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Fine structure of the red seaweed *Hypnea musciformis* (Wulf.) Lamour. (Hypneaceae, Rhodophyta)

K. Sivakumar*

Department of Botany, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, India,

ABSTRACT

Characters of the red seaweed *Hypnea musciformis viz.*, cellular structure of the thallus, cuticle, pit plug and cell wall ultrastructure and morphology of plastids, floridean starch, Golgi bodies, mitochondria and nucleus are described. Anomalous plastids with thylakoid disorganization were found in medullary cells. The significance of this thylakoid disposition is unclear. The golgi complex was found to lay in close proximity to the nucleus. This is a pioneer study that focuses on the fine structure of a red alga recorded in India.

Keywords : anomalous plastids, cell wall, floridean starch, *Hypnea musciformis*, pit connection.

INTRODUCTION

The genus *Hypnea* (Gigartinales, Rhodophyta) includes 50 species distributed in warm waters (Masuda *et al.*, 1997). Many of them are of economic importance in several countries (Critchely and Ohno, 1998). In Brazil, *Hypnea musciformis* (Wulfen in Jacgu.) J.V. Lamour is the main raw material for carrageenan production (Oliveira, 1998). Morphological and anatomical studies of different species of *Hypnea* were made by Diannelidis and Kristen (1988) and Rama Rao (1992). and comprehensive information on its various aspects were given by Dixon (1973) and Cole and Sheath (1990). In general, the cell wall of red algae is made up of an inner microfibrillar framework and the outer of more amorphous component consisting of mucilage (Kloareg and Quadrano, 1988; Delivopolous and Diannelidis, 1990). The reserve food material, floridean starch, occurs as oval, ellipsoidal or basin like electron translucent bodies (Cole and Sheath, 1990). Aghajanian (1979) reported that the starch mitochondrion-dictyosome association might be important in rapid utilization of starch to power the activity of Golgi.

Plastids of red algae are stellate, discoid, spherical or highly lobed structures (Pueschel and Cole, 1985). Occurrence of osmiophilic globules in the plastids of red algae is not uncommon (Dixon, 1973; Cole and Sheath, 1990). These globules possess phenolic substances (Hill *et al.*, 1980). Plastids have unstacked parallel thylakoids. Phycobilins occur on the surface of the thylakoidal membrane (Cole and Sheath, 1990). Rough and smooth endoplasmic reticulum of typical eukaryotic form is present in red algal cells. Generally, they occur in the cell periphery and around the nucleus. Endoplasmic reticulum forms direct connection with the plasmalemma (Goff, 1982). Pit plugs in the Florideophycideae and 'conchocelis phase' of Bangiales have been studied by various workers (Ramus, 1969; Lee, 1971 and Pueschel, 1977). A fine structure survey of pit plugs with their taxonomic implications was carried out by Pueschel and Cole, (1982). In contrast, information about fine structural aspects of the genus *Hypnea* is scarce. This is the first time description of the fine structure of vegetative thalli in this genus *Hypnea*, based on the studies on field grown thalli from natural population of *Hypnea musciformis* collected from the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS

Vegetative thalli of *Hypnea musciformis* were collected from areas adjacent to the Murugan temple, Tiruchendur (Lat. 8°30'N; Long. 78°8'E) South East Coast of Tamil Nadu (Fig. 1), at a depth of 0.5-1.0 m during low tide. They were cut into 1-2 mm bits and fixed on the spot with 3% glutaraldehyde in 0.1 M Na Cacodylate buffer (pH 7.0) containing 0.25 M sucrose as an osmoregulator and kept over night at 4°C. Then they were washed thoroughly with buffer and dehydrated in an upgraded series of acetone (Hayat, 1986). Infiltration and embedding were performed with araldite. Ultra thin sections of 60-90 nm were taken with a Reichert Jung Ultramicrotome using glass knives. The sections were double stained with uranyl acetate and lead citrate (Reynolds, 1963). Electron Micrographs were taken under CM10 Philips transmission Electron Microscope.

