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Valve Ultrastructure of *Didymosphenia geminata* (Lyngbye) M. Schmidt: An Invasive Diatom Species in the Running Waters of Iceland

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Abstract: The stalked diatom *Didymosphenia geminata* is an invasive species in the running waters of Iceland since the 1990s. Here we present an analysis of the morphology of this species from the River Elliðaár catchment, southwestern Iceland by scanning electron microscopy together with its nomenclatural history.

Key words: Didymo, ultrastructure, invasive species, the River Elliðaár catchment, Iceland

Introduction

In recent decades, aquatic and terrestrial alien species have been spreading across Europe and many of these species are potentially dangerous to European biodiversity, economy and human health (EASIN 2017). Anthropogenic activities are the main cause for the enhanced spreading of alien species in environments. However, changing climatic conditions may facilitate the spreading of alien species due to the change of environmental conditions, e.g. warmer temperatures (Parmesan 2006).

Didymo, *Didymosphenia geminata* (Lyngbye) M. Schmidt, is a freshwater diatom which has historically been found in cool, oligotrophic waters of northern Europe and northern North America (Blanco & Ector 2009). Since the mid-1980s it has increasingly been observed in new areas, e.g. New Zealand, Iceland and high elevation Alpine areas (Jónsson et al. 2000, Kilroy et al. 2009, Spaulding et al. 2010). Recently, individual cells have also been recorded in high-mountain areas in southern Europe (Ognjanova-Rumenova et al. 2009). Although *D. geminata* does not present a significant human health risk it can form massive blooms and have negative impact on stream habitats, hampering fishery and degrading the recreational value of streams (Jónsson et al. 2000. Spaulding et al. 2010).

Specimens of the type material of *Echinella geminata* Lyngbye, 1819 (= *Didymosphenia geminata*) have been studied for the first time by Metzeltin & Lange-Bertalot (2014). This is the reference point for various revisions and descriptions of eleven new to science species within genus *Didymosphenia* M. Schmidt.

In the Icelandic rivers, *D. geminata* formed large blooms beginning in the early 1990s (Jónsson et al. 2000). Blooms had no relation to bedrock geology or specific conductance, that is, the distribution and biomass of extensive mats appeared to be unrelated to water chemistry. Since the 1990s, populations of *D. geminata* have decreased, or remained stable. The Icelandic rivers are vital to the salmon fishery and

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there was concern that the masses of *D. geminata* would negatively impact spawning, yet there is no clear evidence of a negative influence of *D. geminata* on fish stock (Beville et al. 2012). Until now, there is only a scarce information on the ultrastructure of the Icelandic specimens of this species (Tuji & Soninkhishing 2008).

The aim of our study was to analyse the ultrastructure of *D. geminata* from the River Elliðaár catchment, southwestern Iceland. Thus, we add new information to existng knowledge about *D. geminata* in the running waters of Iceland.

Materials and Methods

Study area

Iceland is located just on the edge of Artic circle in the North Atlantic Ocean and it is young and still evolving volcanic island (Sæmundsson 1979). Climate in Iceland is determined by the northern position and Gulf Stream flowing along the coast. The summer temperature range between 10 and 13°C, while the average winter temperature is around 0°C. Great amount of precipitation is brought with wet SE and SW winds, and approximately 2000 mm of precipitation fall each year in southern Iceland (Einarsson 1984). Therefore, wet and cold climate causes that water resources in Iceland are abundant. Furthermore, due to high latitude (65°N) of Iceland there is a significant difference between summer and winter day length (Einarsson 1984). In the study area, southwestern Iceland, the summer day lasts approximately 21 hours, whereas in winter time it is around 4 hours long.

The River Elliðaár catchment is located in southwestern Iceland and lies partly within the city boundaries of Reykjavik. The headwaters of the catchment are located in the mountain range east of Reykjavik. Snowmelt and runoff from the headwaters are drained by the rivers Hólmsá and Suðurá, which feed into Lake Elliðavatn. Lake Elliðavatn is located at 76.5 m a.s.l and is drained by the River Elliðaár at the southern side of the lake. The entire catchment area covers about 270 km^2 (Thórðarson 2004). Due to the high bedrock permeability, most of the precipitation percolates and submerges downstream as groundwater (Thórðarson 2004). The River Elliðaár is classified as groundwater-fed (Halldorsdottir et al. 2006).

