青海湖1.8万年以来甲藻Dinoflagellate群落变化及其古气候环境的指示意义

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摘 要:近年来,有学者通过古DNA方法成功地重建了海洋沉积记录中古微生物群落变化,并借此反演了当地古环境—气候变化。然而,此种方法对于陆地湖泊沉积记录是否适用仍然有待研究。采用聚合酶链式反应(PCR, Polymerase Chain Reaction)、变性梯度凝胶电泳(DGGE, Denaturing Gradient Gel Electrophoresis)与实时荧光定量聚合酶链式反应(Q-PCR, Quantitative PCR)相结合的综合分析技术手段,系统研究青海湖5.8 m(时间跨度为~18 500 a)沉积柱中的甲藻(Dinoflagellate)多样性和丰度变化。研究结果显示,青海湖Dinoflagellate藻18S rRNA基因序列主要与海洋型藻类 *Woloszynskiahalophila* 和 *Scrippsiellahangoei* 相近(~98%序列相似性)。定量Q-PCR结果显示,每克沉积物含有dinoflagellate藻类18S rRNA基因丰度范围为2.27×10^3~8.55×10^6拷贝。另外,Dinoflagellate藻类18S rRNA基因丰度与总有机碳含量成显著正相关($R=0.408$, $p=0.0001$)。对比分析揭示,较高的藻类丰度对应高总有机碳含量和较低的可溶解性盐电导率;反之,较低的藻类丰度对应较高的可溶解性盐电导率和较低的总有机碳含量。在青海湖区,总有机碳指示着季风降雨变化,并间接地指示着外源输入和湖泊营养状况变化,然而可溶性盐电导率则指示着湖泊盐度变化。综上所述,青海湖沉积柱Dinoflagellate藻类丰度可能反应了历史时期湖泊营养状况和盐度波动情况。

关键词:青海湖;沉积物;古DNA;Dinoflagellate藻18S rRNA基因;古气候环境变化
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引 言

近年来,古DNA方法迅速兴起,爆发式地吸引着众多地质学家对地质沉积记录中基因信息的研究[1-2]。该方法针对残留于地质记录中的DNA信息,成功地恢复了历史时期的大型植物和动物(尤其是那些没有化石遗迹的生物)群落变化,并借此重建当地古气候和古环境变化[2-6]。既然大型动植物DNA能很好地保存于地质沉积记录中,那么微生物DNA也有可能保存于沉积记录中(比如湖泊沉积物)[7-8]。微生物具有分布广泛、结构简单、功能多样的特点,维持着生态系统的基础功能。相较于大型动植物,微生物响应环境条件变化更加敏感[9]。因此,通过重建地质沉积记录中的微生物群落变化,进而反演古气候—环境条件变化,也是研究古气候—环境变化的有效技术手段[10-14]。国外学者Coolen等通过恢复沉积记录中真核藻类群落多样性及其丰度,成功地重建了地质历史时期黑海盐度和干湿气候变化[12,15-16]。真核藻类(例如甲藻Dinoflagellate)是光合自养生物,光能对其生存不可或缺[17];而且,真核藻类的群落结构与丰度显著

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地受控于环境条件(盐度、营养状况和温度等)变化[18-24]。如果在无光的沉积记录中发现真核藻类的DNA信息,说明这些DNA片段应该来自地质历史时期的藻类残体。由此凭借这些残留于地质沉积记录的DNA信息,可以重建地质历史时期真核藻类群落变化,进而推演相应时期的气候环境变化情况。

长期以来,古气候研究一直是地质学领域的热点。其中,青藏高原古环境和古气候研究一直备受关注[25-28]。青藏高原具有特殊的环境和气候特征,已成为科学家们研究古环境和古气候变化的理想场所[9]。湖泊作为青藏高原典型地貌类型之一,具有重要的古气候研究意义[25]。前人大量研究显示,青藏高原湖泊沉积物较好地记录着亚洲季风变化及全球性气候变化事件,是研究古气候环境变化的理想地质载体[9,26,29-35]。最近,Hou等通过古DNA方法研究库赛湖3 000 a来的藻类丰度变化,显示藻类丰度很好地响应了地质历史时期湖泊环境条件变化[36]。该研究证实了古DNA方法在青藏高原古环境和古气候研究中的有效性。然而,通过古DNA方法研究上万年时间尺度的湖泊沉积记录(比如青海湖)的文献则相对缺乏。本文将进一步采用古DNA方法恢复青海湖沉积柱18 000 a来Dinoflagellate群落及其丰度,并尝试重建青海湖古环境变化。

