

On the Morphology of five species of *Chara* (Characeae, Charophyta) from West Bengal, India

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भारत के पश्चिम बंगाल से प्राप्त *कारा* (कैरेसी, कैरोफायटा) के पांच जातियों के आकृतिकी पर आलेख

कैलाश मंडल, तुहिन पाल एवं जय प्रकाश केशरी

सारांश

कैरेसी कुल से संबंधी पांच जातियों का अध्ययन पश्चिम बंगाल से प्राप्त वंशों कैरा एल. विज. कैरा फाइब्रोसा सी. अगार्थ एक्स ब्रुजेलियस, कैरा सोकोट्रेन्सिस नॉर्डस्ट्र, कैरा जिलेनिका क्लेन एक्स विल्ड., कैरा वुल्गारिस एल., कैरा हाइड्रोपिथिस रचब. के अंतर्गत किया गया है | यह अध्ययन स्टेरियो जूम एवं फिल्ड इमिशन स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (एफइएसइएम) के अंतर्गत किया गया है | पहली बार पूर्वी भारत में इसके ओस्पोरस के आकृतिकी का परीक्षण एफइएसइएम द्वारा किया गया है | कैरा सोकोट्रेन्सिस नॉर्डस्ट्र का पता पहली बार पूर्वी भारत से चला है |

ABSTRACT

Five representatives belonging to the family Characeae have been studied under the genus *Chara* L. viz., *Chara fibrosa* C. Agardh ex Bruzelius, *Chara socotrensis* Nordst., *Chara zeylanica* Klein ex Willd., *Chara vulgaris* L. and *Chara hydrophythys* Rchb. occurring in West Bengal. The studies have been done under stereo zoom and Field emission Scanning Electron Microscope (FeSEM). FeSEM observations for the morphology of oospores are the first time in Eastern India. The occurrence of *Chara socotrensis* Nordst. reported for the first time from Eastern India.

Keywords: *Chara*, Diversity, FeSEM, Morphology, Oospores

INTRODUCTION

Charophytes are macroscopic algae with unique morphology and are sometimes easily confused with aquatic angiosperms like *Ceratophyllum* when observed in vegetative conditions. They mostly grow in fresh water in submerged conditions and prefer hard waters. *Chara* L. grows in calcium-rich waters and often accumulates CaCO_3 . That's why it is called stoneworts; group as a whole. However, its unique morphological features are easily distinguishable from other aquatic macrophytes. They are branched and uniseriate, differentiated into nodes and internodes. Branches are of two types, viz. limited growth, and unlimited growth. They bear particular sex organs are called antheridium or globule and the oogonium or nucule; its structure and arrangement are

essential in distinguishing and identifying the genera. Despite studies made earlier, detailed knowledge of its species in various regions of India is incomplete (Pal & al., 1962; Subramanian, 2002; Gupta, 2012). Knowledge of detailed morphology in light of modern equipment is lacking. SEM observations of the oospore wall ornamentation pattern are presently considered to be one the most important taxonomic characters that have been taken into consideration. Only a few reports of SEM studies are available in India (Balakrishnan & Rani, 2015).

Some taxonomic and cytotoxic studies on different charophytes have been done in West Bengal in scattered manner (Agharkar & Kundu, 1937; Chatterjee, 1975, 1976, 1979a, 1979b; Ray & Chatterjee, 1986, 1987, 1988, 1994;

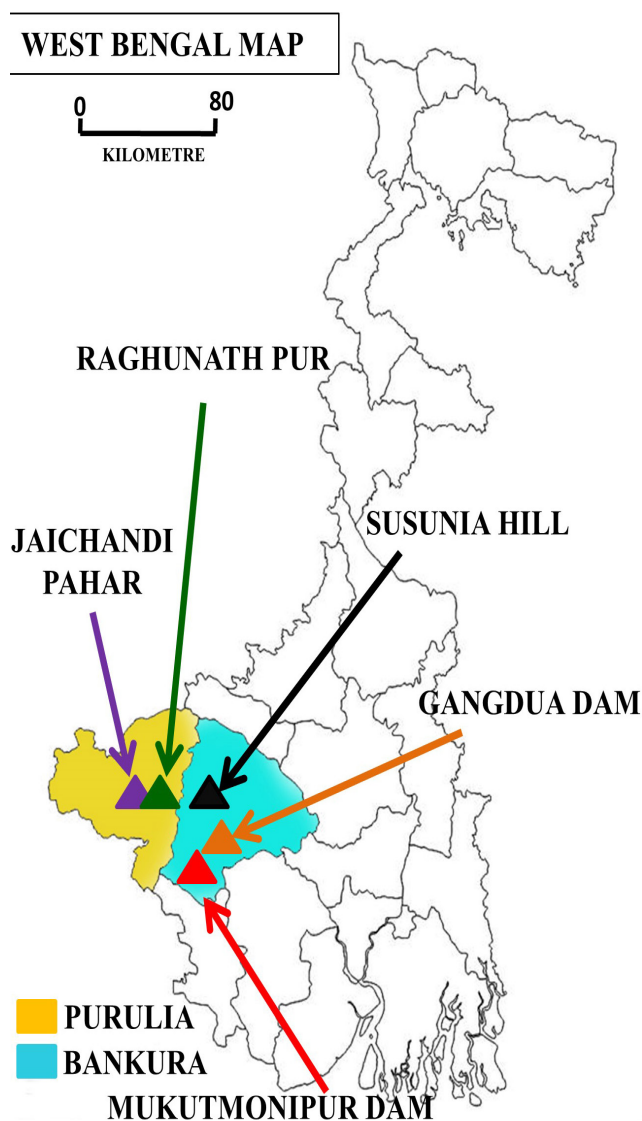


Fig.1. Showing the different collection spots in West Bengal

Pal & al., 1962; Mukhopadhyay & Ray, 1995; Chakrabarty & Ray, 2016 and Halder & Sinha, 2016). The main objective of this study was to explore the charophycean diversity of West Bengal. In this investigation, five dominant species belonging to the family Characeae have been studied in detail along with FeSEM studies on the oospore wall.

