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COMPARATIVE STUDY OF THE ENDEMIC FRESHWATER FAUNA OF LAKE BAIKAL—II. UNUSUAL LIPID COMPOSITION OF TWO SPONGE SPECIES *BAICALOSPONGIA BACILLIFERA* AND *BAICALOSPONGIA INTERMEDIA* (FAMILY LUBOMIRSKIIDAE, CLASS DEMOSPONGIAE)

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Abstract—1. Lipid and phospholipid compositions of endemic freshwater sponges, belonging to the Family Lubomirskiidae, Class Demospongiae *Baicalospongia bacillifera* and *Baicalospongia intermedia* were studied.

2. The fatty acids of total lipids, neutral, glyco- and phospholipid fractions were investigated by capillary gas chromatography–mass spectrometry.

3. One hundred and eighty-five fatty acids were identified: 46 saturated (both iso-, anteiso-, branched, cyclic), 55 monoenoic, 37 dienoic, 25 trienoic and 22 polyenoic.

4. A number of unusual fatty acids, such as br_7–12–17:1, br_9–6–17:1, c-19–26:0, and very-long-chain polyunsaturated 6,9,12,15,18,21–24:6, 15,18,21,24–30:4, and 15,18,21,24–30:5 acids, were found.

INTRODUCTION

Freshwater and marine sponges, the most primitive multicellular animals, have been known for many years to contain a high percentage of unusually very-long-chain fatty acids (Dembitsky, 1981a,b; Dembitsky and Chelomin, 1985; Ayanoglu *et al.*, 1982, 1983; Bergquist *et al.*, 1984; Lawson *et al.*, 1984; Litchfield *et al.*, 1976; Morales and Litchfield, 1976; Walkup *et al.*, 1981; and see recent reviews by Rezanka, 1989; Djerassi and Lam, 1991).

There are about 150 freshwater sponge species known (Pennak, 1978), including six endemic sponge species living only in Lake Baikal (Kozhov, 1930; Rezvoi, 1936). The unique nature of the Baikalian fauna, including sponges, may be judged by the difficulty of determining its position in the existing zoogeographical zone Vereshchagin (1940) considered that the inclusion of Baikal at all into the system of zoogeographical divisions existing for ordinary continental waters was essentially incorrect. The author felt that the distinctions of the Baikalian fauna from those of the surrounding country were not only very pronounced but also rested on a different genetic basis. He equated the distinctions with those between marine fauna and continental freshwater fauna, and suggested it would be more correct to regard the Baikalian fauna as a particular biogeographical unit intermediary between marine and freshwater units.

Recently, phospholipid, fatty acid compositions and biosynthesis pathways of freshwater sponges have been studied (Dembitsky *et al.*, 1991; Hahn *et al.*, 1989; Latyshev *et al.*, 1992; Vysotskii *et al.*, 1990; Rezanka and Dembitsky, 1993).

In the present investigation, we are reporting the results of a detailed study of lipids, phospholipids and fatty acids of two freshwater sponges of Lake Baikal. This paper continues our earlier study on Baikalian sponges (Dembitsky, 1981a,b, 1982).

MATERIALS AND METHODS

Collection of samples

Baicalospongia bacillifera Dyb. and *Baicalospongia intermedia* Dyb., were sampled in the south-western

Table 1. Lipid composition of freshwater sponges

Family, Species	TL	NL	GL	PL
Lubomirskiidae				
Baicalospongia bacillifera	3.7	59.6	12.0	28.4
Baicalospongia intermedia	13.6	54.5	18.9	26.6

Abbreviations—TL, total lipids (mg/g dry wt, %); NL, neutral lipids; GL, glycolipids; PL, phospholipids. NL, GL and PL as % of TL.

Table 2. Phospholipid composition of sponges

Phospholipid classes	*B. intermedia*	*B. bacillifera*
Phosphatidylcholine	25.0 ± 0.7	21.2 ± 0.6
Phosphatidalcholine (plasmalogen form)	6.8 ± 0.4	7.5 ± 0.3
Lysophosphatidylcholine	11.1 ± 0.5	11.9 ± 0.4
Choline-containing phospholipids	42.9	40.6
Phosphatidylethanolamine-1	13.7 ± 0.5	9.5 ± 0.4
Phosphatidalethanolamine-1 (plasmalogen form)	13.4 ± 0.6	8.7 ± 0.5
Phosphatidylethanolamine-2 (alkyl-acyl form)	5.8 ± 0.4	8.9 ± 0.3
Lysophosphatidylethanolamine	4.8 ± 0.3	10.0 ± 0.4
Phosphatidylserine	9.5 ± 0.4	10.1 ± 0.5
Amino-containing phospholipids	47.2	47.2
Phosphatidylinositol	5.2 ± 0.3	5.7 ± 0.2
Phosphatidic acid	4.7 ± 0.2	6.5 ± 0.5