RESULTS

Morphology of the thallus

Vegetative thalli of *H. musciformis* are very bushy, often entangled; texture is some what fragile, fleshy; colour is dull purplish red; the bases are disc like, ill defined; errect branches are 10-20 cm tall, about 1-2 mm diameter; the leading branches are divided several times and abundantly covered with short branches on ramuli;

*Corresponding Author
email: *kshivam69@yahoo.com*

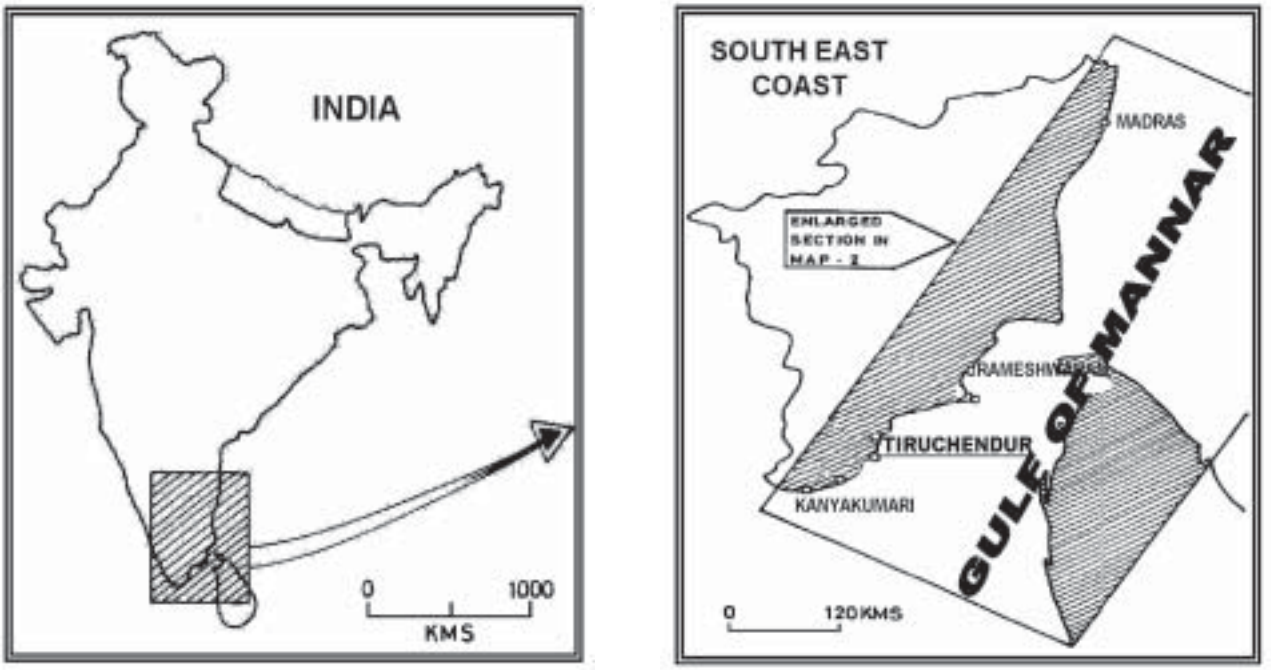

Figure 1. Map showing the location of the study area

terminal portion of the branches twisted as tendrils. The thallus is uniaxial in construction and the apex is terminated by a single apical cell (Pl-I; Figs. 1,2,4).

1. Light microscopy

The outer cortical region consists of a single layer of small densely pigmented cortical cells. Each cell is about 10-25 µm in diameter. The subcortical region consists of 1-2 layers of cells that are 30-50 µm in diameter. The medullary region consists of 2-3 layers of larger cells. Each cell is measuring about 100-300 µm in diameter. The cortical, sub cortical and medullary cells are progressively larger in size towards the centre. At the centre a distinct axial cell is present which is about 25-40 µm in diameter (Pl-I; Fig. 3).

2. Transmission electronmicroscopy

In its ultrastructure, the cell wall had two distinct layers; the outer amorphous electron dense layer 1.0 to 1.5 µm thick and the inner electron translucent layer 1.75 to 2.50 µm thick (Pl-I; Fig.5); cross wall is about 0.3 to 0.5 µm thick. Each cell is with dense cytoplasm packed with spherical plastids.

3. Cortical cell

Cortical cells are irregular in shape, without any vacuole. Floridean starch grains are freely distributed throughout the cytoplasm. Each cortical cell has 10-15 starch grains that are small and are spherical to ovoid structures (Pl-II; Fig. 6).