MATERIAL AND METHODS

Collection of samples

All the samples were collected from different places of Bankura and Purulia districts viz. Gangdua reservoir, Mukutmanipur dam, paddy fields of Susunia, Raghunathpur, and Jaichandi pahar area (Fig. 1). Samples were washed vigorously by gentle cleaning in running water and preserved in 4 % formalin. Water temperature, pH, and habitat etc. were noted at the time

of collection. Multi-parameter PCSTestTM 35 device was used to measure ecological data, and collection spots were tracked using GPS (GPSMAP 78s tracker).

Identifications of the samples

A detailed taxonomic study was made by microscopic observations using Zeiss Stemi 508 stereo zoom research microscope for identification of species and further referred standard monographs (Pal & al., 1962; Wood & Imahori, 1964-1965; Subramanian, 2002; Krause, 1997; John & al., 2011).

For FeSEM studies, 'nucules' were removed from the plant body and cleaned properly following the standard protocol. This has been done by treating the samples with 10N HCl for softening the ectosporangiate layer that was then removed with the help of a fine needle and forceps. After removing the ectosporangiate layer, the samples were dehydrated in ethanol series and stored in small tubes. Before observation, the nucules were mounted on sticky carbon stubs and sputter-coated with gold-palladium alloy. Surface morphology was observed under FeSEM (Model-Sigma-300, Make-Zeiss, Germany) operated at 5.00 kV field emission.

RESULTS

In this investigation following five species of *Chara* have been studied and enumerated below:

Chara fibrosa C. Agardh ex Bruzelius, Observ. Charae 21.1924; R.D. Wood & Imahori, Monogr. Characeae 284.1965; O.N. Srivast. & M. Srivast. in New Botanist, Int. Quart. J. Pl. Sci. Res. 10: 86. 1983; D. Subram., Monogr. Indian Charoph. 88. 2002. (Fig.2. A-J)

Plants olive green colour, monoecious; axes slender, large on younger internodes, main branch corticated, secondary branches ecorticated; branchlets 12–16 in a whorl, segments 3–5; spines cells solitary; gametangia conjoined at branchlets nodes; oogonia solitary, oospores orange in early and blackened at the mature stage; antheridia globose, deep orange.

FeSEM observation: Under FeSEM, the striae were found highly developed and merged at the apex, 6-7 in number, flange absent, fossa wall smooth, ornamentation absent on the wall. (Fig. 2. h-i)

Dimensions: Plant up to 18 cm high; axes 350–500 µm in diameter; spines cells 50-77 µm long; oogonia 480-619 µm in long; antheridia 336 µm in diameter.

Distribution: Freshwater; Jharkhand, Tamil Nadu West Bengal, Uttar Pradesh.

