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Membrane tetraether lipids of planktonic Crenarchaeota in Pliocene sapropels of the eastern Mediterranean Sea

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Abstract

The distribution of glycerol dibiphytanyl glycerol tetraethers (GDGTs), predominantly derived from planktonic Crenarchaeota, were studied in three age-equivalent Pliocene sapropels as well as in the homogeneous intervals, below and above the sapropel. In both the homogeneous intervals and sapropels the dominance of GDGT-0 and crenarchaeol among the GDGTs indicated their origin from planktonic Crenarchaeota as it was found in many other marine sediments. In the homogeneous intervals highest GDGT abundances (normalized to TOC) were measured at the easternmost study Site 967 of the eastern Mediterranean Sea and decreased at the central and western Sites 969 and 964. Within the three studied sapropels large variations in GDGT abundance were observed. The newly established sea surface temperature (SST) proxy, the TEX_{86} index, derived from GDGTs of planktonic Crenarchaeota was used to estimate past SST. Comparison with previous obtained SST data using the alkenones from haptophyte algae revealed substantial differences. Whereas the $U^{K'}_{37}$-based SSTs were almost constant at ca. 25 °C in both the homogeneous intervals and the sapropels, the TEX_{86}-based SSTs were 26–29 °C in the homogeneous intervals and decreased to 15–17 °C in the sapropels. The TEX_{86}-based SST values outside the sapropel probably reflect summer SST based on a comparison with the present-day Mediterranean Sea. The surprisingly low TEX_{86}-based SST estimates for the sapropels showed similarities with those obtained for the contemporary euxinic Black Sea. The distribution of marine Crenarchaeota in the modern Black Sea, i.e. thriving at the deeper and colder chemocline, suggests that this leads to a reduction in TEX_{86}, resulting in artificially low SST estimates. A similar situation is inferred for the Pliocene eastern Mediterranean during sapropel deposition since the SST anomaly co-occurs with the build-up of a shallow chemocline.

Keywords: Sapropels; Sea surface temperature; GDGTs; Alkenones; Pliocene; Black Sea

1. Introduction

Life on Earth can be classified into three fundamentally different domains: Bacteria, Archaea and Eukaryota (Woese, 1987). Archaea are known to live under extreme environmental conditions, such as high temperature, high salinity, high pressure, anoxia, and low and high pH, that are hostile to most other forms of life.

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Archaea are divided into three kingdoms, Crenarchaeota, Euryarchaeota and Korarchaeota (Woese et al., 1990; Barns et al., 1996; Takai and Sako, 1999). Since the application of molecular techniques to microbial ecology, in particular the use of 16S rRNA genes as a molecular marker, archaea have revealed to be widespread in different ecosystems and have been shown not to be restricted to extremophilic environments as was previously thought (Ward et al., 1992). A particularly exciting result was the discovery of the widespread occurrence of planktonic archaea in marine plankton in the Pacific Ocean and coastal waters of North America (Fuhrmann et al., 1992; DeLong, 1992). Among marine archaea, two major groups originating from the euryarchaeotal and crenarchaeotal group were identified in oceans, lakes and polar waters (e.g. Hershberger et al., 1996; McGregor et al., 1997; Massana et al., 1998). Pelagic euryarchaeota and Crenarchaeota showed different patterns of abundance in the open sea. High abundances of Crenarchaeota accounting for up to 40% of total DNA-containing picoplankton in mesopelagic (150–1000 m) and bathypelagic (1000–3500 m) waters (Karner et al., 2001; Church et al., 2003) were detected. In contrast, pelagic euryarchaeota showed higher abundances in coastal surface waters (Massana et al., 1997; Murray et al., 1999b; DeLong et al., 1999).

Membrane lipids of cultivated hyperthermophilic Crenarchaeota consist of diphytanyl glycerol diethers and glycerol dibiphytanyl glycerol tetraethers (GDGTs) (de Rosa and Gambacorta, 1988). The occurrence of ether-bound acyclic and cyclic biphytanes in particulate organic matter (POM) from the water column and surface sediments (e.g. Hoefs et al., 1997; King et al., 1998) as detected by chemical degradation studies, hinted to the presence of planktonic Crenarchaeota, and, thus confirmed the molecular ecological studies (e.g. Karner et al., 2001). The development of a high performance liquid chromatography/mass spectrometry technique has enabled the characterization of intact GDGTs (Hopmans et al., 2000). Using this technique, marine Crenarchaeota were found to contain GDGTs with zero to four cyclopentane rings (Schouten et al., 2000). A novel GDGT containing four cyclopentane rings and one cyclohexane ring was exclusively found in planktonic Crenarchaeota and was called crenarchaeol (Schouten et al., 2000; Sinninghe Damsté et al., 2002a). The biosynthesis of this GDGT, in particular the cyclohexane ring, is thought to be a membrane adaptation to allow these descendants of (hyper) thermophilic Crenarchaeota to cope with the relatively low temperatures of the ocean (Sinninghe Damsté et al., 2002a). The dominant presence of these membrane lipids confirmed their substantial contribution to the marine picoplankton in modern oceans (Schouten et al., 2000; Sinninghe Damsté et al., 2002a).

The number of cyclopentane moieties in GDGTs of cultivated (hyper)thermophilic archaea increases with increasing growth temperature (Gliozzi et al., 1983). A study of marine surface sediments indicated that non-thermophilic Crenarchaeota also adjust the number of cyclopentane moieties in their GDGT membrane lipids according to temperature (Schouten et al., 2002). By mesocosm studies we were able to show that this is indeed a physiological response (Wuchter et al., 2004). This temperature response was quantified in the so-called TEX_{86} index (Schouten et al., 2002), which revealed a strong linear correlation with sea surface temperature (SST) and can be used to reveal past SST (Schouten et al., 2003). The TEX_{86} derived from POM from the upper 100 m at various sites also showed a linear correlation with in situ temperature, which was nearly identical to the core top equation (Wuchter et al., 2005). This demonstrated that the GDGT signal which reaches the sediment is mainly derived from the upper 100 m of the water column, probably because these small (i.e. $<1\,\mu m$) archaeal cells are heavily grazed upon and their GDGTs are packaged in larger particles that rapidly descend to the sea floor in contrast to GDGTs derived from deep water dwelling archae (e.g. Wakeham et al., 2003). This was confirmed by a sediment trap study in the Arabian Sea; TEX_{86}-derived SSTs in the descending particles tracked seasonal changes in SST with a small delay of ca. 1–2 weeks (Wuchter et al., submitted for publication). Application of the TEX_{86} proxy in sediment cores from the Arabian Sea spanning the last 25 ka revealed that it is able to faithfully reconstruct the glacial/interglacial transition from lower to higher SSTs (Huguet et al., submitted for publication). A study of age-equivalent sediments from three cores from the Murray Ridge (Arbian Sea) at different positions within and below the oxygen minimum zone showed that variable degradation under oxic and anoxic conditions does not appear to affect the GDGT distribution and, thus, does not affect SST reconstructions using TEX_{86} (Schouten et al., 2004).

