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First record of the freshwater sponges (Spongilla) in the inland rivers of Bulgaria

Lidiya Taneva, Dimcho Zahariev, Dimitar Doychev

Konstantin Preslavski University of Shumen, Faculty of Natural Sciences,

115 Universitetska Str., 9700 Shumen, Bulgaria

Abstract: *Records from freshwater sponges on Bulgarian territory are scarce. Only two exist in the scientific literature, one from the Zhrebchevo Dam and one from the Bulgarian part of the Danube River near the border with Serbia. Inland lotic ecosystems such as channels, streams, and rivers, as well as lentic ecosystems, were not studied for this potential biological indicator. Due to the existing knowledge gap, studies of entire rivers and other aquatic ecosystems focused on the monitoring of biological or physicochemical quality elements should be vigilant for Spongillidae family representatives since even their random discovery and identification could be of great importance. Such records can be considered interesting for gaining knowledge of their distribution, species richness, habitat preference and particular environmental variables dependencies. We investigated macroinvertebrates and macrophytes from 16 sampling sites in a river with a length of about 200 km and a watershed of 1600 km^2 to register two Spongilla lacustris individuals at two sites without any significant hydro-morphological pressure from big dams. Our results could serve for monitoring program optimization that aims to investigate rare protected species dependent on freshwater sponges and on seeking more localities with established Spongilla populations. This can be achieved by directing sampling efforts on sampling sites that have similar habitats and have a hydraulic connection with the found specimens.*

Keywords: Freshwater sponges, Spongillidae, *Spongilla lacustris*, Luda Kamchia River, Bulgaria

Introduction

Freshwater sponges are globally distributed and cannot be found only at the poles [1]. Those metazoans from the Phylum Porifera are ancient invertebrate filter-feeding organisms [2] that are important for hydro-morphological heterogeneity, macroinvertebrates, microorganisms [3] and algae biodiversity [4].

Corresponding author: d.doychev@shu.bg

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Suborder Spongillina includes 7 families: Spongillidae, Lubomirskiidae, Malawispongiidae, Metaniidae, Metschnikowiidae, Potamolepidae and Palaeospongillidae. The latter is a fossil. Spongillidae is cosmopolitan, whereas the other 2 families in Europe are endemic to Ochrid Lake and the Caspian Sea [5].

Currently, in Bulgaria, this group of organisms was recorded from the scientific literature solely in the Danube River and Zhrebchevo Reservoir in studies related to invasive Bryozoans and benthic diatoms. In the dam, only gemmuscleres were found, and the species was not identified, while in the Danube River, *Spongilla lacustris* was recorded without any further specifications on the identification procedure or the morphology of the cells [6, 7].

The Spongillidae family can outgrow the invasive Bryozoans and could be their main competitor. Nevertheless, sponges remain a highly neglected assemblage in aquatic studies not only in Bulgaria but globally because of the difficulties of identification by morphological features since they vary in shape, colour and size. Additionally, concurrency may occur from freshwater mussels which need substrate for attachment as the sponges [3]. Although freshwater Porifera is observed as capable of eliminating *Dreissena polymorpha* in direct interaction with them, the population of this economically and ecologically threatening invasive bivalve could be no more than 10% affected [8].

In addition, the distribution of the species and individuals from the Spongillidae family is important from a conservation point of view for Bulgaria, since the endangered species *Sisyra terminalis* Curtis, 1854 parasitizes on it during larvae development of the three instars [9]. In addition, Spongillaflies are very weakly studied in the Balkan peninsula and sporadic occurrences of the superorder are recorded [10]. Consequently, this article could direct future studies related to the Sysiridae family and the establishment of protected areas with more strengthened conservation regimes since at least 33% of protected areas or about 10% of EU land, should be subject to stricter conservation regimes for all members states of the EU [11].

A recent study in northern Italy demonstrated that the Spongillidae family can occupy a wide range of habitats that differ in morphology from concrete and bricks to stone and gravel, from shaded to more exposed zones, only if the water quality is with good chemical status [3]. The water quality expressed by physical and chemical parameters is inextricably linked to the ecological status connected to the quality of the irreplaceable dividends, also called "ecosystem services", provided free of charge by aquatic ecosystems [12] as from the studied river. One of the many ecosystem services is the self-purification capacity of the water. This is achieved thanks to filtration and biological accumulation by microorganisms, algae, terrestrial and aquatic plants, freshwater sponges and their epiphytic assemblages that include aquatic animals [13].