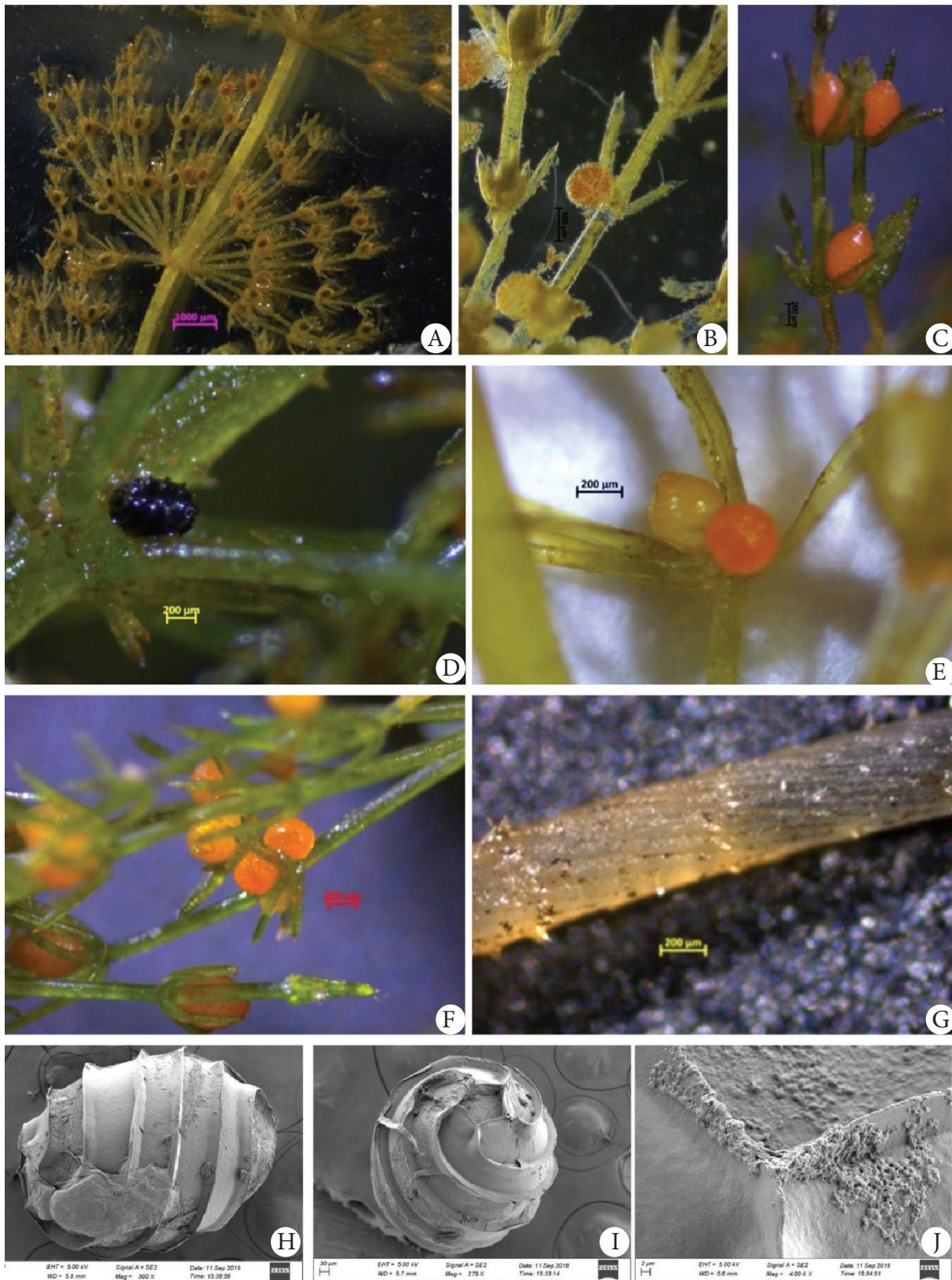


Fig. 2. *Chara fibrosa* C. Agardh ex Bruzelius: **A.** Showing a whorl of branchlet; **B-C.** Gametangia; **D.** Stipules and mature oogonia; **E-F.** Position of antheridia and oogonia on the same branchlet node; **G.** Axis and axile cortex; **H-I.** A general view of an oospore with showing well developed, flanged striae and **J.** fossa.

Specimen examined: India, West Bengal, Bankura District, Chhatna, Susunia, 22°54' 36.35" N; 87°10' 41.63"E, Kailash Mondal, KM 1001 (BURD).

Habitat parameters: Temperature (°C) -25.4°C, pH- 7.87; Conductivity (µS) -150.5; Salinity (ppm) -67.5; TDS (ppm)- 107.

Chara socotrensis Nordstedt in M. Kuhn, Ber. Deutsch. Bot. Ges. 241. 1883; R. D. Wood & Imahori, Monogr. Characeae 279.1965; R. D. Wood & Imahori, Monogr. Characeae 119.1964; John & al., Freshwat. Alg. Fl. Brit. Isles: ident. guide freshwat. terr. Alg., 756. 2011.

(Fig. 3. A-J)

Plants monoecious; axes moderately stout, ecorticated; spine cells absent; stipulodes present in 1 tier, well developed, acuminate; branchlets 8–11 in whorl, segments 3–5, smooth, apical cells very small, mucornate but sometimes acute; nucule and globule conjoined at lowest branchlet nodes; globule brown in color; nucule present above the globule.

FeSEM observation: The mature black oospore elliptical in shape, striae prominent but not flanged and ornamented, 9–10 in number, fossa wall smooth.

Dimensions: Plants up to 16 cm high; axes 600–950 µm in diameter; stipulodes 259–306 µm in long; Globule 635–640 µm in diameter; Nucule 580–670 µm long.

Distribution: Freshwater; Tamilnadu.

This is the new report of the taxon from Eastern India.

Specimen examined: India, West Bengal, Bankura District, Gangdua Dam, 22°54'36.35" N; 87°10'41.63"E, Kailash Mondal, KM 1002(BURD).

Habitat parameters: Temperature (°C) - 30.8°C; pH- 8.97; Conductivity (µS) - 176.0; Salinity (ppm) - 78.3; TDS (ppm) - 125.

Chara zeylanica Klein ex Willdenow, Mém. L'Acad. Roy. Sci. et Belles-Lett. Dep.L'Avén. Fréd. Guill. III, 86. 1805; Pal & al., Monogr. Charoph.. 105, 1962; R. D. Wood & Imahori, Monogr. Characeae 220.1965; R. D. Wood & Imahori, Monogr. Characeae 9.1964; D. Subram., Monogr. Indian Charoph. 93. 2002.

(Fig.4. A-I)

Plants monoecious; highly corticated, axes moderately stout; branchlets 8–11 in whorl, segments 7–10, corticated, longer than internodes; spine cells present on the main stem, well developed, lanceolate, solitary; stipulodes in two tiers, prominent, upper tier is longer than lower ones; bract cells 4–6 in number, well developed, longer than nucule, gametangia conjoint at branchlet nodes; oogonia

ellipsoidal, coronula well developed; oospore black at mature stage; antheridia present below the oogonia.

FeSEM observation: The mature oospore black ovoid structure, 9–10 prominent striae present, flange, well developed, fossa wall irregularly ornamented, some simple pores found on the fossa wall.

