

## Gas chromatographic analysis to compare the fatty acid composition of fifteen lichen species, with a focus on *Stereocaulon*

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**Abstract:** The composition of fatty acids (FAs) is known to discriminate families within genera of bacteria, fungi, octocorals and algae. Here, we applied the more sensitive gas chromatographic (GC) analysis to investigate the taxonomic value of using FA composition to discriminate 15 species of lichen, including three chlorolichens (two *Cladonia* species and *Stereocaulon scutelligerum*), nine tripartite lichens (*Stereocaulon* species) and three cyanolichens (*Lichina pygmaea*, *Collema cristatum*, *Peltigera membranacea*). One macroscopic cyanobacterium (*Nostoc* sp.), corresponding to the photobiont partner of *Peltigera* and *Collema* cyanolichens, was included for comparison. Five lipid extraction methods were evaluated, using *Stereocaulon scutelligerum*. Shaking at 80 °C with chloroform/methanol (2:1, v/v) was the most efficient and reproducible method. The total FA composition of the 16 species was compared, using GC coupled with flame ionization detection or mass spectrometry. A statistical hierarchical cluster analysis was used to determine the similarity between the FA profiles, based on total, saturated, unsaturated and branched-chain FAs (BCFAs). The BCFA profile allowed a distinction between the *Stereocaulon* species and the cyanolichens, in contrast to all the other types of FAs. A detailed characterization of eight FA subclasses, provided by the GC analysis, suggested a preferential pathway for the biogenesis of unsaturated FAs through desaturases, which was especially favoured in the cyanolichens.

**Keywords:** biosynthetic pathways, branched-chain fatty acids, chemotaxonomy, cyanolichens, *Nostoc*

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### Introduction

Total lipids, although hard to define, include lipids containing fatty acids (FAs), as well as fat-soluble vitamins (A, D, E, K), carotenoids and sterols (Cyberlipid Center; AOCs Lipid Library). FAs are found in lipids such as triglycerides, which are not only implicated in energy storage, but also in the phospholipids of membranes. The biosynthesis of FAs is via condensation of malonyl coenzyme A units by a FA synthase complex. They usually contain even numbers of carbon atoms in straight or branched chains, and may be saturated or unsaturated.

Thin-layer chromatography (TLC) and gas chromatography (GC) are the most common and most powerful analytical procedures for separating and analyzing lipids (Cyberlipid Center). TLC, followed by the isolation of simple lipids (e.g. sterols, carotenoids, total phospholipids, glycolipids), is the method usually applied to lichen lipid analysis (Nylander 1866; Culbertson 1969; Hawksworth 1976; Solberg 1987; Tabacchi *et al.* 1987; Bachelor *et al.* 1990; Gonzalez *et al.* 1992;

**Abbreviations:** BCFA: Branch-chained fatty acid; FAs: Fatty acids; FAMES: Fatty acid methyl esters; GC: Gas Chromatography; GC-FID: Gas Chromatography coupled with Flame Ionization Detection; GC-MS: Gas Chromatography coupled with Mass Spectrometry detection; HAC: Hierarchical Ascendant Classification; PUFAs: Polyunsaturated fatty acids.

**Nomenclature:** i/ai-C<sub>x</sub>:y n-z denotes a fatty acid containing x carbon atoms and y double bonds, where z indicates the position of the double bonds (i.e. the first double bond starting from the methyl terminal), i is an iso-fatty acid, and ai is an anteiso-fatty acid; Δ is a desaturase enzyme; ε is an elongase enzyme.

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Sasaki *et al.* 1999). As such, TLC has been a valuable tool for the systematics of lichens (Spribille *et al.* 2011). An alternative approach is GC, which is more sensitive and allows the separation and identification of individual fatty acids that are less abundant. However, intact lipids containing bound and free FAs are not volatile enough to be injected into the GC and are therefore usually first derivatized by saponification, followed by methylation to increase the volatility of the compounds, prior to analysis. After derivatization, the lipid's skeleton (glycerol, for example, in triacylglycerol) and the volatile FA methyl esters (FAMES) are obtained.

Since each phylum has a typical FA signature (Fig. 1), their FA profiles are increasingly being used as a chemotaxonomic tool for the identification and classification of fungi (Kock & Botha 1998), bacteria (Kaneda 1991), micro- and macroalgae (Kumari *et al.* 2013; Sahu *et al.* 2013) and octocorals (Imbs & Dautova 2008). While FA profiles are generally reported as FAMES in lichens (Dembitsky 1992; Rezanka & Dembitsky 1999; Dembitsky & Srebnik 2002), only one study has considered the relationship between FA profiles and lichen systematics (Sasaki *et al.* 2001).

To extend these results, we focused on the lichen genus *Stereocaulon*, which is challenging because it contains a large number of species and relatively few distinguishing phenolic substance profiles. To address this we obtained, from different regions of the world, ten species of *Stereocaulon*, which represented four sections described by Lamb (1951, 1977). Six other species were also included in this study to evaluate the discriminatory strength of FAME analysis. Two *Cladonia* species were included to allow a comparison with previously published results (Sasaki *et al.* 2001). Three cyanolichens (*Lichina pygmaea*, *Collema cristatum*, *Peltigera membranacea*), along with a macroscopic form of a cyanobacterium (*Nostoc* sp.), corresponding to the photobiont of the *Collema* and *Peltigera* species, were also added for comparison.

Several reports on lichen FAs describe the composition of various genera (see

Supplementary Material Text S1, available online). However, they are not easy to compare because a variety of lipid extraction protocols were used. We therefore first developed an optimization procedure for lipid extraction, using *Stereocaulon scutelligerum*. We compared the extraction duration, volume and composition of solvent mixture, extraction yields and diversity of FAMES identified by gas chromatography coupled with flame ionization detection (GC-FID). Moreover, an internal standard was used to quantify the extraction yield for FAs in the fraction, to avoid considering other liposoluble compounds that were co-extracted. The selected method was then checked for reproducibility, using a second species submitted to the same protocol. Finally, we applied the selected method to the 16 species for a comparative analysis of the FAMES profile.

After injection into the GC, the FAMES are quantified by flame ionization and characterized by their retention time. They are then identified after an additional injection on a GC coupled to a mass spectrometric (MS) detector and compared with a database. The GC-FID-MS reveals the total FA profile, which includes the FA chain length (C8, C18, C23, etc.), the number and the position of double bonds of the unsaturated FAs (C18:2n-6, C22:6n-3). The analysis also distinguishes the iso- and anteiso-series for the branched-chain FAs (BCFAs). Thus the high sensitivity, selectivity and resolution of the GC-FID-MS method allowed an unambiguous determination of the FA composition and a quantification of total FAs, thanks to the internal standard heneicosanoic acid (C21:0).

Our goals were therefore to 1) analyze the FA content in the form of FAMES of 15 lichens and one cyanobacterium, 2) compare the FA profiles of chlorolichens and cyanolichens and 3) determine, using hierarchical ascendant classification, if FA profiles are of taxonomic value for differentiating lichen genera, as well as *Stereocaulon* species, with regard to the classification scheme of Lamb (1951, 1977). We applied the above, using a qualitative and quantitative approach based on FA analysis by GC-FID-MS, in order to elucidate lichen chemotaxonomy.

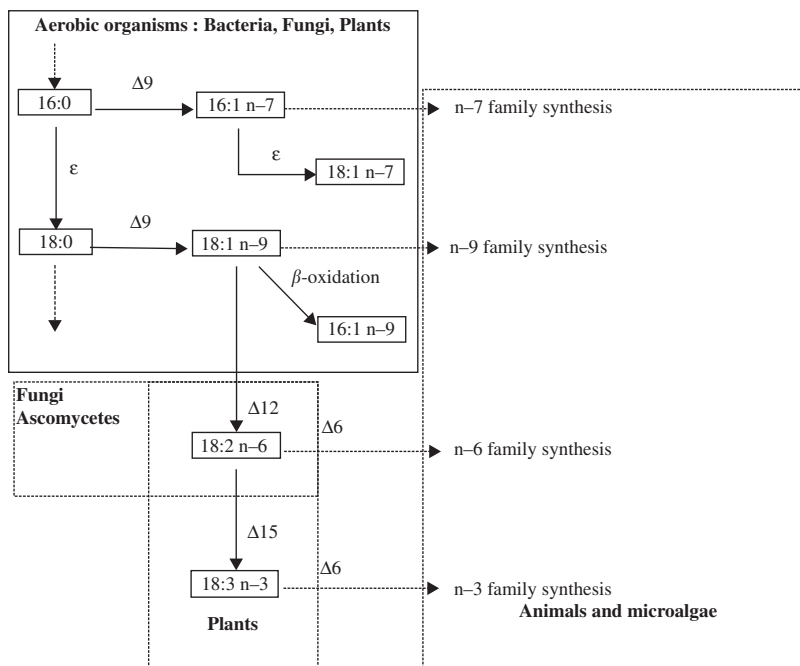


FIG. 1. Simplified biosynthetic pathways for major unsaturated fatty acids.  $\Delta x$  = desaturase where  $x$  indicates the position of the cis double bond introduced in the chain;  $\epsilon$  = elongase;  $C_x:y n-z$  = a fatty acid containing  $x$  carbon atoms and  $y$  double bonds, where  $z$  indicates the position of the first double bond starting from the methyl terminal.