Previous studies of the water chemistry, provided for the Environment and Health Department of Reykjavik indicated that the River Elliðaár catchment is nutrient poor (Thórðarson 2004). The phosphate (PO_4) concentration in the River Hólmsá was 5.4 µg/l, whereas in the Elliðaár and its stream the phosphate concentration ranges between 6.3 and 7.2 µg/l. The ammonia concentration (NH_4) increases downstream in the catchment. In the River Suðurá NH_4 concentration equals to 5.8 µg/l, while in the Hólmsá, Elliðaár and Vesturkvísl equals to 5.4, 6.3 and 7.4 µg/l, respectively (Thórðarson 2004).

Sampling methods

The diatom samples were collected on two occasions, on 20 July 2015 and 4 November 2015, from seven sampling locations: the River Hólmsá upstream/ downstream; the River Suðurá upstream/ downstream; Lake Elliðavatn; the River Elliðaár upstream/ downstream. The methods for epilithon sampling followed those described by Cameron (1997).

Laboratory methods

The diatom samples were cleaned according to the standard methods of Battarbee (1986). The cleaned diatoms were identified and counted under oil immersion, at magnification of ×800 or ×2000, using an Jenaval, Carl Zeiss, Jena light microscope. A minimum of 500 valves was counted in each sample (Renberg 1990).

The preparation for scanning electron microscopy followed Hasle & Fryxell (1970) and samples were examined on a Jeol JSM-5510.

In general, the nomenclature followed Krammer & Lange-Bertalot (1986-1991), Round et al. (1990), Lange-Bertalot & Metzeltin (1996), and Hofmann et al. (2011). For those taxa not listed by them, other taxonomic works were used, e.g. Reichardt (1999, 2001) for some *Gomphonema* taxa and Lange-Bertalot et al. (2011) for the *Eunotia* taxa.

The terminology is recommended by Anonymous (1975), Round et al. (1990), and Metzeltin & Lange-Bertalot (1995, 2014).

Results and Discussion

***Didymosphenia geminata* (Lyngbye) M. Schmidt**

Basionym: *Echinella geminata* Lyngbye, 1819

Synonym: *Gomphonema geminata* (Lyngbye) Agardh, 1824

Type locality: Faroe Archipelago, Streymoy Island

Lectotype: Collection H. C. Lyngbye in the Museum of Natural History Copenhagen, Denmark; Access No CAT 2299 represented by Fig.1:3 (Metzeltin & Lange-Bertalot 2014)

