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## Insights into the diatom world using electron microscopy images

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### Abstract

One group of ubiquitous microscopic organisms with a siliceous cell wall demonstrating structural diversity in morphology, are diatoms. Complex 3D nano and micro-scale silica structures produced by the frustule might be highly significant in the nanotechnological field. Advancement in studies of diatoms include application of electron microscopy (SEM), which allows the profound examination and detailed study of structures at different magnifications. In the present work, ultrastructural characterization and morphological features of diatoms as revealed through scanning electron microscope were described. This investigation in the form SEM micrographs will serve as a guide to morphological diversity of diatoms.

**Keywords:** Diversity, Nanostructure, Morphology, Scanning

### Introduction

Diatoms are efficient single celled photosynthetic organisms actively involved for a quarter of oxygen as well as biomass release on the surface of the earth. Diatoms constitute a diverse class of microorganisms with stiff durable exoskeletons which are composed of hydrated amorphous silica and exhibits 3D nanostructures with hierarchial architectures upto the scale of tens of micrometers. The frustule exhibits optical properties because of silica and structural complexity. Diatoms form an essential component of the global ecosystem with immense

ecological relevance and exhibit certain curious, puzzling or suggestive geographical distributions and serves as an indicator of ecological conditions in lakes and streams. The intricately detailed nanostructures generated by diatoms are ideal for varied applications including biosensing, biophotonics, filtration, microfluidics, optics, and drug delivery (Drum and Gordon, 2003; Rosi *et al.* 2004; Fuhrmann *et al.* 2004; Hamm, 2005). Moreover, such materials provided by diatoms can be a potential source for biotechnological manipulation.

Nanostructures are difficult to manufacture, quite expensive and usually produced through layer-by-layer lithographic technology. However, use of diatom frustules directly is limited because the chemical as well as physical properties of silica have not been able to provide the desired refractive index or optimum chemistry for use in various fields. Silica nanoparticles form the shells of diatoms and are therefore, best described as living cells inside a glass house (Siver, 2005). Structural, chemical, optical and mechanical features of the silica shell of diatoms possess a precedence over the existing microparticle delivery systems which might also overcome the susceptible challenges associated to traditional delivery of therapeutic agents. Diatoms serve as an excellent biological material for drug delivery operations due to presence of enormous surface area, pill-box structures and 3D micro and nanoscale silica structures (Gordon and Drum, 1994).

Several modern visualization techniques provide higher resolution imaging of frustule morphology. Diatoms form suitable objects for undergoing SEM studies as it is composed mainly of hydrated organics such as polysaccharide case, chloroplasts, fats, proteins and mineral silica components. Light microscopic resolution was extensively used for determination of size, shape and relatively incapable to visualize the frustule in detail. However, SEM identifies the pores and curvatures in frustule architecture and allows 3D view of internal content and various nanostructures with higher magnification. Imaging of diatoms applying electron microscopy provides a higher magnification and in depth knowledge of the silica frustule. These naturally designed materials offer great variety in morphology, structure and shape as revealed under the electron microscope. Further, nanoporous silica deposited over the frustules of diatoms is geometric, biologically stable, cost-effective and eco-friendly.

### **Materials and Methods**

Collected samples from aquatic and semi-aquatic habitats of Assam were transferred to culture medium (Beakes *et al.* 1988). The culture procedures were carried out under sterile conditions to avoid contamination. The organic components of the diatom frustule were removed successfully by employing acid digestion method (Hasle and Fryxell, 1970). In SEM

chamber, in addition to cleaning with the help of strong acids, vacuum conditions require proper hot air drying. Metal sputtering is applied when low voltage, and low vacuum regimes do not permit to reach higher resolution of the images. The frustule morphology was imaged using JEOL JSM - 6360 and identified to genus level by consulting various standard literatures (Taylor *et al.* 2007; Buczko, 2016; Borgohain and Tanti, 2018; Salimonet *al.* 2020).

