International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979; Impact Factor: RJIF 5.72
Received: 17-01-2021; Accepted: 09-02-2021; Published: 08-03-2021
www.allsubjectjournal.com
Volume 8; Issue 3; 2021; Page No. 25-33

Fresh water diatom (Bacillariophyceae) diversity in the Willingdan lake Cuddalore district of Tamil Nadu in India

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Abstract
The diversity of diatoms in the river has been investigated for a period of one years ( ) collection of algal samples from willingdon lake surface water and algal samples were collected in velar river The present paper deals with 18 taxa of freshwater diatoms collected from Willingdon lake cuddalore district of Tamil Nadu (Lat. 11° 25' 32.91" N and Long. 79° 6' 12. 89" E) light microscope analysis *Actinocyclus normanii, Tabellaria flocculosa, Synedra famelica, Navicula schroeteri var. Escambia, Synedra acus, Synedra ulna, Navicula pupula, Navicula nunivakiana, Navicula radiosa, Navicula reinhardtii, Frustulia vulgaris, Pinnularia joculata, Pinnularia brevicostata, Encyonema hustedtii Nitzschia palea, Caloneis bacillum, Stauroneis anceps, Diatoma vulgare. And* Scanning electron microscopic (SEM) Studies Discostella asterocostata, Discostella stelligera, Surirella robusta, Navicula symmetrica, Gomphonema biceps, Discostella stelligera, Cyclotella meneghiniana, all the taxa were recorded for the first time from this lake

Keywords: bacillariophyceae, freshwater, Willigdon Lake, light microscope, scanning electron microscopic

Introduction

The bacillariophyceae are unicellular algae characterized by having a cell wall of silica. The wall consists of two vales that have more or less flat surfaces, held together by a band or girdle. The are found in freshwater and marine habitats, and also on moits soil surface. They are only few published recoreds on the systematic account of freshwater diatom flora of the Indian sub-continent.

Taxonomy of Indian Fresh-water diatoms from a literature survey on freshwater diatoms it is seen that algal studies are put to a number of different purposes like taxonomy, diversity, pollution etc., Purely taxonomic works with proper descriptions and taxonomic positions are limited. Many deal with lists of diatoms from a particular region. Various indices like the Margalef index, Nygaard's trophic status index, Shannon species diversity inden, Pilelou's evenness index, Species richness, Palmer's pollution tolerant index etc., have been used by different workers. Studies pertaining to the freshwater diatom flora of different parts of India are not regular. Ehrenberg (1851, 54) was the first to give the floristic account of diatoms some parts of India and Nicobar Islands. West & West (1902, 1907) described for the first time 59 freshwater diatoms from the Punjab & Kashmir regions well as from Bengal and Madras. Carter (1926) added 49 species. Abdul Majeed (1935) gave an account of 62 diatoms from the undivided Punjab. Skvortzow (1935) listed the diatoms from Calcutta, Chennai region (Composite Madras state):- Venkatraman (1939) [35] gave the first systematic account of diatoms in his paper "A systematic account of some south Indian diatoms". He had described in all 98 taza in 32 genera with 6 new varieties and 6 new forms collected from Madras (Chennai), OOty, Kodaikanal Hills, Vandalur in Chingleput district. Krishnamurthy (1954) recorded 58 species of which 2 species, 4 varieties and 4 forms were new reports. His work did not contain those already published by Venkatraman. Suzena studied the Kodaikanal diatoms besides other algae and reported 46 forms in “Bibliotheca Phycologica" (1983). Gajarat-Rajasthan-Bombay, Mysore (Karnataka) regions: Gonzalves and Gandhi (1952, 53, 454) listed the diatoms from Bombay and Saisette Islands. H.P. Gandhi (1955-1998) had published papers on the freshwater diatom flora of different parts of South India like Sagar, Jog Falls, Hirebhaskar Dam Area, Kolhapur in Karnataka state, Vasna Village near Ahmedabad Baroda -Ahmedabad regions of Gujarat. He had written a book on “Fresh water diatoms of Central Gujarat with a review and some others"(1998). He had reported the occurrence of diatoms from Bombay and Salsette islands and was the first to study the freshwater diatoms from Rajasthan. He had recorded many new forms and species from all these places. His publications are full of neatly hand-drawn diagrams with scale. Rashmi Pareek et al., (2011) investigated freshwater diatoms from Galta Kund in Jaipur and reported the occurrence of 24 taxa of diatoms. These diatoms appear to be commonly present in many of the freshwater collections from South India as well. Uttar Pradesh region: - Singh (1961-1963) studied the diatom flora from 3 ponds situated at Banarus Hindu University campus and another permanent pond at Deoria district of Uttar Pradesh as well as from the rivers Ganges & Barna at Varanasi, Uttar Pradesh. Pratap Singh et al., (1978) [26] have worked on the Freshwater diatom flora of Kankawati river in Gujarat. They have recorded 8 genera and 9 species. Vidharba region: - From Vidharba, Sarode and Kamat recorded 119 species of diatoms. (1983) Andhra-Pradesh region: -Venkateswarlu (2006) gave a special lecture on algae of aquatic environments. He has described and classified the freshwaters into two basic types flowing (lentic) and nonflowing (lotic). Benthic attached forms of diatoms predominate in lentic waters and planktonic floating diatoms and other unicellular forms of algae in from the Moosi River lotic waters. Venkateswarlu recorded