Table 3. Saturated fatty acids of sponges

	B. intermedia				*B. bacillifera*			
	TL	NL	GL	PL	TL	NL	GL	PL
i-12:0	0.05	0.08	0.04	0.03	0.05	0.08	0.04	0.03
12:0	0.32	0.16	0.46	0.35	0.32	0.15	0.46	0.35
i-13:0	0.04	0.00	0.07	0.05	0.04	0.00	0.07	0.05
ai-13:0	0.09	0.08	0.11	0.08	0.09	0.08	0.11	0.08
13:0	0.23	0.25	0.25	0.19	0.22	0.23	0.25	0.19
i-14:0	0.19	0.33	0.14	0.11	0.19	0.31	0.14	0.11
14:0	2.22	4.86	1.03	0.78	2.38	4.57	1.46	1.11
4,8,12-triMe-13:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
i-15:0	0.12	0.25	0.07	0.05	0.12	0.23	0.07	0.05
ai-15:0	0.16	0.41	0.04	0.03	0.15	0.39	0.04	0.03
15:0	0.41	0.66	0.32	0.24	0.39	0.62	0.32	0.24
i-16:0	0.43	0.99	0.18	0.13	0.42	0.93	0.18	0.14
pristanic	0.16	0.49	0.00	0.00	0.15	0.46	0.00	0.00
16:0	4.23	10.13	2.44	3.72	5.78	11.53	2.77	3.05
i-17:0	0.13	0.33	0.04	0.03	0.13	0.31	0.04	0.03
ai-17:0	0.20	0.49	0.07	0.05	0.19	0.46	0.07	0.05
phytanic	0.14	0.41	0.00	0.00	0.13	0.39	0.00	0.00
17:0	0.54	1.07	0.32	0.24	0.52	1.01	0.32	0.24
i-18:0	0.32	0.66	0.18	0.13	0.31	0.62	0.18	0.14
18:0	3.67	7.41	1.52	2.09	2.21	8.51	1.81	2.32
i-19:0	0.13	0.33	0.04	0.03	0.13	0.31	0.04	0.03
br_{11}-19:0	0.05	0.08	0.04	0.03	0.03	0.08	0.00	0.00
a1-19:0	0.08	0.25	0.00	0.00	0.10	0.23	0.04	0.03
19:0	0.28	0.66	0.11	0.08	0.27	0.62	0.11	0.08
i-20:0	0.11	0.33	0.00	0.00	0.10	0.31	0.00	0.00
20:0	2.00	3.79	0.50	0.38	1.00	3.56	0.60	0.46
i-21:0	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
ai-21:0	0.11	0.33	0.00	0.00	0.10	0.31	0.00	0.00
21:0	0.31	0.74	0.11	0.08	0.30	0.70	0.11	0.08
i-22:0	0.11	0.33	0.00	0.00	0.10	0.31	0.00	0.00
22:0	0.75	1.89	0.21	0.16	0.72	1.78	0.21	0.16
i-23:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
23:0	0.22	0.58	0.04	0.03	0.20	0.54	0.04	0.03
i-24:0	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
24:0	0.30	0.66	0.14	0.11	0.29	0.62	0.14	0.11
i-25:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
ai-25:0	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
25:0	0.11	0.33	0.00	0.00	0.10	0.31	0.00	0.00
c-19-26:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
26:0	0.19	0.49	0.04	0.03	0.18	0.46	0.04	0.03
27:0	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
28:0	0.11	0.33	0.00	0.00	0.10	0.31	0.00	0.00
29:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
30:0	0.08	0.25	0.00	0.00	0.08	0.23	0.00	0.00
31:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
32:0	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
Total saturated	19.00	41.65	8.51	9.23	18.0	42.72	9.66	9.22

Abbreviations—i, iso-; ai, anteiso-; br, branched; c, propane ring.