1 材料与方法

1.1 主要试剂和仪器

FastDNA SPIN for Soil Kit(美国MP BIO);Agarose Gel DNA purification Kit Ver.2.0(日本TaKaRa);pGEM-T Easy Vector Systems(日本TaKaRa);Bio-Rad T100™PCR仪(美国Bio-Rad);Bio-Rad GelDoc XR凝胶成像仪(美国Bio-Rad);Dcode™ DGGE仪(美国Bio-Rad);7500 Real Time PCR System(美国Applied Biosystems);Centrifuge 5430R高速台式离心机(德国Eppendorf);DDS-307A电导仪(中国上海精密科学仪器有限公司)以及2400 Series II CHNS/O元素分析仪(美国PerkinElmer)等。

1.2 样品的采集和保存

本文使用的样品来自2011年8月在青海湖(36°39.6′N,100°36.0′E)钻取的沉积物岩芯。具体样品采集和分装情况参见已发表文献[37-38]。简单地说,在野外将岩芯分割成~35 cm长度的柱子,并迅速放入干冰中运送至中国地质大学(北京)地质微生物实验室。岩芯运回到实验室后,在超净工作台分别按照2 cm采样间隔取样,并分装于1.5 mL的灭菌离心管中,用于古DNA提取;同时,采集5 g沉积物于50 mL离心管,用于离心提取孔隙水及其它地化分析。样品编号采用青海湖名字和岩芯深度表示,例如QHLS007代表青海湖岩心7 cm处沉积物样品。此外,样品采集使用的器具均被提前灭菌处理,而且整个分样过程在无菌环境下进行,以避免样品外源污染。

1.3 地化参数测试

所有用于地化分析的沉积物样品均需在5 000 g下离心并收集孔隙水,然后将原始孔隙水稀释50倍后,采用DDS-307A电导仪测试孔隙水电导率。另外,沉积物样品总有机碳含量测试如下。1)将沉积物样品用一个当量盐酸酸化去除碳酸盐;2)将酸化的沉积物用去离子水润洗3遍直到pH中性,然后烘干样品;3)将烘干的沉积物样品磨碎,并使用2400 Series II CHNS/O元素分析仪测试总有机碳含量。

1.4 样品DNA的提取及PCR扩增

采用FastDNA SPIN Kit for soil试剂盒,提取沉积物样品的总DNA,所有操作均参照试剂盒使用说明书。采用聚合酶链式反应(PCR)方法,扩增样品中的dinoflagellate藻18S rRNA基因片段。PCR引物为Dinoflagellate藻18S rRNA基因的特异性引物EukA-1f:5′-AACCTGGTT GAT CCT GCC AGT-3′和Dino_Rev:5-′ACAAGACATGGATGCCCT-3′[39]。PCR扩增长度是503 bp左右。由于下一步需要进行变性梯度凝胶电泳分析,故需在引物Dino_Rev的

5′端加上一个 40 bp 的 GC 夹子。PCR 反应条件参照已发表文献[40]。

1.5　变性梯度凝胶电泳分析及 18S rRNA 基因片段分析

Bio－Rad Dcode™ DGGE 仪用于变性梯度凝胶电泳(DGGE)分析。变性梯度凝胶制备采用 6% 聚丙烯酰胺和 30% ~60% 变性剂(尿素和去离子甲酰胺)浓度梯度[40]。将带 GC 夹子的 PCR 产物加到制备好的变性梯度凝胶中,于 100 V 的电压下电泳 12 h;然后取出凝胶进行染色照相,并在凝胶成像仪紫外光下,用无菌手术解剖刀切割 DNA 目标条带,分装于 1.5 mL 的无菌离心管;最后加入 75 μL 的无菌水过夜保存于 4 ℃冰箱洗脱 DNA。第 2 天以洗脱完备的 DNA 为模板,采用不带 GC 夹子的引物 EukA－1f 和 Dino_Rev 再次进行 PCR 扩增。采用 Agarose Gel DNA purification Kit Ver. 2.0(日本 TaKaRa)试剂盒纯化所得 PCR 扩增产物。纯化后的产物通过质粒连接进入大肠杆菌(JM109)体内进行构建克隆文库,具体方法参照相关文献[41]。克隆完成后将阳性克隆子送至测试中心测序,测序引物为 M13F:5′－TGTAAAACGACGGCCAGT－3′。测序所得原始基因序列首先采用 BioEdit 软件(http://www.mbio.ncsu.edu/bioedit/bioedit.html)进行剪切,然后在 NCBI 基因库(http://www.blast.ncbi.nlm.nih.gov/Blast.cgi)中进行 BLAST 比对,并选取最相似的参考序列。最后将参考序列与样品序列合在一起,采用 MEGA 6.0 软件(http://www.megasoftware.net/index.php)和邻接点法构建系统进化树[42]。

1.6　荧光定量分析(Q－PCR)