Hyderabad 78 species of diatoms (1983). At Pakistan: - Syed Tariq-Ali et al., (2006) made a taxonomic study of 10 Cymbella species from Punjab and Azad Kashmir. Syed Tariq-Ali et al., (2006) identified 9 species of Nitszchia from Kasur and Lahore districts of Pakistan. Orissa region: - Jena et al., (2006) [17] reported the freshwater diatom flora from Orissa state and neighbouring regions. They reported the presence of 78 diatom taxa belonging to 26 genera. Of these nearly 22 taxa of diatoms are new reports. Jena et al., (2008) studied the Algal diversity in Rushikullya River, Orissa. They reported on the occurrence of 42 taxa, 29 genera of algae; 10genera and 11 species of diatoms reported. Diadesmis confervacea Kutzing. Is a new name. Maharashtra region:- Dhande &Gunale (2008): studied two genera namely Fragilaria and Synedra from Artak Lake, Majharashtra. Sudhir et al., (2009) reported 58 taxa of freshwater diatoms in 17 genera without figures and descriptions from Aurangabad in Maharashtra. More recently, Andhale, S.B,, S.S. Harane and G.B. Kohle (2012) reported the presence of freshwater diatoms from Jayakwadi Bird Sanctuary of the Godhavari river at Pathan, Maharashtra. Common genera such as Gomphonema, Fragilaria, Synedra, Achnanthes, Stauroneis, Neidium, Mastogloia, Licmophora, Nitszchia and Surirella are reported by them. Thisparticular reference shows diagrams and descriptions of species. Himalayan Region: - P.K. Mishra, et al., (2009): Reported on the freshwater diatoms from Terai mountains and Himalaya regions of Eastern Nepal. They recorded about 36 taxa of diatoms in 9 genera with 20 new reports'. Subha et al., (2009) reported on some freshwater diatoms from the Trans-Himalaya region at Mustang numbering 19 taxa with beautiful colour photographs but without description. Central high land region: - Jyoti Verma and Praksh Nautiyal (2010) [36] have studied the floristic composition of the epilithic diatoms belonging to Thallassiosiraceae, Fragilariaceae, Eunotiaceae and Achnanthaceae. of Central Highland region: They have recorded a total of 293 species, varieties and forms of which only 3 species are from the centric. A total of 47 genera and 290 species belong to the Pennate groups. They were concerned more with percentage composition of each group. No descriptions of species were given. Calcutta region: - Bhattacharya et al., (2011): Reported on the Freshwater diatoms from Kolkata with special reference to their taxonomy. A total of 15 genera and 61speices were recorded by them. There is a new report of Amphiprora sp., in freshwater. Tamilnadu region: - Sridharan (former Head, Dept. of Botany, National College, Trichy) has been working on the freshwater algae of different parts of South India (1986-2012) Karthick & al. (2009, 2010, 2013), like Trichy City -Karumandapam, Pirattiyur, surrounding areas of Bharathidasan University (Suriyur Campus); Pudukkottai district. Pudukkottai City, Keeranur, Narthamalai, Kudumiyanmalai; Karaikudi, Kundrakudi, Pillaiyarpatti and surroundings; Dharmapuri district -Kaveri Falls at Hogenakkal; Pollachi District- Parambikulam areas; Namakkal district- Kolli Hills; Dindugal District- Udumalpet, Thirumoorthy Hills, Perambalur District - Ariyalur, Nakkambadi village etc., He has studied several new reports for the different collection localities. Similarly, there are many reports of freshwater algae and diatoms from different parts of India published all these years. Several papers are without figures or photographs of diatoms as well as without descriptions of species. Hence it is difficult to consider them as purely taxonomic.

Materials and Methods

Description of study area Willingdon Lake

willingdon Lake is a lake situated in the keelachiruvoi village tittakudi taluk of the cuddalore district in the india state of tamilnadu.it is the second-largest lake in tamilnadu after the Willingdon lake, which also is situated in the cuddalore district. The Willingdon Lake also has about 27 arcillary lakes and together They from the primary source of irrgaton for nearly 25,000 actes (10,000) hectares of land and over 67 village depondent on it for cultivation.

