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Communication

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SEM STUDY OF PLANKTONIC CHLOROPHYTES FROM THE AQUATIC HABITAT OF THE INDIAN SUNDARBANS AND THEIR CONSERVATION STATUS

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Abstract: Scanning electron microscopy (SEM) is the most modern technique for plankton research. The present paper deals with the taxonomy and morphology of some rare and endangered planktonic chlorophytes in relation to scanning electron microscopy. Water samples from the distinct water body of the Sundarbans have been concentrated and examined by scanning electron microscopy. A total of 45 species, of which 17 species of Scenedesmaceae, 10 species each of Hydrodictyaceae and Desmidiaceae, five species of Chlorococcaceae, two species of Selenastraceae and only one species of Chlorellaceae were recorded from the study site. Some species were recorded as new and rare from the study area. About 18 species including nine extremely rare, seven occasional, six frequent, four sporadic and one abundant was recorded in the present study. A detailed taxonomic description with line drawings is also included in the present communication.

Keywords: Conservation, morphology, plankton, scanning electron microscopy, Sundarban, taxonomy, water samples.

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Author contribution: Both GGS and RP conceived and designed the study concept, while GGS implemented monitoring and data collection in the field. GGS lead the writing of the manuscript with significant guidance and contribution from RP.

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INTRODUCTION

Planktonic chlorophytes play an important role in the aquatic ecosystem. They act as the primary producer of the aquatic food chain. Several reports have emphasized the importance of small planktonic chlorophytes in the aquatic habitat such as pond (Anand 1998), river, and sea (Vaulot et al. 2008). They also function as the progenitor of higher plants because of their photosynthetic pigments, starch as storage reserve food and chloroplast morphology (Lewis & McCourt 2004). They possess simple structural organization and reproduction. They also belong to unicellular and multicellular form.

The planktonic flora of the Indian Sundarbans was greatly diversified due to salinity gradient. Phytoplanktons play an important role in protecting the mangrove vegetation of the planet. In our earlier reports a few micro- and macro-chlorophytes were found from different habitats of the Indian Sundarbans (Satpati & Pal 2015, 2017). The habitats such as mud surface, tree bark, pneumatophore surface, stone surface, forest floor, water surface, and stilt root surface were surveyed for filamentous algae collection. A few reports were also available from other parts of the Sundarbans (Prain 1903; Naskar & Santra 1986; Sanyal & Bal 1986; Maity et al. 1987; Pal et al. 1988; Santra & Pal 1988; Santra et al. 1991; Mandal & Naskar 1994; Mukhopadhyay & Pal 2002). Changes in atmospheric temperature and global warming remarkably affect mangrove vegetation including micro- and macro-flora. The algal species are diminishing with changes in temperature and increased salinity. Micro- and pico-planktons are becoming rare and endangered and not functioning as primary producer of the ecosystem.

The present study was undertaken in order to identify some rare and endangered planktonic chlorophytes in different water bodies of the Indian Sundarbans. All taxa were documented on SEM and illustrations were also made for the same.

MATERIAL AND METHODS

Study area

The Sundarbans is the largest tiger inhabiting mangrove biosphere reserve in India and also a world heritage site designated by UNESCO. It is the largest chunk of mangrove ecosystem of the World encompassing many islands and rivers interconnected with creeks and canals. The deltaic appearance of the mangrove is formed by the confluence of three rivers, Ganges, Brahmaputra and Meghna in the Bay of Bengal. The major part of the Sundarbans (60%) lies in the Bangladesh and the remaining portion (40%) in India. The Hooghly River flows over India's state of West Bengal comprising mudflats, multiple tidal streams, open and closed mangrove systems. The Indian part of the Sundarbans is distinctive in terms of its vegetation, marine ecosystem and salinity. Continuous inundation of saline water into the fresh water ecosystem, greatly affect the floral diversity. The study area lies between 21.516–22.883 ^{0}N and 88.616–89.150 ^{0}E of the southeastern part of Bay of Bengal (Image 1).

Sampling site

The sampling site varies from fresh water to brackish water. A total number of 23 sites were studied in detail. All the sampling stations belong to the 24 Parganas (South and North) of the state of West Bengal, India. The name of the sampling sites and their physico-chemical parameters are given in Table 1.

Collection of samples

The phytoplanktons were collected from aquatic habitats during tidal action and also from the brackish water areas with the help of a truncated plankton net of 25µ mesh size. The samples thus collected were thoroughly washed with running tap water or saline water and then with double distilled water to remove soil particles and other debris. The sample material was then washed with phosphate buffer saline (PBS) 2–3 times and centrifuged at 8000rpm (Satpati & Pal 2017).

Measurement of Physicochemical Parameters

Physicochemical parameters like air and surface water temperature, pH, and salinity were recorded using digital thermometer (Eurolab ST9269B), pH meter (Eco testr) and Refractometer (Erma, Japan).

Preparation of voucher specimens

Samples were preserved in 4% (v/v) formalin and stored as voucher specimen in Calcutta University Herbarium (CUH) for further study.

Scanning Electron Microscopy (SEM)

One drop of washed material was put on a glass cover slip (Blue Star) and dried at 20°C. The samples were repeatedly washed with ethanol grade and dried at room temperature. After complete dehydration the cover slips were placed on carbon tape and put in Quorum (Q 150 TES) gold coater to coat the samples with gold. The scanning electron microscopic (SEM)

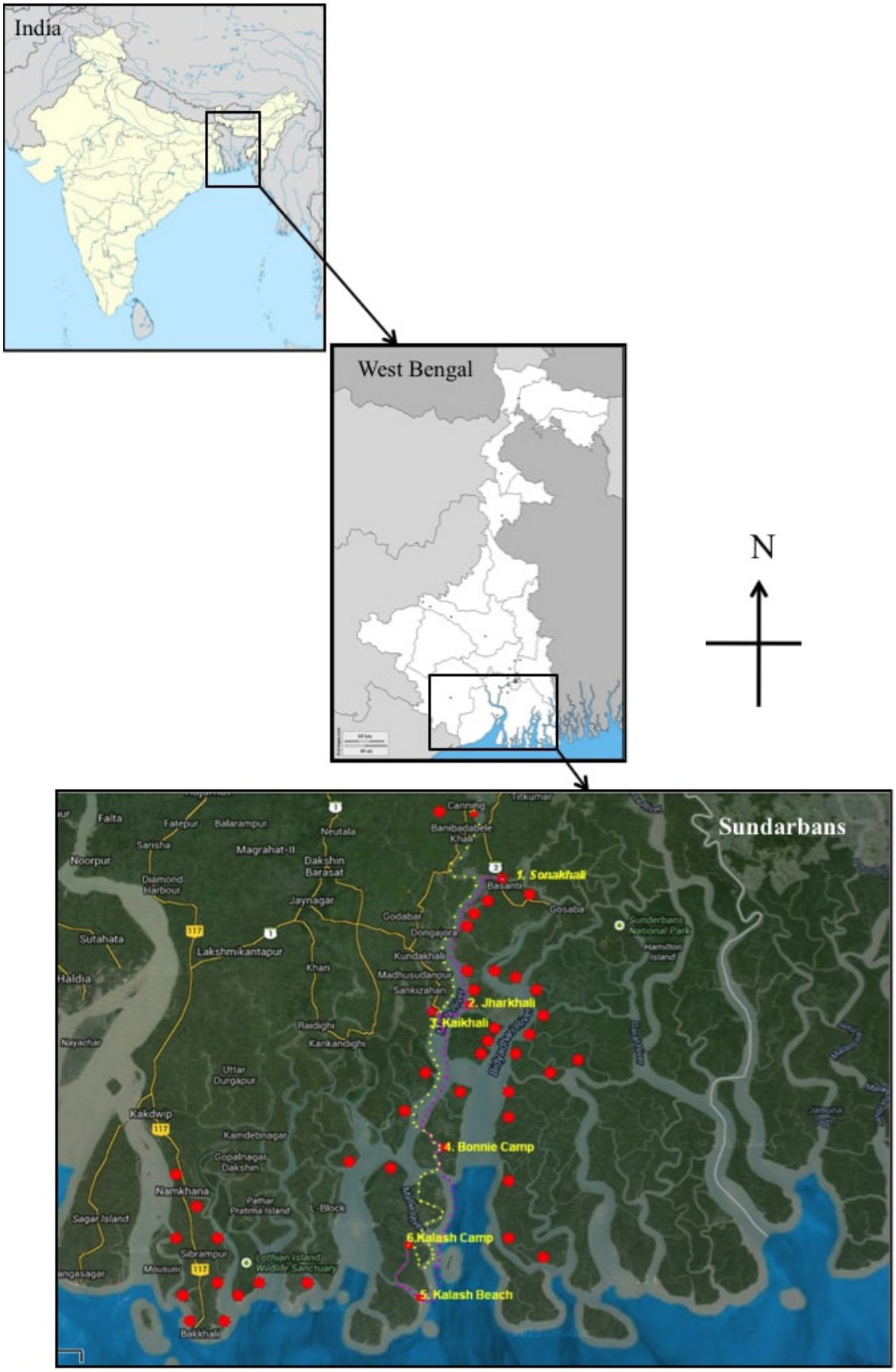

Image 1. Study area along different parts of Sundarbans (Google image, Red bullets indicating various sampling sites).

Table 1. Name of the sampling station and their physicochemical parameters.

	Sampling station	Physicochemical parameters			
		Air temperature (°C)	Surface water temperature (°C)	pH	Salinity (ppt)
1	Basanti	30.8	31.2	8.2	1.2
2	Jaigopalpur	31.3	31.7	7.8	1.5
3	Jharkhali	29.7	30.2	8.7	4.9
4	Rajbari	28.6	27.8	6.6	6.9
5	Malancha	27.4	26.5	6.2	7.4
6	Minakha	30.3	30.8	8.3	0.8
7	Canning	29.4	29.7	9.4	7.8
8	Bhagabatpur	27.6	28.2	9.6	3.4
9	Sandeshkhali	28.8	28	9.4	8.7
10	Namkhana	27.7	26.8	8.7	6.7
11	Fraserganj	28.6	28	8.6	11.6
12	Patibunia Island	27.6	27.4	9.8	12.3
13	Dabu	26.5	26.3	9.2	18.4
14	Hamanbere Island	27.5	26.8	9.6	12.5
15	Bakkhali	26.5	26	8.6	11.2
16	Sushni Island	30.5	31.7	8.4	22.1
17	Suryamoni Island	27.8	27.3	6.8	12.5
18	Kala Jungle	28.5	28.2	6.8	11.5
19	Morahero Island	27.5	27.2	9.3	12.2
20	Narayanitala	28.4	28.2	9.1	19.6
21	Cheramatla	30.2	31.2	8.7	19.6
22	Jammudwip	31.2	32	9.6	16.8
23	Aamarboni Island	28.8	28	9.2	15.2

images have been taken with Carl Zeiss EVO 18 (EDS 8100) microscope and Zeiss Inca Penta FETX 3 (Oxford instruments) attachment. Photographs were taken in different magnifications.

