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Genomic identification of the long-chain alkenone producer in freshwater Lake Toyoni, Japan: implications for temperature reconstructions

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ABSTRACT

Identifying the lacustrine haptophyte species that produce long-chain alkenones (LCAs) is essential prior to down-core temperature reconstructions. Here, we investigated the identity of LCA-producing species from Lake Toyoni, Japan using 18S ribosomal DNA (rDNA) and organic geochemical analyses. The rDNA analyses identified eighteen operational taxonomic units (OTUs) of which only one fell within the haptophyte phylotype. This haptophyte belongs to the Group I phylotype, as supported by the LCA distribution found in surface and down-core sediments, and is closely related to a haptophyte found in Lake BrayaSø (Greenland). The inferred temperature using the Greenland calibration is very close to the Lake Toyoni surface temperature recorded during the spring/early summer season, when the LCA-producing haptophyte is likely to bloom. We therefore suggest that the temperature calibration from the Lake BrayaSø, Greenland is a suitable calibration for down-core temperature reconstructions at Lake Toyoni.
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1. Introduction

Long-chain alkenones (LCAs) are a class of C_{35}–C_{42} unsaturated ketones that are produced by three distinct phylogenetic groups of haptophyte algae within the Isochrysidales order (e.g., Theroux et al., 2010). Group I includes haptophyte species restricted to freshwater and oligohaline lakes, whereas Group II includes *Ruttnera lamellosa* and *Isochrysis galbana*, as well as related species found in brackish waters and saline lakes. Group III includes the marine producers *Emiliania huxleyi* and *Gephyrocapsa oceanica*. Since the degree of unsaturation of C_{37} LCAs varies as a function of environmental temperature (Brassell et al., 1986; Prahl and Wakeham, 1987), LCAs have been used as temperature proxies based on the alkenone unsaturation indices U^{K}_{37} and $U^{K'}_{37}$ (e.g., Brassell et al., 1986; Prahl and Wakeham, 1987). Since the first report of the occurrence of LCAs in lakes by Cranwell (1985), a number of significant calibrations of the U^{K}_{37} and $U^{K'}_{37}$ indices have been established in lacustrine settings (e.g., Zink et al., 2001; Chu et al., 2005; D'Andrea and Huang, 2005; Sun et al., 2007; Pearson et al., 2008; Toney et al., 2010, 2012; D'Andrea et al., 2011; Theroux et al., 2013; Longo et al., 2016). However, the presence of multiple species within and among lakes has complicated the development of LCA-based proxies for temperature reconstructions since different species may produce different temperature calibrations (Theroux et al., 2010; Randlett et al., 2014). Identifying the LCA-producing haptophyte species present prior to lacustrine down-core temperature reconstructions is therefore essential for selecting the most appropriate calibration.

The identification of lacustrine haptophytes through microscopy is challenging due to their relatively small size and seasonal production (Zink et al., 2001). In addition, LCA distribution parameters such as the C_{37}/C_{38} ratio do not give an accurate indication of haptophyte species identity (Theroux et al., 2010), even though recent studies by Longo et al. (2016, 2018) have shown that Group I haptophytes feature a highly specific LCA distribution with the

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presence of tri-unsaturated isomers ($C_{37:3b}$, $C_{38:3b}$ and $C_{39:3b}$) and C_{38}Me LCAs. Environmental genomics using haptophyte-specific oligonucleotide primers targeting 18S ribosomal DNA (rDNA) coding regions has thus become the prominent technique to unambiguously identify LCA-producing haptophyte species (Simon et al., 2000; Coolen et al., 2004; D'Andrea et al., 2006; Theroux et al., 2010; Randlett et al., 2014; Longo et al., 2016).

In the present study, we use genomic analyses to target the haptophyte 18S rRNA gene and to identify the LCA-producer(s) in Lake Toyoni, Japan. Lake Toyoni represents a key natural archive to reconstruct past climate variability because it is under the influence of Arctic Oscillation and Pacific Decadal Oscillation (Tsuji et al., 2008) and is situated at the boundary of the northern edge of the East Asian Summer Monsoon (Xu et al., 2010) making it sensitive changes to the global climate system such as changes in El Niño–Southern Oscillation (e.g., Zhang et al., 1996; Wang et al., 2003; Hong et al., 2005). Genomic analyses, coupled with LCA profiles, allow us to clearly identify the LCA-producing species in Lake Toyoni and to discuss which temperature calibration is most appropriate for down-core temperature reconstructions.

