Biogeosciences Discuss., 12, 12455–12484, 2015 www.biogeosciences-discuss.net/12/12455/2015/ doi:10.5194/bgd-12-12455-2015 © Author(s) 2015. CC Attribution 3.0 License.



This discussion paper is/has been under review for the journal Biogeosciences (BG). Please refer to the corresponding final paper in BG if available.

Contribution of Marine Group II *Euryarchaeota* to cyclopentyl tetraethers in the Pearl River estuary and coastal South China Sea: impact on the TEX₈₆ paleothermometer

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Received: 15 July 2015 – Accepted: 25 July 2015 – Published: 7 August 2015

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Published by Copernicus Publications on behalf of the European Geosciences Union.





Abstract

TEX₈₆ (TetraEther indeX of glycerol dialkyl glycerol tetraethers (GDGTs) with 86 carbon atoms) has been widely applied to reconstruct (paleo-) sea surface temperature (SST). While Marine Group I (MG I) *Thaumarchaeota* have been commonly believed to be the source for GDGTs, Marine Group II (MG II *Euryarchaeota*) have recently been suggested to contribute significantly to the GDGT pool in the ocean. However, little is known how the MG II *Euryarchaeota*-derived GDGTs may influence TEX₈₆ in marine sediment record. In this study, we characterize MG II *Euryarchaeota*-produced GDGTs and assess the likely effect of these tetraether lipids on TEX₈₆. Analyses of

- ¹⁰ core lipid (CL-) and intact polar lipid (IPL-) based GDGTs, 454 sequencing and quantitative polymerase chain reaction (qPCR) targeting MG II *Euryarchaeota* were performed on suspended particulate matter (SPM) and surface sediments collected along a salinity gradient from the lower Pearl River (river water) and its estuary (mixing water) to the coastal South China Sea (seawater). The results showed that the community
- ¹⁵ composition varied along the salinity gradient with MG II *Euryarchaeota* as the second dominant group in the mixing water and seawater. qPCR data indicated that the abundance of MG II *Euryarchaeota* in the mixing water was three to four orders of magnitude higher than the river water and seawater. Significant linear correlations were observed between the gene abundance ratio of MG II *Euryarchaeota* vs. total archaea and the
- relative abundance of GDGTs-1, -2, -3, or -4 as well as the ring index based on these compounds, which collectively suggest that MG II *Euryarchaeota* may actively produce GDGTs in the water column. These results also show strong evidence that MG II *Euryarchaeota* synthesizing GDGTs with 1–4 cyclopentane moieties may bias TEX₈₆ in the water column and sediments. This study highlights that valid interpretation of TEX₈₆
- ²⁵ in sediment record, particularly in coastal oceans, needs to consider the contribution from MG II *Euryarchaeota*.





1 Introduction

TEX₈₆ is a popular temperature proxy in paleoclimatological studies (Schouten et al., 2013; Pearson and Ingalls, 2013). It is based upon relative distribution of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs; see Supplement Fig. S1 for structures;

Schouten et al., 2002), which ubiquitously occur in marine and terrestrial environments. The global core-top calibrations of TEX₈₆ are empirically correlated with annual mean sea surface temperature (SST; Schouten et al., 2002; Kim et al., 2008, 2010; Tierney and Tingley, 2014), with the assumption that the majority of sedimentary GDGTs are produced by planktonic *Thaumarchaeota* (Schouten et al., 2002; Kim et al., 2008).
 However, mounting evidence indicates anomalies or discrepancies in TEX₈₆-derived SST in coastal seas and open ocean, which have been interpreted as additional contribution of GDGTs from, such as, terrestrial input (e.g. Weijers et al., 2006), subsurface (e.g. Lee et al., 2008) and/or marine sediment production (e.g. Liu et al., 2011).

A great deal of efforts has been made to assess TEX_{86} accuracy in marine and lake sediments. For example, TEX₈₆ values are cautioned when branched and iso-15 prenoid tetraether (BIT) index is > 0.2 (Zhu et al., 2011), ratio of GDGT-2/crenarchaeol > 0.4 (Weijers et al., 2011b), Methane Index > 0.5 (Zhang et al., 2011), ratio of GDGT-0/crenarchaeol > 2 (Blaga et al., 2009), or %GDGT-2 > 45 (Sinninghe Damsté et al., 2012). Recently, based on assessment of the relationship between the weighted average number of cyclopentane rings in all GDGTs (ring index, RI) and TEX₈₆ from the published core-top sediments, Zhang et al. (2015) established a significant correlation between TEX₈₆ and RI, given by RI = $3.32 \times (TEX_{86})^2 - 0.77 \times TEX_{86} + 1.59$ $(\pm 2\sigma \sim 0.3)$. This relationship reflects the physiological adaptation of marine archaea by synthesizing GDGTs with more rings (higher RI values) when their ambient environment is warm (high TEX₈₆ values). Deviations from this relationship suggest that 25 temperature is no longer the dominant factor governing the GDGT distribution under the circumstances when the response of GDGTs to temperature is different from the modern analog as defined by the global core-top dataset.