In this study, we analyzed GDGTs in three age-equivalent Pliocene sapropels to obtain insight in temporal and spatial changes in the abundances and distribution pattern of these biomarkers. Additionally, the newly established palaeo proxy for SST reconstruction, the TEX_{86}, was compared with previous results (Menzel et al., 2003) obtained from a more commonly used proxy for SST reconstruction in

palaeoceanography, the $U^{K'}_{37}$, which derives from the long-chain unsaturated alkenones of haptophyte algae (Brassell et al., 1986). These two SST palaeo proxies give divergent results for the three studied Pliocene sapropels. Whereas the $U^{K'}_{37}$-based SST showed values of ca. 25 °C, the TEX_{86} revealed a significant trend to lower temperatures down to 15–17 °C. Possible causes are discussed.

2. Materials and methods

2.1. Samples

Core samples were taken during ODP Leg 160 in the eastern Mediterranean Basin (Emeis et al., 1996). Site 967 (Eratosthenes Seamount) and Site 969 (Mediterranean Ridge) are located in the Levantine Basin. Site 964 is located in the Ionian Basin (Fig. 1a). The detailed chronology for these cores obtained by astronomical tuning enabled us to select three laterally equivalent sapropels with an age of 2.943 Ma (Lourens et al., 1996). Relevant core sections (967C-8H-4, 111–134 cm; 969E-6H-6, 23–43 cm; 964D-10H-1, 100.5–113 cm) were sub-sampled in 0.5–1.5 cm slices above, through and below the sapropel. Total organic carbon (TOC) was reported previously and showed distinct differences between these sapropels (Nijenhuis et al., 1998; Fig. 1b).

2.2. Lipid analyses

Ground, freeze-dried sediment samples (0.1 to 3 g) were Soxhlet extracted with DCM/methanol (1:1, v/v)

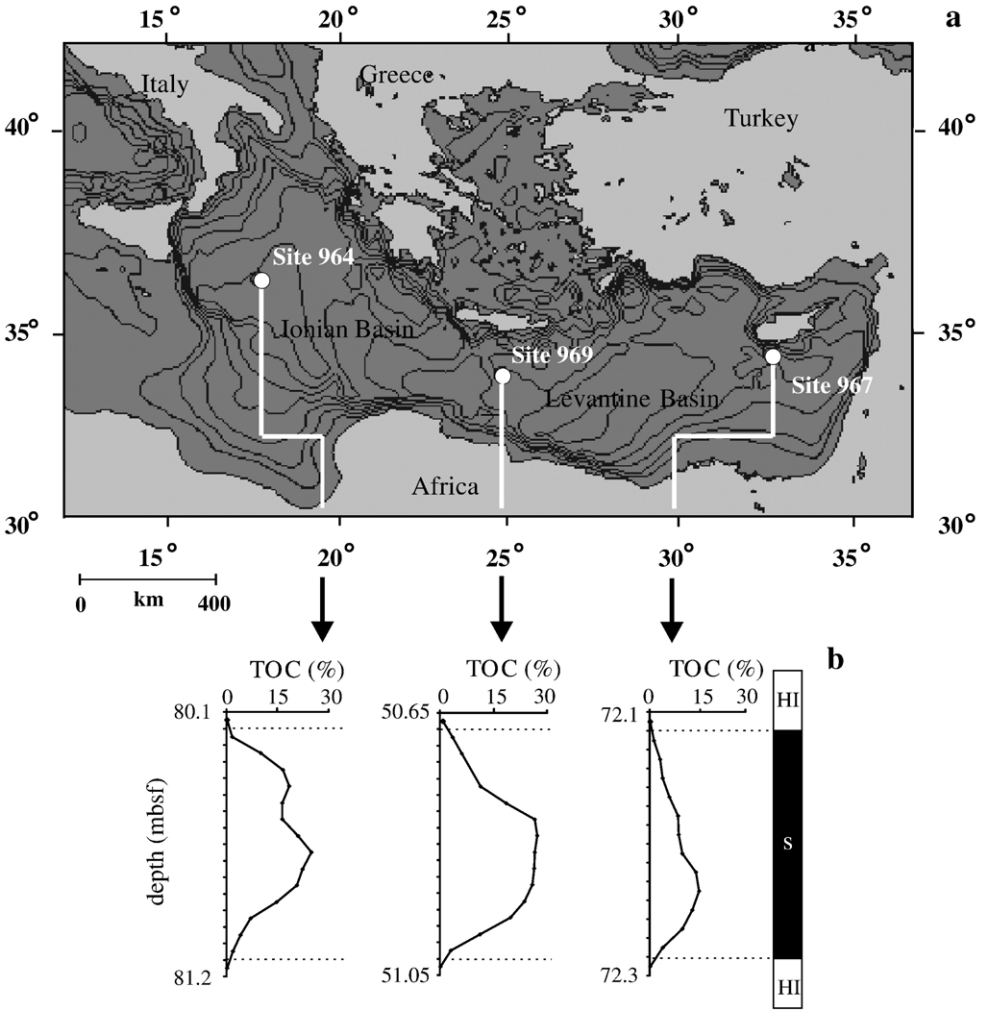

Fig. 1. (a) Location of ODP drilling sites and (b) lithological description (HI=homogeneous interval, S=sapropel) and TOC profiles of the investigated Pliocene sapropel (data from Nijenhuis et al., 1998). Depth scale is in meters below sea floor (mbsf).