2. Methods

2.1. Site and sampling

Lake Toyoni (42°05′N; 143°16′E) is located in Hokkaido (Japan) (Fig. 1) and is a small (0.3 km^2), dimictic, freshwater (0 g/kg) lake. It is a closed basin with an average water depth of 12 m and a maximum water depth of 19 m. The lake is ice-covered from the end of November until early April. The nearest weather station is located in Hiroo, approximately 25 km north of Lake Toyoni. In situ lake parameters, including temperature, conductivity, pH, depth and dissolved oxygen, were measured each month between September 2010 and October 2011 during the ice-free season at 1 m depth intervals for the entire water column using a Horiba Multiparameter Water Quality meter (Table 1). Water probe data show that: (1) the mean lake surface temperature is 8.3 °C, 17.2 °C and 13.7 °C in spring, summer and autumn, respectively (Table 1); (2) the water column is well mixed in April and November, suggesting that the lake overturns in spring and autumn (Supplementary Fig. S1); (3) the bottom water becomes hypoxic (O_2 < 2.0 mg/L) between July and October due to thermal stratification (Supplementary Fig. S1) and a lack of mixing within the water column; (4) the lake is slightly acidic (pH: 5.5–6.8; Table 1). A surface sediment sample (upper 0–1 cm) was retrieved using an Ekman grab sampler in September 2011, and kept at 4 °C until analysis. A 250 cm long sediment core was also recovered for palaeoclimate reconstruction from a water depth of 19 m using a square-rod Livingstone piston corer from a floating platform. Ten subsamples of this core were selected at different depths and analysed in the present study in order to compare surface and down-core LCA profiles.

2.2. rDNA analyses

Metagenomic DNA was extracted from wet surface sediment (±0.5 g) using a FastDNA™ Spin Kit for soils and sediments (MP Biomedicals) according to the manufacturing specifications and quantified using a QUBIT™ (Invitrogen) fluorescence assay.

An 887 bp region of the haptophyte 18S ribosomal RNA gene was amplified via a standard polymerase chain reaction (PCR) using the eukaryotic-specific forward primer EukA(F) designed by Medlin et al. (1988) and a modified haptophyte reverse primer (887Deg(R); DVA ATA CGA RTR CCC CYR AC, where D = A/G/T; V = A/C/G; R = A/G; Y = C/T). The reverse primer was a modification of the original Prym887R designed by Simon et al. (2000) and Coolen et al. (2004) by adding degenerate bases at genomic positions that varied between alignments of the different groups of haptophytes. As Lake Toyoni is a novel environment with potentially undiscovered haptophytes, incorporating degenerate bases into Prym887R presented us with the opportunity to capture any new and phylogenetically diverged haptophyte sequences from these environmental samples. However, to ensure that specificity for haptophytes was still retained, the primer set (EukA (F)/887Deg(R)) was benchmarked using polymerase chain reaction (PCR) amplifications on genomic DNA extracted from isolated strains of known haptophyte algae (*Emiliania huxleyi*; CCAP 920/8 and *Ruttnera lamellosa*; CCAP 918/1). Following confirmation, PCR amplifications were performed using a BIOTAQ PCR kit (bioline BIO-2107; including the BIOTAQ DNA Polymerase) and the following thermal cycle conditions: 4 min of initial denaturing at 95 °C, 35 cycles of denaturing for 30 sec at 95 °C, 40 sec of primer annealing at 57 °C, and 40 sec of primer extension at 72 °C, with a final

