Freshwater Sponges – Skeletal Structure Analysis Using Light Microscopy and Scanning Electron Microscopy

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Abstract

Freshwater sponges, compared to their marine counterparts have not been studied extensively in the past. The standard approach in their identification is microscopy analysis of the sponge skeletal structures, combined with the, ever more popular, genetic analysis. The aim of this paper is to briefly describe the use of light microscopy and scanning electron microscopy (SEM), two methods applied in sponge ultrastructure analysis, as well as to present preliminary results on identification of samples from the Danube River. Based on the reviewed material, the species *Spongilla lacustris* (Linnaeus 1759) and *Ephydatia fluviatilis* (Linnaeus 1759) were identified. This paper describes sample preparation and highlights some of the skeletal characteristics crucial for the identification of these two freshwater sponge species, widespread in Europe, Asia and North America.

Keywords: Freshwater sponges, spicules, light microscopy, SEM.

Introduction

Freshwater sponges, formed of 219 species belonging to 45 genera and grouped into 6 families (Manconi & Pronzato, 2008), are sessile organisms that represent an important component of the aquatic ecosystem with considerable filtering potential and filter particles of a smaller size range than other benthic invertebrates (Francis & Poirrier, 1986; Frost, 1978). These organisms are of ecological importance due to their role in water purification. They could also be used as bioindicators for their widespread distribution in a variety of habitats as well as their capacity to tolerate different levels of pollution (Covich, Palmer, & Crowl, 1999; Mahaut et al., 2013; Rao, Srikanth, Pallela, & Rao, 2009).

In central Europe six species have been recorded: *S. lacustris, E. fragilis, E.fluviatilis, Ephydatia mülleri, Trochospongilla horrida* and *Heteromeyenia stepanowii* (Dröscher & Waringer, 2007). There are no published data dealing with freshwater sponges for the territory of Serbia and this paper aims to shed some light on this subject using light microscopy and SEM in spicule analysis. Three types of spicules, the main elements of the sponge skeleton, were observed: megascleres, microscleres and gemmuloscleres, which envelope gemmules and represent the most valuable identification structure (Cocchiglia Kelly-Quinn, & Lucey, 2013).

Materials and Methods

Samples

Samples were collected during the research of the Danube River in the framework of the Joint Danube Survey 3 investigation (JDS-3) in the period August-September 2013 and carried out with the support of the International Commission for the Protection of Danube River (ICPDR). A total length of 2.581 km was explored and sponges were collected at 68 locations. Samples were transported to the laboratory either in river water or different preservation agents (4% formaldehyde and 70% ethanol). Fragments from each sample were taken for spicule preparation and skeletal structure analysis.

Spicule Preparation for Light Microscope Analysis

The nitric acid technique was used to dissolve sponge tissue as described by Jakhalekar and Ghate (Jakhalekar & Ghate, 2013). Briefly, 2-5mm sponge fragments were washed with ethanol, dried and fed

into labeled glass tubes. They were then carefully topped with approximately 2ml of concentrated nitric acid (HNO_3) and left to decompose for 24h. The acid was then removed by pipette and the spicule residues were washed repeatedly with distilled water. Finally, the spicules were rinsed with and resuspended in 96% ethanol. A drop of suspension was then placed on a cover slip. When the alcohol dried the cover slip was placed over the microscope slides with a drop of Canada balsam and heated to complete the preparation. The spicule preparations were observed under 25x and 40x magnification (microscope model BIM-312T) and a Zeiss camera was used to make micrographs (model AxioCam ERc5s).

Electron Microscopy

To better visualize microscopic details of the morphological structure of sponge spicules, some samples were prepared for SEM. Drops of spicule suspension in ethanol were placed on specimen holders and coated with gold in a gold sputter at 18mA for 1 minute. The specimens were analyzed and photographed in a VEGA TS 5133MM scanning electron microscope, in high vacuum mode using the SE detector with accelerating voltage.

Results and Discussion

Sponge skeleton is usually calcareous, siliceous, or composed of spongin. Freshwater sponges belong to the Class Demospongiae, which is characterized by silica skeletal structures. The examination of slides revealed all three types of spicules: megascleres, the main support structures of sponges, microscleres - structures scattered throughout sponge tissue, and gemmuloscleres that form the gemmule walls and are the most important identification character (Boury-Esnault & Rutzler, 1997; Hooper e Van Soest, 2002). Based on the analysis of skeletal elements, two sponge species dominated in the examined sample: *Spongilla lacustris* (Linné 1759) and *Ephydatia fluviatilis* (Linné 1759). One type of megascleres were smooth and slightly curved or straight, pointy at both ends, about 210-318μm in length and 5-16μm in width, typical for *S. lacustris*. In addition, *S. lacustris* was also characterized by the presence of microscleres and gemmuloscleres that were identical, curved and pointy at both ends, with thorn-like structures along the spicule body, with the size ranging from 50-116 x 2-6μm as seen in Fig. 1 (light microscopy) and Fig. 2 (SEM). *E. fluviatilis*, on the other hand, was identified by the specific birotule shape of gemmulosclere (Fig. 3). Namely, the birotule is deeply cleaved with radially arranged thorns and with the stem significantly longer than the width of the rotules (Schletterer & Eggers, 2006; Økland & Økland, 1996). This structure is essential for distinguishing the two species, since they have similar megascleres and microscleres. Scanning electron microscopy, as a powerful technique, enabled us to see in greater detail the smallest elements of spicule morphology and to confirm the findings of light microscopy. This approach was also suggested by several authors, concluding that, increased magnification provided by SEM can be an accurate method for skeletal structure measurements and sponge identification (Evans & Kitting, 2010; Jakhalekar & Ghate, 2013).