The TEX₈₆-related GDGTs (GDGT-1, -2, -3, and crenarchaeol regioisomer) in marine water column are commonly derived from the Marine Group I (MG I) *Thaumarchaeota* (e.g. Schouten et al., 2008; Pitcher et al., 2011a), as it is one of the dominant groups of planktonic archaea in the ocean (Karner et al., 2001). In particular, crenarchaeol, con-

- taining one cyclohexane and four cyclopentane moieties, is considered as a specific biomarker for MG I *Thaumarchaeota* (Sinninghe Damsté et al., 2002; Schouten et al., 2008). Marine Group II *Euryarchaeota* are another abundant planktonic archaea inhabiting predominantly coastal water and (near) surface water of open oceans (e.g. Delong et al., 1992; Galand et al., 2010; Hugoni et al., 2013). Recently, this cosmopolitan group be be supported by the participation of OPOTe (including predominant planktonic archaea and planktonic).
- ¹⁰ has been suggested to be another major source of GDGTs (including crenarchaeol) in the ocean (Lincoln et al., 2014a), which supported an earlier hypothesis about GDGTproducing MG II *Euryarchaeota* (Turich et al., 2007); however, concrete evidence, such as culturing study focusing on MG II *Euryarchaeota*, has been lacking (Schouten et al., 2014; Lincoln et al., 2014b).
- Because of the absence of MG II *Euryarchaeota* enrichments or cultures, little is known about the characteristic of the MG II *Euryarchaeota*-produced GDGTs in water column and how these tetraethers might bias TEX₈₆ in marine sediment. Recently, unusually low TEX₈₆ values in the estuarine and coastal region of South China Sea (SCS) are hypothesized to relate to the production of GDGTs by MG II *Euryarchaeota* Wang et al. 2015) However, the lack of direct link between archaeal links and DNA
- ²⁰ (Wang et al., 2015). However, the lack of direct link between archaeal lipids and DNA prevented the drawing of a more concrete conclusion.

In this study, we investigate the archaeal community compositions, present the distribution of TEX₈₆ and Ring Index (RI), and evaluate the relationship between MG II *Euryarchaeota* and core lipid (CL-) and intact polar lipid (IPL-) based GDGTs in the suspended particulate matter (SPM) and surface sediments collected along a salinity gradient from the lower Pearl River (PR) and its estuary to the coastal South China Sea. Our goal was to test the hypothesis that a large portion of the isoprenoid GDGTs are produced by MG II *Euryarchaeota*, which might influence the applicability of TEX₈₆ in coastal marine environments where MG II *Euryarchaeota* are abundant. In addition



to total CL-GDGTs, total IPL-GDGTs obtained through acid hydrolysis (IPL-H or total IPL), and IPL-GDGTs with only phosphate head groups from base hydrolysis (IPL-OH or phospho IPL), were also examined in order to better correlate with the qPCR data of MG II *Euryarchaeota* and total *Archaea*. The results indicate that MG II *Euryarchaeota* may produce GDGTs having 1–4 cyclopentane moieties. Contribution of the MG II *Euryarchaeota*-produced GDGTs with more cyclopentane rings appears to bias TEX₈₆, shown by their GDGT distribution deviating from the RI-TEX₈₆ relationship identified from the global core-top dataset used for TEX₈₆-SST calibration (Zhang et al., 2015). As a result, the TEX₈₆-derived temperature in these samples significantly deviates from the actual SSTs. Our results have important implications for a better understanding of the production of marine GDGTs, and the use of TEX₈₆ as a pale-othermometer for probing past changes of climate.

2 Material and methods

2.1 Sample collection

- ¹⁵ The sampling locations and sampling information for SPM and surface sediments are shown in Fig. 1 and Table 1, respectively. SPM samples (n = 18) and surface sediments (n = 8) were collected along a salinity gradient from the lower Pearl River and its estuary to the coastal South China Sea in the summer of 2011. River water SPM samples were collected from the surface (station R1 to R6) and the bottom (station R1
- and R2) of the water column in the lower Pearl River. Mixing water SPM samples were collected from three water layers (surface, middle and bottom) and from three tidal periods (high tide, slack tide and low tide) at station M located in the PR estuary. Seawater SPM samples were collected from four water layers (surface, subsurface, middle and bottom) at station S in the coastal SCS (Fig. 1). The depth of the sampling layers in the water column is given in Table 1. About 4 to 103 liters of water were filtered onto





ashed (450°C, overnight) glass-fiber filters (Whatman GF/F, 0.7 µm, 142 mm diameter)

using an in situ submersible pump. The pH, temperature, and salinity were determined in situ by a Horiba instrument (W-20XD, Kyoto, Japan; Table 1).

Surface sediments (top ca. 10 cm) were collected at all stations using a grab sampler (Fig. 1; Table 1). All samples were frozen immediately in liquid nitrogen and kept at -80° C in the laboratory before analysis.

2.2 GDGT analysis and indices

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2.2.1 GDGT extraction and separation

The SPM samples (n = 18) and surface sediments (n = 8) were freeze dried and extracted using a modified Bligh and Dyer method (Blight and Dyer, 1959); the separation of core lipids and intact polar lipids followed the procedure described in Weijers et al. (2011a). Briefly, the total lipid extract (TLE) was obtained by extraction (10 min each, 6 times) of SPM (1 filter) or sediments (5 g) with a single-phase solvent mixture of methanol, dichloromethane (DCM) and phosphate buffer (2 : 1 : 0.8, v/v/v; pH 7.4). The TLE was separated over an activated silica gel column eluted with *n*hexane/ethylacetate (1 : 1, v/v) and methanol for CL and IPL, respectively. For GDGT quantification, a known amount of an internal C₄₆ GDGT standard was added into the CL fraction or IPL fraction (Huguet et al., 2006).