Camera Lucida drawing

The hand drawing was made under a compound microscope with the help of a prism and 0.1- and 0.2- Rotring isograph pen (Germany). The drawing was done on transparent tracing paper (A4 size). The cellular details of vegetative and reproductive parts of different species were outlined and scale measurement was given under 10X, 40X and 100X objectives with proper magnification. The cell length and breadth was measured with ocular lens as ocular division (O.D.) and standardized using stage micrometer.

Species identification

Identification of taxa was done using standard research articles and monographs (Smith 1950; Randhawa 1959; Prescott 1982; Anand 1998; Jaiswal & Tiwari 2003; Sen & Naskar 2003; Shukla et al. 2008; Bellinger & Sigee 2010; Das & Adhikary 2012; Tripathi et al. 2012; van Geest & Coesel 2012; Baruah et al. 2013; Keshri & Mallick 2013).

RESULTS

Taxonomic descriptions

A total of 45 species were identified and detailed descriptions of the species are enumerated below:

Family: Hydrodictyaceae

1. *Pseudopediastrum boryanum* (Turpin) E. Hegewald (Image 2A-B and Figure 1A)

Synonym: *Pediastrum boryanum* (Turpin) Meneghini

Basionym: *Helierella boryana* Turpin

[Prescott 1982; Day et al. 1995; Hu & Wei 2006; Kim & Kim 2012].

Coenobium entire; cells 5–6 sided with smooth or granular walls; peripheral cells with outer margins extended into two blunt-tipped processes, cells up to 14µ in diameter and 21µ long; 36 celled colony 85–90 µ wide. Cells are well ornamented with pores and wavy margins.

Occurrence: aquatic; voucher number: CUH/Al/MW-171.

2. *Pseudopediastrum boryanum* var. *perforatum* (Raciborski) Nitardy (Image 2C and Figure 1B)

Basionym: *P. boryanum* subsp. *perforatum* Raciborski [Cambra-Sánchez et al. 1998; Kim & Kim 2012].

Coenobia are circular in outline and with well perforations. Coenobia composed of 4–32 cells. Incisions are wide and V-shaped. Each cell extended to two processes. Cell wall ultrastructure is very distinctly granular having honey comb like appearance. Diameter of the coenobia is 70–120 µ, cells 3–20 µ wide and 4–20 µ long.

Occurrence: aquatic; voucher number: CUH/Al/MW-172.

3. *Stauridium tetras* (Ehrenberg) Hegewald var. *apiculatum* (Fritsch) Keshri et Mallick comb. nov. (Image 2E and Figure 1C)

Synonym: *Pediastrum tetras* (Ehrenberg) Ralfs var. *apiculatum* Fritsch.

[Keshri & Mallick 2013].

Coenobia 4-celled, less rectangular with cells without intercellular spaces; marginal cells divided into two lobes with deep linear to cuneate incision on the outer side reaching the middle of the cell and are trapezoidal in shape; each lobe further divided into two lobes terminating in an apical nodular thickening; cells 5–15 µ in diameter and colony of four cells up to 14–28 µ in diameter. Cell wall ultrastructure varies being irregular net-like or warty.

Occurrence: aquatic; voucher number: CUH/Al/MW-199.

4. *Pediastrum obtusum* Lucks (Image 2F and Figure 1D)

[Prescott 1982; Kim & Kim 2012].

Coenobia nearly entire, with minute interstices formed by the retuse margins; Coenobia oblong to nearly star shaped. Coenobia 8–32 celled with deep narrow sinus forming two major lobes, lobes incised to form bluntly rounded lobules. The sinus outwardly closed due to the contact of two central lobules. The ultrastructure of cell wall shows dotted appearance having minute pores.

Cells are 5–10 µ in diameter and 6–12 µ in length. Coenobia 15–40 µ in diameter.

Occurrence: aquatic; voucher number: CUH/Al/MW-200.

5. *Pediastrum duplex* Meyen (Image 2G and Figure 1F)

Synonym: *P. napoleonis* Ralfs; *P. pertusum* Kützing; *P. duplex* var. *reticulatum* Lagerheim

[Bruhl & Biswas 1926; Prescott 1982; Day et al. 1995; Buchheim et al. 2005; Hu & Wei 2006; Kim & Kim 2012].

Coenobia 16-celled, arranged more or less compactly, semicircular in outline. The outer margin is smooth, concave and extended into two blunt tapering processes. Cells 10–15 µ in diameter and coenobia are 40–80 µ in diameter. The ultrastructure of cell wall is smooth with tiny pores.

Occurrence: aquatic; voucher number: CUH/Al/MW-201.

6. *Pediastrum araneosum* (Raciborski) Raciborski (Image 2H and Figure 1G)

Synonym: *P. angulosum* Ehrenberg ex Meneghini

[Prescott 1982; Day et al. 1995; John & Tsarenko 2002].

Coenobia entire with minute interstices. Central cells packed and peripheral cells with two minute lobes; margin concave between two lobes. Cell wall smooth and with reticulate ridges. The ultrastructure of cell wall shows tiny pores. Cells are 8–12 µ in diameter.

Occurrence: aquatic; voucher number: CUH/Al/MW-173.

7. *Pediastrum integrum* Nägeli (Image 2I and Figure 1E)

[Prescott 1982; McManus & Lewis 2005; Hu & Wei 2006; Tsarenko 2011; Kim & Kim 2012].

Coenobia 4, 8, 16 and 32 celled, without or little perforations. Cell wall reticulates with tiny granules. Shapes of the inner cells are similar to the peripheral cells. Outer margins of the peripheral cells with two truncate short processes. The tip of the processes is unequal. Coenobia 14–18 µ in diameter and cells are 4–8 µ in diameter.

Occurrence: aquatic; voucher number: CUH/Al/MW-174.

8. *Parapediastrum biradiatum* (Meyen) E. Hegewald (Image 2J and Figure 1H)

Basionym: *Pediastrum biradiatum* Meyen

[Prescott 1982; Menezes 2010; McManus & Lewis 2011; Tsarenko 2011].

Coenobia perforated, 16-celled; peripheral cells

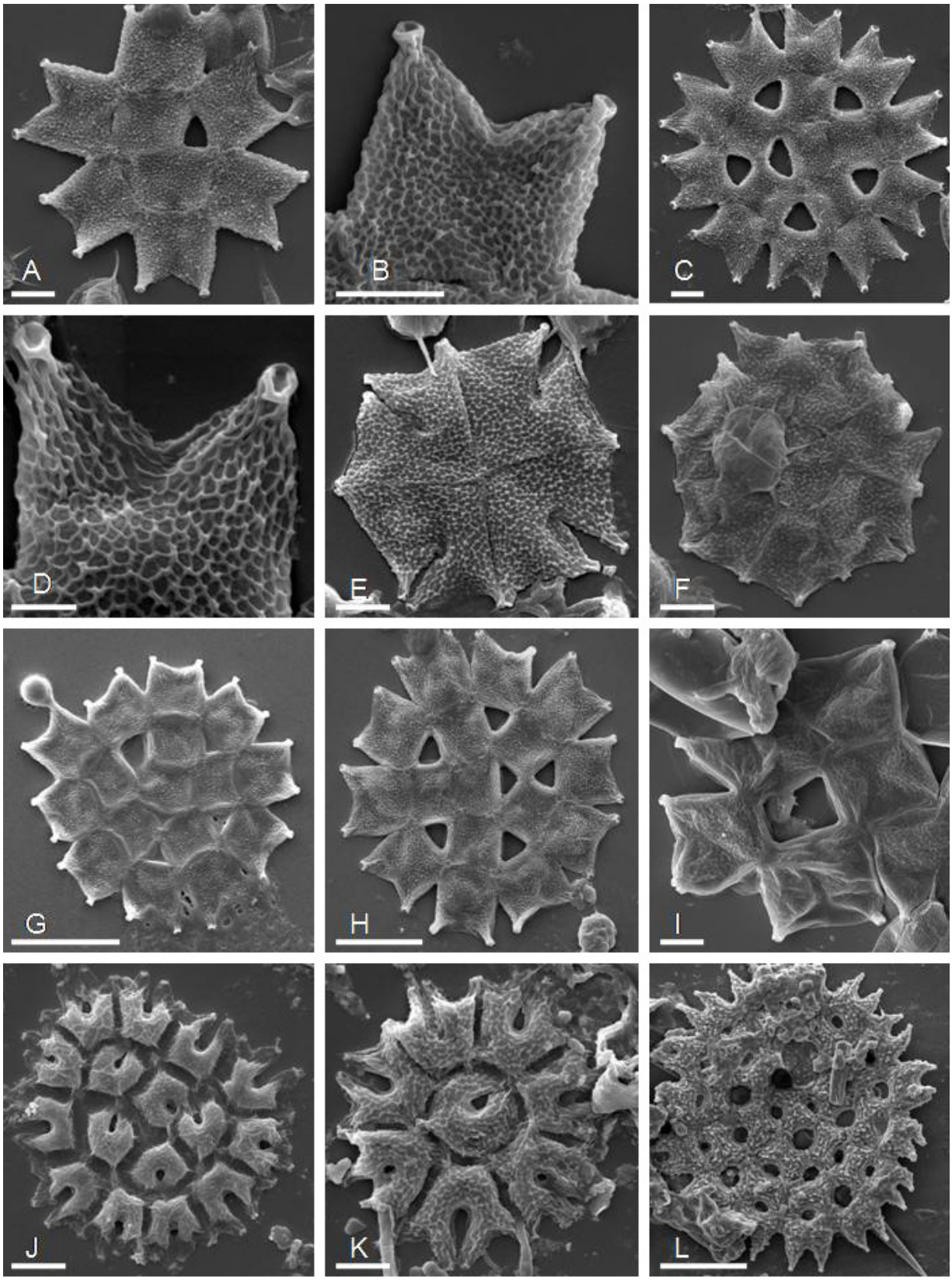

Image 2A–L. Scanning electron micrographs: A–B—*Pseudopediastrum boryanum* (×6.00KX and ×25.00KX) | C–D—*P. boryanum* var. *perforatum* (×6.00KX and ×25.00KX) | E—*Pediastrum tetras* var. *apiculatum* (×10.00KX) | F—*P. obtuosum* (×8.00KX) | G—*P. duplex* (×2.00KX) | H—*P. arneosum* (×4.25KX) | I—*P. integram* (×5.00KX) | J—*Parapediastrum biradiatum* (×7.50KX) | K—*Stauridium tetras* (×7.50KX) | L—*Pediastrum duplex* var. *duplex* (×1.75KX). Scale bar: A–B—3µ | C–L—2µ.

deeply bilobed, the lobes incised. Each cell is bilobed two times. Coenobia 12–16 µ in diameter; cells are 2–6 µ in diameter. The cell wall is reticulate and tiny pores are present on it.