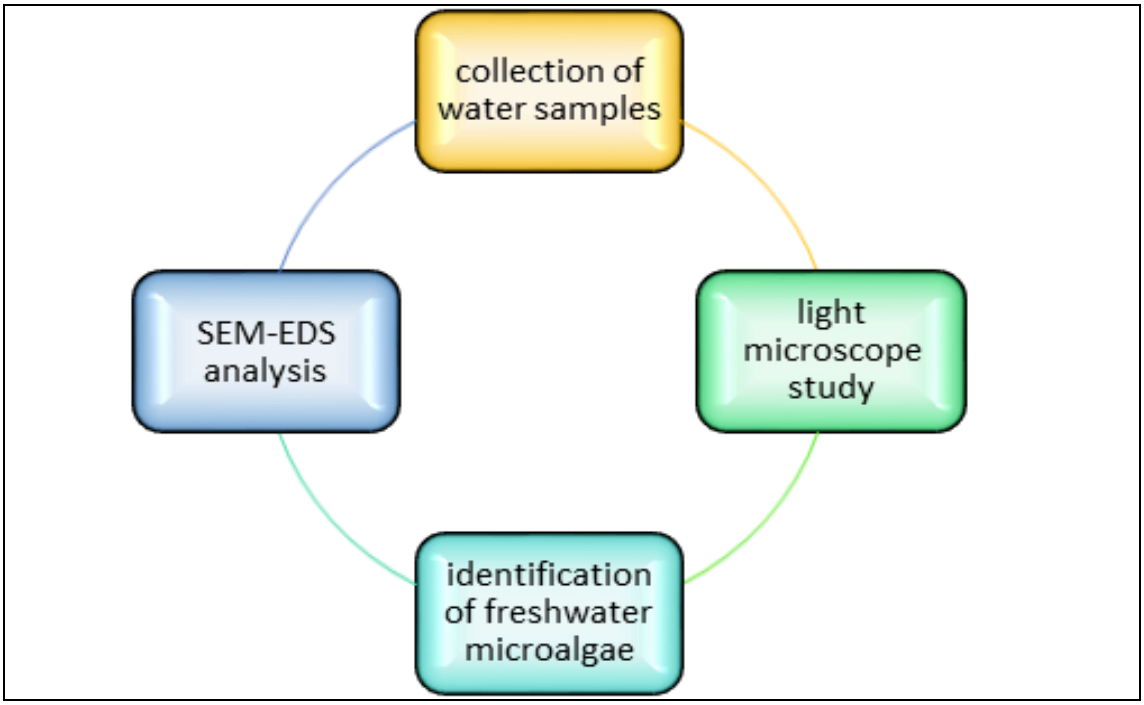

Fig 1: flow chart of Analysis

Sample collection from the study area

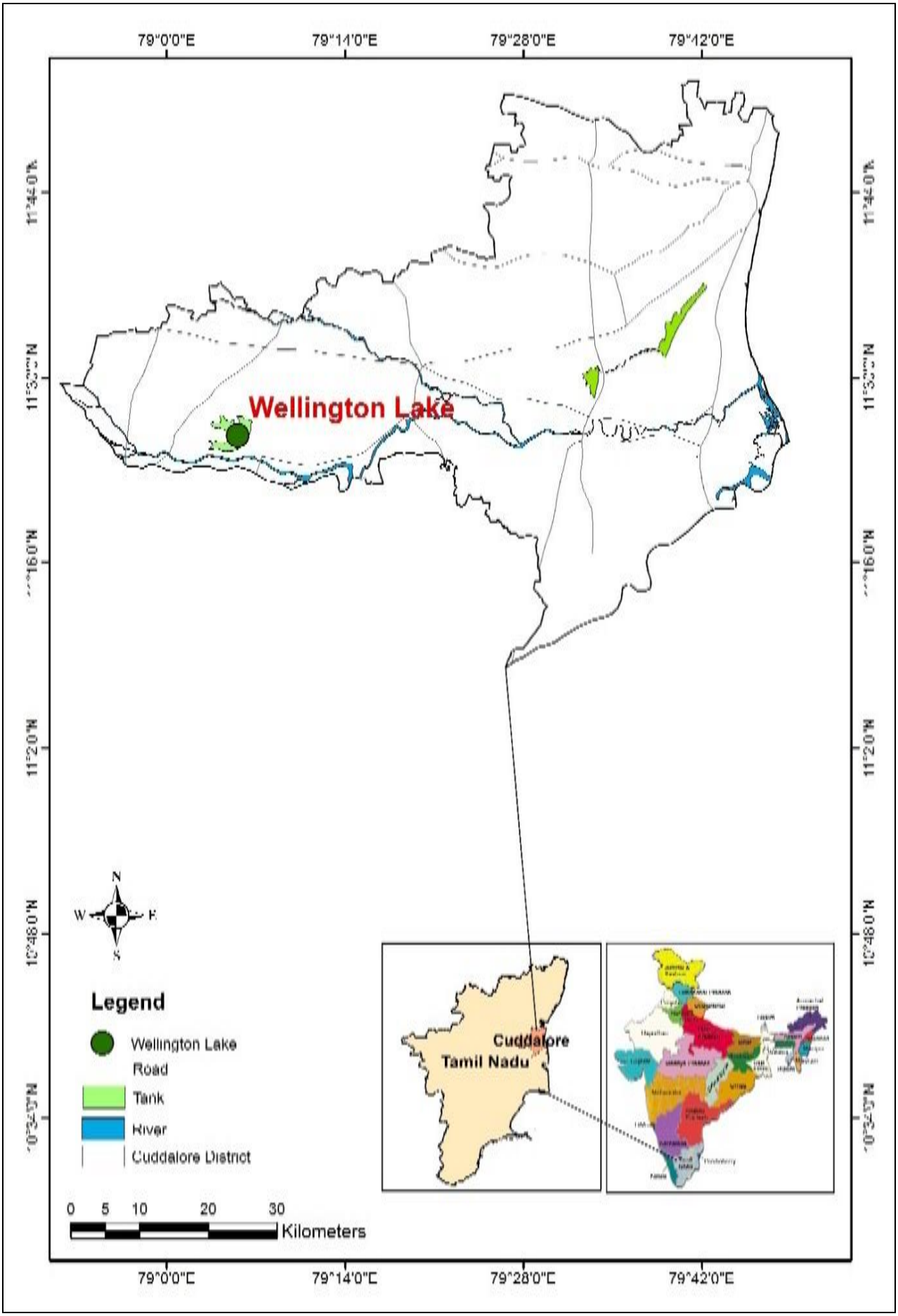

Fig 2: Map showing the sampling station of the Willingdon lake Cuddalore district (Tamil Nadu).

