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Evidence of Freshwater Sponges (Porifera: Spongillidae) in the Upper Volga River (Russia)

by

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S y n o p s i s : Microscopical observations of diatom mounts, processed from material of the Upper Volga Expedition 2005, revealed the presence of sponge spicules that corresponded to *Spongilla lacustris* (LINNAEUS 1758), *Ephydatia mülleri* (LIEBERKÜHN 1855), *Trochospongilla horrida* WELTNER 1893, and likely *Heteromeyenia baileyi* (BOWERBANK 1863). These species have three different zoogeographical distributions, namely, cosmopolitan, holarctic and holarctic-amphiatlantic. In the paper illustrations for determination of sponge spicule are given and the possibility to use the biogenic opal from the spicules for paleotemperature reconstructions is discussed.

1. Introduction:

Considering evolution, sponges (Parazoa) are the most primal group of the Metazoa, therefore sponges were used as model organisms for several approaches in developmental biology (WEISSENFELS 1989, VOS et al. 1991). Most of the freshwater sponges of the world belong to the family Spongillidae (Porifera, Demospongia) (ADDIS & PETERSON 2005). They appear in stagnant as well as in running water bodies, usually in waters with little or no particulate matter, which would affect on the pores and close channels. Sponges are active filter feeders on plankton, bacteria and dissolved organic matter, due to this they play a specific role for water-purification. The filter capacity of *Spongilla lacustris* amounts about 6ml h^{-1} mg^{-1} dry mass (FROST 1980), thus a finger sized sponge can filter over 125 liters of water within 24 h (FROST 1991). Sponges also provide a habitat for different invertebrates, e.g. caddisflies of the genera *Ceraclea* (RESH 1976) and the larvae of the spongillaflies (Neuroptera: Sisyridae) live associated with freshwater sponges in lentic and lotic habitats. The Sisyridae larvae are planktonic till they find a sponge host, where they remain until the last larval stage. Then they crawl out of the water, pupate and emerge within some days (BOUCHARD 2004). Porifera are producing skeleton-elements, like Bacillariophyceae,

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Rhizopoda and Chrysophyceae, consisting of biogenic silica (BSi), by polymerising the dissolved silicic acid (H_4SiO_4 * n H_2O). Approximately 90 % of the spicule consist of SiO_2; other components are water and variable concentrations of C, K, Na, Mg and N. This needle-like spicule, which form the body of the animal can be classified by their size and shape, are kept together by spongin (see Fig. 1). Two days after sprouting of the gemmule, archeocytes differentiate to scleroblasts, which are producing the basic unit for a spicule – on this axial fibre a spicula is developed and under optimal conditions, e.g. a spicula of *Ephydatia fluviatilis* is developed within 40 hours (accession of 5μm/h) (WEISSENFELS 1989). Within the Spongillidae different spicule types can be distinguished: (1) "Oxe" are pinnacled on both endings, (2) "Strongyle" have rounded endings and (3) "Amphidiscs" look like spools. The skeleton is composed by macroscleres with a size of 180-350 μm and a diameter of 10μm, while the smaller microscleres with a size of 70-150 μm are located in the sponge body.

In temperate climates freshwater sponges develop gemmules (reproductive bodies, diameter about 500μ) which are placed directly in the body of the sponge or as a layer on the substratum, to outlast unfavourable conditions (withering of the water body or the cold

