus* species. In fact, this mean value is of the same order of magnitude as that usually found between conspecific populations of cavernicolous organisms of various groups (Sbordoni 1982). However, the mean value of D among the three remaining populations is higher (D mean = 0.232); there is greater genetic homogeneity between MUS and VER (D = 0.100) while PIT is the most differentiated (D mean = 0.300). Nevertheless, the genetically heterogeneous populations of this group can still clearly be considered related to one another (Fig. 2). The value of D = 0.167 found between the two close Tuscan populations of *S. racovitzai* examined by Messana *et al.* (1995) indicates a low degree of gene flow. It may be hypothesised that, in these organisms, processes of genetic divergence may easily occur even at a small geographical scale. Indeed, we intend to assess the magnitude and significance of the observed genetic differentiation by examining a larger number of populations. On the basis of the genetic relationships expressed in Fig. 2, it can be concluded that *S. nuragicus* is distributed to the east and west of the Piana del Campidano, while *S. assorgiai* is present along the branch of the Campidano that heads toward Iglesias. The problem remains whether the cladogenetic events that led to the current levels of divergence were contemporaneous with the phase of formation of the island of Sardinia or occurred subsequently.

With regard to the *virei-racovitzai* group, we can suppose a progenitor in the Pyrenean area at the moment of separation of the Sardinia-Corsica microplate. In the same area and in the same period, there must also have been an already differentiated progenitor of the *nuragicus-assorgiai* group. Unfortunately this progenitor has not left any traces in central Europe, even though the two species have affinities with a group currently present in the Balkan area (Magniez 1981). Therefore, at the moment we do not have a comparable, central European taxon with which to relate our findings to the origin of the island of Sardinia.

Nevertheless, assuming a time/genetic divergence relationship given by $T = D \times 5 \times 10^6$ (Nei 1975), one can estimate, very approximately and indirectly (Sbordoni *et al.* 1991), that the cladogenetic event separating the two species occurred about 3 My ago. This is quite a long time after the separation of the Sardinia-Corsica microplate, indicating that the speciation process took place much later on the island itself.

The events responsible for the present level of differentiation among the populations of *S. assorgiai* and those of *S. nuragicus* would have occurred even more recently, around 1.5 My and 0.71 My ago, respectively.

It is very difficult to identify single well-dated events in the palaeogeological history of Sardinia that would provide a time reference from which to calibrate the rate of accumulation of the observed genetic divergence. The evolution of Sardinia is extraordinarily complex, and this especially applies to the area involving the two species under study. The Piana del Campidano (of Plio-Pleistocene origin) is superimposed on a rift of Oligo-Miocene origin that cuts the whole of Sardinia from north to south. Sea level fluctuations have caused continuous ingressions and regressions of the sea along this rift during the past 15 My (Cherchi & Montadert 1982, Boccaletti *et al.* 1990) up until the glacial phenomena. From time to time, this created insurmountable geographical barriers for various organisms (especially the strictly freshwater ones), thus favouring phenomena of local differentiation. It can reasonably be supposed that the first interruption of gene flow between populations of the Sardinian progenitor was related to the Pliocene marine ingressions (from 5 to 3 My ago). Thus, the two species would have become differentiated, *S. nuragicus* to the east of the Piana del Campidano and *S. assorgiai* to the west.

From the preceding discussion, it can be inferred (and the genetically based dating confirm it) that *S. nuragicus* broadened its area of distribution in an east-west direction by exploiting the underground water systems of the Sardinian rift during the successive periods of its emersion, thus determining the present geographical contiguity between the distribution areas of the two species. During this process of westward dispersion, the populations that gradually formed probably underwent a process of genetic differentiation, as a result both of stochastic phenomena such as genetic drift, founder effect, etc. and of selective pressures due to the characteristics of the newly occupied environments. The possibility of active dispersal in conditions of a connection between underground strata across lithologically favoura-

ble zones, such as sandstone or limestone, has been widely documented by Magniez (1981, 1996) for *S. virei* in France. From this point of view, the Piana del Campidano, with a geological morphology of sediments, alluvial gravel and fallen debris, and dissected by a series of watercourses (Pracchi & Terrosu Asole 1971), can be considered an excellent dispersal path for the interstitial and hyporheic fauna. We have begun further research on the levels of genetic differentiation among a larger number of populations sampled along the Piana del Campidano whose results could lead to an even more clear comprehension of the Sardinian evolutionary history of this relict group of isopods.

