DNA Barcoding, phylogeny and phylogeography of green seaweed *Ulva* from Indian subcontinent

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BY

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JULY, 2019

CERTIFICATE

I declare that the dissertation entitled "**DNA Barcoding, phylogeny and phylogeography of green seaweed *Ulva* from Indian subcontinent**" has been prepared by me under the guidance of Dr. Felix Bast, Assistant Professor, Centre for Biosciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

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Genus *Ulva* (*Ulvales*, Chlorophyta) consists of green intertidal marine algae and are cosmopolitan in distribution. *Ulva* exists in compressed filamentous, tubular and foliose forms. It may be monostromatic or distromatic, branched or unbranched and multiseriate. In this study, 48 isolates were analyzed from different marine, estuarine and limnetic habitats of India. Samples were amplified at five different loci (ITS1, 18S, tufA, atpB, and rbcL). Eleven different species of *Ulva* were revealed in this study, including one new species. Two species were compressed filamentous (*Ulva intestinalis* and *Ulva uniseriata* sp. nov.), four species were tubular (*Ulva prolifera*, *Ulva paschima*, *Ulva sapora*, and *Ulva shanxiensis*), while five species were foliose (*Ulva fasciata*, *Ulva reticulata*, *Ulva ohnoi*, *Ulva taeniata* and *Ulva linza*). *Ulva sapora* (reported earlier only from Australia) and *Ulva shanxiensis* (reported earlier only from China) were reported for the first time from Indian coast. In addition, this study reported the existence of *Ulva shanxiensis*-previously thought to be a freshwater species-from a marine habitat for the first time in the world. This study also revealed the existence of a new estuarine algal species, *Ulva uniseriata* sp. nov., based upon morphological and molecular synapomorphy. This is the first species in genus *Ulva* with uniseriate filamentous morphology. Red microalgae *Sahlingia subintegra*, *Erythrocladia irregularis*, *Erythrotrichia carnea* and *Porphyridium* were also found to be growing on various *Ulva* species as either epiphytic or endophytic algae; none of these epi-endophytic red microalgae had previously been reported from Indian coastal regions. In addition, all of the generated data including DNA sequences and voucher collections were made accessible via public repositories: DBIndAlgae, NCBI-GenBank, and Central National Herbarium of Botanical Society of India.

Pooja Rani (Dr. Felix Bast)

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S. No.	Abbreviation	Full Form
1.	BLAST	Basic Local Alignment Sequence Tool
2.	Bp	Base pairs
3.	BI	Bayesian Inference
4.	dNTPs	Deoxyribonucleotide triphosphates
5.	DNA	Deoxyribonucleic acid
6.	NCBI	National Centre for Biotechnology Information
7.	ML	Maximum likelihood
8.	nrITS	Nuclear internal transcribed spacer
9.	SDS	Sodium dodecyl sulphate
10.	Ml	Microliter
11.	mM	Millimolar
12.	PCR	Polymerase Chain Reaction
13.	^{0}C	Degree Celsius
14.	Ng	Nanogram
15.	U	Unit
16	V	Volt
17.	MUSCLE	Multiple Sequence Comparison by Log- Expectation
18.	PSU	Practical Salinity Unit

DNA barcoding, Phylogeny and Phylogeography of green seaweed *Ulva* from Indian subcontinent.

Chapter 1: Introduction

Genus *Ulva* (Ulvales, Chlorophyta) consists of green intertidal marine and freshwater algae. *Ulva* exists in tubular and foliose form. Usually *Ulva* is monostromatic or distromatic, branched or unbranched, but all the described species of this genus were multiseriate (Liu *et al*., 2010). Currently, 131 *Ulva* species are recognized worldwide as distinct taxonomic entities (Guiry and Guiry, 2018). Foliose thallus of *Ulva* was firmly attached to the hard substratum with the aid of rhizoids whereas filamentous thallus attached to the substratum either with the rhizoids or freely floats in the water (Melton III *et al*., 2015; Tan *et al*., 1999). It is very challenging to identify the species only based on morphology due to very limited defining features.

