Institute of Zoology, University of Rome
and Institute of Zoology, University of L'Aquila

Genetic differentiation and variability in cave dwelling and brackish water populations of Mysidacea (Crustacea)

By E. De Matthaeis, R. Colognola, V. Sbordoni, M. Cobolli Sbordoni and G. L. Pesce

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Introduction

The Mysidae are an order of sea-originated crustaceans which currently includes species to be found in widely differing environments such as the open sea, brackish waters, the interstitial coastal belt and fresh surface and groundwater habitats.

Most groundwater or cave dwelling taxa have been placed in the Lepidomyidae and Stygiomyidae families (Gordon 1960). However, the disjunct, world-wide distribution of some genera, such as *Spelaemysis* and *Stygiomysis*, and their taxonomic assessment based chiefly on characters which might reflect convergent or parallel evolution (e. g. the absence of statocyst) raise the question of whether monophyly is well-established in these genera or whether different cave forms originate from different marine ancestors. Difficulties in identifying epigean relatives of the more specialized subterranean species also stem from the lack of good taxonomic characters not related to adaptation to cave habitats. Relationships between subterranean species and their epigean relatives have been thoroughly assessed only in a few taxa belonging to the Mysidae family, which still exhibit statocystis (Pesce 1976).

The present paper reports genetic divergence and variability data obtained by electrophoretic analysis of enzymatic proteins from Apulian (s. eastern Italy) populations of *Spelaemysis bottazzii* Caroli and *Diamysis baibrensis* (Sars).

*Spelaemysis bottazzii* is a hypogean endemic of Southern Apulia showing blindness, depigmentation and other features typical of most troglobites (i. e. obligatory cave dwelling animals). *Diamysis baibrensis* is an epigean species which has adapted to several aquatic habitats and is widely distributed along the coasts of the Mediterranean basin. Both species are markedly euryhaline as shown by the fact that the former may be found in waters whose salinity varies from 0.2% to 9% (Ruffo 1958) and the latter in fresh, brackish and salt waters (salinities: 10–13 and 40% respectively.

Our study aims:
1. to establish the degree of genetic differentiation among conspecific populations and the possible occurrence of gene flow among them;
2. to detect the genetic relationships between the two species in order to test their present taxonomic arrangement in different families, and
3. to assess their respective degrees of polymorphism.

Both divergence and variability sets of data provide meaningful information in estimating the antiquity of cave colonization by *S. bottazzii*. 
Materials and methods

*Stenacomysis botazzii* (fam. Lepidomysidae) occurs in several caves and wells near Bari, Lecce, Taranto and in the Apulian coast between Otranto and S. Maria di Leuca (Peschl et al. 1978; Peschl and Cicolani 1979). Our study populations came from the following sampling sites (Fig. 1):

1. MOL: an artificial well near Mola di Bari (Bari): depth, 6.5 m; average water level, 0.50 m; water temperature, 16.9–18.5°C; pH, 6.9; salinity, 1.5–1.7%; specimens sampled, 95.
2. ABI: Abisso cave near Castromarina (Lecce); a natural cave with an underground lake whose level varies seasonally from 0.50 to 5.50 m; temperature, 12.8–13.5°C; pH, 6.8; salinity, 2.5–2.9%; specimens sampled, 34.
3. BBA: an artificial well near Porto Badisco (Lecce); depth, 14.5 m; water level, 0.80 m; temperature, 14.5°C; pH, 6.9; salinity, 2.4–2.6%; specimens sampled, 14.
4. GAI: a natural well near Gallipoli (Taranto); depth, 3.5 m; water level, 0.30 m; temperature, 14.5–15.6°C; pH, 6.9; salinity, 1.7–1.8%; specimens sampled, 60.

All sampling sites were coastal, three of them on the Adriatic and the fourth, near Gallipoli, on the Ionian.

*Diamesis babirenisi* (fam. Mysidae). On the other hand, is a circummediterranean species with a relict distribution in the coastal brackish waters of France, Tunisia, Israel, Sicily, Apulia, Dalmatia; in fresh waters near Trieste and Padua, and, occasionally, in open sea waters (Tattersall 1927; Bacescu 1941; Holmquist 1955; Genovese 1956; Ariani 1966). The species has also been found in the Black Sea where the *mezzukowski* subspecies has been sited (Bacescu 1954). Our study samples, which belong to an unnamed Adriatic subspecies (Ariani 1979, 1981), were collected in the following localities along the Apulian coastal belt:

1. DCM: Morello River, near Torre Canne (Brindisi); a stream with mesoaline waters; average salinity, 10–12%; average annual temperature, 18°C; at, 11–12°C; pH, 7.4–7.5. Habitat conditions are most stable in spring. Specimens sampled, 196.
2. DCP: Piccolo River, a stream near Torre Canne (Brindisi); temperature, 18°C; salinity, 11%; pH, 7.1; specimens sampled, 31.
3. DQW: a brackish lagoon near Quartine (Lecce); salinity, 20–25%; specimens sampled, 18.

