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The Middle Pleistocene fossiliferous sequence of Grotta dei Fiori (Sardinia, Italy): multidisciplinary analysis

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KEY WORDS - Arvicolid, Biochronology, Micromorphology, Stable Isotope, Pleistocene, Sardinia.

*ABSTRACT - A multidisciplinary methodological approach (palaeontological, sedimentological and stable isotope analyses) was performed to analyse the fossiliferous sequence exposed in the "Grotta dei Fiori" cave (South Western Sardinia, Italy), where several small mammal remains (*Microtus (Tyrrhenicola) henseli, Rhagamys orthodon, "Nesiotites" similis *and* Prolagus sardus*) were discovered together with scanty remains of the endemic canid* Cynotherium*, and birds. Morphology and morphometry of first lower molars (M_1) of* Microtus (Tyrrhenicola) henseli *from Grotta dei Fiori show that primitive and advanced morphotypes are present in each fossiliferous level. The relative frequency of morphotypes indicates that the population from "Grotta dei Fiori" might be regarded as more advanced than* Microtus (Tyrrhenicola) sondaari *from Xg3 fissure of Monte Tuttavista (Eastern Sardinia, late Early Pleistocene) and more primitive than the most of ?Middle and Late Pleistocene populations of* Microtus (Tyrrhenicola) henseli *from Sardinia. All in all, the data thus far provided by sedimentology and geochemistry indicate that the fossiliferous sequence of "Grotta dei Fiori" was deposited under climatic conditions characterized by an alternation of wet and dry periods. Taking into account the evolutionary degree of voles, $^{13}C/^{12}C$ and $^{16}O/^{18}O$ ratio and micromorphology of sediments, the hypothesis that the "Grotta dei Fiori" succession would be not older than the marine isotope stage (MIS) 11 cannot be ruled out. The results obtained stress once more the difficulty of a precise chronologic setting for deposits filling caves when no absolute date is available, and highlight the usefulness of a multidisciplinary approach to define environmental context and, perhaps, constrain chronology.*

RIASSUNTO - [La sequenza fossilifera di Grotta dei Fiori del Pleistocene Medio (Sardegna, Italia): analisi multidisciplinari] *- I risultati di uno studio multidisciplinare (paleontologia, geochimica isotopica e micromorfologia) vengono utilizzati nel tentativo di precisare l'intervallo cronologico in cui si sono accumulati i depositi fossiliferi di una successione sedimentaria presente nella grotta carsica di Grotta dei Fiori (Sardegna sud-occidentale, Italia). La Grotta dei Fiori si sviluppa nei calcari paleozoici del Gruppo di Gonnesa: l'evoluzione speleogenetica è caratterizzata da vari cicli di sedimentazione, erosione e fasi di collasso. I sedimenti plio-pleistocenici sono piuttosto abbondanti. In tutti i livelli fossiliferi della Sezione C sono presenti numerosi resti di micromammiferi, mentre scarsi resti del canide endemico* Cynotherium sardous *ed una avifauna diversificata provengono da uno dei livelli terminali della sequenza. Lo studio dei primi molari inferiori (M_1) dell'arvicolide endemico* Microtus (Tyrrhenicola) henseli *ha evidenziato come nell'insieme la popolazione di Grotta dei Fiori, per la frequenza relativa dei morfotipi (prevalenza del morfotipo 2) e per i valori dell'indice di differenziazione dello smalto (SDQ), sia meno avanzata rispetto a popolazioni sarde provenienti da altre località ascritte al Pleistocene Medio/Superiore, e risulta più evoluta di quella a* Microtus (Tyrrhenicola) sondaari *ritrovata nella fessura carsica Xg3 di Monte Tuttavista (Sardegna orientale, Italia). I risultati relativi all'analisi della micromorfologia dei depositi ed i valori isotopici ($\delta^{18}O$, $\delta^{13}C$) ricavati dall'analisi del carbonato della bioapatite dei denti e delle ossa lunghe dei micro- e dei macromammiferi, forniscono indicazioni di condizioni ambientali subtropicali con alternanza di periodi umidi e periodi più aridi. L'insieme dei dati paleontologici, micromorfologici e isotopici indicherebbe per la successione di Grotta dei Fiori un'età tardo medio pleistocenica (?MIS11). I risultati ottenuti sottolineano la difficoltà di un preciso inserimento cronologico dei depositi di riempimento di grotte carsiche, specie in mancanza di datazioni assolute.*

INTRODUCTION

The reconstruction of the environmental evolution and of the action of biotic and non-biotic factors on the structure of land mammalian palaeocommunities is a challenging study, which requires the cultural and methodological support of disciplines of apparently remote specialized sectors, such as geochemistry, palaeontology and sedimentology. A micromorphological analysis of sediments provides details of palaeo-environmental and climatic characteristic. Isotopic analyses (O, C) allows us to obtain detailed palaeo-ecological and palaeoenvironmental results (cf. Palmqvist et al., 2003). The application of these studies to small mammal fossil remains from short and continuous clastic continental sequences is of particular interest because small mammals are important chronological and ecological markers because of their rapid evolution, their wide distribution and the great number of specimens sometimes recovered. On the other hand, the study of the *Microtus (Tyrrhenicola)* endemic lineage is of particular interest because its first lower molar (M_1) shows morphological and morphometrical trends which could be a useful support in defining the chronologic setting of the Middle and Late Pleistocene fossiliferous deposits of Sardinia (e.g. Mezzabotta et al., 1996; Marcolini et al., 2006).

The present paper is aimed at finding chronological constrains for the fossiliferous deposits exposed in the karstic cave of Grotta dei Fiori (South-western Sardinia,

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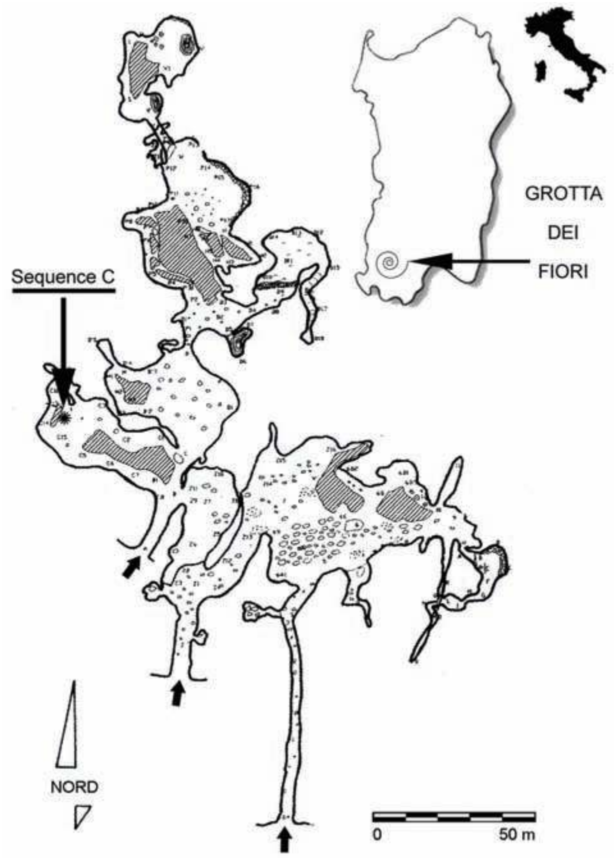

Fig. 1 - Localization and plan of Grotta dei Fiori, from: Elenco Catastale delle grotte del Comune di Carbonia, Gruppo Ricerche Speleologiche "E. A. Martel" Carbonia (1977).

Italy) by means of different disciplinary approaches (micromorphological, paleontological and stable isotope analysis).