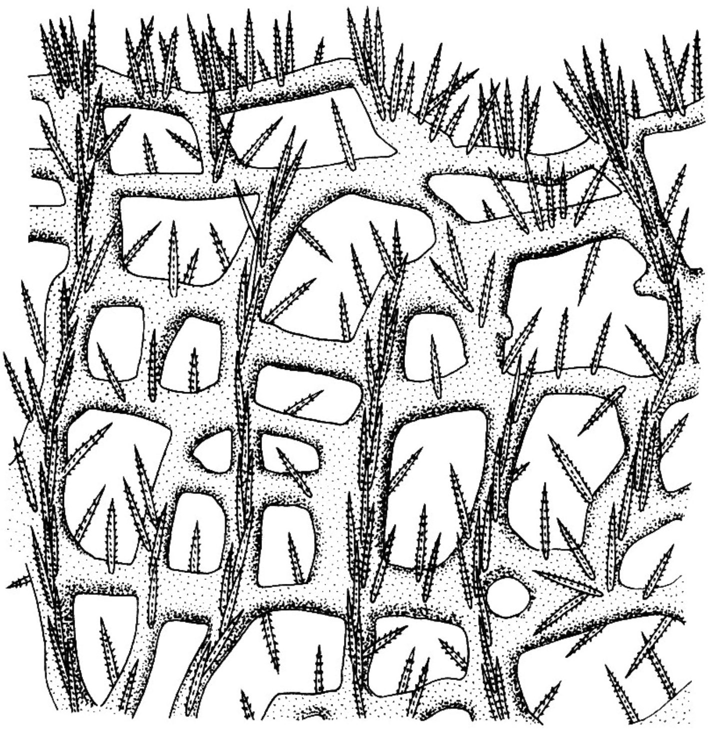

Fig. 1: Spongin fibers with spicule – microscleres (from WESTHEIDE & RIEGER 2004, with permission).

season = winter) or to disperse into new habitats by spreading vectors such as current or animals. Spongin (in the spongin layer gemmoscleres and amphidiscs are located) covers thesocytes, a cell type that is rich in yolk, and under better conditions a new sponge may develop out of this (van Soest 2004). In spring and early summer freshwater sponges are releasing pelagic larvae, which are settling on the surface of a convenient substratum after two days of swarming. Young sponges of the same species, either formed from gemmule or larvae, can coalesced and form one individuum with a joint osculum. For determination of sponges microscopic diagnosis of spicule and gemmule is needed (Arndt 1928, Wesenberg-Lund 1939). Thus the best time for collecting sponges is late summer or autumn, when gemmules were already produced (Gee 1931).

This paper is intended to present an actual evidence of sponge species in the Upper Volga River and to discuss the appearance of this group throughout Europe. For a quick determination of the common species, illustrations are arranged. Finally scopes and limits of the use of biogenic opal for paleotemperature reconstructions are characterised and discussed.

2. Investigation area:

The research area is located in the administrative region of Tver, which spreads over 84586 km^2 of gently undulating landscape, with temperate decidious and mixed forests. The temperate continental climate in Western Russia indicates cold winters and mild summers, the precipitation is about 690 mm per year (Ryzhavskij 1985, Gravenhorst et al. 2000).

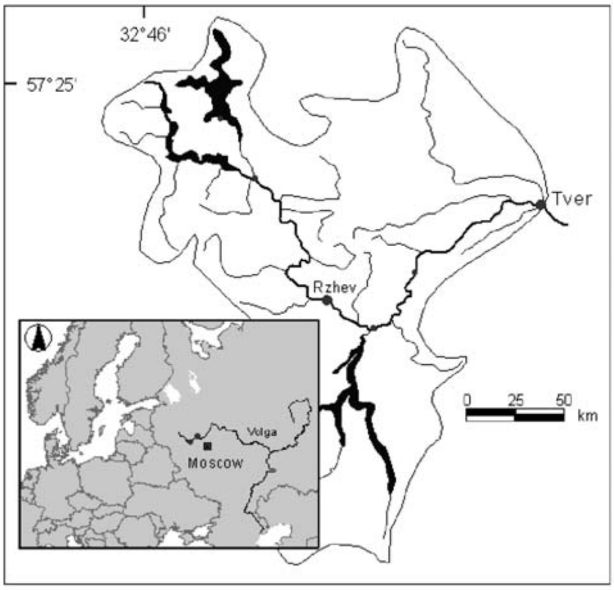

Fig. 2: Research area on the Upper Volga River.