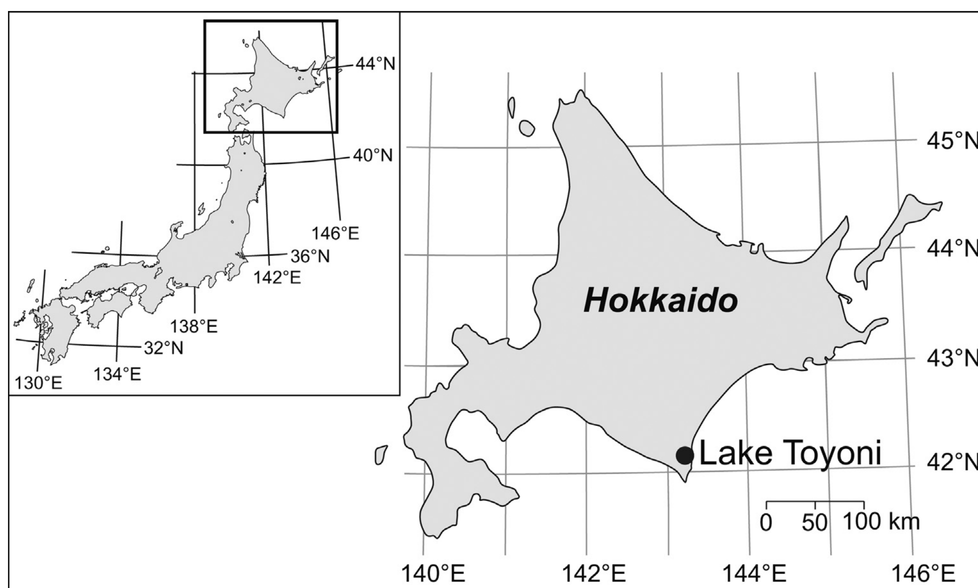

Fig. 1. Location of Lake Toyoni, Japan.

Table 1
In situ lake parameters of Lake Toyoni measured during the ice-free period.

Date/period of measurement	Water temp. (°C)	Surface water temp. (°C)	pH	Conductivity (mS/cm)	Dissolved oxygen (mg/L)
19/09/2010	12.9	19.7	6.8	0.04	14.2
14/10/2010	7.6	7.7	6.7	0.04	17.4
16/04/2011	5.9	7.1	6.2	0.04	14.7
17/05/2011	7.6	9.6	5.9	0.04	16.0
06/06/2011	9.3	13.8	5.7	0.04	15.5
25/06/2011	10.6	16.0	5.9	0.04	17.4
25/07/2011	11.9	19.4	6.4	0.04	15.2
25/08/2011	12.8	19.8	5.8	0.04	12.3
19/09/2011	11.9	15.8	5.5	0.04	10.1
25/11/2011	10.7	11.6	5.9	0.04	12.5
Spring (April–May)	6.7	8.3	6.1	0.04	15.4
Spring/early summer (April–June)	8.3	11.6	5.9	0.04	15.9
Summer (June–August)	11.2	17.2	5.9	0.04	15.1
Autumn (September–November)	10.8	13.7	6.2	0.04	13.6

extension of 10 min at 72 °C. The primer concentrations were 10 μM. Genomic DNA extracted from isolated strains of the haptophyte algae were used for positive control during all PCR amplification.

The amplicon was then resolved on a 0.8% agarose gel, extracted (NuceloSpin® Extract II; Macherey-Nagel), and cloned using the TOPO TA Cloning® Kit (Invitrogen), after which 100 colonies were screened for unique inserts using a restriction digest with Hpa II. Unique clones were mini-prepped (Mini-Prep spin kit, Qiagen) and sequenced via the Sanger method from both the 5′ and 3′ directions using standard T7F and T3 primers (Source biosciences; Nottingham, UK). Forward and reverse sequence for each environmental clone were trimmed by deleting T7F and T3 linking sites and assembled via CLC Genomics Workbench. A complete overlap was observed between both forward and reverse sequences, which were designated operational taxonomic units (OTU) 1-18. Closest relatives of these 18 OTUs were curated via a BLAST search from the GenBank database (NCBI). To make sure all sequences were the same length and equally aligned, the OTUs were aligned using MAFFT v7.040 (Katoh and Standley, 2013).

Phylogeny was estimated via a Bayesian inference performed using MrBayes v.3.2.2 under the GTR model of nucleotide base substitution considering invariants and a gamma-shaped distribution of rates across sites. Markov Chain Monte Carlo simulations were performed at 1,000,000 generations with trees sampled every 100 generations and the first 10,000 trees discarded as burn-in for both the tree topology and posterior probability. *Cyclonexis annularis*, *Chrysoxys* sp., *Ochromonas danica*, *Odontella sinensis* and *Thraustochytrium multirudimentale* were selected as outgroups after de Vargas et al. (2007). OTUs were analysed to infer species' identity along with full-length 18S rRNA gene sequences from reference taxa.