## Materials and Methods

### Lichen material

Fifteen lichens and one cyanobacterium were obtained from different locations (Table 1), representing a wide range of temperate and tropical climates. Three contained a chlorococcoid alga (*Stereocaulon scutelligerum*, *Cladonia rangiferina*, *C. portentosa*) and three contained a cyanobacterium only, two of these having *Nostoc* (*Collema cristatum* var. *marginale*, *Peltigera membranacea*) and one *Calothrix* (*Lichina pygmaea*). The nine remaining lichen species (*Stereocaulon vulcani*, *S. vesuvianum*, *S. dactylophyllum*, *S. evolutum*, *S. pileatum*, *S. alpinum*, *S. graminosum*, *S. halei*, *S. montagneanum*) contained both a chlorococcoid alga and an additional cyanobacterium in cephalodia. They are listed according to the taxonomic order, family, genus, section and subsection to which they belong. *Stereocaulon* species were identified and authenticated by HJM Sipman (Berlin Museum, Germany) and the other species (*Cladonia*, *Peltigera*, *Collema*, *Lichina*) by L. Massé (Rennes, France). Voucher specimens were deposited in the herbarium of Rennes1, as a code JB/year/number of species.

The material was air-dried for one day at room temperature, in the shade, and stored in darkness until used for experimentation.

### Reagents

Boron trifluoride ( $BF_3$ , 14% (by wt) in methanol), saturated, unsaturated, iso- and anteiso-FAs, the internal standard (heneicosanoic acid; C21:0), and other reagents and solvents, were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

### Sample preparation and optimization of lipid extraction

After cleaning, several whole thalli of air-dried lichens (200 mg) were ground with a mixer mill to ensure sufficient pulverization and homogenization, which allowed impregnation by the solvents during the extraction process. For the tripartite lichens, whole thalli included pseudopodetia, phyllocladia and cephalodia; cephalodia were unambiguously present and in large quantity for *S. halei*, *S. montagneanum* and *S. graminosum*. To optimize the extraction method, lichen thalli (*S. scutelligerum*) were tested using three systems: 1) a test tube rotator wheel (Labinco LD-79, Fisher Scientific); 2) a parallel synthesis apparatus (Heidolph Parallel Synthesis Apparatus<sup>®</sup>-Instruction Synthesis 1, France); 3) a Soxhlet Extraction Apparatus. Lipids were extracted according to the method described by Folch *et al.* (1957), with a slight modification for the solvent system, as extraction was carried out in parallel using chloroform/methanol

TABLE 1. List of species, sampling locations, dates and altitudes for the lichens and other species used in the study.

Species	Order	Family	Subgenus/Section/Subsection according to (Lamb 1951)	Sampling site, altitude, collection date	Voucher code
<b>Lichens</b>					
<i>Stereocaulon alpinum</i> Laur.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Stereocaulon</i>	Savoie (France), 2000 m, August 2012	JB/12/45
<i>S. dactylophyllum</i> Flörke	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Stereocaulon</i>	Brittany, (France), 260 m, July 2010	JB/10/120
<i>S. evolutum</i> Graewe	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Stereocaulon</i>	Brittany, (France), 260 m, July 2010	JB/10/121
<i>S. graminosum</i> Schaer.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Denudata/Denudata</i>	Sumatra (Indonesia), 2800 m, November 2009	JB/09/118
<i>S. halei</i> Lamb	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Holostelidium/Holostelidium/Aciculisporae</i>	Sumatra (Indonesia), 2800 m, November 2009	JB/09/117
<i>S. montagneanum</i> Lamb	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Holostelidium/Holostelidium/Holostelidium</i>	Sumatra (Indonesia), 1500 m, November 2009	JB/09/119
<i>S. pileatum</i> Ach.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Stereocaulon</i>	Brittany (France), 200 m, September 2012	JB/12/44
<i>S. scutelligerum</i> Th. Fr.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Denudata/Denudata</i>	Reunion (Indian Ocean), 1000 m, March 2011	JB/11/123
<i>S. vesuvianum</i> Pers.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Denudata/Denudata</i>	Sicily (Italy), 1350 m, August 2001	JB/02/01
<i>S. vulcani</i> (Bory) Ach.	<i>Lecanorales</i>	<i>Stereocaulaceae</i>	<i>Stereocaulon/Denudata/Denudata</i>	Reunion (Indian Ocean), 1000 m, March 2011	JB/11/122
<i>Cladonia portentosa</i> (Dufour) Coem.	<i>Lecanorales</i>	<i>Cladoniaceae</i>		Lot (France), 260 m, November 2005	JB/05/54
<i>C. rangiferina</i> (L.) Weber ex F.H. Wigg.	<i>Lecanorales</i>	<i>Cladoniaceae</i>		Pyrénées (France), 1500 m, August 2002	JB/02/64
<i>Collema cristatum</i> var. <i>marginale</i> (L.) Weber ex F.H. Wigg.	<i>Peltigerales</i>	<i>Collemataceae</i>		Lot (France), 260 m, October 2004	JB/04/31
<i>Lichina pygmaea</i> (Lightf.) C.Agardh.	<i>Lichinales</i>	<i>Lichinaceae</i>		Brittany (France), intertidal, December 2007	JB/07/98
<i>Peltigera membranacea</i> (Ach.) Nyl.	<i>Peltigerales</i>	<i>Peltigeraceae</i>		Savoie (France), 1200 m, August 2011	JB/11/130
<b>Cyanobacterium</b>					
<i>Nostoc</i> sp.	<i>Hormogonales</i>	<i>Nostocaceae</i>		Morbihan (France), 40 m, January 2012	JB/13/166

(2:1, v/v) and chloroform/methanol (2:1, v/v) + 5% acetic acid (v/v). The following five methods were tested in triplicate to evaluate the reproducibility of the extractions.

**Method 1.** A rotator wheel apparatus with a capacity to simultaneously extract 32 samples was used. Samples were extracted for 12 h with chloroform/methanol (2:1, v/v, 6 ml) at room temperature, under constant rotation at 10 rpm.

**Methods 2 and 3.** A Heidolph Parallel Synthesis apparatus with a capacity to simultaneously extract 24 samples was used. Samples were extracted three times, for 1 h each, with chloroform/methanol (2:1, v/v, 6 ml) with (Method 2) or without (Method 3) acetic acid, at 80 °C, under constant shaking at 700 rpm.

**Methods 4 and 5.** A Soxhlet apparatus was used, which allowed for only one extraction at a time. Samples were extracted once, at 60 °C, for 4 h, with chloroform/methanol (2:1, v/v, 250 ml) with (Method 4) or without (Method 5) acetic acid.

To standardize the method, we initially used 100 µg of margaric acid (C17:0), added at the beginning of the extraction process. However, after we detected endogenous C17:0 in the 16 samples, we replaced this standard with 100 µg of heneicosanoic acid (C21:0), thus removing any doubts about the performance of this quantitative analysis.

Samples treated with Methods 1 to 3 were centrifuged at low speed (470 g) for 20 min at room temperature to recover the organic phase containing the total lipids, whereas solvents of samples treated with Methods 4 and 5 were first reduced under pressure. All were then evaporated to dryness under a nitrogen flow. The final extracts were stored at -20 °C under nitrogen until derivatization.

After selection of the most accurate method (as described in the Results), reproducibility was again tested using thalli of another *Stereocaulon* species, *S. vesuvianum*.

### Sample derivatization and GC-FID/GC-MS analysis

Total extracts were derivatized by saponification, using sodium hydroxide (0.5 M) in MeOH for 30 min at 70 °C, then methanolized in 14% BF<sub>3</sub> in MeOH for 15 min at the same temperature. After hydrolysis, the resulting FAMES were extracted from this reaction mixture with *n*-hexane. They were then analyzed by GC (Agilent 6890N, Toulouse, France) using a split injector (10:1) at 250 °C, a bonded silica capillary column (BPX 70, 60 m × 0.25 mm, 0.25 µm film thickness; SGE, Villeneuve-St Georges, France) and helium as the carrier gas (constant flow: 1.5 ml/min). The column temperature program started at 150 °C, increased by 1.3 °C/min to 220 °C and held at 220 °C for 10 min. The FID temperature was 250 °C (air: 450 ml/min; hydrogen: 40 ml/min). Identification of FAMES was based on retention times obtained for FAMES, prepared from FA standards and detected by FID.

For calculations based on identification of FAMES by GC-FID, peak areas were determined using ChemStation software (Agilent, version B04.02) and results were expressed as % of total identified FAs (Equation 1):

$$\%_i = \frac{AUC_i \times 100}{\sum AUC}$$

where %<sub>*i*</sub> represents the percentage of total identified FAs, AUC<sub>*i*</sub> is the FAME peak area, and ∑AUC is the sum of FA peak areas on the chromatogram. The quantity of total FAs was calculated using the internal standard and expressed as mg g<sup>-1</sup> dry weight of lichen (Equation 2):

$$M = \frac{\sum AUC \times m_{C_{21:0}}}{AUC_{C_{21:0}}} \times \frac{1}{m}$$

where M represents the total FA content expressed as mg g<sup>-1</sup> dry weight of lichen; m<sub>C<sub>21:0</sub></sub>, as an FA, is the weight of the internal standard in mg; AUC<sub>C<sub>21:0</sub></sub> is the area of the peak of the internal standard on the chromatogram; and m is the weight of the lichen from which the lipids were extracted (in g).

The unambiguous identification for most of the FAMES was performed by comparing their retention times with those prepared from standard FAs, including iso- and anteiso-FAs (11-methyldodecanoic acid (iso-C13:0)), 12-methyltetradecanoic acid (anteiso-C15:0), 13-methyltetradecanoic acid (iso-C15:0), 14-methylhexadecanoic acid (anteiso-C17:0) and 19-methyleicosanoic acid (iso C21:0). When ambiguous identifications were obtained by GC-FID, samples were analyzed by GC-MS using a 7890N chromatograph (Agilent) fitted with the same column as above and connected to a 5975C MSD working in the EI mode (70 eV). Mass spectra of FAMES were compared to those in the NIST database (v2.0, 2008).

### Data analysis

For reproducibility among experiments, values of extraction yields are expressed as means ± 1SD. Statistical significance of the differences was assessed, using GraphPad Prism (GraphPad Software Inc., La Jolla, CA), by analysis of variance followed by the Student-Newman-Keuls post-hoc test.

