

Micro-morphological Characterization of In-Vivo Diatoms Using ESEM

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Diatoms (Bacillariophyta) are one of the most distinctive and successful groups of unicellular photosynthetic algae [1]. They are ecologically widespread inhabiting mainly fresh, brackish and marine waters, often dominant in given microhabitats and contribute about one fourth to the world's productivity (in terms of carbon fixation) [2]. Diatoms are also the most species-rich algal group represented by 50 – 200 thousand species. Diatom taxonomy is predominantly based on the morphology of ornate silicified cell wall called frustule which is composed of two overlapping parts (thecae). This frustule displays intricate patterns and designs unique to each species [3]. For these studies conventional scanning electron microscopy (SEM) was and still is widely used. This method requires cleaning of diatom frustules in strong acids and peroxides followed by conductive coating. This aggressive procedure removes protoplast and damages delicate structures. Environmental scanning electron microscopy (ESEM) brings advantages of observation of fresh diatom material that are presence of: 1) whole intact diatom cells, not only empty diatom frustules, 2) extracellular mucilaginous diatom secrets (e.g. pads, stalks, tubes), 3) whole diatom assemblages directly in situ together with other algal assemblages (e.g. cyanobacteria or green algae). However, in diatom research, ESEM is used mainly for elimination of conductive coating [4]. Observation of these aquatic organisms in their native wet state is not yet common even if it allows study of diatoms adhered directly on the host plant [5]. The ESEM observation of native aquatic samples is usually affected with radiation damage that can be lowered by the use of special methods like the Low Temperature Method for the ESEM (LTM) [6], ideally in a combination with advanced low noise detectors with very high detection efficiency.

Using the LTM we observed epiphytic diatom assemblages on a stem epidermis of common reed (*Phragmites australis*). Samples were observed using the FEI Peltier stage equipped with a special flat cylindrical brass sample holder. Epidermis was cut to 4 – 8 mm² segments and placed into a drop of 2 µl of water, to obtain better thermal contact between the sample and the Peltier stage. The observation conditions were adjusted according to [6]: the air pressure was 250 Pa, sample temperature -20°C. All experiments were performed on FEI ESEM QUANTA 650FEG and carried out under constant operating conditions. The beam energy 20 kV, probe current 35 pA and working distance was 8.5 mm.

Traditional diatom taxonomy according to [7] based on the symmetry of a frustule distinguishes centric diatoms with radial symmetry (Figure 1 D) and pennate diatoms with bilateral symmetry (Figure 1 A, B, C). Figures 1 A, B show a common benthic species *Achnantheidium minutissimum* able to attach to the surface with a gelatinous stalk. Both pictures show a frustule of this pennate diatom oriented up with its rapheless convex valve face ornamented with lines of pores called striae that are radially oriented around pseudoraphe. Figure 1 C shows three individuals of the heteropolar genus *Gomphonema* attaching to the substrate with a mucilaginous stalk. Figure 1 D shows a common planktic centric diatom *Puncticulata balatonis* known from mesotrophic and eutrophic European lakes and rivers with well visible radial striae at the marginal zone of the valve face and fultoportulae in the central area of the valve face.

The work shows high applicable potential of the LTM ESEM to observe aquatic plants and algae under conditions of reduced gas pressure and low relative humidity with higher resolution as well as capability to observe susceptible structures like mucilaginous stalks that are destroyed during the preparation for SEM.

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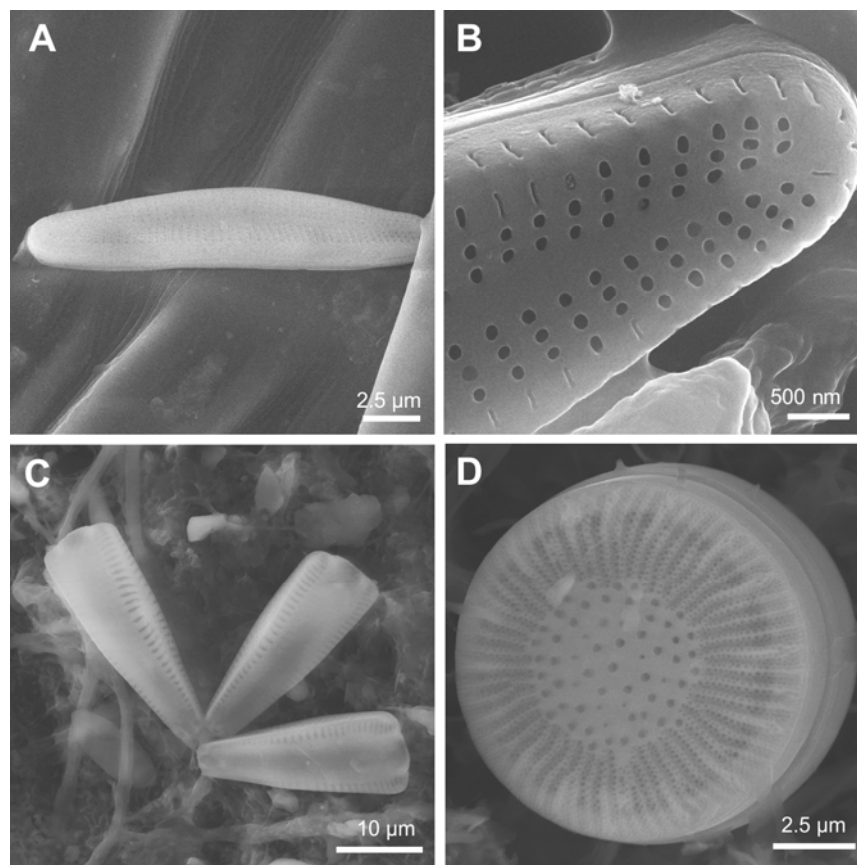


Figure 1. A) Benthic pennate diatom *Achnantheidium minutissimum* on a stem of common reed (*Phragmites australis*) B) *Achnantheidium minutissimum* – detail of valve end C) three individuals of a benthic pennate diatom *Gomphonema* sp. attached to the surface of common reed (*Phragmites australis*) with a mucilaginous stalk D) Planktic centric diatom *Puncticulata balatonis*.