## Results and Discussion

Clean frustules of freshwater diatoms were obtained by treating with sulphuric acid, which aided the removal of organic matter. Further, the frustules were analyzed for the interpretation of their structure and properties using SEM. Highly complex siliceous frustule morphology were formed with hierarchical organization, ranging from micrometers to nanometers. Under SEM analysis, the treated diatom frustules showed distinct raphe, forams and the valve view of the diatom. The silica nanopores appeared as distinct holes. Highly ornated valve views with intricate structure exhibited greater morphological variability compared to girdle views. Valve views show variously angled, linear, lanceolate, rhombic, panduriform or sigmoid forms. The outline of girdle views was simple, tend to appear square or rectangular in shape, rarely cuneate and shows distinct distinguishing features. The ornamentations were confined only to the valve position of the silica wall. These unique morphological patterning consists of rows of pores arranged in a definite pattern which are quite distinctive to individual species. Though these natural organisms seem to be quite simple, yet they have an array of sophisticated structures which can be visualized using various imaging techniques and thus forms a base of identification.

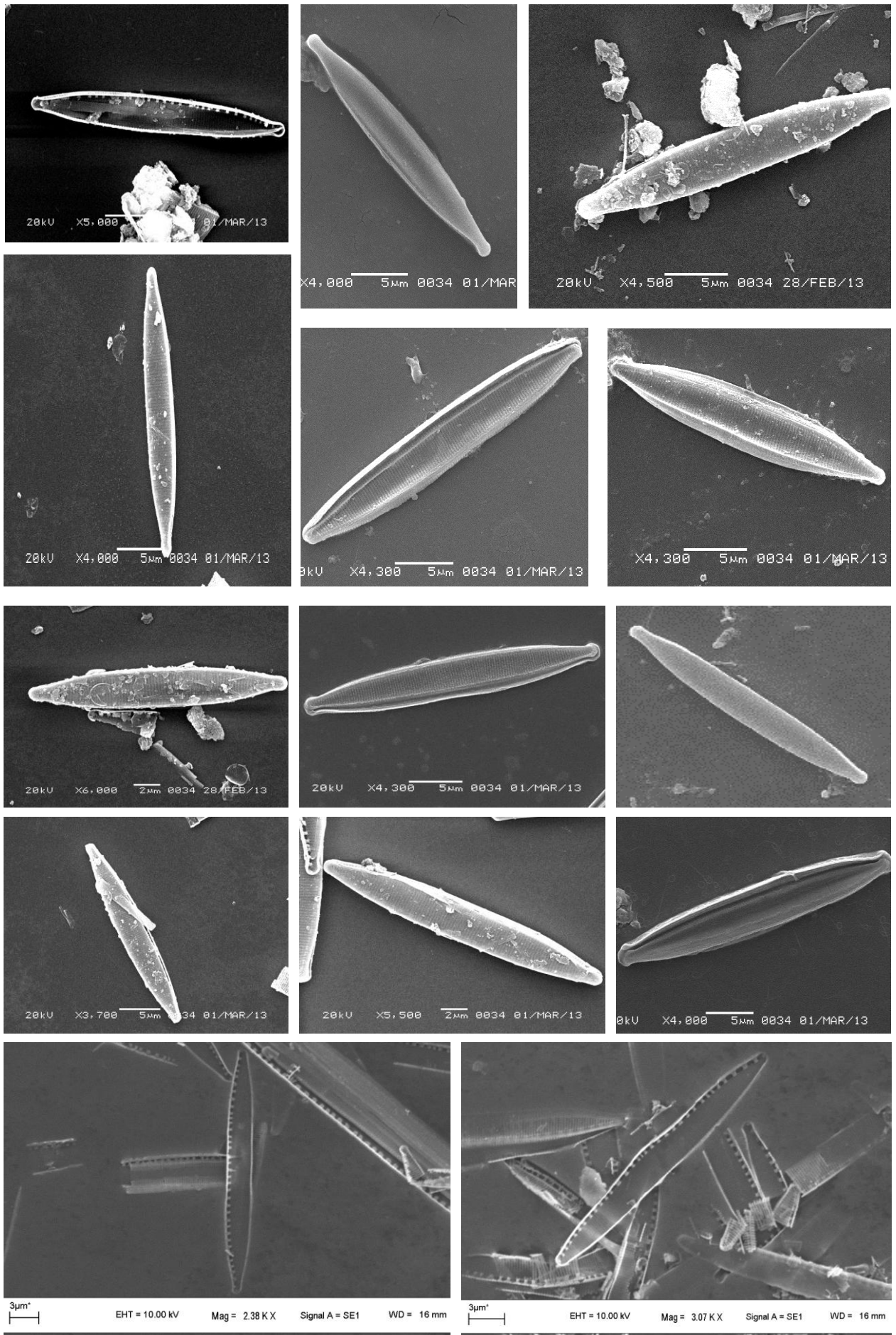


Fig. 1. SEM of *S. ...*

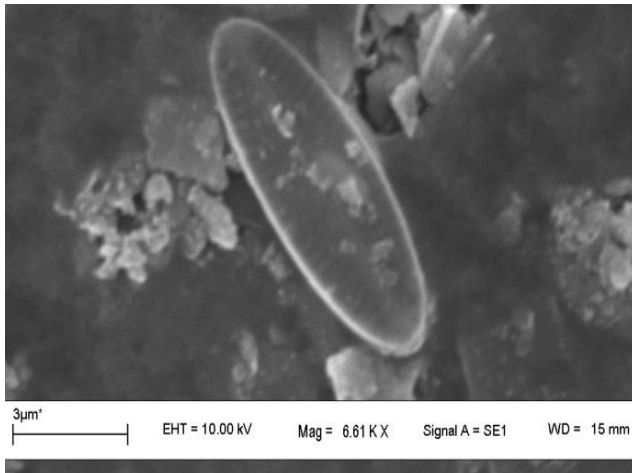


Fig 2: SEM image of *Achnanthes*

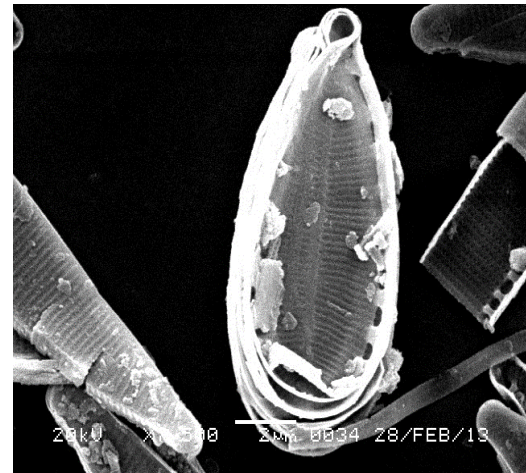


Fig 3: SEM image of *Surirella*

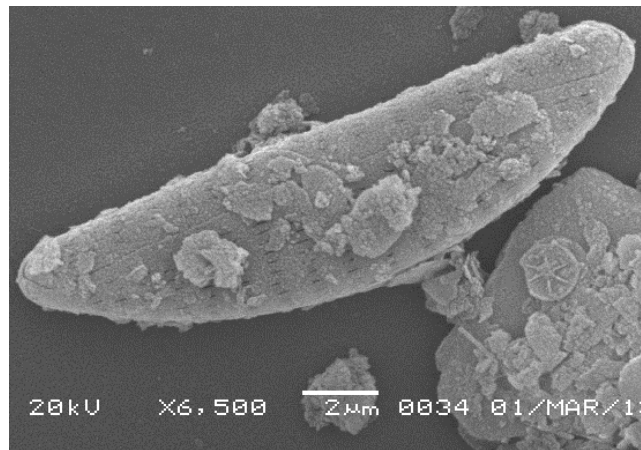
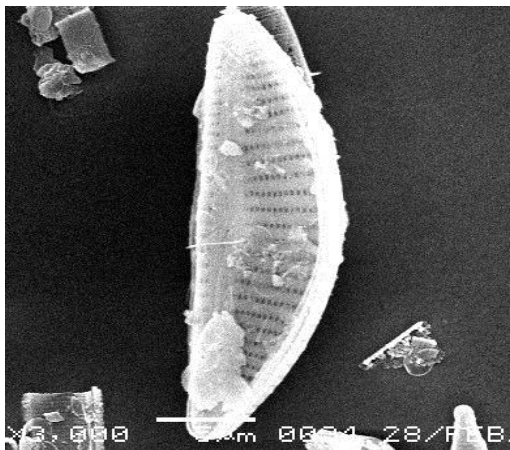