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REFERENCES

Alvarez W 1972. Rotation of the Corsica-Sardinia microplate. *Nature* 235 : 103-105.

Argano R 1968. Due nuovi *Stenasellus* di Sardegna (Crustacea, Isopoda, Asellota). *Fragm Entomol* 6 : 1-22.

Argano R, Messana G, Matthaeis De E, Cobolli M, Ketmaier V 1998. Uso di marcatori biochimici per lo studio del popolamento stigobio di Sardegna : gli Asellota (Crustacea Isopoda). *Biogeographia* 19 : 157-171.

Ayala FJ 1983. Enzymes as taxonomic characters. *In* Protein polymorphism : adaptive and taxonomic significance, Edited by GS Oxford & D. Rollinson, Academic Press, London.

Baccetti B 1983. Biogeografia sarda venti anni dopo. *Lav Soc It Biogeogr* 8 : 859-872.

Boccaletti M, Ciaranfi N, Cosentino D, Deiana G, Gelati R, Lentini F, Massari F, Moratti G, Pescatore T, Ricci Lucchi F, Tortorici L 1990. Palinspastic restoration and paleogeographic reconstruction of the peri-Tyrrhenian area during the Neogene. *Palaeogeography, Palaeoclimatology, Palaeoecology* 77 : 41-50.

Cherchi A, Montadert L 1982. Oligo-Miocene rift of Sardinia and the early history of the western Mediterranean basin. *Nature* 298 : 736-739.

Hedgecock D, Tracey ML, Nelson K 1982. Genetics. *In* The biology of Crustacea, Edited by DE Bliss, 2, Academic Press, New York.

Magniez G 1974. Données faunistiques et écologiques sur les Stenasellidae (Crustacea Isopoda Asellota des eaux souterraines). *Int J Speleol* 6 : 1-80.

Magniez G 1981. Biogeographical and paleogeographical problems in Stenasellids (Crustacea, Isopoda, Asellota of underground waters). *Int J Speleol* 11 : 71-82.

Magniez G 1983. Biogéographie et paléobiogéographie des Sténasellides (Crustacea Isopoda Asellota des eaux souterraines continentales). *Mém Biospéol* 10 : 187-191.

Magniez G 1996. Histoire des Stenasellidae Ibéro-Aquitains (Crustacea, Isopoda, Asellota, Aselloidea). *Mém Biospéol* 23 : 173-179.

Manicastri C, Baldari F, Argano R 1983. Aspetti biogeografici del popolamento isopodologico della Sardegna. *Lav Soc It Biogeogr* 8 : 251-269.

Messana G, Cobolli M, De Matthaeis E, Ketmaier V, Argano R 1995. A preliminary essay on the biogeography of Italian Stenasellids (Crustacea, Isopoda, Asellota). *Mém Biospéol* 22 : 20-26.

Nei M 1975. Molecular Population Genetics and Evolution. Amsterdam, North-Holland.

Nei M 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89 : 583-590.

Pracchi R, Terrosu Asole A 1971. Atlante della Sardegna. La Zattera Editrice. Cagliari.

Sbordoni V 1982. Advances in speciation in cave animals. *In* Mechanisms of speciation. Edited by C Barigozzi Alan R Liss New York.

Sbordoni V, Baldari F, Caccone A 1991. Tempi relativi e assoluti dell'evoluzione. *Atti Convegni Lincei* : 197-244.

Sneath PHA, Sokal RR 1973. Numerical taxonomy. The principles and practice of numerical classification. WH Freeman & Co San Francisco.

Stewart BA 1993. The use of protein electrophoresis for determining species boundaries in amphipods. Proceed First European Crustacean Conf. *Crustaceana* 65 : 265-277.

Swofford DL, Selanderr B 1981. BIOSYS-1 : a computer program for the analysis of allelic variation in genetics. University of Illinois Urbana.

Thorpe JP 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. *In* Protein polymorphism : adaptive and taxonomic significance, Edited by GS Oxford and D Rollinson, Academic Press, London.

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