Surface water samples were collected from Willingdon Lake in the second week of every month during October 2018 to September 2019. A liter of water sample was collected every month separately for the qualitative and quantitative estimation of phytoplankton and studies of physical and chemical properties were also made.

Sample preparation for light microscopic studies of phytoplankton

Lugol's solution

This was prepared by dissolving 10 gm of iodine and 20 gm of potassium iodine in 200 mL of water. To this was added 20 mL of glacial acetic acid. Lugol's iodine solution prepared in this method was stored in a darkened bottle; it was mixed with plankton samples to yield a final concentration of 1% to preserve the sample better. Lugol's iodine fixed samples were mixed with equal amount of formalin glycerine preservative.

Formalin-acetic acid-Alcohol mixtures: Rawlin's formula (F.A.A.)

Ethyl alcohol 95%: 50 mL
Glacial acetic acid: 5 mL
Formalin: 10 mL
Water: 35 mL
Recommended for algal materials for histological studies.

Diatom Cleaning

5-15 ml each sample containing freshwater diatoms was taken in a centrifuge tube, concentrated and given distilled water wash twice by centrifuging and decanting the supernatant solutions in a laboratory centrifuge at 1500 rpm. To the pellet in the centrifuge tube, 5-10 ml of 100 volumes of hydrogen peroxide solution was added and kept in a hot-air oven set at 60° C for 4-8 hours. The material is allowed to cool to room temperature and washed with distilled water by a similar method 5-6 times to remove every trace of hydrogen peroxide. Now the sample is said ti be cleaned or free from organic matter leaving only the silica-shell of diatom frustules that only are useful in diatom identification. Finally, the cleaned sample were stored in 70% alcohol in suitable airtight containers.

Freshwater algae studies

Freshwater microalgae samples were collected towing a plankton net (mouth diameter 0.35 M) made up of bolting silk (No. 30 mesh size 48 □m) for half an hour. The samples were in black polythene bags and immediately preserved with 4% formalin for quantitative analysis. Photomicrographs were taken selectively from computer screen fresh water algae was identified by referring to the standard keys of Desikachary (1959), Prescott (1964), Bongale and Bharathi (1980), Gonzalves (1981), Cox (1996), Anand (1998) [1] and Siva kumar and Senthil kumar (2008).

Scanning electron microscope with Energy Dispersive Spectroscopic analysis (EDS)

Phytoplankton samples were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8. Specimens were dehydrated through a graded series of alcohol at 12-15 minutes interval at 4°C up to 70% of alcohol. Then dehydrated phytoplankton sample was treated with critical point drier (CPD) and fixed on a stub and the specimens were coated to examine in Joel JSM-56010 LV (INSA-EDS). Electron micrographs was taken selectively from the computer screen. Simultaneously selected portions of micrograph was subjected to Energy Dispersive Spectroscopic analysis (EDS). This was conducted with an EDS 700 series interfaced with a data general $NOVA_2$ computer and a Texas instrument silent 700 ASR. The EDS X-ray spectrometer was interfaced with a scanning electron microscope (20 kV) stage. The area of different components such as cell wall and cellular inclusion was analysed. To find out the fluxes of particular mineral, both the counts per second (S^{-1} or CPS) value and the apparent relative atomic percentage of weight in different components of the cell wall and cellular inclusion details were documented.

Results and Discussion

1. ***Actinocyclus normanii*** (Gregory) Hustedt 1957 (**Plate:1; Fig.1)**

Centric in outline with a relatively shallow mantle. Diameter 68 m, marginal tubules visible, hexagonal aeriolae are equally spaced in rows of variable length.

2. ***Tabellaria flocculosa*** **(Roth) Kützing 1844 (Plate:1; Fig.2)**

Valves are linear with inflation in the center of the valve and capitate to slightly capitate apices. The medial inflation of the valve is wider than the apices. Striae are usually parallel but can be slightly radiate in the medial inflation. The st riae are alternate. The axial area is narrow and linear. The central area is small and rhombic. Short marginal spines are present.

3. ***Synedra famelica*** **Kützing 1844(Plate: 1; Fig.3)**

Valves are narrow and linear, not swollen in the middle, with gradually tapered and rounded apices; ranges from 18-65µm in length and 2.4-3.1 µm in width. Axial area is straight and very narrow. Central area is ovoid, often offset to one side of the valve and with ghost striae. Short striae are typically found at one or both sides of the central area; 18-20 in 10 µm. Striae are parallel throughout.

4. ***Navicula schroeteri var. escambia*** **Patrick 1959 (Plate: 1; Fig.4)**

Valves are linear-elliptical with rounded apices, length 28.1-48.6 m, width 6.3-9.1 m with 12-13 striae per 10 m. Axial area is narrow, linear and distinct; a distinct central nodule is present. The central area is asymmetrically rounded, wider on the secondary side of the valve, the raphe is filiform with enlarged proximal raphe ends deflected towards the primary side of the valve, and distal fissures are distinctly hooked towards the primary side extending from the valve face onto the mantle. Striae are radiate.

5. ***Synedra acus*** **Kützing, 1844 (Plate: 1; Fig.5)**

Valves are narrow and linear ranges from 90-130 µm in length and 3-5µm in width; narrowly lanceolate in outline, with a central area that is longer than broad;valves tapering to rounded or sub-capitate apices. Distinct rectangular central area, reaching to the valve margin, a little longer than broad.