4. Medullary cell

Ultrastructurally, the cells of the medullary region are highly irregular and have vacuolated. Plastids linked to each other and forming chain like structures. Each plastid is 5.0-10.0 µm wide. The vacuolar sap is without any fibrillar or membranous material (Pl-II; Figs. 6 and 7). Floridean starch granules are frequently present near the plastids and endoplasmic reticulum. They are freely distributed throughout the cytoplasm and are 3-5 µm long and 6-10 µm wide (Fig. 8). Discoid plastids that occupy almost the entire volume of the cell, have a single peripheral encircling thylakoid and a few inner parallel thylakoids (Pl-II Fig. 7). These plastids are parietally arranged in close association with the mitochondria.

5. Pit connection

Pit connections or cytoplasmic connections had a pit plug and pit core (Pl-II; Fig. 9) with an electron dense layer present near the margin of the plug core. The length and width of pit plugs are 1.5 to 2.5 µm and 0.3-0.5 µm, respectively. The plug core is encircled by a cap membrane. Cap membranes are observed in the pit plugs of all cells; but they were not clearly defined in the flat surfaces of this structure. Immediately underneath, there has been a well defined and clear layer, conspicuously distinct from the rest of the core. Outside the cap membrane, no outer cap layer is found. Cytoplasmic fusion between two adjacent cells has also been observed (Pl-II; Fig. 10).

The plastids (2-4) were peripheral, with many central thylakoids (4-7) and are encircling thylakoid. Large vesicles derived from Golgi bodies were located against the plasmalemma (Pl-II; Fig. 12), with, some of them fusing with the plasma membrane. Mitochondria are spherical in shape, generally situated near the plastids and the mitochondrion membrane is 6-10 nm thick. Each mitochondrion is with 10-15 cristae. Nucleus is situated at the periphery of the cytoplasm, spherical or ovoid in shape due to vacuolation, 20-25 nm thick and has no folding of the outer nuclear membrane. The nucleus has a typical morphology with distinct nucleolus in the center (Pl-II; Fig. 12). Nucleolus is 2-5 µm in diameter.

The development of the vacuolar system during cell differentiation is related to the intense hydration of the cytoplasm. The phenomenon of membrane coalescence is initiated by a protrusion from one vacuole deforming the adjacent vacuole. Medullary cells are with a large central vacuole filled with confluent material. As a result of high vacuolation, cytoplasmic inclusions become compressed and form a thin layer (Pl-II; Fig. 11).

DISCUSSION

Vegetative thallus of the red alga *Hypnea musciformis* displayed a uniaxial construction of growth with a clear distinction of cortical and medullary layer similar to other genera of Gigartinales (Bold and Wynne, 1985; Coomans and Hommersand, 1990; van den Hoek *et al.*, 1995).

Cuticle is a widespread feature in red algae (Hanic and Craigie, 1969; Gerwick and Lang, 1977; Craigie *et al.*, 1992; Flores *et al.*, 1997) and protects the thallus during adverse conditions, such as desiccation, herbivore grazing and bacterial degradation (Gerwick and Lang, 1977). Usually, the cuticle is composed by a multilayered structure (Brawley and Wetherbee, 1981; Hommersand and Fredericq, 1987; Foltran *et al.*, 1996), but *H. musciformis* presents a single and unstratified one, like in *Porphyra umbilicalis* (L.) and *J. agardh* (Hanic and Craigie, 1969). One adaptative advantage, if any, of the multilayered cuticle is that it could be more resistant to the marine environment than the monolayered one.

The cell wall showed a distinct inner cellulosic layer and an electron dense layer which consisted of an amorphous matrix (Dixon, 1973). Brody and Vatter (1959) reported that *Porphyridium cruentum* showed a fine fibrillar structure and appeared to be uniform rather than differentiated. In the present investigation the cell wall of *H. musciformis* showed two distinct wall layers, the outer electron dense and an inner electron translucent layer. Fluoridean starch grains are reported to often occur in proximity of the nucleus or other structures when pyrenoids are absent (Lee, 1974; Borowitzka, 1978; and Tsekos, 1982). In the present study, floridean starch granules were found to occur in groups in the cortical cell. Aghajanian (1979) observed that the starch-mitochondrian-dictyosome association might be important in rapid utilization of starch to enhance the activity of Golgi. In the present study also, Golgi complex and plastids were seen in close proximity to the nucleus.