Phylum Porifera is a significant component of aquatic ecosystems participating in self-purification processes and enhancing its effectiveness. They are sessile filter-feeding organisms that can filter zoo-phyto-pico plankton, bacteria, and even smaller-sized particles, which have the potential to be used as bioindicators

Corresponding author: d.doychev@shu.bg
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and are gaining popularity in this direction [14].

Although habitat preference was well studied in artificial channels in northern Italy [3], one major drawback encompasses most of the studies and this one. This concerns the absence of systematic studies on species richness, distribution and species-specific habitat preference [15]. Nevertheless, due to the lack of information about the distribution of freshwater sponges in inland rivers in Bulgaria, even their random discovery and identification could be considered interesting to gain knowledge about their distribution.

Material and Methods

Study area

Luda Kamchia River is the biggest tributary of the Kamchia River with a length of 200.9 km and a watershed area above 1600 km^2 [16]. The river is of strategic importance having in mind the built Kamchia and Tsonevo dams in the riverbed, which supply water for multiple uses and purposes [17]. Except that the complex anthropogenic pressure is related to diffuse pollution too, since 61.7% of the Kamchia River catchment is occupied by agricultural land [18]. In addition, municipal point source pollution induces pressure on the ecosystem diversity, function and services.

Along the river, three protected areas from the ecological network Natura 2000, were announced in connection with the implementation of Directive 92/43/EEC. These are BG0000136 "Gorna Luda Kamchia River", announced by Order No. RD-985 of December 10, 2020, of the Ministry of Environment and Waters (SG, No. 4/2021), BG0000137 "Dolna Luda Kamchia River", announced by Order No. RD-1015 of December 17, 2020, of the Ministry of Environment and Waters (SG, issue 16/2021) and BG0000139 "Luda Kamchia", announced by Order No. RD-1023 of December 17, 2020, of the Ministry of Environment and Waters (SG, issue 18/2021).

Fieldwork

During the 2024 survey, in all protected areas in the Luda Kamchia River, 16 sampling sites with lengths between 100 and 150 m were established (Fig. 1). Initially sampling sites were chosen for macrophytes and macroinvertebrates and were distributed in about 10 km. Sampling sites downstream from the dams were located at smaller distances to investigate the longitudinal macroinvertebrate recovery as in Doychev [19] and Doychev and Taneva [20], since the sites downstream from the dam's river reaches can be divided into 5 classes based on the impact of the hydro-morphological alterations [21].

Macrophytes were studied at all sites by wading transects 100 m long. Transverse transects for aquatic plant investigation were done every 10 m. All aquatic plants were recorded in the watercourse and along the banks [22]. Benthic macroinvertebrates were sampled from a 100 m long sampling site, too, and 20 subsamples distributed according to the microhabitat's availability were collected [21].

Corresponding author: d.doychev@shu.bg
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Combining those two methodologies in slightly different transects in terms of location allowed the detailed survey of about 150 m at every sampling site and facilitated the registration of Spongillidae representatives.

In addition, the Index of River Habitat Quality (IHF) was calculated to characterize hydrological and morphological settings at all sites during sampling events. The IHF has proven its effectiveness for riverbed assessment in Spain. Its scores range from 0 to 100, with the final sum formed by 7 types of variables: (1) rapids and pools, (2) frequency of rapids, (3) substrate, (4) speed and depth regime, (5) shade, (6) morphological heterogeneity, (7) plant cover. The overall result falls into 5 classes for evaluating the environmental status [20].