For the Hierarchical Ascendant Classification (HAC) analysis (package *stats*, function *hclust*), normality of data was firstly assessed on the FA composition using the multivariate Shapiro-Francia test (package *mvsf*; Delmail *et al.* 2011), with software R 2.13.0. The HAC analysis, the biplot analysis (package *stats*, function *biplot*), and the explained-discrimination assessment were performed on these data to highlight the variance between total and saturated FA contents. Similar analyses were then carried out on the iso- and anteiso-FA compositions of these lichens.

HAC is used here, instead of a Principal Component Analysis (PCA), to better visualize distances between species, especially because close relationships occur between several species, thus rendering PCA less informative. The explained-discrimination assessment is calculated using the function *prcomp* (package *stats*) on the data matrix to obtain all explained variances and the subfunction *\$rotation* is used to obtain the weights of parameters on the factorial axis.

## Results

### Optimization of the extraction method

Five extraction methods were applied to 200 mg of dried thallus powder from *Stereocaulon scutelligerum* in order to select the fastest, lowest-cost method that would result in a complete extraction, reproducible results and a large FA diversity. Extractions were carried out using a rotating mixing wheel, a Soxhlet apparatus and a parallel synthesis robot. Extraction solvents included the usual lipid solvent (chloroform/methanol; 2:1, v/v) with and without acetic acid (5%, v/v); an exception was the rotating wheel, where only the acid-free lipid solvent was used. Statistical analyses were performed to compare the results of each method. The various extraction methods resulted in different yields and FA (in the form of FAMES) profiles (Table 2). The highest extraction yields were obtained with Methods 4 and 5 (2.1% and 1.9%, respectively), but they were nevertheless excluded because of: 1) a lack of reproducibility; 2) a low unsaturated FAs extraction rate; and 3) an unacceptably long extraction time. Method 1 was also discarded because of a lower content of minor polyunsaturated FAs (PUFAs). Method 3 was preferred over Method 2, as acetic acid did not substantially improve extraction yields. Thus, Method 3 was selected for all analyses on the 16 species, namely extraction using the Heidolph Parallel Synthesis Apparatus, three extractions for 1 h each, with chloroform/methanol (2:1, v/v, 6 ml), at 80 °C, under constant shaking at 700 rpm. The selected method, applied to two lichen species (*S. scutelligerum* and *S. vesuvianum*), gave good reproducibility for extraction yields ( $0.77 \pm 0.03 \text{ mg g}^{-1}$  dried lichen and  $0.82 \pm 0.02 \text{ mg g}^{-1}$  dried lichen, respectively) and

FA profiles (see Supplementary Materials Table S1, available online). A fourth extraction with the same protocol did not result in any additional FAs (data not shown), indicating a complete lipid extraction.

### Fatty acid profile of the lichens

#### *Fatty acids of the 16 species*

The FA composition of the 16 species examined by capillary GC-FID is shown in Table 3. Total FAs varied from 0.3 to  $2.7 \text{ mg g}^{-1}$  of dried lichen. The higher content of FAs was observed in *S. pileatum* ( $2.7 \text{ mg g}^{-1}$ ) and *S. dactylophyllum* ( $2 \text{ mg g}^{-1}$ ), compared to *S. graminosum* ( $0.3 \text{ mg g}^{-1}$ ), *S. montagneanum* ( $0.5 \text{ mg g}^{-1}$ ) and both *Cladonia* species (0.4 and  $0.5 \text{ mg g}^{-1}$ , respectively).

Forty-five FAs were identified, including 24 saturated FAs, corresponding to a 30% ratio, except for *S. graminosum* (45.0%) and *Cladonia rangiferina* (50.8%). The major saturated acids were palmitic acid (C16:0; 12.6–27.4%) and stearic acid (C18:0; 3.0–17.2%). However, the content of C18:0 was low (<8%) for *Stereocaulon dactylophyllum*, *S. pileatum*, *Peltigera membranacea*, *Lichina pygmaea*, *Nostoc* sp. and *Collema cristatum* var. *marginale*. The proportion of BCFAs was highest for *C. cristatum* (7.9%), *S. vesuvianum* (6.1%), *C. rangiferina* (5.7%) and *Nostoc* (5.2%). These were distributed between iso-acids (C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0) and anteiso-acids (C14:0, C15:0, C16:0, C17:0, C18:0) in a ratio of 1:2.

Each lichen species contained significant levels of unsaturated FAs. Total monoene FAs varied from 11.2 to 34.4%; the major one was C18:1(n-9) (28.8%), although levels of C16:1(n-7) and C18:1(n-7) were also significant for some lichen species, such as *C. cristatum* (12.2% and 8.0%), *Nostoc* (5.1% and 4.6%) and *L. pygmaea* (5.1% and 3.1%). Other monounsaturated FAs were also detected, but at trace levels, for example C14:1(n-5), C16:1(n-9), C20:1(n-9), C22:1(n-9), C24:1(n-9). The unique diene C18:2(n-6) varied between 12.4% (*C. cristatum* var. *marginale* and *S. vesuvianum*) and 52.2%

TABLE 2. Comparison of five fatty acid extraction methods, based on the extraction yield ( $\text{mg g}^{-1}$  dry weight) and FA profiles (% of total) in the lichen *Stereocaulon scutelligerum* (mean  $\pm$  1 SD,  $n = 3$ ). Total values are given in bold.