Description: Cells large, colonial, forming

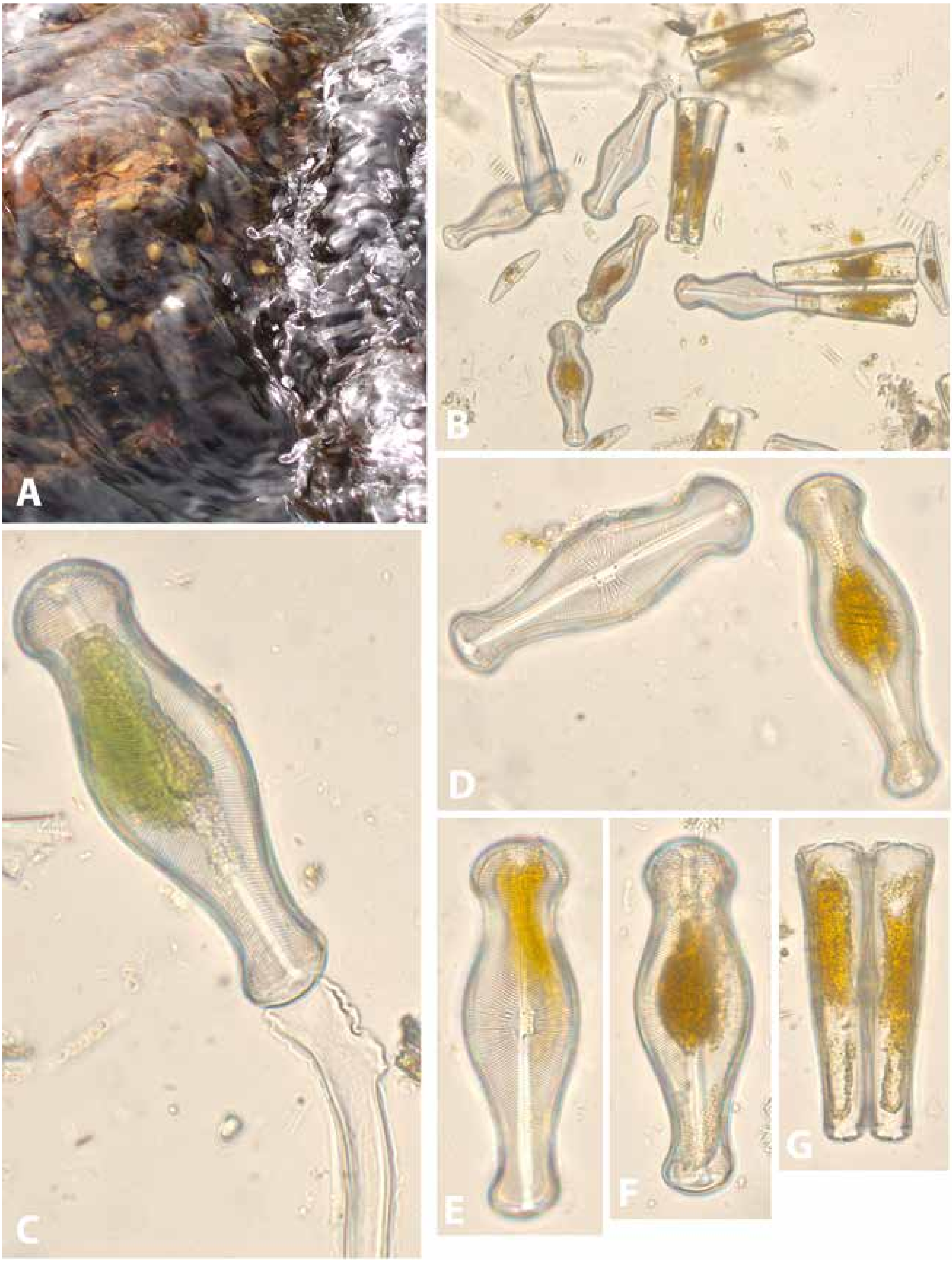

Fig. 1 (A-G). Live specimens from the River Elliðaár, Iceland, showing the mucilaginous stalk and form and position of the plastids