用 7500 Real Time PCR System 定量分析沉积物的 dinoflagellate 藻 18S rRNA 基因丰度,定量 PCR 的引物为 EukA－1f 和 Dino_Rev。SYBR Premix Ex Taq™(日本 TaKaRa)试剂用于荧光定量 PCR 反应。PCR 扩增条件为 95 ℃预变性 30 s,然后直接进行 40 个循环 PCR 扩增(95 ℃变性 5 s,56 ℃退火 34 s,72 ℃延伸 60 s)。另外,标准曲线的制作和定量结果分析的方法参见已发表文献[38]。

1.7　DNA 序列在 Gene Bank 中登录号

本次研究获得的所有序列均已提交至 GenBank(NCBI),其登录号为 KU295756 ~ KU295760。

2　结果与分析

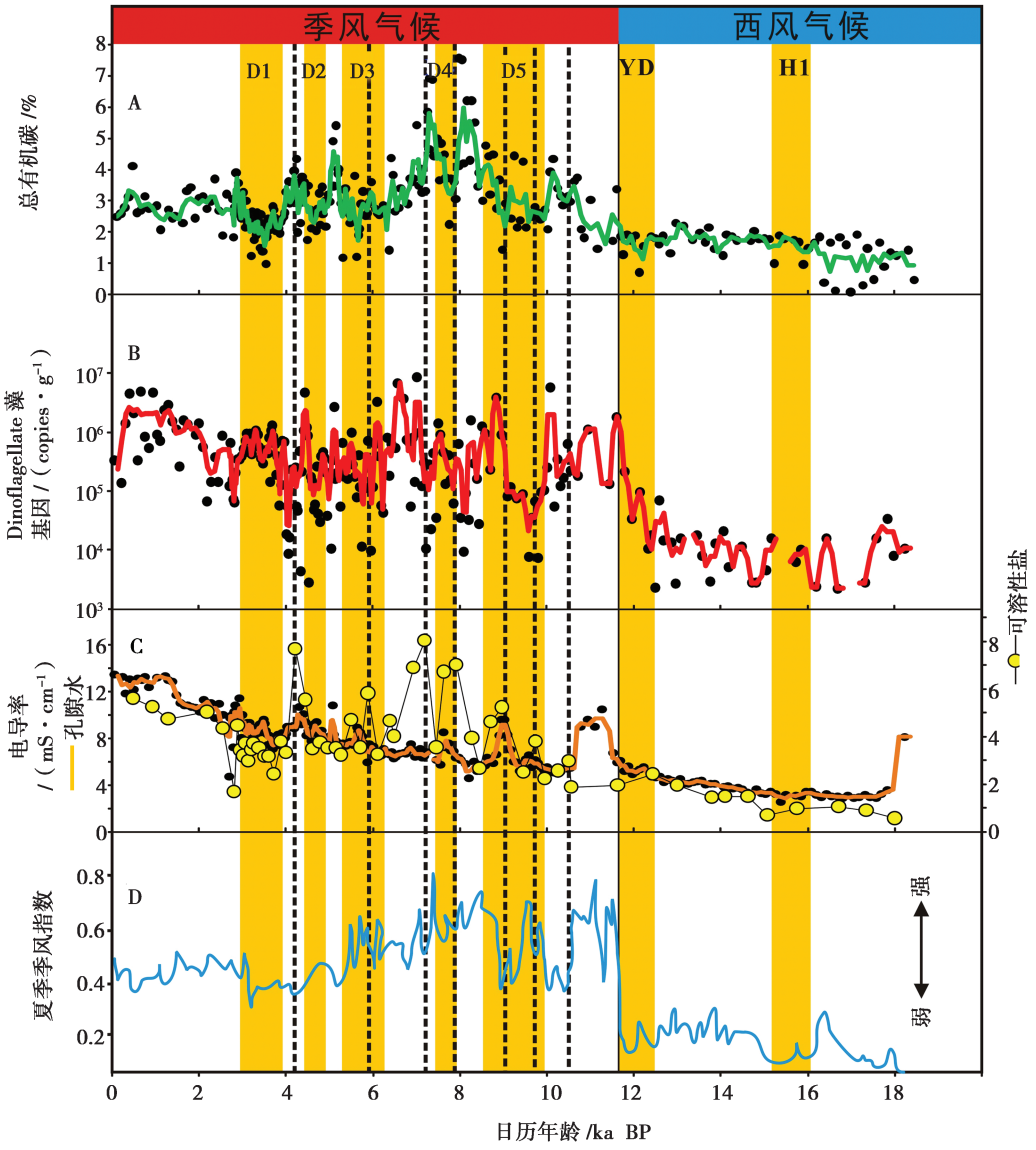
2.1　年龄模型和地球化学参数测试结果

^{14}C 测年数据和沉积柱年龄模型参见已发表文献[38]。测年结果显示本次研究使用的湖泊沉积物岩芯年龄跨度为 ~18 500 a。沉积柱总有机碳含量范围为 0.1% ~7.6%,孔隙水电导范围为 2.3 ~13.4 mS/cm(图 1)。研究结果发现岩芯中段(距今 10.5 ~4.0 ka)沉积物总有机碳含量较高(大于 2%),岩芯底段(18.5 ~10.5 ka)沉积物总有机碳含量较低(小于 2%)。前人研究指出,青海湖沉积记录总有机碳含量指示着湖区夏季季风降雨强弱[29－32]。根据总有机碳的含量变化情况和前人研究结果,本文沉积岩芯柱包含了 7 个典型的地质历史时期干冷事件,D1 ~ D5,以及 YD 和 H1(如图 1 黄色方框所示),它们都对应着较低的总有机碳含量。

2.2　Dinoflagellate 18S rRNA 基因定量 PCR 结果

定量 Q－PCR 结果显示,青海湖沉积柱 dinoflagellate 藻类 18S rRNA 基因丰度范围为每克沉积物具有 2.27×10^3 ~8.55×10^6 基因拷贝(图 1)。另外,Dinoflagellate 藻类 18S rRNA 基因丰度与总有机碳成显著正相关($R=0.408$, $p=0.0001$)。An 等[29]指出青海湖区在距今 11.5 ka 左右夏季季风陡然增强。