Ulva shows high morphological plasticity and frequently changes the morphology of foliose and tubular thallus under the influence of environmental factors. It is hard to classify *Ulva* on the basis of limited morphological traits that were used in classical species concepts. According to classical morphological species concept, a species is characterized by body shape and structural features, which is not reliable for *Ulva* (Schnittler and Mitchell, 2000). Biological species concept is the most widely accepted species concept because it explained that the species are group of interbreeding population that is reproductively isolated from other groups of the population (Donoghue, M.J., 1985). There are indefinite numbers of cryptic and pseudo-cryptic species existing within the literature. Cryptic species are the group of species that contains morphologically identical individuals but belong to different species. Pseudo-cryptic species are species among which morphological differences recognized upon re-examining the separated lineages using molecular data (Hofmann *et al*., 2010). Cells of *Ulva* are commonly arranged in longitudinal and transverse rows, but some are irregular. Cells are rectangular in shape with rounded corner but rarely polygonal. Chloroplast either lies completely inside the cell or restricted to one side of the cell (Coat *et al*., 1998; Dion *et al*., 1998). The number of pyrenoids varies in different *Ulva* species. Malta *et al*., (1999)

explained that the number of pyrenoids in cells is not useful for species identification because it might be easily changeable under different environmental conditions (Malta *et al*., 1999). Life history of *Ulva* shows both sexual and asexual reproduction. Rate of growth of *Ulva* depends on various biotic and abiotic factors. Abiotic factors include seasonal variations, salinity and temperature of the surrounding environment. Biotic factors include the interaction with the surrounding bacteria and diatoms. Different bacteria control the growth of thallus and diatoms control the folding of the foliose thallus (Luo *et al*., 2012).

Avise and Wollenberg defined phylogeography as a study of historical events at spatial and temporal context that might be responsible for the current geographic distribution and evolution of the organisms (Avise and Wollenberg, 1997). This terminology is used to describe the geographically distributed genetic signals within the population. Phylogeography combines the phylogenetics and classical population genetics (Teske *et al.*, 2011). Different factors, such as migration, population bottlenecks, and biogeographical barriers influence the distribution of the population (Avise and Wollenberg, 1997). Phylogeographic studies of widely distributed species are always an area of keen interest for an evolutionary scientist. Different geographical barriers can be identified which might have led to the reproductive isolation among the populations. New species may arise due to the establishment of reproductive barrier or isolation (Baker, 2005). Climatic factors produce changes in gene frequencies within the gene pool of a population. Various changes in chemical composition take place due to variation in environmental factors. Such changes were instantaneously expressed in the changed behavior of a conserved gene. These circumstances may lead to the formation of multiple alleles at a given locus (Vamosi *et al.*, 2009).

Phylogeography helps in explaining the extinction, recolonization and patterns of distribution of species (Emerson *et al.*, 2011). Even in presence of biogeographical barriers, size of the population and its dispersal potential throughout the geographical area are strong determinants of the phylogeographic distribution of species (Manel *et al.*, 2003). Migration is regarded as gene flow between the populations of a species.

Biodiversity or biological diversity defined as the variety of living beings in a particular area. Marine biodiversity includes coastal and marine animals, plants their species, habitat, ecology and genetic diversity. It is related to the number and abundance of plants, animals and microorganisms in the marine environment (Wafar *et al.*, 2011). The oceans are a significant source of oxygen for our planet and were instrumental in the capture and storage of carbon dioxide (Law and Owens, 1990). An ocean provides the suitable ecosystem for the growth of all marine organisms (Worm *et al*., 2006). Seaweeds exist in coastal as well as deep-sea regions. There are about 9, 300 species of seaweeds (Huisman *et al.*, 1998). On basis of pigment in thallus, they are divided into red algae (6,000 species), brown algae (1,800 species) and green algae (1,500 species). Indian coastline (Rath and Adhikary, 2006) harbor 844 species distributed among 217 genera. Rhodophyta (434 species), followed by Chlorophyta (216 species), Phaeophyta (191 species) and Xanthophyta are representing only three species. Most of the reports are based on morphology.

There is very limited information on DNA barcoding and phylogeographic studies on *Ulva* species from Indian coastal regions. Apart from the report of *U. paschima* by Bast *et. al.* (2014a), there is no phylogeographic study of *Ulva* from Indian coasts. This study attempts to analyze the morphology of thallus and arrangement of cells of the *Ulva* throughout the east and west coast of India. DNA barcoding was performed using nuclear (ITS, 18S), chloroplast (rbcL, tufA) and mitochondrial (atpB) region for accurate taxonomic identification.