The polar head groups were cleaved off through hydrolyzation, which allows indirect analysis of IPL as CL (Pitcher et al., 2009; Weijers et al., 2011a). Briefly, 1/3 IPL frac-

- tion (non-hydrolyzed IPL fractions) was directly condensed to determine any carryover of CL into the IPL fraction; another 1/3 IPL fraction was hydrolyzed (2 h) in 1.5 N HCl in methanol, which is called acid-hydrolyzed IPL fraction (IPL-H). DCM and MilliQ water were added, and the DCM fraction was collected (repeated 4 times). The DCM fraction was rinsed (6 times) with MilliQ water in order to remove acid and dried under N₂
- ²⁵ gas. The last 1/3 IPL fraction was subjected to base hydrolysis (2 h) in a 1N KOH in methanol/H₂O mixture (95 : 5, ν/ν), which is called base-hydrolyzed IPL fraction (IPL-OH). The recovery of IPL-OH was similar to that of IPL-H. Together with the condensed





CL fraction, the four fractions were dissolved in *n*-hexane/isopropanol (99 : 1, v/v), and filtered using a PTFE filter that had a pore diameter of 0.45 µm. IPL-H reflects total IPL, which includes both phosphate head groups and glycosidic head groups; IPL-OH represents the IPL with phosphate head group only (phospho IPL; Weijers et al., 2011a).

⁵ The analysis of non-hydrolyzed IPL fractions is to determine any carryover of CL into the IPL fraction.

2.2.2 GDGT analysis

GDGTs from all treatments were analyzed using high performance liquid chromatography/atmospheric pressure chemical ionization-tandem mass spectrometry (HPLC/APCI-MS/MS), which was performed with an Agilent 1200 liquid chromatography equipped with an automatic injector coupled to QQQ 6460 MS and Mass Hunter LC-MS manager software using a procedure carried out in the same way as described by Zhang et al. (2012). Separation was achieved by using a Prevail Cyano column (2.1 mm × 150 mm, 3 µm; Alltech Deerifled, IL, USA) with *n*-hexane (solvent A) and a mixture of *n*-hexane/isopropanol 90/10 (v/v; solvent B). The (M+H)⁺ ions of each core isoprenoid GDGT (m/z 1302, 1300, 1298, 1296, 1294, 1292) was monitored via selected ion monitoring (SIM) mode (Schouten et al., 2007).

Indices based on the fractional abundance of GDGTs were calculated as follows:

 $TEX_{86} = ([GDGT-2] + [GDGT-3] + [Cren.iso]) / ([GDGT-1] + [GDGT-2] + [GDGT-3])$

²⁰ +[Cren.iso]) (Schouten et al., 2002)

Ring $Index_1(RI_1) = ([GDGT-1] + 2 \times [GDGT-2] + 3 \times [GDGT-3] + 4 \times [Cren.]$

+ 4 × [Cren.iso])/100 (Zhang et al., 2015)

 $Ring Index(RI) = ([GDGT-1] + 2 \times [GDGT-2] + 3 \times [GDGT-3] + 4 \times [GDGT-4]$

+ 4 × [Cren.iso])/100

with the GDGT numbers corresponding to the GDGT structures in Fig. S1. Note that RI_1 is originally proposed by Zhang et al. (2015); RI is modified from RI_1 , in which



(1)

(2)

(3)

the fractional abundance of crenarchaeol is replaced by GDGT-4 in order to eliminate the influence of crenarchaeol on weighted average number of cyclopentane rings in GDGTs (see Sect. 3.2 for more details).

2.3 DNA analysis

5 2.3.1 DNA Extraction and qPCR of archaeal 16S rDNA

The SPM samples (n = 12) and surface sediments (n = 3) from station R1 (river water), station M (mixing water), and station S (seawater) were selected for the DNA analysis. The frozen filters were washed 3 times by phosphate buffered saline (pH 7.4). The supernatants were centrifuged under 11000 g for 10 min. The sediments were collected and transferred into the FastDNA SPIN Kit tubes 10 (MP Biomedical, OH, USA). The DNA was extracted following the protocol of FastDNA SPIN Kit. The DNA samples were dissolved with a final elution in $100\,\mu\text{L}$ de-ionized water and were preserved at -80° until further processing. The quantitative PCR primers were Arch_334F (5'ACGGGGCGCAGCAGGCGCGA3') /Arch 518R (5'ATTACCGCGGCTGCTGG3') for archaeal 16S gene quantification 15 (Bano et al., 2004) and GII-554-f (5'GTCGMTTTTATTGGGCCTAA3') and Eury806r (5'CACAGCGTTTACACCTAG3') for MG II Euryarchaeota 16S gene quantification (Massana et al., 1997; Teira et al., 2004). The qPCR analysis of this gene was performed at 95° for 30 s and 40 cycles at 94° for 30 s, 55° for total Archaea (53° for MG II Eurvarchaeota) for 30 s and 68° for 1 min. 20

2.3.2 454 sequencing

SPM samples (n = 3), which respectively represent river water, mixing water and seawater, are selected to conduct pyrosequence targeting archaeal 16S rDNA. Different from qPCR primers, we chose the primers targeting longer sequences to get more confident phylogenetic composition of those sam-





ples. The primer were Arch_344F (5'ACGGGGCGCAGCAGGCGCGA3')/Arch_915R (5'GTGCTCCCCGCCAATTCCT3'; Gantner et al., 2011). The pyrosequencing was conducted on the Roche GS FLX + (454) system by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). We use the Mothur (version 1.29.2; Schloss et al., 2009) to filter the raw pyrosequencing data. The selected sequences were analyzed using QIIME standard pipeline (Caporaso et al., 2010). Taxonomy was assigned using the

Ribosomal Database Project (RDP) classifier 2.2 (minimum confidence of 80%; Cole et al., 2009).