"Grotta dei Fiori" is a karstic cave located in Southwestern Sardinia, on the hydrographic right of Rio Cannas, near Carbonia (Cagliari, Italy) (Fig. 1). This cave developed in a Cambrian limestone ("Gruppo di Gonnesa") slightly metamorphosised by Ercinic tectonic. The hypogean karstification started in the Lower Cambrian, and continued at different times until the Quaternary (Melis et al., 2002). Morphological features of galleries suggest they had a phreatical origin. Various cycles of sedimentation, erosion and collapsing phases characterised the complex speleogenetical evolution of this cave. The Plio-Pleistocene deposits, mostly fine and coarse sediments, are the most abundant. Only the fossiliferous deposits on the first cavity of "Grotta dei Fiori" have been sampled and analysed so far (Fig. 1).

MATERIALS

The studied sedimentary sequence (sequence C in Melis et al., 2002) includes several fossiliferous levels from which a rich sample of small mammals, *Talpa tyrrhenica*, *"Nesiotites" similis, Microtus (Tyrrhenicola) henseli, Rhagamys orthodon* and *Prolagus sardus* were retrieved (Fig. 2). Scanty remains of the endemic canid *Cynotherium sardous* and some birds (*Ciconia nigra*, *Gypaetus barbatus*, *Aquila* sp., *Columba livia*, *Pyrrhocorax pyrrhocorax*, *Pyrrhocorax graculus*, *Corvus corone*, *Corvus corax*, *Emberiza cirlus/schoeniclus*) were found in the uppermost fossiliferous level of the sequence (Melis et al., 2002; Pavia & Bedetti, 2002).

Micromorphological analysis of the sediments were made on undisturbed samples taken from C1, C2, C3, C6, C7, C10, C13, C14 and C15 Levels of the sequence C.

Isotope Analysis of stable isotopes (O, C) were performed on teeth and long bones of *Microtus (Tyrrhenicola), Ragamys orthodon* and *Prolagus sardus* from C1, C3, C5, C11, C13 and C14 Levels. Small mammals from Dragonara cave (Northwestern Sardinia, late Pleistocene, Malatesta, 1970; Caloi & Malatesta, 1974; Palombo, 2006) and Cava Alabastro quarry (Iglesiente, Southwestern Sardinia, late Middle Pleistocene, Gliozzi et al., 1986; Minieri et al., 1995) were also analysed for comparison.

38 first lower molars (M_1) of *Microtus (Tyrrhenicola)* from C2, C5, C10, C11, C12, C13 and C14 Levels have been studied. Comparison was made with sample of *M. (Tyrrhenicola) sondaari* and *M. (Tyrrhenicola) henseli* from various Sardinian localities ranging in age from the late Early Pleistocene to the Last Glacial (data from Mezzabotta et al., 1995; Minieri et al., 1995; Marcolini et al., 2005, 2006; Palombo, 2009).

METHODS

Sedimentological and Micromorphological analysis

Samples for chemical and granulometric analyses were dried at room temperature and sieved (<2 mm). Analysis of granulometry was done using the Pipette method (Violante, 2000). Calcium carbonate was determined with a Dietric-Fruhling Calcimeter following the procedure of Violante (2000). Mineralogical on bulk powder (randomly oriented) and clay fractions (parallel oriented specimens separated by pipette) was carried out using a Spectro X-Lab XRF machine (Philips PW 1710) with Cuka radiation. Qualitative clay-mineral identification of <2mm fraction was obtained by search match ADP software (Laviano, 1987). Undisturbed samples were collected for micromorphological study. Thin sections were analysed using a polarising microscope in plane and crossed polarised light and micromorphological features were described according to Bullock et al. (1985).

Carbon and Oxygen isotope analysis

In order to measure the carbon isotope composition of hydroxyapatite carbonate, about 100 mg of powdered sample were treated with 2% NaOCl solution for 1 day. The sample was then repeatedly rinsed with distilled water and leached with 1 M acetic-acid buffer solution for 20 h to remove diagenetic calcium carbonate (Lee-Thorpe et al., 1989; Bocherens et al., 1991). After washing and drying, the sample was reacted overnight at 50°C in vacuo with 100% H_3PO_4 to release carbon dioxide. The CO_2 was purified and analysed for its carbon and oxygen

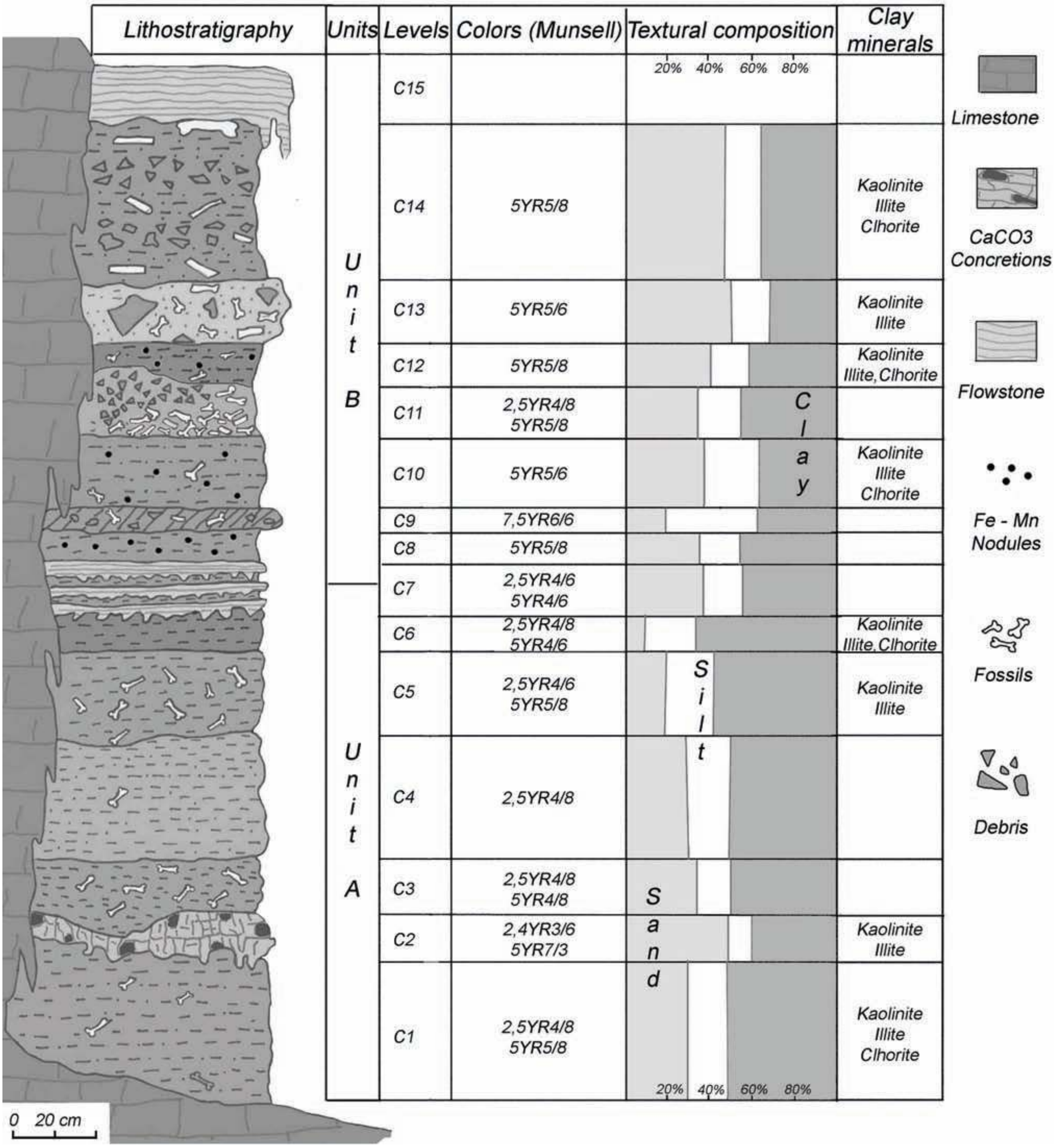

Fig. 2 - Litostratigraphy of Sequence C from Grotta dei Fiori (modified from Melis et al., 2002).

isotope composition by means of a mass spectrometer. Isotopic ratios are reported relative to the isotopic standard PDB-1 for C and V-SMOW for O using the conventional notation = (Rsample / Rstandard - 1) x 1000.