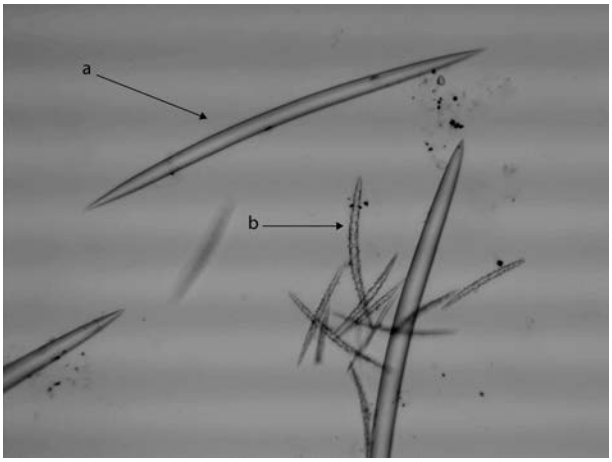

Figure 1: Light microscopy slide of spicules from *S. lacustris*: (a) smooth megasclere and (b) spine-covered microsclere (magnification 25X).

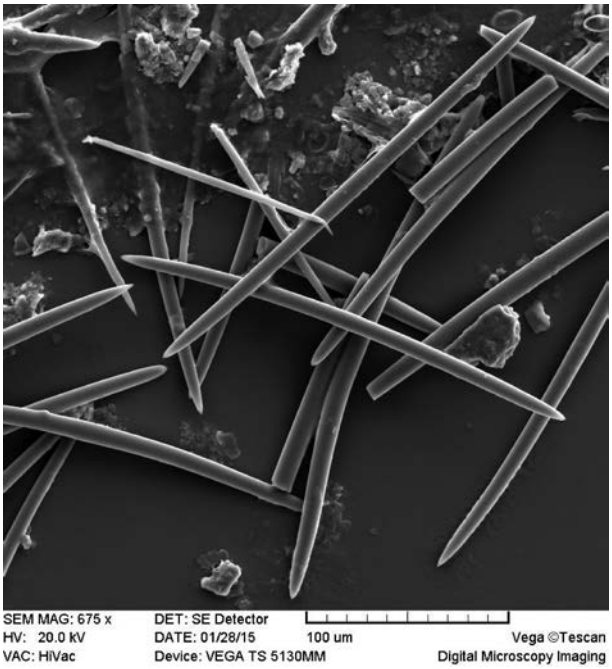

Figure 2: SEM micrograph of smooth pointed megascleres of *S. lacustris* seen in higher resolution.

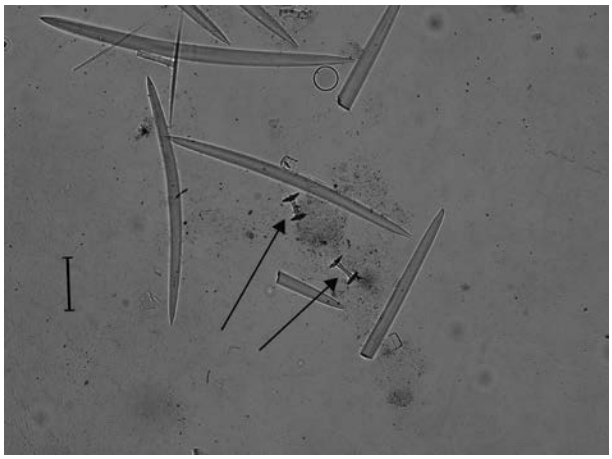

Figure 3: Light microscopy slide of the typical birotule shape of gemmuloscleres from *E. fluviatilis* (arrows), showing considerably greater stem length compared to rotule width.

Conclusions

Freshwater sponges are poorly explored in Serbia and represent a good ground for further scientific work both in taxonomy as well as in the fields of ecology and biochemistry. Light microscopy includes a relatively simple methodology for spicule slide preparation and provides us with details sufficient for basic taxonomy work. The use of SEM is an important addition to the research process as it allows observation, in far greater resolution, of the miniscule details of the sponge skeletal structures that are often crucial in species determination.

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