The part of the Upper Volga River from the source to Tver can be divided into three morphological reaches: reach 1 (rkm 3531 - 3520), the source region from Volgoverkhovje (228m asl) to Kokovkino village. The Volga's source in the Valdaian hills is a limnokrene, located in a swamp, from where the water seeps towards two lakes: the small and the big Werchit. The – seminatural – Upper Volga Lakes are classified as reach 2 (rkm 3520 - 3426): these 4 basins (Sterzh, Vselug, Peno and Volgo) are originally natural lakes, but since 1843 the water level of the lakes is raised by a dam (Bejshlot), to ensure the nautic depth between Reshev and Tver. Reach 3 (rkm 3426 - 3085) leads from Bejshlot to the begin of Ivankovskoye Reservoir. Most of the tributaries can be assigned to low stream orders (SCHLETTERER 2006). Boulders which were deposited during the ice-age in the Upper Volga River Basin (BEHNING 1928) provide today a suitable hard substratum for freshwater sponges. Anthropogenic activity has provided additional habitats (e.g. bridges or the bank fixation in Tver).

3. Methods:

a) Sampling:

During the Upper Volga Expedition 2005 an assessment of hydrological, limnochemical and biological parameters was carried out. Within the procedure for sampling attached algae (phytobenthos), 23 diatom samples were taken in the Volga River and some tributaries. Rocks were scraped with a brush and in some cases parts of macrophytes or some sediment was taken. This material was stored in 10ml plastic tubes in Ethanol (50%). Within the "Upper Volga Survey" in August 2006, on the strech Rzhev to Tver, a specific focus was put on living sponges. The collected sponges were dryed and stored.

b) Sample processing:

For quick determination water mounts are sufficient, but for permanent microscopic slides a treatment of the material is needed. ARNDT (1928) suggested to cook the material in HCl and wash it afterwards in dest. H_2O. Within the present study the diatom samples were prepared with H_2O_2 (protocol acc. to KINGSTON 1985 cited in SCHIEDELE 1987, modified): The material was pretreated with 2m HCl (reacts with carbonat), which was added to the boiling samples. Afterwards the sample had to be washed three times, it was centrifuged 15 minutes at 2500g and the supernatant was decanted. In the next step 10ml H_2O_2 were added and the material was boiled again at 100°C for about 20 minutes. Then some $K_2Cr_2O_7$ was added, to accelerate the consumption of the organic matter. Due to this reaction the colour changes and the sample turns dark purple and after some time the solution turns green-yellow and precipitation of amorphous BSi gets visible. After washing the samples again for 3 times, two mounts per sample were made on an object slide: 2 drops of the white precipation were placed on 2 round cover slips an object slide and some dest. H_2O was added to distribute the material. Then the object slide was transferred into a dry box at 70°C until the water on the cover slips evapourated. Afterwards the BSi was sintered to the cover slips by heating them on a plate at about 100°C. Later on a drop of Naphrax™ was placed on an object slide and the cover slip with the diatoms was placed on it. Then the ready mount was placed again on the plate for about a minute, to ensure the release of air bubbles. The ready mounts dried within some hours and can be used for many years. The material which was not used for preparation was stored in glass tubes with a plastic cover in water with some drops of Ethanol (90%) to prevent fungal infestation.

c) Identification:

The sponge spicules we found in the diatom mounts, were identified with different determination keys (ARNDT 1928, REZVOJ 1936, RUDESCU 1975, IVANOVA 1994, PRONZATO & MANCONI 2001, EGGERS 2004). For determination of the freshwater sponges of Western Russia, illustrations are given in this paper. Species which were suitable to find in the investigation area were selected by the Limnofauna Europeae (SIMON 1978). The relative abundance of spicule in the mount is displayed with following scale, as used by ANDRI et al. (2001), additionally the paramter L is used where living sponges were found: A = abundant (>100 spicules), C = common (10–100 spicules), U = uncommon (1–9 spicules), N = not found (0 spicules) and L = living sponge.

Tab. 1: Different spicule types from selected, for Western Russia relevant, species.

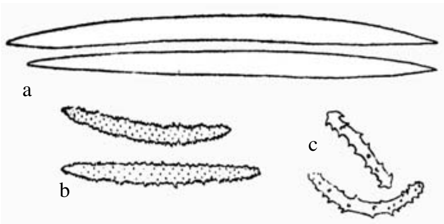

Fig. 3: Spicule of *Spongilla lacustris* (syn. *Euspongilla*): **(a)** macroscleres, **(b)** microscleres and **(c)** gemmoscleres (from LIPIN 1950).