6. ***Synedra ulna*** **(Nitzsch) Ehrenberg 1832 (Plate: 1; Fig.6)**

Cells robust; linear or sometimes linear-lanceolate valves

narrowing toblunt sub-rostrate or rostrate apices; ranges from 12–250 µm in length and 5-6µm in length.Central area is distinct, roughly square in outline and usually reaching the valve margin, striae visible within the central area.

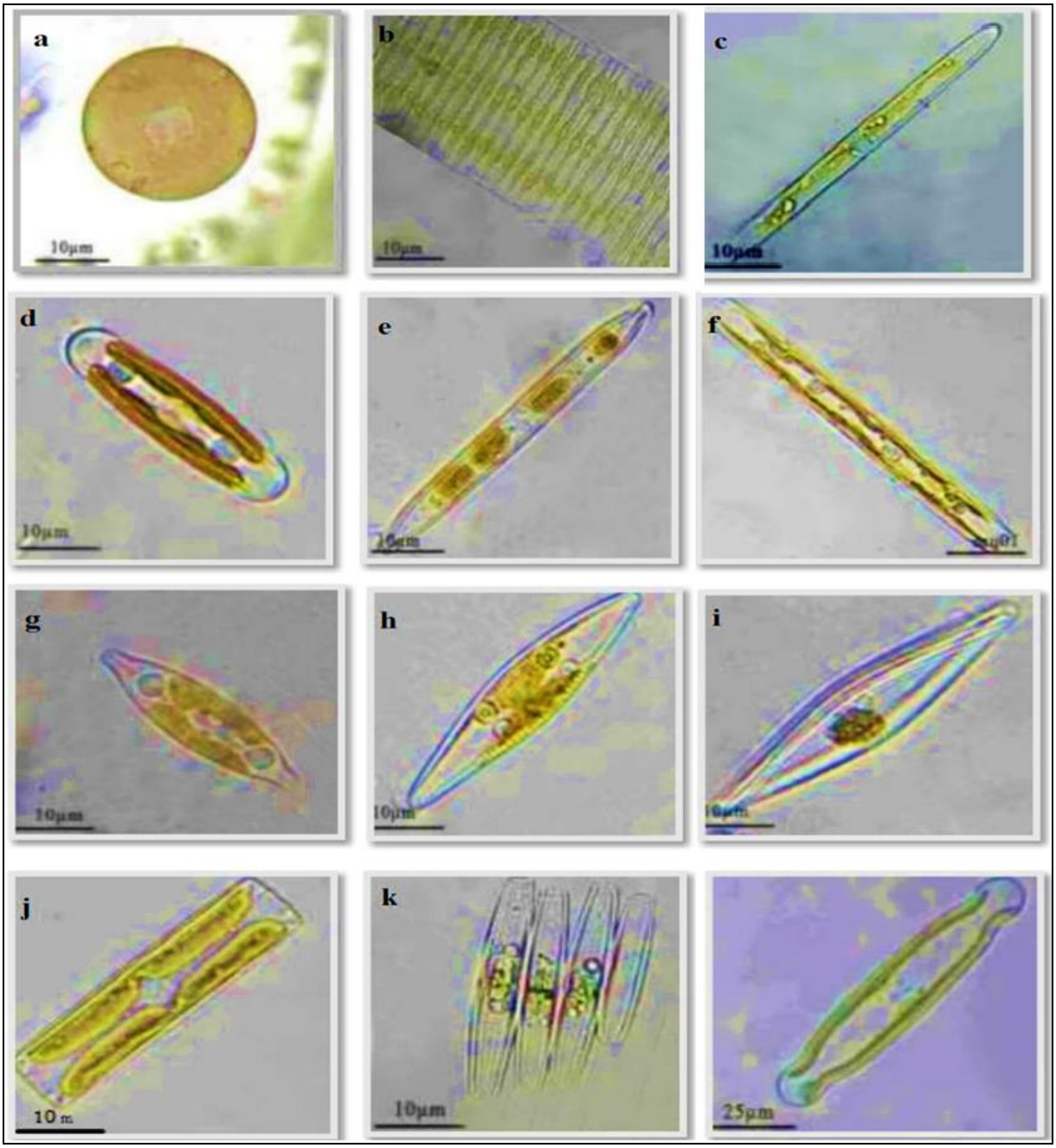

Fig 3: Light Microscope Image

7. *Navicula pupula* Kützing 1844 (Plate: 1; Fig.7)

Valves are linear-lanceolate with convex margins and constricted to produce round capitate ends. Raphe is mostly thin, straight. Central nodule is distinct. Axial area is narrow and central area is elliptical. Striae radiate; 8m broad, 28.8 m long, striae 22 in 10 m.

8. *Navicula nunivakiana* Patrick and Freese 1961 (Plate: 1; Fig.8)

Valves are lanceolate with rounded central margins and apices, 84–96m long and 23–27 m broad. Striae are radiate throughout the valve. The central area is asymmetrically rectangular and the axial area is broad and straight. Striae on one or both sides of the central area vary in length and are irregularly spaced. The striae are formed between internal costae giving a light interstriae and darker striae appearance

9. *Navicula radiosa* Kützing 1844 (Plate: 1; Fig.9)

The valves are narrow and lanceolate, with acutely rounded ends. The axial area is narrow and linear. The central area is rhombic. The raphe is straight with proximal ends that are hooked to the secondary side. The transapical striae are strongly radiate, bent in the valve center and convergent near the poles.