Occurrence: aquatic; voucher number: CUH/Al/MW-175.

9. *Stauridium tetras* (Ehrenberg) E. Hegewald (Image 2K and Figure 1I)

Synonym: *Pediastrum tetras* (Ehrenberg) Ralfs; *Helierella renicarpa* Turpin; *Stauridium bicuspidatum* Corda

Basionym: *Micrasterias tetras* Ehrenberg

[Bruhl & Biswas 1926; Prescott 1982; Tsarenko 2011].

Coenobia oval or circular, 8-celled, marginal cells are deeply incised and form two lobes, each lobes truncate, generally further divided into two lobes and are trapezoidal in shape, inner cells 4–6 sided with a single linear or cuneate incision; cells 5–8 µ in diameter, eight celled colonies 12–18 µ in diameter. The ultrastructure of cells and coenobia shows presence of granules throughout. The cell surface is well wrinkled and folded.

Occurrence: aquatic; voucher number: CUH/Al/MW-81.

10. *Pediastrum duplex* (Meyen) var. *duplex* (Image 2L and Figure 1J)

[Prescott 1982; Anand 1998].

Coenobia circular, 40–70 µm in diameter; 16-32-64 celled. The peripheral cells are deeply incised to form V-shaped processes. The central and peripheral cells are of different sizes. The central cells are 5–8 µ in diameter and marginal cells are 8–12 µ in diameter. The central cells joined with each other and leave fine gaps within the coenobia. The ultrastructure of coenobia shows fine sculpture and granules throughout the cell wall.

Occurrence: aquatic; voucher number: CUH/Al/MW-202.

11. *Desmodesmus abundans* var. *brevicauda* G.M. Smith (Image 3A-B and Figure 1K–L)

[Menezes 2010; Tsarenko 2011; Tsarenko & John 2011; Gopalakrishnan et al. 2014].

Coenobium composed of four cells, cells smaller with relatively smaller spines. Cells are 2–4 µ in length and 1–2 µ in diameter. Spines fewer, 1–3 µ long. The ultrastructure of the cells shows smooth cell wall with slightly wavy margins.

Occurrence: aquatic; voucher number: CUH/Al/MW-187.

12. *Desmodesmus bicaudatus* (Dedusenko) P.M. Tsarenko (Image 3C and Figure 1M)

Basionym: *Scenedesmus bicaudatus* (Dedusenko)

[Tsarenko 2011; Tsarenko & John 2011].

Coenobium 2–4 celled, with linear or slightly alternate in arrangement, cells elongated, outer cells with a long curved spine at alternate poles; inner cells without spines, oval to cylindrical. Cells are 8–12 µ in length and 4–8 µ in width. The electron microscopic study revealed folded sculptured wall outside the cell with fine pores.

Occurrence: aquatic; voucher number: CUH/Al/MW-186.

13. *Desmodesmus serratus* (Corda) S.S. An, T. Friedl & E. Hegewald (Image 3D and Figure 2B)

Synonym: *Scenedesmus serratus* (Corda) Bohlin

Basionym: *Arthrodesmus serratus* Corda

[Prescott 1982; Fawley et al. 2011; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of four ovate, oblong cells arranged in a single series; the outer and inner cells with longitudinal teeth; apices of all cells bearing 3–4 small teeth. Cells are 6-10 µ in length and 2–4 µ in width. The electron microscopic study revealed presence of beads like structure throughout the cell wall.

Occurrence: aquatic; voucher number: CUH/Al/MW-165.

14. *Desmodesmus armatus* (R. Chodat) E. Hegewald (Image 3E and Figure 2D)

Synonym: *Scenedesmus armatus* (Chodat) G.M. Smith

Basionym: *Scenedesmus hystrix* var. *armatus* R. Chodat

[Prescott 1982; Verschoor et al. 2004; Matusiak-Mikulin et al. 2006; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of four cells and arranged in single series. Cells are elongated, ellipsoid; each cell with abundant uneven spines; each pole of the individual cell contains 3–6 uneven spines; each cell contains a longitudinal ridge. Cells are 4–8 µ in length and 3–5 µ in width. The sculptured cell wall with folded margin and granules are shown under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-166.

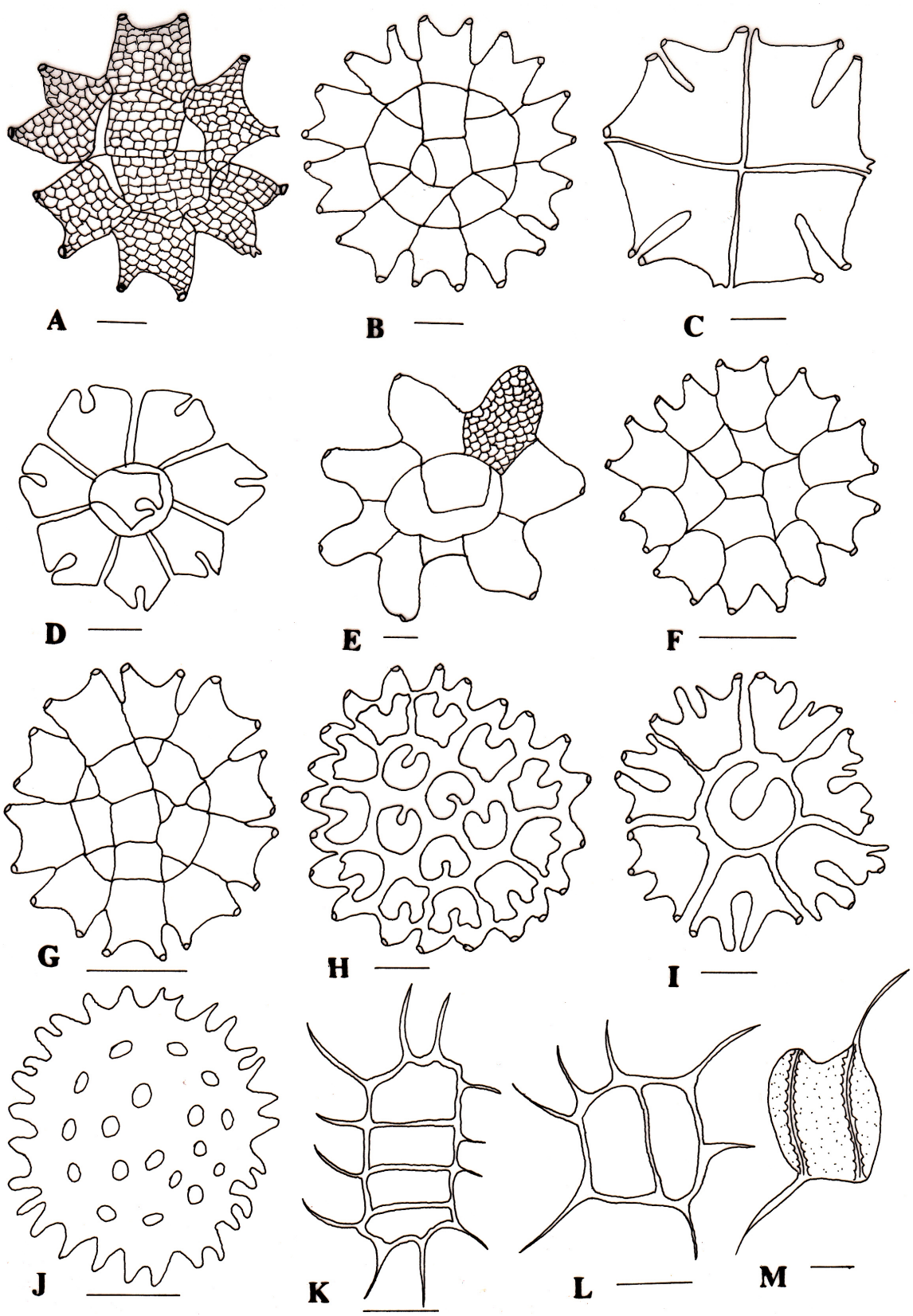

Figure 1A–M. Line drawings: A—*Pseudopediastrum boryanum* | B—*P. boryanum* var. *perforatum* | C—*Pediastrum tetras* var. *apiculatum* | D—*P. obtuosum* | E—*P. integram* | F—*P. duplex* | G—*P. arneosum*; |H—*Parapediastrum biradiatum* | I—*Stauridium tetras* | J—*Pediastrum duplex* var. *duplex* | K–L—*Desmodesmus abundans* var. *brevicauda* | M—*D. bicaudatus*. Scale bar: A, M—3µ | B–E, H–I, K–L—2µ | F, J—20µ | G—10µ.