本文在距今 11.5 ka 也发现 Dinoflagellate 藻类 18S rRNA 基因丰度显著增高。结合前人研究结果,本文以距今 11.5 ka 为时间分界点,将岩芯年龄划分为两个主要时期,夏季季风气候主导时期和西风带气候主导时期[29](图 1)。较高的 Dinoflagellate 藻类基因丰度出现在夏季季风气候主

导时期;而西风带气候主导时期(距今 18.5 ~ 10.5 ka)Dinoflagellate 藻类基因丰度明显偏低(图 1)。此外,本研究还发现,可溶性盐电导率高值对应 dinoflagellate 藻类基因丰度低值(图 1 虚线)。



YD: Younger Dryas; H1: Heinrich 事件 1

图 1 A 青海湖沉积柱总有机碳含量;B 青海湖沉积柱 dinoflagellate 18S rRNA 基因丰度;C 青海湖沉积柱孔隙水和可溶性盐电导率;D 夏季季风指标数[29]

Fig. 1 A: Total organic carbon of the Qinghai Lake core; B: Abundance of Dinoflagellate 18S rRNA gene from this study as quantified by Q-PCR; C: Conductivities of sediment pore water and soluble salts; D: Summer monsoon index of the Qinghai Lake region from a previous study

2.3 DGGE 分析结果及 Dinoflagellate 18S rRNA 基因序列分析

变性梯度电泳(DGGE)分析显示,青海湖沉积物样品中 Dinoflagellate DGGE 带型很少,共有 5 个带型(如图 2 中数字标注)。DGGE 2 型条带几乎分布于所有测试样品。系统进化树分析显示,这些 DGGE 序列主要与海洋型 Dino-

flagellate 藻 *Woloszynskiahalophila* 和 *Scrippsiella-hangoei* 18S rRNA 基因(~98% 序列相似性)相近[43](图 3)。