10. *Navicula reinhardtii* (Grunow) Grunow 1880 (Plate: 1; Fig.10)

Valves are elliptical with broadly rounded ends. 34-48 m long and 14-18 m broad.The axial area is narrow and the central area is transverse, formed by alternating short and long striae. Proximal raphe ends are straight, small and bulbous. The striae are wide and radiate, becoming nearly parallel at the ends.

11. *Frustulia vulgaris* (Thwaites) De Toni 1891(Plate: 1; Fig.11)

Cell body 51.7-57.5 µm long, 11-12.5 µm broad, the ribs is slightly
Constricted at the central nodule. The longitudinal striae are wavy, but not prominent.

12. *Pinnularia joculata* (Manguin) Krammer, 2000 (Plate: 1; Fig.12)

Valves 70-120 m long, 10-65 m broad,linear with very slightly inflated margins in the middle part with capitate ends; raphe thin and straight with unilaterally bent central pores and curved terminal fissures; axial area wide, central area large, reaching the margins; striae 7- 8 in 10 m, slightly radial in the middle and convergent at the ends.

13. *Pinnularia brevicostata* Cleve. 1891 (Plate: 1; Fig.13)

Cells 83-92m long and 13-15m broad; striae 8-9 in 10m.

14. *Encyonema hustedtii* Krammer 1997(Plate: 1; Fig.14)

Valves are strongly dorsi-ventral. The dorsal margin is broadly arched, while the ventral margin is only slightly expanded, valves 50-84 µm long; 22-27 µm wide. The valve apices are rounded. The axial area is relatively broad in the central part, narrowing at the valve ends.

15. *Nitzschia palea* (Küt.) W.Smith 1856 (Plate: 1; Fig.15)

Frustule is distinctly sigmoid. Walls parallel to the apices are small and capitate. The keel of the raphe is strongly excentric. Fibula density is 7-12 in 10 m. Valves lack an expanded central gap in the fibulae.

16. *Caloneis bacillum* (Grunow) Cleve 1894(Plate: 1; Fig.16)

Valves linear to linear-lanceolate. Axial area linear, expanded at the central valve to form a broad transverse fascia. The fascia is typically asymmetric

17. *Stauroneis anceps* Ehrenberg 1843 (Plate: 1; Fig.17)

Valves are moderately large lanceolate to linear-lanceolate, 12-15 m wide. Apices are distinctly protracted, narrow and rostrate or relatively narrow and rectangular; proximal raphe ends are weakly expanded and deflected.

18. *Diatoma vulgare* Grunow in Van Heurck, 1881 (Plate: 1; Fig.18)

Frustules are rectangular tapering towards the ends in girdle view. Valves are elliptical to elliptical-lanceolate with broadly rounded subrostrate ends, 8-10 m wide, and 20-46 m long. Transapical ribs 8-12 in 10 m.