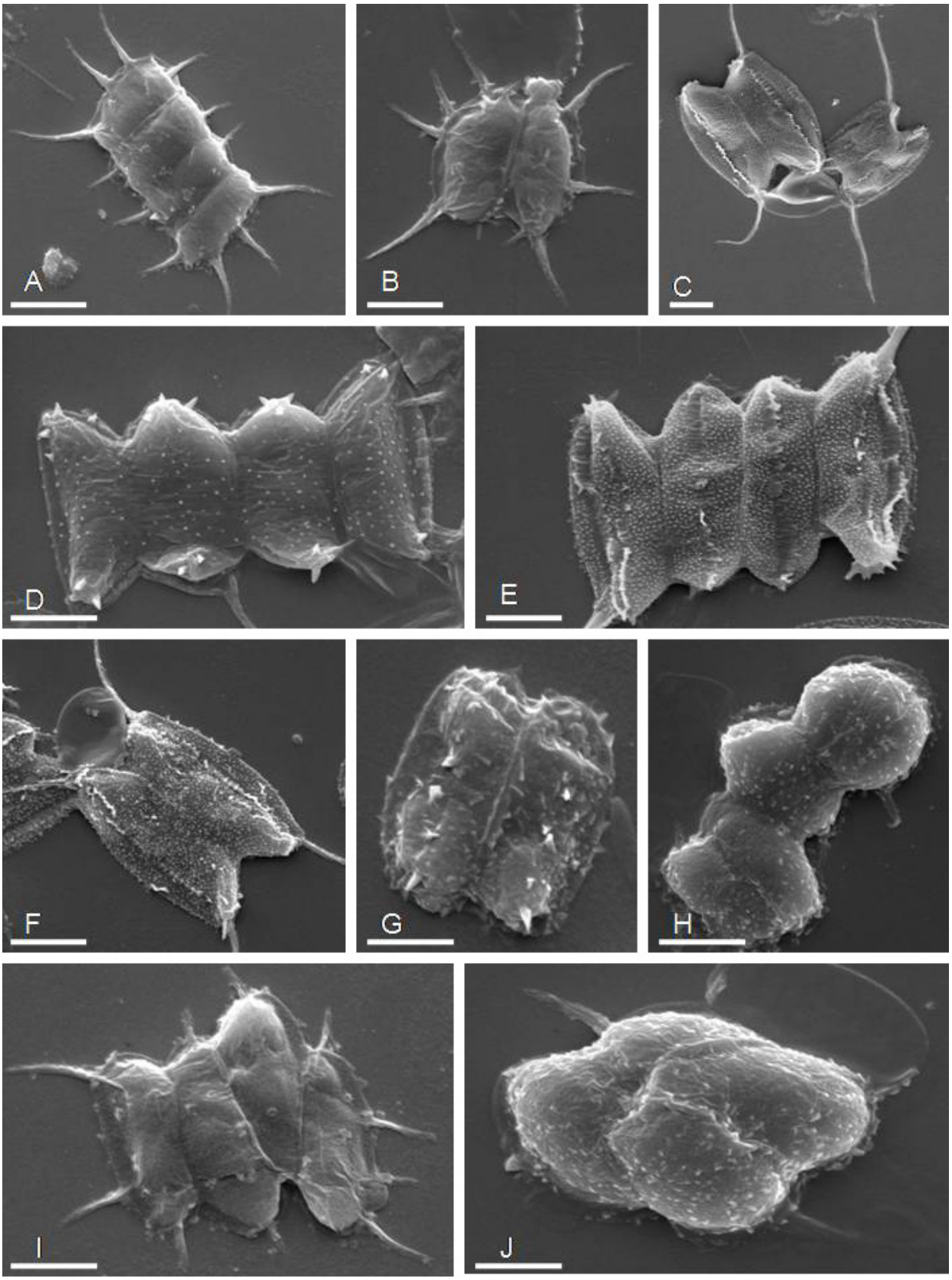

Image 3A–J. Scanning electron micrographs: A–B—*Desmodesmus abundans* var. *brevicauda* (×12.50KX and ×12.50KX) | C—*D. bicaudatus* (×5.00KX) | D—*D. serratus* (×15.00KX) | E—*D. armatus* (×12.50KX) | F—*Scenedesmus quadricauda* (×10.00KX) | G—*S. ellipticus* (×20.00KX) | H—*S. bijuga* (×17.50KX) | I—*Desmodesmus denticulatus* (×15.00KX) | J—*D. opoliensis* (×15.00KX). Scale bar: A–B—2µ | C—3µ | D–J—2µ.

15. *Scenedesmus quadricauda* (Turpin) de Brébisson (Image 3F and Figure 2A)

Basionym: *Achnanthes quadricauda* Turpin

[Bruhl & Biswas 1926; Prescott 1982; Day et al. 1995; Hu & Wei 2006].

Coenobia 2–4 celled, cylindrical, sometimes ellipsoid arranged in a single series. Cells are 6–12 µ long and 2–4 µ width; cells contain long spines at two opposite poles. Each cell contains a longitudinal ridge covered by small teeth. Under electron microscope the cell wall showed several granules and spines.

Occurrence: aquatic; voucher number: CUH/Al/MW-82.

16. *Scenedesmus ellipticus* Corda (Image 3G and Figure 2C)

Synonym: *S. ecornis* var. *flexuosus* Lemmermann; *S. linearis* Komárek

[John & Tsarenko 2002; Verschoor et al. 2004; Tsarenko 2011; Tsarenko & John 2011].

Coenobia 2-celled, arranged in a single row, cells cylindrical, bean shaped. Cells are 6–10 µ long and 2–4 µ width. Cell contains numerous small spines throughout the body. Under electron microscope the cell wall of each cell showed convoluted margins with numerous granules.

Occurrence: aquatic; voucher number: CUH/Al/MW-191.

17. *Scenedesmus bijuga* (Turpin) Lagerheim (Image 3H and Figure 2F)

Basionym: *Achnanthes bijuga* Turpin

[Prescott 1982; Cambra Sánchez et al. 1998].

Coenobia composed of four cells; cells alternately arranged; cells round to oblong to cylindrical, without teeth or spines; cells 2–6 µ in diameter and 3–8 µ long. Electron microscopic study revealed smooth cell wall with a few convoluted margins and granules.

Occurrence: aquatic; voucher number: CUH/Al/MW-83.

18. *Desmodesmus denticulatus* (Lagerheim) S.S. An, T. Friedl & E. Hegewald (Image 3I and Figure 2E)

Basionym: *Scenedesmus denticulatus* Lagerheim

[Prescott 1982; Day et al. 1995; Hu & Wei 2006; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of 4 cells; cells alternately arranged in a single series; cells are ellipsoid to cylindrical, 4–6 µ long and 1–3 µ width; each cell with 1–4 small spines and teeth. Under electron microscope the cells showed wavy margins on the cell wall and a few apertures.

Occurrence: aquatic; voucher number: CUH/Al/MW-167.

19. *Desmodesmus opoliensis* (P.G. Richter) E. Hegewald (Image 3J and Figure 2G)

Basionym: *Scenedesmus opoliensis* P.G. Richter

[Prescott 1982; Hu & Wei 2006; Menezes 2010; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of 2–4 celled arranged in a single series; cells 2–6 µ long and 1.5–3 µ width; cells with long spines at the apices. Cell wall is granulated and slightly folded as shown under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-168.

20. *Desmodesmus subspicatus* (Chodat) E. Hegewald & A. Schmidt (Image 4A and Figure 2H)

Basionym: *Scenedesmus subspicatus* Chodat

[Verschoor et al. 2004; Tsarenko 2011; Tsarenko & John 2011; Hilt (nee Korner) et al. 2012].

Coenobia 2-celled arranged in a single row; cells 6–8 µ in length and 2–3 µ width; apices of the cells contain 2–4 small teeth or spines. The cell surface showed numerous small teeth and granules under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-169.

21. *Desmodesmus brasiliensis* (Bohlin) E. Hegewald (Image 4B)

Basionym: *Scenedesmus brasiliensis* Bohlin

[Bruhl & Biswas 1926; Prescott 1982; Menezes 2010; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of four cells arranged in a single row; cells 6–10 µ in length and 1–2 µ in width; apices of each cell with 1–4 small teeth and with a longitudinal median ridge extending between the apices of each cell. Median ridge of each cells are surrounded by folded margins. The outer two cells are covered with crown like structure shown under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-170.

22. *Comasiella arcuata var. platydisca* (G.M. Smith) E. Hegewald & M. Wolf (Image 4C and Figure 2J)

Synonym: *Tetrachlorella nephrocellularis* Komárek

Basionym: *S. arcuatus* var. *platydiscus* G.M. Smith

[Prescott 1982; Day et al. 1995; Menezes 2010].

Coenobia composed of eight cells arranged in a flat double series; no intercellular spaces between

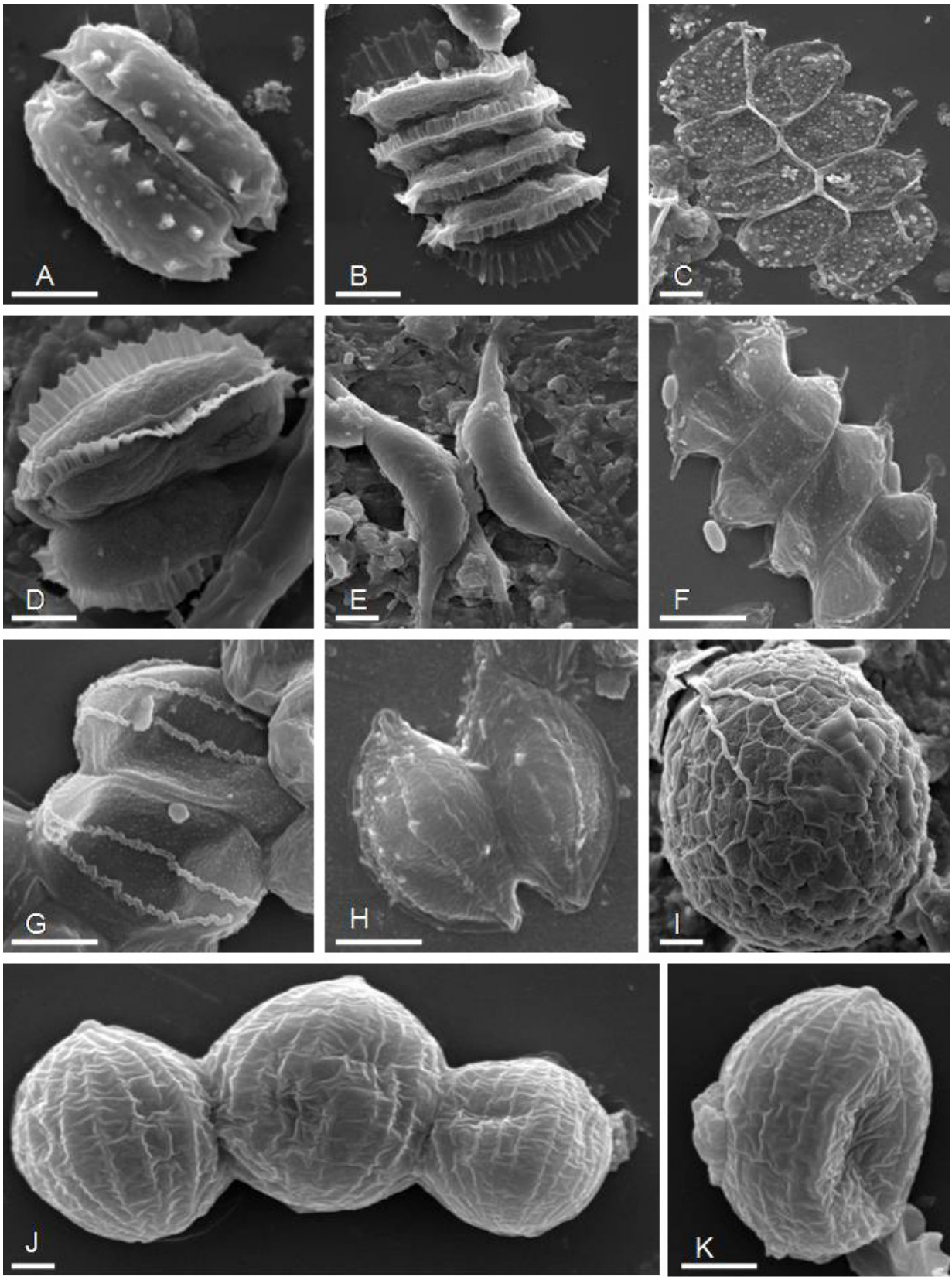

Image 4A–K. Scanning electron micrographs: A—*Desmodesmus subspicatus* (×20.00KX) | B—*D. brasiliensis* (×12.50KX) | C—*Comasiella arcuata* var. *platydisca* (×7.50KX) | D—*Scenedesmus acutiformis* (×15.00KX) | E—*Acutodesmus acuminatus* (×7.50KX) | F—*S. magnus* (×15.00KX) | G—*S. bijuga* var. *alternans* (×15.00KX) | H—*S. raciborskii* (×15.00KX) | I–K—*Chlorococcum infusionum* (×7.00KX, ×10.00KX and ×15.00KX). Scale bar: A–K—2µ.

