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Signature pigments of Green Sulfur Bacteria in ancient sediments from the Banyoles lacustrine area

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Keywords: sediments, bacteriochlorophyll *e*, biomarkers, isorenieratene, Green Sulfur Bacteria, paleolimnology, xanthophylls.

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Abbreviations: BChl – Bacteriochlorophyll; BChl e_F – Bacteriochlorophyll *e* esterified with farnesol; BChlide *e* – Bacteriochlorophyllide *e*; BPheo *e*– Bacteriopheophorbide *e*; Car – carotenoids; Chl – chlorophyll; GSB – Green Sulfur Bacteria; Isr – Isorenieratene; t_R – Retention time.

Abstract

Signature pigments from photosynthetic Green Sulfur Bacteria (GSB) were found in ancient sediments collected from an abandoned clay quarry located in the Banyoles lacustrine area. The sediments belong to the Interglacial Waalian of the Inferior Pleistocene (0.7–1.5 millions years old) and were deposited after a marshy event occurring at that period. Reverse phase HPLC analyses of acetone:methanol sediment extracts revealed that the main pigments found were carotenoids of both eukaryotic and prokaryotic origin. In particular, Isorenieratene (Isr) and β-Isorenieratene (β-Isr) constituted the larger bacterial fraction (35–40% of the total carotenoid content) whereas okenone, a signature pigment for Purple Sulfur Bacteria, accounted for less than 2%. Xanthophylls from oxygenic photosynthetic organisms accounted for the rest of the total carotenoid content. Traces of chlorophyll degradation products, especially GSB bacteriochlorophylls (BChls), were also detected. The finding of signature pigments for brown-colored species of GSB (Isr, β-Isr and degradation products of BChl *e*) in the sediments suggests an ancient aquatic environment where GSB were present and where episodes of sulfide-rich anoxia reaching the photic zone could be envisaged. Other anoxygenic phototrophs like Purple Sulfur Bacteria were nearly absent as suggested by the hardly traceable amounts of their signature pigments (BChl *a* and carotenoids as okenone or spirilloxanthin). The large percentage of xanthophylls and the presence of chlorophyll degradation products suggest that algae could also be and important part of the microbial photosynthetic community in the ancient ecosystem.

Introduction

Several photosynthetic pigments are regarded as biomarkers since they can be uniquely assigned to a particular group of organisms. For instance, isorenieratene (Isr) and its derivatives (diagenetic products) are unambiguously assigned to brown-colored species of Green Sulfur Bacteria (GSB), a group of obligate anaerobic bacteria carrying out anoxygenic photosynthesis. Accordingly, the presence of signature photosynthetic pigments from brown-colored GSB in aquatic systems has been used not only to trace back the occurrence of these microorganisms but also as a clear indication of water column stratification, anoxia and sulfide accumulation (Summons & Powell 1986; Summons & Powell 1987). Likewise, the finding of Isr and its diagenetic products in naturally occurring sediments is also a valuable tool to reconstruct (paleo)enviroments that in the past have experienced photic zone anoxia (Sinninghe Damsté et al. 1993; Koopsman et al. 1996; Koopsman et al. 1996; Clifford et al. 1998).

The Banyoles lacustrine system consists of a main lake (1.5 km^2 and 17 Hm^3) and several minor water bodies with a common karstic origin but exhibiting different physical, chemical and biological characteristics. A common feature in most of these environments is the seasonal occurrence of anoxia accompanied by sulfide accumulation in hypolimnetic waters, which allow the development of dense populations of photosynthetic sulfur bacteria (Vila & Abella 1993; Vila & Abella 1994; Garcia-Gil et al. 1996). About 1 km north from the Banyoles lacustrine area there is a clay quarry originated by a local marshy event different from the global lacustrine one that resulted in the formation of Lake Banyoles. The sediments from the clay quarry have been dated back to the Interglacial Waalian of the Inferior Pleistocene, and so suggested to be between 0.7 and 1.5 million years old (Leroy 1986).

The aim of this work has been to analyze the clay quarry ancient sediments by HPLC and search for pigment biomarkers from Green Sulfur Bacteria so as to evaluate whether or not these anoxygenic photosynthetic microorganisms were present in the Banyoles lacustrine area since then.

Material and Methods

Study Site. The clay quarry of Ordis (42º 08', 2º 45') is located 1 km north of Lake Banyoles (Girona, Spain) and is part of its lacustrine system. The quarry, which is currently abandoned, was exploited for brick manufacturing in the past resulting in an exposed abrupt wall of about 50 m high and 200 m wide. This wall consists of dark-grey, compact sediments with no clear lateral continuity (Bech 1970). The sediment color is presumably a consequence of both anaerobic reducing conditions in the past that led to sulfide formation and carbonization processes (i.e. lignite and turf formation).

Sample collection. Sediment sample blocks of about 0.2–1.0 dm^3 were extracted with a shovel from the main clay wall. The sediments from the wall surface were discarded for further analyses. So that, only 30–50 cm-deeper, blackish layers were collected in sterile 1L plastic containers and stored in a portable icebox. In the laboratory, the samples were stored until use in a Heraeus Instruments WA6100 anaerobic chamber under a 75% N_2 and 25% CO_2 atmosphere to avoid oxidation.

Fresh sediment samples used for pigment comparison were collected from a 10 m depth meromictic basin of Lake Vilar (adjacent to Lake Banyoles) using a modified Birge-Ekman dredge (KAHLSICO 214WA180). Sample wash-out and contamination with biological material from the water column during the retrieval of the box sampler was negligible since the hinged overlapping lids of the grab were tightly closed while it was being raised. At the surface, the excess of water in the sample was removed and the sediment was transferred to completely-filled sterile 50 ml Falcon tubes, which were immediately stored on ice in the dark. After transfer to the laboratory the samples were stored at –30 °C.