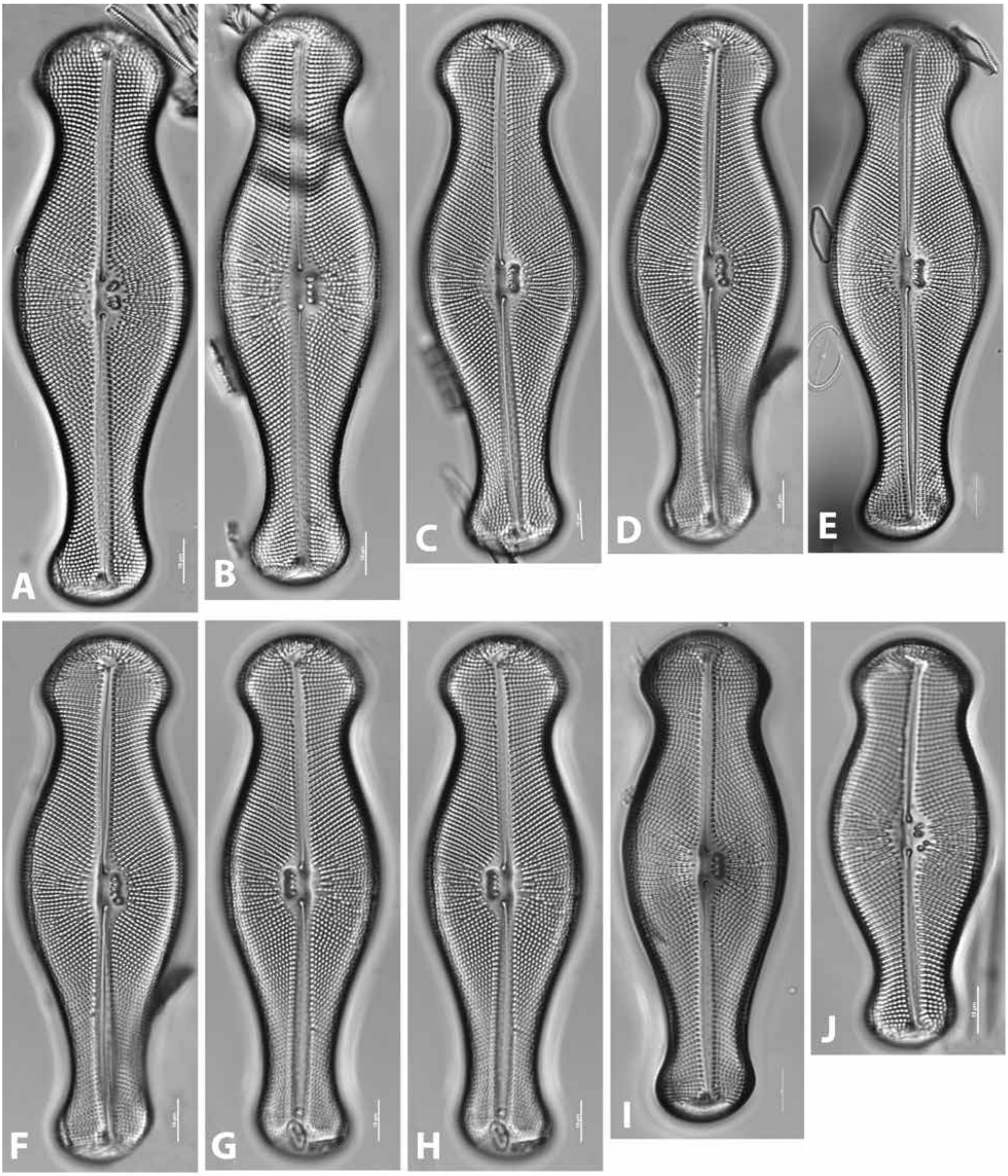

Fig. 2 (A-J). *Didimosphenia geminata*, light microscopy (LM) – specimens of the investigated populations

branched mucilaginous stalks by which they are attached to a substrate (Fig. 1: A-C). The extracellular stalk of *D. geminata* is a complex, multi-layered structure, resistant to degradation in streams (Kilroy & Larned 2016).

Frustule heteropolar in valve and girdle view (Fig. 1: B, G). The valves are large sized, robust, heteropolar and slightly asymmetric to the apical axis (Fig.1: D-F; Fig. 2: A-J). The poles are capitates, but the footpole is narrower. Valve length 96-135 μm, width at mid-valve 30-42 μm. The valve face is flat with a marginal ridge at the junction with the mantles (Fig. 3: A, C, D; Fig. 4: B, D). The ridges end in small spines at the headpole (Fig. 4: A, C).