Dimensions: Plants up to 20 cm high; axes 447–600 µm in diameter; spines 280–332 µm in length; bract cells 1380–1410 µm long; oogonia 892–900 µm long, 390–450 µm wide, coronula 115–130 µm high; antheridia 320–330 µm in diameter.

Distribution: Freshwater, Andhra Pradesh, Arunachal Pradesh, Jharkhand, Maharashtra, Tamil Nadu, Uttarakhand, Uttar Pradesh, West Bengal.

Specimen examined: India, West Bengal, Bankura District, Mukutmonipur, 22°57'49.88"N; 86°47'19.62"E, Kailash Mondal, KM 1003(BURD).

Habitat parameters: Temperature (°C) - 22.4°C, pH - 6.95, Conductivity (µS) - 190.3, Salinity (ppm) - 87.4, TDS (ppm) -176.

Chara vulgaris L. Pal & al., Monogr. Charoph. 102, 1962; R. D. Wood & Imahori, Monogr. Characeae 73.1965; R. D. Wood & Imahori, Monogr. Characeae 2, 1964; John & al., The Freshwat. Alg. Fl. Brit. Isles: ident. guide freshw. terr. Alg., 755. 2011; Krause Süßwasserfl. Mitteleur., 18, Charoph. 81, 1997. (Fig. 5. A-H)

Plants monoecious, corticated bright green in colour, axes slender, stipules in two tiers, well developed, varying from globose to elongated cells; branchlets 7–10 in a whorls, branchlets segments 7–10, gametangia present at the base of branchlet and the segment of branchlet; bract cells 2–4 not exceeding the mature oogonia, globule and nucule are present at the same node in earlier condition, solitary, nucule are broad at the equatorial region; oospore dark brown to almost black in colour.

FeSEM observation: The striae are very prominent, 12–13 in number (Fig. 5-f), the striae and fossa are covered with small granulate, very tiny pores are occur in the fossa wall, small crest are present at the tip of the striae.

(Fig. 5-H)

Dimensions: Plants up to 26 cm high; axes 450–520 µm in diameter; globule 330–370 µm in diameter; nucule 650–700 µm long and 390–450 µm broad at equatorial region.

Distribution: Freshwater, Jharkhand, West Bengal, Uttar Pradesh.

Specimen examined: India, West Bengal, Purulia District, Jaichandipahar paddy field, 23°31'00"N; 88°40'51"E,