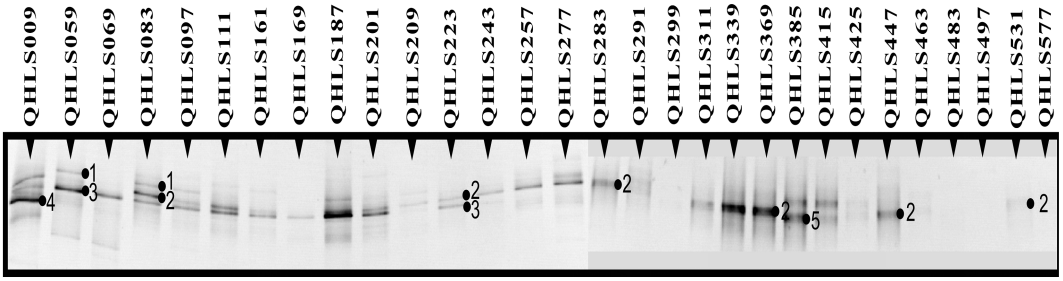
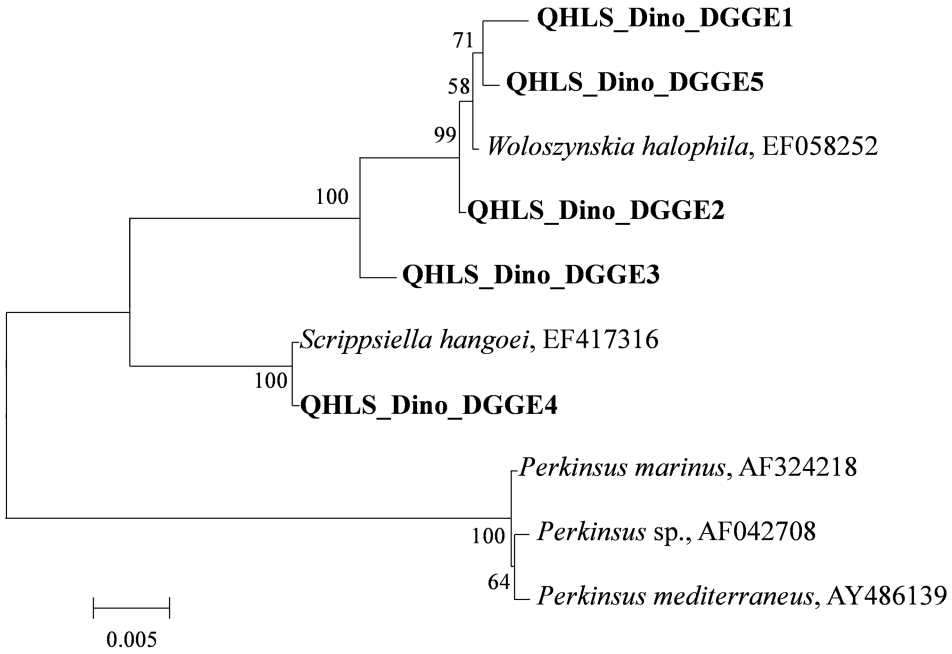


图 2　青海湖沉积物 Dinoflagellate 藻 18S rRNA 基因 PCR 产物 DGGE 图

Fig. 2　Image showing PCR-amplified ancient 18S rRNA genes of dinoflagellate algae in the Qinghai Lake sediment core



采用 Poisson 模型构建的邻接进化树,进化树中只显示大于 50% 的 Bootstrap 值(1 000 个重复运算)。本次研究所得的 DGGE 序列在进化树中均加粗表示,例如 QHLS_Dino_DGGE1 指代青海湖岩芯样品 dinoflagellate 藻类 18S rRNA 基因第 1 号 DGGE 条带序列。所有 DGGE 条带数字编号均来自图 2

图 3　Dinoflagellate 藻类 18S rRNA 基因序列系统进化树

Fig. 3　Neighbor-joining tree showing the phylogenetic relationships of Dinoflagellate18S rRNA gene sequences obtained from this study to their closely related sequences from the GenBank database

3　讨　论

大量海洋型 Dinoflagellate 藻(比如 *Woloszynskia halophile*)长期栖息于青海湖。前人已有报道青海湖分布着大量海洋型古菌[44]。因此,本文观察到海洋型 Dinoflagellate 藻类存在于内陆青海湖是可能的,同时也说明这些藻类并非只存在于海洋环境,内陆咸水湖泊环境也同样存在。另外,本文从青海湖沉积柱获得的 Dinoflagellate 藻类基因型比较单一,并没有发现其基因型对环境变化有响应。

青海湖沉积岩芯样品 Dinoflagellate 藻类基因丰度可能响应着历史时期湖泊营养状况和盐度变化。大量前人研究结果显示,夏季季风增强会导致湖区降雨增加,同时湖泊外源输入增强,湖泊营养提高,相应光合藻类繁盛,湖泊光合生产力增强,使沉积物总有机碳含量增加,且环境温度也会相应提高;相反,弱的夏季季风(比如西风带气候)则会导致寡营养湖泊、低沉积物总有机碳含量和相对低的环境温度[29-30,32]。因此,观察到总有机碳和 Dinoflagellate 藻类丰度成正相关,较高 Dinoflagellate 藻类丰度出现在强夏季季风时期,偏低的藻类丰度出现在干冷气候时期等现象是合理的。主要原因是由于气候导致的湖泊营养条件变化引起 Dinoflagellate 藻类丰度变化,这与大量现代环境研究结果一致[18-19,22-24]。同时,研究进一步暗示了 Dinoflagellate 藻类是青海湖泊沉积物总有机碳重要贡献源。

前人研究还显示,盐度是影响 Dinoflagellate 藻类群落结构和丰度的重要环境因素,盐度越高藻类丰度越低[20-21,43]。历史时期的湖泊盐度波动主要受控于湖泊蒸发强度,沉积物可溶性盐含量指示湖泊蒸发强度[45]。因此,在强夏季风主导时期也会出现许多藻类丰度低值区,这主要是由于湖泊盐度波动导致 Dinoflagellate 藻不能快速适应,从而丰度变低。