2.4 Satellite-derived surface water temperature (SWT)

¹⁰ The satellite-derived SWT was derived from the data sets on a spatial resolution of 4km from the NOAA advanced very-high-resolution radiometer (AVHRR; version 5.2; http://www.nodc.noaa.gov/SatelliteData/pathfinder4km/). The June mean SWT was obtained from the daily averaged values of 30 days in June 2011 (sampling month). The annual mean SWT and winter mean SWT represent 8-years mean values of annual mean temperature (2004–2011) and monthly mean temperature (December– February), respectively, as the surface sediment (top ca. 10 cm) collected in this study may represent a deposition of 6–10 yr based on an estimation from Strong et al. (2012).

2.5 Statistical analysis

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Cluster analysis was performed on CL-GDGTs and phospho IPL-GDGTs in the SPM
 samples collected from the lower Pearl River, the PR estuary and coastal South China
 Sea using the base program in R 2.12.1. The fractional abundance of GDGTs from
 all samples was imported into R and the Euclidean method was used to compute the
 distance matrix and to generate a hierarchical clustering tree. The linear regression
 analysis was conducted from iPython Notebook. The Canoco software (version 4.5)
 was used to perform the redundancy analysis (RDA), which was to assess the rela-



Discussion **BGD** 12, 12455-12484, 2015 Paper **Contribution of** Marine Group II Eurvarchaeota to **Discussion** Paper cyclopentyl tetraethers J. X. Wang et al. **Title Page** Abstract Introduction **Discussion** Paper Conclusions References Tables **Figures** Back Close **Discussion** Pape Full Screen / Esc **Printer-friendly Version** Interactive Discussion

tionship between archaeal tetraether lipids, GDGT-based indices and environmental parameters.

3 Results and discussion

3.1 Archaeal community composition

- The phylogenetic classification of archaeal sequences exhibited that methanogens 5 were the predominant group in the fresh water (station R1) and MG I Thaumarchaeota mainly occurred in the salty water (stations M & S; Fig. 2). A significant proportion of MG II Euryarchaeota was present at station M in the PR estuary and station S in the coastal SCS; whereas they were absent at station R1 in the lower Pearl River (Fig. 2). These results are in agreement with the previous observations showing that 10 MG I Thaumarchaeota and MG II Euryarchaeota dominated in the planktonic archaeal communities in the marine-influenced region of the Pearl River estuary (Liu et al., 2014; Wang et al., 2015), the Yangtze River estuary (Liu et al., 2011), and the Jiulong River estuary (Hu et al., 2015). This is also supported by cluster analysis based on fractional abundances of CL- and phospho IPL-GDGTs in the water column along the salinity 15 gradient, as GDGT-0 and crenarchaeol were the dominant archaeal tetraether lipids in the river water and mixing/sea water, respectively (Fig. S2). The overwhelming change
- in archaeal community composition from the lower Pearl River to the PR estuary was suggested to be predominantly controlled by salinity (Xie et al., 2014). Since the pur-20 pose of this study was to explore how MG II *Euryarchaeota* influenced TEX₈₆ in the
- water column and surface sediments, the following results and discussion focus on the PR estuary and coastal SCS where MG II *Euryarchaeota* are present.

3.2 TEX_{86}-derived temperature and Ring Index

The TEX₈₆-derived temperature was calculated based on the calibration of Kim et al. (SST = $68.4 \times \log(\text{TEX}_{86})$ + 38.6; 2010). The results exhibited that CL-TEX₈₆ tem-

peratures from either SPM or surface sediments were close to the satellite-based annual mean SWT in the lower Pearl River and its estuary; whereas those from the coastal SCS were lower than the winter mean SWT (Fig. 3). The correspondence between the CL-TEX₈₆ temperature in the SPM and sediment along the salinity gradient indicates that the TEX₈₆ signal in the sediment is predominantly from water column, which is consistent with previous studies in the PR estuary (Wang et al., 2015) and other coastal settings (Herfort et al., 2006; Zell et al., 2014).

Total IPL-TEX₈₆ temperatures in the water column were consistently below either June mean SWT or in situ instrumental measurements, although the sampling season was surgery $(\Sigma = 0)$. In the DD extreme and exactly $\Sigma = 0$.

- ¹⁰ was summer (Fig. 3). In the PR estuary and coastal SCS, the phospho IPL-TEX₈₆ (IPL-OH-TEX₈₆) was lower than the total IPL-TEX₈₆ (IPL-H-TEX₈₆); whereas, it was very close to the CL-TEX₈₆ (Fig. 3; Table 1). Since phosphate head groups can be degraded faster than the glycosidic head groups, the phospho IPL is considered to be a better reflection of the living microbes (Harvey et al., 1986; Schouten et al., 2010);
 ¹⁵ on the other hand, the CL may be mostly derived from the easier degrading phospho
- IPL, thus causing their TEX₈₆ values to be similar. Furthermore, in the same study area, variation in TEX₈₆ has been suggested to attribute to the change in archaeal community composition in the water column, in which the unusually low TEX₈₆-derived temperature in the coastal SCS was speculated to link to MG II *Euryarchaeota* (Wang et al., 2015).

Since the TEX₈₆ can be influenced by factors other than temperature as shown in Fig. 3, the Ring Index was proposed to be able to evaluate the accuracy of TEX₈₆ in the marine sediments (Zhang et al., 2015). Here, the CL- and IPL-TEX₈₆ values were plotted against RI (RI₁, Eq. 2) using the SPM and surface sediments in this study and from previous studies (Wei et al., 2011; Ge et al., 2013; Zhang et al., 2013; Wang et al., 2015). The results exhibited that SPM and surface sediments from the open South China Sea were predominantly assembled within the RI₁-TEX₈₆ -confined zone (RI₁ = $3.32 \times (TEX_{86})^2$ –0.77 × TEX₈₆+1.59, ±2 σ = 0.3; Zhang et al., 2015); whereas, the majority of samples from coastal SCS and the PR estuary fell outside the calibration



zone (Fig. 4). Furthermore, all the SPM samples in the mixing water and seawater were scattered above the confined zone (Fig. 4), suggesting that ring index values in the PR estuary and coastal SCS were unexpectedly elevated. This implies that more cyclopentane-containing GDGTs seem to contribute to the GDGT pool in the water
 ⁵ column of the PR estuary and coastal SCS.