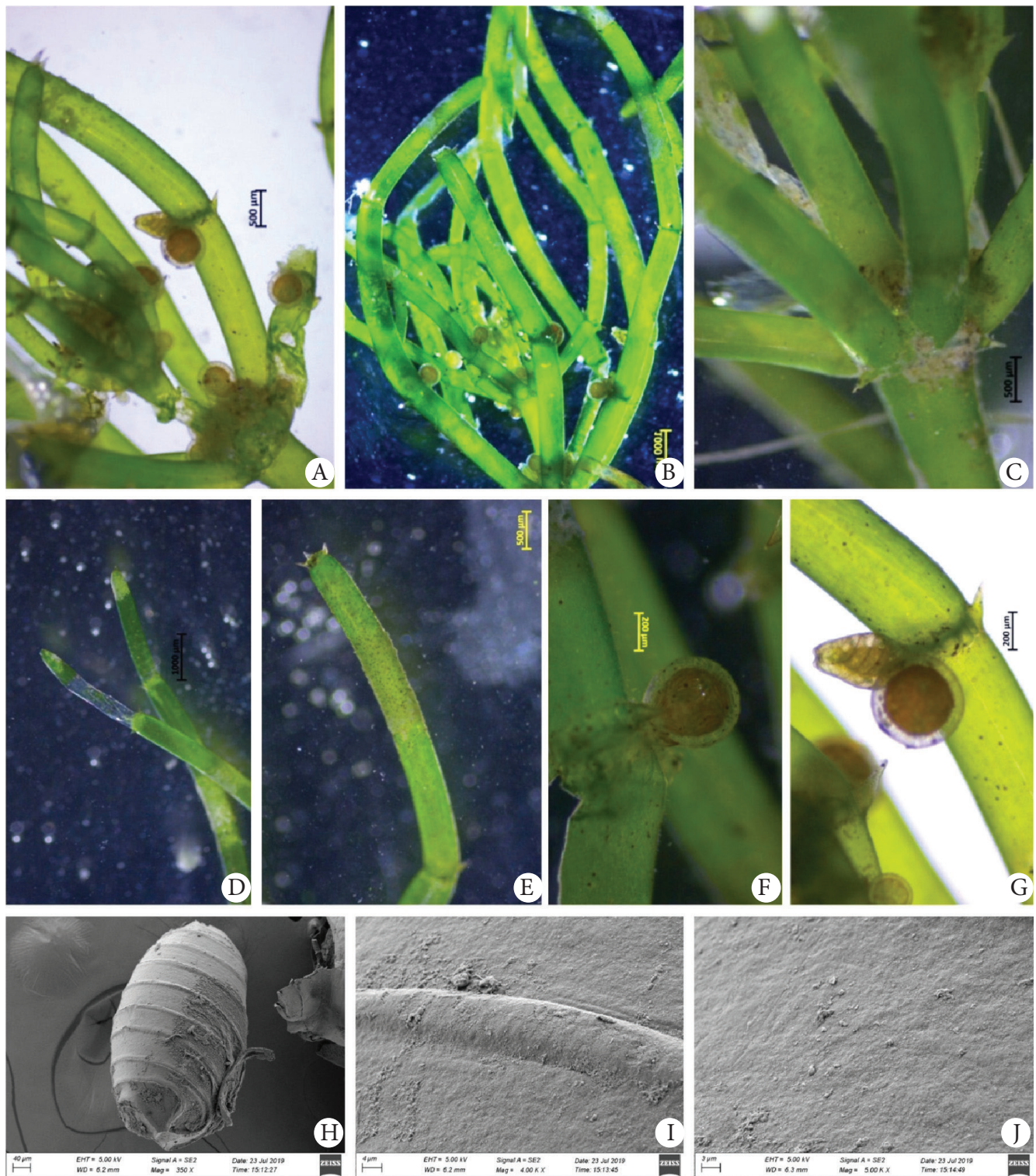


Fig. 3. *Chara socotrensis* Nordstedt in M. Kuhn Fig. A - B. Axis and base of whorl; C. Branch node with stipule; D-E. Dactyl; F. Antheridia; G. Antheridia and oogonia; H. A general view of oospore showing striae; I. Fossa and J. Showing smooth wall ornamentation.

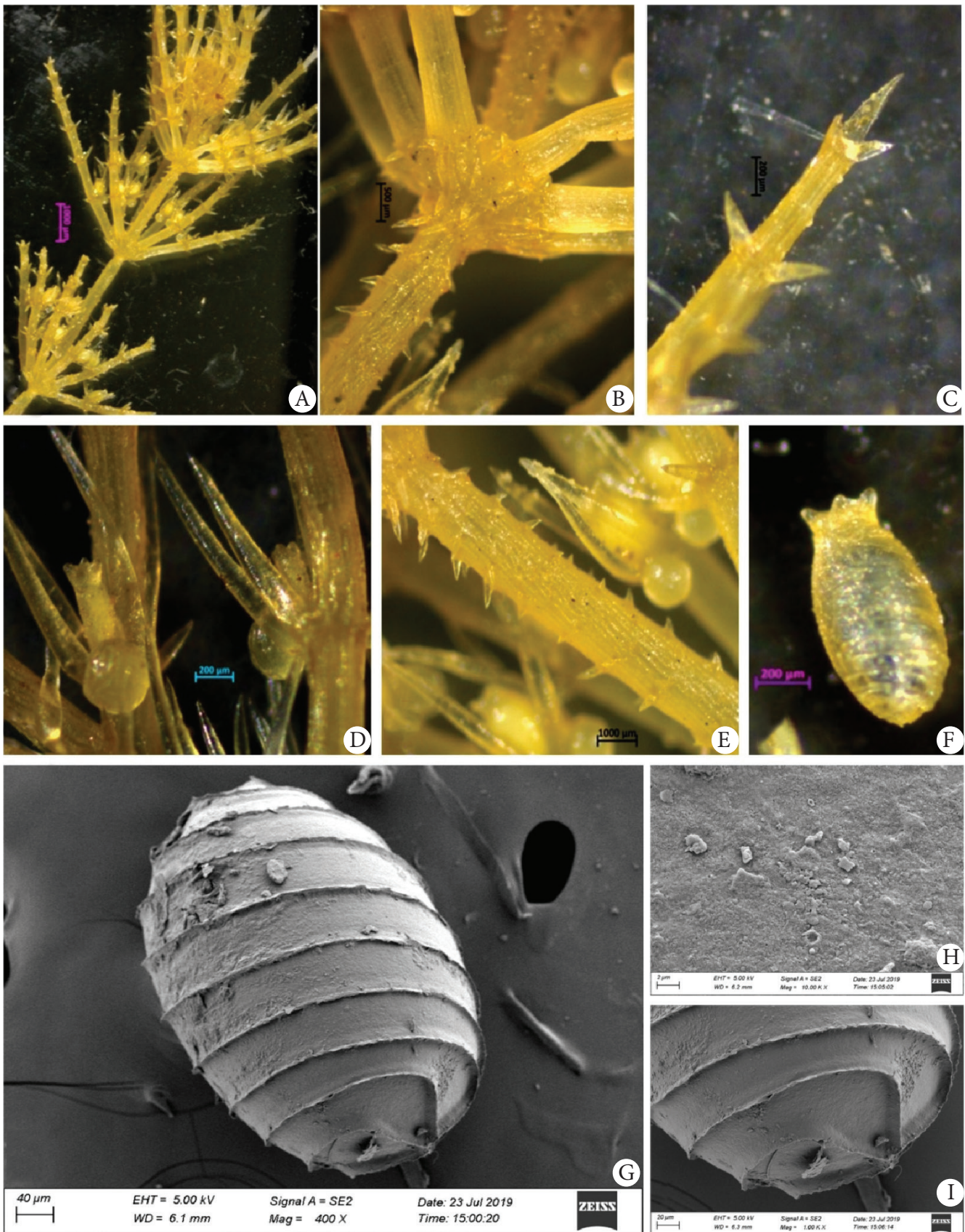


Fig. 4. *Chara zeylanica* Klein ex Willd. **A.** Showing a whorl of branchlets; **B.** A branch node with stipules; **C.** Dactyl; **D.** Well developed bract cells; **E.** Axis with spine cells; **F.** Mature oospore with coronal cells; **G.** A general view of an oospore having striae; **H.** Pustular ornamentation and **I.** Basal part of the oospore, fossa wall.