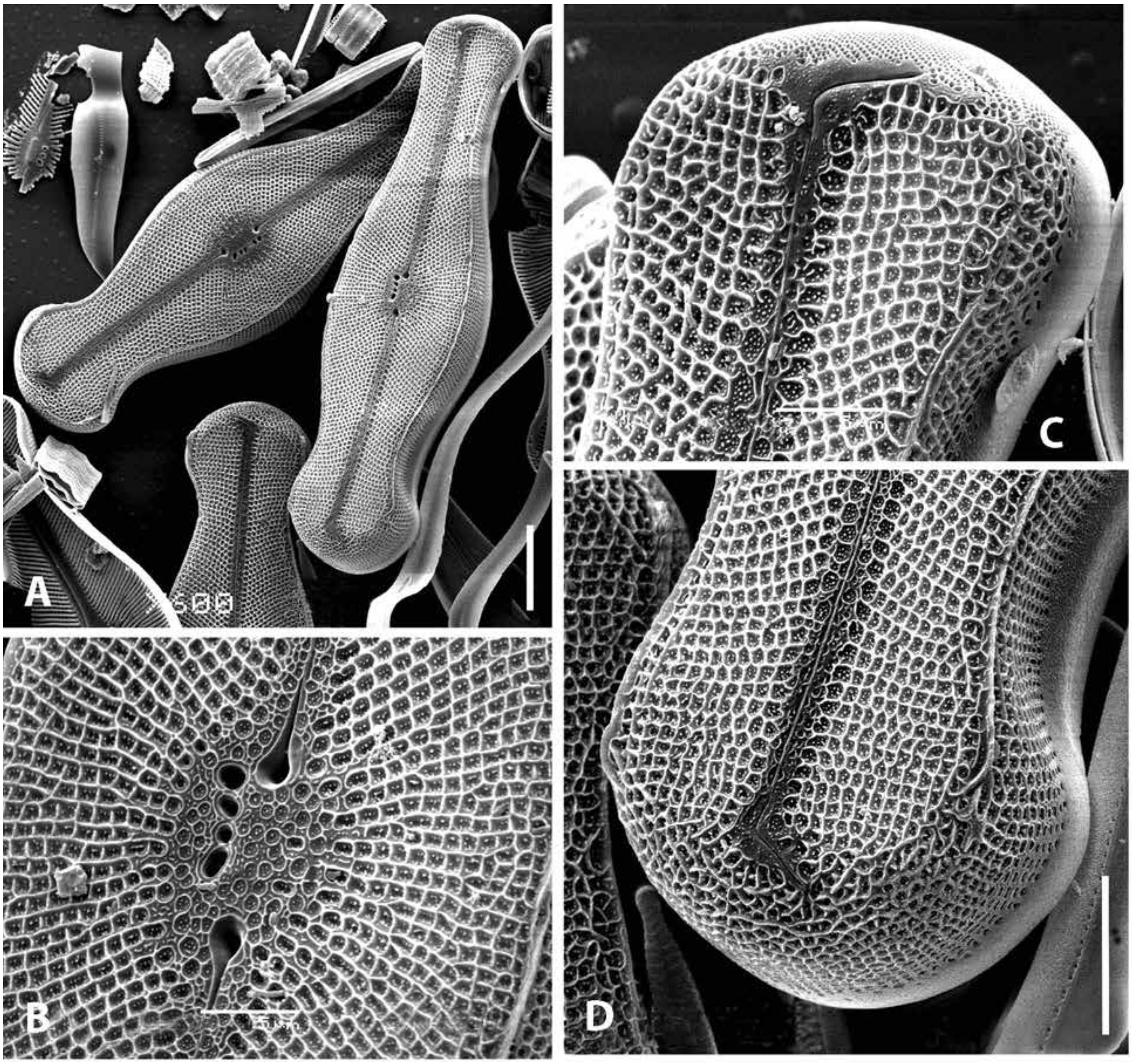

Fig. 3 (A-D). *Didimosphenia geminata* scanning electron microscopy (SEM): A. General outline; B. Detail of the central part with central raphe endings and several central stigmata; C. Detail of the footpole with the apical pore field; D. Detail of the headpole with the hooked raphe end; Scale bars: A = 20 µm; D = 10 µm

Raphe lateral, straight. The axial area is relatively narrow (Fig. 3: A; Fig. 4: A-B). The central area is expanded – oval, with several stigmata (2-6), their number is positively related to the valve size. Externally, they have a simple round aperture, but open internally through convoluted spongy bosses of silica (Fig. 3: B; Fig. 5: C). Terminal fissures present, hooked (Fig. 3: C, D). The central raphe endings expanded externally into pores (Fig. 3: B). Internally, they obscured by a nodular outgrowth of silica on the primary side of the raphe sternum (Fig. 5: C). Helictoglossae deflected to one side (Fig. 5: A-B). Striae uniseriatae, coarse, 8-10 in 10 µm. At the valve centre, the striae are radiate and of different length (Fig. 3: B; Fig. 4: A-B). Towards each pole, the striae become parallel (Fig. 3: C, D). Areolae distinguishable in LM, 9-12 in 10 µm. Externally, the areolae occluded by several volae, internally, opened between transverse ribs. (Fig. 4: D; Fig. 5: B-C). The narrower footpole has an area of small, unoccluded, round pores, structurally different from the areolae present at the headpole (Fig. 3: C). Girdle composed of open bands (Fig. 4: B).