To further assess the distribution of RI in the SPM and to explore other contributor(s) to the cyclopentyl GDGT pool in the study area, mean values of CL-, total IPL-, and phospho IPL-RI were examined (Fig. 5). Note that crenarchaeol was excluded from the ring index calculation (RI, Eq. 3) in order to limit its overwhelming influence on the index. The re-defined RI equation makes it more sensitive to the variation of cyclopentane-containing GDGTs that may be contributed from other archaeal group(s). This is also in agreement with the cluster analysis based on the same dataset, showing

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that the GDGTs with 1–4 cyclopentane moieties belonged to the same cluster group (Fig. S2). Compared with the river water and seawater, the highest ring index value for either CL (avg. 0.39 ± 0.08) or IPL (avg. 0.48 ± 0.07 for total IPL; avg. 0.47 ± 0.10 for

- phospho IPL) occurred at station M in the mixing water (Fig. 5; Table 1), with the PR estuary (station M) appears to be a hot spot of the additional input of GDGTs with cyclopentane moieties. Further confirmation comes from the comparison of the fractional abundance of the CL- and phospho IPL-GDGTs in the cluster analysis, showing that
- ²⁰ the sum of the GDGTs with 1–4 cyclopentane moieties in the mixing water cluster was significantly higher than that from the seawater cluster or river water cluster (Fig. S2). In the mixing water station, the mean values of total IPL-RI and phospho IPL-RI had no significant difference; and both were higher than the CL-RI (Fig. 5). However, in the seawater station, the total IPL-RI (0.34 ± 0.07) was more elevated than the phospho
- ²⁵ IPL-RI (0.22 \pm 0.01) and the CL-RI (0.24 \pm 0.04; Fig. 5). This distribution pattern of RI at station S in the seawater corresponded to the TEX₈₆-temperature distribution in the SPM and sediments (Fig. 3), suggesting that cyclopentane-containing GDGTs appear to be able to alter TEX₈₆ record in the water column, or even in the surface sediment,





where the vertical transportation of GDGTs from the water column predominantly occurs.

3.3 Relationship between MG II *Euryarchaeota* and cyclopentane-containing GDGTs

⁵ The abundances of MG II *Euryarchaeota* 16S rRNA gene and total *Archaea* 16S rRNA gene were examined in the SPM and surface sediments of the river water (station R1), mixing water (station M), and seawater (station S; Table 1). In the water column, the abundance of MG II *Euryarchaeota* 16S rRNA gene in the mixing water station averaged 5.4 ± 6.5 × 10⁸ copies L⁻¹ (*n* = 5), which was three orders of magnitude higher
¹⁰ than that in the river water (avg. 1.5 ± 2.1 × 10⁵ copies L⁻¹, *n* = 2) and seawater (avg. 1.8 ± 3.0 × 10⁵ copies L⁻¹, *n* = 3; Fig. 5). In the surface sediment, however, no significant difference in the abundance of MG II *Euryarchaeota* was observed (Table 1). Although it is unclear what factors exactly control the distribution of MG II *Euryarchaeota Euryatchaeota* in the water column and sediments, salinity apparently played an important role (Table 1) and perhaps competition for nutrients (e.g. ammonium and phos-

phate; Fig. S3) ought to be considered as well.

Furthermore, the ratio of the abundances of MG II *Euryarchaeota* 16S rRNA gene to total *Archaea* 16S rRNA gene ([MG II *Euryarchaeota*]/[Archaea] ratio) in the mixing water station (avg. 0.23 ± 0.08 , n = 5) was significantly higher than that in the seawater

²⁰ (avg. 0.09 ± 0.07 , n = 3); yet, the [MG II *Euryarchaeota*]/[Archaea] ratio in the river water was negligible (avg. < 0.0001) (Fig. 5). This observation further confirms that the PR estuary (mixing zone, salinity avg. 16.6) is a hot spot for the occurrence of MG II *Euryarchaeota* along the salinity gradient from the low Pearl River to the coastal SCS. The above observations provide the opportunity to evaluate the relationship between

²⁵ Ring Index and MG II *Euryarchaeota*. On one hand, the presence of (more labile) phospho IPL-GDGTs (Table 1) implies that in situ production of isoprenoid GDGTs occurs in the water column along the Pearl River and its estuary to the coastal SCS. The elevated value of phospho IPL-RI in the mixing water, thus, indicates that higher relative





proportions of GDGTs with 1–4 cyclopentane moieties were produced in situ in the PR estuary by the source microorganism(s). On the other hand, the MG II *Euryarchaeota* 16S rRNA gene copy numbers and the [MG II *Euryarchaeota*]/[Archaea] ratio follow practically identical patterns with the distribution of the CL- and IPL-RI along the salin-