2.3. LCA analyses

Homogenised freeze-dried sediments (±2 g) were extracted using sonication (3×) with dichloromethane (DCM):methanol (MeOH) (3:1, v:v). The total lipid extracts were then separated into neutral and acid fractions by elution through a LC-NH_2 SPE column using DCM:isopropyl alcohol (1:1, v:v) followed by 4% acetic acid in ether (v:v) as eluents, respectively. The neutral fractions were further separated into four fractions of increasing polarity by chromatography over a silica gel column packed with 35–70 μm particles using hexane, DCM, ethyl acetate:hexane (1:3, v:v) and MeOH as eluents.

LCAs, contained in the second fraction (DCM), were detected using gas chromatography with a flame-ionization detector (GC-FID). GC-FID analyses were performed on an Agilent 7890B Series GC system configured with an Agilent VF-200 ms capillary column (60 m length, 0.25 mm i.d., 0.10 μm film thickness) (Longo et al., 2013). Hydrogen was used as the carrier gas at a 36 cm/s column flow rate. The GC method used splitless injection (320 °C), and the oven temperature was programmed from 50 °C (hold for 1 min) to 255 °C at 20 °C/min, then to 300 °C at 3 °C/min, followed to 10 °C/min increase to 320 °C and hold for 10 min.

Samples were also run with the same temperature program on an Agilent 7890B Series GC coupled with a 5977A GC–EI mass spectrometer (GC–MS) to confirm the identity of the LCAs using the known ion chromatograms and by comparison of mass spectral data and GC retention times with published data (de Leeuw et al., 1980; Marlowe et al., 1984). The alkenone unsaturation index U^K_{37} was calculated following Brassell et al. (1986). LCA distribution parameters such as $\%C_{37:4}$ (Rosell-Melé, 1998) and RIK_{37} (ratio between $C_{37:3a}$ and $C_{37:3b}$ isomers) (Longo et al., 2016) were also calculated.

3. Results

3.1. 18S rDNA haptophyte identification

Eighteen OTUs were identified in Lake Toyoni, of which only OTU7 fell within the haptophyte phylotype (Fig. 2 and Supplementary Fig. S2), and was 96% similar to a haptophyte found in Lake BrayaSø (Greenland) based on a BLAST analysis (Table 2 and Supplementary Table S1). The second closest BLAST hit for OTU7 was a haptophyte species from the freshwater Lake Esch-sur-Sûre in Luxembourg (Supplementary Table S1). The remaining 17 OTUs did not occupy the haptophyte phylotype and were 95 to 99% related to other groups of algae, such as *Chlamydomonas* sp., *Chlorophyta* sp., *Dinobryon sertularia*, and uncultured alveolate and *Woloszynskia* clones (Table 2 and Supplementary Fig. S2). OTU7 grouped within the Group I phylotype along with previously published sequences from the Greenland lakes (D'Andrea et al., 2006), Chinese lakes (Tso Ur and Keluke Hu), North American lakes (Skoal Lake and Medicine Lake) and a Canadian lake (Upper Murray Lake) (Theroux et al., 2010) (Fig. 2). The OTU7 sequence has been deposited in GenBank (accession number: MH119763).

3.2. LCA distribution at Lake Toyoni

Consistent with the identification of one haptophyte species, C_{37}–C_{39} LCAs were detected in the surface sediment of Lake Toyoni. The LCA distribution was also specific to Group I haptophyte (Longo et al., 2016, 2018), with abundant $C_{37:4}$ (48.8% of the total C_{37} LCAs), the presence of $C_{38:3}$Me LCAs, and the full suite of