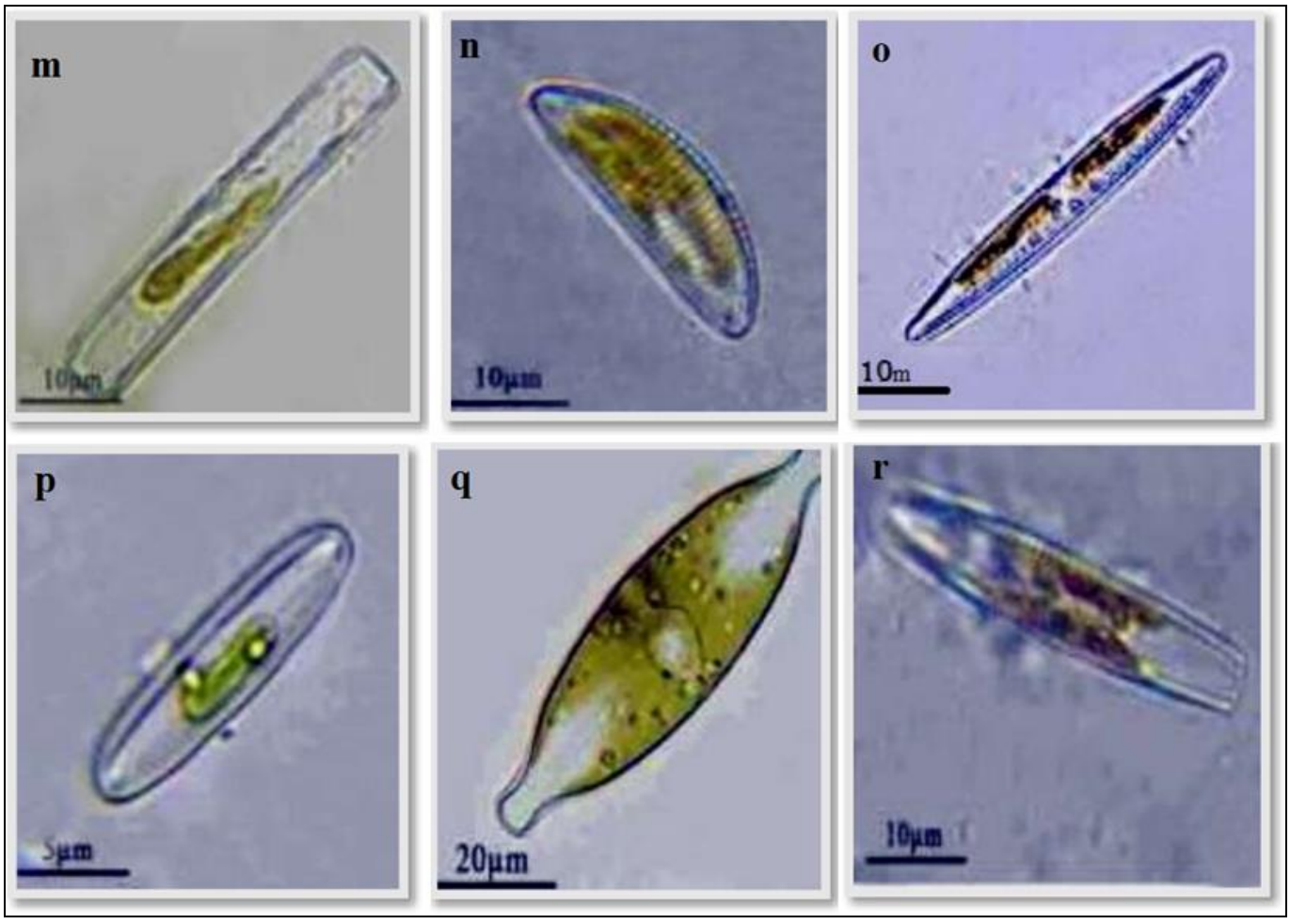

Fig 4: light microscope image

Scanning Electron Microscope Analysis

A. Discostella asterocostata (Lin, Xie & Cai) Houk & Klee 2004

External view of vale face shows concave central area and radiality arranged atriae. Note the short tubule morphology of the external opening of fultoportulae.

Single discoid cell usually seen in valve view. Valves with a concentric undulation, 12-20 µm in diameter. Concave central area occupied by radially arranged striae surrounding a central pore, which may sometimes be lacking. Central and marginal area separated by a rougher hyaline ring. Marginal striae, 12-16 in 10 µm, with two rows of areolae. Fultoportulae locted at the valve face/mantle junction, between every 2-3 costae; the external opening of the fultoportulae shows short tubules with branched rather Then a domed pore. One rimoportila with projection located between striae.

B. Discostella stelligera (Cleve & Grunow in Van Heurck &klee

External view of valve shows a convex area, central and marginal striae and external opening of the fultoportulae

Single, cylindrical cell strongly silicified, large form (> 10 µm diameter) usually undulate. Valves with a concentric undulation, but occasionally flat. Valve edge without shadow lines. Centre of the valve with a distinct star-shaped structure and as central areola. Marginal spines and valve face fultoportulae absent, and 9-13 distinctly alveolate marginal striae in 10 µm. valve circular measuring 3-31 µm in diameter, mantle depth <1 µm, striae resolvable, density 7-16 in 10µm.

C. **Surirella robusta Ehrenberg 1841**

Internal vive of the valve shows the alar canals, subdivided into canaliculi, portulae occur in between them, portulae subdivided of second order

Frustules in gridle view slightly wedge- shaped and linear. Valves linearly elliptical, with broadly rounded heteropolar apices. Valves 146-291 µm in length and 56-94 µm in breadth. Costae broad, parallel centrally to radiate apically, 1-1.5 in 10 µm. striae of similar orientation, finely punctate, 22-27 in 10 µm. pseudoraphe broad, not the observable groove-like nature of the longitudinal axis.

D. Navicula symmetrica Patrick 1944

Valve view shows proximal raphe ends with central pores, both deflected opposite to the hooked distal raphe; central area larger, asymmetric and linear axial area. Stria looks like apically elongated ridges due to apically widen areolae and relatively small costae.

Valves linearly elliptical to linearly lanceolate with narrow rounded ends. Valves 30-47 µm in length 5-8 µm in breadth. Raphe filiform, proximal raphe ends with small rounded central pores and weakly unilaterally deflected. The distal raphe ends distinctly deflected from the valve face onto the mantle, with the deflection to the second. A thickened central nodule present on the primary side, positioned between the deflected proximal raphe ends. Striae strongly radiate at the center and radiate throughout the valve. The space between each stria equal pr wider to the width of the stria. Striae radiate throughout, 14-17 in 10 µm. areolae lineate, 23-27 in 10 µm.

E. E) Gomphonema biceps Maeister 1935

Valve view shows undulated raphe, broad centerl area with a single stigma and apical pore fields. Note the distal raphe ends curved on same direction.

Valve center enarged to show proximal raphe ends with central pores, proad central area with a round stigma. Note redial striae with 'C' and 'I' shaped areolae. Valves eliptic or rhombic-lanceolate with rostrate or borad rounded end; valves 20-28 µm in length and 5-7 µm in breadth. Axial area linear-lanceolate, narrow at the end and widened at the center, central area large and appear rhomboidal to rectangular with a single stigma.raphe wavy, slightly lateral in position, proximal end with rounded central pores and deflected towards stigma; distal raphe ends curved in opposite direction to stigma. Striae radial, 11-13 in 10 µm.