Fig 4: SEM images of *Encyonema*

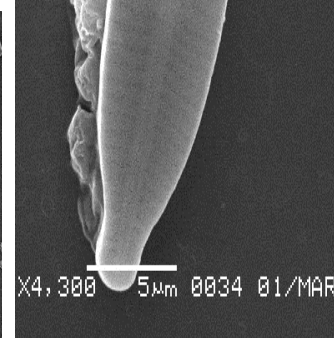
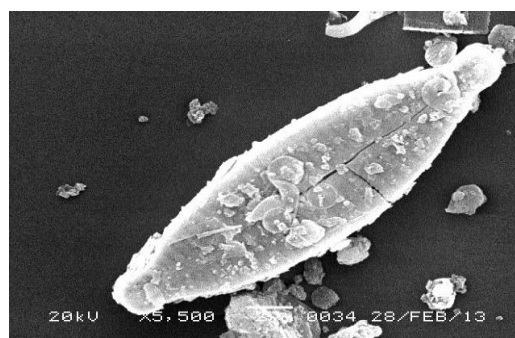
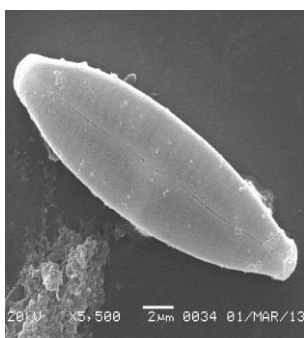
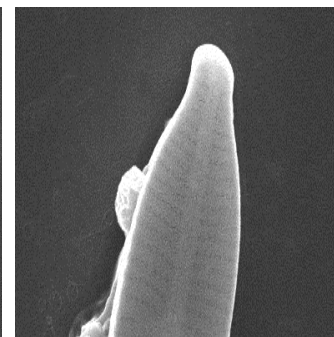
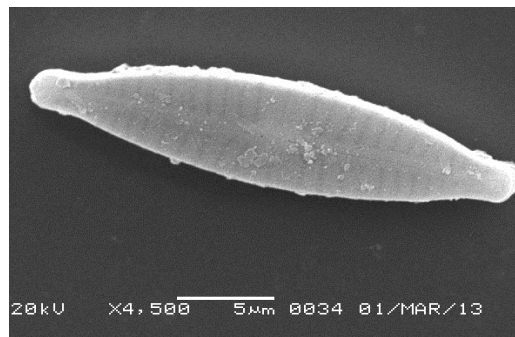
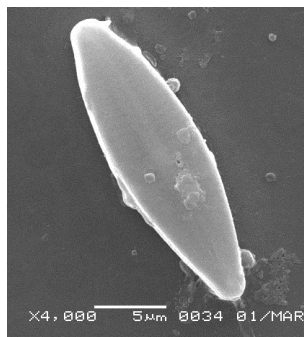