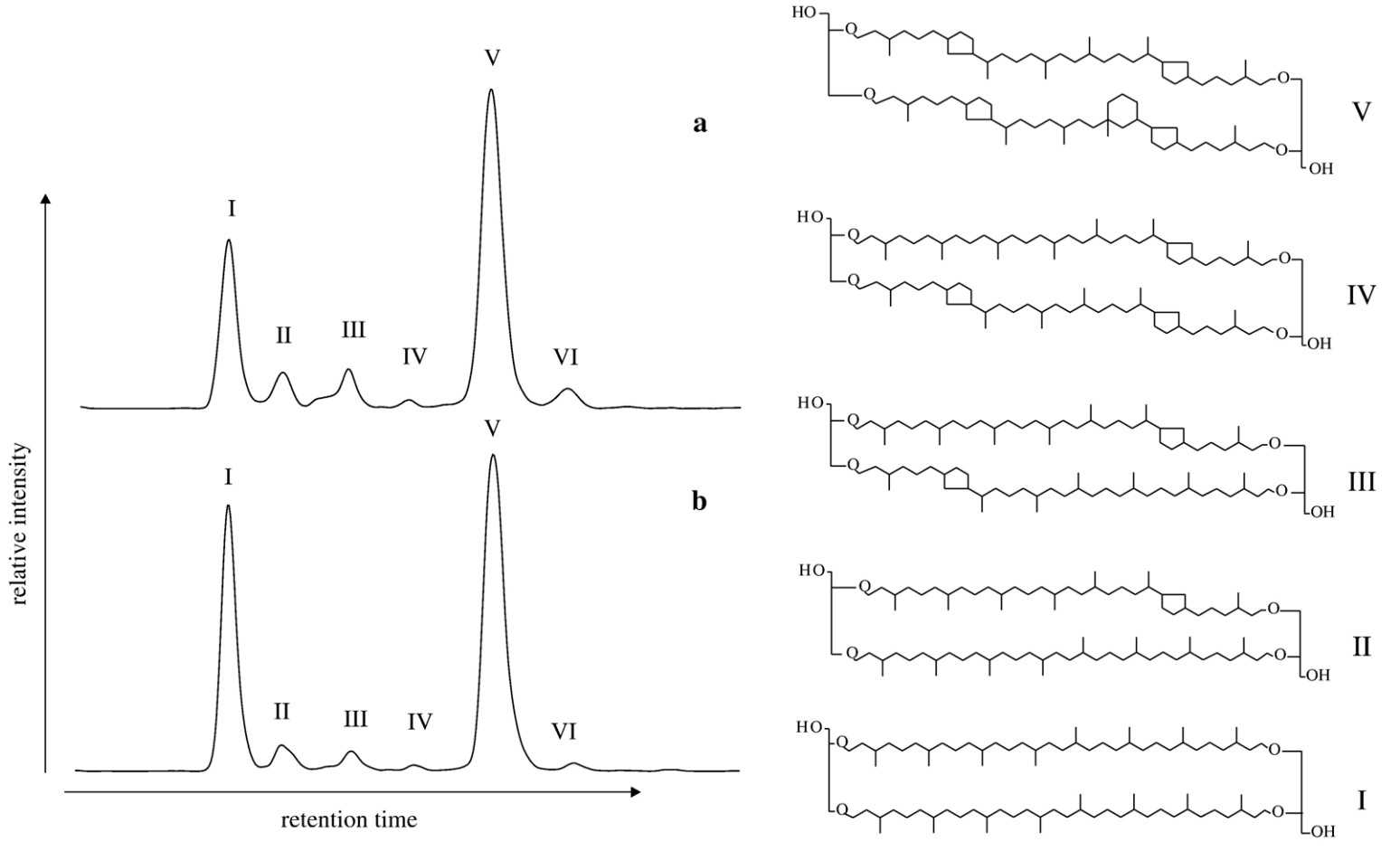

Fig. 2. HPLC/MS base peak chromatograms of (a) the homogeneous interval of ODP Site 160-967C-8H-4, 130–134 cm and (b) the sapropel of ODP Site 160-967C-8H-4, 121.5–122.5 cm. GDGT series were identified as: GDGT-0=I, GDGT-1=II, GDGT-2=III, GDGDT-3=IV, crenarchaeol=V and the regio-isomer of crenarchaeol=VI (cf. Wuchter et al., 2004).

mixture for 24 h. The extracts were concentrated with a rotary evaporator at 30 °C. An aliquot of the total extract (1.5 to 3 mg) was fractionated using a Pasteur pipette (150 mm length) packed with Al_2O_3 (activated for 2.5 h at 120 °C). The apolar fraction (F1) was eluted using a mixture of hexane/DCM (9:1, v/v; 4 ml). Subsequently, the ketone fraction (F2) was eluted using hexane/DCM (1:1, v/v; 4 ml). Finally, the polar fraction was obtained using DCM/methanol (1:1, v/v; 4 ml). The polar fraction was used to identify and quantify glycerol dibiphytanyl glycerol tetraethers (GDGTs) by high performance liquid chromatography/mass spectrometry analyses (HPLC/MS).

2.3. High performance liquid chromatography/mass spectrometry (HPLC/MS)

Analyses were performed using an HP (Palo Alto, CA, USA) 1100 series LC-MS equipped with an auto-injector and Chemstation chromatography manager software. Separation was achieved on a Prevail Cyano column (2.1 × 150 mm, 3 μm; Alltech, Deerfield, IL, USA), maintained at 30 °C. Injection volumes varied from 1 to 5 μl. GDGTs were eluted isocratically with 99% A and 1% B for 5 min, followed by a linear gradient to 1.8% B in 45 min, where A = hexane and B = propanol. Flow rate was 0.2 ml min^{-1}. After each analysis the column was cleaned by back-flushing hexane/propanol (90:10, v/v) at 0.2 ml min^{-1} for 10 min. Detection was achieved using atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS) of the eluent. Conditions for APCI-MS were as follows: nebulizer pressure 60 psi, vaporizer temperature 400 °C, drying gas (N_2) flow 6 l min^{-1}, and temperature 200 °C, capillary voltage −3 kV, corona 5 μA (~3.2 kV). Positive ion spectra were generated by scanning *m/z* 950–1450 in 1.9 s.

2.4. Determination of accumulation rates and sea surface temperature estimates

Absolute GDGT amounts (μg g^{-1} dry weight sediment) were used to calculate accumulation rates (ARs), assuming (i) a synchronous start and end of sapropel deposition at the three locations, (ii) a duration of sapropel deposition of 7000 years (Nijenhuis and de Lange, 2000) and (iii) a constant sedimentation rate during sapropel deposition (Menzel et al., 2002). ARs in the homogeneous intervals were not calculated because sedimentation rates are expected to be substantially different during times of deposition of sapropel and the homogeneous intervals.

The TEX_{86} index (TetraEther indeX of GDGTs consisting of 86 carbon atoms; see Fig. 2 for GDGT structures) defined as:

$$TEX_{86} = \frac{([III] + [IV] + [VI])}{([II] + [III] + [IV] + [VI])} \quad (1)$$

was used to reveal past SST (Schouten et al., 2002) using the calibration line:

$$TEX_{86} = 0.015SST + 0.28 \quad (2)$$

3. Results

3.1. GDGT distribution

The HPLC/MS base peak chromatograms revealed the presence of the GDGTs I–VI (Fig. 2) in both the sapropels and the homogeneous sediments below and above the sapropel at the three sites. GDGT-0 (I) and crenarchaeol (V) were the dominant GDGTs (Fig. 2). A comparison of the GDGT distribution of the homogeneous sediment and the sapropel of Site 967 indicated internal variations in the relative distribution of the analyzed GDGTs. In the homogeneous intervals of the sapropels (Fig. 2a) higher relative abundances of GDGT-2, GDGT-3 and crenarchaeol (V + VI) were detected, compared with the GDGT distribution in the sapropel (Fig. 2b). Smaller variations in the relative distribution of GDGTs within the three age-equivalent sapropels were also observed as is expressed by variations in TEX_{86} values (see below).

3.2. Temporal and spatial distribution pattern of tetraether lipid abundance

The total GDGT content below the sapropel at Site 967 showed values of 1670 μg g^{-1} TOC and reached a maximum of 2790 μg g^{-1} TOC when the TOC content was the highest (Fig. 3). After the maximum was reached, the GDGT content decreased substantially in the central part of the sapropel to values of about 100 μg g^{-1} TOC and increased again in the upper part of the sapropel to 1600 μg g^{-1} TOC. Above the sapropel the GDGT content was 550 μg/g TOC. Site 969 showed below the sapropel GDGT amounts of 640 μg g^{-1} TOC (Fig. 3). In the lower and central part of the sapropel the values remained relatively constant and then increased to maximum value of 2150 μg g^{-1} TOC. After the maximum was reached, the GDGT content decreased noticeably towards the top of the sapropel to values of about 150 μg g^{-1} TOC. Above the sapropel the GDGT