Morphological analysis of Microtus (Tyrrhenicola)

Morphological and morphometrical analyses of M_1 were performed focusing on characters regarded as the most useful for discriminating among primitive and advanced morphotypes: relative length of the anteroconid complex (ACC), morphology of the anterior cap (AC), degree of pinching of the neck, development of sixth triangle (T6), occurrence of seventh and ninth triangles, T7 and T9.

Measurements of first lower molars (M_1) of *Microtus (Tyrrhenicola)* (32 linear measurements and 8 indexes) were taken following Van der Meulen (1973), Marcolini et al. (2006) methods and Boldrini's (2005) further modifications (Fig. 3a). Principal Component Analysis (PCA) was carried out first using all the measurements, then using as input selected variables (13 linear measurements).

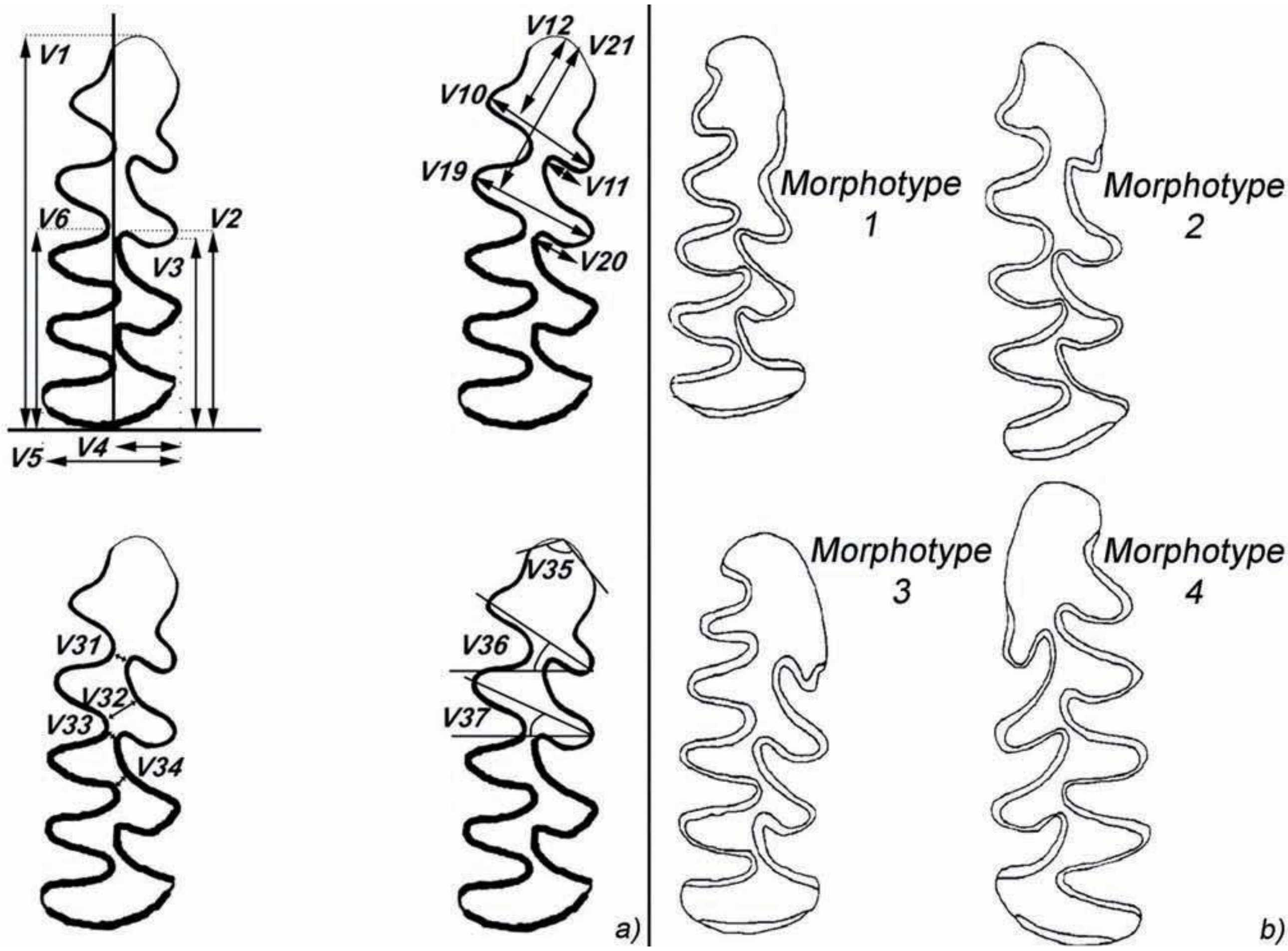

Fig. 3 - a) Sketch of main measurements of M_1 *Microtus (Tyrrhenicola)*; V1: Total length; V2: Length of posterior part of BRA2; V3: Length of posterior part of BSA2; V4: Width of mesial part; V5: Total width; V6: Elongation of TTC; V10: Distance T6-T7; V11: Width of mesial part T6-T7; V12: Maximum length between T6T7 and AC; V19: Outer distance T4T5; V20: Outer width of mesial part T4-T5; V21: Maximun length between T4T5 Ex and AC; V31: Anterior cap; V32: Minimum distance T4-T5; V33: Minimum distance T3-T4; V34: Minimum distance T2-T3; V35: Angle AC; V36: Angle T6T7; V37: Angle T4T5. b) Main classes of morphotypes of M_1 *Microtus (Tyrrenicola) henseli* (after Mezzabotta et al., 1996).

M_1 of *Microtus (Tyrrhenicola)* show a wide morphologic variability which makes the identification of specific morphotypes very difficult. For instance, Minieri et al. (1995) described eleven morphotypes, while Mezzabotta et al. (1995) grouped in four morphotypes the twenty different morphologies by these authors identified analysing some Middle and Late Pleistocene endemic voles from Sardinia and Corsica. According to Mezzabotta et al. (1995), the morphotype 1 is the most primitive and includes small M_1, with a simple AC, T6 absent or not well developed, and large neck. In contrast, morphotype 4 includes the most derived M_1, characterised by large size, long asymmetric AC, T6 fairly developed and nearly parallel to the axis of the tooth, T7 always present, and neck narrow. Morphotypes 2 and 3 include M_1 with intermediate features.

M_1 from Grotta dei Fiori have been attributed to the main morphotypes recognized by Mezzabotta et al. (1996) (Fig. 3b), and compared the frequency percentages have been calculated for each level. The SDQ (Schmelzband-Differenzierungs-Quozient) analysis was performed on images of M_1 made by SEM in low vacuum mode. The thickness of the enamel was measured on M_1 triangles according to the Heinrich method (1978) (Fig. 4), the ratio of posterior versus anterior band of enamel in each triangle was calculated. The SDQ index corresponds to the means of all measured ratios.

RESULTS

Lithostratigraphy and Micromorphology

The sequence C, exposed on the cave wall of western chamber (Fig. 1), is formed by sub-horizontal levels and ends with a flowstone about 25 cm thick. This sequence, about 5 m thick, is divided into two units: lower unit (*Unit A*), characterised by fine sediments and upper unit (*Unit B*) defined by predominantly coarse deposits. Lithostratigraphical data of the section C are synthesised in Fig. 2.