Starch gel electrophoresis was carried out to assay eight enzymes, namely: aldehyde oxidase (AO), alkaline phosphatase (APH), esterase (EST), glucose-6-phosphate dehydrogenase (G6PD), glutamate-oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM). For buffer systems and staining techniques, see Sordoni et al. 1979. 12 staining zones with independent variation, each presumably controlled by a single gene locus, were scored. When a given enzyme is encoded by more than one locus, the symbol of the enzyme is followed by a number to designate each locus: the locus coding for faster allozymes is designated 1 (e.g. Est-1), the next on is designated 2, and so on. At each locus the most common allele was assigned the number 100 while other alleles were numerically designated according to their mobility with respect to allele 100. Allelic designations and frequencies for the twelve loci are summarized in Table 1.

Genetic identity (I) and genetic distance (D) were calculated using Nei’s method (1972). The dendrogram in Fig. 2 was built up from the

**Fig. 1.** Collecting sites of *Stenacomysis botazzii* (black circles): 1 = MOL; 2 = ABI; 3 = BBA; 4 = GAI and *Diamesis babirenisi* (white circles): 1 = DCM; 2 = DCP; 3 = DQW study populations.
### Table 1

Allele frequencies at 12 gene loci coding for enzymes in 4 populations of *S. bottazzii* (from MOL to PBA) and in 3 of *D. bahirensis* (from DCM to DQU)

- **N** is the number of the genome sampled.
- **He** is the expected frequency of heterozygous individuals assuming Hardy-Weinberg equilibrium and **Ho** is the observed heterozygosity.

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Table 2

Estimates of genetic identity (above) and distance (below) between populations and species of Spelaeomysis and Diamysis

<table>
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<tr>
<th></th>
<th>MOL</th>
<th>GAL</th>
<th>ABI</th>
<th>PBA</th>
<th>DCM</th>
<th>DCP</th>
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<td>.622</td>
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</table>

Matrix of genetic distances (Table 2) using the unweighted pair-group method (UPGMA, Sneath and Sokal 1973). D may be considered as an approximate measure of the average number of amino acid substitutions per locus accumulated by two populations since they formed a single ancestral population. Evolutionary divergence times between populations and species have been computed according to the following formula (Nei 1975):

\[ t = \frac{D}{2\alpha} = \frac{D}{2cnr_{l_x}} \]
where \( t \) stands for the time lapsed since the two populations separated, \( D \) for genetic distance, \( c \) for the proportion of amino acid replacements electrophoretically detectable, \( n \) for the average number of amino acids per protein and \( \lambda \) for the rate of amino acid replacements per polypeptide per year. Two different values of \( \alpha \) were used, namely: \( \alpha = 10^{-7} \) (N"i 1975) and \( \alpha = 1.136 \times 10^{-7} \), calculated on the basis of available biochemical information for the tested proteins.

Allele frequency data were analysed by the RQ factorial method [designated also as 'correspondence analysis' (Obléct 1975)] to obtain deeper insight into genetic relationships among populations and species.

Results and discussion

Genetic differentiation between populations and species

Table 2 gives the coefficients of genetic identity, \( I \), and of genetic distance, \( D \), for all pairwise comparisons between populations of *Spelaemysis bottazzii* and *Diamaemysis bahirensis*.

Genetic identity and genetic distance values between populations of *S. bottazzii* (Table 2) vary within the limits usually observed in intraspecific comparisons for a great number of animal species (N"i 1975; Ayala 1975; Avise 1976). The genetic distances in Table 2 range from 0.011 (\( I = 0.989 \)) to 0.116 (\( I = 0.891 \)). As may be seen these values are rather low in comparison with other troglobitic species (*Astyanax mexicanus*, \( D = 0.17 \), Avise and Selander 1972; *Ptomaphagus birtus*, \( D = 0.21 \), Lang et al. 1976). The degree of genetic divergence between populations is very high in other troglobitic crustaceans (*Niphargus longicaudatus*, \( D = 0.3 \), Sbordoni et al. 1979). Genetic distances between *S. bottazzii* populations are close to values for troglobitic organisms such as the *Meta menardi* cave spider \( D = 0.026 \), Laing et al. 1976).