此外,值得注意的是,在 6 个干冷时期,Dinoflagellate 藻类基因丰度普遍处于低值区(图 1)。但是在 D1 时期,我们观察到 Dinoflagellate 藻丰度明显走高。这种现象可能是由于盐度波动差异性导致。D1 时期是典型的干冷时期[30],此时期青海湖区温度较低,因此蒸发很弱,故盐度并没有剧烈波动(可溶性盐和孔隙水电导值平缓)。由于盐度并没有剧烈波动,因此在 D1 时期 Dinoflagellate 藻类丰度可能并未明显下降或减少。

4 结 论

本文通过恢复沉积记录中光合藻类基因丰度,重建了青海湖 18000 a 来古环境条件变化。依赖光能的 Dinoflagellate 藻类偏好富营养和低盐环境,故其丰度变化间接反映了湖泊的营养和盐度波动情况。因此,通过恢复沉积物光合藻类古 DNA 信息重建地质历史时期湖泊环境条件变化的方法切实可行,并且这些光合藻类古 DNA 有希望发展成为一种有效的古环境—气候替代指标。

参考文献:

[1] Willerslev E, Cooper A. Review Paper. Ancient DNA [J]. Proceedings of the Royal Society of London B: Biological Sciences, 2005, 272 (1558): 3-16.

[2] Anderson-Carpenter L L, McLachlan J S, Jackson S T, *et al*. Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics[J]. BMC Evolutionary Biology, 2011, 11 (1): 30.

[3] Pedersen M W, Ginolhac A, Orlando L, *et al*. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa [J]. Quaternary Science Reviews, 2013, 75: 161-168.

[4] Willerslev E, Cappellini E, Boomsma W, *et al*. Ancient biomolecules from deep ice cores reveal a forested southern Greenland[J]. Science, 2007, 317(5834): 111-114.

[5] Willerslev E, Hansen A J, Binladen J, *et al*. Diverse plant and animal genetic records from Holocene and Pleistocene sediments[J]. Science, 2003, 300(5620): 791-795.

[6] Willerslev E, Davison J, Moora M, *et al*. Fifty thousand years of Arctic vegetation and megafaunal diet[J]. Nature, 2014, 506 (7486): 47-51.

[7] Coolen M J, Overmann J. Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment[J]. Applied and Environmental Microbiology, 1998, 64(11): 4513-4521.

[8] Coolen M J, Overmann J. 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment [J]. Environmental Microbiology, 2007, 9 (1): 238-249.

[9] Dong H, Jiang H, Yu B, *et al*. Impacts of environmental change and human activity on microbial ecosystems on the Tibetan Plateau, NW China [J]. GSA Today, 2010, 20 (6): 4-10.

[10] Coolen M J, Muyzer G, Rijpstra W I C, *et al*. Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake [J]. Earth and Planetary Science Letters, 2004, 223 (1): 225-239.

[11] D'Andrea W J, Lage M, Martiny J B, et al. Alkenone

producers inferred from well-preserved 18S rDNA in Greenland lake sediments[J]. Journal of Geophysical Research: Biogeosciences, 2006, 111(G3):G03013.

[12] Coolen M J, Orsi W D, Balkema C, *et al*. Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene[J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110 (21): 8609 - 8614.

[13] Coolen M J. 7000 years of Emiliania huxleyi viruses in the Black Sea[J]. Science, 2011, 333 (6041): 451 - 452.

[14] Boere A C, Damsté J S S, Rijpstra W I C, *et al*. Source-specific variability in post-depositional DNA preservation with potential implications for DNA based paleoecological records[J]. Organic Geochemistry, 2011, 42(10):1216 - 1225.

[15] Coolen M J, Saenz J P, Giosan L, *et al*. DNA and lipid molecular stratigraphic records of haptophyte succession in the Black Sea during the Holocene [J]. Earth and Planetary Science Letters, 2009, 284 (3): 610 - 621.

[16] Coolen M J, Boere A, Abbas B, *et al*. Ancient DNA derived from alkenone-biosynthesizing haptophytes and other algae in Holocene sediments from the Black Sea[J]. Paleoceanography, 2006, 21 (1): PA1005.

[17] Hiller R G, Broughton M J, Wrench P M, *et al*. Dinoflagellate light-harvesting proteins: genes, structure and reconstitution, in the chloroplast: from molecular biology to biotechnology[M]. Argyroudi-Akoyunoglou J H, Senger H. Dordrecht: Springer, 1999:3 - 10.

[18] Chen B, Liu H. Temporal stability of marine phytoplankton in a subtropical coastal environment[J]. Aquatic Ecology, 2011, 45(3):427 - 438.

[19] Godhe A, Norén F, Kuylenstierna M, *et al*. Relationship between planktonic dinoflagellate abundance, cysts recovered in sediment traps and environmental factors in the Gullmar Fjord, Sweden [J]. Journal of Plankton Research, 2001, 23 (9): 923 - 938.