	Method 1	Method 2	Method 3	Method 4	Method 5
<b>Extraction yield (<math>\text{mg g}^{-1}</math> DW)</b>	<b>1.40 <math>\pm</math> 0.07</b>	<b>1.30 <math>\pm</math> 0.21</b>	<b>1.3 <math>\pm</math> 0.07</b>	<b>2.10 <math>\pm</math> 0.01</b>	<b>1.90 <math>\pm</math> 0.01</b>
<b>Saturated</b>					
C12:0	0.06 $\pm$ 0.01	0.03 $\pm$ 0.04	0.05 $\pm$ 0.02	0.52 $\pm$ 0.23	0.51 $\pm$ 0.04
C14:0	0.39 $\pm$ 0.01	0.44 $\pm$ 0.00	0.43 $\pm$ 0.05	1.34 $\pm$ 0.26	1.26 $\pm$ 0.25
C15:0	0.22 $\pm$ 0.01	0.21 $\pm$ 0.01	0.23 $\pm$ 0.03	0.72 $\pm$ 0.02	0.76 $\pm$ 0.05
C16:0	14.40 $\pm$ 0.04	16.83 $\pm$ 0.50	15.58 $\pm$ 1.14	23.30 $\pm$ 4.94	20.19 $\pm$ 3.28
C18:0	9.21 $\pm$ 0.13	10.83 $\pm$ 0.32	9.76 $\pm$ 0.24	16.48 $\pm$ 4.59	13.02 $\pm$ 3.69
C20:0	0.52 $\pm$ 0.01	0.65 $\pm$ 0.05	0.58 $\pm$ 0.09	0.97 $\pm$ 0.17	0.99 $\pm$ 0.14
C22:0	0.39 $\pm$ 0.01	0.41 $\pm$ 0.02	0.37 $\pm$ 0.02	1.49 $\pm$ 0.07	1.65 $\pm$ 0.07
C24:0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.06 $\pm$ 0.05	1.25 $\pm$ 0.25
<b>Total saturated</b>	<b>25.17 <math>\pm</math> 0.10</b>	<b>29.41 <math>\pm</math> 0.86</b>	<b>27.01 <math>\pm</math> 1.60</b>	<b>45.89 <math>\pm</math> 9.75</b>	<b>39.62 <math>\pm</math> 6.76</b>
<b>Branched-chain</b>					
iC12:0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.01 $\pm$ 0.02	0.01 $\pm$ 0.02
iC13:0	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.01	0.07 $\pm$ 0.05	0.06 $\pm$ 0.04
iC14:0	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.17 $\pm$ 0.02	0.22 $\pm$ 0.00
aiC14:0	0.16 $\pm$ 0.00	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.39 $\pm$ 0.16	0.26 $\pm$ 0.11
aiC15:0	0.11 $\pm$ 0.02	0.10 $\pm$ 0.01	0.11 $\pm$ 0.04	0.15 $\pm$ 0.02	0.20 $\pm$ 0.02
iC16:0	0.36 $\pm$ 0.01	0.33 $\pm$ 0.00	0.35 $\pm$ 0.01	0.29 $\pm$ 0.03	0.33 $\pm$ 0.03
aiC16:0	1.21 $\pm$ 0.01	1.11 $\pm$ 0.08	1.08 $\pm$ 0.07	0.81 $\pm$ 0.15	0.94 $\pm$ 0.08
iC17:0	0.09 $\pm$ 0.00	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.12 $\pm$ 0.00	0.13 $\pm$ 0.01
aiC17:0	0.42 $\pm$ 0.02	0.45 $\pm$ 0.05	0.43 $\pm$ 0.06	0.85 $\pm$ 0.47	0.68 $\pm$ 0.20
iC18:0	0.31 $\pm$ 0.01	0.32 $\pm$ 0.00	0.32 $\pm$ 0.01	0.32 $\pm$ 0.11	0.11 $\pm$ 0.05
iC20:0	0.28 $\pm$ 0.01	0.23 $\pm$ 0.02	0.22 $\pm$ 0.00	0.19 $\pm$ 0.09	0.19 $\pm$ 0.05
iC21:0	0.00 $\pm$ 0.00	0.25 $\pm$ 0.04	0.26 $\pm$ 0.12	1.82 $\pm$ 0.02	1.84 $\pm$ 0.86
<b>Total iso</b>	<b>1.24 <math>\pm</math> 0.02</b>	<b>1.42 <math>\pm</math> 0.02</b>	<b>1.45 <math>\pm</math> 0.16</b>	<b>2.99 <math>\pm</math> 0.14</b>	<b>2.88 <math>\pm</math> 0.74</b>
<b>Total anteiso</b>	<b>1.90 <math>\pm</math> 0.03</b>	<b>1.81 <math>\pm</math> 0.14</b>	<b>1.75 <math>\pm</math> 0.02</b>	<b>2.19 <math>\pm</math> 0.56</b>	<b>2.08 <math>\pm</math> 0.41</b>
<b>Total branched-chain saturated</b>	<b>3.15 <math>\pm</math> 0.04</b>	<b>3.23 <math>\pm</math> 0.12</b>	<b>3.20 <math>\pm</math> 0.18</b>	<b>5.19 <math>\pm</math> 0.42</b>	<b>4.96 <math>\pm</math> 1.15</b>
<b>Monoenes</b>					
C14:1 n-5	0.12 $\pm$ 0.01	0.13 $\pm$ 0.00	0.13 $\pm$ 0.02	0.17 $\pm$ 0.03	0.16 $\pm$ 0.03
C16:1 n-9	0.41 $\pm$ 0.01	0.45 $\pm$ 0.02	0.46 $\pm$ 0.05	0.33 $\pm$ 0.00	0.64 $\pm$ 0.39
C16:1 n-7	0.79 $\pm$ 0.02	0.75 $\pm$ 0.01	0.74 $\pm$ 0.04	0.45 $\pm$ 0.04	0.60 $\pm$ 0.07
C18:1 trans	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.15 $\pm$ 0.08	0.18 $\pm$ 0.04
C18:1 n-9	13.87 $\pm$ 0.07	13.67 $\pm$ 0.07	13.56 $\pm$ 0.17	9.19 $\pm$ 0.36	10.45 $\pm$ 0.62
C18:1 n-7	1.98 $\pm$ 0.02	1.95 $\pm$ 0.00	1.93 $\pm$ 0.03	1.38 $\pm$ 0.25	1.62 $\pm$ 0.09
C20:1 n-9	0.62 $\pm$ 0.01	0.69 $\pm$ 0.01	0.67 $\pm$ 0.04	1.49 $\pm$ 1.44	1.69 $\pm$ 0.07
C22:1 n-9	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	0.02 $\pm$ 0.02	2.79 $\pm$ 3.95	2.60 $\pm$ 0.91
C24:1 n-9	0.04 $\pm$ 0.01	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.04 $\pm$ 0.06	0.07 $\pm$ 0.05
<b>Total monoenes</b>	<b>17.88 <math>\pm</math> 0.12</b>	<b>17.69 <math>\pm</math> 0.08</b>	<b>17.56 <math>\pm</math> 0.04</b>	<b>16.00 <math>\pm</math> 6.15</b>	<b>18.01 <math>\pm</math> 2.07</b>
<b>Dienes</b>					
C18:2 n-6	43.84 $\pm$ 0.10	39.88 $\pm$ 0.68	41.86 $\pm$ 1.78	24.58 $\pm$ 1.78	27.96 $\pm$ 2.82
C20:2 n-6	1.60 $\pm$ 0.01	1.58 $\pm$ 0.04	1.61 $\pm$ 0.13	1.31 $\pm$ 0.03	1.43 $\pm$ 0.13
<b>Total dienes</b>	<b>45.45 <math>\pm</math> 0.09</b>	<b>41.45 <math>\pm</math> 0.72</b>	<b>43.47 <math>\pm</math> 1.91</b>	<b>25.90 <math>\pm</math> 1.81</b>	<b>29.39 <math>\pm</math> 2.95</b>
<b>Polyunsaturated</b>					
C20:3 n-9	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.02 $\pm$ 0.03	0.00 $\pm$ 0.00
C18:3 n-6	0.30 $\pm$ 0.01	0.27 $\pm$ 0.01	0.25 $\pm$ 0.01	0.21 $\pm$ 0.08	0.22 $\pm$ 0.04
C20:3 n-6	0.06 $\pm$ 0.01	0.58 $\pm$ 0.11	0.37 $\pm$ 0.32	0.09 $\pm$ 0.02	0.15 $\pm$ 0.08
C20:4 n-6	0.07 $\pm$ 0.03	0.06 $\pm$ 0.01	0.05 $\pm$ 0.00	0.02 $\pm$ 0.03	0.04 $\pm$ 0.03
C22:4 n-6	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.07 $\pm$ 0.04	0.05 $\pm$
C16:3 n-3	1.24 $\pm$ 0.01	1.12 $\pm$ 0.05	1.26 $\pm$ 0.05	0.73 $\pm$ 0.07	0.83 $\pm$ 0.08
C18:3 n-3	6.48 $\pm$ 0.05	5.69 $\pm$ 0.20	6.46 $\pm$ 0.16	4.94 $\pm$ 1.52	4.85 $\pm$ 0.96
C20:3 n-3	0.04 $\pm$ 0.01	0.18 $\pm$ 0.01	0.13 $\pm$ 0.03	0.72 $\pm$ 0.93	0.58 $\pm$ 0.46
C18:4 n-3	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00.00	0.88 $\pm$ 1.24
C20:5 n-3	0.00 $\pm$ 0.00	0.03 $\pm$ 0.04	0.03 $\pm$ 0.04	0.00 $\pm$ 0.00	0.34 $\pm$ 0.14
C22:5 n-3	0.07 $\pm$ 0.01	0.14 $\pm$ 0.01	0.10 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01
C22:6 n-3	0.09 $\pm$ 0.03	0.15 $\pm$ 0.01	0.11 $\pm$ 0.02	0.18 $\pm$ 0.21	0.03 $\pm$ 0.01
<b>Total polyunsaturated</b>	<b>8.36 <math>\pm</math> 0.07</b>	<b>8.22 <math>\pm</math> 0.10</b>	<b>8.76 <math>\pm</math> 0.18</b>	<b>7.04 <math>\pm</math> 1.69</b>	<b>8.01 <math>\pm</math> 2.89</b>
<b>Total unsaturated</b>	<b>71.68 <math>\pm</math> 0.12</b>	<b>67.36 <math>\pm</math> 0.74</b>	<b>69.79 <math>\pm</math> 1.77</b>	<b>48.93 <math>\pm</math> 7.31</b>	<b>55.41 <math>\pm</math> 7.91</b>

TABLE 3. Total fatty acid (as FAMES) composition (% of total FAs) of 15 species of lichen in the genera *Stereocaulon*, *Cladonia*, *Peltigera*, *Lichina*, *Collema*, and one cyanobacterium (*Nostoc* sp.). Total values are given in bold.

	<i>Ste ves</i>	<i>Ste gra</i>	<i>Ste vul</i>	<i>Ste dac</i>	<i>Ste evo</i>	<i>Ste hal</i>	<i>Ste mon</i>	<i>Ste pil</i>	<i>Ste alp</i>	<i>Ste scu</i>	<i>Cla ran</i>	<i>Cla por</i>	<i>Pel mem</i>	<i>Lic pyg</i>	<i>Col cri</i>	<i>Nos sp</i>
<b>FA content (as methyl esters; mg g<sup>-1</sup> DW)</b>	<b>0·8</b>	<b>0·3</b>	<b>0·6</b>	<b>2·0</b>	<b>1·1</b>	<b>0·9</b>	<b>0·5</b>	<b>2·7</b>	<b>1·9</b>	<b>0·8</b>	<b>0·4</b>	<b>0·5</b>	<b>1·3</b>	<b>1·4</b>	<b>0·7</b>	<b>0·9</b>
Saturated																
C10:0	0·00	0·28	0·77	0·09	0·17	0·05	0·00	0·09	0·15	0·97	0·44	0·25	2·11	0·00	0·00	0·45
C12:0	0·20	0·28	0·33	0·07	0·09	0·09	0·00	0·12	0·05	0·21	0·21	0·93	0·20	0·06	0·25	0·54
C14:0	0·68	1·05	0·55	0·54	0·68	0·60	0·68	0·64	0·29	0·55	0·90	1·13	0·36	0·26	1·26	0·49
C15:0	0·17	1·14	0·32	0·29	0·29	0·75	0·51	0·34	0·28	0·29	1·41	0·59	0·44	0·83	0·35	0·53
C16:0	17·94	24·87	14·29	14·07	16·61	20·10	21·50	12·58	13·27	16·99	27·37	16·47	20·13	14·38	22·53	17·69
C17:0	0·89	0·77	1·11	0·59	0·52	0·94	0·83	0·69	0·39	1·19	1·94	0·79	0·38	0·89	0·54	0·70
C18:0	12·43	14·51	10·43	6·16	10·23	9·26	14·61	5·66	9·05	11·24	17·24	7·72	3·00	6·14	3·82	4·81
C20:0	1·56	0·98	0·42	0·51	0·64	0·47	0·73	0·79	0·46	0·57	1·34	1·52	0·34	0·31	0·77	0·57
C22:0	0·10	1·07	0·37	0·34	0·87	0·55	0·57	0·62	0·44	0·46	0·00	2·69	1·66	1·28	0·92	1·30
C24:0	0·14	0·00	0·00	0·04	0·08	0·06	0·00	0·07	0·08	0·00	0·00	0·18	0·00	0·00	0·00	0·00
<b>Total saturated</b>	<b>34·12</b>	<b>44·96</b>	<b>28·60</b>	<b>22·70</b>	<b>30·18</b>	<b>32·87</b>	<b>39·42</b>	<b>21·59</b>	<b>24·45</b>	<b>32·48</b>	<b>50·84</b>	<b>32·27</b>	<b>28·62</b>	<b>24·16</b>	<b>30·44</b>	<b>27·07</b>
Branched-chain																
iC12:0	0·18	0·20	0·32	0·04	0·05	0·09	0·00	0·09	0·03	0·12	0·20	0·00	0·00	0·00	0·13	0·00
iC13:0	0·12	0·22	0·13	0·08	0·12	0·12	0·14	0·07	0·06	0·10	0·51	0·18	0·25	0·16	0·22	0·11
iC14:0	0·16	0·00	0·35	0·04	0·11	0·13	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
iC15:0	0·26	0·43	0·19	0·25	0·30	0·15	0·22	0·36	0·16	0·15	0·40	0·33	0·12	0·13	0·43	0·48
iC16:0	0·11	0·22	0·00	0·03	0·13	0·00	0·00	0·06	0·04	0·07	0·11	0·00	0·07	0·10	0·23	0·44
iC17:0	0·07	0·23	0·14	0·15	0·15	0·18	0·13	0·24	0·10	0·13	0·18	0·13	0·08	0·10	0·71	0·47
iC18:0	0·00	0·00	0·35	0·15	0·19	0·49	0·50	0·29	0·14	0·29	0·36	0·48	0·07	0·18	0·38	0·47
iC20:0	0·11	0·27	0·00	0·03	0·06	0·00	0·00	0·03	0·00	0·18	0·00	0·00	0·04	0·09	0·16	0·12
aiC12:0	0·15	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
aiC14:0	0·00	0·52	0·37	0·25	0·31	0·21	0·20	0·50	0·14	0·30	0·71	0·36	0·46	0·37	0·71	0·36
aiC15:0	0·33	0·60	0·27	0·11	0·10	0·10	0·27	0·08	0·07	0·10	0·19	0·13	0·10	0·15	0·32	0·59
aiC16:0	0·62	1·32	1·22	1·07	1·36	0·91	0·86	0·90	0·64	1·17	2·99	1·28	2·29	2·26	4·23	1·95
aiC17:0	3·64	0·00	0·00	0·00	0·08	0·00	0·00	0·06	0·00	0·04	0·00	0·00	0·00	0·05	0·18	0·17
aiC18:0	0·31	0·00	0·22	0·46	0·23	0·39	0·19	0·99	0·12	0·91	0·10	0·10	0·21	0·12	0·18	0·06
<b>Total iso</b>	<b>1·01</b>	<b>1·58</b>	<b>1·49</b>	<b>0·77</b>	<b>1·11</b>	<b>1·17</b>	<b>0·99</b>	<b>1·14</b>	<b>0·53</b>	<b>1·04</b>	<b>1·75</b>	<b>1·12</b>	<b>0·64</b>	<b>0·75</b>	<b>2·26</b>	<b>2·08</b>
<b>Total anteiso</b>	<b>5·05</b>	<b>2·44</b>	<b>2·09</b>	<b>1·89</b>	<b>2·09</b>	<b>1·61</b>	<b>1·52</b>	<b>2·53</b>	<b>0·97</b>	<b>2·52</b>	<b>3·99</b>	<b>1·87</b>	<b>3·07</b>	<b>2·95</b>	<b>5·62</b>	<b>3·13</b>