Pigment extraction. For sediments blocks collected in the quarry, the inner layers of the blocks were scratched with a sterile stainless steel spatula and the slices were pooled and weighed until reaching 20 g. The material was then collected in sterile, 50 ml glass tubes

previously wrapped in aluminum foil. Extraction of pigments was accomplished by mixing 1.5 volumes of acetone:methanol (7:2, v/v) per gram of sediments followed by vortexing. The same extraction procedure was applied to fresh sediment samples collected from Lake Vilar. All the manipulations were carried out at room temperature and under dim light to prevent further changes in the naturally occurring pigments. The mixture was stored overnight at 4 ºC and then centrifuged at 17,000 × *g* in a Sorvall RC-5B centrifuge. The supernatant was collected and the pellet re-used twice to achieve a complete pigment extraction. The supernatants were pooled and evaporated in a rotary evaporator at 40 ºC. The desiccated material was then thoroughly dried under a stream of N_2 and kept in an inert atmosphere at −80º C until use.

Chemical preparation of Bacteriochlorophyllide e and Bacteriopheophorbide e. BChl *e* was extracted from fresh cell pellets of *Chl. phaeobacteroides* using acetone:methanol 7:2 (vol:vol). The pigment mixture was centrifuged at 5,000 rpm in a bench centrifuge and the supernatant collected. Then, phase separation was performed to bring the bulk of pigments to the non polar epiphase, consisting of light petroleum:dichloromethane, 9:1 (vol:vol). The epiphase was collected with care and dried under stream of N_2. A crude separation between BChl *e* and carotenoids was achieved by washing out the dried pigments with hexane. Bacteriochlorophyllide *e* (BChlide) was obtained by dissolving BChl *e* in 1% KOH methanol and left overnight at room temperature. As a result of the chemical reaction, the esterifying alcohols of the BChl *e* molecules (both farnesol from the main homologues and other alcohols from the secondary ones (Senge & M. 1995; Airs & Keely 2002) were swapped for methanol. When obtaining bacteriopheophorbide *e* (BPheo *e*), diluted HCl was added to remove the central Mg ion of the pool of BChlide. The HCl addition was stopped when changes in the color of the BChlide solution was observed by eye, suggesting the formation of BPheo *e*.

HPLC analyses. The HPLC system and sample preparation were the same as described by Borrego and Garcia-Gil (1994) with the following modifications. Dry pigments were dissolved

in 1 ml of acetone:methanol (7:2, v/v) (Scharlau, HPLC grade) and filtered through a 0.2 μm, 25 mm polycarbonate membranes (MSI Micron Separations Inc.) prior to analysis. To improve the resolution on pigment separation, 1 % of 1M ammonium acetate was added to each sample as ion pairing agent (Mantoura & Llewellyn 1983; Borrego & Garcia-Gil 1994). The complete separation of carotenoids was accomplished by using the following solvent gradient: a 40 min linear increase of solvent B from 50 to 100%, a hold at 100% of solvent B for 34 min, and a return to initial conditions in 1 min (method A). For the separation of (bacterio)chlorophylls and derivatives the gradient was modified as follows: a 30 min linear increase of solvent B from 0 to 100%, a hold at 100% of solvent B for 44 min, and a return to initial conditions in 1 min (method B). In both cases the re-equilibration of the system took 15 min. In both gradients the total run time was of 90 min.

The identification of pigments was done according to their absorption spectra and their retention times (Borrego & Garcia-Gil 1994). Quantification of pigments was performed by measuring the areas of the corresponding peaks in the chromatograms at the λ_{max} for each pigment. The molar extinction coefficients used were: 107,000 l $mol^{-1}cm^{-1}$ for Isr in ethanol, 111,000 l $mol^{-1}cm^{-1}$ for auroxanthin, 134,000 l $mol^{-1}cm^{-1}$ for okenone, an averaged value of 140,000 l $mol^{-1}cm^{-1}$ for all xanthophylls and β–carotene (Britton 1995), and 41,000 l $mol^{-1}cm^{-1}$ for BChl *e* in ethanol (Borrego et al. 1999).

Results

The HPLC trace of the photosynthetic pigments extracted from the clay quarry sediments and analyzed using method A is shown in Fig. 1. The chromatogram reveals no less than 20 peaks, most of them assigned to carotenoid molecules based on their respective UV-visible absorption features (see below). On comparing their retention time, they were clustered in three main groups. Other pigments found in trace amounts were (bacterio)chlorophylls and their degradation products. They were studied in more detail using the HPLC method B.

The first group of carotenoids eluted from 21 min to 37 min and mainly consisted of xanthophylls with high polarity (epoxide-containing xanthophylls, dihydroxy carotenoids, and dioxocarotenoids). The most polar xanthophyll of this group (t_R =21.4 min) was identified as auroxanthin (5,8,5',8'-diepoxy-7,8,7',8'-tetrahydro-β,β-carotene-3,3'-diol). This xanthophyll has seven conjugated double bonds and its spectroscopic properties resemble to those of the precursor ζ–carotene (i.e. visible absorption peaks approximately at 385 (I), 409 (II) and 434 (III) nm and a peak height ratio III/II slightly higher than 100 (Fig. 2A). The other main xanthophylls of this group were antheraxanthin (t_R =28.8 min), lutein (t_R =30.6 min) and zeaxanthin (t_R =31.0) (Fig. 1).The latter three xanthophylls were identified by comparing their respective retention times and absorption features with the ones obtained from plant pigments (data not shown). Although other carotenoids were present in this first cluster, their identification was not straightforward due to different reasons, namely: i) they appeared in trace amounts, ii) peaks overlapped with (bacterio)chlorophyll degradation products, and iii) they presented di-*cis* or poly-*cis* conformations due to different degradation stages. The xanthophylls eluted in the first group approximately accounts for about 50% of the total carotenoid content in the clay quarry sediments.