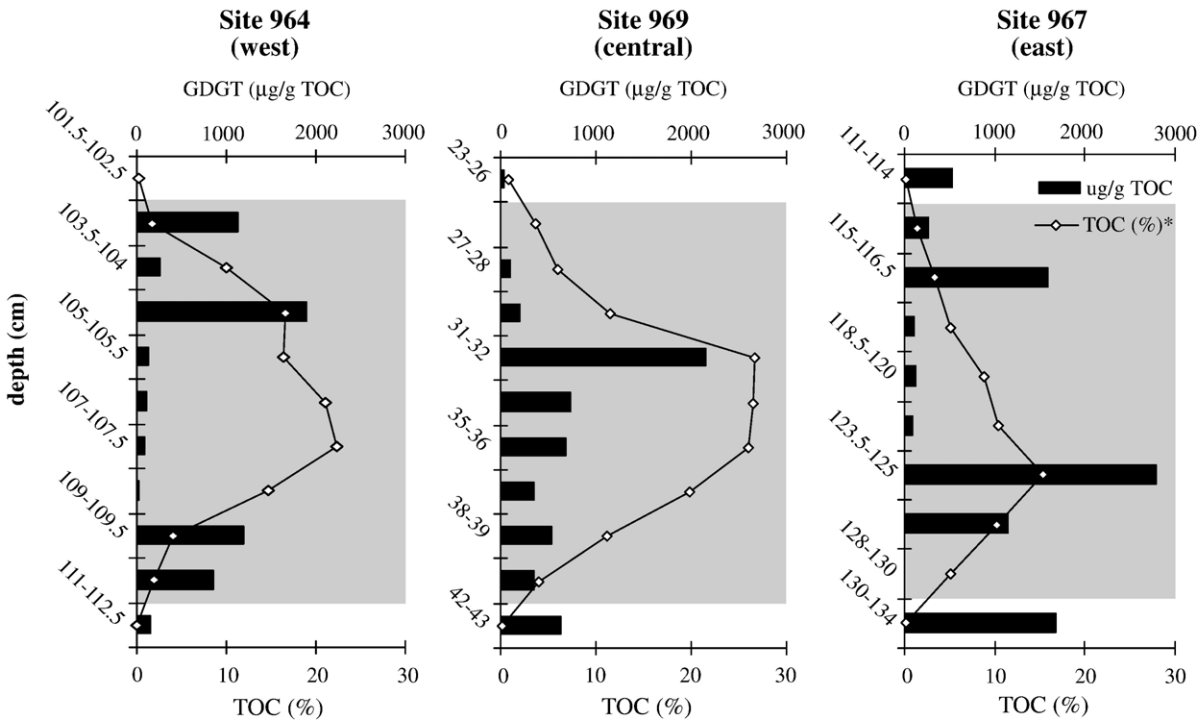

Fig. 3. Summed GDGT concentrations (normalized to TOC) and TOC content (∗ data from Nijenhuis et al., 1998) plotted against depth (in the specific core, see Materials and methods) for the three sapropels at ODP Sites 967, 969 and 964. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze.

content decreased to values of 40 µg g^{-1} TOC. Site 964 revealed below the sapropel GDGT values of 160 µg g^{-1} TOC (Fig. 3). At the base of sapropel the GDGT content increased to values of 1200 µg/g TOC, but then decreased considerably to values of about 100 µg g^{-1} TOC in the central part of the sapropel. Maximum concentrations of GDGT occurred in the upper part of the sapropel with 1900 µg g^{-1} TOC. Above the sapropel the GDGT content was negligible.

In the lower part of sapropel at Site 967, GDGT accumulation rates (ARs) (see Materials and methods for details) reached a maximum value of 12.3 mg m^{-2} yr^{-1} and collapsed to values ranging between 0.2 and 0.4 mg m^{-2} yr^{-1} at the central part of the sapropel where these low values remained up to the upper part of the sapropel (Fig. 4). At Site 969, GDGT ARs increased steadily reaching a maximum value at the central part of the sapropel with values of 17.2 mg m^{-2} yr^{-1} (Fig. 4). After the maximum was reached, GDGT ARs decreased considerably to values of 0.7 mg m^{-2} yr^{-1} at the upper part of the sapropel. Site 964 showed the lowest GDGT ARs with a slight increase in the lower part of the sapropel of 0.8 mg m^{-2} yr^{-1}. Through the entire central part of the sapropel low ARs of about 0.3 mg m^{-2} yr^{-1} were observed, whereas in the upper part of the sapropel a maximum value of 4.6 mg m^{-2} yr^{-1} was reached (Fig. 4). After the GDGT AR reached the maximum, it decreased dramatically as was observed at the other two sites.

3.3. SST estimation with the TEX_{86} proxy

The TEX_{86} showed distinct changes in SST between the homogeneous intervals and Pliocene sapropels at all three studied ODP sites (Fig. 5). In the homogeneous intervals below the sapropel, the TEX_{86} revealed highest SST at Site 969 of 29 °C and slightly lower SSTs at Site 967 and 964 of 27 °C and 25 °C, respectively. In all sapropels, the TEX_{86}-based SST showed a substantial decrease. The largest drop (15 °C) relative to the TEX_{86} temperatures of the homogeneous calcareous sediments was observed at Site 967 in the lower part of the sapropel (Fig. 5). In the upper part of the sapropel the SST slowly increased to values of 22 °C. In the homogeneous interval above the sapropel the SST reached 26 °C. At Site 969, the SST dropped within the sapropel to values around 17 °C in the central part of the sapropel and increased also slowly towards the top of the sapropel to values of 21 °C. In the homogeneous interval above the sapropel the SST remained at 21 °C

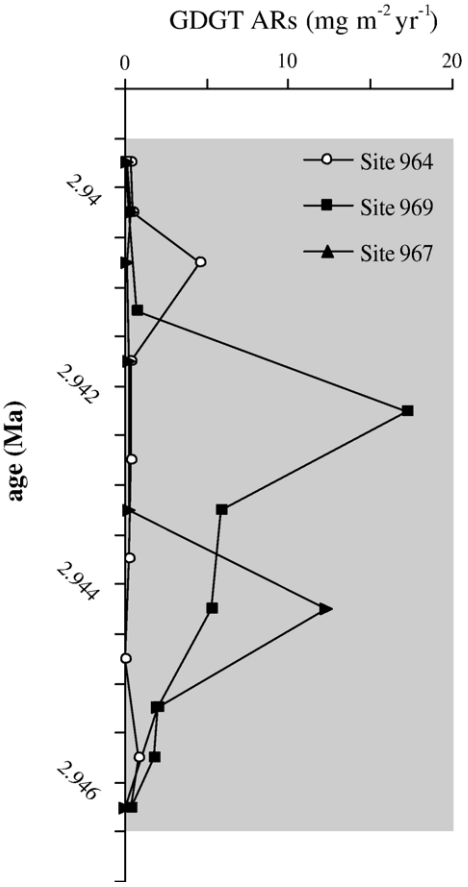

Fig. 4. Summed GDGT ARs in the three sapropels of the three ODP Sites 967, 969 and 964. Note that the ARs are plotted versus age (see Materials and methods for the involved assumptions).

(Fig. 5). In the sapropel at Site 964 the SST at the base of the sapropel was the highest with 33 °C and then decreased towards the top of the sapropel to values of 18 °C. In the homogeneous interval above this sapropel, some GDGT isomers were below the detection limit and no TEX_{86} values could be obtained (Fig. 5).