Table 4. Monoenoic fatty acids of sponges

	B. intermedia				*B. bacillifera*			
	TL	NL	GL	PL	TL	NL	GL	PL
7–12:1	0.16	0.41	0.04	0.03	0.15	0.39	0.04	0.03
8–13:1	0.05	0.08	0.04	0.03	0.05	0.08	0.04	0.03
7–14:1	0.23	0.49	0.11	0.08	0.22	0.46	0.11	0.08
9–14:1	0.26	0.33	0.25	0.19	0.25	0.31	0.25	0.19
5–15:1	0.11	0.25	0.04	0.03	0.10	0.23	0.04	0.03
7–15:1	0.09	0.08	0.11	0.08	0.09	0.08	0.11	0.08
5–16:1	0.22	0.41	0.14	0.11	0.21	0.39	0.14	0.11
7–16:1	1.45	1.48	1.06	0.81	1.23	1.39	1.03	0.79
9–16:1	3.92	7.41	2.48	1.88	3.87	7.74	2.20	1.68
3t–16:1	0.09	0.08	0.11	0.08	0.09	0.08	0.11	0.08
11–16:1	0.30	0.41	0.28	0.21	0.30	0.39	0.28	0.22
i–9–17:1	0.05	0.08	0.04	0.03	0.05	0.08	0.04	0.03
i–12–17:1	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
ai–9–17:1	0.08	0.25	0.00	0.00	0.08	0.23	0.00	0.00
br_7–12–17:1	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
br_9–6–17:1	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
7–17:1	0.09	0.16	0.07	0.05	0.09	0.15	0.07	0.05
9–17:1	0.23	0.33	0.21	0.16	0.23	0.31	0.21	0.16
5–18:1	0.27	0.25	0.32	0.24	0.26	0.23	0.32	0.24
7–18:1	0.42	0.33	0.53	0.40	0.48	0.31	0.64	0.49
9–18:1	8.90	7.41	6.09	7.41	9.00	10.06	5.54	8.11
11–18:1	0.98	1.07	1.06	0.81	1.11	1.01	1.31	1.00
13–18:1	0.87	1.48	0.64	0.48	0.65	1.39	0.32	0.24
15–18:1	0.41	0.66	0.32	0.24	0.37	0.62	0.28	0.22
19:1	0.05	0.08	0.04	0.03	0.05	0.08	0.04	0.03
11–19:1	0.33	0.74	0.14	0.11	0.32	0.70	0.14	0.11
5–20:1	0.25	0.49	0.14	0.11	0.24	0.46	0.14	0.11
7–20:1	0.25	0.25	0.28	0.21	0.24	0.23	0.28	0.22
9–20:1	0.45	1.15	0.11	0.08	0.42	1.08	0.11	0.08
11–20:1	1.81	4.86	0.32	0.24	1.48	3.87	0.32	0.24
13–20:1	0.68	0.91	0.64	0.48	0.68	0.85	0.68	0.52
11–21:1	0.19	0.49	0.04	0.03	0.18	0.46	0.04	0.03
5–22:1	0.25	0.25	0.28	0.21	0.24	0.23	0.28	0.22
7–22:1	0.30	0.58	0.18	0.13	0.29	0.54	0.18	0.14
9–22:1	0.59	1.15	0.35	0.27	0.57	1.08	0.36	0.27
11–22:1	1.41	3.29	0.53	0.40	1.16	2.48	0.57	0.43
13–22:1	1.16	0.99	1.42	1.07	1.17	0.93	1.46	1.11
15–22:1	0.54	0.82	0.46	0.35	0.49	0.77	0.39	0.30
5–23:1	0.13	0.33	0.04	0.03	0.13	0.31	0.04	0.03
13–23:1	0.29	0.74	0.07	0.05	0.27	0.70	0.07	0.05
14–23:1	0.11	0.25	0.04	0.03	0.10	0.23	0.04	0.03
17–23:1	0.09	0.16	0.07	0.05	0.09	0.15	0.07	0.05
9–24:1	0.22	0.41	0.14	0.11	0.21	0.39	0.14	0.11
13–24:1	0.83	1.48	0.57	0.43	0.84	1.39	0.64	0.49
15–24:1	0.79	1.15	0.60	1.39	1.05	1.08	0.64	1.43
17–24:1	0.70	1.65	0.25	0.19	0.66	1.55	0.25	0.19
19–24:1	0.31	0.74	0.11	0.08	0.30	0.70	0.11	0.08
15–25:1	0.05	0.08	0.04	0.03	0.05	0.08	0.04	0.03
17–25:1	0.22	0.58	0.04	0.03	0.20	0.54	0.04	0.03
5–26:1	0.48	1.32	0.07	0.05	0.56	1.55	0.07	0.05
9–26:1	0.31	0.74	0.11	0.08	0.30	0.70	0.11	0.08
15–26:1	0.17	0.25	0.14	0.11	0.16	0.23	0.14	0.11
17–26:1	0.30	0.66	0.14	0.11	0.29	0.62	0.14	0.11
27:1	0.03	0.08	0.00	0.00	0.03	0.08	0.00	0.00
28:1	0.05	0.16	0.00	0.00	0.05	0.15	0.00	0.00
Total monoenoic	32.73	50.70	21.20	19.81	31.81	50.37	20.61	20.53