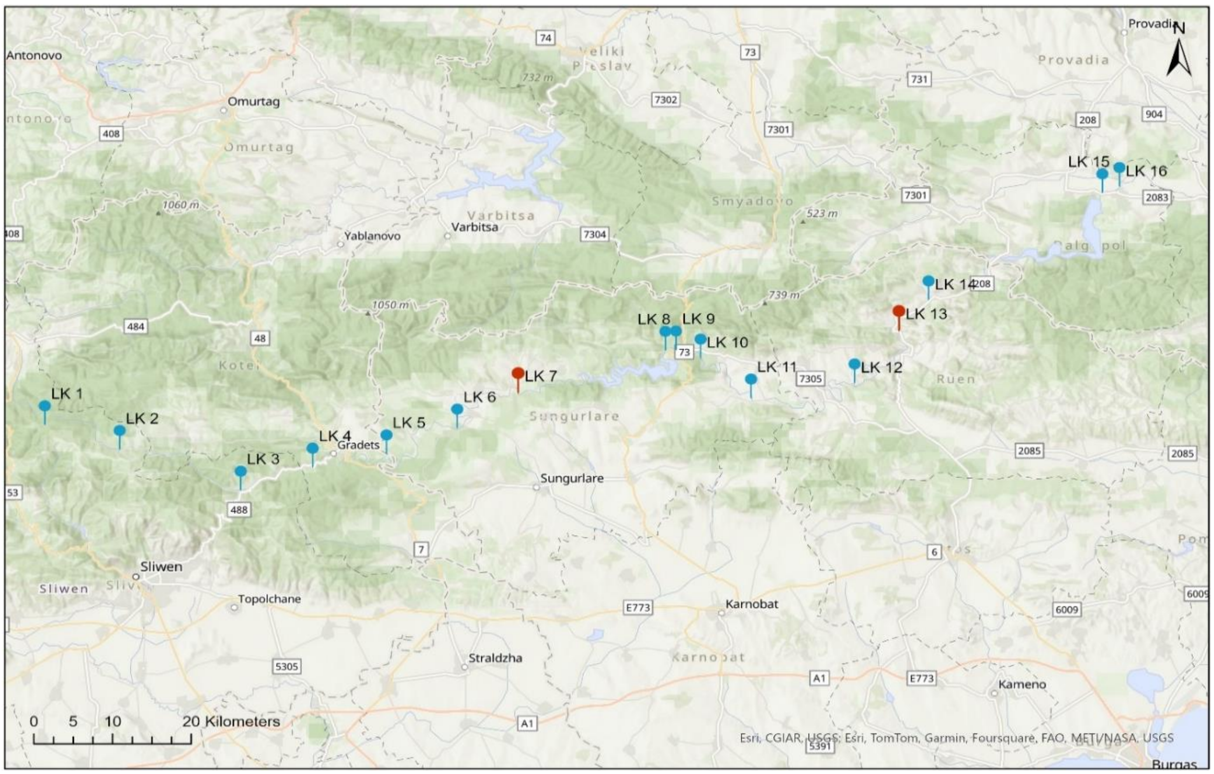

Figure 1. Map of Luda Kamchia River with all studied sampling sites

Riparian habitat quality was assessed using the QBR index. Its results range from 0 to 100 and allow the determination of environmental status in 5 classes. Both shores are being explored. Shrub and tree species are considered. Four separate assessment blocks help to form the score: (1) per cent cover of banks with trees and shrubs, (2) habitat structure, (3) quality of cover (number of native and non-native species), (4) naturalness of the river trough [24]

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In situ measurements related to the water quality included temperature (T), pH, and conductivity (CD). The parameters were measured with a multiprobe Senso Direct 150 Lovibond (Tintometer GmbH, Dortmund, Germany).

Laboratory work

Freshwater sponge samples were collected and conservated in ethanol (95%) and were subjected to nitric acid (69%) digestion of the tissue to separate siliceous spicules in preparation for microscopy. In a glass test tube, 2 ml of nitric acid was added using an automatic dispenser. Afterwards, a sponge tissue with a length of about 20 mm was dried on absorbent paper to remove the ethanol and to reduce the risk of explosive reaction. The sponge tissue was added to the test tubes under the fume cupboard and left to be digested for 24 hours. After that three washes with distilled water were conducted to remove the acid and leave the spicules behind. As a final step 2 ml of ethanol was added to each sample and left there for 10 minutes. Then most of the ethanol was pipette out and permanent slides were prepared. Spicules were examined using an Optica B-360 microscope at_400x magnification. Images were acquired with the eyepiece camera Dino-Eye AM4023X (Dino-Lite ANMO Electronics, Tayvan) [25].

Results and Discussion

Freshwater sponges are difficult to identify by morphological features due to their variable shape, colour and sizes (Fig. 2). It is easier to rely on the skeletal structure [22].

A **B**

Figure 2. A - *Spongilla lacustris* at about 30 cm depth with a colony length above 20 cm, B - *Spongilla lacustris* colonizing a stone (Photos by L. Taneva)

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Photography of the skeletal structure of collected specimens from LK7 and LK13 (Fig. 1) revealed that megascleres, microscleres and gemmuscleres are representative of the cosmopolitan species *Spongilla lacustris*, according to the descriptions of skeletal features (Fig. 3, 4, 5) [15, 25, 26].