- ⁵ ity gradient (Fig. 5). Linear regression analysis then confirmed the positive relationship between phospho IPL-derived RI and the [MG II *Euryarchaeota*]/[Archaea] ratio along the salinity gradient ($R^2 = 0.61$, P < 0.01; Fig. 6f). Therefore, it is reasonable to hypothesize that MG II *Euryarchaeota* preferentially synthesize GDGTs with 1–4 cyclopentane moieties in this region, resulting in an elevated value of RI. However, since no more
- samples to quantify the abundance of MG I *Thaumarchaeota* in this study, further work will focus on comparing the proportion of cyclopentane-containing GDGTs contributed between MG I *Thaumarchaeota* and MG II *Euryarchaeota* in water column and sediment.
- The correspondence between MG II *Euryarchaeota* and RI in the water column along
 the salinity gradient is an important observation in this study. To further constrain the characteristic of MG II *Euryarchaeota Euryarhcaeota*-produced GDGTs, linear regression analysis was conducted between fractional abundance of GDGTs and [MG II *Euryarchaeota*]/[Archaea] ratio in the SPM along the salinity gradient. In respect to the RI-related GDGTs (Eq. 3), our data exhibited a significantly positive linear correlation between [MG II *Euryarchaeota*]/[Archaea] ratio and the fractional abundance of phospho IPL-based GDGT with 1–4 cyclopentane moieties (Fig. 6a–d); whereas it appears to be less correlated with the fractional abundance of crenarchaeol regioisomer (Fig. 6e). Similar trends of the linear correlations were also shown between [MG II *Euryarchaeota*]/[Archaea] ratio and the CL- and total IPL-GDGT with 1–4 cyclopentane
- ²⁵ moieties, with a less significant correlation between [MG II *Euryarchaeota*]/[Archaea] ratio and total IPL-based crenarchaeol regioisomer (Table 2). In contrast, with respect to phospho IPL-based GDGT-0 or crenarchaeol, our data exhibited no correlation between [MG II *Euryarchaeota*]/[Archaea] ratio and the fractional abundance of these membrane lipids (Fig. 6g, h; Table 2). Crenarchaeol has been proved to be a biomarker





of MG I *Thaumarchaeota* (Schouten et al., 2008, 2012; Pitcher et al., 2011a, b). Although MG II *Euryarchaeota* were suggested to be another source of crenarchaeol in the ocean (Lincoln et al., 2014a), our study shows the absence of a significant correlation between the distribution of MG II *Euryarchaeota* and crenarchaeol (Fig. 6h), suggesting that MG II *Euryarchaeota* may not be a major source of crenarchaeol in Pearl River estuary.

On the other hand, comparison of the slopes and the R^2 values of the regression equations (Fig. 6a–e) show that GDGT-1 has a stronger correlation with MG II *Euryarchaeota Euryarhcaeota* in the study area. If MG II *Euryarchaeota* preferentially synthesize CDCT1 additional contribution of the CDCT1 to the water column of the DD

- ¹⁰ thesize GDGT-1, additional contribution of the GDGT-1 to the water column of the PR estuary and the coastal SCS is capable of causing a substantial decrease of the TEX₈₆ value. Moreover, Wang et al. (2015) suggested that the decreased ratio of GDGT-2 to GDGT-3 contributes to the offset of TEX₈₆ in the surface sediments of this area; on the contrary, the increased ratio of GDGT-2 over GDGT-3 in the deep-water column seems
- to be responsible for a warm bias of TEX₈₆-derived temperature in other marine environments (Taylor et al., 2013; Hernandez-Sanchez et al., 2014). A recent study by Kim et al. (2015) suggested that the co-variation of an increase in GDGT-2 & crenarchaeol regioisomer and a decrease in GDGT-1 & GDGT-3 altered TEX₈₆-derived temperature toward higher values in the deep-water surface sediments of the Mediterranean Sea.
- ²⁰ Considering the above, along with the new observations in this study (Fig. 5 & 6), it seems feasible that planktonic *Euryarchaeota* bias TEX₈₆ by changing the distribution of TEX₈₆-related GDGTs (especially the GDGTs with 1–3 cyclopentant rings) in the estuary and coastal area.

4 Conclusions

²⁵ This study assesses the relationship between GDGTs with cyclopentane moieties and MG II *Euryarchaeota* along a salinity gradient from river water to seawater. The correlation between the percentage of MG II *Euryarchaeota* over total archaeal population





and GDGT-1, -2, -3, or -4 as well as RI implies that MG II *Euryarchaeota* can produce GDGTs with 1–4 cyclopentane moieties. The production of GDGTs from MG II *Euryarchaeota* changes the proportion of ringed GDGTs in the total GDGT pool, which may bias TEX₈₆. On the other hand, apparent evidence shows that MG II *Euryarchaeota* do not seem to be a significant source of crenarchaeol. However, validation of the production of GDGTs by MG II *Euryarchaeota* may have to wait until a pure culture is available.

The Supplement related to this article is available online at doi:10.5194/bgd-12-12455-2015-supplement.

- Acknowledgements. We thank Huangmin Ge, Geng Wu, and Chao Li for helping with the sampling. Songze Chen helped performing the qPCR analysis. We appreciate Ding He for commenting on an earlier version of the manuscript. This research was supported by the National Key Basic Research Program of China grant #2013CB955703 (CLZ), the South China Sea-Deep program of the National Science Foundation of China Grant # 91028005 and 91428308
- ¹⁵ (CLZ), and the "National Thousand Talents" Program (CLZ) through the State Key Laboratory of Marine Geology at Tongji University.

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Interactive Discussion

Discussion Paper **BGD** 12, 12455-12484, 2015 **Contribution of** Marine Group II Eurvarchaeota to **Discussion** Paper cyclopentyl tetraethers J. X. Wang et al. **Title Page** Abstract Introduction **Discussion** Paper Conclusions References Tables **Figures** Back Close **Discussion** Paper Full Screen / Esc **Printer-friendly Version** Interactive Discussion



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Table 1. Basic information, abundance of isoprenoid GDGTs, TEX₈₆, Ring Index, and 16S rRNA gene for suspended particulate matters (SPM) in the water column and surface sediments collected from the lower Pearl River, the Pearl River estuary, and coastal northern South China Sea. Basic information includes location, sampling date, water depth, temperature (Temp.), and salinity (Sal.).