part of Lake Baikal, near Bolshye Koty (Biological Station of Irkutsk University) in July 1992, at depths 8–10 m; the temperature of the water was 4°C. The animals were put into tanks under water at the site of collection and transported to the laboratory. To promote lipid extraction, the tissues of the sponges were thoroughly cleansed of any impurities.

Lipid analysis

The species were treated immediately in the laboratory as described previously (Dembitsky *et al.*, 1991). Lipids were extracted and separated by column and thin-layer chromatography, as described elsewhere (Dembitsky *et al.*, 1991).

Methyl esters of fatty acids analysis

Fatty acids ME esters were analysed by general experimental procedures.

The semi-preparative Gradient LC System G-I (Shimadzu, Kyoto, Japan) with two LC-6A pumps (2 ml/min), an SCL-6A system controller, an SPD UV detector (210 nm), an SIL-1A sample injector and a C-R3A data processor, with an analytical column, 250 × 4 mm i.d., packed with SGX C18 with 5 μm

particles (Tessek, Prague, Czech Republic) was used for RP-HPLC. The C-NMR (75 MHz) and H-NMR (300 MHz) spectra were measured by means of a Brucker AM 300 instrument in CDCI. For scanning infrared spectroscopy of the methyl esters after RP-HPLC (in CCI) a Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT) infrared spectrophotometer was used. The HR–MS were determined by the Finnigan MAT 90 mass spectrometer with EI mode. All GC–MS separations were performed by using a Finnigan MAT (San José, CA) 1020 B apparatus with electron impact. Injection temperature (splitless injection) was 100°C and a fused-silica capillary column (Supelcowax 10) (60 m × 0.25 mm i.d. 0.25 μm film thickness) was used. The temperature programme was as follows: 100°C for 1 min, then increased at 20°C/min to 180°C and at 2°C/min to 280°C, which was maintained for 1 min. The carrier gas was hydrogen at the linear velocity of 60 cm/sec. All spectra were scanned within the range m/z 70–450. With the exception of GC–MS, where we used picolinyl esters, the other values were measured for FAME.

RESULTS AND DISCUSSION

Baikalian sponges, belonging to the Family Lubomirskiidae, originated in the ancient Mediterranian sea, Tetis, which covered vast territories of middle and south-eastern Eurasia, in the Mesosoic and the first half of Tertiary times (Kozhov, 1962). According to Gureeva (1975) and Efremova (1981) the Family of Lubomirskiidae are the most ancient group, followed by Spongillidae.

The information on lipids and phospholipid composition of Baikalian freshwater sponges is rather sparse and involves only a few representatives of this animals group (Dembitsky, 1982).

Total lipid extracts were separated on a silica gel column into three fractions: neutral, glyco- and phospholipids. Total lipids varied from 3.8 (*B. bacillifera*) to13.6% dry wt (*B. intermedia*) (Table 1). Neutral lipids in the examined species varied between 59.6 and 54.5% and phospholipids varied from 28.4 to 26.6%, while glycolipids varied from 12.0 to 18.9%, respectively.