Diatom community in the River Elliðaár catchment

A total of 132 diatom taxa were recorded in the samples from the River Elliðaár catchment in 2015. The complete list of species occurring in the studied new materials is given in Jónsson et

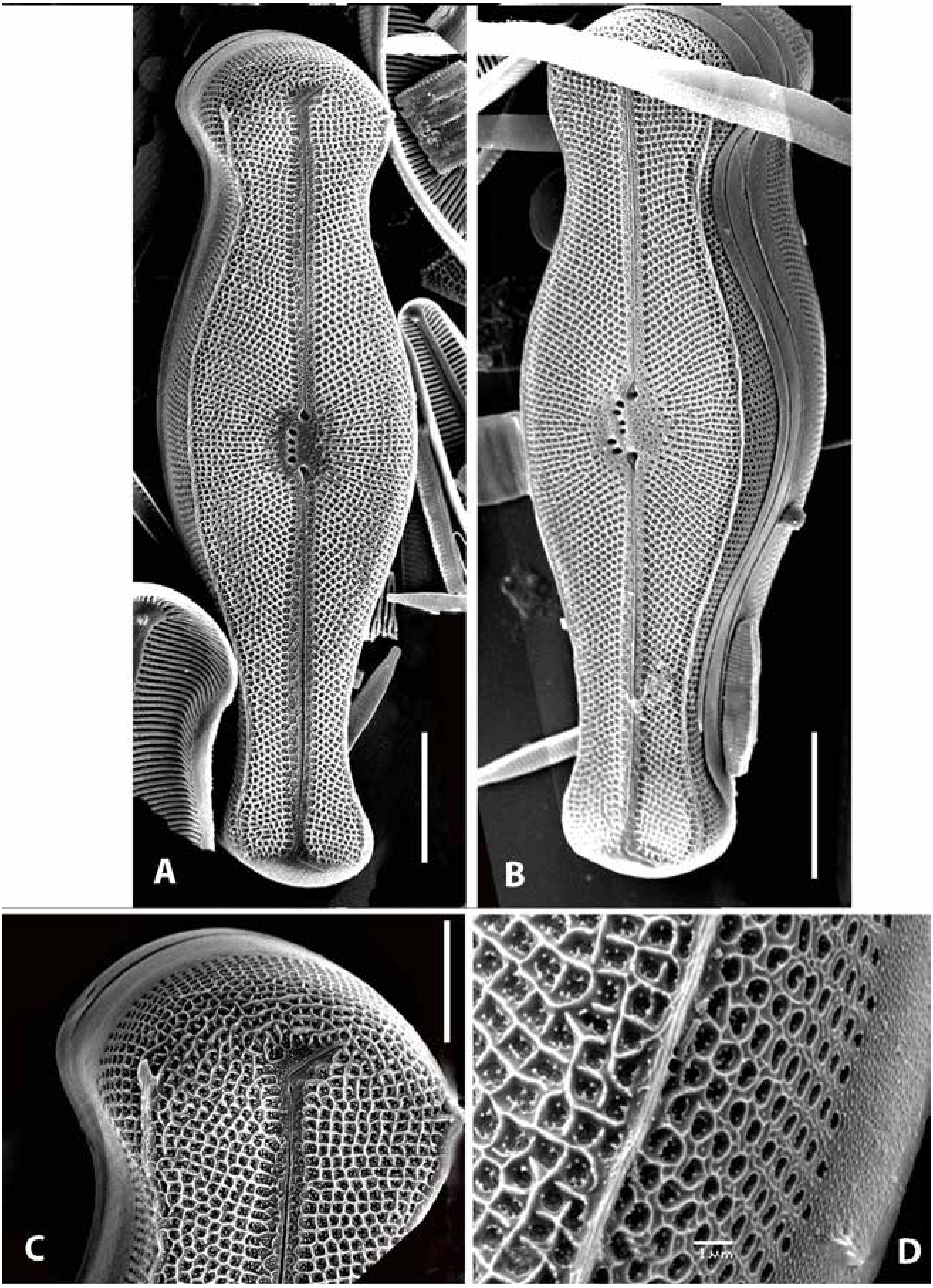

Fig. 4 (A-D). *Didimosphenia geminata* (SEM): A, B. General outline; C. Detail of the headpole with the hooked raphe end; the marginal ridge ends in a small spine; D. Detail of the areolae structure; the marginal ridge at the junction face/mantle; Scale bars: A, B = 20 µm; C = 10 µm