TABLE 3. Continued

	<i>Ste ves</i>	<i>Ste gra</i>	<i>Ste vul</i>	<i>Ste dac</i>	<i>Ste evo</i>	<i>Ste hal</i>	<i>Ste mon</i>	<i>Ste pil</i>	<i>Ste alp</i>	<i>Ste scu</i>	<i>Cla ran</i>	<i>Cla por</i>	<i>Pel mem</i>	<i>Lic pyg</i>	<i>Col cri</i>	<i>Nos sp</i>
<b>Total branched-chain</b>	<b>6·06</b>	<b>4·02</b>	<b>3·58</b>	<b>2·66</b>	<b>3·20</b>	<b>2·77</b>	<b>2·50</b>	<b>3·67</b>	<b>1·50</b>	<b>3·56</b>	<b>5·74</b>	<b>2·99</b>	<b>3·71</b>	<b>3·70</b>	<b>7·88</b>	<b>5·22</b>
Monoenes																
C14:1 n-5	0·15	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·01	0·06	0·14
C16:1 n-9	0·62	0·71	0·10	0·48	0·23	0·24	0·39	0·06	0·14	0·25	0·80	0·24	0·04	0·08	0·21	0·21
C16:1 n-7	1·11	0·65	0·83	0·68	0·70	0·60	0·65	0·80	0·89	0·77	0·64	0·68	1·78	5·08	12·17	5·08
C18:1 n-9	28·44	21·03	17·66	29·34	14·70	15·64	19·90	28·83	11·93	15·84	11·21	22·70	19·18	2·89	7·60	6·55
C18:1 n-7	2·94	2·79	2·39	2·31	2·86	2·60	2·77	2·59	2·01	2·17	2·39	2·34	1·48	3·05	7·97	4·57
C20:1 n-9	0·96	0·84	0·72	0·26	0·28	0·14	0·94	0·36	0·24	0·65	0·12	0·34	0·95	0·10	0·10	0·21
C22:1 n-9	0·18	0·00	0·00	0·09	0·21	0·06	0·00	0·05	0·11	0·00	0·00	0·15	0·10	0·05	0·07	0·20
C24:1 n-9	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·02	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
<b>Total monoenes</b>	<b>34·41</b>	<b>26·02</b>	<b>21·71</b>	<b>33·15</b>	<b>18·97</b>	<b>19·28</b>	<b>24·65</b>	<b>32·70</b>	<b>15·33</b>	<b>19·67</b>	<b>15·17</b>	<b>26·44</b>	<b>23·54</b>	<b>11·25</b>	<b>28·18</b>	<b>16·94</b>
<b>Total n-9</b>	<b>30·21</b>	<b>22·58</b>	<b>18·48</b>	<b>30·16</b>	<b>15·41</b>	<b>16·08</b>	<b>21·22</b>	<b>29·32</b>	<b>12·42</b>	<b>16·73</b>	<b>12·14</b>	<b>23·43</b>	<b>20·27</b>	<b>3·11</b>	<b>7·98</b>	<b>7·16</b>
Polyunsaturates																
C18:2 n-6	17·66	21·61	38·50	32·51	39·50	39·48	30·26	29·66	52·22	38·48	20·52	32·82	37·68	49·83	12·44	42·94
C18:3 n-6	0·29	0·61	0·39	0·30	0·36	0·25	0·41	0·40	0·08	0·28	0·30	0·25	0·17	0·10	0·11	0·06
C20:3 n-6	0·00	0·00	0·15	0·31	0·08	0·34	0·17	0·14	0·84	0·20	1·74	0·50	0·20	0·05	0·11	0·13
C20:4 n-6	0·07	0·00	0·28	0·17	0·22	0·00	0·00	0·24	0·10	0·00	0·00	0·24	0·33	0·08	0·37	0·12
C22:4 n-6	0·23	0·00	0·00	0·24	0·00	0·00	0·00	0·10	0·00	0·00	0·37	1·30	0·00	0·00	0·84	0·00
C22:5 n-6	0·14	0·00	0·00	0·02	0·00	0·00	0·00	0·03	0·00	0·00	0·00	0·00	0·00	0·03	0·00	0·00
<b>Total n-6</b>	<b>18·37</b>	<b>22·22</b>	<b>39·33</b>	<b>33·55</b>	<b>40·16</b>	<b>40·07</b>	<b>30·83</b>	<b>30·58</b>	<b>53·24</b>	<b>38·96</b>	<b>22·93</b>	<b>35·12</b>	<b>38·39</b>	<b>50·09</b>	<b>13·87</b>	<b>43·26</b>
C16:3 n-3	0·00	0·25	0·50	0·07	0·33	0·28	0·27	0·06	0·20	0·69	0·55	0·45	0·15	0·25	0·34	0·24
C18:3 n-3	4·37	0·85	5·25	6·75	5·71	3·56	1·15	10·13	4·23	4·21	3·12	0·63	2·36	9·29	17·60	6·68
C18:4 n-3	0·84	0·00	0·00	0·06	0·10	0·05	0·32	0·08	0·06	0·00	0·00	0·00	2·78	0·18	0·13	0·04
C20:3 n-3	0·97	0·18	0·29	0·21	0·26	0·20	0·00	0·47	0·22	0·13	0·48	1·35	0·17	0·27	0·93	0·30
C20:5 n-3	0·67	0·35	0·21	0·30	0·31	0·17	0·13	0·27	0·25	0·09	0·46	0·21	0·08	0·61	0·22	0·06
C22:5 n-3	0·06	0·00	0·00	0·00	0·00	0·12	0·00	0·02	0·11	0·05	0·00	0·00	0·12	0·11	0·23	0·00
C22:6 n-3	0·12	1·14	0·54	0·56	0·78	0·65	0·74	0·41	0·41	0·16	0·71	0·54	0·08	0·10	0·18	0·18
<b>Total n-3</b>	<b>7·03</b>	<b>2·78</b>	<b>6·79</b>	<b>7·94</b>	<b>7·49</b>	<b>5·02</b>	<b>2·60</b>	<b>11·45</b>	<b>5·48</b>	<b>5·32</b>	<b>5·31</b>	<b>3·18</b>	<b>5·74</b>	<b>10·80</b>	<b>19·63</b>	<b>7·51</b>
<b>Total polyunsaturated FAs</b>	<b>25·41</b>	<b>25·00</b>	<b>46·12</b>	<b>41·49</b>	<b>47·65</b>	<b>45·08</b>	<b>33·43</b>	<b>42·03</b>	<b>58·72</b>	<b>44·28</b>	<b>28·25</b>	<b>38·30</b>	<b>44·13</b>	<b>60·89</b>	<b>33·50</b>	<b>50·77</b>
<b>ratio (n-6)/(n-9)</b>	<b>0·61</b>	<b>0·98</b>	<b>2·13</b>	<b>1·11</b>	<b>2·61</b>	<b>2·49</b>	<b>1·45</b>	<b>1·04</b>	<b>4·29</b>	<b>2·33</b>	<b>1·89</b>	<b>1·50</b>	<b>1·89</b>	<b>16·08</b>	<b>1·74</b>	<b>6·04</b>
<b>ratio (n-3)/(n-6)</b>	<b>0·38</b>	<b>0·12</b>	<b>0·17</b>	<b>0·24</b>	<b>0·19</b>	<b>0·13</b>	<b>0·08</b>	<b>0·37</b>	<b>0·10</b>	<b>0·14</b>	<b>0·23</b>	<b>0·09</b>	<b>0·15</b>	<b>0·22</b>	<b>1·41</b>	<b>0·17</b>
<b>Total unsaturated FAs</b>	<b>59·81</b>	<b>51·02</b>	<b>67·83</b>	<b>74·64</b>	<b>66·63</b>	<b>64·36</b>	<b>58·07</b>	<b>74·73</b>	<b>74·05</b>	<b>63·95</b>	<b>43·41</b>	<b>64·74</b>	<b>67·66</b>	<b>72·14</b>	<b>61·67</b>	<b>67·71</b>