The main phospholipid classes in the examined sponge species were PE and PC. According to micro-TLC analysis, PE contained three spots: PE-1 (diacyl

Table 5. Dienoic fatty acids of sponges

	B. intermedia				*B. bacillifera*			
	TL	NL	GL	PL	TL	NL	GL	PL
6,9–14:2	0.16	0.16	0.18	0.13	0.16	0.15	0.18	0.14
9,12–16:2	0.65	0.08	1.06	0.81	0.88	0.08	1.46	1.11
5,9–16:2	0.12	0.25	0.07	0.05	0.12	0.23	0.07	0.05
6,9–18:2	1.90	1.65	2.30	1.75	1.87	2.09	1.99	1.52
6,11–18:2	0.04	0.00	0.07	0.05	0.04	0.00	0.07	0.05
9,12–18:2	5.03	3.29	5.67	6.13	4.28	2.32	3.84	6.69
12,15–18:2	2.25	0.66	3.47	2.63	2.07	0.62	3.16	2.42
6,11–20:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
8,11–20:2	0.33	0.00	0.57	0.43	0.38	0.00	0.64	0.49
11,14–20:2	0.19	0.00	0.32	0.24	0.19	0.00	0.32	0.24
11,15–20:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
14,17–20:2	0.12	0.00	0.21	0.16	0.12	0.00	0.21	0.16
i–5,9–21:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ai–5,9–21:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10,13–22:2	0.06	0.00	0.11	0.08	0.06	0.00	0.11	0.08
13,16–22:2	0.10	0.00	0.18	0.13	0.11	0.00	0.18	0.14
5,9–24:2	0.16	0.00	0.28	0.21	0.19	0.00	0.32	0.24
15,18–24:2	0.15	0.00	0.25	0.19	0.15	0.00	0.25	0.19
17,20–24:2	0.16	0.00	0.28	0.21	0.17	0.00	0.28	0.22
24:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9–25:2	0.10	0.00	0.18	0.13	0.11	0.00	0.18	0.14
16,19–25:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
i–5,9–26:2	0.12	0.00	0.21	0.16	0.12	0.00	0.21	0.16
ai–5,9–26:2	0.16	0.00	0.28	0.21	0.17	0.00	0.28	0.22
5,9–26:2	0.62	0.00	1.06	0.81	0.79	0.00	1.35	1.03
17,20–26:2	0.08	0.00	0.14	0.11	0.08	0.00	0.14	0.11
19,22–26:2	0.16	0.00	0.28	0.21	0.17	0.00	0.28	0.22
i–5,9–27:2	0.06	0.00	0.11	0.08	0.06	0.00	0.11	0.08
ai–5,9–27:2	0.12	0.00	0.21	0.16	0.12	0.00	0.21	0.16
5,9–27:2	0.19	0.00	0.32	0.24	0.19	0.00	0.32	0.24
i–5,9–28:2	0.62	0.00	1.06	0.81	0.56	0.00	0.96	0.73
ai–5,9–28:2	0.08	0.00	0.14	0.11	0.08	0.00	0.14	0.11
5,9–28:2	0.35	0.00	0.60	0.46	0.35	0.00	0.60	0.46
br_{22}–5,9–29:2	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9–29:2	0.19	0.00	0.32	0.24	0.19	0.00	0.32	0.24
5,9–30:2	0.33	0.00	0.57	0.43	0.31	0.00	0.53	0.41
5,9–31:2	0.08	0.00	0.14	0.11	0.08	0.00	0.14	0.11
Total dienoic	14.78	6.09	20.84	17.62	14.24	5.59	19.05	18.31

Table 6. Trienoic fatty acids of sponges

	B. intermedia				*B. bacillifera*			
	TL	NL	GL	PL	TL	NL	GL	PL
6,9,12–16:3	0.25	0.00	0.43	0.32	0.27	0.00	0.46	0.35
4,7,10–16:3	0.13	0.08	0.18	0.13	0.13	0.08	0.18	0.14
7,10,13–16:3	0.42	0.00	0.71	0.54	0.63	0.00	1.07	0.81
6,9,12–18:3	3.55	0.00	5.53	5.12	3.55	0.00	4.98	5.67
9,12,15–18:3	6.75	0.33	8.15	11.77	6.37	0.31	7.46	11.34
5,8,11–20:3	1.11	1.15	1.24	0.94	0.90	0.93	1.00	0.76
8,11,14–20:3	0.50	0.00	0.85	0.64	0.54	0.00	0.92	0.71
11,14,17–20:3	0.33	0.00	0.57	0.43	0.38	0.00	0.64	0.49
7,10,13–22:3	0.58	0.00	0.99	0.75	0.63	0.00	1.07	0.81
10,13,16–22:3	0.35	0.00	0.60	0.46	0.31	0.00	0.53	0.41
13,16,19–22:3	0.37	0.00	0.64	0.48	0.42	0.00	0.71	0.54
24:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
15,18,21–24:3	0.37	0.00	0.64	0.48	0.29	0.00	0.50	0.38
25:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9,17–26:3	1.67	0.00	4.96	3.76	3.13	0.00	5.33	4.07
5,9,19–26:3	0.83	0.00	1.42	1.07	1.05	0.00	1.78	1.36
17,20,23–26:3	0.06	0.00	0.11	0.08	0.06	0.00	0.11	0.08
5,9,19–27:3	0.31	0.00	0.53	0.40	0.35	0.00	0.60	0.46
5,9,20–27:3	0.33	0.00	0.57	0.43	0.35	0.00	0.60	0.46
27:3	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
5,9,19–28:3	0.70	0.00	1.20	0.91	1.05	0.00	1.78	1.36
5,9,21–28:3	1.04	0.00	1.77	1.34	1.25	0.00	2.13	1.63
5,9,23–28:3	0.42	0.00	0.71	0.54	0.48	0.00	0.82	0.62
5,9,23–29:3	0.33	0.00	0.57	0.43	0.42	0.00	0.71	0.54
5,9,23–30:3	0.35	0.00	0.60	0.46	0.38	0.00	0.64	0.49
Total trienoic	20.81	1.56	33.04	31.59	23.00	1.32	34.10	33.57