F. Discostella stelligera (Cleve & Grunow in Van Heurck &klee

Oblique view of thvalve show a concave central area and external opening of the fltoportulae

Single, cylindrical cell strongly silicified, large form (> 10 µm diameter) usually undulate. Valves with a concentric undulation, but occasionally flat. Valve edge without shadow lines. Centre of the valve with a distinct star-shaped structure and as central areola. Marginal spines and valve face fultoportulae absent, and 9-13 distinctly alveolate marginal striae in 10 µm. valve circular measuring 3-31 µm in diameter, mantle depth <1 µm, striae resolvable, density 7-16 in 10µm

G. Cyclotella meneghiniana (Cleve & Grunow in Van Heurck 1882

Oblique view of the valves shows a concave central area and external opening of the fultoportulae (arrow)

Single, cylindrical cell strongly silicified, large form (> 10 µm diameter) usually undulate. Valves with a concentric undulation, but occasionally flat. Valve edge without shadow lines. Centre of the valve with a distinct star-shaped structure and as central areola. Marginal spines and valve face fultoportulae absent, and 9-13 distinctly alveolate marginal striae in 10 µm. Valve circular measuring 3-31 µm in diameter, mantle depth <1 µm, striae resolvable, density 7-16 in 10µm.

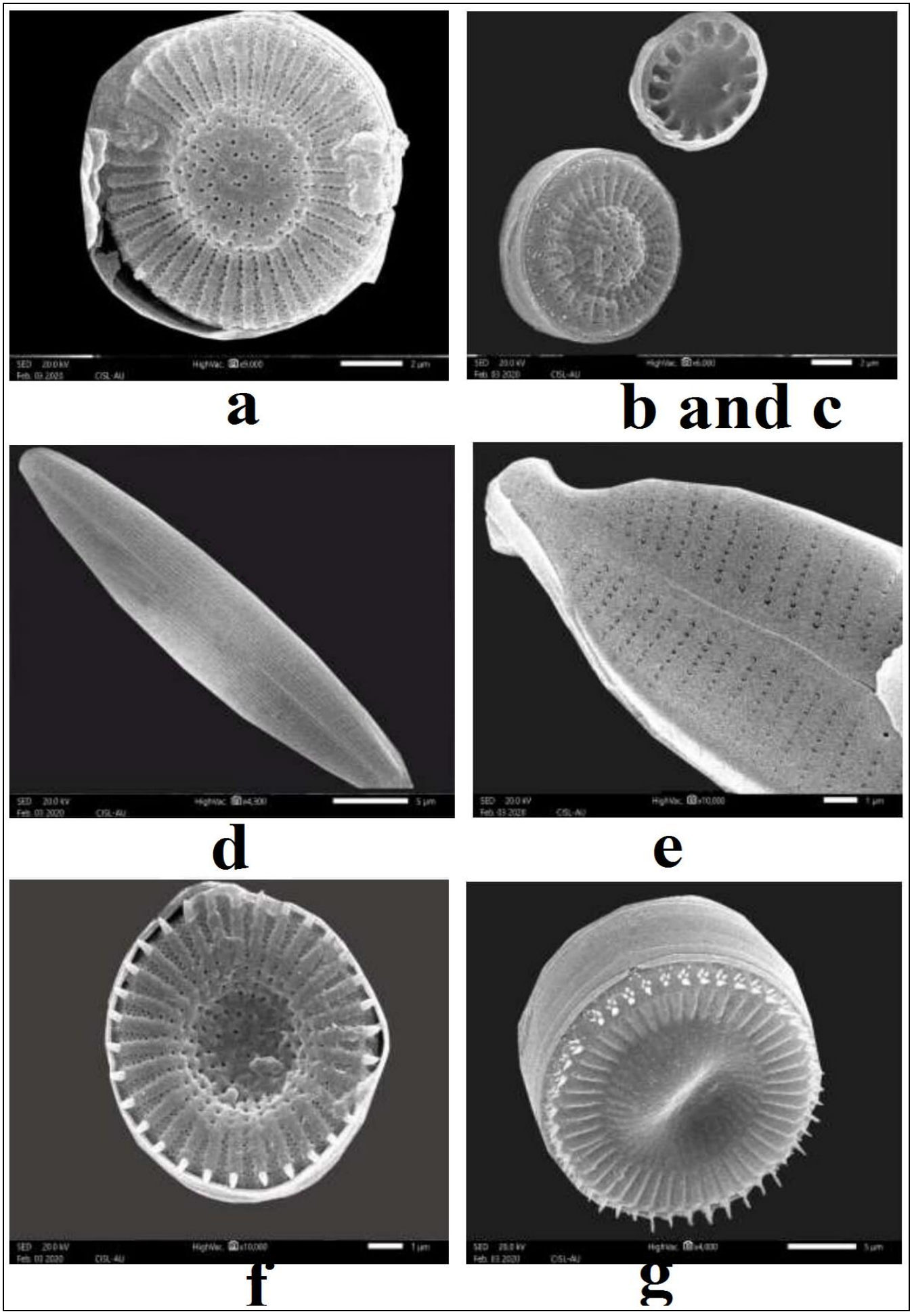

Fig 5: Some phytoplankton species as seen under electron microscope

Potentially, the river is a valuable environmental asset. Taking appropriate measures in important sensitive watershed areas will help to prevent damage to biodiversity and other natural values. Studies of diatom flora would provide additional information leading toward a better ecological approach of lotic ecosystem for human welfare. The diatom flora of the present river.

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