The second cluster of carotenoids grouped xanthophylls with an intermediate polarity that eluted from 43 to 50 min. They were mono- or diepoxide-containing xanthophylls, monohydroxy carotenoids and monooxocarotenoids that accounted for 12% of the total carotenoid content in the clay quarry sediments (Table 1). In this group, most xanthophylls were identified as algal ones, namely, cryptoxanthin-type such as β-cryptoxanthin diepoxide (t_R = 43.4 min), β-cryptoxanthin monoepoxide (t_R = 46.5 min), α–cryptoxanthin (t_R = 46.8 min), and β–cryptoxanthin (t_R = 47.5 min). Minor amounts of okenone, presumably derived from ancient Purple Sulfur Bacteria, were also detected (<2% of the total pigment content).

The third cluster consisted of non-polar carotenoids from GSB together with early metabolic precursors of both bacterial carotenoids and xanthophylls. Diaryl carotenoids as Isr and derivatives were unambiguously associated with GSB, α–carotene with plants (algae and

higher plants), and β–carotene with both plants and GSB. The carotenoids of this group were identified as all-*trans* Isr (t_R = 53.1 min), *cis* Isr (t_R = 54.1 min), all-*trans* β–Isr (t_R = 56.8 min), *cis* β–Isr (t_R = 57.9 min), all-*trans* α–carotene (t_R = 60.5 min), all-*trans* β–carotene (t_R = 61.4 min), and *cis* β–carotene (t_R = 62.7 min). The *cis* forms of the different carotenoids were identified by their *cis* peak in the UV region (Fig. 2B) and, based on the intensity of this peak, they were supposed to be central-*cis* isomers (15–*cis*) (Briton, 1995). The relative amount of the 15–*cis* isomers with regard to their all-*trans* counterparts was approximately 40–45% for Isr and β-Isr. Interestingly, this ratio is similar to that calculated for pure cultures of the brown-colored GSB, *Chlorobium phaeobacteroides* strain CL1401, growing under saturating light conditions in the laboratory (unpublished data). The relative content of the carotenoids derived from GSB (Isr and β–Isr) was 35% of the total carotenoid content in the clay quarry sediment (Table 1).

For the detection of (bacterio)chlorophyll derivatives it was necessary to modify the HPLC elution gradient program to improve the resolution since they elute together at the beginning of the run using method A. With this modification (named as method B), we were able to detect traces of the degradation products of (bacterio)chlorophylls, such as (bacterio)chlorophyllide and (bacterio)phorbide. It is worth noting that BChls from GSB consisted of a series of homologues with different substituents in the chlorin ring (Senge & Smith 1995). This feature results in a high variety of homologues from a single BChl type in either a laboratory culture or a natural population of GSB (Borrego et al. 1998; Airs et al. 2001)

All the (B)Chl degradation products eluted within the first 30 min using the HPLC method B (Fig. 3A). Despite the differences between the retention times of these degradation products to those of their correspondent BChl *e* homologues, their respective absorption spectra showed very similar features. To confirm the identity of these BChl degradation products, BChl *e*, BChlide *e* and BPheo *e* were used as external standard (see Material and Methods) and analyzed using the same HPLC method. By doing so, BChlide *e* homologues eluted

between 18 and 23 min. When comparing both their t_R and their absorption spectra with those of the peaks eluted at the same retention time in samples from the clay quarry sediments, both sets of HPLC peaks exhibited identical retention times and absorption properties. This observation suggested that BChlide *e* was actually present in the clay quarry sediments as a result of a chemical degradation of BChl *e*. Similarly, BPheo *e* homologues lacking both the central Mg ion and the esterifying alcohol, were also detected in clay quarry sediments after comparison of their correspondent retention times —between 24 and 29 min— and their absorption spectra with those obtained after the HPLC analysis of external BPheo *e* homologues. Interestingly, each BChl e homolog yielded its correspondent BPheo *e* counterpart, eluted in the same polarity order but at lower t_R (Table 2). The presence of BChl *e* homologues were tracked down in the sediment samples only when HPLC elution profiles of a laboratory culture of *Chl. phaeobacteroides* CL1401 were used for comparison. Fig. 3B shows the absorption spectra of BChlide *e*, BPheo *e*, and BChl *e* found in the analyzed sediments.

Although traces of degradation products of chlorophylls were also detected, their identification was very difficult due to their extremely low amounts and their overlapping with the carotenoid peaks along the HPLC elution profile.

Discussion

The finding of pigments from anoxygenic and oxygenic photosynthetic organisms in the clay quarry sediments supports former reports that describe an ancient aquatic system in the Ordis area (Solé Sabarís 1957). It was suggested to have its origin in a local swamp episode rather than in a large-scale lacustrine phenomenon, as the one giving rise to the karstic Lake Banyoles (Bech 1970). The existence of this ancient aquatic system has also been confirmed by the discovery of fossils of aquatic fauna in Ordis (Leroy 1986). The analyzed clay quarry sediments were found under the limestone of Pla d'Usall, with which contacted after a series of tectonic processes. Both fossils of aquatic fauna and geological events have led to date

back the clay quarry sediments of Ordis to the Waalian period of the lower Pleistocene (Leroy 1986).