4. Discussion

4.1. Origin of GDGT lipids

GDGT-0 (I) is a general archaeal core membrane lipid (e.g. Koga et al., 1998) and occurs not only in planktonic Crenarchaeota but also in methanogens (Koga et al., 1993). Crenarchaeol (V) has been assigned as a specific core membrane lipid of planktonic Crenarchaeota (Schouten et al., 2000; Sinninghe Damsté et al., 2002a). The dominance of GDGT-0 and crenarchaeol in both the homogeneous intervals and the sapropels (Fig. 2) is characteristic for the GDGTs of planktonic Crenarchaeota (DeLong et al., 1998; Sinninghe Damsté et al., 2002b). Such GDGT membrane lipid distributions were also observed in many other marine sediments (Schouten et al., 2002), marine POM (Sinninghe Damsté et al., 2002b; Wuchter et al., 2003, 2005) and descending particles (Wuchter et al., submitted for publication) from various locations. GDGTs in the water column of the Black Sea also showed a predominance of GDGT-0 and crenarchaeol in the upper 400 m (Wakeham et al., 2003). By contrast, GDGTs derived from anaerobic, methane-oxidizing archaea, which reside in the deeper anoxic zone (>1000 m water depth) have a distinct distribution of carbon isotopically depleted GDGTs. A dominance of GDGT-0 and crenarchaeol in GDGT distributions was found in sinking particles collected with sediment traps in the Black Sea. The GDGTs derived from methane-

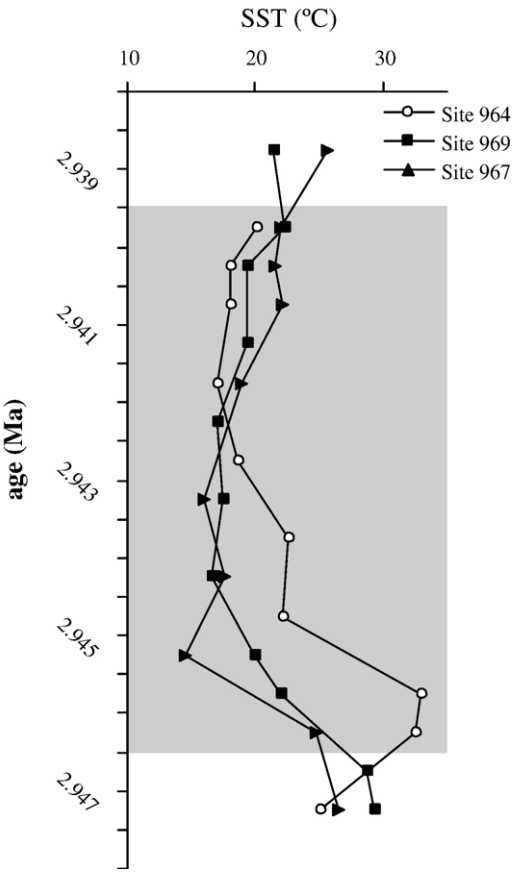

Fig. 5. SST (°C) reconstruction based on the TEX_{86} proxy (after Schouten et al., 2002) of three time-equivalent Pliocene sapropels of the eastern Mediterranean Sea. Light grey shaded regions represent the sapropel. White regions represent the homogeneous calcareous ooze. Note that the SSTs are plotted versus age (see Materials and methods for the involved assumptions).

oxidizing archaea were not detected in sediment traps or underlying surface sediments, showing that the sedimentary GDGTs are predominately derived from surface waters. This is apparently caused by efficient transport mechanism (e.g. grazing and production of fecal pellets) of GDGTs in surface waters (Wakeham et al., 2003).

Coolen et al. (2002) proposed that metabolically active marine chemoorganotrophic Crenarchaeota are present in late Pleistocene sapropels of the eastern Mediterranean Basin. These authors suggested that, in comparison with the homogeneous intervals, sapropels exhibit elevated microbial cell numbers with Crenarchaeota constituting ca. 16% of the total cells. Increased enzyme activities of anaerobic microbial degradation using carbon substrates originating from the sapropel organic matter were measured and ascribed, in part, to metabolically active Crenarchaeota (Coolen et al., 2002). This indicates that the GDGTs found in our sapropels could derive from physiologically active Crenarchaeota in the sapropels and do not represent chemical fossils derived from planktonic Crenarchaeota. However, the GDGT abundances of a sapropel showed large fluctuation on a cm scale (Fig. 3), which is not consistent with chemoorganotrophic Crenarchaeota using sapropel organic matter as carbon source as the source of these GDGTs. The ^{13}C contents of biphytanyl moeities of crenarchaeol from both present-day and ancient marine environments (Hoefs et al., 1997; Kuypers et al., 2001) and their ^{14}C content (Pearson et al., 2001) indicated that marine Crenarchaeota probably fix bicarbonate. ^{13}C label experiments confirmed a light-independent bicarbonate uptake by crenarchaeol-producing Crenarchaeota (Wuchter et al., 2003), indicating chemoautotrophy and not chemoorganotrophy (cf. Coolen et al., 2002) by marine planktonic Crenarchaeota. In addition to the large variations in the absolute abundances of GDGTs, also large variations in the GDGT distributions within the sapropel are observed. As these represent physiological adaptation of their membrane to temperature (see detailed discussion below), this provides an additional argument for their origin from planktonic Crenarchaeota and not from living chemoorganotrophic Crenarchaeota within the sapropel, which would encounter identical sedimentary temperature conditions. This is consistent with the recent finding that the marine Crenarchaeota found in Mediterranean sapropels (Coolen et al., 2002) fall in a phylogenetic cluster of Crenarchaeota that do not produce crenarchaeaol (Coolen and Sinninghe Damsté, unpublished results).

4.2. SST reconstructions using $U^{K'}_{37}$ and TEX_{86}

$U^{K'}_{37}$-based SST derived from the haptophyte algae revealed almost similar SST values of 24–26 °C obtained in the three age-equivalent Pliocene sapropels and their corresponding homogeneous intervals (Fig. 6) (Menzel et al., 2003). In contrast, the TEX_{86}-based SST estimates showed substantial differences between the homogeneous intervals and the sapropels as well as between these sapropels (Fig. 6). The reason for this large difference most likely lies in the fact that both SST proxies originate from two different organisms occupying different habitats in the eastern Mediterranean Basin. Alternatively, the archaeal GDGTs could derive from terrestrial sources (e.g. soils, lakes or rivers; Schouten et al., 2000; Powers et al., 2004; Weijers et al., in press, submitted for publication). It is generally assumed that the fresh water runoff from the continent to the Mediterranean was substantially increased during times of sapropel deposition. However, we have recently shown that contribution from terrestrial sources only affects the GDGT distribution in marine sediments if relatively high amounts of non-isoprenoidal GDGTs derived from soil bacteria (Weijers et al., in press) are detected in marine sediments (Weijers et al., submitted for publication). This can be expressed using the so-called Branched and Isoprenoid Tetraether (BIT) index, based on the relative abundance of terrestrially derived tetraether lipids versus crenarchaeol (Hopmans et al., 2004). If BIT values are <0.30, no major interference of terrestrial GDGTs on the TEX_{86}-based SSTs is apparent (Weijers et al., submitted for publication). BIT indices for the Pliocene sapropels were <0.1 and for the homogenous intervals were <0.2 and, thus, indicate that TEX_{86}-derived SSTs are not significantly affected by input of terrestrial organic matter.