Sample	Longitude (E)	Latitude (N)	Sampling date	Depth ^b	Temp.	Sal.	pН	iGD	GTs (ng	L ⁻¹)°	TEX ₈₆		Ring Index (RI)			Archaeal 16S	MG II Euryarchaeota 165	
ID ^a			(dd/mm/yyyy)	(m)	(*)			CL	IPL-H	IPL-OH	CL	IPL-H	IPL-OH	CL	IPL-H	IPL-OH	(copies L ⁻¹)	(copies L ⁻¹
River water SPM																		
R1_sur	113*34.249'	22°52.647′	21/06/2011	1.5	29.7	0.2	7.25	188.6	201.5	16.7	0.59	0.50	0.57	0.19	0.15	0.12	2.1E+09	3.0E+05
R1_bott	113°34.249′	22°52.647'	21/06/2011	6.0	29.6	0.2	7.25	221.3	128.1	8.9	0.60	0.56	0.54	0.16	0.12	0.15	3.6E+08	1.2E+00
R2_sur	113*36.680'	22°56.338'	21/06/2011	1.5	29.0	0.1	6.95	64.8	82.5	6.8	0.56	0.42	0.49	0.16	0.15	0.11	_d	-
R2_bott	113*36.680'	22°56.338'	21/06/2011	6.0	29.0	0.1	6.90	266.6	426.5	27.9	0.60	0.45	0.38	0.14	0.10	0.12	-	-
R3	113*28.726'	23°04.339'	22/06/2011	1.5	29.4	0.1	7.46	79.8	150.2	8.0	0.57	0.41	0.38	0.07	0.09	0.14	-	-
R4	113°33.507′	22°58.409'	22/06/2011	1.5	29.6	0.2	7.28	19.1	30.6	3.3	0.63	0.57	0.37	0.26	0.13	0.20	-	-
R5	113°29.941'	22°53.588'	22/06/2011	1.5	28.5	0.1	7.28	20.0	30.4	2.0	0.61	0.48	0.35	0.37	0.40	0.34	-	-
R6	113*33.088'	22°44.811′	22/06/2011	1.5	27.8	0.1	6.92	49.1	21.8	1.2	0.62	0.68	0.53	0.15	0.19	0.25	-	-
Mixing water SPM																		
M_lt	113*45.098'	22°27.206'	18/06/2011	1.5	-	-	-	25.9	105.0	2.4	0.66	0.55	0.53	0.52	0.47	0.43	2.0E+07	6.1E+06
M_st	113*45.098'	22°27.206'	18/06/2011	1.5	-	-	-	35.3	97.3	1.4	0.58	0.65	0.60	0.36	0.61	0.61	1.3E+09	3.1E+08
M_ht	113*45.098'	22°27.206'	18/06/2011	1.5	-	-	-	21.9	86.8	2.8	0.59	0.56	0.55	0.43	0.46	0.38	6.9E+07	1.2E+07
M_sur	113*45.098'	22°27.206'	18/06/2011	1.5	28.7	11.1	8.03	18.9	67.0	1.9	0.58	0.60	0.63	0.36	0.44	0.65	-	7.2E+07
M_mid	113*45.098'	22°27.206'	18/06/2011	5.0	28.3	15.6	7.93	102.6	73.4	5.2	0.61	0.64	0.59	0.35	0.47	0.36	6.0E+09	7.9E+08
M_bott	113*45.098'	22°27.206′	18/06/2011	9.0	27.6	23.0	7.89	107.4	76.7	6.0	0.56	0.60	0.58	0.30	0.42	0.38	5.1E+09	1.6E+09
	Sea water SP	М																
S_sur	113*70.448'	22°05.165'	15/06/2011	1.5	29.6	29.5	8.63	1.1	0.7	0.1	0.52	0.65	0.58	0.28	0.39	0.22	1.0E+05	4.8E+03
S_subs	113°70.448'	22°05.165'	15/06/2011	5.0	29.5	29.7	8.64	2.1	1.1	0.1	0.56	0.63	0.58	0.27	0.33	0.24	3.1E+06	5.3E+05
S_mid	113°70.448'	22°05.165'	15/06/2011	10.0	28.6	31.7	8.45	12.6	13.5	0.6	0.49	0.55	0.50	0.20	0.24	0.23	9.8E+04	4.4E+03
S_bott	113*70.448'	22°05.165′	15/06/2011	18.0	25.4	33.7	7.92	21.4	9.6	0.7	0.53	0.59	0.49	0.20	0.39	0.21	-	-
	Sediment																	
Sedi-R1	113*34.249'	22°52.647′	21/06/2011	8.0	-	-	7.46	461.8	142.8	9.5	0.58	0.37	0.30	0.27	0.38	0.41	2.8E+10	1.2E+06
Sedi-R2	113*36.680'	22°56.338'	21/06/2011	7.0	-	-	7.68	687.5	289.2	17.0	0.56	0.35	0.31	0.23	0.22	0.26	-	-
Sedi-R3	113°28.726'	23°04.339'	22/06/2011	9.0	-	-	7.29	800.3	233.5	19.6	0.57	0.43	0.36	0.15	0.23	0.30	-	-
Sedi-R4	113°33.507'	22°58.409'	22/06/2011	7.0	-	-	7.50	881.0	149.7	9.7	0.58	0.51	0.38	0.33	0.75	0.62	-	-
Sedi-R5	113*29.941'	22°53.588'	22/06/2011	8.0	-	-	7.58	621.7	84.2	7.3	0.62	0.52	0.37	0.33	0.66	0.43	-	-
Sedi-R6	113°33.088′	22°44.811'	22/06/2011	8.0	-	-	7.66	120.2	103.8	6.2	0.67	0.35	0.42	0.42	0.65	0.74	-	
Sedi-M	113*45.098'	22°27.206'	18/06/2011	12.0	-	-	7.60	200.4	67.7	2.3	0.62	0.52	0.50	0.38	0.69	0.42	1.1E+10	1.7E+06
Sedi-S	113*70.448'	21°95.165′	15/06/2011	20.0	-	-	7.40	5003.6	267.3	20.8	0.50	0.64	0.50	0.19	0.73	0.34	1.3E+10	4.8E+06
P. D. Divers (4)	a James Danel Diversi	under la faitheur and	hu tha station such a	and a second second be		-				da a contra da la fala a	Daniel Dive		In Law Alata and	بماما والمامي	he black ald	ter mini menera	e estable le reconstant	C. Castron (another and Casth Ch