[20] Matsubara T, Nagasoe S, Yamasaki Y, *et al*. Effects of temperature, salinity, and irradiance on the growth of the dinoflagellate Akashiwo sanguinea[J]. Journal of Experimental Marine Biology and Ecology, 2007, 342 (2): 226 - 230.

[21] Ellegaard M. Variations in dinoflagellate cyst morphology under conditions of changing salinity during the last 2000 years in the Limfjord, Denmark [J]. Review of Palaeobotany and Palynology, 2000, 109 (1): 65 - 81.

[22] Lee Y W, Kim G. Linking groundwater-borne nutrients and dinoflagellate red-tide outbreaks in the southern sea of Korea using a Ra tracer[J]. Estuarine, Coastal and Shelf Science, 2007, 71(1):309 - 317.

[23] Delmas D, Herbland A, Maestrini S. Environmental conditions which lead to increase in cell density of the toxic dinoflagellates Dinophysis spp. in nutrient-rich and nutrient-poor waters of the French Atlantic coast[J]. Marine ecology progress series, 1992, 89(1):53 - 61.

[24] Watson S B, McCauley E, Downing J A. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status[J]. Limnology and Oceanography, 1997, 42(3):487 - 495.

[25] Colman S M, Yu S Y, An Z, *et al*. Late Cenozoic climate changes in China's western interior: a review of research on Lake Qinghai and comparison with other records[J]. Quaternary Science Reviews, 2007, 26(17):2281 - 2300.

[26] Shen J. Spatiotemporal variations of Chinese lakes and their driving mechanisms since the Last Glacial Maximum: A review and synthesis of lacustrine sediment archives [J]. Chinese Science Bulletin, 2013, 58 (1):17 - 31.

[27] Zheng B, Xu Q, Shen Y. The relationship between climate change and Quaternary glacial cycles on the Qinghai-Tibetan Plateau: review and speculation [J]. Quaternary International, 2002, 97 - 98: 93 - 101.

[28] Owen L A. Latest Pleistocene and Holocene glacier fluctuations in the Himalaya and Tibet[J]. Quaternary Science Reviews, 2009, 28 (21 - 22): 2150 - 2164.

[29] An Z, Colman S M, Zhou W, *et al*. Interplay between the Westerlies and Asian monsoon recorded in Lake Qinghai sediments since 32 ka[J]. Scientific Reports, 2012, 2: 619.

[30] Shen J, Liu X, Wang S, *et al*. Palaeoclimatic changes in the Qinghai Lake area during the last 18,000 years[J]. Quaternary International, 2005, 136 (1): 131 - 140.

[31] Ji J, Shen J, Balsam W, *et al*. Asian monsoon oscillations in the northeastern Qinghai-Tibet Plateau since the late glacial as interpreted from visible reflectance of Qinghai Lake sediments[J]. Earth and Planetary Science Letters, 2005, 233 (1): 61 - 70.

[32] Ji J, Balsam W, Shen J, *et al*. Centennial blooming of anoxygenic phototrophic bacteria in Qinghai Lake linked to solar and monsoon activities during the last 18,000 years[J]. Quaternary Science Reviews, 2009, 28(13):1304 - 1308.

[33] Liu X, Dong H, Yang X, *et al*. Late Holocene forcing of the Asian winter and summer monsoon as evidenced by proxy records from the northern Qinghai-Tibetan Plateau [J]. Earth and Planetary Science Letters, 2009, 280 (1): 276 - 284.

[34] Lai Z, Kaiser K, Brückner H. Luminescence-dated aeolian deposits of late Quaternary age in the southern Tibetan Plateau and their implications for landscape history[J]. Quaternary Research, 2009, 72 (3): 421 - 430.

[35] Yang X, Kamenik C, Schmidt R, *et al*. Diatom-based conductivity and water-level inference models from eastern Tibetan (Qinghai-Xizang) Plateau lakes [J]. Journal of Pale-

olimnology,2003,30(1):1 - 19.

[36] Hou W,Dong H,Li G,*et al*. Identification of photosynthetic plankton communities using sedimentary ancient DNA and their response to late-Holocene climate change on the Tibetan Plateau[J]. Scientific Reports, 2014,4:6648.

[37] Wang H, Dong H, Zhang C L, *et al*. Water depth affecting thaumarchaeol production in Lake Qinghai, northeastern Qinghai-Tibetan plateau: Implications for paleo lake levels and paleoclimate[J]. Chemical Geology, 2014, 368: 76 - 84.

[38] Yang J, Jiang H, Dong H, *et al*. Sedimentary archaeal *amoA* gene abundance reflects historic nutrient level and salinity fluctuations in Qinghai Lake, Tibetan Plateau[J]. Scientific Reports, 2015, 5:18071.