In the contemporary oligotrophic eastern Mediterranean Sea the annual phytoplankton bloom occurs in winter as soon as deep water mixing occurs, providing nutrients to the photic zone, and ceases when phosphate is depleted (Krom et al., 2003). After the winter bloom, water column stratification occurs in March–April, which results in the formation of a deep chlorophyll maximum that is characteristic for the remainder of the year (Krom et al., 2003). Haptophytes live in the upper 200 m and are the most common eukaryotes followed by diatoms and dinoflagellates (Kimor et al., 1987). An $U^{K'}_{37}$-based SST of ca. 19 °C was estimated from suspended matter collected in sediment traps (Ternois et al., 1997) and surface sediments of the Mediterranean Basin (Emeis et al., 2000), reflecting the SST in winter when haptophyte production was most pronounced and

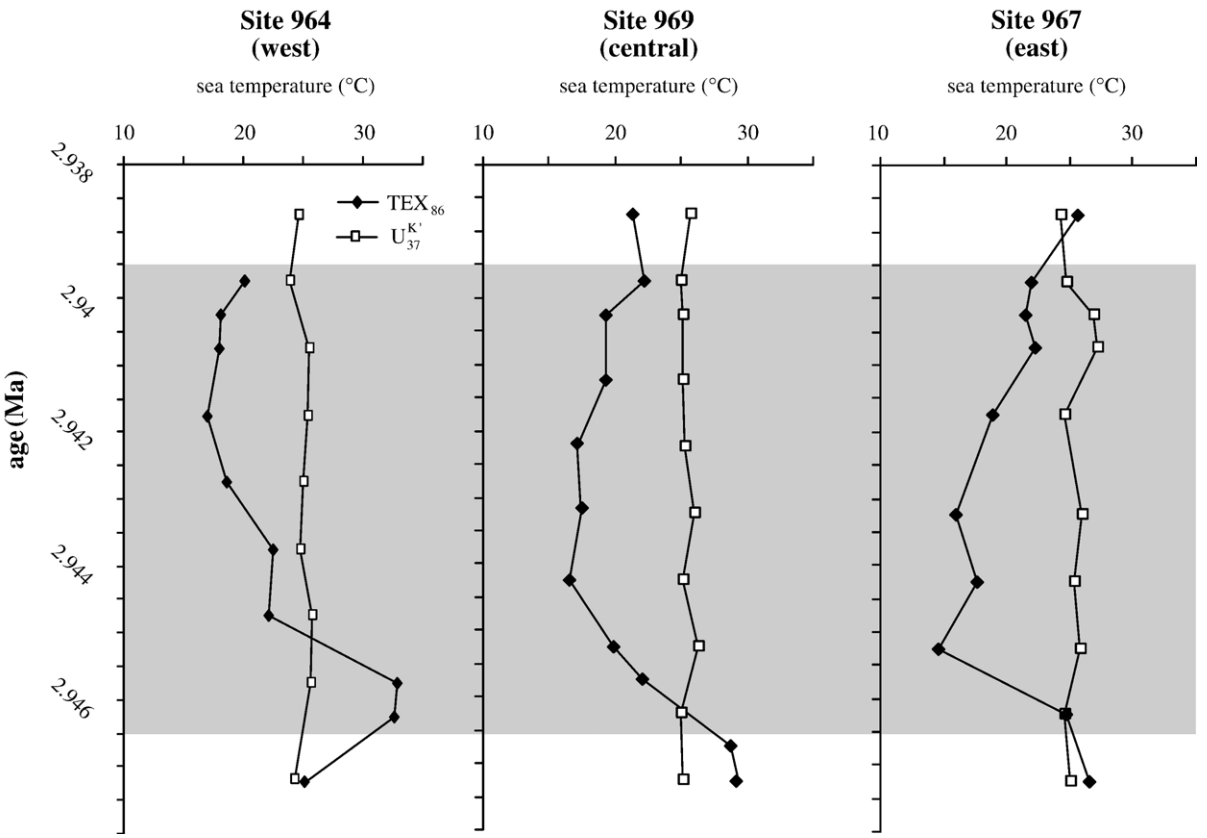

Fig. 6. Reconstruction of the SST (°C) during Pliocene sapropel deposition using two different SST proxies $U^{K}_{37}{}'$ and TEX_{86}, in the sapropels at ODP Sites 967, 969 and 964. Note that the SSTs are plotted versus age (see Materials and methods for the involved assumptions).

when water temperatures were cooler compared to annual mean SST. Planktonic Crenarchaeota typically have their main phase of growth during the annual cycle outside the main period of phytoplankton blooms. A negative correlation was obtained between the abundance of chlorophyll *a* and particulate organic carbon and the abundance of Crenarchaeota for Antarctic coastal waters and Southern Ocean (polar regions) (Murray et al., 1998; Murray et al., 1999b), Santa Barbara Channel (Murray et al., 1999a) and the North Sea (Wuchter et al., submitted for publication). Schouten et al. (2002) also inferred different growing seasons for haptophytes and marine Crenarchaeota in the eastern South Atlantic. Indeed, TEX_{86} values in surface sediments of the eastern Mediterranean Sea indicate an SST of ca. 26 °C (Sinninghe Damsté et al., unpublished data), which reflects the SST during summer in the Mediterranean Sea.

Thus, the $U^{K'}_{37}$- and TEX_{86}-based SSTs in the homogeneous intervals (thought to reflect periods in the Pliocene comparable with the modern oligotrophic eastern Mediterranean Sea) are likely reflecting the winter and summer SST, respectively. These SST estimates are realistic values considering the warmer climate conditions during the Pliocene, compared to the present-day conditions (e.g. Poore and Sloan, 1996).

During sapropel deposition, the precession minimum caused warmer and especially more humid climate conditions with a stronger seasonal climate contrast (e.g. Rossignol-Strick et al., 1982; Rossignol-Strick, 1985; Prell and Kutzbach, 1987; Hilgen, 1991). Due to warmer summer and colder winter periods, lower $U^{K'}_{37}$-based and higher TEX_{86}-based SSTs could be expected, if the timing of the bloom seasons of the haptophytes and Crenarchaeota in the past was not different from that in the present-day Mediterranean. The $U^{K'}_{37}$ values in the sapropel, compared with those in the homogeneous intervals, indicated an increase in SST of 1 °C at maximum. $U^{K'}_{37}$-based SSTs obtained for other Pliocene sapropels showed a slight decrease of 1 °C in SST (Rinna et al., 2002). $U^{K'}_{37}$ values of late Pleistocene to Holocene sapropels, relative to those of the homogeneous intervals also only revealed a small change in SST of a few degrees (Emeis et al., 1998). In strong

contrast with the $U^{K'}_{37}$-based SST estimates in the Pliocene sapropels, the TEX_{86}-based SST revealed an unexpected cooling, i.e. a drop of 10–12 °C in SST (Fig. 6), which seems to be at odds with the reported climatic change. This must indicate that either the ecological niche of the planktonic Crenarchaeota or the timing of their growth season must have been fundamentally different at the time of sapropel deposition.