Fig 5: SEM images of *G. sp.*

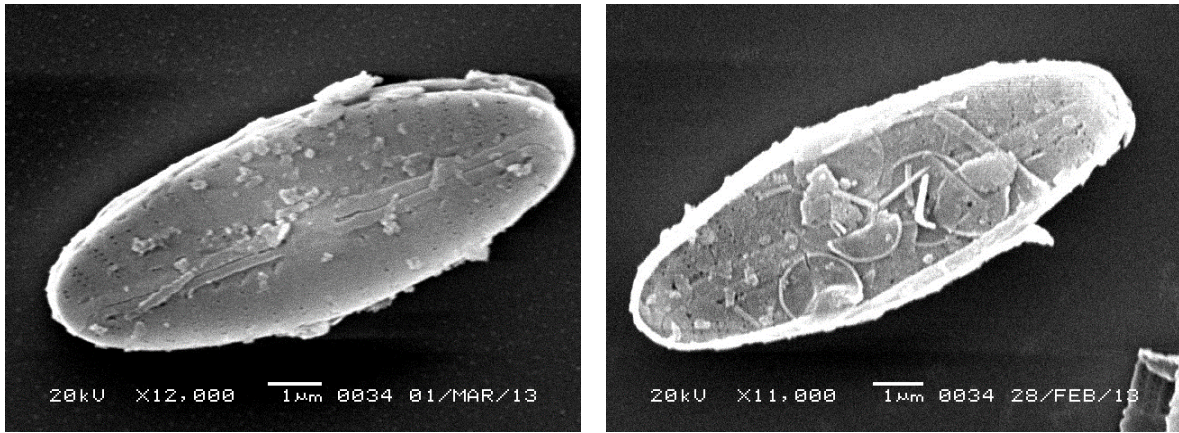


Fig 6: SEM images of *Psammothidium*

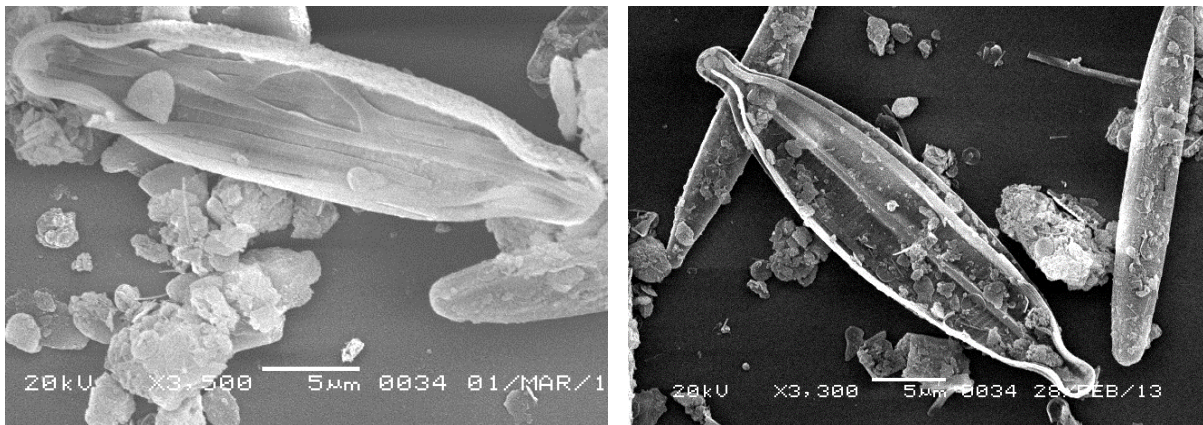


Fig 7: SEM images of *Kobayasiella*

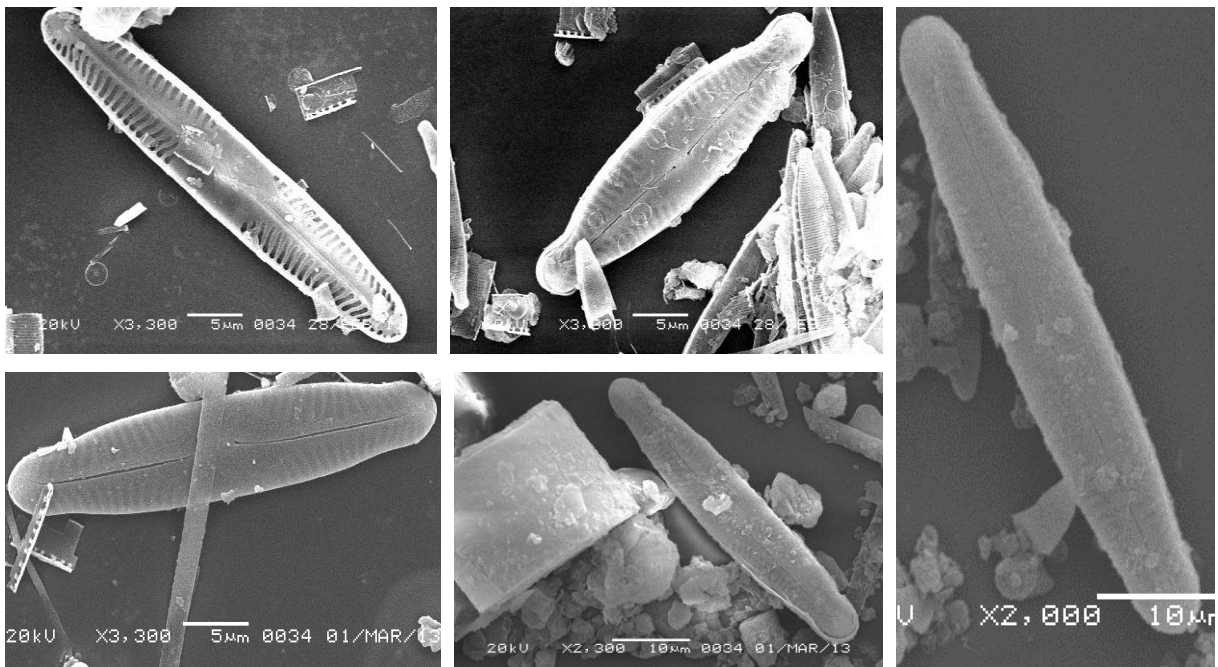


Fig 8: SEM images of *Pinnularia*

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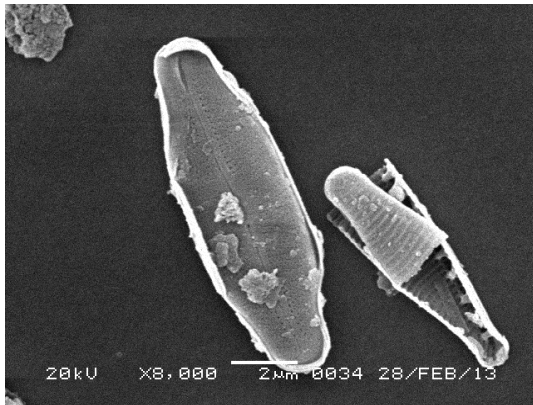