- Microscleres present, with small spines
- Macroscleres smooth
- Gemmoscleres rod shaped, spined

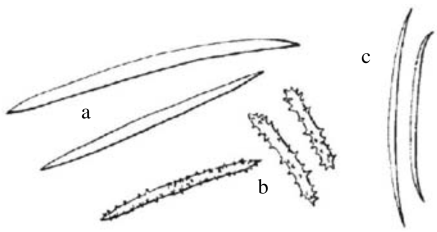

Fig. 4: Spicule of *Eunapius fragilis*: **(a)** macroscleres, **(b)** gemmoscleres (from ARNDT 1928, modified) and **(c)** gemmoscleres from *E. carteri* (RUDESCU 1975).

- Microscleres absent
- Macroscleres smooth
- Gemmoscleres rod shaped; spined (*E. fragilis*) or smooth (*E. carteri*)

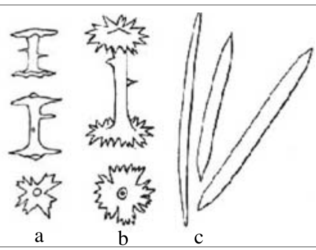

Fig. 5: Spicule of *Ephydatia fluviatilis*: **(a)** amphidiscs (GEE 1931), **(b)** also amphidiscs and **(c)** macroscleres (from ARNDT 1928)

- Microscleres absent
- Macroscleres smooth
- Amphidiscs with long shaft; no deep incisions

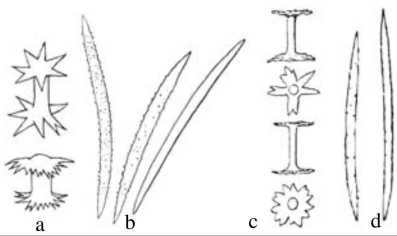

Fig. 6: Spicule of *Ephydatia mülleri*: **(a)** amphidiscs, **(b)** microscleres of *E. mülleri*, **(c)** amphidiscs and **(d)** microscleres (from ARNDT 1928) of *E. mülleri* var. *behningi*

- Microscleres absent
- Macroscleres rough, with small spines
- Amphidiscs with short shaft; deep incisions

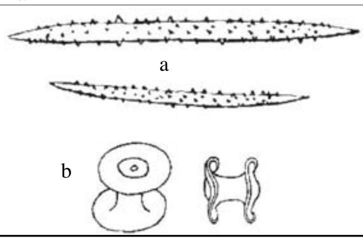

Fig. 7: Spicule of *Trochospongilla horrida*: **(a)** acanthoxeas megascleres, **(b)** spherical gemmules (from ARNDT 1928).

- Microscleres absent
- Macroscleres with big spines
- gemmoscleres amphidiscs with smooth margins

4. Results:

On the diatom mounts, four species of freshwater sponges were identified: *Spongilla lacustris* (LINNAEUS 1758), *Ephydatia müllleri* (LIEBERKÜHN 1855), *Trochospongilla horrida* WELTNER 1893 and likely *Heteromeyenia baileyi* (BOWERBANK 1863). Because of its spicule *Ephydatia mülleri* could be considered as *E. mülleri* var. *behningi* (KIRKPATRICK 1915) – however, in modern taxonomy the subspecies are summarized in *Ephydatia mülleri*. Also the microscleres of *Heteromeyenia baileyi* resemble with *H. baileyi* var. *repens* (POTTS 1881), but the subspecies are as well abolished. Singular smooth macroscleres, found in the mounts, could not be related to one certain species. They are listed in Tab. 2 as "Spongillidae indet." and reached a relative proportion of up to 30%. These macroscleres could belong to the species: *Spongilla lacustris*, *Eunapius fragilis*, *Ephydatia mülleri* or even *E. fluviatilis*. Samples 4 (Lk. Big Vetrits), 11 (Ostashkov) and 12 (Spring at Shirkovo) were also checked, but no spicule were found – these samples are not listed in Tab. 2, because these locations are not directly connected with the Upper Volga River. Living sponges (*E. mülleri*) were found during a survey in August 2006, on a landing stage and bank fixation near Tver.