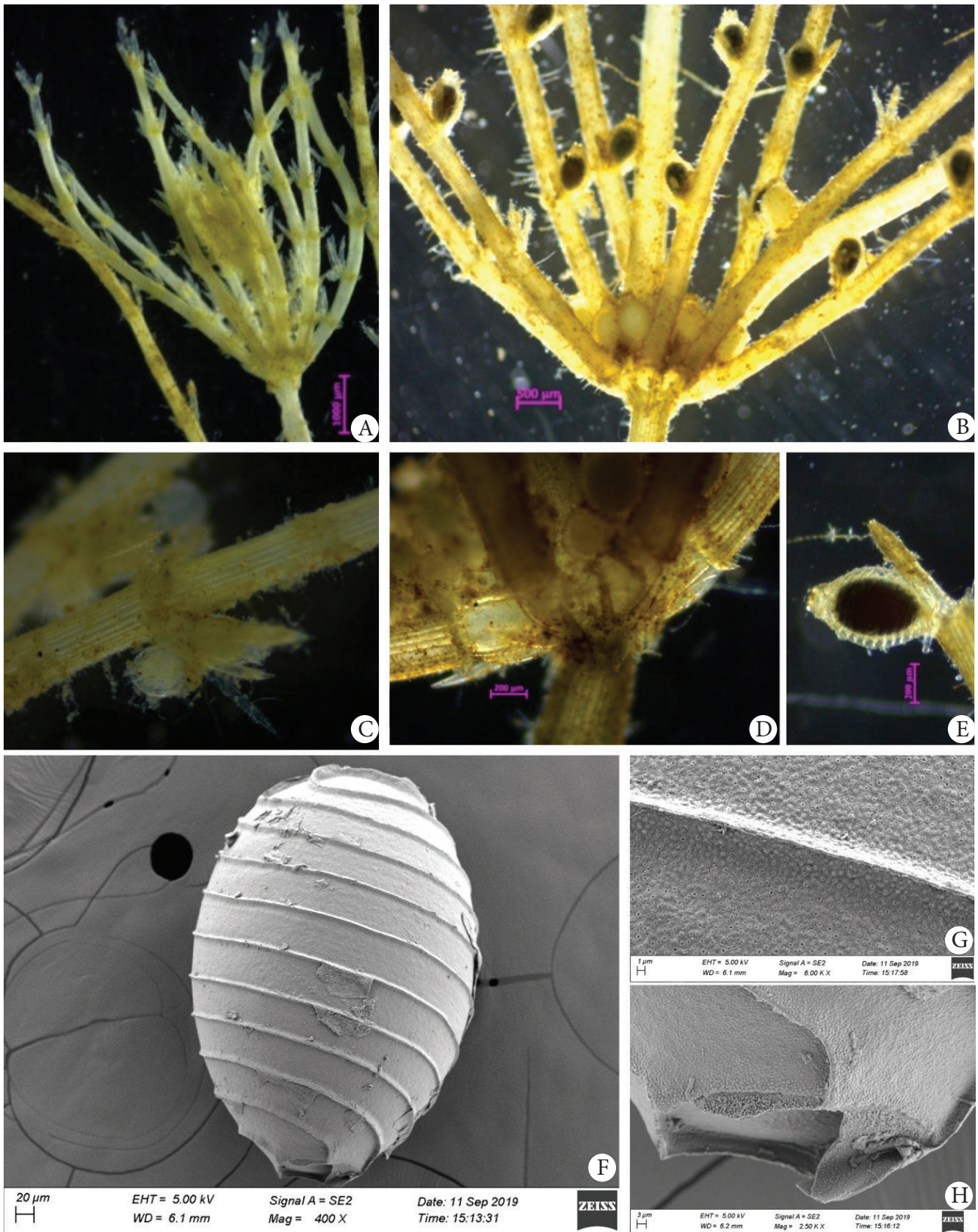


Fig. 5. *Chara vulgaris* L. A. Branchlet whorl; B. Basal node of branchlet whorl with oogonia; C. Gametangia; D. Stipule; E. Mature oogonia; F. Details of the oospore; G. Part of the oospore wall ornamentation and H. Basal portion of the oospore.