S. lacustris framework of the skeleton is the megascleres (Fig. 3). They are smooth and slightly curved with typical size classes. Microscleres are present too (Fig. 4). All of them have dense spines and are curved with size classes typical for the species between 2 to 8 times smaller than megascleres [25]. Bent gemmuscleres are found in the samples with much lesser spines than microscleres (Fig. 5).

Both sites where freshwater sponges were recorded are not influenced by heavy hydro-morphological alteration from the dams. LK7 is a reference site or class 0 site, for Luda Kamchia River, considering the hydrological and morphological alteration from the dams and LK13 is class 1, more than 30 km away [21]. Therefore, LK7 and LK13 are not subjected to serious alteration on the aqueous and solid runoff from the existing strategic hydro-technical facilities.

Macrophytes

Aquatic plants were investigated at all sampling sites (Fig. 1). We present information about the two sites with registered *S. lacustris*. At LK7 were registered 23 macrophyte species and dominancy is held by *Carex riparia* Curt., which was recorded at 10 transversal transects. Other very well-established species were *Phalaris arundinacea* and *Fontinalis antipyretica*. At LK13 the diversity of aquatic plants was higher since 29 species in total were recorded. Here the dominancy of a single species was not so well pronounced. The species found at most transversal transects was again *Carex riparia* Curt., followed by *Carex hirta* Huds. The rest of the aquatic plants were very evenly distributed along all transversal transects in the 100-meter reach that was investigated.

IHF, QBR and water quality

Both sampling sites were studied in the summer. LK7 was sampled on 27.07.2024 and LK13 was sampled on 03.08.2024. The fluvial index – IHF for the two sites accomplished good ecological status with a slightly higher score at LK13 of 84.5 points against 77 points. This means that hydrological and morphological conditions are good enough to support various organisms within the riverbed.

The maximum result of 100 points was accomplished by the QBR on LK7 and 80 points at LK13. The result of LK7 corresponds to high ecological status and at LK13 to good ecological status. The differences come from the location of the sites at first, because the main road is parallel to the river around the LK13, better distributed non-indigenous species Amorpha fruticosa at both riverbanks are present. At the same time, at LK7 only the left one is occupied by Amorpha fruticosa and the road crosses the river perpendicularly.

Salix alba is the dominant tree species at LK13, where 5 more native tree species were present. At LK7 the dominance is held by *Alnus Glutinosa* and 3 more native trees are evenly distributed at both banks.

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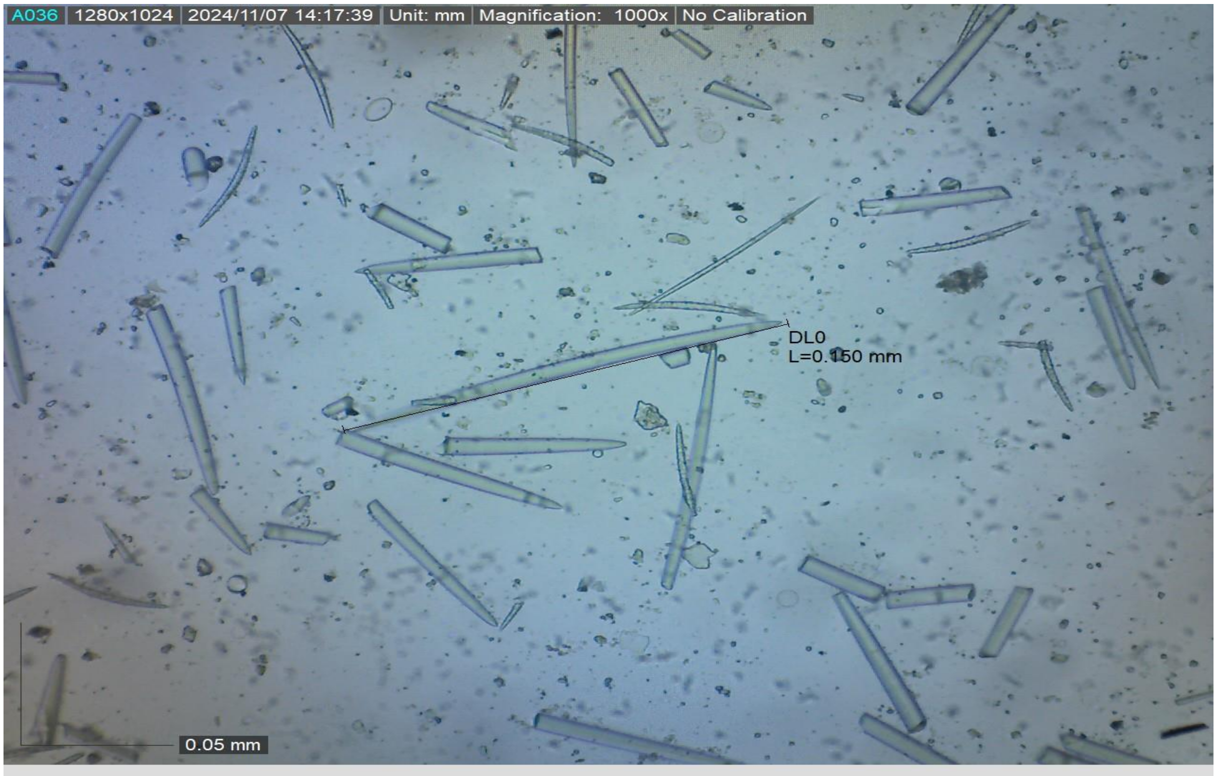