During Pliocene sapropel deposition a substantial increase in primary productivity, compared to the situation before and after sapropel deposition (represented by the homogeneous intervals), is indicated by the enhanced Barium flux (Nijenhuis and de Lange, 2000) and was caused by increased nutrient delivery to the basin (Passier et al., 1999). The increased primary productivity is also revealed by increasing accumulation rates of specific biomarkers for the major groups of phytoplankton (diatoms, haptophytes, dinoflagellates, eustigmatophytes) towards the centre of the sapropel (Menzel et al., 2003). The enhanced organic matter flux to the sea floor would lead to oxygen depletion, sulphate reduction and ultimately to the presence of sulphide in the photic zone of the water column. The presence of such a shallow chemocline was indicated by the presence of isorenieratene derivates (Passier et al., 1999) and intact isorenieratene (Menzel et al., 2002), the characteristic pigment of anaerobic, photolithotrophic green sulfur bacteria, which require both sulphide and light. Such conditions are currently found in the Black Sea (Repeta et al., 1989), the world's largest euxinic basin. Therefore, the Black Sea is a good model to compare environmental situations related to sapropel deposition in the Mediterranean Sea. Marine primary productivity in the Black Sea

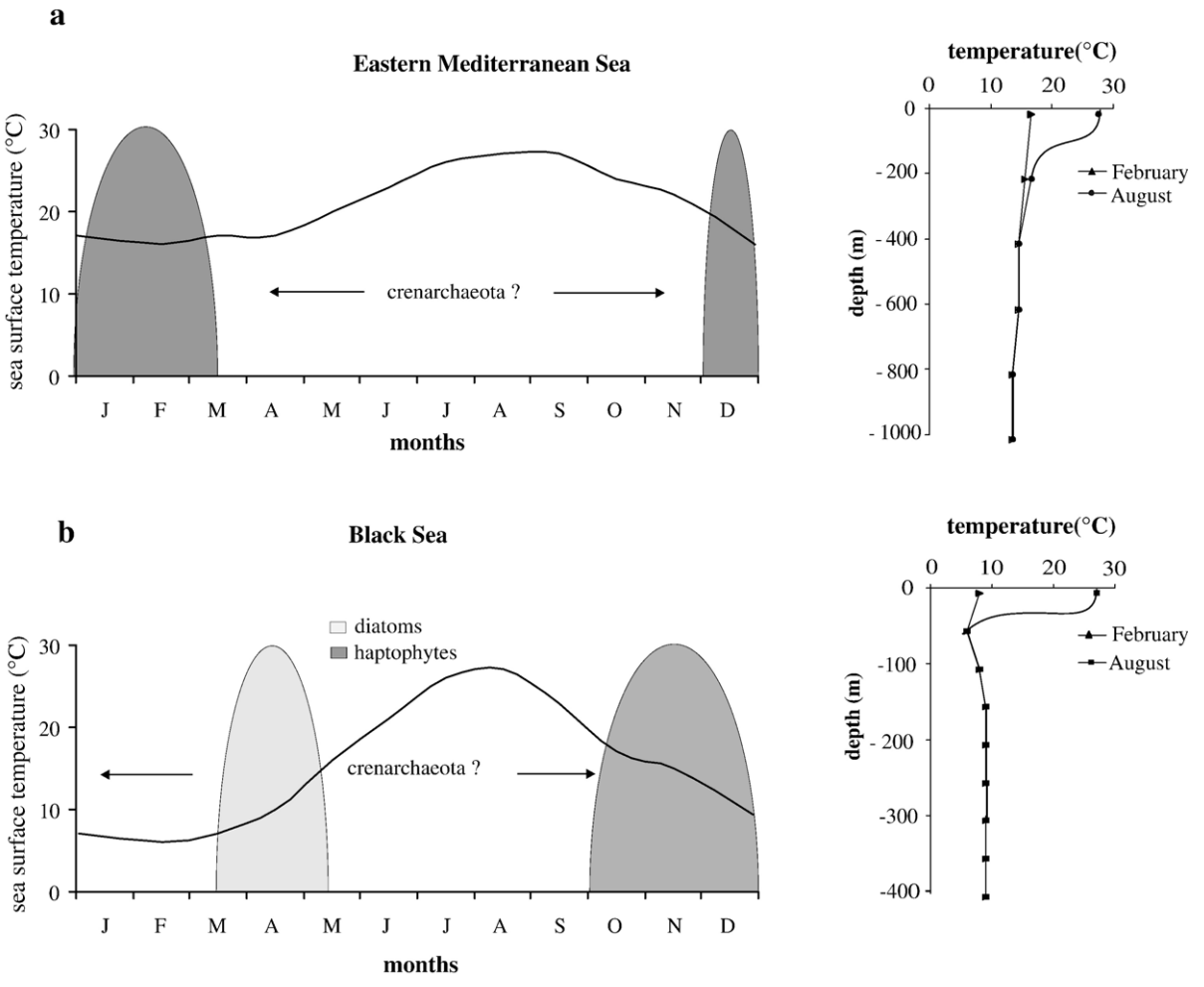

Fig. 7. Illustration of monthly (average) sea surface temperature (SST, °C), water temperature depth profile and the seasonal cycle of the major phytoplankton bloom in (a) the eastern Mediterranean Sea and (b) the Black Sea based on data from (www.nemoc.navy.mil/images/climo/med).

(210 g C m^{-2} yr^{-1}; Karl and Knauer, 1991) is substantially higher than in the oligotrophic eastern Mediterranean Sea (26 g C m^{-2} yr^{-1}; Bethoux, 1989), and rather similar with that estimated for the eastern Mediterranean Sea during Pliocene sapropel deposition (ca. 150 g C m^{-2} yr^{-1}), based on Barium ARs (Passier et al., 1999). The Black Sea shows in general two major phytoplankton blooms, a diatom and silicoflagellate bloom in spring and a haptophyte bloom in autumn (Honjo et al., 1987).

The $U^{K'}_{37}$-based SST of 13 °C obtained from the Black Sea surface sediments (Freeman and Wakeham, 1992) reflects autumn SSTs. A TEX_{86}-based SST of 5 °C was determined for settling particles collected with a sediment trap (Wakeham et al., 2003) and 11 °C for surface sediment (0–1 cm) (Schouten et al., 2002). This TEX_{86} value gives an even lower SST than observed with the $U^{K'}_{37}$-based SST. Both values are low in comparison to annual mean SST in the Black Sea (Fig. 7). The low TEX_{86}-based SST, compared with the $U^{K'}_{37}$-based SST, shows similarities of SST data obtained from the studied Pliocene sapropels (Fig. 8). It should be noted that the SSTs reported from the Black Sea show in general colder SSTs, compared to the Mediterranean Sea, because of its location at higher latitude.

Water column studies of the Black Sea could provide insight in the observed TEX_{86}-based SST "anomaly". In the Black Sea water column, a sharp peak in GDGT concentrations at the chemocline (ca. 100 m) was revealed for both summer (July 1988) (Wakeham et al., 2003) and winter (December 2001) without a substantial difference in absolute abundance between the summer and winter situation (Sinninghe Damsté et al., unpublished data), suggesting that there is no large contrast in the seasonal abundance of the Crenarchaeota. If the GDGT distribution would reflect the annual average SST, temperatures of ca. 17 °C would be expected. However, average TEX_{86}-based SSTs of 9 °C in July 1988 (Schouten et al., 2002) and 6 °C in December 2001 (Sinninghe Damsté et al., unpublished data) for water column POM from 0 to 400 m were measured, in line with the relatively low TEX_{86}-based estimates for sediment traps and the surface sediment. The relatively low TEX_{86}-based SST estimates may be related to the specific distribution of Crenarchaeota in the Black Sea (i.e. peaking at the chemocline) in combination with the strong thermal stratification in summer (Fig. 7). This stratification results in low temperatures (ca. 8 °C) at the chemocline throughout the annual cycle. It is likely that this population of Crenarchaeota also contributes to the GDGT flux to the sediments since, in contrast to the much deeper dwelling population of methane-oxidizing Crenarchaeota, these archaea can still be grazed upon. This may explain the anomalously low TEX_{86}-based SST estimates in the Black Sea.