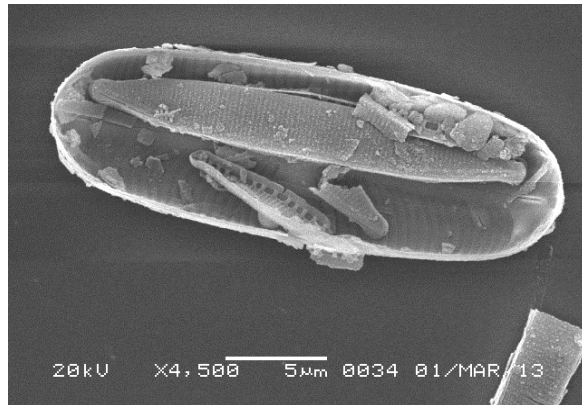
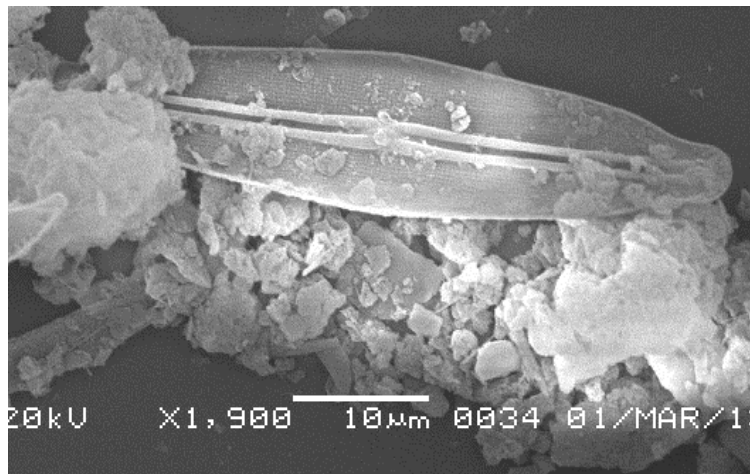
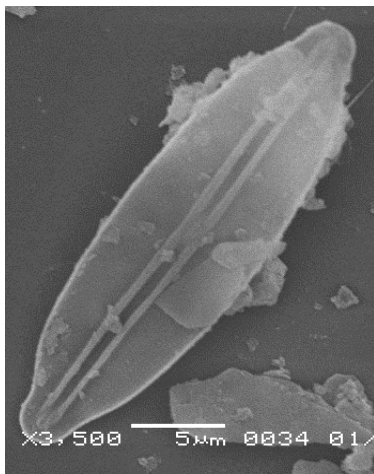
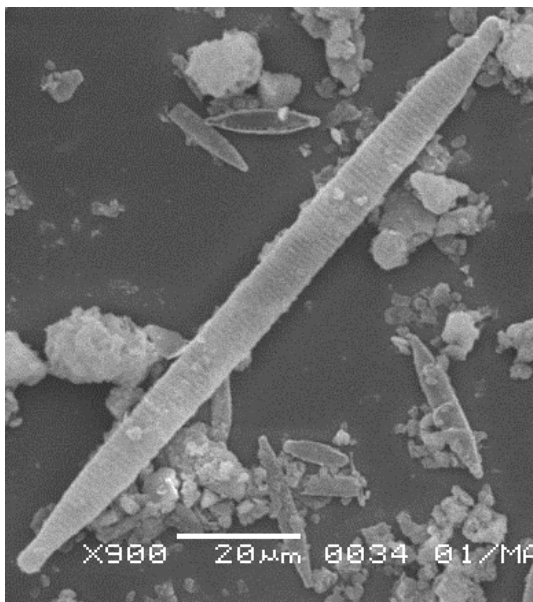
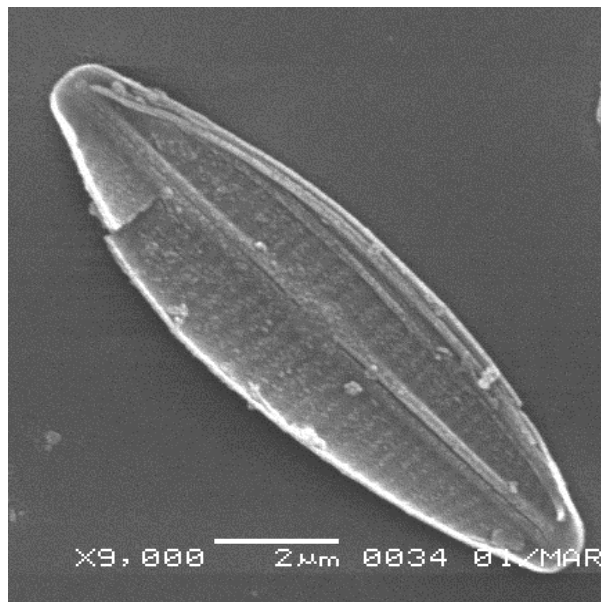
Fig 9: SEM image of *Luticola*Fig 10: SEM image of *Navicula*Fig 11: SEM images of *Frustulia*