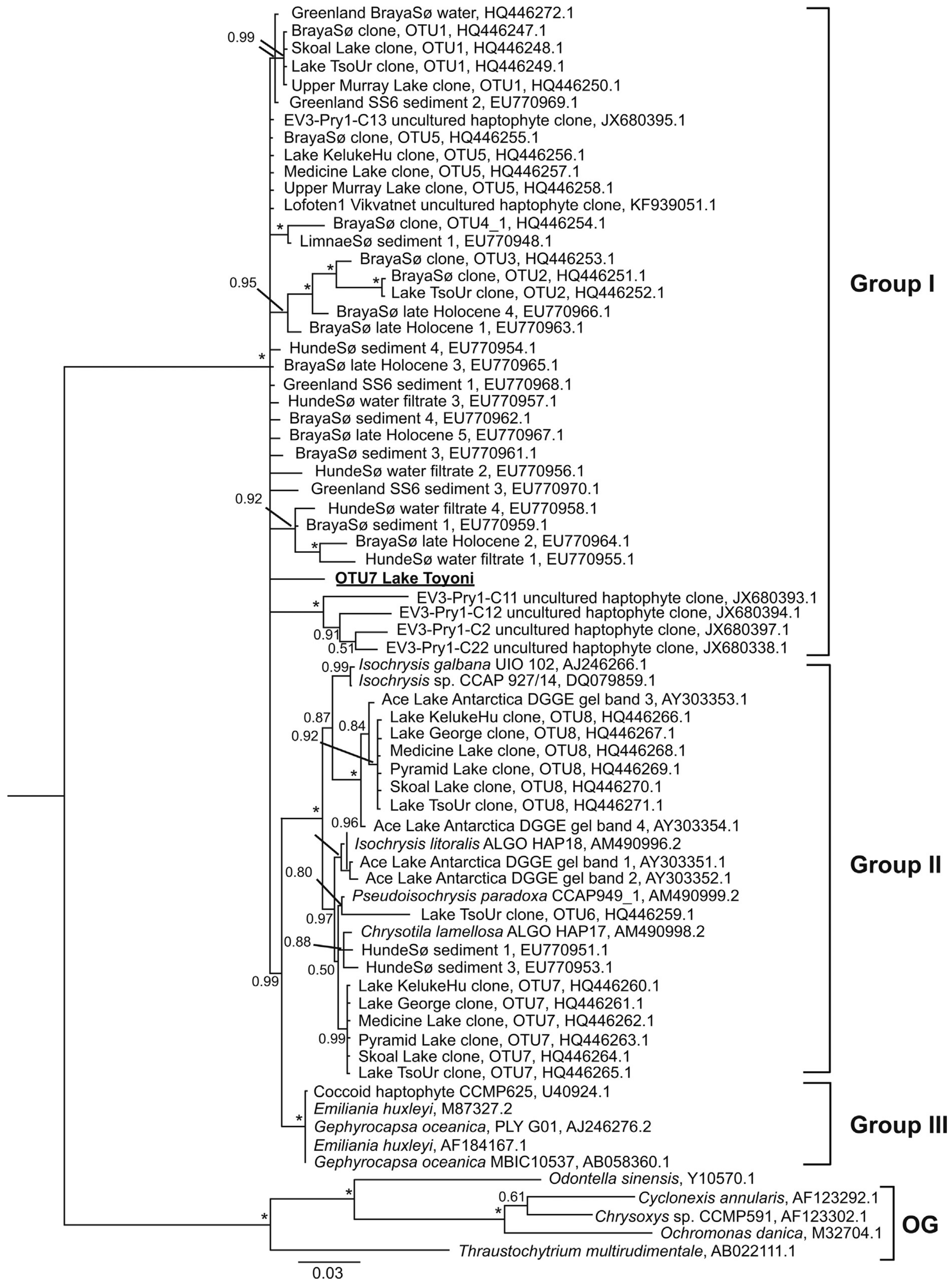

Fig. 2. A phylogenetic tree depicting 18S rRNA gene-inferred relationships among haptophyte algae. A Bayesian inference was used to generate this consensus tree from publicly available partial length sequence fragments. GenBank accession numbers follow all species names. An asterisk (*) indicates posterior probability values of 1.00 at branching nodes; all other values are as shown. The evolutionary distance for the number of changes per site is represented by the scale bar. Brackets mark the LCA-producing haptophyte groups I, II, III, and the outgroup (OG). The phylogenetic position of the Group I LCA-producer in Lake Toyoni (OTU7 Lake Toyoni) is shown in bold and underlined (GenBank accession number: MH119763).

Table 2
Closest BLAST hits to OTUs identified at Lake Toyoni.