¹ R, Hive (the lower Pearl River), which is followed by the station numbers; sur and bott present surface water and bottom water. (M, Mixing water (the Pearl River estuany); II, low tide; st, slack tide; ht, high tide; mit means middle layer water. (Sa evaluer (contern South China See); subs represents subsurface water. ² For the SPM samples collected from the water collumn, the depth is referred to the sampling water (depth; For the sediments, the depth indicates the river water depth.² CL, core liptid; (PL-H, intact polar liptid (PL) derived core liptid upon add (H) phytohysis; IPL-OH; IPL-derived core liptid series are not available on or teamined.

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Contribution of Marine Group II <i>Euryarchaeota</i> to cyclopentyl tetraethers J. X. Wang et al.									
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	16S gei	ne Ratio vs. CL*	16S gen	e Ratio vs. IPL-H	16S gene Ratio vs. IPL-OH			
	R^2	P value	R^2	P value	R^2	P value		
%GDGT-0	0.43	0.04	0.47	0.03	0.44	0.04		
%GDGT-1	0.52	0.02	0.63	0.01	0.68	0.00		
%GDGT-2	0.54	0.02	0.47	0.03	0.53	0.02		
%GDGT-3	0.43	0.04	0.54	0.02	0.57	0.01		
%GDGT-4	0.52	0.02	0.62	0.01	0.51	0.02		
%Cren.	0.32	0.09	0.39	0.05	0.30	0.10		
%Cren.iso	0.48	0.03	0.02	0.72	0.35	0.07		
RI	0.54	0.02	0.61	0.01	0.61	0.01		
GDGT 2/3	0.16	0.25	0.34	0.08	0.06	0.49		

Table 2. Regression analysis between the ratio of MG II *Euryarchaeota* to Archaeal 16S rRNA genes and the fractional abundance of GDGTs, RI, and the ratio of GDGT-2 to GDGT-3.

* 16S gene ratio, the ratio of MG II *Euryarchaeota* to archaeal 16S rRNA genes. CL, core lipids; IPL-H, intact polar lipids derived upon acid hydrolysis; IPL-OH, intact polar lipids derived upon base hydrolysis.







Figure 1. Map showing location of sites (dark circle) in the lower Pearl River (PR), the PR estuary, and coastal South China Sea (SCS). Abbreviations: R, River water, which is followed by the station #; M, mixing water; S, seawater. Station S includes four water layers (surface, subsurface, middle, and bottom). Station M includes three water layers (surface, middle and bottom). The surface water at station M was also collected during the high tide-, slack tide-, and low tide-periods. Stations R1 and R2 include two water layer (surface and bottom). Surface sediments were collected at each sampling site.







Figure 2. The distribution of archaeal community composition based on 454 sequencing along the salinity gradient at the lower Pearl River (river water, station R1), The PR estuary (mixing water, station M), and coastal South China Sea (seawater, station S).



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Figure 3. Mean values of TEX₈₆-derived temperatures in SPM and surface sediments from the lower Pearl River (R), the PR estuary (M), and coastal SCS (S). CL, core lipids; IPL-H, intact polar lipid based upon acid hydrolysis; IPL-OH, intact polar lipid based upon base hydrolysis. Dashed lines A, June mean surface water temperature (SWT; 28.4 ± 0.07 °C); dashed line B, in situ instrumental temperature (29.1 °C, in the river water; 28.2 °C in the mixing water and seawater); dashed line C, annual mean SWT (24.71 ± 0.11 °C); dashed line D, winter SWT (20.54 ± 0.10 °C).







Figure 4. TEX₈₆ of the SPM samples and surface sediments plotted against RI_1 . The blue dashed line represents the RI-TEX₈₆ calibration from Zhang et al. (in review). The SCS SPM/sediments and coastal SCS SPM/sediments (black points) are from Wei et al. (2011), Ge et al. (2013), Zhang et al. (2013), and Wang et al. (2015).



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Figure 5. Distribution of the mean values of the Ring Index (bars) calculated from CL, IPL-H and IPL-OH, the abundance of 16S rRNA genes (purple marked line), and the ratio of MG II Euryarchaeota 16S to Archaeal 16S (green marked line) along the salinity gradient from the river water to seawater. Individual value is shown in Table 1.



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Figure 6. Fractional abundance (%) of GDGTs, RI, and ratio of GDGT-2 to GDGT-3 vs. the ratio of MG II *Euryarchaeota* 16S to Archaeal 16S. SPM samples collected from the lower Pearl River, the PR estuary, and the coastal SCS.