The degree of genetic similarity between these populations may be due to either 1. some gene flow among populations as a result of groundwater mixing, as suggested by the structural homogeneity of the Apulian limestone table land; and/or 2. a recent colonization of caves and phreatic waters by *S. bottazzii*. This view is in contrast with earlier speculations (Ruffo 1955).

As for Hypothesis 1, we tested the relationship between geographic distance and degree of genetic divergence (Fig. 3), but found no significant correlation between genetic and geographic distances. The highest values of \( D \) were found in pairwise comparisons between the Ionian population (GAL) and the Adriatic ones, whereas lower values of \( D \) were found within the Adriatic populations, in spite of their greater geographic dis-
placement (see also Fig. 4). These findings suggest that the populations along the two coasts of the Salentine peninsula are geographically isolated, possibly because of two groundwater basins are distinctly separated.

Evidence in favour of Hypothesis 2 is given by the comparatively low genetic variability in all tested populations of *S. bottazzii* (see following pages).

There is a substantially greater genetic distance between the *D. bahirensis* study populations of DQU and either of the two Torre Canne populations (DCM and DCP) than between the latter two populations alone, which are only 70 km from DQU. Average genetic distance between DQU and the two Torre Canne populations is = 0.126, a higher value than observed between populations of *S. bottazzii* (D = 0.063). This figure for genetic distances between *D. bahirensis* populations may partially be due to population isolation as a result of the relict distribution of *D. bahirensis* (Ariani 1981).

The mean genetic distance between *S. bottazzii* and *D. bahirensis* may be seen to be 0.7, a value frequently recorded between congeneric species (Nil 1975; Avise 1976) and yet *Spelaecomyys* and *Diamysus* are currently classified in different families.

An interpretation of allele frequency data resulting from RQ factorial analysis provides further information on the genetic relationships between populations of the two species (Figs. 4 and 5). Fig. 4 reports data for the *S. bottazzii* populations. The GAL population is
Fig. 5. A two-dimensional plot of *Spelaeomys bottazzii* and *Diamysis bahirensis* populations based on allele frequency RQ analysis. Points referring to populations (circles) and to alleles (squares) are plotted against the first two principal axes. Due to space requirements the coordinates of some points have been shortened to a third of their real value.

separated from the others by the second axis which explains 29% of the overall variance, indicating its relative degree of genetic differentiation and isolation in agreement with the previously discussed data on genetic distance. The PBA population is separated from GAL by the second axis and from MOL and ABI populations by the first one which explains 56% of the overall variance. Common alleles, which occur with similar frequencies in the various populations, and which contribute only slightly to inter-population differentiation, are plotted around axis origin. Other alleles are situated near a particular population, and contribute substantially to its genetic differentiation and characterize it. For instance, alleles Pgm100, Est-1100, Pgi100, Est-2100 and Ao100 do not differentiate *S. bottazzii* populations whereas Lap-2null, Lap-2100 and Est-3100 are peculiar to GAL and G6pd38, Ao37, G6pd38 are peculiar to PBA and so on.

Fig. 5 reports data on both *S. bottazzii* and *D. bahirensis*. The four *Spelaeomys* populations form a very homogeneous group, which is separated from the *Diamysis* populations by the second axis which explains 24% of the overall variance. The relative distances between the *Spelaeomys* populations shown in Fig. 5 are modified and diminished by the presence of *Diamysis*. *Diamysis DQU* population isolation from the other two (DCP and DCM) is stressed by its displacement with respect to the first axis, which explains 52% of the overall variance.
Variability estimates and inference as to the evolutionary history of Speleomysis bottazzii

Table 3 shows several estimates of genetic variability for the study populations. All values reflect a low degree of genetic variability in *S. bottazzii*. In fact, expected heterozygosity for the *S. bottazzii* populations was found to be 0.081 on the average and 0.159 for *D. bahirensis* (DCM) population, for which a comparable set of loci were assayed. *S. bottazzii* genetic variability ranks among the lowest values so far observed for aquatic cave dwelling species, such as *Astrayx mexicanus* (AVISE and SELANDER 1972) and other species, with relatively recent cave colonization and isolation.