It is likely that the observed TEX_{86}-based SST "anomaly" in the Pliocene sapropels results from the

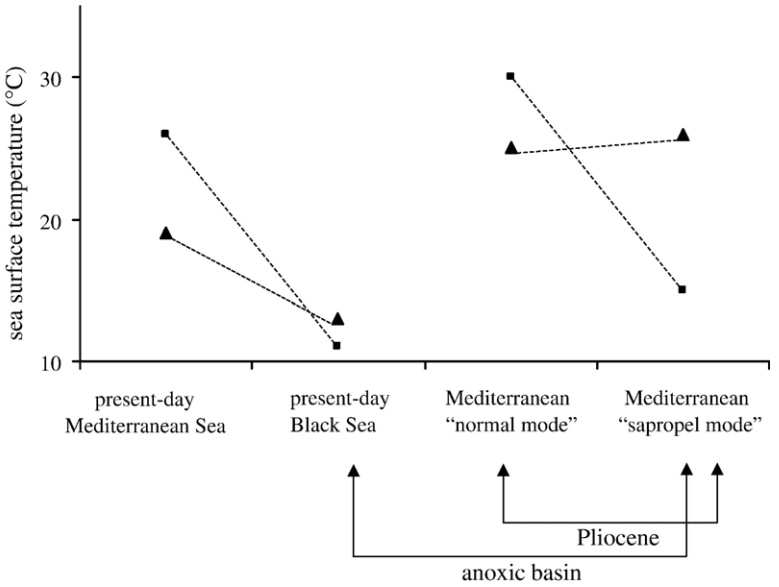

Fig. 8. Sea surface temperature reconstruction using the $U^{K'}_{37}$ (▲) and TEX_{86} (■) between the Mediterranean Sea at present-day, during homogeneous intervals and sapropel deposition as well as the present-day Black Sea.

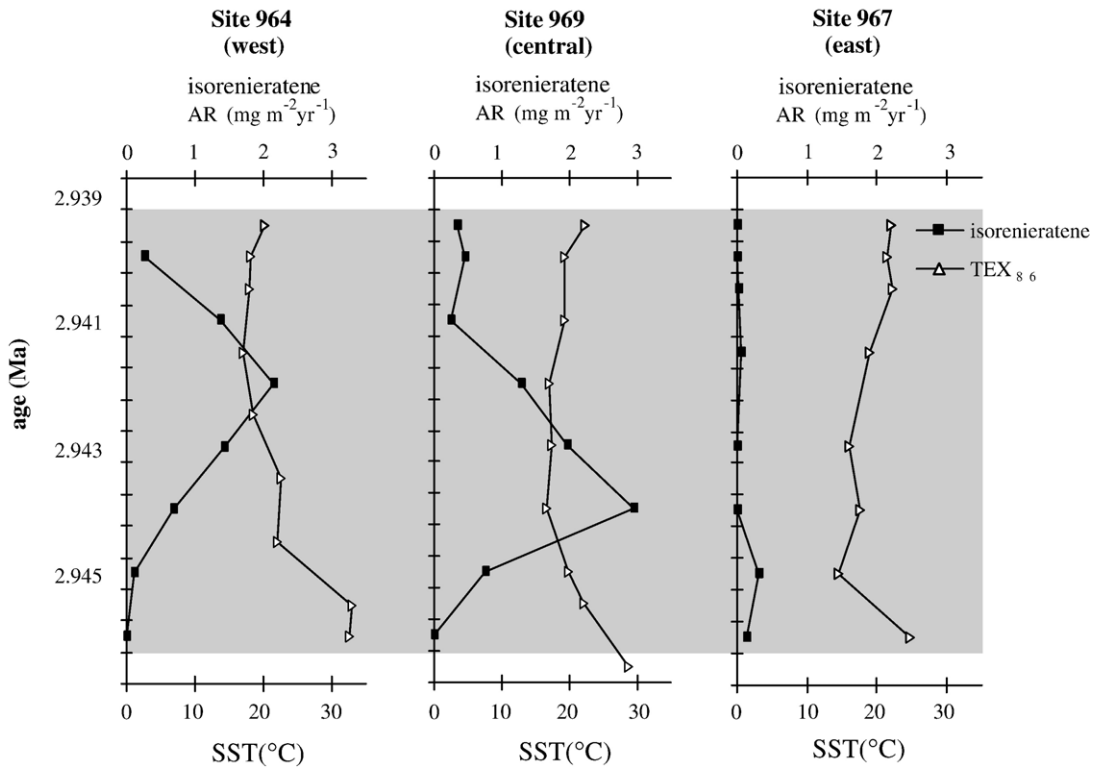

Fig. 9. Comparison of isorenieratene accumulation rates and TEX_{86}-based SST estimates in the sapropel at Sites 967, 969 and 964. Note that the data are plotted versus age (see Materials and methods for the involved assumptions).

same mechanism as in the Black Sea. Indeed, the large drop in TEX_{86}-based SST is in all sapropels associated with the shoaling of the chemocline which is revealed by increased levels of isorenieratene, the characteristic pigment of green sulfur bacteria (Fig. 9). It seems that the availability of nutrients drives the planktonic Crenarchaeota to a different ecological niche, in contrast with the oligotrophic Mediterranean Sea, where the TEX_{86}-based SST derives from the upper water column during the summer (Fig. 7). However, at present, the ecophysiological factors determining the abundance of planktonic Crenarchaeota are still unknown to fully understand this relationship.

5. Conclusions

The dominance of GDGT-0 and crenarchaeol among the GDGTs and the large variations in absolute abundances of GDGTs and their distribution (expressed as in the TEX_{86}-index) within the three Pliocene sapropels and their corresponding homogeneous intervals indicated the presence of fossil GDGTs derived from planktonic Crenarchaeota. Differences in $U^{K}_{37}{}'$- and TEX_{86}-based SST estimates obtained in both, the homogeneous intervals and sapropels are due to the fact that both SST proxies originate from two different organisms occupying different habitats in the eastern Mediterranean Basin. Whereas the $U^{K'}_{37}$-based SST estimates showed hardly any differences between the homogeneous intervals and the sapropels, the TEX_{86}-based SST estimates revealed a cooling of 10–12 °C during sapropel deposition. TEX_{86}-based SST estimates obtained in the homogeneous intervals showed similarities with the modern oligotrophic Mediterranean Sea, while the TEX_{86}-based SST estimates in the sapropels showed similarities with the contemporary euxinic Black Sea. It is assumed that this TEX_{86}-based SST trend is caused by a shallowing chemocline during sapropel deposition, which has driven planktonic Crenarchaeota to a different ecological niche compared to the situation during the deposition of homogeneous intervals. This change of niche of the pelagic Crenarchaeota may perhaps have implications for the application of TEX_{86} in determining past SSTs of marine systems with a shallow chemocline. Ecophysiological studies on planktonic Crenarchaeota are required to further unravel this TEX_{86}-based SST "anomaly".

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