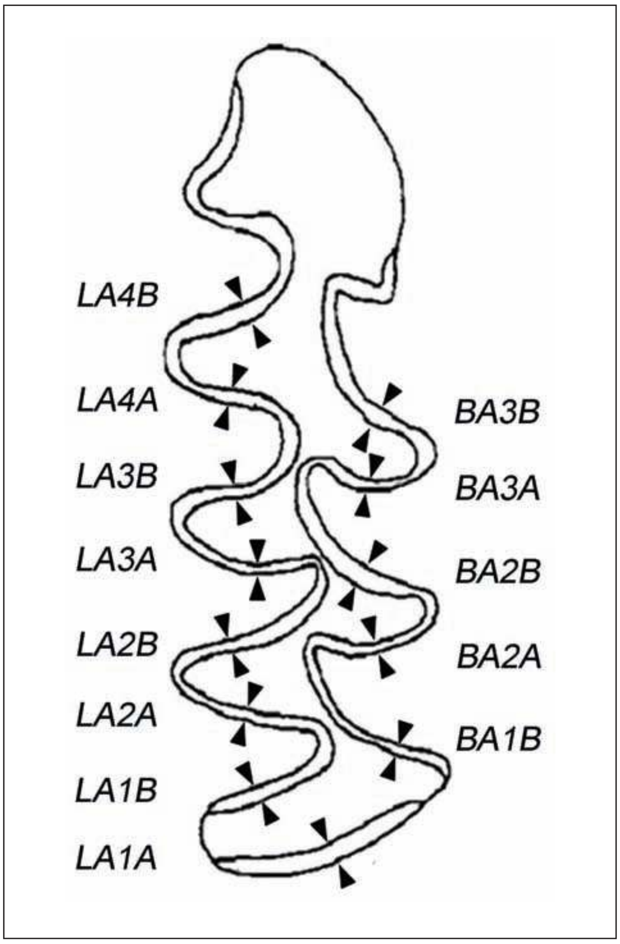

Fig. 4 - Points of measurement of the thickness of the enamel on the M_1 of *Microtus (Tyrrhenicola)*.

Unit A - Unit A includes 6 clay levels, from red (2,5YR 4/8) to yellowish red (5YR 5/8) colors, except C2 Level where the red clay sediments appear to be incorporated within the "*network*" white calcitic concretion. This level resulted from cracks, caused by desiccation of clay sediment, subsequently filled by calcite.

Granulometric analysis shows clayey texture in all levels (Fig. 2). Kaolinite and illite are dominant in C1, C2, C5 and C6 Levels, while scarce chlorite is present in C1 and C6 Levels. In thin sections, the matrix of all levels consists of reddish-brown clay with some fine sand-sized quartz grains. Metamorphic lithorelicts, round black iron-manganese nodules and rounded pedorelicts, were also observed. These pedorelicts, characterized by yellow-red clay coatings, derived from outside red soils, which developed on limestone bedrock under subtropical climate with wet and arid periods. Bone remains are observed in all layers except for C6 Level.

The transition from the Unit A) to the Unit B) is marked by C7 Level, characterised by three thin flowstones alternated by levels of red clay sediments about 10 cm thick. Micromorphological analysis reveals that calcification is pervasive, filling extension cracks with coarse sparite and microsparite coronas with bone fragments and grains of sediments. Rounded Mn nodules and pedorelicts are abundant.

By and large, unit A consists of fine gradient, red clay sediments in close association with bone fragments and pedorelitcts. These sediments came from erosion of soils developed under subtropical climate.

Unit B - The upper unit is characterised by fine sediments levels interbedded with coarse clastic deposits dominated by angular fragments enclosed in a red (2,5YR 4/8) to yellowish red (5YR5/8) fine grained matrix. The matrix consists of quartz-rich sand and lithorelicts of limestone, schist and quartzite. The coarse clastic deposits were poorly sorted and contain bone fragments.

Textural analysis show dominance of sandy clay texture (Fig. 2). Clay fraction is characterised by kaolinite and illite. Chlorite is poorly present only in C12 and C14 levels.

Thin sections show that the clay-rich areas consist of reddish-brown clay with small amounts of silt and some fine sand sized quartz grains. Black mottles are frequent in C13 and C14 levels. Their diffusive boundaries suggest in situ formation in local wet conditions. Conversely, pedorelicts within the matrix and the concentration of detrital, rounded and concentric iron-manganese nodules present in fine sediments, derives from the erosion of soils present outside the cave.

In Unit B, poorly to well-developed internal layering, relatively lenticular, and angular to subangular shape of clasts, indicates that sediments were washed into the cave in a chaotical fashion, possibly during arid phases. The

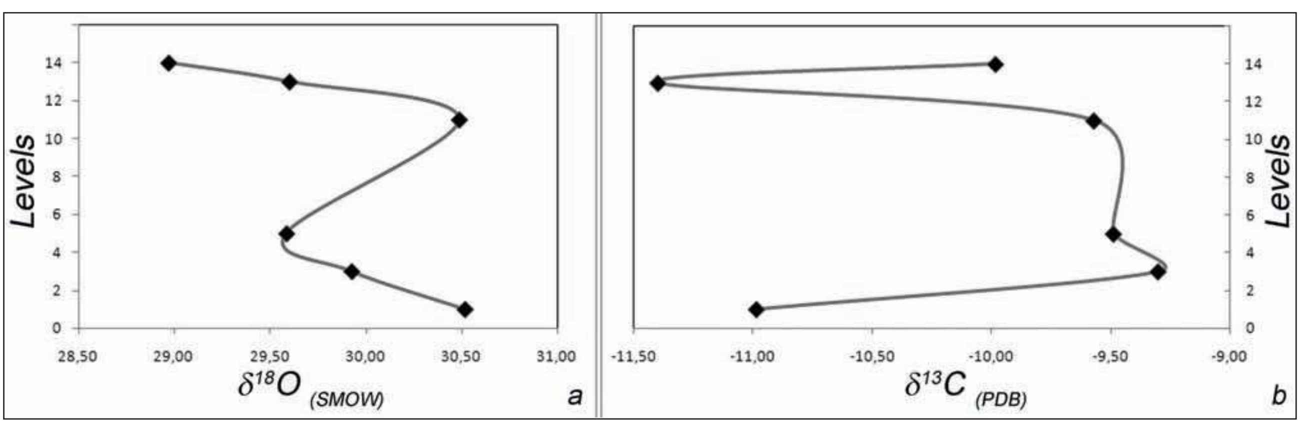

Fig. 5 - $\delta^{18}O$ (a) and $\delta^{13}C$ (b) of samples from selected levels of Grotta dei Fiori.

angularity of the clasts suggests short-distance transport. Large amount of pedorelicts in the fine matrix with coarse sediments suggests that there deposits were produced when soils from outside the cave were suddenly washed down the slope and deposited into the cave. The presence of strial fabric and disrupted clay coatings in the pedorelicts indicates soils that were repeatedly exposed to shrink-swell conditions (McCarthy and Plint, 1998; McCarthy et al., 1999b), suggesting prolonged stability of soil profile.

All in all, the presence of pedorelicts and metamorphic lithorelicts in the fine fraction of several levels of the analysed section, indicates that the sediments result from allogenic deposits of eroded soils, which had developed outside the cave under subtropical conditions, characterised by alternation of wet and dry periods.

Carbon and Oxygen isotope analysis

The $\delta^{18}O$ (Mean =27.94) and $\delta^{13}C$ (Mean= -14.63) values obtained for *Cynotherium sardous*, coming from the C14 level, indicates that the average temperature at the time during which this level deposited where not far from the present days, but the humidity was higher. Conversely, the $\delta^{18}O$ and $\delta^{13}C$ values obtained for the micromammals (Tab. 1, Fig. 5) would indicate that the average temperature at time of deposition of "Grotta dei Fiori" fossiliferous levels, calculated following the methodology as in Iacumin et al. (1996) and Longinelli et al. (2003), was sensibly higher than the present days. This could depends by several reasons, first of all by the size of micromammal skeletal remains is quite small and caves are generally wet so that diagenetical processes may have affected the isotopical composition of skeletal carbonate. Accordingly, the isotope data have been utilised only in order to compare the climatic signal given by the Grotta dei Fiori remains each other as well with those given by Sardinian small mammals for which a similar taphonomic history has confidently been inferred.The comparison of the Grotta dei Fiori values suggests that

	$\delta^{13}C_{(PDB)}$	$\delta^{18}O_{(V-SMOW)}$
GDFC1PRA	-11,55	30,21
GDFC1PRB	-11,64	30,12
GDFC1RG	-10,50	30,50
GDFC1TY	-10,26	31,24
GDFCC3PR	-9,30	29,93
GDFCC5BPR	-9,20	31,18
GDFC5PR	-8,59	28,48
GDFC5RG	-9,95	29,58
GDFC5TY	-9,92	30,70
GDFCC5TR	-8,03	30,29
GDFC5SOFTR	-9,65	29,66
GDFCC10TR	-9,05	28,64
GDFCC10PT	-10,36	29,99
GDFC11PR	-8,83	29,75
GDFC11RG	-9,63	31,36
GDFC11TY	-10,25	30,35
GDFCC13TY	-12,00	30,46
GDFCC13DTY	-10,76	28,78
GDFC14PR	-9,06	28,70
GDFC14RG	-12,37	29,35
GDFC14TY	-8,51	28,86
GDFCC14INFPR	-11,31	30,54

Tab. 1 - Isotope values of samples from Grotta dei Fiori.