According to BARR (1968), genetic variability in a cave population increases as it progresses from a troglobitic to a troglobitic stage of adaptation. BARR’s model predicts low variability in a newly isolated cave population, due to the founder effect. According to his hypothesis, variability should then increase as a result of extensive epigenotype reorganization which improves population adaptation to the cave environment.

POULSON and WHITE (1969), on the other hand, hypothesize low variability in cave populations as the result of strong stabilizing selection pressures in the cave environment. The low variability exhibited by *S. bottazzii* would seem to agree with both hypotheses. Nevertheless, high values of heterozygosity have been reported in other troglobitic species, mostly in aquatic Amphipodan and Isopodan crustaceans such as several populations of *Niphargus longicaudatus* (H = 0.298, SBORDONI et al. 1979), *Crangonyx antennatus* (H = 0.118, DICKSON et al. 1979) and several species of *Monolistra* (H = 0.314, SBORDONI et al. 1980). These values are often higher than those shown by similar epigean forms such as amphipods [*Echinogammarus tibaldii*: H = 0.07, *Echinogammarus stammeri*: H = 0.1, *Gammarus aequicauda*: H = 0.1 (SBORDONI 1980; SBORDONI et al., unpubl.)] and isopods [*Sphaeroma hookeri*: H = 0.21 and *Sphaeroma serratum*: H = 0.28 (SBORDONI et al. 1980)]. These findings contradict POULSON and WHITE's hypothesis. The high heterozygosity values found in *Niphargus* and in *Monolistra* are more probably the result of an ancient colonization by these hypogean populations (SBORDONI 1980), and support BARR's model.

Thus, the low genetic variability and the high genetic similarity between populations of *S. bottazzii* suggest they are young cavernicolous that colonized caves after a relatively recent isolation from a sea ancestor.

Assuming a monophyletic origin of *Speleomysis* and *Diamyris* (Fig. 2), and starting from genetic distance values, we estimated divergence times between the two taxa, according to NIE’s method (1975) and found the values for the two tested species to range from 3,100,000 to 3,550,000 years, according to the two different values of α (see ‘Materials and methods’).

<table>
<thead>
<tr>
<th>Species and population</th>
<th>H̄e</th>
<th>H̄o</th>
<th>A</th>
<th>P</th>
<th>No. of loci scored</th>
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<tbody>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>DCM</td>
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<td>.131</td>
<td>1.75</td>
<td>.67</td>
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</table>

H̄e, average heterozygosity (expected under Hardy-Weinberg equilibrium). H̄o, average observed heterozygosity. A, mean number of alleles per locus. P, frequency of polymorphic loci (those with a frequency of the most common allele < .99).
Divergence times between single allospecific populations range from 2,290,000 (or 2,016,000) to 4,400,000 (or 3,873,000) years.

On the basis of our rough estimates and assuming neutral evolution of protein polymorphism, gene flow interruption between *Spelaeomysis* and a possible sea-ancestor — similar say to *Diarmysis* — may be dated to the late Pliocene. These estimates fit present palaeogeographic evidence well (GRIDELLI 1950; PASHA 1953; TERMIER and TERMIER 1952). According to these authors, during most of the Pliocene Apulia was either completely submerged or limited to a few small isles that do not in any way coincide with the present distribution of *Spelaeomysis* (Fig. 6), thereby suggesting that cave colonization by the *Spelaeomysis* ancestor did not occur until the late Pliocene.

![Fig. 6. A palaeogeographic map of Apulia, according to LEONARDI (1940) and to GRIDELLI (1950), and the locations of all known populations of *Spelaeomysis bottazzii*](image)

Moreover, the idea that *S. bottazzii* may only recently have adapted to the cave environment agrees with findings from other subterranean crustaceans belonging to such groups as Thermosbenaceae, Isopoda (Microparasellidae, Cirolanidae), and Amphipoda. These organisms are thought to have colonized underground freshwater systems as the result of "stranding" and subsequent "uplifting" of their marine coastal ancestors during the Mio-Pliocene regressions of the Mediterranean Sea. Such a colonization model, referred to as a "Regression Model" by STROCK (1977), would explain the present distribution of several hypogean crustaceans ("thalassoid" groups according to DANIELopol 1978) that are generally limited to subterranean environments not far from coasts and characterized by variable salinity, temperature and dissolved oxygen conditions.