form), PEP-1 (plasmalogen-acyl form) and PE-2 (alkyl-acyl form); PC contained one spot only (Table 2). The major phospholipids in sponges were amine-containing lipids at concentrations of 47.2% in both species.

Most marine (Dembitsky and Chelomin, 1985; Dembitsky and Nebylitsyn, 1980) and freshwater sponges (Dembitsky, 1982; Dembitsky *et al.*, 1989) as shown earlier, are characterized by a high content of amino-phospholipids. Sponges are marked by a high content of lysophospholipids, e.g. in *B. intermedia* the content of lyso-PC equals 11.1% and in *B. bacillifera*, 11.9%.

Fatty acid composition of Baikalian sponges is most interesting. Fatty acids were examined in the total lipid extract and in the neutral, glyco- and phospholipid fractions.

We have identified 185 fatty acids, including 46 saturated, 55 monoenoic, 37 dienoic, 25 trienoic and 22 tetra-, penta- and hexaenoic acids. Among saturated acids (Table 3), which show a considerable variation in fractions, palmitic acid is found to have the highest content. Neutral fraction is found contain 10.13 and 11.53% palmitic acid in *B. intermedia* and *B. bacillifera*, respectively. Glyco- and phospholipid fractions have yielded low amounts of 2.44–3.72%. Among the saturated fatty acids, two unusual acids, namely br_{11}–19:0 and c-19–26:0 (may be propane rings) were identified. Several acids, 14:0, 16:0, 18:0 and 20:0, were present in amounts exceeding 2% of total lipids.

Among the monoenoic fatty acids, the major acids were 7–16:1, 9–16:1, 9–18:1, 11–20:1, 11–22:1 and 13–22:1 in *B. intermedia* and 7–16:1, 9–16:1, 9–18:1, 11–18:1, 11–20:1, 11–22:1, 13–22:1 and 15–24:1 in *B. bacillifera* (Table 4). We have identified four isomers for 23- and 26-carbon acids; five isomers for 16-, 20- and 24-carbon acids; six isomers for 18- and 22-carbon acids; and seven isomers, including iso-, anteiso- and branched, for 17:1 acid. The greatest amount of monoenic acid (over 50%) is found in neutral fractions.

Thirty-seven of the dienoic fatty acids were identified mainly in glyco- and phospholipid fractions (Table 5). The major acids among these were in glyco- and phospholipid fractions (over 1%): 9,12–16:2, 6,9–18:2, 9,12–18:2, 12,15–18:2, 5,9–26:2 and iso-5,9–28:2. The range of isomers was widest for acids 26:2, 20:2 and 24:2 and 18:2, with five and four isomers, respectively.