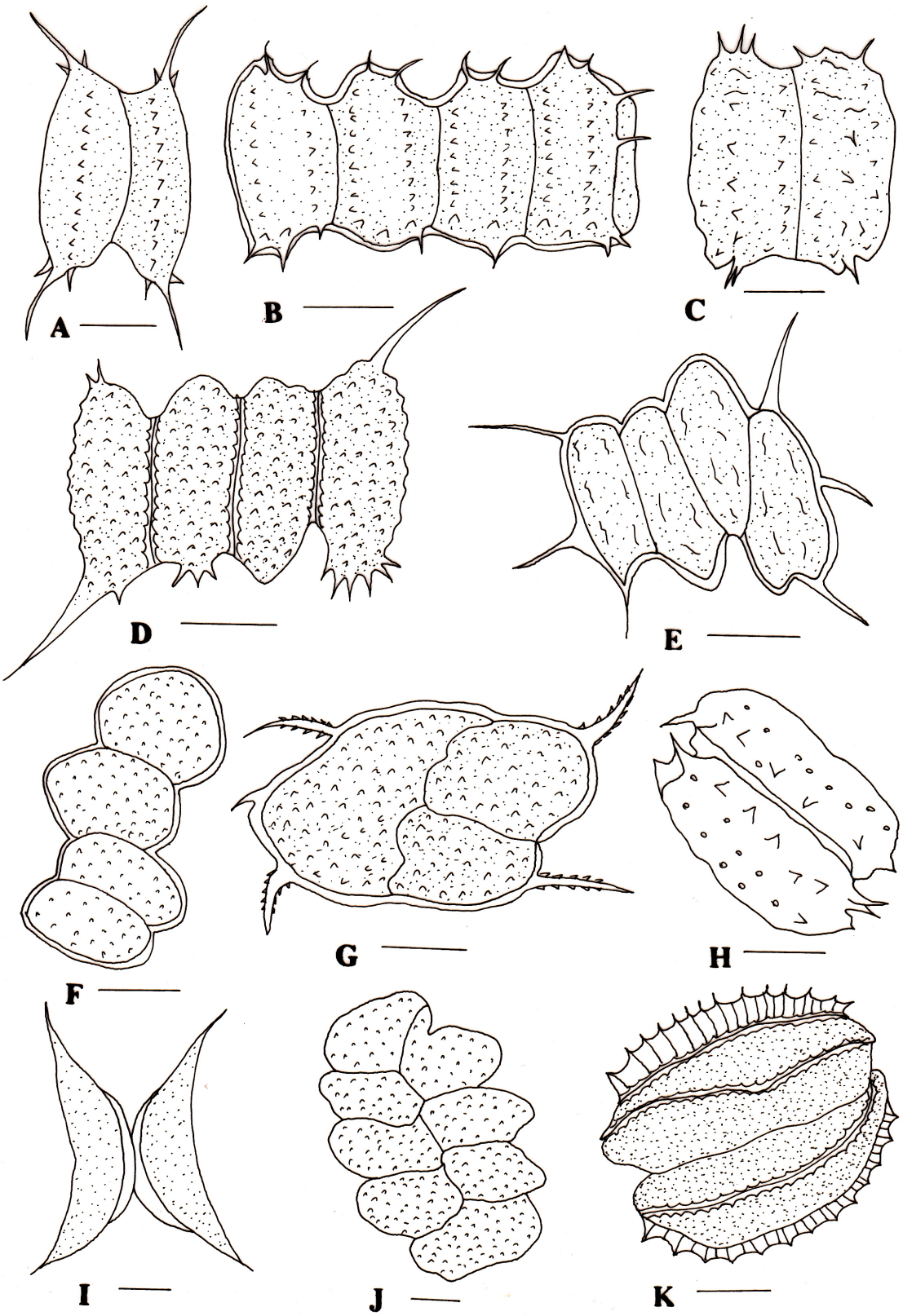

Figure 2A–K. Line drawings: A—*Scenedesmus quadricauda* | B—*S. serratus* | C—*S. ellipticus* | D—*Desmodesmus armatus* | E—*D. denticulatus* | F—*Scenedesmus bijuga* | G—*Desmodesmus opoliensis* | H—*D. subspicatus* | I—*Acutodesmus acuminatus* | J—*Comasiella arcuata* var. *platydisca* | K—*Scenedesmus acutiformis*. Scale bar: A–K—2µ.

the joining of cells; cells are 6–10 µ long and 3–6 µ width. Numerous small teeth and granules are found throughout the cell surface of each cell under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-196.

23. *Scenedesmus acutiformis* Schröder (Image 4D and Figure 2K)

Synonym: *Acutodesmus acutiformis* (Schröder) Tsarenko & D.M. John

[Prescott 1982; Day et al. 1995; Cambra Sánchez et al. 1998; John & Tsarenko, 2002; Verschoor et al. 2004; Hu & Wei 2006].

Coenobia of 2-celled arranged in a single row; cells 10–16 µ long and 6-8 µ broad; each cell having 2–3 facial longitudinal ridges covered by folded margins. The crown-like folded cell wall with smooth surface was shown under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-192.

24. *Acutodesmus acuminatus* (Lagerheim) Tsarenko (Image 4E and Figure 2I)

Synonym: *Scenedesmus acuminatus* (Lagerheim) Chodat

Basionym: *Selenastrum acuminatum* Lagerheim

[Bruhl & Biswas 1926; Prescott 1982; Tsarenko 2011; Tsarenko & John 2011].

Coenobia composed of two cells arranged in a curved series; cells strongly lunate with sharply pointed apices; cells 12–20 µ long and 2–4 µ width; the concave faces of the cells directed outward. The smooth cell wall is observed under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-195.

25. *Scenedesmus magnus* Meyen (Image 4F and Figure 3A)

Synonym: *Desmodesmus magnus* (Meyen) Tsarenko; *Scenedesmus longus* Meyen

[Prescott 1982; John & Tsarenko 2002].

Coenobia composed of four cells arranged in a single row; cells cylindrical 2–6 µ long and 1–3 µ width; apices of both inner and outer end of each cell with 1–2 sharp spines. Cells are compactly arranged and contain a median ridge. The convoluted cell wall with tiny pores was observed under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-193.

26. *Scenedesmus bijuga* var. *alternans* (Reinsch) Hansgirg (Image 4G and Figure 3B)

[Prescott 1982; Caraus 2002].

Coenobia 2-celled arranged in a single row; cells oval 6–10 µ long and 4–6 µ width. Under electron microscope the ridges were shown well and cell wall covered with tiny pores.

Occurrence: aquatic; voucher number-:CUH/Al/MW-198.

27. *Scenedesmus raciborskii* Woloszynska (Image 4H and Figure 3C)

Synonym: *Acutodesmus raciborskii* (Woloszynska) Tsarenko & D.M. John; *Scenedesmus incrassatulus* var. *mononae* G.M. Smith

[Prescott 1982; John & Tsarenko 2002; Tsarenko 2011].

Coenobia 2-celled arranged in a single row; cells elliptical or spindle shaped 6–8 µ long and 2–4 µ width; the cells are swollen in the middle and tapered at the two ends. Fine ridges and folds were found on the cell wall under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-194.

Family: Chlorellaceae

28. *Chlorella vulgaris* Beyerinck [Beijerinck] (Image 5A)

Synonym: *C. pyrenoidosa* var. *duplex* (Kützing) West; *Pleurococcus beijerinckii* Artari

[Shihira & Krauss 1965; Prescott 1982; Krientez et al. 2004; Rindi & Guiry 2004].

Unicellular, green, free floating planktonic, single or aggregated form, cells small spherical, single prominent, cup shaped parietal chloroplast, cells 4–8 µ in diameter.

Occurrence: aquatic; voucher number: CUH/Al/MW-80.

Family: Chlorococcaceae

29. *Chlorococcum infusionum* (Schrank) Meneghini (Image 4I-K)

Synonym: *Cystococcus humicola* Nägeli; *Lepra infusionum* Schrank; *Chlorococcum humicola* (Nägeli) Rabenhorst

Basionym: *Lepraria infusionum* Schrank

[Smith 1950; Prescott 1982; Chrétiennot-Dinet 1990; John & Tsarenko 2011].

Free living, unicellular, green, cells are solitary or sometimes in colonial form; striking variation in size shows between various cells when the alga grows in an expanded stratum, young cells are thin walled and spherical or somewhat compressed, old cells have thick

walls that are often irregular in outline, chloroplasts of young cells are parietal massive cups, completely filling the cell except for a small hyaline region at one side, they contain one pyrenoid, as a cell increases in size, the chloroplast usually becomes diffuse and contains several pyrenoids, young cells are 50–125 µ in diameter and mature cells are 120–210 µ in diameter. Under electron microscope several groves and ridges were found on the cell surface. Many tiny pores were also observed on the cell walls.

Occurrence: aquatic, endozoic; Voucher number: CUH/Al/MW-190.

30. *Tetraëdron caudatum* (Corda) Hansgirg (Image 5B and Figure 3D)

Synonym: *Polyedrium pentagonum* Reinsch; *Tetraëdron caudatum* var. *punctatum* Lagerheim

Basionym: *Asteriscium caudatum* Corda

[Hindák 1980; Prescott 1982; Cambra Sánchez et al. 1998; Hu & Wei 2006; Tsarenko 2011; Tsarenko & John 2011].

Cells flat, irregular, 5-sided, the angles rounded and tipped with a short, sharp spine; the sides between the angles concave; margins of the cells were narrowly and deeply incised; cells 6–12 µ in diameter. Granulated cell wall with honey comb like pores were found under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-176.

31. *Tetraëdron minimum* (A. Braun) Hansgirg (Image 5C-D and Figure 3E–G)

Synonym: *T. platyisthmum* (W. Archer) G.S. West; *T. quadratum* (Reinsch) Hansgirg

Basionym: *Polyedrium minimum* A. Braun

[Hindák 1980; Prescott 1982; Andreyeva 1998; Hu & Wei 2006; Tsarenko & John 2011].

Cell flat, tetragonal, the angles rounded and without spines and processes, sometimes very minute process were found on each angles; cell margin concave; cells 8–16 µ in diameter. Various apertures and undulating margins were observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-177.