The pigment composition found in Ordis sediments is quite similar to that found in fresh sediments collected from a stratified lagoon of the Banyoles lacustrine area except for the presence of Chl *a*, BChl *a* and okenone, and BChl *e* (Fig. 4). Chemical degradation of labile photosynthetic pigments such as chlorophylls is a rapid process in sediments under oxidizing, acidic or high temperature conditions, whereas more stable pigments, such as carotenoids, degrade much slowly. This explains why it is easier to find traces of carotenoids in depositional environments than those of chlorophylls. Interestingly, the only chlorophyll degradation products found in the analyzed sediments were those coming from brown-colored GSB whereas those derived from photosynthetic eukaryotes were hardly found. A possible explanation could rely on the chemical behavior of these bacteriochlorophylls (BChls), that spontaneously form large aggregates inside the antenna complexes of GSB, the so-called chlorosomes (Blankenship et al. 1995). It has been demonstrated that acidic treatment of aggregated BChl *c* in chlorosomes results in a slower degradation of this pigment (i.e. formation of BPheo *c*) than that observed on monomeric BChl *c* (Steensgaard et al. 1997). Besides, several authors suggest that aggregated BChls are more resistant to (photo)chemical oxidation than eukaryotic chlorophylls since they possess a very poor triplet quantum yield, thereby avoiding the photosensitization of singlet oxygen very efficiently (Krasnovsky et al. 1994; Arellano et al. 2002). Despite that the results of these experiments cannot be easily extrapolated to sediments, especially after several million years of deposition, the great prevalence of BChlide *e*, BPheo *e* and BChl *e* itself over eukaryotic chlorophyll by-products in the quarry sediments suggests that the former could overcome the first fast chemical degradation by remaining in the sediment in an aggregate state.

The major pigments found in the clay quarry sediments of Ordis were carotenoids from both oxygenic and anoxygenic photosynthetic organisms. Two out of the three pools of carotenoids consisted mostly of xanthophylls. The first one, clustering highly polar dihydroxy-

and epoxydihydro-carotenoids, could have their origin in both algae and higher plants. Lutein and zeaxanthin were easily identified in the HPLC chromatogram, but the presence of auroxanthin was less trivial to explain, since this carotenoid is found more often in marine algae or flowers and ripe fruits of plants. It is known, however, that acidic treatments promote the conversion of either 5,6-epoxy-5,6-dihydro or 5',6'-epoxy-5',6'-dihydro-carotenoids (or both) into 5,8-epoxy-5,8-dihydro and 5',8'-epoxy-5',8'-dihydro-carotenoids, respectively (Barua & Olson 2001). Violaxanthin is highly present in oxygenic photosynthetic organisms and the finding of auroxanthin in the clay quarry sediments of Ordis might be explained as a chemical conversion of violaxanthin into the latter under acidic conditions. In the second pool of carotenoids, monohydroxy- and monooxocarotenoids were tentatively associated with *Cryptophyceae* based on the number of identified cryptoxanthin derivatives (Kingsley, 1989). This is not surprising assuming that *Cryptomonas* spp. are, nowadays, usual inhabitants in the metalimnion of several lagoons of the Banyoles lacustrine area (Gasol et al. 1991; Gasol et al. 1993). Okenone was also detected in this second pool of carotenoids, but its small percentage in the ancient sediments of Ordis allows us to infer that purple photosynthetic bacteria were scarce in the ancient aquatic system. This is especially evident when compared to okenone and BChl *a* concentrations found in fresh sediments collected from a meromictic basin of Lake Vilar during a sampling campaign recently carried out by our group (Fig. 4).

Concerning signature carotenoids from GSB, Isr, β-Isr and derivatives were the dominant forms found. Although no quantitative conclusions may be taken from our analyses, the relative abundance of these pigment biomarkers and the appearance of BChl *e* and its degradation products supports the hypothesis that brown species of GSB were present in the ancient aquatic system. Besides, since no traces of pigment markers from green-colored species of GSB (i.e. BChl *c*, *d*, or carotenoids as γ–carotene or chlorobactene) were observed and assuming a similar degradation rate to BChl *e* and Isr molecules, we conclude that in the past brown-colored species overcome their green-colored counterparts. This

scenario is not very far from the one that prevails nowadays in the meromictic basins of Lake Banyoles (Garcia-Gil et al. 1996; Borrego et al. 1997).

Since Isr and its diagenetic and catagenetic derivatives have been used as markers of photic zone anoxia (Summons & Powell 1986; Sinninghe Damsté et al. 1993; Koopsman et al. 1996; Koopsman et al. 1996), their presence in the clay quarry sediments of Ordis provides some clues about the physical and chemical conditions of the ancient aquatic environment. Based on the physiological requirements of brown species of *Chlorobia*, we can infer that anoxia was accompanied by sulfide accumulation and a severely restrictive light regime in the oxygen-depleted meromictic water layers, natural conditions that confer some advantage to brown-colored photosynthetic bacteria over their green and purple counterparts (Vila & Abella 1994; van Gemerden & Mas 1995).

In conclusion, our results support the hypothesis that GSB have been inhabitants of the Banyoles lacustrine area at least since mid-Pleistocene. The presence of this group of bacteria in the past also might prove that episodes of anoxia accompanied by sulfide accumulation reaching up to photic water levels can be regarded as a common phenomenon that not only persisted over time but still prevails in most of the water bodies of the region.