OTU	Closest Blast hit with accession number	Max identity	%query
OTU1	*Chlamydomonas* sp. SAG 75.94 18S ribosomal RNA gene, partial sequence; AF514399.1	99%	97%
OTU2	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU3	*Chlorophyta* sp. I-155 clone A1 18S ribosomal RNA gene, partial sequence; EF432529.1	98%	99%
OTU4	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU5	Uncultured *Woloszynskia* clone ESS220206.046 18S ribosomal RNA gene, partial sequence; GU067825.1	96%	99%
OTU6	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU7	Uncultured haptophyte clone BrayaSø_water_18S 18S ribosomal RNA gene, partial sequence; HQ446272.1	96%	99%
OTU8	*Dinobryon sertularia* small subunit ribosomal RNA gene, partial sequence; AF123289.1	99%	99%
OTU9	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	96%	99%
OTU10	Uncultured *Woloszynskia* clone ESS220206.046 18S ribosomal RNA gene, partial sequence; GU067825.1	96%	99%
OTU11	Uncultured *Woloszynskia* clone ESS220206.046 18S ribosomal RNA gene, partial sequence; GU067825.1	98%	99%
OTU12	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU13	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU14	Uncultured eukaryote clone Ch8A2mF4 18S ribosomal RNA gene, partial sequence; JF730784.1	97%	99%
OTU15	Uncultured *Banisveld* eukaryote clone P2-3m3 18S ribosomal RNA gene, partial sequence; EU091850.1	98%	99%
OTU16	Uncultured eukaryote clone KRL01E15 18S ribosomal RNA gene, partial sequence; JN090875.1	99%	99%
OTU17	Uncultured *alveolate* clone PAA8SP2005 18S ribosomal RNA gene, partial sequence; EU162629.1	95%	99%
OTU18	*Chlorophyta* sp. I-155 clone A1 18S ribosomal RNA gene, partial sequence; EF432529.1	99%	99%

tri-unsaturated isomers ($C_{37:3b}$, $C_{38:3b}$ and $C_{39:3b}$) (Fig. 3). The U^K_{37} ratio value was −0.49 and the RIK_{37} was at 0.53 (Table 3).

The highly specific Group I LCA distribution was also consistently observed in the studied down-core sediments (Fig. 3) with U^K_{37} values ranging from −0.39 to −0.56 and RIK_{37} values from 0.48 to 0.57 (Table 3).

4. Discussion

4.1. Haptophyte DNA amplification at Lake Toyoni

Haptophyte-specific primers (Prym429F and Prym887R) are commonly used to identify haptophyte species in lacustrine environments (e.g., Coolen et al., 2004; Theroux et al., 2010; D'Andrea et al., 2016). Although these primers target the most variable region of the haptophyte 18S rRNA gene, they produce relatively short sequences (461 bp). Using short sequences limits the ability to match the haptophyte DNA to other haptophyte

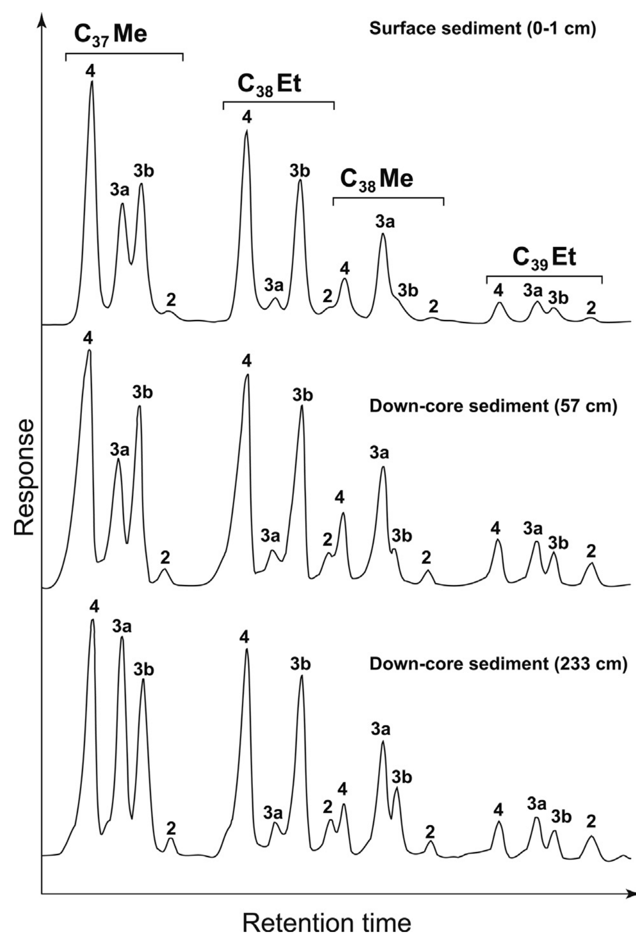

Fig. 3. LCA distribution in surface sediment and down-core sediments of Lake Toyoni Lake showing the consistent presence of $C_{37:3b}$, $C_{38:3b}$ and $C_{39:3b}$ isomers and C_{38}Me alkenones, highly specific of Group I haptophytes.