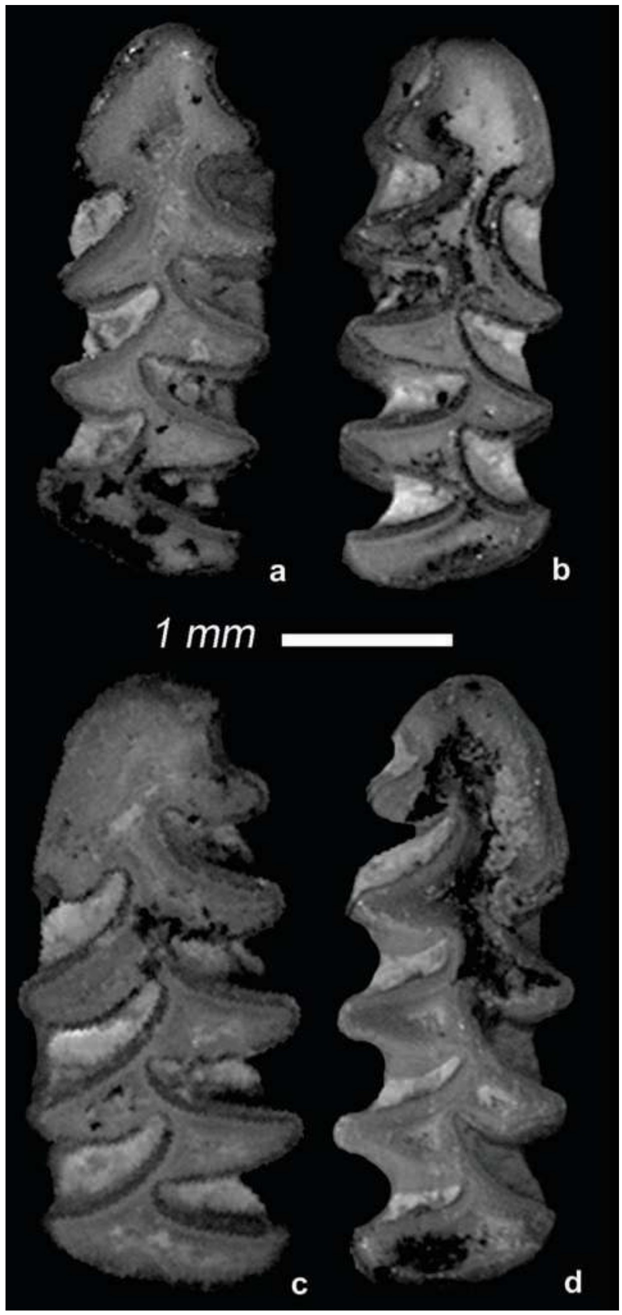

Fig. 6 - Morphotypes 1-4 of *Microtus (Tyrrhenicola)* first lower molars (M_1) from "Grotta dei Fiori" Sequence C: a) C13-26 specimen; b) C14-22 specimen; c) C13-07 specimen; d) C13-18 specimen.

		V1	V5	V10	V31	ACC	A/L	W/L	B/W
Level C14 (n=13)	min	2,58	0,48	1,07	0,08	1,20	39,87	0,38	0,11
	max	3,54	0,79	1,39	0,03	1,81	48,02	0,45	0,20
	mean	3,05	0,59	1,26	0,21	1,36	44,34	0,42	0,16
Level C13 (n=13)	min	2,64	0,45	1,06	0,13	1,16	42,42	0,39	0,13
	max	3,47	0,62	1,42	0,34	1,60	47,35	0,47	0,25
	mean	3,06	0,78	1,28	0,23	1,37	44,77	0,42	0,19
Level C11 (n=8)	min	2,47	0,41	0,86	0,15	1,23	46,67	0,33	0,12
	max	3,23	0,63	1,43	0,27	1,61	51,44	0,44	0,22
	mean	2,99	0,88	1,19	0,21	1,47	49,14	0,40	0,18
Level C5 (n=2)	min	3,09	0,59	1,22	0,17	1,48	46,48	0,39	0,12
	max	3,27	0,81	1,35	0,24	1,52	47,90	0,40	0,19
	mean	3,18	1,02	1,29	0,21	1,50	47,19	0,41	0,16
Level C2 (n=2)	min	2,96	0,93	1,26	0,18	1,60	51,37	0,43	0,12
	max	3,29	1,14	1,45	0,25	1,69	54,05	0,43	0,15
	mean	3,13	1,04	1,36	0,22	1,65	52,71	0,44	0,13

Tab. 2 - Descriptive statistics of variables and morphological indices of M_1 from Grotta dei Fiori. V1: total length; V5: width; V10: distance T4-T5; V31: width of the neck; ACC: length of ACC; A/L: length of ACC against total length; W/L: width against total length.

during the time interval of the sedimentation from level C1 to C14 temperature parametri fluctuated even decreasing on average. In particular, at the time of the C1 and C13 Levels deposition the most humid conditions occurred. Moreover, C1 and C11 Levels indicate warmer conditions than Levels C5, C13 and C14 (Fig. 5a). Eventually, the average values of $\delta^{18}O$ and $\delta^{13}C$ of Grotta dei Fiori are inferior than those obtained for Cava Alabastro ($\delta^{18}O$ = 32.96; $\delta^{13}C$ = -7.94) and Dragonara ($\delta^{18}O$ = 32.55; $\delta^{13}C$ = -9.34).

Morphology, morphometry and enamel index (SDQ) of Microtus (Tyrrhenicola)

The morphology of *Microtus (Tyrrhenicola)* M_1 from "Grotta dei Fiori" stresses again the great variability of this Sardinian endemic vole. ACC shows the highest morphological variability ranging from primitive types where T6 is absent or just barely outlined, to the most derived types with well outlined T7 and T9. Conversely, all specimens share T1 and T2 weakly confluent, and buccal reentrant angles (BRA) more elongated than the lingual re-entrant ones (LRA). Smallest M_1 can be ascribed to morphotype 1 of Mezzabotta et al. (1995). Their length falls in the range of *Microtus (Tyrrhenicola) sondaari,* a new primitive species described by Marcolini et al. (2006), close in morphology with morphotypes 1, on the basis of the reach sample from Xg3 fissure fillings of Monte Tuttavista (Marcolini et al., 2006). They also have simple ACC, short AC, and narrow neck, T6 is weakly outlined as in a few of paratypes of *M. sondaari* (Marcolini et al., 2006, fig. 10), but look averagely more advanced. Scanty specimens of this morphotypes are recovered from Levels C11, 13 and 14 (Fig. 6). Larger specimens ascribed to morphotypes 2, 3 are quite common in every fossiliferous level. By comparison to the morphotype 1, these latter share a complex ACC, a narrower neck, more or less outlined T6 and T7, whereas a poorly outlined T9 may be occasionally present. The most advanced M_1 ascribed to morphotypes 4 are present in the Levels C5, C11, C13 and C14. These specimens are characterised by long and complicated ACC, large and asymmetrical AC, very narrow neck, and outlined T9.