Key to lichen species abbreviations: *Ste ves*, *Stereocaulon vesuvianum*; *Ste gra*, *S. graminosum*; *Ste vul*, *S. vulcani*; *Ste dac*, *S. dactylophyllum*; *Ste evo*, *S. evolutum*; *Ste hal*, *S. halei*; *Ste mon*, *S. montaganum*; *Ste pil*, *S. pileatum*; *Ste alp*, *S. alpinum*; *Ste scu*, *S. scutigerum*; *Cla ran*, *Cladonia rangiferina*; *Cla por*, *C. portentosa*; *Pel mem*, *Peltigera membranacea*; *Lic pyg*, *Lichina pygmaea*; *Col cri*, *Collema cristatum* var. *marginale*; *Nos sp*, *Nostoc* sp.

(*S. alpinum* and *L. pygmaea*). The triene FAs found belong to the families n-3 (C16:3 (n-3), C18:3(n-3), C20:3(n-3)) and *w*-6 (C18:3(n-6), C20:3(n-6)). FAs with four, five and six double bonds were identified, but at low levels (0.3–3.4%). The n-3 FAs were present at high levels (10–19%) for *C. cristatum*, *L. pygmaea* and *S. pileatum*, but in low quantities for *S. graminosum* (2.8%) and *S. montagneanum* (2.6%). A high content of n-6 FAs was found in *L. pygmaea* (50.1%) and *S. alpinum* (53.2%), whereas they were at lower levels in *C. cristatum* (13.9%) and *S. vesuvianum* (18.4%). The n-9 FA levels were low for *L. pygmaea* (3.1%), but higher (*c* 30%) for *S. vesuvianum*, *S. dactylophyllum* and *S. pileatum*.

#### Multivariate analysis

Four HAC analyses were carried out to compare the 16 species with respect to total FAs, saturated FAs, unsaturated FAs (see Supplementary Material Figure S1, available online) and BCFAs (Fig. 2). The

correspondence observed between the total-, saturated- and unsaturated-FA distributions in the chlorolichens and the cyanolichens, as well as in the *Stereocaulon* species, was poor (see Supplementary Material Figure S1, available online), except when BCFAs were selected for HAC analysis. No experiment was conducted on a tripartite *Stereocaulon* to observe if a pseudopodetium without cephalodia would give a different quantitative profile to a pseudopodetium with a cephalodium attached to it, but preliminary results do not support this. Cyanobacteria of *S. montagneanum* and *S. halei* were very much alike (*Scytonema* type, even if the type of cephalodium is different: sacculate for *S. montagneanum* and protosacculate for *S. halei*). Despite this, dendrograms derived from total-, saturated- and unsaturated-FA data failed to correlate with either species: *S. montagneanum* seems to be closer to *S. graminosum* (*Nostoc* type in the cephalodia) than to *S. halei*. These preliminary results have to be confirmed by other studies. Both anteiso-C16:0 and C17:0 contributed in a

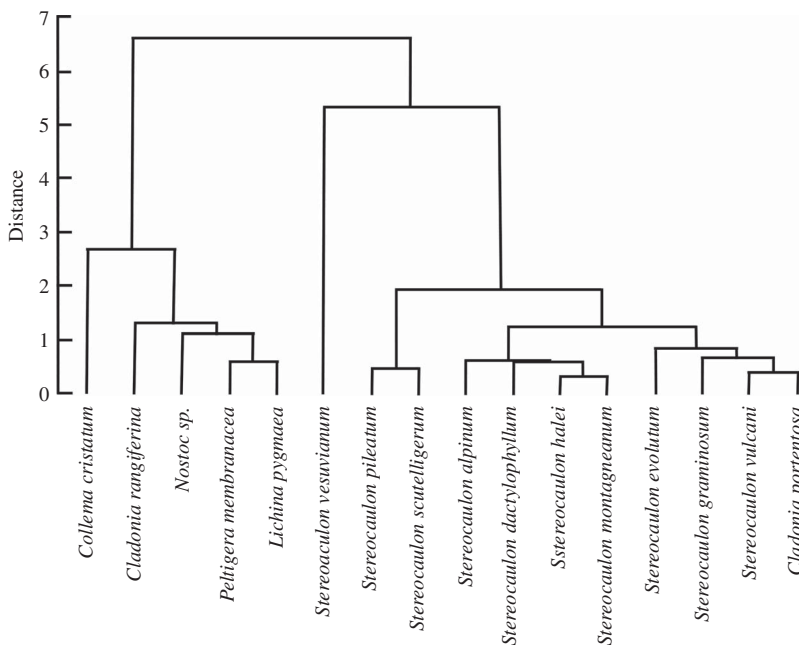


FIG. 2. Dendrogram obtained by hierarchical cluster analysis of 15 species of lichens in the genera *Stereocaulon*, *Cladonia*, *Peltigera*, *Collema*, and one cyanobacterium (*Nostoc* sp.), based on iso- and anteiso-fatty acids (FAs).

large part (26% and 45% on factorial axis PC1, and 47% and 29% on factorial axis PC2) to the discrimination between the cyanolichens and the genus *Stereocaulon* (Fig. 3). *Stereocaulon vesuvianum* was very distinct on the biplot because it contained a high value of aiC17:0 (3.5%). Moreover, the *Stereocaulon* species *S. halei* and *S. montagneanum*, as well as *S. graminosum* and *S. vulcani*, had similar profiles.

### Discussion

Before evaluating if FAs have a taxonomic value in discriminating among lichen species,

we evaluated five methods for extracting the highest yield and diversity of FAs. Of the several solvent systems used for extracting lipids from lichens, we chose to optimize the classical method developed by Folch *et al.* (1957). The method was modified by the use of acetic acid, as it ensures phospholipid extraction (Hanus *et al.* 2008; Temina *et al.* 2010). However, the FA profiles and extraction yields were unchanged, whether acetic acid was used or not. Although lichen lipids are most commonly extracted, after maceration, using a Soxhlet apparatus, we chose to use the Parallel Synthesis Apparatus (Method 3: three extractions, for 1 h each, with chloroform/methanol (2:1, v/v, 6 ml), at

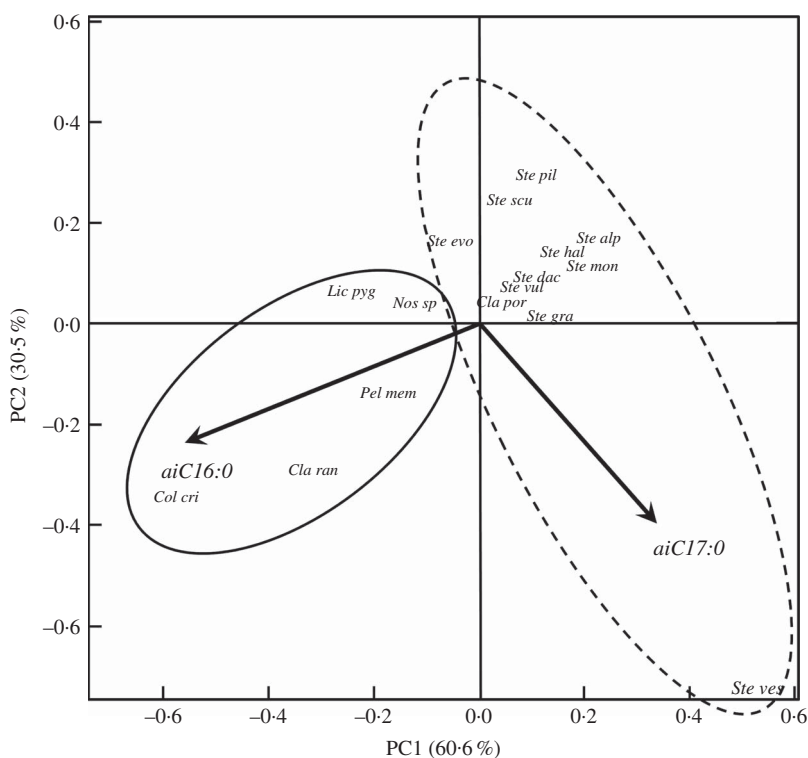


FIG. 3. Biplot of principal component analysis of the 15 species of lichen and one cyanobacterium (*Nostoc* sp.), based on iso- and anteiso-FAs. When vectors are too close, they are merged and so unreadable; they are insignificant in the discrimination. The explained variances of factorial axes (PC1 and PC2) are indicated in parentheses near the respective axes. Fatty acid abbreviations: ai = anteiso-fatty acid; C $x$ : $y$  where  $x$  = number of carbon atoms and  $y$  = number of double bonds. Key to lichen species abbreviations: *Ste ves*, *Stereocaulon vesuvianum*; *Ste gra*, *S. graminosum*; *Ste vul*, *S. vulcani*; *Ste dac*, *S. dactylophyllum*; *Ste evo*, *S. evolutum*; *Ste hal*, *S. halei*; *Ste mon*, *S. montagneanum*; *Ste pil*, *S. pileatum*; *Ste alp*, *S. alpinum*; *Ste scu*, *S. scutelligerum*; *Cladonia rangiferina*; *Cladonia portentosa*; *Pel mem*, *Peltigera membranacea*; *Lic pyg*, *Lichina pygmaea*; *Col cri*, *Collema cristatum* var. *marginale*; *Nos sp*, *Nostoc* sp.