[39] Boere A, Abbas B, Rijpstra W, *et al*. Late-Holocene succession of dinoflagellates in an Antarctic fjord using a multiproxy approach:Paleoenvironmental genomics, lipid biomarkers and palynomorphs[J]. Geobiology, 2009,7(3):265 - 281.

[40] Boere A C, Rijpstra W I C, de Lange G J,*et al*. Exploring preserved fossil dinoflagellate and haptophyte DNA signatures to infer ecological and environmental changes during deposition of sapropel S1 in the eastern Mediterranean[J]. Paleoceanography, 2011, 26 (2): PA2204.

[41] Yang J, Jiang H, Dong H, *et al*. *amoA*-encoding archaea and thaumarchaeol in the lakes on the northeastern Qinghai-Tibetan Plateau, China[J]. Frontiers in Microbiology, 2013, 4: 329.

[42] Tamura K,Stecher G, Peterson D, *et al*. MEGA6:molecular evolutionary genetics analysis version 6.0[J]. Molecular Biology and Evolution,2013,30(12):2725 - 2729.

[43] Kremp A, Elbrchter M, Schweikert M,*et al*. *Woloszynskia halophila* (Biecheler) comb. nov.: A bloom-forming cold-water dinoflagellate co-occurring with Scrippsiella hangoei (Dinophyceae) in the Baltic Sea[J]. Journal of Phycology, 2005, 41 (3): 629 - 642.

[44] Jiang H, Dong H, Yu B, *et al*. Dominance of putative marine benthic Archaea in Qinghai Lake, north-western China [J]. Environmental Microbiology, 2008, 10 (9): 2355 - 2367.

[45] Yu J. Lake Qinghai, China: A multi-proxy investigation on sediment cores for the reconstructions of paleoclimate and paleoenvironment since the Marine Isotope Stage 3[C]// Darmstadt:Faculty of Materials and Geoscience, Technical University of Darmstadt,2005.

Ancient DNA Derived from Dinoflagellates in Qinghai Lake Sediments and their Implications for Paleoclimate and Paleoenvironment Constructions

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Abstract:In recent years, ancient DNA-basedmicrobial communities were retrieved from marine sedimentaryrecords, which were successfully applied to reconstruct local paleo-environmental changes. However, it is poorly known whether ancient DNA-basedmicrobial studies could be possible for reconstructing historic limnic environmental conditionsin lacustrine sedimentary records. Here, we investigatedthe diversity and abundance of light-dependent dinoflagellate algaein a 5.8 m sediment core(spanning the last 18,500 years) of Qinghai Lake using an integrated approach including polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and quantitative PCR (qPCR). Our phylogenetic results showed that the dinoflagellate 18S rRNA gene sequences from this study were closely (~98% identity) related to marine algalspecies*Woloszynskiahalophila* and *Scrippsiellahangoei*. qPCR results showed that the dinoflagellateabundance in the sedimentsalong the Qinghai Lake core was 2.27×10^3 ~ 8.55×10^6 copies per gram sediment, which wassignificantly ($R=0.408$, $p=0.0001$) correlated withtotal organic carbon

(TOC) content. Parallel analyses revealed that lowdin of lagellate 18S rRNA gene abundance corresponded to low TOC and high conductivities of soluble salt, whereas high dinoflagellate gene abundance corresponded to high TOC and low conductivities of soluble salt. In the Qinghai Lake region, TOC can be served asan indicator of paleo-precipitation, which is related to historic nutrient input; while the conductivities of soluble salt indicated salinity fluctuation. Therefore, our data suggested that temporal variation of dinoflagellate 18S rRNA gene abundance preserved inthe Qinghai Lake sediments mightreflect the variations innutrient level and salinity sincethe latePleistocene in the Qinghai Lake region.

Key words: Qinghai Lake; Sediment; Ancient DNA; Dinoflagellate 18S rRNA gene; qPCR; Paleo-climate and-environment variations

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Advances in Paleoclimate Proxies Based on Microbial Glycerol Dialkyl Glycerol Tetraether Lipids on the Qinghai-Tibet Plateau

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Abstract: The microbial glycerol dialkyl glycerol tetraethers (GDGTs) are increasingly popular in the field of organic geochemistry. These high-molecular-weight compounds are sensitive to environmental variables, and therefore they contain important information on environmental parameters in paleoclimatic studies. On the Qinghai-Tibet Plateau, studies of GDGTs have just begun in the early 2010s but progressed rapidly. Here we reviewed advances in paleoclimate proxies based on microbial GDGTs on the Qinghai-Tibetan Plateau. Our aim was to provide some useful references for paleoclimatic studies in this region.

Key words: GDGTs; Soils; Lakes; Paleoclimatic proxies; Qinghai-Tibet Plateau; GDGTs