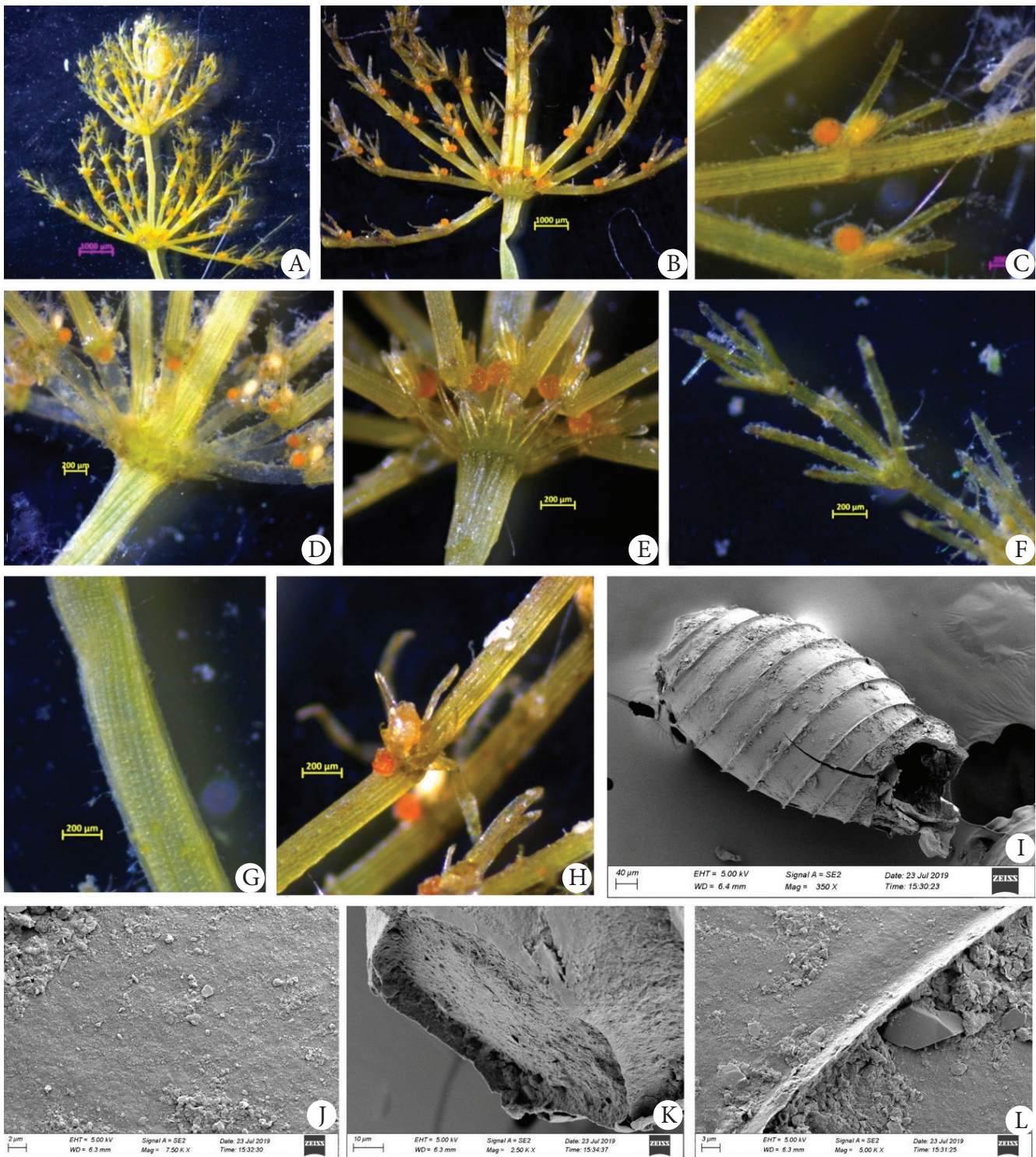


Fig. 6. *Chara hydropithys* Rchb. A-B. A whorl of branchlets C. Gametangia with bract cells; D. Ecorticated basal branchlet node; E. Well developed spine cells; F. Single branchlet with whorl of bracts; G. Corticated axil; H. Antheridia below the oogonia; I. General view of oospore having prominent striae; J. Smooth wall ornamentation; K. Basal portion of the oospore and L. Striae.

Kailash Mondal, KM 1004(BURD).

Habitat parameters: Temperature (°C) - 30.3°C; pH - 8.28; Conductivity (µS) - 320; Salinity (ppm) - 134; TDS (ppm) - 228;

Chara hydropithys Reichenbachin Mössl. Handb. Gewächsk. 1600. 1829; Pal & al., Monogr. Charophy.. 98, 1962; R. D. Wood & Imahori, Monogr. Characeae 302.1965; R. D. Wood & Imahori, Monogr. Characeae 146, 1964; D. Subram., Monogr. Indian Charoph. 90. 2002. (Fig. 6. A-L)

Plants monoecious, axes slender, highly corticated, bright green in colour, stipulodes in one tier, well developed, acute; branchlets 8–11 in a whorls, corticated but lower segment which are attached to the branchlet ecorticated, segments 5–7; gametangia are present at branchlet nodes; spine cells very small, solitary; bract cells well developed, 4–6 in number, exceeding the oogonia; globule and nucule present at the same node, in earlier globule are light brown in color, coronula cells are presents above the nucule.

FeSEM observation: Oospores cylindrical, striae well developed, 11–12 in number, a small pustular elevation present along with striae (Fig. 6-l), fossa smooth, ridges of the striae sharp (Fig. 6-l).

Dimensions: Plants up to 20 cm in high; axes 340–410 µm in diameter; Stipulodes 480–520 µm long globule 180–214 µm in diameter; nucule 293–315 µm long and coronula 60–70 µm in high.

Distribution: Freshwater; Maharashtra, Punjab & Tamil Nadu, Uttarakhand, Uttar Pradesh, West Bengal.

Specimen examined: India, West Bengal, Purulia District, Raghunathpur Rice field, 23°31'00"N; 88°40'51"E, Kailash Mondal, KM 1005(BURD).

Habitat parameters: Temperature (°C) - 22°C; pH - 8.97; Conductivity (µS) - 331; Salinity (ppm) - 142; TDS (ppm)- 197.