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Table 1. Composition and t_R of the main carotenoids obtained from the HPLC elution profile of the clay quarry sediments of Ordis near Lake Banyoles.

	t_R (min)	[Car]* ($\mu g\ g^{-1}$)	[Car] (%)
Dihydroxy carotenoids and xanthophyll epoxides	28.3–36.9	11.92	50
Auroxanthin	21.4	0.04	–
Antheraxanthin	28.8	1.67	–
Lutein	30.6	1.03	–
Zeaxanthin	31.0	2.44	–
Monohydroxy carotenoids and			
β–Cryptoxanthin diepoxide.	43.4	0.40	–
Okenone	45.2	0.43	1.8–2.0
β–Cryptoxanthin monoepoxide	46.5	0.16	–
α–Cryptoxanthin	46.8	0.09	–
β–Cryptoxanthin	47.5	0.26	–
Non-polar Carotenoids			
all *trans*-Isr	53.1	4.79	–
cis-Isr	54.1	2.49	–
all *trans*-β-Isr	56.8	0.60	–
cis-β-Isr	57.9	0.19	–
all *trans*-α-Carotene	60.5	0.06	–
all *trans*-β-Carotene	61.4	0.44	–
cis-β-Carotene	62.7	0.06	–

*The extinction coefficient used to determine the carotenoid concentrations was 140,000 l $mol^{-1}cm^{-1}$ for all of them except for auroxanthin (111,000 l $mol^{-1}cm^{-1}$), okenone (134,100 l $mol^{-1}cm^{-1}$) and Isr and β–Isr (107,000 l $mol^{-1}cm^{-1}$) (Britton 1995; Borrego et al. 1999).

Table 2. Composition and t_R of BChl *e* and its derivatives found in the clay quarry sediments of Ordis near Lake Banyoles.

	t_R (min)	[BChl]* (μg g^{-1})
BChlide *e*		–
[Pr,E] BChlide *e*	19.4	
[I,E] BChlide *e*	21.3	
[N,E] BChide *e*	22.9	
BPheo e_H		–
[Pr,E] BPheo e_H	25.5	
[I,E] BPheo e_H	26.8	
[N,E] BPheo e_H	27.9	
BChl e_F		0.153
[E,E] BChle e_F	35.2	
[Pr,E] BChle e_F	35.7	
[I,E] BChle e_F	36.2	
[N,E] BChle e_F	36.8	

*The BChl *e* extinction coefficient was 41,000 l $mol^{-1}cm^{-1}$ (Borrego et al. 1999).

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Figure Legends

Figure 1. HPLC elution profile for the pigment solvent extraction of Ordis sediments detected at 453 nm (black line) and at 433 nm (gray line). Peak identification: (1) Auroxanthin, (2) Antheraxanthin, (3) Lutein, (4) Zeaxanthin, (5) *cis* Zeaxanthin, (6) β-Cryptoxanthin diepoxide, (7) Okenone, (8) β-Cryptoxanthin monoepoxide, (9) Cryptoxanthin, (10) *all-trans* Isr, (11) *cis* Isr, (12) *all-trans* β-Isr, (13) *cis* β-Isr, (14) *all-trans* α-Carotene, (15) *all-trans* β-Carotene, and (16) *cis* β-Carotene. (*) Not identified. The box shows the region where the BChl *e* derivatives eluted. Circled numbers corresponds to peaks which absorption spectra are shown in Fig. 3.

Figure 2. Absorption spectra of the main peaks eluted in the first pool of carotenoids (A) and in the third one (B) showed in Fig. 2. Identification: (1) Auroxanthin, (2) Antheraxanthin, (3) Lutein, (4) Zeaxanthin, (10) all-*trans* Isr, (11) *cis* Isr, (12) all-*trans* β–Isr, and (13) *cis* β–Isr. (*) *Cis* peak.

Figure 3. (A) HPLC trace of the pigment extract from Ordis sediments recorded at 473 nm (black line) and 448 nm (gray line). Identification: (1–3) BChlide *e* homologues, (4–7) BPheo *e* homologues, and (8–11) BChl *e* homologues. (B) Absorption spectra of the peaks circled in A.

Figure 4. Comparison between HPLC traces of the clay quarry sediments (A, same as in Fig. 1) and fresh sediments from Lake Vilar (B). For common peaks in both samples the numbering used in figure 1 has been conserved. Distinct peaks in B and C have been properly named. Detection channels in A: 453 nm (black line) and 433 nm (gray line). Detection channels in B: 453 nm (black line) and 771 nm (gray line). Some peak labels in A have been omitted for better comparison.