80 °C, under constant shaking at 700 rpm). Historically, the ratio of lichen powder weight to solvent volume for lipid extraction has ranged between 3 mg ml<sup>-1</sup> (Reis *et al.* 2005) and 100 mg ml<sup>-1</sup> (Sasaki *et al.* 2001). In this case, the ratio was *c* 30 mg ml<sup>-1</sup> for Methods 1, 2 and 3, and 1 mg ml<sup>-1</sup> for Methods 4 and 5, which could explain our better extraction yields. Total FAs are composed of lipids from cellular membranes and energy storage deposits. Thus their content can vary widely, making comparisons with literature values difficult. In this study we therefore targeted total FAs derived only from phospholipids and glycolipids, which also had a better chance of being conserved between species. These were successfully quantified by using an internal standard (heneicosanoic acid; C21:0). The alternative was to calculate the weights of total extracts without derivatizing the samples. However, this would include fatty acids covering lipids (triglycerids and phospholipids), other lipids without FAs (sterols, liposoluble vitamins) but also depsides and depsidones (Dertien *et al.* 1977; Dembitsky *et al.* 1991, 1992a).

*Stereocaulon* species collected in tropical or hot climates, such as *S. graminosum*, *S. montagneanum* and *S. vulcani* (Table 1), contained lower levels of FAs (<1 mg g<sup>-1</sup> dry weight) than those from temperate locations. Stress conditions, such as a decrease in temperature of only a few degrees, nitrogen starvation and increased light intensity, are known to favour FA accumulation (Dembitsky *et al.* 1994; Reis *et al.* 2005; Goss & Wilhelm 2009). This would be consistent with the higher FA content of the temperate *S. pileatum* and *S. alpinum*. However, *S. evolutum* and *S. dactylophyllum* were both collected from the same temperate location (Brittany, France; Table 1), yet there is a twofold difference in FA content between these two species (Table 3). Thus, growth at a given temperature is not necessarily a unique parameter that determines FA content, but rather genetics, combined with environmental conditions (e.g. altitude, air pollution, seasonal effects), must also be considered (Temina *et al.* 2010).

The use of gas chromatography, with a capillary column and flame ionization detection, allowed a complete and detailed FA record. This approach complements and contributes to completing the classical analyses (e.g. TLC) performed on lichen terpenoids and steroids. The FA profiles retrieved for the lichens are in agreement with the literature for the major FAs, that is, a high content of C16:0, C18:0, C18:1(n-9) and C18:2(n-6). This is supported in the literature by FAME analyses of 69 species of lichens (see Supplementary Material Text S1, available online). The total FA profile was reported for four *Collema* species (Temina *et al.* 2010): both *C. fuscovirens* and *C. flaccidum* exhibited a profile different to that of *C. cristatum* and *C. callopismum*. These differences were attributed to their growth substratum: epiphytic for *C. fuscovirens* and *C. flaccidum*, and lithophilic for *C. cristatum* and *C. callopismum*. Our *C. cristatum* var. *marginale* sample was collected on rocks and reinforces this observation, as its FA profile is closer to that of *C. cristatum* and *C. callopismum*. The FA profile of *Peltigera membranacea* is different to that of *P. didactyla* (syn. *P. spuria*) and *P. canina*, both of which contain the cyanobacterium *Nostoc* as the main photobiont. The main difference is the high content of the diene C18:2(n-6) and the low levels of polyene in *P. membranacea*, as previously observed (Dembitsky *et al.* 1992b; Režanka & Dembitsky 1999). Three other samples of *Cladonia rangiferina* were previously analyzed (Yamamoto & Watanabe 1974; Dembitsky *et al.* 1991; Režanka & Dembitsky 1999; Sasaki *et al.* 2001), and their profiles are similar to our current data. An exception is Yamamoto & Watanabe (1974), who reported a lower diversity of saturated FAs. However, this could be attributed to the use of a low-resolution column. Guschina *et al.* (2003) found that those lichen species that harbour *Nostoc* contain more C18:3(n-3) than those that contained a green lga. Furthermore, they did not produce FAs with chains longer than C18 (Ratledge & Wilkinson 1988). In our case, all the cyanolichens produced minor quantities of unsaturated FAs longer than C18.

The biosynthetic pathway for FAs uses two types of enzyme: elongases ( $\epsilon$ ), for adding two carbons to the FA at the carboxyl end, and desaturases ( $\Delta x$ ), for adding *cis*-double bonds at specified positions ( $x$ ). These desaturases are rate-limiting enzymes. Two major desaturases,  $\Delta 12$  and  $\Delta 15$ , are involved in the biosynthesis of the (n-6) and (n-3) families, respectively. The ratios (n-6)/(n-9) and (n-3)/(n-6) reflect the activity of each enzyme (Fig. 1); the higher the ratio, the higher the enzyme activity (Table 3). Most lichens have a high (n-6)/(n-9) ratio (i.e.  $>1.5$ ), especially the cyanolichen *L. pygmaea* (16.1), the chlorolichen *S. alpinum* (4.3), and also the cyanobacterium *Nostoc* sp. (6.1). This could be attributed to their very low content of (n-9) monoenes and their high content of C18:2(n-6), indicating a possible high activity of the  $\Delta 12$  desaturase. However, even if the four cyanolichens and the cyanobacterium do not share a similar unsaturated FA profile, certain parts of their metabolic pathways appear to be particularly active, especially for *C. cristatum* var. *marginale*. Its high (n-3)/(n-6) ratio, combined with a lower (n-6)/(n-9) ratio, is in agreement with its high content of C18:3(n-3), C20:3(n-3) and C20:5(n-3), and its lower content of C18:2(n-6). For this species, the biosynthetic pathway upstream of C18:3(n-3) is enhanced due to the activity of the  $\Delta 15$  desaturase, which results in a larger diversity and content of trienes. These metabolic pathways are also used by the cyanolichens *L. pygmaea* and *C. cristatum* var. *marginale*, and the cyanobacterium *Nostoc* sp. These species also contain a high level of (n-7) monoene FAs ( $>5\%$ ), in equal quantity to C18:1(n-9), which is the common precursor for unsaturated FAs. The biosynthetic pathway for FAs in the cyanolichens is different to that in the chlorolichens, as the former had a higher activity of desaturases. These results are in agreement with the FAME profiles reported for the cyanolichens *Peltigera didactyla* (syn. *P. spuria*) and *Leptogium saturninum* (Rezanka & Dembitsky 1999) and the tripartite lichen *Peltigera aphthosa* (Dembitsky et al. 1992b). However, they differ to those for the *Collema* species, which were harvested from rocks or bark (Temina et al. 2010).

Dendrograms derived from total-, saturated- and unsaturated-FA data failed to separate *Stereocaulon*, *Cladonia* and the cyanolichens into distinct groups. Despite the small amounts of iso- and anteiso-FAs in lichens and in *Nostoc* sp. (0.5–2.3% and 1.0–5.6%, respectively), their presence appears to be informative for *Stereocaulon* species, given that our method distinguished this genus from the cyanolichens. Excluding *S. vesuvianum* from the PCA and HCA had no influence on the results. Nevertheless, this profile did not consistently separate the close genera *Cladonia* and *Stereocaulon*, nor the *Stereocaulon* species in sect. *Denudata*. Thus it would be premature to conclude that the value of FA profiles for lichen taxonomy is as high as it is for well-documented bacteria. The chemotyping study of Sasaki et al. (2001) had previously shown that total FAME data were of limited use for elucidating the taxonomy of *Cladonia* species. For example, *C. miniata* was positioned outside the group, whereas *Parmotrema delicatum* was included. Separate analyses of phospholipids (membrane structure) and triglycerides, including glycolipids (energy storage), should be considered to improve and confirm the value of FA profiles in the chemotaxonomy of lichens.

BCFAs are found in many bacterial strains (Zelles 1999). These are synthesized in bacteria by the deamination of valine, leucine or isoleucine, giving 2-methylpropionyl-CoA, 3-methylbutyryl-CoA and 2-methylbutyryl-CoA, followed by elongation to yield iso- and anteiso-FAs (Kaneda 1963, 1977, 1991). Interestingly, bacterial-fungal interactions occur in many contexts (e.g. in plankton, mixed biofilms, intrahyphal colonization) as ectosymbiotic and endosymbiotic relationships (Frey-Klett et al. 2011). Lichens also exhibit this relationship with bacteria (Finegold et al. 1990; Dembitsky et al. 1992a; Matsumoto et al. 2004; González et al. 2005). Indeed, the non-photoautotrophic bacteria of the lichen microbiome are increasingly regarded as significant components of lichen thalli (Grube et al. 2009). Alphaproteobacteria are believed to dominate the bacterial communities of the chlorolichens

(Hodkinson *et al.* 2012). Bacteria from this class were likewise found on the thalli of *C. rangiferina* (Cardinale *et al.* 2006) and *L. pygmaea* (Parrot *et al.* 2014), along with dominant populations of Firmicutes and Actinobacteria, respectively. However, their biomass contribution within the lichen thallus appears to be too low (Anderson 2014) to be correlated with the percentage of BCFAs obtained. This suggests that such a symbiosis between multiple partners (e.g. fungi, algae, bacteria) could modify the FA synthesis and distribution pattern via a complex molecular communication that has yet to be investigated thoroughly.