32. *Tetraëdron trigonum* (Nägeli) Hansgirg (Image 5E and Figure 3H–I)

Basionym: *Polyedrium trigonum* Nägeli

[Prescott 1982; Day et al. 1995; Hu & Wei 2006].

Cell triangular, the angles narrower and tapering at each corner, each angle was terminated to a small spine or processes, each arm of the triangle is straight, margins convex; cells 12–20 µ in diameter. Smooth and wavy cell surface was observed under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-178.

33. *Tetraëdron gracile* (Reinsch) Hansgirg (Image 5F and Figure 3J)

Synonym: *T. trigonum var. gracile* (Reinsch) DeToni

Basionym: *Polyedrium gracile* Reinsch

[Prescott 1982; Day et al. 1995; Hu & Wei 2006].

Cell triangular, the angles narrower and more curved like starfish, the angles tapering and terminated to spines; cells 10–22 µ in diameter; the arms of triangle are not straight and form V-shaped structure. The electron micrograph showed wrinkled margins and a triangular ridge on the cell surface.

Occurrence: aquatic; voucher number: CUH/Al/MW-179.

Family: Selenastraceae

34. *Selenastrum gracile* Reinsch (Image 5G-H and Figure 4A)

[Prescott 1982; Hindák 1988; Day et al. 1995; John & Tsarenko 2002; Hu & Wei 2006; Tsarenko 2011; Tsarenko & John 2011; Das & Keshri 2012].

Cells are found in colonies; cells are sickle shaped and in irregular arrangement; cells 2–8 µ in diameter; apices of the cells are sharply pointed. Electron micrograph showed folded and wrinkled cell surface.

Occurrence: aquatic; voucher number: CUH/Al/MW-180.

35. *Selenastrum bibraianum* Reinsch (Image 5I and Figure 4B)

Basionym: *Kirchneriella bibraiana* (Reinsch) E. G. Williams; *Ankistrodesmus bibraianus* (Reinsch) Korshikov

[Prescott 1982; Hindák 1988; Day et al. 1995; John & Tsarenko 2002; Hu & Wei 2006; Tsarenko 2011; Tsarenko & John 2011; Das & Keshri 2012].

Cells are found in colonies; cells lunate to sickle shaped; the apices of the cells are not sharply pointed; cells 12–20 µ long and 2–6 µ width. Small teeth-like projections on the cell surface were observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-181.

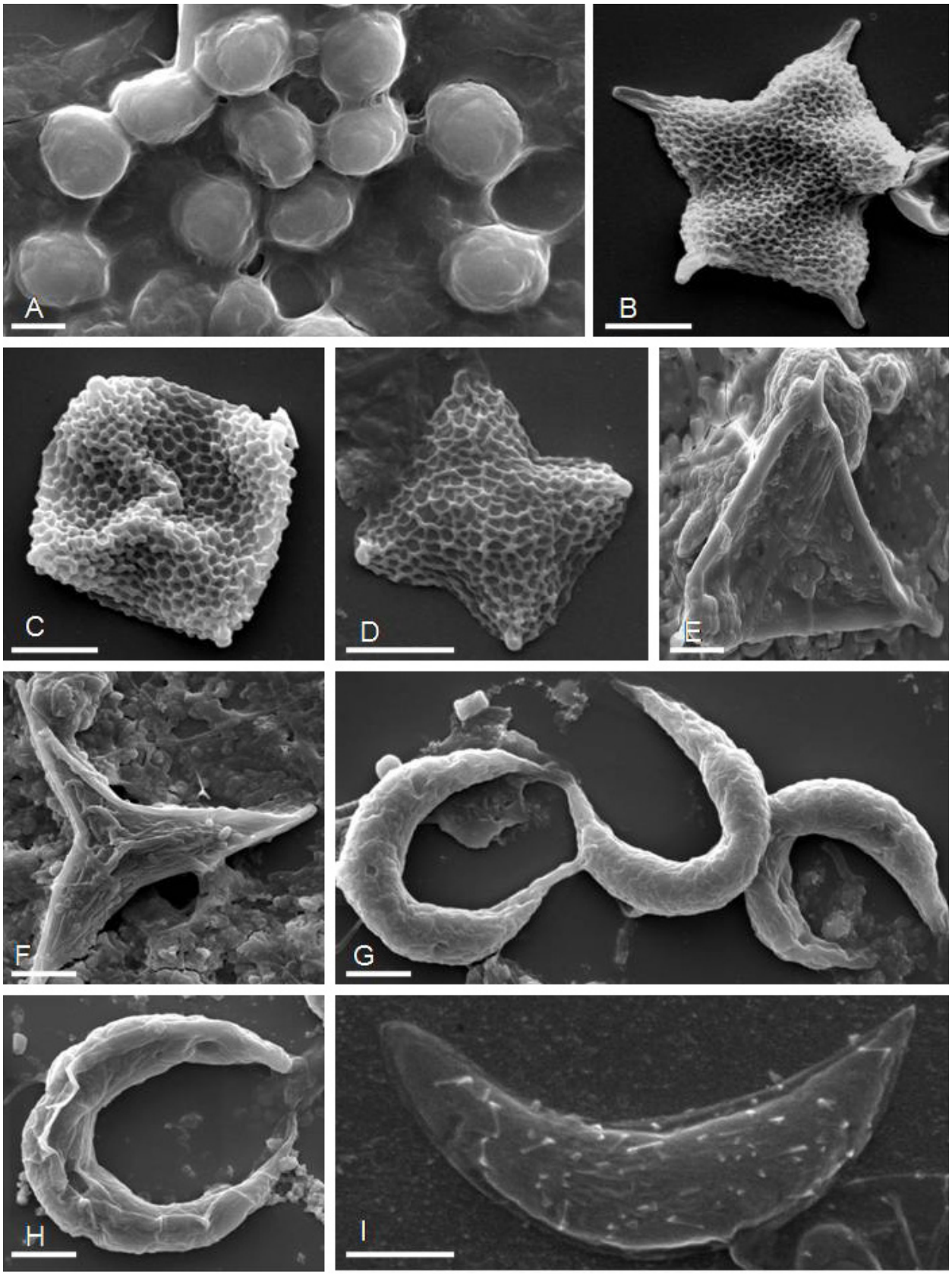

Image 5A–I. Scanning electron micrographs: A—*Chlorella vulgaris* (×10.00KX) | B—*Tetraedron caudatum* (×12.50KX) | C–D—*T. minimum* (×15.00KX and ×20.00KX) | E—*T. trigonum* (×7.50KX) | F—*T. gracile* (×10.00KX) | G–H—*Selenastrum gracile* (×10.00KX) | I—*S. bibraianum* (×20.00KX). Scale bar: A–I—2µ.

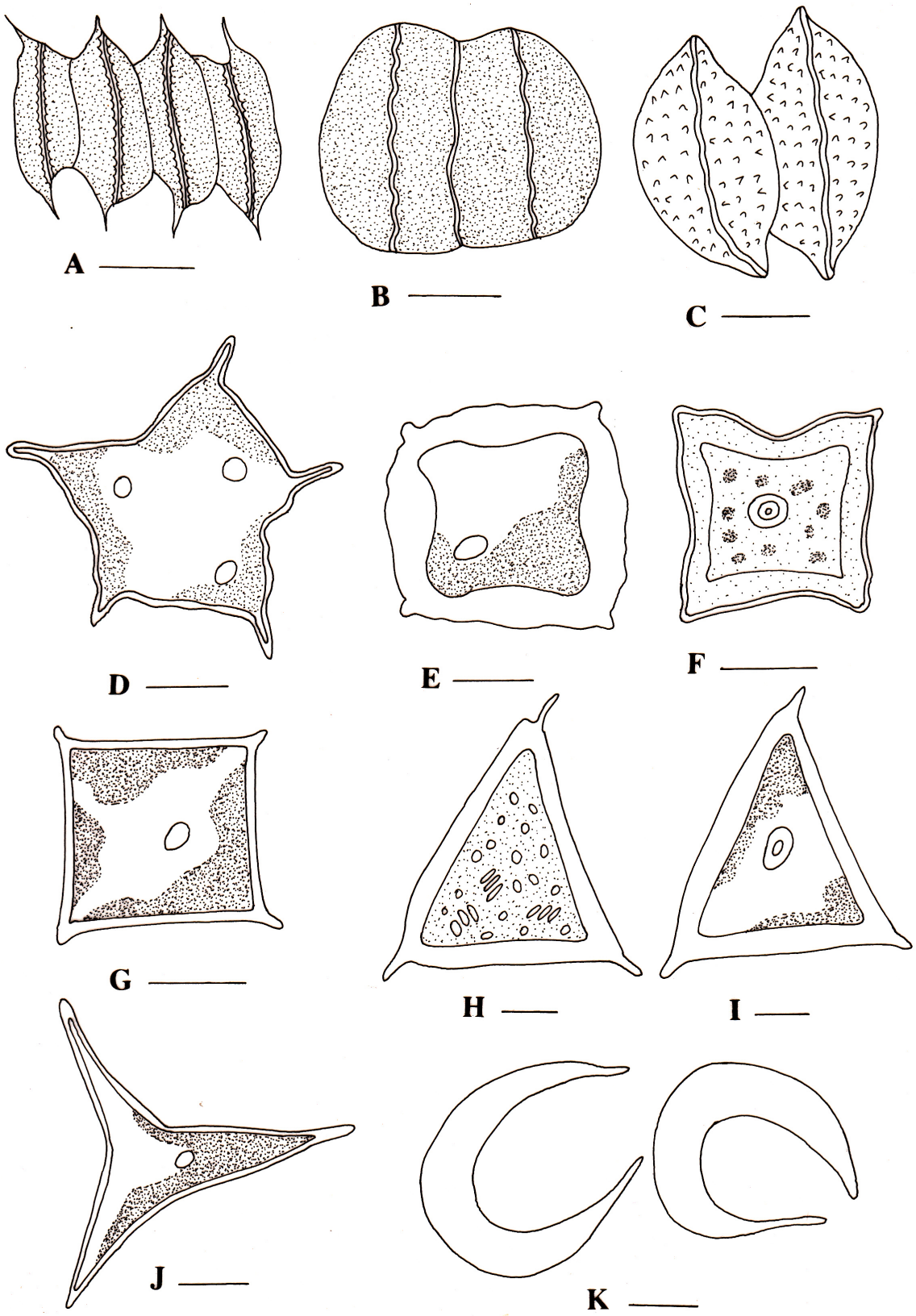

Figure 3A–K. Line drawings: A—*Scenedesmus magnus* | B—*S. bijuga* var. *alternans* | C—*S. incrassatulus* var. *monorae* | D—*Tetraedron caudatum* | E–G—*T. minimum* | H–I—*T. trigonum* | J—*T. gracile* | K—*Selenastrum gracile*. Scale bar: A–K—2µ.