Fig 12: SEM image of

Fig 13: SEM image of *Geissleria*

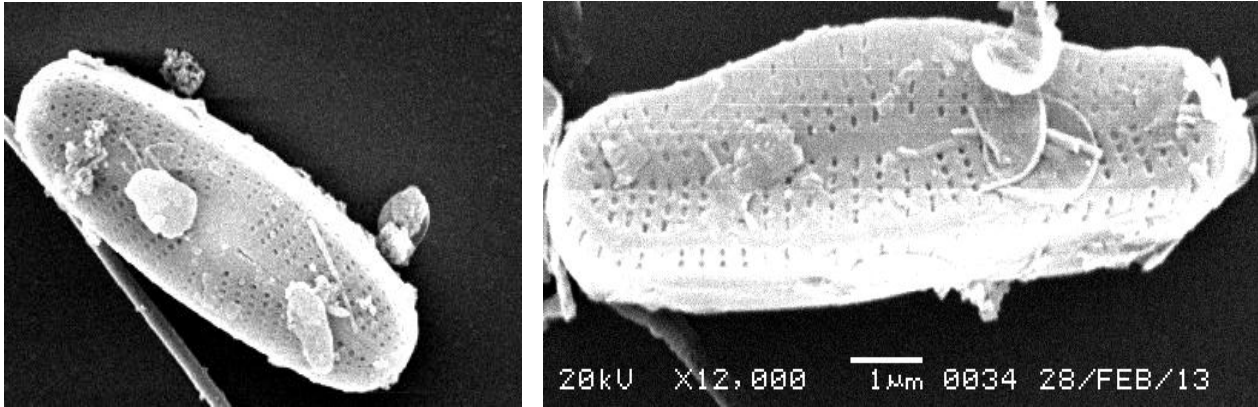


Fig 14: SEM images of *Achnantheidium*

### Conclusion

Diatoms are precisely engineered materials of nature with extensive variety in their morphology, shape, size and structure as observed through imaging techniques. The usage of scanning electron microscopy provided a panoramic, non-corrosive visualization of their biological framework. The present work is an attempt to provide a platform for researchers working in this field to serve as a guide and help identify diatoms through SEM micrographs.

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