DISCUSSION

Since several species of *Chara* L. accumulate CaCO₃ due to its unique physiological adaptations related to CCM (Carbon Concentrating Mechanism), its distribution leads to interesting inferences. The habitats in several localities of West Bengal, like Bankura & Purulia support the growth of Charophyte species due to its characteristic limnological conditions, relatively undisturbed water bodies, and low human interference. The distribution record says that charophytes have not been adequately studied throughout the country.

On the other hand, a few reports are available on the molecular studies on Charophytes (Abrol & Bhatnagar, 2006). Considering the importance of the group thorough biodiversity study of Charophytes in the Country is needed. Habitats of West Bengal and other states supporting this group of algae are rapidly degrading. Various reports of this utilization are coming; for example, *Chara* L. have been projected in mosquito control (Gonzalves & Vaidya, 1963).

CONCLUSION

This work is focused on the detailed morphology of the species of *Chara* L. in light of modern equipments. From this beginning, it appears that there is a vast scope of exploration concerned with the morphology, biogeography, and biodiversity of Charophytes in India. Their limnological preferences may be utilized in the typification of habitats. The applied potentials as mentioned above are also extremely significant in present context.

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REFERENCES

- AGHARKAR, S.P. AND B. C. KUNDU 1937. Charophytes of Bengal. *J. Dept. Sci. Calcutta Univ.* 1(1): 1–23.
- ABROL, D. AND S.K. BHATNAGAR 2006. Biodiversity of few Indian Charophytes taxa based on molecular characterization and construction of phylogenetic tree. *African J. Biotech.* 5(15): 1511–1518.
- BALAKRISHNAN, S. AND G. RANI 2015. SEM Studies on the Compound oospore ornamentation of some Members of Charales from South India. *Int. J. Sci. Res.* 6(8): 2154–2160.
- CHAKRABARTY, R. AND S. RAY 2016. Chromosomal variations and cytotaxonomical considerations in two populations of *Nitella hyalina* (Charophyceae, Characeae) from West Bengal, India. *Phykos* 46(2): 14–19.
- CHATERJEE, P. 1975. Some additions to the Charophytes of West Bengal. *Bull. Bot. Soc. Bengal* 29: 105–109.
- CHATERJEE, P. 1976. Cytotaxonomical studies of West Bengal Charophyta: Karyotype analysis in *Chara braunii*. *Hydrobiol.* 49: 171–174.

- CHATERJEE, P. 1979a. *Chara fibrosa* var. *fibrosa* f. *longicoronata*, a new record for India and its cytology. *Curr. Sci.* 48(12): 545–547.
- CHATERJEE, P. 1979b. Karyological investigation of *Chara wallichii* A. Br. from West Bengal. *Cell & Chromosome Newsletter* 2(1): 21–23.
- GONZALVES, E.A. AND B. S. VAIDYA 1963. On the larvicidal properties of charophytes. *Hydrobiol.* 21: 188–192.
- GUPTA, R.K. 2012. *Algae of India, Volume 2. A checklist of Chlorophyceae, Xanthophyceae, Chrysophyceae and Euglenophyceae.* Botanical Survey of India. Kolkata.
- HALDER, N. AND S.N. SINHA 2016. *Nitella flagelliformis* A. Br. and *Chara braunii* Gm. two new records of Charophytes from freshwater bodies in Hooghly district, West Bengal, India. *Trop. Plant Res.* 3(2): 354–340
- JOHN, D. M., B. A. WHITTON AND A. J. BROOK 2011. *The Freshwater Algal Flora of British Isles: An Identification guide to Freshwater and Terrestrial Algae.* 2nd Edition, Cambridge.
- KRAUSE, W. 1997. *Süßwasserflora von Mitteleuropa, Band 18, Charales (Charophyceae).* Gustav Fischer, Jena, Germany.
- MUKHOPADHYAY, A. AND S. RAY 1995. Karyology and cytotaxonomy of *Chara wallichii* and *Chara braunii* from West Bengal, India. *Acta Bot. Gall.* 142(7): 787–791.
- PAL, B. P., B.C. KUNDU, V.S. SUNDRALINGHAM AND G. S. VENKATARAMAN 1962. 'Charophyta.' Indian Council of Agricultural Research: New Delhi.
- RAY, S. AND P. CHATTERJEE 1986. Karyotype analysis in *Chara braunii* Gm. f. *braunii* (Characeae). *Nucleus* 29:33–36.
- RAY, S. AND P. CHATTERJEE 1987. Karyomorphological Investigation of Three Population of *Chara corallina* var. and f. *corollina* in west Bengal. *Cytologia* 52: 455–458.
- RAY, S. AND P. CHATTERJEE 1988. Cytotaxonomical study of West Bengal charophyta: a new chromosome number count of *Nitella stuartii* and its karyotypes. *Acta Bot. Neerl.* 37(4): 523.
- RAY, S. AND P. CHATTERJEE 1994. Cytotaxonomy of Charophytes in West Bengal, India: Karyotype analysis of three subspecies of *Nitella furcata*. *Acta Bot. Gall.* 141(1):43–48.
- SUBRAMANIAN, D. 2002. *Monograph on Indian Charophyta.* Bishen Singh Mahendra Pal Singh, Dehradun.
- WOOD, R. D. AND K. IMAHORI 1964. Vol. II, *Inconograph of the Characeae.* Verlag Von J. Cramer, Weinheim, West Germany
- WOOD, R. D. AND K. IMAHORI 1965. A revision of Characeae: Vol. I, *Monograph of the Characeae.* Verlag Von J. Cramer, Weinheim, West Germany.