The relative frequency of morphotypes in C2 and C5 levels has not been calculated due to the scantiness of remains thus far retrieved (Tab. 3). It is worth noting that in the richest samples (Levels C11, 13, 14) the morphotypes 2 is always the most abundant. Thus, it appears that no evolutionary trend is detectable in the fossil record of the "Grotta dei Fiori" sequence as confirmed by morphometric data (Tabs. 2 and 3). For instance, some specimens falling in the dimensional field of *Microtus (Tyrrhenicola) sondaari* are present in nearly all the examined fossiliferous levels and no increase in average size (V1) of M_1 is detectable from the bottom to the top of the stratigraphical succession. Moreover, most of the specimens cannot be separated on the basis of the level they were found, neither plotting A/L index (expressing the ACC relative elongation) versus the width of the neck (V31), nor using PCA analysis (Fig. 7). Two specimens from the C2 Level set apart possibly because of their

		Level C11 (n=8)	Level C13 (n=13)	Level C14 (n=13)
Morphotype 1	n	1	3	3
	%	13	23	23
Morphotype 2	n	5	5	8
	%	63	38	62
Morphotype 3	n	1	4	0
	%	13	31	0
Morphotype 4	n	1	1	2
	%	13	8	15

Tab. 3 - Percent distribution of the main morphological classes (morphotypes) within each level from Grotta dei Fiori.

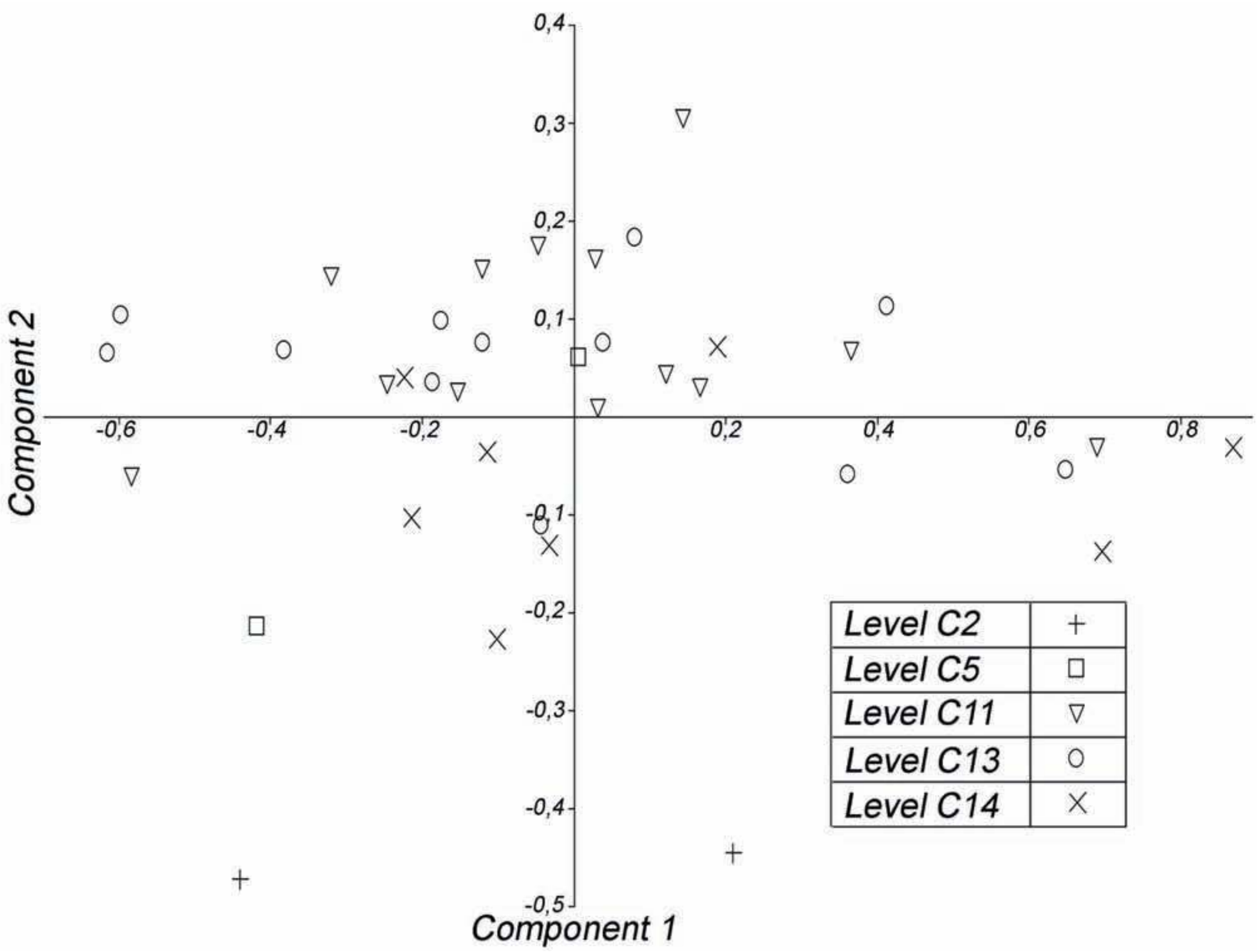

Fig. 7 - Scatter diagram of Principal Component Analysis on occlusal surface measurements of M_1.

anomalous total width. PCA has been performed both taking into account all the linear measurements, and only the variables regarded as the most significant in describing the *Microtus* (*Tyrrhenicola)* evolutionary trend variables (Tab. 4). Variance and Eigenvalues of PCA and Factor Loadings of principal components are reported in Tab. 4.

PC	Eigenvalue	% Variance		PC 1	PC 2
1	0,1372	67,0300	V1	-0,6386	0,0692
2	0,0247	12,0480	V2	-0,2537	0,0031
3	0,0130	6,3575	V3	-0,2965	0,3712
4	0,0110	5,3913	V4	-0,0585	-0,2318
5	0,0056	2,7215	V5	-0,2618	-0,7250
6	0,0036	1,7447	V6	-0,3483	0,4575
7	0,0031	1,5328	V10	-0,3015	-0,1046
8	0,0019	0,9298	V11	-0,2081	-0,0345
9	0,0016	0,7936	V12	-0,3300	-0,2130
10	0,0014	0,6846	V31	-0,0468	-0,0046
11	0,0007	0,3578	V32	-0,0261	0,0165
12	0,0006	0,2791	V33	-0,0218	-0,1043
13	0,0003	0,1287	V34	-0,0113	0,0022

Tab. 4 - Eigenvalues and Variance values of Principal Component Analysis of M_1 and Factor Loadings of principal components.

Scatter diagram does not show a clear separation among specimens coming from the individual levels. Comparison with M_1 from other sites of Sardinia (Tab. 5), puts in evidences the average small size of "Grotta dei Fiori" M_1 (Fig. 8), likely due to the occurrence of the specimens ascribed to morphotype 1, whose size is close to that of *Microtus (Tyrrhenicola) sondaari* from Xg3 fissure (Marcolini et al., 2006) (Fig. 8). Moreover, despite the large size of some specimens matching that of specimens from Monte San Giovanni, Siniscola E, Corbeddu and Capo Figari, M_1 from Grotta dei Fiori have on average ACC less developed than samples from the other examined sites holding *Microtus (Tyrrhenicola) henseli*.

SDQ values range from 74,9 to 118,4 with a mean value of 96,9 and are lower than those of Monte Tuttavista, with the exception of those of "VI Banco 6" fissure (Late Pleistocene) (Marcolini et al., 2006b), but surprisingly higher than that of M_1 of Cava Alabastro (Fig. 9, Tab. 6).