5. Discussion:

In benthic surveys smaller animals (like bryozoans or sponges) are usually not considered, due to difficulties in sampling and quantification. Fortunately the identification of diatoms revealed the existence of sponge needles. Thus intensive search in the diatom mounts resulted in a knowledge about species distribution and an estimation of their relative abundance in a large river. Due to this it is possible that the spicules, recorded in the

Tab. 2: Sponge species of the Upper Volga River, identyfied from spicule. The abundance of the spicule is specified according to ANDRI et al. (2001), whereas the designation for none spicule (N) is not written down, for better overview.

	1 Wolgowerchowie	2 Crossing (End of Reach 1	3 Novinka (L 1)	5 Lk. Peno (Pionerlag)	6 Lk. Vselug-Peno	7 R. Runa	8 Lk. Peno (Peno)	9 Lk. Volgo 1/1 (Ilinskoe)	10 Lk. Volgo 1/2 (Zaneprechje)	13 Bejshlot bank	14 Volgo 2 (near Bejshlot)	15 Bejshlot dam	16 Selizharovo	17 R. Locha	18 Rzhev	19 Rublevo (L12)	20 R. R. Iruzha	21 Nr. 210 (Borovaja)	22 Molokovo (L 13)	23 Staritsa (downstream bridge)	24 Tver (upstream 1^{st} bridge)
Spongilla lacustris				U		U				U		U		U			U			U	
Ephydatia mülleri		U		U						U	U									U	L
Trochospongilla horrida						U		U		U	U	U		U	U		U	C		U	
Heteromeyenia baileyi				U																	
Spongillidae indet.		U		U		U	U			C	U		U	C	C		U	C		U	

diatom mounts, do not belong a priori to recent specimens, as they could also be subfossile and originate from eroded sediment layers, but considering the good state of the material, without hints of transportation or erosion, it is likely that it is from recent organisms. During the Upper Volga Survey 2006, living sponges could be collected from hard substrates near the city of Tver. Nevertheless all the records offer valuable information for setting up a checklist for the Upper Volga Basin (SCHLETTERER, in preperation).