sequences within GenBank. In this study, we used a combination of a eukaryotic-specific primer (EukA(F)) and a re-designed haptophyte reverse primer (887Deg(R)) that allows for longer sequence amplification (887 bp) and the identification of potentially diverged haptophyte species. However, since the degenerate bases increase variability, this combination can result in amplification of eukaryotic species other than just haptophyte algae. In fact, only one of the 18 OTUs identified in Lake Toyoni occupied the haptophyte phylotype (OTU7; Fig. 2 and Supplementary Fig. S2; Table 2).

Ideally the best primers for haptophyte amplification would be eukaryotic-specific primer (EukA(F) and EukB(R)) as the size of the sequence obtained (1792 bp) increases the posterior probability (Theroux et al., 2010). Amplification of the Lake Toyoni surface sediment was, however, unsuccessful using generic eukaryote-specific primers. Empirical obstacles, such as low haptophyte abundance, primer mismatches and a high guanine (G) and cytosine (C) content (up to 57%) could explain this unsuccessful amplification (Liu et al., 2009; Stoeck et al., 2010). We therefore suggest that the original haptophyte-specific primers (Prym429F and Prym887R) are still more suitable to target haptophyte sequences, especially where LCA concentrations are low.

4.2. LCA-producer at Lake Toyoni

The combined genomic and LCA analyses revealed the presence at Lake Toyoni of a new LCA-producing haptophyte species belonging to the Group I phylotype (Figs. 2 and 3). Previous studies have

Table 3
LCA data for surface and down-core sediments of Lake Toyoni with inferred temperatures using the three in situ temperature calibrations from lakes with phylogenetically confirmed Group I haptophyte.

Depth (cm)	U^K_{37}	$\%C_{37:4}$	RIK_{37}	RIK_{38E}	Longo et al. (2016) (Alaska)	D'Andrea et al. (2016) (Norway)	D'Andrea et al. (2011) (Greenland)
					T (°C)[a]	T (°C)[b]	T (°C)[c]
0–1	−0.49	48.8	0.53	0.16	9.05	5.81	11.7
26	−0.42	44.1	0.54	0.25	12.2	8.11	14.5
56	−0.50	47.7	0.49	0.19	8.66	5.53	11.3
57	−0.56	55.5	0.50	0.18	5.85	3.45	8.85
80	−0.54	54.1	0.48	0.15	6.57	3.98	9.49
88	−0.34	38.2	0.48	0.13	15.9	10.9	17.9
149	−0.53	57.8	0.50	0.20	7.24	4.47	10.1
167	−0.48	52.8	0.54	0.17	9.59	6.21	12.2
201	−0.48	48.4	0.48	0.16	9.45	6.11	12.1
214	−0.51	52.5	0.52	0.18	8.10	5.11	10.8
233	−0.39	42.5	0.57	0.17	13.8	9.32	15.9

$U^K_{37} = [C_{37:2} - C_{37:4}]/[C_{37:2} + C_{37:3} + C_{37:4}]$ (Brassell et al., 1986).
$\%C_{37:4} = 100 \times ([C_{37:4}]/[C_{37}\ LCAs])$ (Rosell-Melé, 1998).
$RIK_{37} = [C_{37:3a}]/[C_{37:3a} + C_{37:3b}]$ (Longo et al., 2016).
$RIK_{38E} = [C_{38:3a}Et]/[C_{38:3a}\ Et + C_{38:3b}Et]$ (Longo et al., 2016).
[a] $T = (U^K_{37} + 0.68)/0.021$ (Longo et al., 2016).
[b] $T = (U^K_{37} + 0.655)/0.0284$ (D'Andrea et al., 2016).
[c] $T = (U^K_{37} \times 40.8) + 31.8$ (D'Andrea et al., 2011).

shown the presence of Group I haptophyte species in freshwater and oligohaline lakes from Greenland (BrayaSø, HundeSø and LimnaeSø), North America (Skoal and Pyramid), Canada (Upper Murray), France (Étang des Vallées), and Alaska (Toolik) (Theroux et al., 2010; Simon et al., 2013; Longo et al., 2016). In parallel, recent studies (Longo et al., 2016, 2018) have shown the widespread occurrence of Group I haptophytes in freshwater and oligohaline lakes based on their highly specific LCA distribution (with abundant $C_{37:4}$, the presence of C_{38}Me LCAs, and the full complement of 16 C_{37}–C_{39} LCAs including four tri-unsaturated isomers).