Family: Desmidiaceae

36. *Euastrum denticulatum* F. Gay (Image 6A and Figure 4C)

Synonym: *E. denticulatum* var. granulatum West; *E. amoenum* F. Gay

[Ruzicka 1981; Day et al. 1995; Kouwets 1999; Wei 2003; Martello 2004; Coesel & Meesters 2007; Brook et al. 2011].

Cell solitary, green, longer than broad, small spine like projections are found on the surface of the cells; sinus narrow and linear; cells 18–26 µ long and 13–17 µ broad. The convoluted cell surface with wrinkled margins was observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-182.

37. *Euastrum dubium* Nägeli (Image 6B and Figure 4D)

Synonym: *E. dubium* var. *triquetrum* Nägeli

[Ruzicka 1981; West & West 1905; Kouwets 1999; Wei 2003; Hu & Wei 2006; Coesel & Meesters 2007; Brook et al. 2011].

Cell solitary, green, semi-cells trapezi-form, basal angles broadly rounded, four very small spines like projections or processes were shown at each corner of the cell; the margins were denticulate; cells 18–22 µ long and 12–16 µ broad. Ornamented cells with wavy margin were observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-183.

38. *Teilingia wallichii* (D.L. Jacobsen) Bourrelly (Image 6C and Figure 4H)

Basionym: *Sphaerozosma wallichii* J. Jacobsen

[Day et al. 1995; Kouwets 1999].

Colonies thread like attached by apices into long filaments or cells attached by spherical apical processes; apical processes of cells are very short; individual cells are 'X' shaped; cells 2–8 µ in diameter. Under electron microscope, smooth cell walls with minute processes were observed.

Occurrence: aquatic; voucher number: CUH/Al/MW-184.

39. *Staurastrum pantanale* K.R.S. Santos, C.F. da Silva Malone, C. Leite Sant'Anna & C.E. de Matos Bicudo (Image 6D and Figure 4E)

[Santos et al. 2013].

Cells 3-radiate, 18–24 µ long, 8–12 µ broad with processes of 8–10 µ long; isthmus 4–8 µ wide; median constriction deep; sinus acute, angular; margins deeply crenate. Cell wall provided with minute acute granules in concentric series on the processes, observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-185.

40. *Staurastrum johnsonii* West & G.S. West (Image 6E and Figure 4F)

Synonym: *S. leptocladum* L.N. Johnson

[Kouwets 1999; Coesel & Meesters 2013].

Cells 3-radiate, composed of two halves called semi-cells; cells 20–24 µ long and 10–14 µ wide; the processes 10–16 µ long with small spine like projections. Cell wall with crenate margins and acute granules were studied under the scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-159.

41. *Staurastrum simonyi* var. *semicirculare* Coesel (Image 6F and Figure 4K)

[Coesel & Meesters 2007; Coesel & Meesters 2013].

Cell triangular, 14–18 µ long and 8–12 µ wide; cell wall smooth, small minute apertures were present throughout the surface, observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-160.

42. *Staurastrum oxyacanthum* W. Archer (Image 6G and Figure 4G)

[Kouwets 1999; Coesel & Meesters 2007; Brook et al. 2011; Coesel & Meesters 2013].

Cells 3-radiate, composed of two halves of semi-cells, cells 30–44 µ long and 20–30 µ broad and isthmus 8–12 µ in diameter; the processes are deeply incised, 20–30 µ long; cell margin dentate with spine like projections; each spine is bifurcated to for two daughter spines. Several spines and wavy margins were observed under electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-161.

43. *Cosmarium dubium* Borge (Image 6H and Figure 4I)

[Day et al. 1995].

Cell solitary, green, 30–50 µ long and 20–25 µ broad; isthmus 8–10 µ; the connection between two semi-cells is smooth; the cell wall is well ornamented with small rounded projections, observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-162.

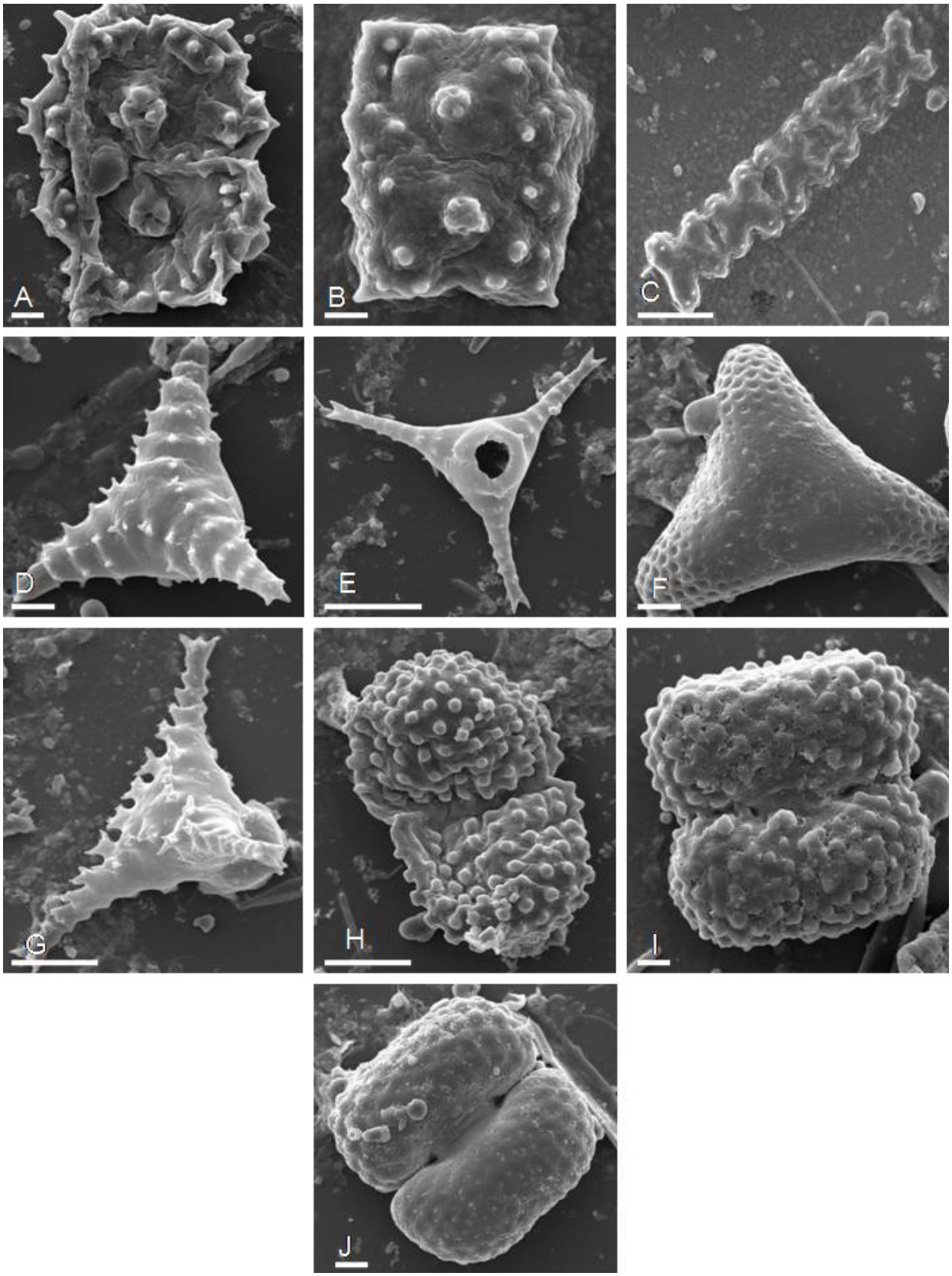

Image 6A–J. Scanning electron micrographs: A—*Euastrum denticulatum* (×5.00KX) | B—*E. dubium* (×7.50KX) | C—*Teilingia wallichii* (×3.50KX) | D—*Staurastrum pantanale* (×9.00KX) | E—*S. johnsonii* (×4.50KX) | F—*S. simonyi* var. *semicircularae* (×6.00KX) | G—*S. oxyacanthum* (×4.00KX) | H—*Cosmarium dubium* (×3.25KX) | I—*C. punctatum* (×5.00KX) | J—*C. reniforme* (×5.00KX). Scale bar: A–B, D, F, I–J—2µ | C, E, G–H—10µ.