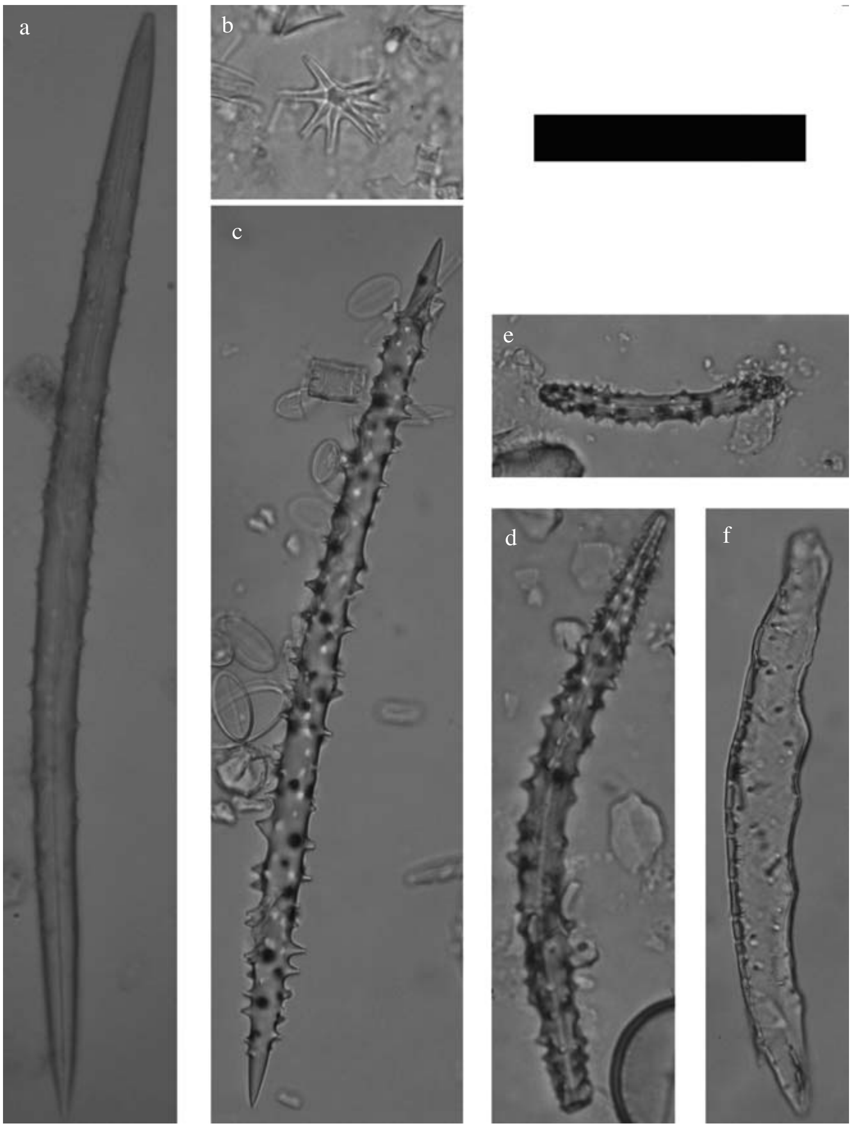

Fig. 8: **(a)** Macrosclere and **(b)** amphidisc of *E. mülleri*, **(c)** macrosclere of *T. horrida*, **(d)** microsclere and **(e)** gemmosclere of *S. lacustris* and **(f)** microsclere of *H. baileyi* (Scale = 50μ).

There is no historical data about sponge species for the research area on the Upper Volga River, but former investigations covered the stretch from the town Tver to the mouth of Volga River in the Caspian Sea. The Biological Volga Station in Saratov, which was run at the begin of the 20th century, identified *Spongilla lacustris*, *Eunapius carteri* (former *Spongilla rotundacuta*, *E. rotundacuta*), *Ephydatia fluviatilis* and *E. mülleri* in the Volga and its main tributaries. It is mentioned that Traxler found 1894 two more species, but *Eunapius fragilis* (former *Spongilla fragilis*) and *Trochospongilla horrida* could not be found during the investigations of the Biological Volga Station (Behning 1924). Records of *Ephydatia fluviatilis* are known from rivers in Moscow Region (Falk 1786) and from the Volga River near Volgograd (Rossinskij 1892). *Eunapius fragilis* is known from River Jaran near Jaransk and *T. horrida* was found in River Koksha, a tributary of the Middle Volga (Traxler 1894). During a study on the Ukrainian sponge fauna between 1990 and 1995 seven sponge species were recorded: *Spongilla lacustris*, *Ephydatia fluviatilis*, *E. mülleri*, *Eunapius fragilis*, *E. carteri*, *Trochospongilla horrida* and *Heteromeyenia stepanovii* (Trylis & Shcherbak 1996). Within the present study *Trochospongilla horrida* turned out as most common species in the Upper Volga River. This species is also known from other large rivers like the Rhine (Gugel 2000), Elbe (Eggers 2006) or Danube (Literáthy et al. 2002). Autecological characteristics of all relevant species are notified in Tab. 3 together with physico-chemical data from the research area and in Tab. 4 the range of important microcomponents is notified.

Zoogeographically the freshwater sponge, found in the Upper Volga River can be divided into three groups: cosmopolitan, holarctic and holarctic-amphiatlantic species. The dominant sponge species, *Trochospongilla horrida*, is a cosmopolitan species (Penney & Racek 1968). Recent records from this species in Middle Europe were mainly made in large rivers, stagnant waters in their floodplain or navigable canals. *Spongilla lacustris*, which occoures in cold temperate regions, and *Ephydatia mülleri*, which prefers cold- to warm-temperate regions, are described as holarctic species (Økland & Økland 1996). Only a few records of the holarctic-amphiatlantic species *Heteromeyenia baileyi* are known from NE-America and W-Europe (Pronzato & Manconi 2001), thus the actual record from the Upper Volga River is very valuable evidence of this species in Eastern Europe. The second indigenous species from the genus *Heteromeyenia* should also occour in the region, but up to now *H. stepanowi* was not found. Some more species could be expected in permanent waterbodies of the catchment area from the Upper Volga, including the Volga River itself, and its biogeographical region (Continental Eastern Europe). *Eunapius fragilis* and *Ephydatia fluviatilis* are known from former investigations in the region (Kirkpatrick 1915, Behning 1924). Records of *Eunapius carteri*, which has its main distribution in South Europe, are only known from the Volga Delta (Behning 1924, Rezvoj 1926, 1936).