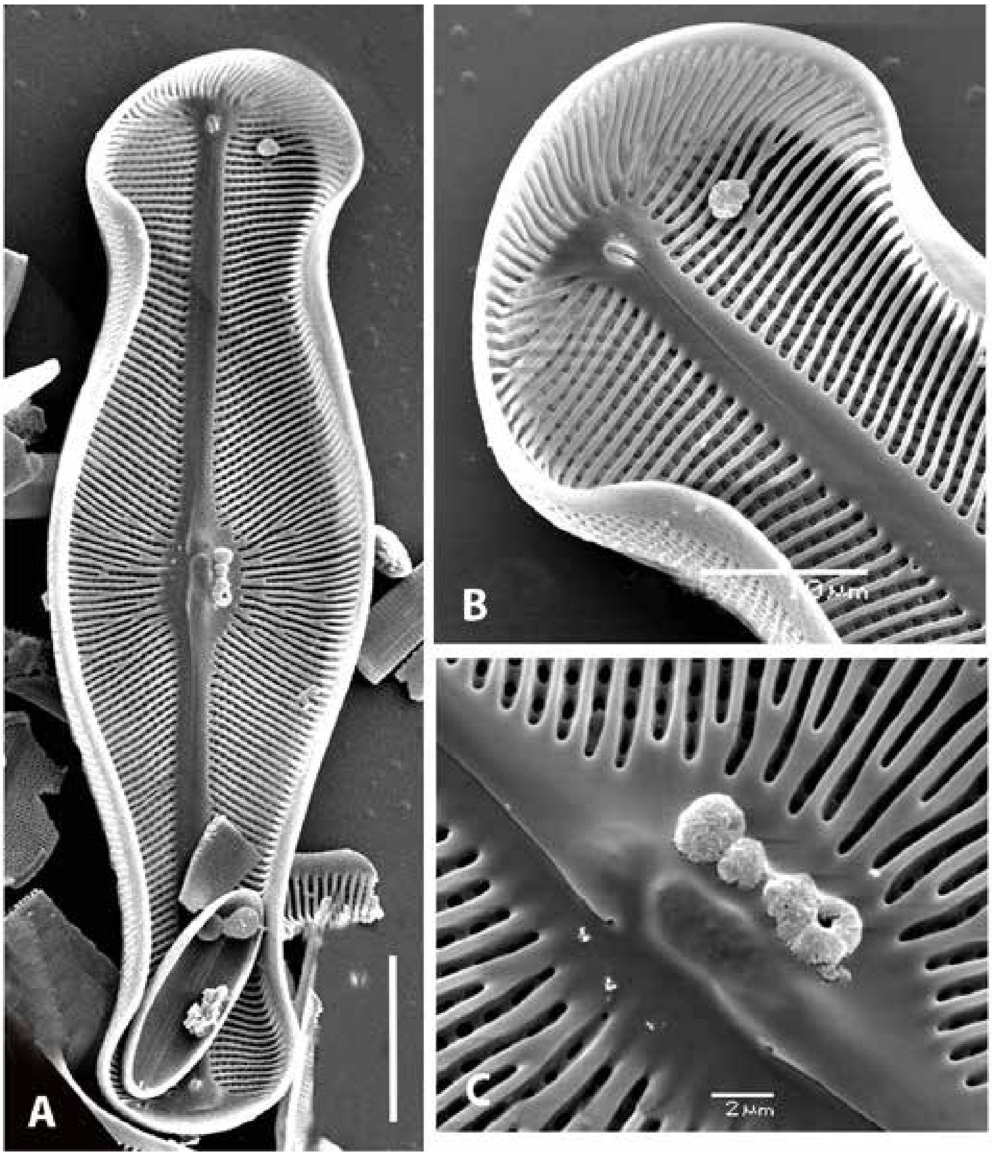

Fig. 5 (A-C). *Didimosphenia geminata* (SEM): A. General inline; B. Inside view of the headpole with the raphe end with helictoglossa deflected to one side; C. Stigmata - internally through convoluted spongy bosses of silica; Scale bars: A = 20 μm

al. (2017). The highest relative abundance of *D. geminata* and the lowest taxon richness was found in the sample collected from the upstream of the River Suðurá, in July 2015, where it was 40.1%. Subdominant species were: *Pseudostaurosira pseudoconstruens* (Marciniak) D. M. Williams & F. E. Round, *Fragilaria radians* (Kutzing) Lange-Bertalot, *Pseudostaurosira brevistriata* (Grunow) D. M. Williams & F. E. Round, *Encyonema minutum* (Hilse) D. G. Mann, and *Staurosira construens* var. *venter* (Ehrenberg) Hamilton.

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