Figure 3. Skeletal structure of *Spongilla lacustris* visualizing megascleres

Figure 4. Skeletal structure of *Spongilla lacustris* visualizing microscleres

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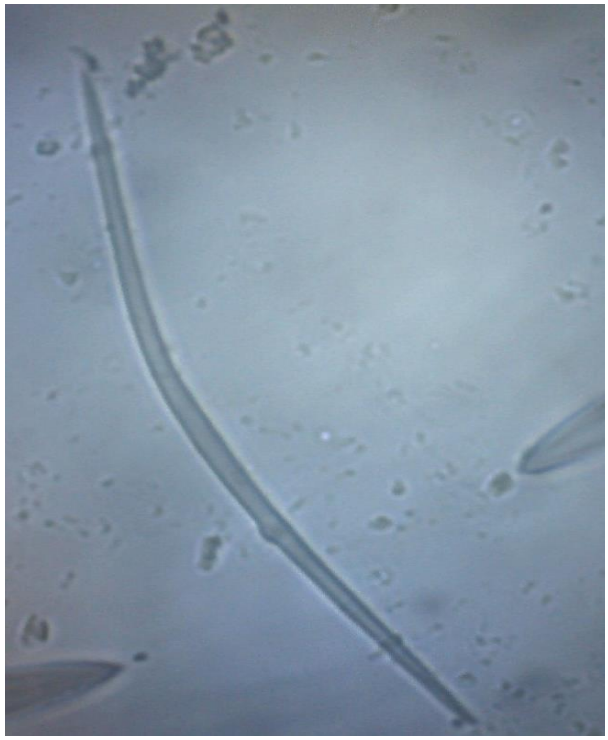

Figure 5. Skeletal structure of *Spongilla lacustris* visualizing gemmuscleres

In situ measurements revealed that pH was almost identical, registered 7.4 at LK7 and 7.34 at LK13. CD was higher at LK13, reaching 404 µS/cm^2 and 340 µS/cm^2 at LK7. Electrical conductivity scores correspond to high environmental status, and pH to good ecological status [27].

Conclusions

Spongilla lacustris was registered at two sites of the biggest tributary of the Kamchia River [16]. This is the first record of the species in the intra-territorial Bulgarian rivers.

Sampling sites of their registrations (LK7 and LK13) had similar IHF and QBR results and therefore possess analogous fluvial and riparian habitats. They were alike in terms of dominating macrophyte species, pH and conductivity scores. Bearing in mind that the Spongillidae family widely tolerate diverse physicochemical conditions [28] and the small differences in pH and CD between sites, sponges may not be of great importance for further research of the group as a water quality bioindicator for Bulgarian rivers.

Nevertheless, future studies on freshwater sponges are certainly needed for understanding better the species richness and distribution, the habitat preferences and the dam regulation tolerance not only in the Kamchia River basin since *Spongilla lacustris* was found only at sites with minor or lacking hydro-morphological pressure.

Corresponding author: d.doychev@shu.bg
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