Trienoic fatty acids are more interesting among all fatty acids (Table 6). Early studied fatty acids from the genus Baikalospongia, and found only *cis* isomers of 26:2 and 26:3 acids (Dembitsky, 1981b). Recently, Vysotskii *et al.* (1990) reported that three Baikalian sponges contain *trans* isomers of 26:3 acid from 3.3 to 9.4% of total acids. The major acids among trienoic acids in phospholipid fractions of two sponges were 9,12,15–18:3 (11.77 and 11.34%), 6,9,12–18:3 (5.12 and 5.67%), 5,9,17–26:3 (3.76 and 4.07%), 5,9,19–26:3 (1.07 and 1.36%), 5,9,21–28:3 (1.34 and 1.63%) and 5,9,19–28:3 (0.91 and 1.36%) in *B. intermedia* and *B. bacillifera*, respectively. The content of trienoic fatty acids in various species was found varying from 31.59 to 33.57% in phospholipids; in glycolipids from 33.04 to 34.10%.

Dembitsky *et al.* (1991) examined phospholipid and fatty acid compositions of freshwater sponge

Table 7. Tetra-, penta- and hexaenoic fatty acids of sponges

	B. intermedia				*B. bacillifera*			
	TL	NL	GL	PL	TL	NL	GL	PL
4,7,10,13–16:4	0.19	0.00	0.32	0.24	0.19	0.00	0.32	0.24
6,9,12,15–16:4	0.16	0.00	0.28	0.21	0.17	0.00	0.28	0.22
18:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
6,9,12,15–18:4	0.52	0.00	0.89	0.67	0.63	0.00	1.07	0.81
19:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
3,6,9,12,15–18:5	0.62	0.00	1.06	0.81	0.67	0.00	1.14	0.87
5,8,11,14–20:4	2.59	0.00	2.30	5.47	1.89	0.00	2.13	3.53
6,9,12,15–20:4	0.62	0.00	1.06	0.81	0.65	0.00	1.10	0.84
8,11,14,17–20:4	0.31	0.00	0.53	0.40	0.23	0.00	0.39	0.30
5,8,11,14,17–20:5	1.99	0.00	2.34	3.64	1.78	0.00	2.49	2.84
6,9,12,15,18–21:5	0.15	0.00	0.25	0.19	0.15	0.00	0.25	0.19
7,10,13,16–22:4	0.31	0.00	0.53	0.40	0.21	0.00	0.36	0.27
10,13,16,19–22:4	0.16	0.00	0.28	0.21	0.17	0.00	0.28	0.22
4,7,10,13,16–22:5	0.42	0.00	0.71	0.54	0.44	0.00	0.75	0.57
7,10,13,16,19–22:5	0.25	0.00	0.43	0.32	0.23	0.00	0.39	0.30
4,7,10,13,16,19–22:6	2.90	0.00	2.83	5.87	4.07	0.00	3.20	5.26
9,12,15,18–24:4	0.19	0.00	0.32	0.24	0.19	0.00	0.32	0.24
6,9,12,15,18–24:5	0.14	0.00	0.28	0.21	0.17	0.00	0.28	0.22
6,9,12,15,18,21–24:6	0.23	0.00	0.39	0.30	0.21	0.00	0.36	0.27
15,18,21,24–30:4	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
15,18,21,24,27–30:5	0.02	0.00	0.04	0.03	0.02	0.00	0.04	0.03
Unknown (from total)	0.85	0.00	1.45	1.10	0.82	0.00	1.39	1.06
Total polyenoic	12.68	—	16.41	21.75	12.95	—	16.58	18.37

Euspongilla lacustris collected in the Volga river estuary, belonging to class Spolgillidae which contain demospongial fatty acids 5,9–26:2 (4.7% of TL) and 5,9,19–26:3 (10.3%). Hahn *et al.* (1989) found the same acids 26:2 (27.2%) and 26:3 (14.9%) in freshwater sponge *Ephydatia fluviatilis*, collected from Lake Lagunita at Stanford University.

Tetra-, penta- and hexaenoic acids were not found in neutral fractions (Table 7). The major polyenoic fatty acids in phospholipids were arachidonic (5,8,11,14–20:4, 5.47 and 3.53%), eicosapentaenoic (5,8,11,14,17–20:5, 3.64 and 2.84%) and docosahexaenoic (4,7,10,13,16,19–22:6, 5.87 and 5.26%). The content of polyenoic acids was found vary from 21.75 to 18.37% in phospholipids, and from 16.41 to 16.58% in glycolipids.

Some novel polyisoprenoids fatty acids were identified from glycolipid fractions of four Baikalian freshwater sponges (Rezanka and Dembitsky, 1993).

Thus, the present study has demonstrated that lipid and fatty acid compositions of the endemic Baikalian freshwater sponges are more diverse but essences in their lipid and fatty acid compositions from marine sponge species belonging to the same class.

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