Unfortunately, data on the genetic distances between *S. bottazzii* and other species currently classified in the *Spelaeomysis* genus are not available to establish whether the genus is polyphyletic. On the grounds of our findings, these genetic distances between *S. bottazzii* and other *Spelaeomysis* species could ever be higher than those between *S. bottazzii* and *Diarmysis*. One possible hypothesis to test is that various *Spelaeomysis* species, living in widely different continental areas, might have evolved as the result of local adaptations to the cave environment and that they have independent evolutionary histories similar to *Spelaeomysis bottazzii*. 
Genetic divergence in cave dwelling and brackish water populations of Myisidacea

Acknowledgments

We are grateful to Dr. Antonio Ariani for providing *Diamysis* samples and many useful informations on *Diamysis* biology and ecology. Thanks are also due to Dr. Eugenio Prest for his substantial help in the multivariate analysis of allele frequency data. This study was supported by grants to V. Swordson from the National Research Council, Italy, (CNR), and from the Faculty of Sciences, University of Rome.

Summary

Genetic divergence and variability at 12 gene-enzyme loci were studied in 4 populations of *S. botazzi* and 3 populations of *D. babirenisi* (Crustacea, Mysidacea). Material examined included populations collected in caves, wells, mesosaline and brackish waters of Apulia (south eastern Italy).

Genetic distance (D) values between *S. botazzi* populations range from 0.011 to 0.116, showing greater genetic similarity than other cave organisms. D values between *D. babirenisi* populations range from 0.005 to 0.155 thereby revealing some genetic differentiation between these epigean populations. The average genetic distance between the two species is 0.7.

RQ factorial analysis applied to the gene frequency data enables genetic relationships among populations and species to be assessed and also contributes to determining which loci and alleles are involved in the differentiation of conspecific and allospacific populations.

Expected average heterozygosity (H) is 0.081 in *S. botazzi* populations and 0.159 in a *D. babirenisi* population, for which a comparable set of loci were assayed.

Both genetic divergence and variability estimates suggest recent cave colonization and adaptation by *S. botazzi* populations. The time of this event has been roughly dated back 3,500,000 years. This supports Stock’s hypothesis that Mysidacea and other “thalassoid” hypogean crustacean colonized subterranean waters in connection with Pliocene regressions of the Mediterranean sea.

Zusammenfassung

Genetische Differenzierung und Variabilität von höhlenbewohnenden und Brackwasser-Populationen der Mysidacea (Crustacea)

Genetische Unterschiede und Variabilität in 12 Gen-enzyme-Loci wurden bei 4 Populationen von *S. botazzi* und 3 Populationen von *D. babirenisi* (Crustacea, Mysidacea) untersucht. Das Untersuchungsmaterial umfasste Populationen aus Höhlen, Brunnen, Mesosalinen und brackigen Gewässern Apullens (Südtalien).

Die Werte für die genetischen Abstände (D) zwischen *S. botazzi*-Populationen schwankten zwischen 0,011 und 0,116 und zeigten damit eine größere genetische Ähnlichkeit als andere Höhlenbewohner. Die D-Werte zwischen *D. babirenisi*-Populationen lagen zwischen 0,005 und 0,115, offenbarten also eine gewisse genetische Differenzierung zwischen diesen epigeanen Populationen. Der durchschnittliche genetische Abstand zwischen beiden Arten beträgt 0,7.

Die faktorielle RQ-Analyse der Genfrequenz-Daten ermöglicht die Abschätzung der genetischen Beziehungen zwischen Populationen und Arten und trägt zur Bestimmung der Loci und Allele bei, welche die Differenzierung konspizifischer und allopästischer Populationen bewirken.

Die geschätzte durchschnittliche Heterozygotie (H) beträgt bei *S. botazzi*-Populationen 0,081, bei *D. babirenisi*-Populationen 0,159, was mit einem entsprechenden Satz an Loci in Zusammenhang gebracht wird.


References


STOCK, J., 1967: The taxonomy and zoogeography of the Hadziid Amphipoda with emphasis on the west indian taxa. Studies on the fauna of Curaçao and the other Caribbean Islands, 177.


Authors' addresses: E. De MATTHAES, V. SBORDONI and M. COBOLLI SBORDONI, Institute of Zoology, University of Rome, Viale dell'Universita, 32, I-00185 Rome; R. COLOGNOLA, Laboratory of "Ecologia del Benthos", Punta S. Pietro, I-80070 Ischia Portu (Napoli); G. L. PESCE, Institute of Zoology, University of L'Aquila, Piazza Regina Margherita, 7, I-67100 L'Aquila.