The thylakoid system of many chloroplasts in medullary cells of *G. torulosus* suffered a notable disorganization like in other red algae (Hara and Chihara, 1974; Hara, 1975; Borowitzka, 1978). In *Porphyra leucosticta*, a similar phenomenon was caused by low levels of illumination, both in field and in culture conditions (Sheath *et al.*, 1977). However, in the case of *Griffithsia pacifica* Kylin (Koslowsky and Waaland, 1987) the chloroplast deterioration was explained as a result of a cytoplasmatic incompatibility reaction. In the present study, plastids were found to be linked to each other and form chain like structures in the cortical and medullary regions. The significance of the thylakoid fragmentation and the presence of tubular units in *H. musciformis* is still unclear. The occurrence of plastoglobuli in plastids of (Fig. 9) macroalgae which possibly contain phenolic substances, has been reported by Hill *et al.* (1980). In the present study also, plastids frequently possessed plastoglobuli. They occurred in 2-5 rows each having 4-6 globules.

A distinctive feature of the Florideophycideae is the presence of pit connections, which are neither a pit nor an inter cellular connection but a distinct lens-shaped plug held within a septal aperture by its equatorial groove (Ramus, 1971). Pit connections are membrane bounded plugs with caps that fit smugly against the plasmalemma, thus, sealing off the plug from the cytoplasm. In *Corallina officinalis* the pit plug has two cap layers and the layers are not separated by a cap membrane. In *Palmaria mollis* the pit plug has two cap layers but a cap membrane is present between them (Pueschel, 1989; Cole and Sheath, 1990). The cap membrane appears to provide a membrane barrier between the cells of transport and cellular communication (Pueschel and Magne, 1987). In many species, the plug core shows a gradual increase of electron dense substances from the centre to the margin. Sometimes a distinct dark band is present near the margin of plug core (Pueschel, 1989). In the present study, the pit connections of *H. musciformis* possessed a cap membrane on the plug core as is typical of the *Gigartinales* and the margin of the plug core contained a distinct band of electron dense region.

Vacuoles were most prominent in differentiated cells. The vacuolar envelop is a unit membrane like the plasmalemma (Buvat, 1971). Morphologically, the differentiation of provacuoles into vacuoles is characterized by the continuous inflation and extensive fusion of provacuoles / small vacuoles (Matile and