While a mix of Group I and Group II haptophyte species can occur in oligohaline lakes, freshwater lakes such as Lake Toyoni appear to be immune to species-mixing effects (Longo et al., 2018). Indeed, a single haptophyte sequence has been amplified at Lake Toyoni using our combination of primers. This is also supported by the RIK_{37} value at the surface sediment of Lake Toyoni (0.53; Table 3), since RIK_{37} values of 0.48–0.60 would be indicative of lakes hosting only Group I haptophytes (Longo et al., 2016, 2018). Furthermore, the LCA distribution and RIK_{37} values of the down-core samples (Table 3) strongly suggest that Group I haptophyte was consistently the only LCA-producer at Lake Toyoni in the past.

4.3. Implication for palaeotemperature reconstructions

The presence of a single haptophyte both in surface and down-core sediments is promising for the use of Lake Toyoni as a palaeotemperature archive as it avoids complications linked to multiple LCA-producer species (Theroux et al., 2010). Unfortunately, there is currently no site-specific calibration developed for Lake Toyoni. So far, three in situ temperature calibrations have been developed from three lakes in Greenland, Norway and Alaska with phylogenetically confirmed Group I haptophyte producers (D'Andrea et al., 2011, 2016; Longo et al., 2016). We applied these three calibrations using the U^K_{37} value of the surface sediment of Lake Toyoni (−0.49). The temperatures produced by the Norway calibration (D'Andrea et al., 2016), the Alaska calibration (Longo et al., 2016) and the Greenland calibration (D'Andrea et al., 2011) are 5.8 °C, 9.1 °C and 11.7 °C, respectively (Table 3). The Alaska and Greenland calibration-derived temperatures are within the range of observed lake surface temperature during the ice-free months (April–November; 7.1–19.8 °C) at Lake Toyoni. More precisely, the Alaska calibration derived temperature is close to modern spring (April–May: 9.3 °C) and late-spring (9.6 °C in May 2011) lake surface temperatures (Table 1), while the Greenland calibration derived temperature is particularly close to modern spring/early summer (April–June: 11.6 °C) and mid-autumn (11.6 °C in October 2011) lake surface temperatures (Table 1). Even though the timing of LCA production in Lake Toyoni is currently unknown, LCAs are most likely produced during the ice-free time period (~March–November) and may have a preference for the spring period as seen in other temperate lakes (Toney et al., 2010).

Previous studies have shown that lacustrine LCA-producing haptophytes bloom in the photic zone and produce LCAs during the spring/early summer season, concurrent with the period of lake ice melt (Toney et al., 2010; D'Andrea et al., 2011, 2016; Longo et al., 2018). The U^K_{37}-derived temperatures would thus reflect the lake temperature of the photic zone during the spring/early summer season, which is supported by the application of both Alaska and Greenland temperature calibrations. In addition, a BLAST search indicates that the Lake Toyoni OTU7 is closely related to the haptophyte species from Lake BrayaSø, Greenland (Table 2), further supporting the application of the Greenland temperature calibration by D'Andrea et al. (2011) for down-core temperature reconstructions at Lake Toyoni, which will be used in future studies. In the first instance, preliminary data using the down-core sediment samples indicate that lake temperature varied between 8.8 and 17.9 °C (Table 3).

5. Conclusions

This study is the first to report the presence of a Group I haptophyte in a Japanese lake (Lake Toyoni), which was identified using a combination of a eukaryotic-specific primer and a re-designed haptophyte reverse primer. This LCA-producing species represent a new taxon within the Group I haptophytes, and is closely related to the Greenland LCA-producer. Both genomic and LCA distribution analyses showed that the Group I haptophyte is the only LCA-producer at Lake Toyoni, holding promise for the use of this site as a palaeotemperature archive. Despite a lack of in situ temperature calibrations, we suggest the temperature calibration from the Lake BrayaSø, Greenland is an appropriate calibration for down-core temperature reconstructions at Lake Toyoni, which will be used in future studies to decipher the variability of the East Asian Monsoon in the palaeoclimatic record.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2018.09.011.

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