All in all, voles of "Grotta dei Fiori" can be ascribed to *Microtus (Tyrrhenicola) henseli,* thereby this locality can be regarded as more recent than "Xg3" fissure of Monte Tuttavista. The rare presence of M_1 ascribed to morphotype 1 and the low abundance of morphotype 4 in most of the fossiliferous level, suggest that the voles

	L (M_1)				A/L (M_1)				B/W (M_1)			
	n	min	max	Media	n	min	max	Media	n	min	max	Media
Grotta dei Fiori	38	2,47	3,54	3,05	38	39,87	54,05	46,21	38	11	25	17
Monte Tuttavista Xg3	36	2,32	3,05	2,72	36	39,67	48,4	44,13	36	-	-	-
Capo Figari	6	2,9	3,6	3,27	6	47,4	50,6	48,55	8	14,4	25,4	21,48
Bonaria	39	2,9	3,7	3,32	39	46,1	54,5	51,07	39	11	29,8	19,69
Monte San Giovanni	64	2,61	3,55	3,14	64	46,9	55,6	51,25	66	6	28	17
Cava Alabastro	317	0,13	0,17	3,42	315	42	61	51	317	7,81	45,83	27,6
Dragonara	51	2,7	3,73	3,34	50	47	56	51,5	51	2	37,71	18,1
Siniscola C	8	3,3	3,5	3,4	8	45,9	52,6	48,85	10	14	22,4	18,85
Siniscola E	4	2,9	3,5	3,25	4	48,1	51,7	49,89	6	11	26,8	19,3
Corbeddu	18	2,9	3,5	3,26	18	49	55,3	52,39	18	7,7	22,9	15,77
Su Guano	24	3,1	4	3,54	24	49,2	54,6	51,96	24	8,8	26,5	16,54
MTVX3	92	2,3	3,07	2,72	92	36,84	60,73	45,27	92	10,71	42,11	26,87
MTVIX3	33	2,65	3,42	2,99	33	35,09	53,27	47,77	33	16,35	35,58	23,98
MTVIII1	60	3	3,89	3,36	60	45,27	53,66	49,7	60	4,83	27,19	17,58
MTVIV14	169	3	3,93	3,36	169	46,01	65,02	50,68	169	3,76	29,75	15,32
MTVIV5	13	2,99	3,59	3,34	13	47,21	54,52	50,34	13	7,87	23,15	15,5
MTVVI6	87	2,95	3,77	3,32	87	36,6	59,49	51,42	87	4,17	29,37	13,99
MTVVII2	25	3,07	3,71	3,35	25	48,29	54,45	51,38	25	7,32	25,69	14,03

Tab. 5 - Descriptive statistics of variables and morphological indices of M_1 from localities of Sardinia (data from Mezzabotta et al. 1995; Minieri et al. 1995; Turmes 2003; Marcolini et al. 2005; Marcolini et al. 2006, Boldrini 2008).

from "Grotta dei Fiori" might be on the average regarded as less advanced even than the *Microtus (Tyrrhenicola) henseli* populations from Monte San Giovanni and Cava Alabastro, (Mezzabotta et al., 1995; Minieri et al., 1995). The scarcely advanced features of Grotta dei Fiori voles is supported by their A/L low ratio (Tab. 5). The results thus far obtained by preliminary analyses of M_1 from "Grotta dei Fiori" stress once more the great morphological variably of *M. (Tyrrhenicola)*.The mosaic distribution pattern of morphometrical characters and the

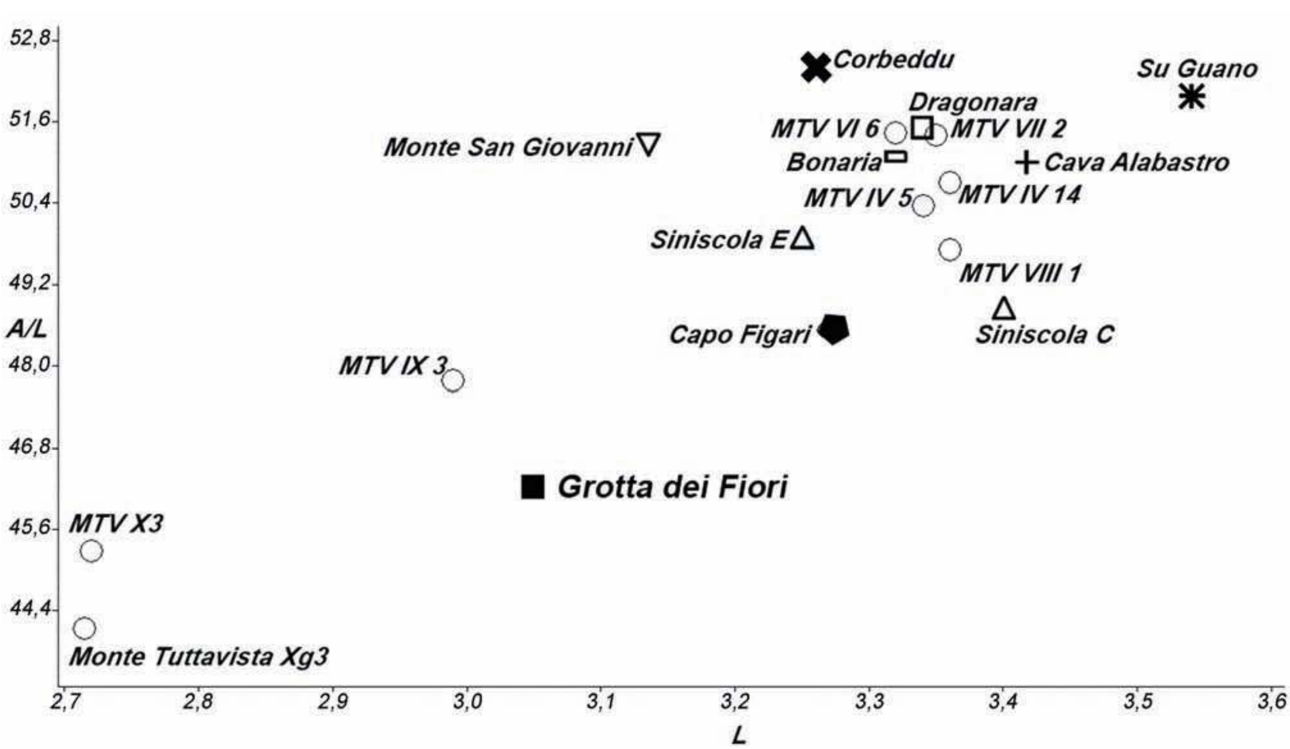

Fig. 8 - L against A/L of M_1 from various localities of Sardinia (data from Mezzabotta et al., 1995; Minieri et al., 1995; Turmes, 2003; Marcolini et al., 2005; Marcolini et al., 2006).

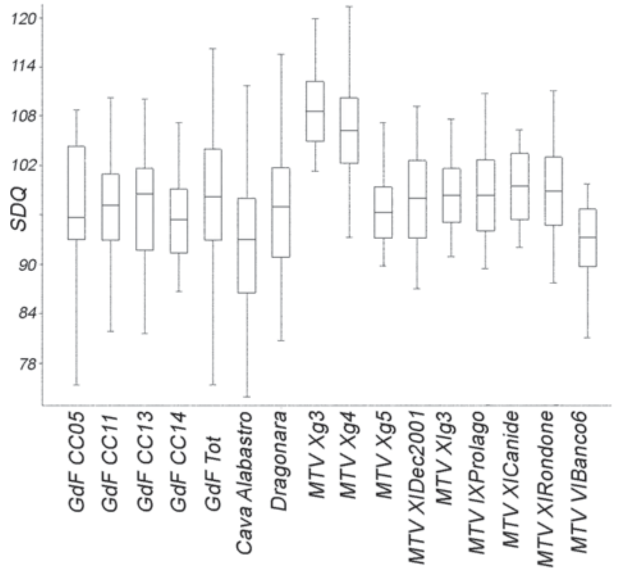

Fig. 9 - Boxplot of SDQ of M_1 from various localities of Sardinia (Monte Tuttavista (MTV) data from Marcolini et al., 2006b).