PLATE – I

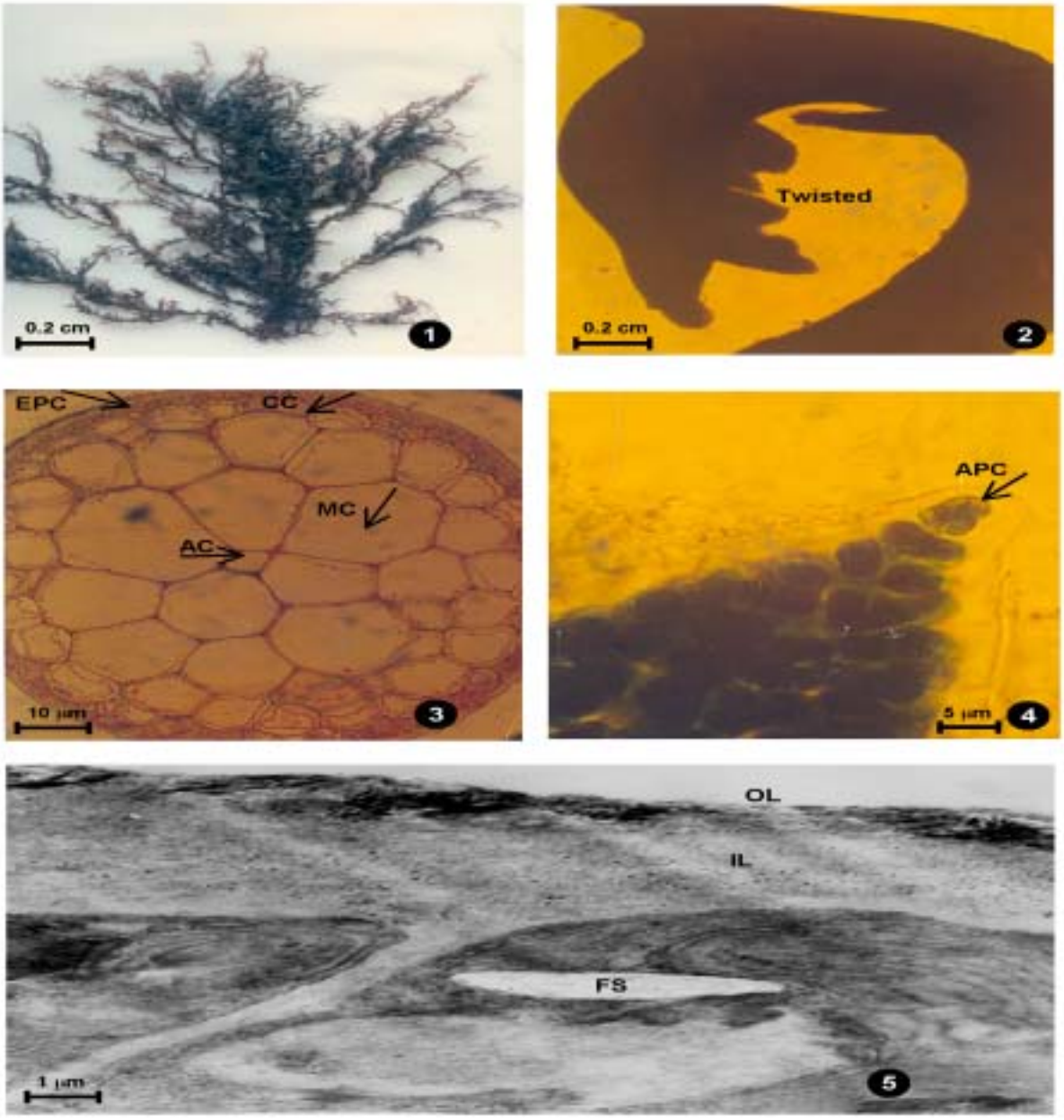

Figure 1. Morphology of Hypnea musciformis Scale bar =0.2cm.

Figure 2. Portion of the branches twisted as tendrils. Scale bar = 0.2cm.

Figure 3. Light photomicrograph of semi thin section of the thallus shows epidermal, cortical and medullary cells. Scale bar = 10µm.

Figure 4. Apex is terminated by an single apical cell. Scale bar = 5 µm.

Figure 5. The fibrillar and the amorphous matrix of the cell wall are both clearly discernible. Scale bar = 1µm.

Epidermal cell, CC - Cortical cell, MC - Medullary cell, AC - Axial cell, APC - Apical cell, OL - outer layer

IL - Inner layer, FS - Floridean starch grains.