Tab. 3: Zoogeographical classification (PRONZATO & MANCONI 2001) and autecology (ØKLAND & ØKLAND 1996) of freshwater sponges, which are relevant in Western Russia.

	Zoogeography					Autecology			
	Cosmopolitan species	**Boreal speciea**	**Holarctic-amphiatlantic**	**Palaearctic**	**Palaearctic and Australian**	Temperature (°C)	pH-Value	Conductivity (min)	Mg content (mg l^{-1})
Eunapius carteri (BOWERBANK, 1863)				X		-	-	-	-
Eunapius fragilis (LEIDY, 1851)	X					-	max 9,6	-	min 0,7
Ephydatia fluviatilis (LINNAEUS, 1759)[1]	X					9,0 – 20,0	6,7-9,2	135,6-292,4	-
Ephydatia mülleri (LIEBERKÜHN, 1855)		X				10-27,0	5,7-9,6	9,9-286,9	-
Heteromeyenia baileyi (BOWERBANK, 1863)			X			-	-	-	-
Heteromeyenia stepanowii (DYBOWSKY, 1884)					X	-	-	-	-
Spongilla lacustris (LINNAEUS, 1759)		X				min 9,0	4,2-9,6	min 5,3	min 0
Trochospongilla horrida WELTNER, 1893[2]		X				6,6-27,1	5,5-8,7	355-1070	8,3-11,1
Physico-chemical data of the Volga; August 2005 (pers. comm. KUZOVLEV & SHAPORENKO)						16,7-20,3	6,0-8,0	48-244	4,6-41,5

[1] Temperature according to GAINO et al. (2003), pH minimum acc. to FRANCIS et al. (1990) and the maximum value was published by RICHELLE-MAURER et al. (1994).

[2] The data listed for the autecology of *T. horrida* just represents the values of a three year study on populations of this freshwater sponge in the Rhine River (GUGEL 2000).

Tab. 4: Physico-chemical parameters of the Upper Volga River (pers. comm. KUZOVLEV & SHAPORENKO).

	Dissolved components (mg l^{-1})					
	C	**K**	**Mg**	**Na**	**Si**	Total N (mg l^{-1})
Mean	21,539	1,664	16,527	3,280	4,356	1,69
Median	19,224	1,333	12,770	2,260	4,379	1,50
Minimum	7,276	0,441	4,576	1,006	2,268	0,07
Maximum	51,015	10,029	41,635	28,998	6,517	3,40

The siliceous sponge spicule can be relevant as non-pollen-palynophorms ("extrafossils") for paleolimnological research, because after death of a sponge its scaffold material sinks to the bottom where it is conserved in the sediment. Non-biological deposits of silicia are not as common as biogenic opal, which can be used for estimating the amount of microfossils in the sediment (esp. diatoms) and as a paleoindicator, for estimating diatom production, to gain informations about changes in paleoproductivity (CONLEY 1998). The ratio between the oxygen isotopes ^{16}O and ^{18}O, which is integrated in biogenic silicia, reflects the water temperature when the animal was alive and allows a reconstruction of the climate in the region. Stabile oxygen isotopes in the SiO_2 from diatom frustles, which depend on changing parameters (T_{H2O} and $\partial 18O_{H2O}$), enabeled the development of a paleothermometer: T_{H2O} (°C) = 190,07 - 5,26 * ($\partial 18O_{Opal}$ - $\partial 18O_{H2O}$) (MOSCHEN 2004, LÜCKE et al. 2005). The biogenic opal from sponge spicule could support this approach, to get a correlation between different organism groups. Because of their annual live cycle, sponges would be an interesting indicator for this approach.

The present study revealed an evidence of four freshwater sponge species in the Upper Volga River and shall provide a basis for further research in this area. Often the sponges are ignored, but in faunistic contexts it is important to consider also this aspect.

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