SDQ				
	n	Media	Min	Max
GdFCC05	9	93,97	74,98	106,01
GdFCC10	17	95,11	67,84	115,79
GdFCC12	6	97,21	89,82	105,51
GdFCC13	17	100,09	88,21	113,81
GdFCC14	13	91,16	76,53	106,22
GdF Media	96	96,08	75,34	116,15
Cava Alabastro	51	96,96	73,96	203,67
Dragonara	62	96,41	66,68	169,24
MTV Xg3	15	108,58	101,30	119,80
MTV Xg4	14	106,21	93,30	121,20
MTV Xg5	13	96,29	89,80	107,20
MTV XIdec2001	15	98,01	87,10	109,10
MTV XIg3	15	98,31	90,90	107,60
MTV IXprolagus	15	98,32	89,40	110,70
MTV XIcanide	8	99,48	92,00	106,30
MTV XIrondone	13	98,89	87,70	111,10
MTV VIb6	15	93,22	81,10	99,70

Tab. 6 - Descriptive statistics of SDQ of M_1 from various localities of Sardinia (Monte Tuttavista (MTV) data from Marcolini et al., 2006b).

anomalous fluctuation in SDQ trend confirm the evolutionary process of this endemic vole was maybe affected by stochastic fluctuations, as suggested by Mezzabotta et al. (1996).

DISCUSSION AND CONCLUSIONS

A chronological setting of deposits filling karstic fissure or caves are usually difficult, especially when absolute dates is not available, because multiple events (karstification, successive phases of sedimentation and erosions of deposits) complicate the reconstruction of sedimentary sequences and their correlation with deposits cropping out in the surrounding area. This might be true also for fossiliferous levels lacking of biochronological markers or containing taxa with a quite large chronological spectrum as the case study of "Grotta dei Fiori" fossiliferous succession.

For instance, among the species recorded at "Grotta dei Fiori", *Talpa tyrrhenica* and *"Nesiotites" simili*s have been cited in a number of Middle and Late Pleistocene localities, the high-crowned *Rhagamys orthodon* has been identified in a number of faunas ranging in age from the Middle Pleistocene (e.g. Santa Lucia of about 450 ka BP) to the Neolithic Age (Grotta del Guano, Grotta Punta del Quadro), *Prolagus sardus* is abundant in ?late Middle Pleistocene-Holocene faunas and is recorded till historical time (Palombo, 2006, 2009 and references therein). Despite the increase in knowledge of *Microtus* (*Tyrrhenicola*) (Marcolini et al., 2006) and *Prolagus sardus* (Angelone et al., 2007) evolutionary trends, a detailed biochronological scheme for the late-Middle and Late Pleistocene LFAs of Sardinia has been not defined (cf. Palombo, 2009), possibly due to the still imperfect knowledge about the actual evolutionary trend of *Microtus (Tyrrhenicola)* lineage. The relative frequency of morphotypes seems at the present the most parsimonious tool to infer the relative age *Microtus (Tyrrhenicola) henseli* population.

Results obtained by preliminary analysis of M_1 of *Microtus (Tyrrhenicola)* confirm the presence of primitive morphotypes 1 and 2 together with advanced morphotypes 3 and 4 in almost all the fossiliferous levels. Samples from Grotta dei Fiori are characterized by ACC less developed than that the other ?Middle and Late Pleistocene populations from Sardinia. Accordingly, the stratigraphical succession of "Grotta dei Fiori" should be tentatively ascribed to the Middle Pleistocene.

Results of isotope analysis of *Cynotherium* remains, even if obtained from scanty specimens, could suggest climatic condition not so different from the present days. The values obtained for small mammals are indicative of relative fluctuations in climate conditions. Although the conversion of oxygen isotopical composition of structural carbonate ($\delta^{18}O_c$) in phosphate ($\delta^{18}O_p$), (Iacumin et al., 1996), provides values of the oxygen isotopical composition of local meteorical water ($\delta^{18}O_w$) too high if compared with the present, these data could be confidently used for inferring temperature and humidity shifts comparing the values obtained each other in the light of the indication given by *Cynotherium* remains.

On the other hand, results obtained from lithostratigraphical and micromorphological analyses enable us to infer the climatic context at the times of the deposition of sediments and maybe to slightly refine its chronological attribution. The presence of pedorelicts of red eroded soils as well as the abundance of illite and kaolinite in nearly all the levels indicates that the sediments outside the cave deposited under subtropical climatic conditions, characterised by alternation of wet and dry periods (see e.g Millot, 1964; Tardy et al., 1973; Duchaufour, 1984; Pedro, 1984; Van Wambeke, 1992). For example, Boero et al. (1992) found that illite and kaolinite are the main clay mineral phases in "terra rossa" from xeric sites and that the chief constituents of the clay fraction of "terra rossa" from Sardinia are illite and kaolinite. Furthermore, the red hues of the fine fractions, in both units, might indicate dramatic climatic contrasts, including pronounced dry seasons (Núñez & Recio, 2007). The hypothesis of an alternation of warm and cool and more or less humidity climatic conditions occurred might be partially supported by isotope data, which indicate alternating changes of climatic variable during the sedimentation of Grotta dei Fiori stratigraphic succession. The trend of the $\delta^{18}O$ and $\delta^{13}C$ suggest that C1 Level was deposited under humid conditions (Fig. 5), whereas the cracks, caused by desiccation of clay sediment, then filled by calcite present the C2 Level, suggest the climate was still warm, but the humidity sensibly decreased. The shift towards more arid climatic conditions are confirmed by the trend of $^{13}C/^{12}C$ ratio of C5 Level (Fig. 5), and by the presence of mud cracks in the C6 Level.

A successive increase of humidity is detectable on the basis of the presence of abundant rounded Mn nodules in the C8, 10, 12 Levels. Sand-sized Fe-Mn nodules are interpreted to have formed by segregation of iron and manganese due to alternating episodes of soil drying and wetting (Bouma et al., 1990; McCarthy et al., 1998, 1999b). A relative moderate increase of humidity and an augment of temperature are also suggested by $^{13}C/^{12}C$ and $^{16}O/^{18}O$ ratio of C11 Level, then temperature notably decreases, but climate became a bit more humid (Fig. 5). The chaotic fabric of sediments in the upper part of the Unit B, that were transported into the cave by water flow with moderate energy that had eroded the soils from the surrounding area, could be regarded as an indirect evidence of this progressive moderate increase of humidity.

All in all, data thus far provided by palaeontology and sedimentology indicate that the stratigraphical succession C of "Grotta dei Fiori" deposited during the Middle Pleistocene under subtropical climatic conditions shifting from warm/humid to cool and less humid. Since during the first climatic oscillations of the Middle Pleistocene (from the marine isotope stages, MIS, 22 to 12), temperature in the Mediterranean area was definitely cooler than today, the hypothesis to suppose that "Grotta dei Fiori" succession deposited not earlier than the MIS 11 cannot be rouled out. At that time (MIS 11), astronomical insolation and some proxy climate indicators were similar to those of today (Loutre & Berger, 2003). On the other hand, *Microtus (Tyrrhenicola) henseli* of "Grotta dei Fiori" seems to be less advanced than other Middle Pleistocene Sardinian voles, such as those from Monte San Giovanni, Capo Figari. For the Capo Figari II local fauna, a ESR date of 366.959±20% is available (Montoja Ikeya fide Made 1999).

Accordingly and taking into account the evolutionary degree of voles, $^{13}C/^{12}C$, $^{16}O/^{18}O$ and sediment micromorphology the "Grotta dei Fiori" succession could be tentatively correlated with MIS 11 (Termination V). Ongoing researches and the absolute datings of some speleothemes, now in progress, either could or could not confirm this hypothesis. This preliminary study stresses once more the difficulty of a precise chronological setting for deposits filling caves when no absolute data are available, and highlight the need of a multidisciplinary approach to better infer the environmental context at the time of the deposition of fossiliferous beds.

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