PLATE – II

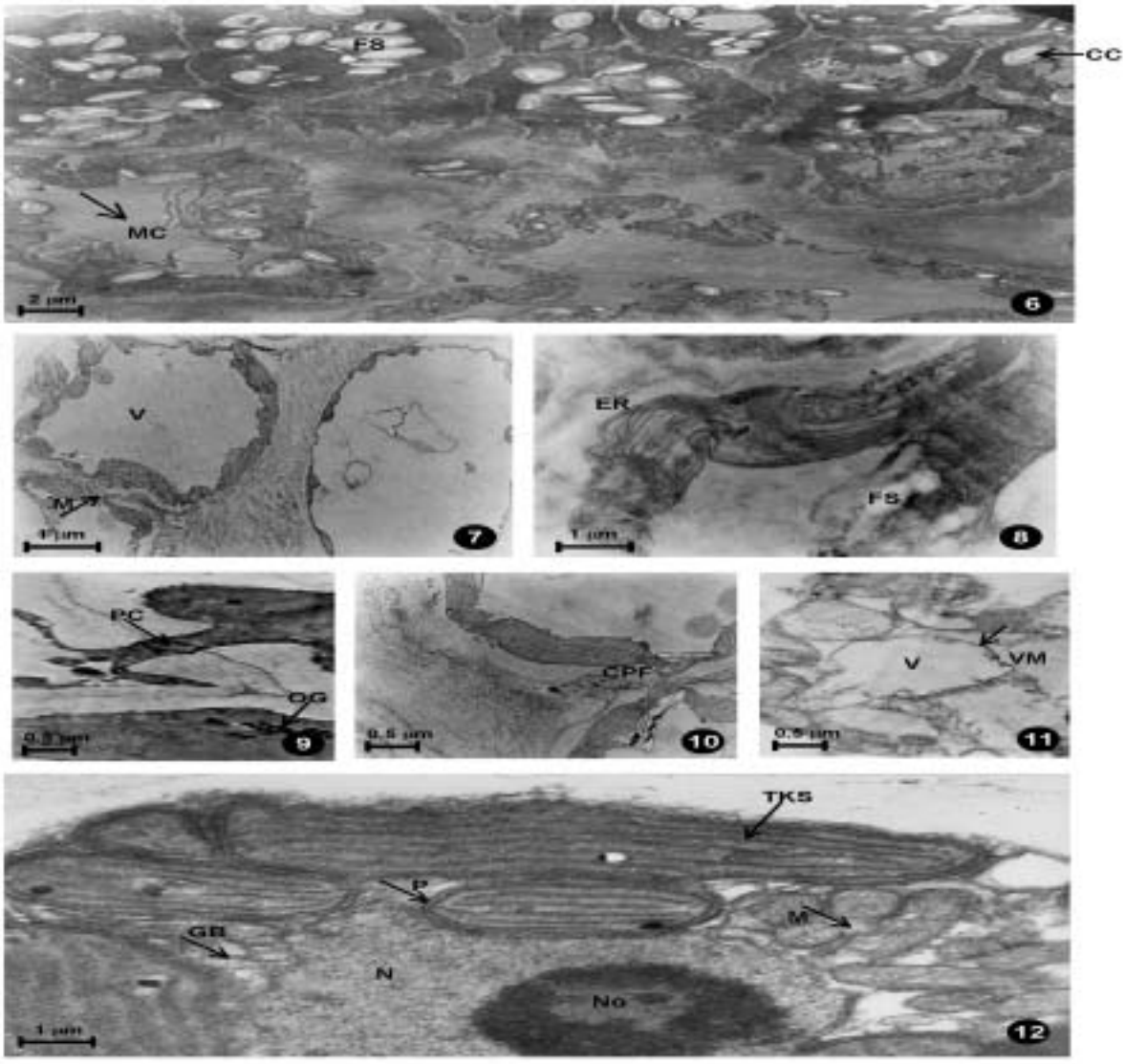

Figure 6. Cortical cell showing peripherally arranged plastids and Floridean starch grain. Scale bar = 2 µm.

Figure7. Enlarged portion of medullary cell showing irregularly arranged various shaped plastids.

Scale bar = 1mm.

Figure 8. ER strands frequently widenout locally into vesicles or chains of vesicles, these are close association with mitochondria and floridean starch grain. Scale bar = 1µm.

Figure 9. Pit connection shows a band of distinct electron dense region at the margin of plug core. The plug core encircled by a cap membrane. Scale bar = 0.5µm.

Figure 10. Cytoplasmic fluid between two adjacent cell was noticed. Scale bar = 0.5µm.

Figure 11. Vacuoles are formed by continuous inflation and coalescence is initiated by a protrusion from one vacuole deforming another adjacent vacuole. Scale bar = 0.5µm.

Figure 12. Nucleus has typical morphology with distinct nucleolus in the centre and also number of plastids and mitchondria were noticed. Scale bar = 1µm.

FS - Floridean Starch grains, MC - Medullary cell , CC - Cortical cell, ER - Endoplasmic reticulum, OG - Osmiophilic globules, CPF - Cytoplasmic fluid, V - Vacuole, VM - Vacular membrane, GB - Golgi bodies, N - Nucleus, P - Plastids, M - Mitochondria, No - Nucleolus, TKS - Thylakoids.

Moor, 1968). Multinucleus condition is common in multicellular red algae. However, species of Corallinales, Cryptonemiales and Gigartinales show uninucleate condition. In the present study *H. musciformis* was found to possess uninucleate cells. In order to understand the origin of this degenerative process of the plastids and certain organelles additional studies of this red seaweed that focus on its growth under controlled conditions are needed.

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