Figure 0

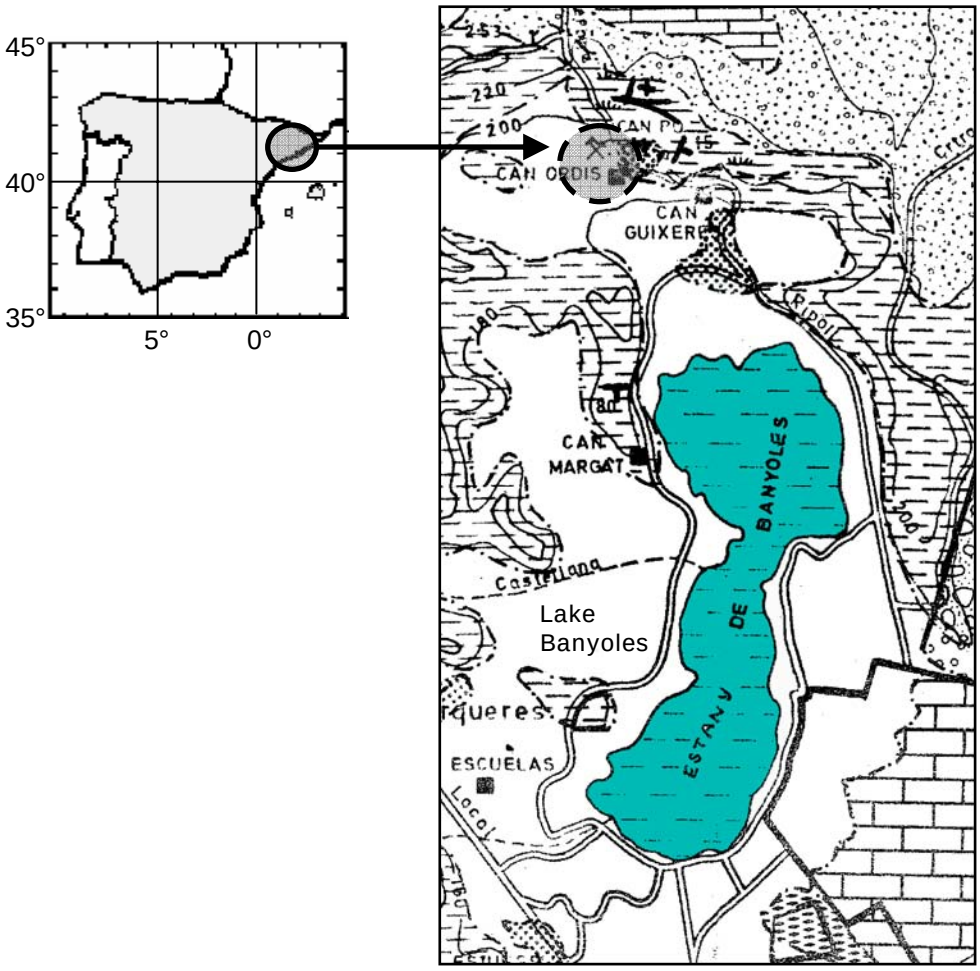

45°
40°
35°
5°
0°
CAN ORDIS
CAN GUIXERE
CAN MARGAT
ESTANY DE BANYOLES
Lake Banyoles
ESCUELAS

Figure 1

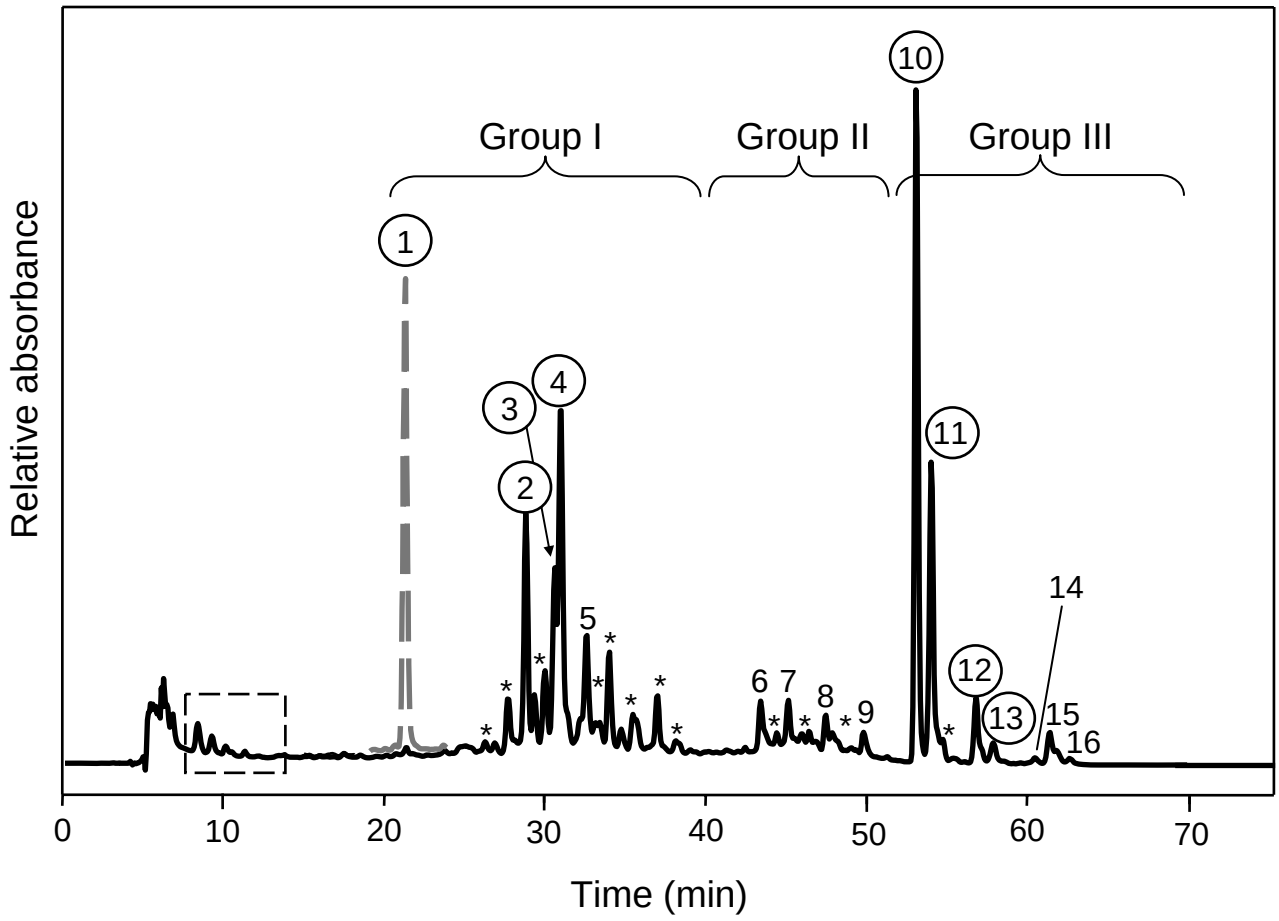

Group I
Group II
Group III
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Relative absorbance
Time (min)
0
10
20
30
40
50
60
70