In memory of Dr Louis Jean-Claude Massé, who introduced us to the world of lichens.

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#### SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper <http://dx.doi.org/10.1017/S0024282916000141>

#### REFERENCES

- Anderson, O. R. (2014) Microbial communities associated with tree bark foliose lichens: a perspective on their microecology. *Journal of Eukaryotic Microbiology* **61**: 1–7.
- AOCS Lipid Library (undated) Available from: <http://lipidlibrary.aocs.org> (accessed 8 October 2015).
- Bachelor, F., King, G. & Richardson, J. (1990) Phlebic acids C and D, lichen acids from *Peltigera aphthosa*. *Phytochemistry* **29**: 601–604.
- Cardinale, M., Puglia, A. M. & Grube, M. (2006) Molecular analysis of lichen-associated bacterial communities. *FEMS Microbiology Ecology* **57**: 484–495.
- Culbertson, W. L. (1969) The use of chemistry in the systematics of the lichens. *Taxon* **18**: 152–166.
- Cyberlipid Center (undated) Available from: <http://www.cyberlipid.org> (accessed 8 October 2015).
- Delmail, D., Labrousse, P. & Botineau, M. (2011) The most powerful multivariate normality test for plant genomics and dynamics data sets. *Ecological Informatics* **6**: 125–126.
- Dembitsky, V. M. (1992) Lipids of lichens. *Progress in Lipid Research* **31**: 373–397.
- Dembitsky, V. M. & Srebnik, M. (2002) Natural halogenated fatty acids: their analogues and derivatives. *Progress in Lipid Research* **41**: 315–367.
- Dembitsky, V. M., Bychek, I. A., Shustov, M. V. & Rozentsvet, O. A. (1991) Phospholipid and fatty acid composition of some lichen species. *Phytochemistry* **30**: 837–839.
- Dembitsky, V. M., Rezanka, T. & Bychek, I. A. (1992a) Fatty acids and phospholipids from lichens of the order. *Lecanorales*. *Phytochemistry* **31**: 851–853.
- Dembitsky, V. M., Rezanka, T. & Bychek, I. A. (1992b) Lipid composition of some lichens. *Phytochemistry* **31**: 1617–1620.
- Dembitsky, V. M., Rezanka, T. & Bychek, I. A. (1994) Seasonal variation of lipids and fatty acids from tree-growing lichens of the genus *Physcia*. *Phytochemistry* **36**: 601–608.
- Dertien, B. K., De Kok, L. J. & Kuiper, P. J. C. (1977) Lipid and fatty acid composition of tree-growing and terrestrial lichens. *Physiologia Plantarum* **40**: 175–180.
- Finegold, L., Singer, M. A., Federle, T. W. & Vestal, J. R. (1990) Composition and thermal properties of membrane lipids in cryptoendolithic lichen microbiota from Antarctica. *Applied and Environmental Microbiology* **54**: 1191–1194.
- Folch, J., Lees, M. & Sloane Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Canadian Journal of Biochemistry and Physiology* **37**: 497–509.
- Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M. & Sarniguet, A. (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews* **75**: 583–609.
- Gonzalez, A. G., Rodriguez Pérez, E. M., Hernandez Padron, C. E. & Barrera, J. B. (1992) Chemical constituents of the lichen *Stereocaulon azureum*. *Verlag der Zeitschrift für Naturforschung C* **47**: 503–507.
- González, I., Ayuso-Sacido, A., Anderson, A. & Genilloud, O. (2005) Actinomycetes isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. *FEMS Microbiology Ecology* **54**: 401–415.
- Goss, R. & Wilhelm, C. (2009) Lipids in algae, lichens and mosses. In *Lipids in Photosynthesis: Essential and Regulatory Functions* (H. Wada & N. Murata, eds): 117–135. Berlin: Springer.
- Grube, M., Cardinale, M., de Castro, J. V., Müller, H. & Berg, G. (2009) Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *The ISME Journal* **3**: 1105–1115.
- Guschina, I. A., Dobson, G. & Harwood, J. L. (2003) Lipid metabolism in cultured lichen photobionts with different phosphorus status. *Phytochemistry* **64**: 209–217.
- Hanus, L. O., Temina, M. & Dembitsky, V. (2008) Biodiversity of the chemical constituents in the epiphytic lichenized ascomycete *Ramalina*

- lacera* grown on difference substrates *Crataegus sumacicus*, *Pinus halepensis*, and *Quercus calliprios*. *Biomedical Papers of the Medical Faculty of the University Palacky Olomouc, Czechoslovakia* **152**: 203–208.
- Hawksworth, D. L. (1976) Lichen chemotaxonomy. In *Lichenology: Progress and Problems* (D. Brown, D. L. Hawksworth & R. H. Bailey, eds): 139–184. London: Academic Press.
- Hodkinson, B. P., Gottel, N. R., Schadt, C. W. & Lutzoni, F. (2012) Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome. *Environmental Microbiology* **14**: 147–161.
- Imbs, A. B. & Dautova, T. N. (2008) Use of lipids for chemotaxonomy of Octocorals (Cnidaria: Alcyonaria). *Biochemistry* **34**: 174–178.
- Kaneda, T. (1963) Biosynthesis of branched chain fatty acids. *Journal of Biological Chemistry* **238**: 1229–1235.
- Kaneda, T. (1977) Fatty acids of the genus *Bacillus*: an example of branched-chain preference. *Bacteriological Reviews* **41**: 391–418.
- Kaneda, T. (1991) Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiology and Molecular Biology Reviews* **55**: 288–302.
- Kock, J. L. F. & Botha, A. (1998) Fatty acids in fungal taxonomy. In *Chemical Fungal Taxonomy* (J. C. Frisvad, ed.): 219–249. New York: CRC Press.
- Kumari, P., Bijo, A. J., Mantri, V. A., Reddy, C. R. K. & Jha, B. (2013) Fatty acid profiling of tropical marine macroalgae: an analysis from chemotaxonomic and nutritional perspectives. *Phytochemistry* **86**: 44–56.
- Lamb, I. M. (1951) On the morphology, phylogeny, and taxonomy of the lichen genus *Stereocaulon*. *Canadian Journal of Botany* **29**: 522–584.
- Lamb, I. M. (1977) A conspectus of the lichen genus *Stereocaulon* (Schreb.) Hoffm. *Journal of the Hattori Botanical Laboratory* **43**: 191–355.
- Matsumoto, G. I., Nienow, J. A., Friedmann, E. I., Sekiya, E. & Ocampo-Friedmann, R. (2004) Biogeochemical features of lipids in endolithic microbial communities in the Ross Desert (McMurdo Dry Valleys), Antarctica. *Cellular and Molecular Biology* **50**: 591–604.
- Nylander, W. (1866) Circa novum in studia *Lichenum critericum* chemicum. *Flora* **49**: 198–201.
- Parrot, D., Intertaglia, L., Grube, M., Suzuki, M. T. & Tomasi, S. (2014) Lichens: a bacterial hot-spot for the production of bioactive compounds. In *Gordon Research Seminar, 1–2 March, 2014, Ventura, California*, pp. 1–2.
- Ratledge, C. & Wilkinson, S. G. (1988) *Microbial Lipids*. London: Academic Press.
- Reis, R. A., Iacomini, M., Gorin, P. A. J., Mera de Souza, L., Grube, M., Cordeiro, L. M. C. & Sasaki, G. L. (2005) Fatty acid composition of the tropical lichen *Teloschistes flavicans* and its cultivated symbionts. *FEMS Microbiology Letters* **247**: 1–6.
- Rezanka, T. & Dembitsky, V. M. (1999) Fatty acids of lichen species from Tian Shan Mountains. *Folia Microbiologica* **44**: 643–646.
- Sahu, A., Pancha, I., Jain, D., Paliwal, C., Ghosh, T., Patidar, S., Bhattacharya, S. & Mishra, S. (2013) Fatty acids as biomarkers of microalgae. *Phytochemistry* **89**: 53–58.
- Sasaki, G. L., Machado, M. J., Tischer, C. A., Gorin, P. A. & Iacomini, M. (1999) Glycosyldiacylglycerolipids from the lichen *Dictyonema glabratum*. *Journal of Natural Products* **62**: 844–847.
- Sasaki, G. L., Cruz, L. M., Gorin, P. A. & Iacomini, M. (2001) Fatty acid composition of lipids present in selected lichenized fungi: a chemotyping study. *Lipids* **36**: 167–174.
- Solberg, Y. (1987) Chemical constituents of the lichens *Cetraria delisei*, *Lobaria pulmonaria*, *Stereocaulon tomentosum* and *Usnea hirta*. *Journal of the Hattori Botanical Laboratory* **63**: 357–366.
- Spribille, T., Klug, B. & Mayrhofer, H. (2011) A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinari* (*Mycoblastaceae*, lichenized Ascomycota) reveals cryptic clades correlated with fatty acid profiles. *Molecular Phylogenetics and Evolution* **59**: 603–614.
- Tabacchi, R., Tsoupras, G. & Huneck, S. (1987) Lichen substances. No. 148. Steroids and nortriterpenoids from lichens. *Journal of the Hattori Botanical Laboratory* **63**: 351–355.
- Temina, M., Levitsky, D. O. & Dembitsky, V. M. (2010) Chemical constituents of the epiphytic and lithophilic lichens of the genus *Collema*. *Records of Natural Products* **1**: 79–86.
- Yamamoto, Y. & Watanabe, A. (1974) Fatty acid composition of lichens and their phyco- and mycobionts. *Journal of General and Applied Microbiology* **86**: 83–86.
- Zelles, L. (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils* **29**: 111–129.