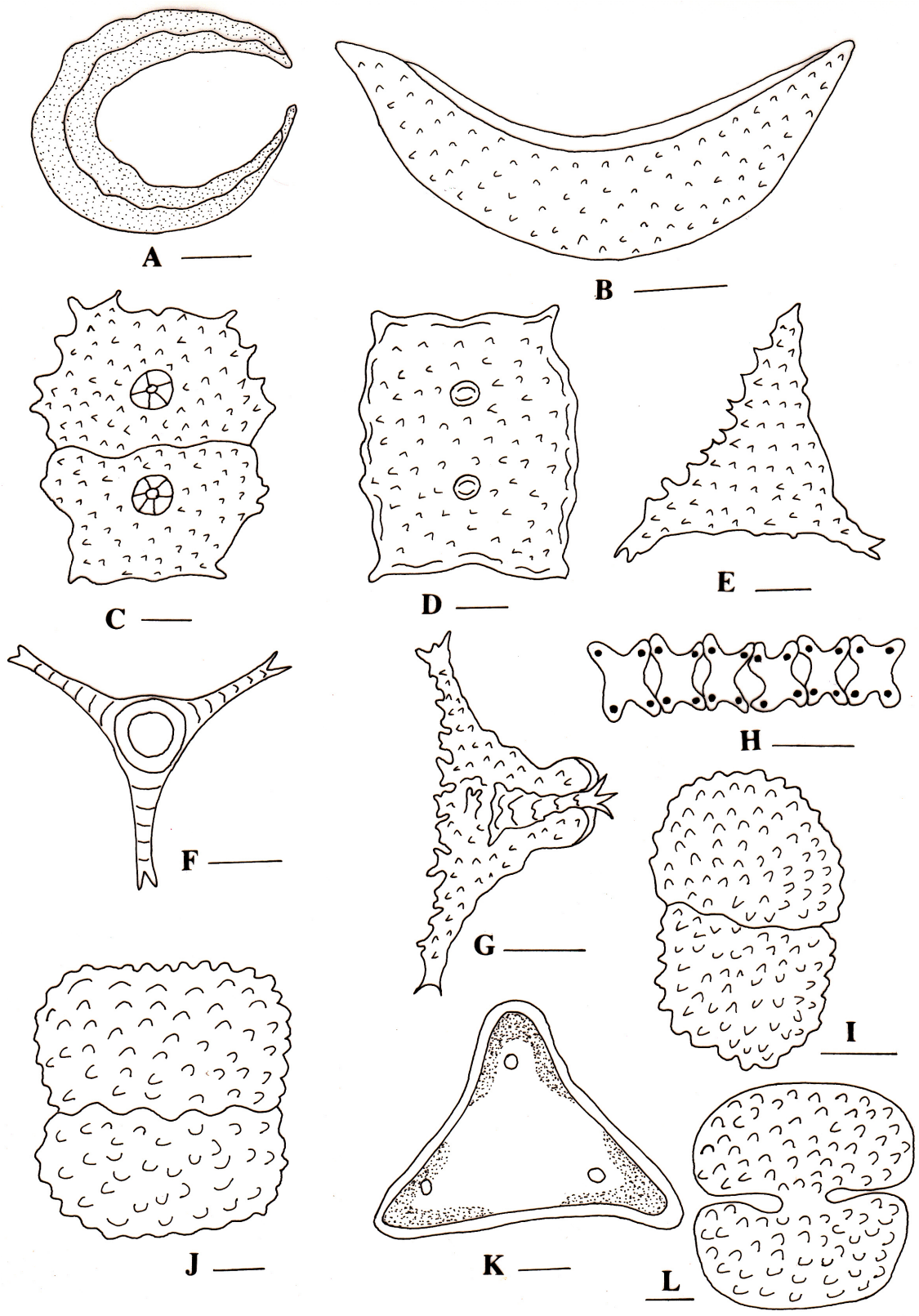

Figure 4A–L. Line drawings: A—*Selenastrum gracile* | B—*S. bibraianum* | C—*Euastrum denticulatum* | D—*E. dubium* | E—*Staurastrum pantanale* | F—*S. johnsonii* | G—*S. oxyacanthum* | H—*Teilingia wallichii* | I—*Cosmarium dubium* | J—*C. punctatum* | K—*Staurastrum simonyi* var. *semicircularae* | L—*C. reniforme*. Scale bar: A–E, J–L—2µ | F–I—10µ.

44. *Cosmarium punctulatum* Brébisson (Image 6I and Figure 4J)

Synonym: *C. punctulatum* var. *granulusculum* (Roy & Bissett) West & West

[West & West 1908; Day et al. 1995; Kouwets 1999; Hu & Wei 2006; Martello 2006; Brook et al. 2011].

Cell solitary, green, 20–26 µ long and 18–22 µ broad; isthmus 4–8 µ; cells 'dumble' shaped. Cell wall rough, well ornamented with small rounded processes observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-163.

45. *Cosmarium reniforme* (Ralfs) W. Archer (Image 6J and Figure 4L)

Basionym: *C. margaritiferum* var. *reniforme* Ralfs

[West &d West 1908; Day et al. 1995; Kouwets 1999; Hu & Wei 2006; Coesel & Meesters 2007; Brook et al. 2011].

Cell solitary, green, 20–24 µ long and 12–16 µ broad; isthmus 6–8 µ; cells 'dumble' shaped. Cell wall not so rough, well ornamented with small rounded globular projections, observed under scanning electron microscope.

Occurrence: aquatic; voucher number: CUH/Al/MW-164.

DISCUSSION

The present investigation reveals that the Indian Sundarbans exhibit rare green planktonic diversity. A few studies on planktonic chlorophytes and diatoms were found in the Gangetic belt and Bhagirathi-Hooghly estuary of the southern coast (Mukhopadhyay & Pal 2002; Chowdhury & Pal 2008). Mukhopadhyay & Pal (2002) have reported nine species of chlorophytes and five species of rhodophytes from the estuarine and coastal region of Bay of Bengal. A detailed systematic account of 19 genera and 32 species of diatoms has been done so far from the coastal belt (Chowdhury & Pal 2008). A new planktonic diatom, *Cocconeis gracilariensis* was investigated from the brackish water ecosystem of the Indian Sundarbans as epiphytic on *Gracilaria* sp. (Satpati et al. 2017). Continuous inundation of marine water in the freshwater ecosystem is the major problem for diminishing these planktonic chlorophytes. A total number of 46 taxa belonging to six groups have been reported from the Sundarban estuarine ecosystem (Manna et al. 2010). They have reported two green algal taxa *Chlorella* and *Dunaliella* in association with cyanobacteria and diatom assemblages. The conservation of these planktonic chlorophytes is suggested to protect the primary food chain of aquatic ecosystem. A few strains were maintained in the laboratory in isolated condition with accession number. Planktonic Eugenophytes were also reported and conserved from different brackish water habitats of the Indian Sundarbans (Satpati & Pal 2017). A total of 41 species of euglenoids were reported in our previous study (Satpati & Pal 2017). Some work was available on planktonic diatoms from the Sundarbans ecoregion (Choudhury & Bhadury 2014; Satpati et al. 2017). Most of the work was conducted in the Bangladesh region of the Sundarbans mangrove (Aziz & Rahman 2011). Based on previous literature, Sarkar (2011) has reported 166 species of phytoplanktons from estuarine ecosystem and associated brackish water wetlands of the Indian Sundarbans. Most of the work has been done on cyanobacteria, diatoms and filamentous chlorophytes. In this study the present group highlighted on rare green planktonic chlorophytes which were not reported in earlier studies.

Riverine fresh water run-off and tidal influx of marine water are two antagonistic hydrological processes resulting in dynamic changes in phytoplanktons and their community structure. The continuous anthropogenic perturbations, nutrient overload, increasing human population density, globalization and economic development causes vulnerability of phytoplanktons in estuarine ecosystems (Roshith et al. 2018). Temporal succession of phytoplankton assemblages of Sundarbans' mangrove was reported in a tidal creek system of the Sundarbans mangrove (Bhattacharjee et al. 2013). The phytoplankton assemblage depends on the physicochemical parameters of water and nutrient availability. The tropical and sub-tropical coastal ecosystems of the World serve as a great carbon sink due to the presence of mangroves and phytoplanktons. The biogeochemistry of carbon regulated by the key functions of genes present in the phytoplanktons reveals to illustrate their diversity (Bhattacharjee et al. 2013). The availability of phytoplanktons and mangroves helps to maintain the aquatic food chains of the coastal environments. Roshith et al. (2018) have reported the most updated information on the green phytoplanktons of Hooghly-Matla estuary. They have reported about 44 species of green chlorophytes of which 32 belong to Chlorophyceae, 11 belong to Trebouxiophyceae and 1 to Prasinophyceae. The Indian part is still less explored and more work is needed to investigate the different areas of the Sundarbans. The detailed morphological study of

Table 2. Name of the identified taxa, their latitude- longitude and conservation status.

	Name of the taxa	Latitude & Longitude (N, E)	Conservation status
1	*Pseudopediastrum boryanum*	21.893, 88.956	Rare
2	*Pseudopediastrum boryanum* var. *perforatum*	21.893, 88.956	Rare
3	*Stauridium tetras* var. *apiculatum*	22.328, 88.820	Rare
4	*Pediastrum obtusum*	22.328, 88.820	Occasional
5	*Pediastrum duplex*	22.328, 88.820	Frequent
6	*Pediastrum araneosum*	21.893, 88.956	Occasional
7	*Pediastrum integrum*	21.893, 88.956	Rare
8	*Parapediastrum biradiatum*	21.893, 88.956	Rare
9	*Stauridium tetras*	21.893, 88.956	Sporadic
10	*Pediastrum duplex* var. *duplex*	22.328, 88.820	Sporadic
11	*Desmodesmus abundans* var. *brevicauda*	22.328, 88.820	Rare
12	*Desmodesmus bicaudatus*	22.328, 88.820	Occasional
13	*Desmodesmus serratus*	22.246, 88.819	Extremely rare
14	*Desmodesmus armatus*	22.246, 88.819	Rare
15	*Scenedesmus quadricauda*	22.246, 88.819	Abundant
16	*Scenedesmus ellipticus*	22.327, 88.818	Frequent
17	*Scenedesmus bijuga*	22.246, 88.819	Frequent
18	*Desmodesmus denticulatus*	22.246, 88.819	Occasional
19	*Desmodesmus opoliensis*	22.246, 88.819	Extremely rare
20	*Desmodesmus subspicatus*	22.246, 88.819	Rare
21	*Desmodesmus brasiliensis*	22.246, 88.819	Rare
22	*Comasiella arcuata var. platydisca*	22.327, 88.818	Extremely rare
23	*Scenedesmus acutiformis*	22.327, 88.818	Frequent
24	*Acutodesmus acuminatus*	22.327, 88.818	Frequent
25	*Scenedesmus magnus*	22.327, 88.818	Rare
26	*Scenedesmus bijuga* var. *alternans*	22.246, 88.819	Rare
27	*Scenedesmus raciborskii*	22.327, 88.818	Extremely rare
28	*Chlorella vulgaris*	22.328, 88.820	Sporadic
29	*Chlorococcum infusionum*	22.055, 88.731	Sporadic
30	*Tetraëdron caudatum*	22.055, 88.731	Occasional
31	*Tetraëdron minimum*	22.055, 88.731	Rare
32	*Tetraëdron trigonum*	22.055, 88.731	Rare
33	*Tetraëdron gracile*	22.055, 88.731	Occasional
34	*Selenastrum gracile*	22.055, 88.731	Frequent
35	*Selenastrum bibraianum*	22.055, 88.731	Occasional
36	*Euastrum denticulatum*	22.055, 88.731	Rare
37	*Euastrum dubium*	22.055, 88.731	Extremely rare
38	*Teilingia wallichii*	22.055, 88.731	Extremely rare
39	*Staurastrum pantanale*	22.055, 88.731	Extremely rare
40	*Staurastrum johnsonii*	22.246, 88.820	Rare
41	*Staurastrum simonyi* var. *semicirculare*	22.246, 88.820	Extremely rare
42	*Staurastrum oxyacanthum*	22.246, 88.820	Extremely rare
43	*Cosmarium dubium*	22.246, 88.820	Rare
44	*Cosmarium punctulatum*	22.246, 88.820	Rare
45	*Cosmarium reniforme*	22.246, 88.820	Rare

phytoplanktons is lacking from the Indian Sundarbans to understand their role in primary productivity.

Conservation Status

In the present study about 18 species including nine extremely rare, seven occasional, six frequent, four sporadic and one abundant were recorded. The most abundant species in the mangrove ecosystem was *Scenedesmus quadricauda*. On the basis of sampling, *Stauridium tetras*, *Pediastrum duplex* var. *duplex*, *Chlorella vulgaris*, and *Chlorococcum infusionum* were found to be sporadic (Table 2). The species recorded as frequent were *Pediastrum duplex*, *Scenedesmus ellipticus*, *Scenedesmus bijuga*, *Scenedesmus acutiformis*, *Acutodesmus acuminatus*, and *Selenastrum gracile*. The details about the conservation status of the species including latitude and longitude are given in Table 2.

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