Figure 2

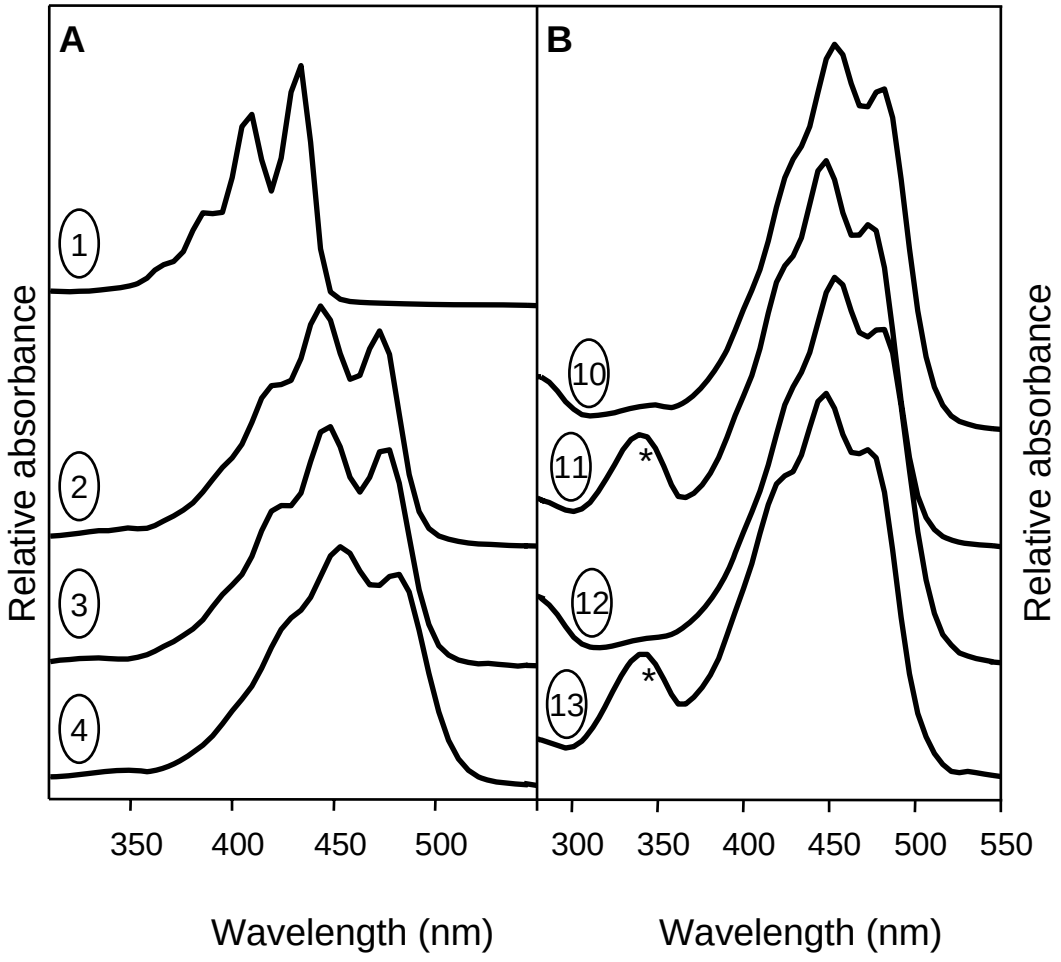

A
B
1
2
3
4
10
11
12
13
*
*
Relative absorbance
Relative absorbance
350
400
450
500
300
350
400
450
500
550
Wavelength (nm)
Wavelength (nm)

Figure 3

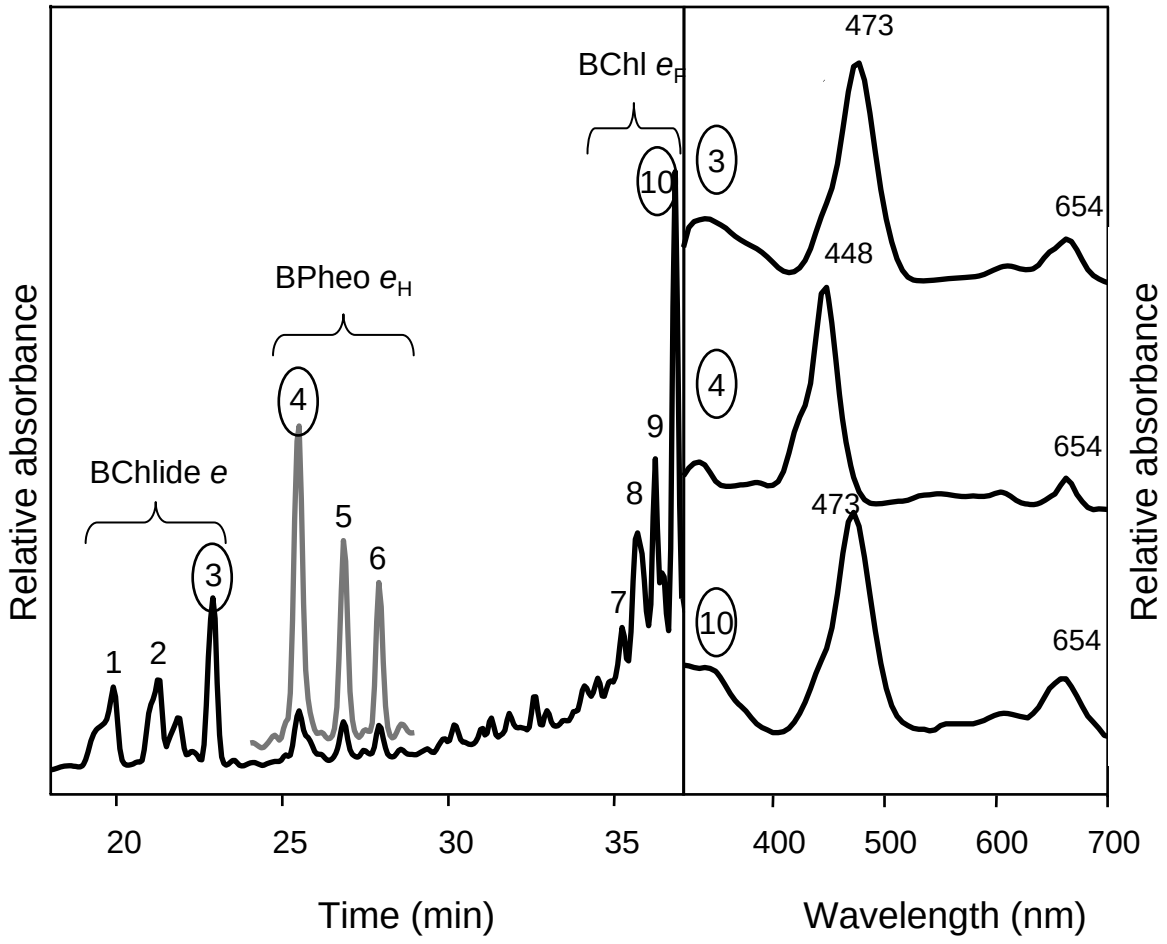
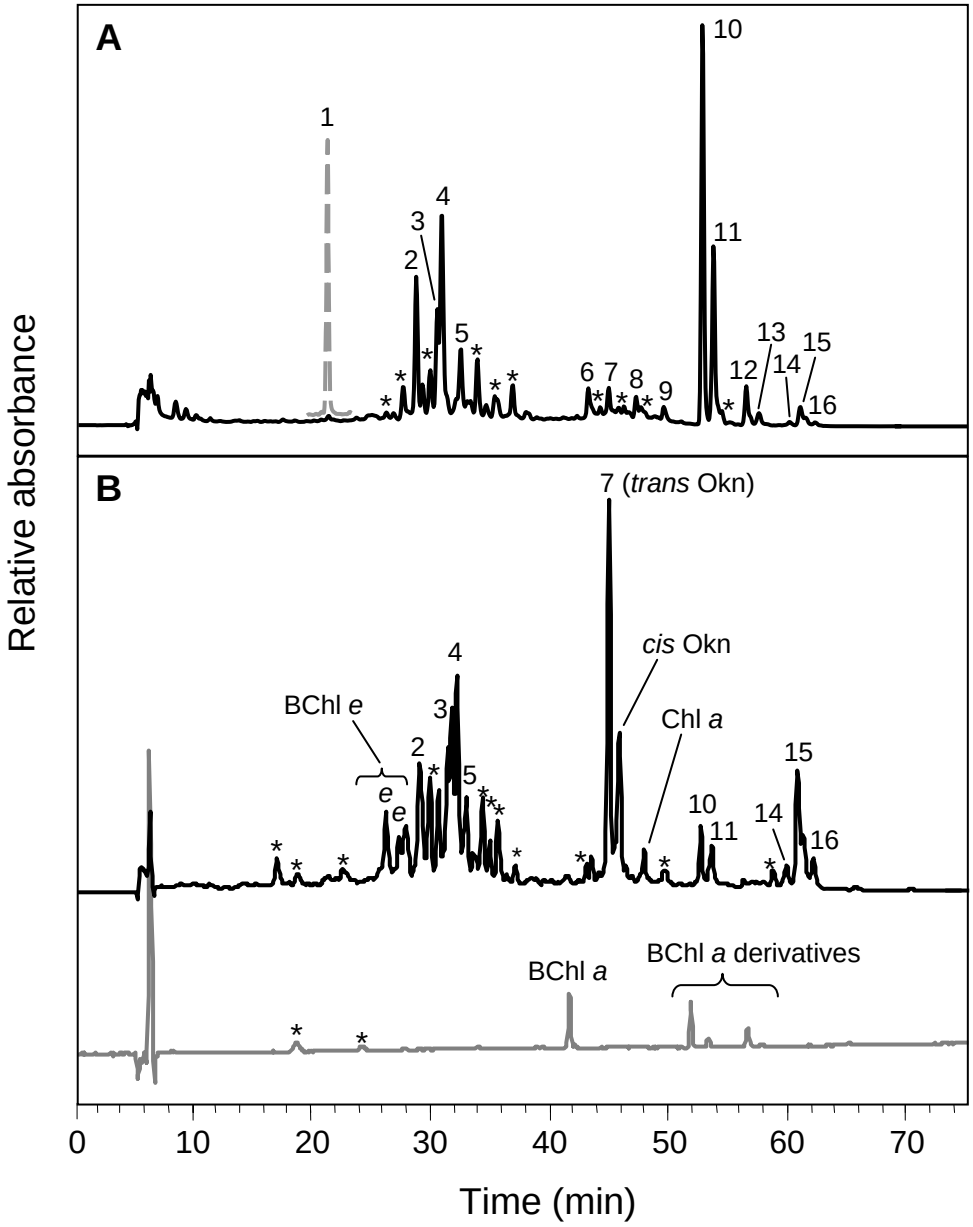

Relative absorbance
BChlide e
BPheo e_H
BChl e_P
1
2
3
4
5
6
7
8
9
10
20
25
30
35
Time (min)
473
654
448
654
473
654
400
500
600
700
Wavelength (nm)
Relative absorbance

Figure 4


A
B
Relative absorbance
Time (min)
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
7 (trans Okn)
cis Okn
Chl a
BChl e
e
e
BChl a
BChl a derivatives