Chapter 2: Review of Literature

2.1 Introduction to *Ulva*

Ulva is a dominant member of rocky intertidal and subtidal habitats (Hayden and Waaland, 2004). Foliose form of *Ulva* is known as “Sea lettuce” while tubular form referred earlier as genus “*Enteromorpha*”, which is now merged with genus *Ulva*, is known as “Green laver” (Hayden *et al*., 2003; Hofman *et al*., 2010; K. Nisizawa, 1987; Wolf *et al*., 2012). Chlorophyta vary in size and structure of the thallus from unicellular motile to colonial flagellates to colonial, coccoid and filamentous forms and macroscopic multicellular seaweeds (Lewis and McCourt, 2004). Chlorophyta (Reichenbach, 1828) consists of three classes, i.e., Ulvophyceae, Trebouxiophyceae and Chlorophyceae. Chlorophytesare evolved from unicellular marine planktonic Prasinophyte algae in the Neoproterozoic era, 729 MYA (Becker, B. 2013). Chlorophyceae and Trebouxiophyceae grow in freshwater and terrestrial habitat while Ulvophyceae dominates in the marine habitat. Genus *Ulva* catalogued under order Ulvales (Blackman and Tansley, 1981). Ulvales are multicellular, distromatic, green to pale green, soft, slimy surface, cup-shaped chloroplast and multiple pyrenoids in thallus. Ulvales possess tubular morphology (as in *Ulva intestinalis*) and membranous distromatic blade (as in *Ulva fasciata*) (Tan *et al*., 1999).

Ulva prolifera and *U. lactuca* are regarded as a bio-indicator of pollution in the coastal zone (Kirkendale *et al*., 2013; Wang *et al*., 2010). *Ulva intestinalis* can be used for the production of ethane (Plettner *et al*., 2005). *Ulva prolifera* species plays a significant role in bioremediation (Lawton *et al*., 2013). *Ulva reticulata* acts as a bio-filter (Masuya *et al*., 2006).

2.2 Morphology of *Ulva*

Ulva species exhibits high morphological plasticity, i.e. the ability of one genotype to produce more than one phenotype when exposed to the different environmental conditions, particularly with variation in salinity (Wang *et al*., 2010). Some reports suggest the role of epiphytic bacteria in the morphological switch (Burke *et al*., 2009; Joint *et al*., 2007; Marshall *et al*., 2006). Studies had

revealed that *Ulva* fails to form its typical morphology in the absence of the appropriate bacteria. It merely proliferates into an undifferentiated clump of callus cells (Burke *et al*., 2009; Joint *et al*., 2007; Marshall *et al*., 2006). There is no species specificity between *Ulva* and bacterial interaction. The microbial community surrounding the *Ulva* is essential for the settlement and growth of *Ulva* zoospore (Braten *et al*., 1975). *Roseobactor* and *Cytophaga* bacteria are colonized on the surface of *Ulva mutabilis* zoospore. *Roseobactor* releases factors similar to cytokinin and *Cytophaga* releases factors similar to auxin (Lachnit *et al*., 2009; Spoerner *et al*., 2012).

2.3 Environmental factors affecting the morphology and growth of *Ulva* algae

Biotic and abiotic factors affect the growth of *Ulva* (Hofmann *et al*., 2010; López *et al*., 2007).

2.3.1 Light

During the summer, sunlight penetrates the entire depth of the water column and its temperature increases greatly. Under sufficient sunlight (100µmol photons/m^2sec at 20°C) and nutrient conditions, algal blooms have an increased probability of development (Duke *et al*., 1986). Pigment level increases in presence of light in *U. curvata* and *U. lactuca*. As a result, the growth of *Ulva* species also increases (Duke *et al*., 1986). Xu *et al*., 2014 reveals that *U. prolifera* exhibit high relative growth rate (RGR) under high light intensity (130µmol photons/m^2sec) as compared to low light intensity (30µmol photons/m^2sec) (Xu *et al*., 2014). Addition of nitrogen at low light density does not affect the growth of *Ulva* but growth increases at high light intensity (Lapointe *et al*., 1981). A study on *U. rigida* exposed that growth rate is reduced in the autumn season despite favorable temperature conditions (De Casabianca *et al*., 1998). It is reported that the metabolic rate is increased in presence of high light intensity in *U. lactuca* (Nejrup *et al*., 2013).

2.3.2 Temperature

A study by Reddy *et al*., 2006 on *Ulva* from Okha coast of India revealed that optimum temperature for its growth is 17°-32°C. *Ulva* is cultivated from the month of October to March in laboratory conditions. An optimum temperature for the growth of *U. fasciata* is 24°C. An increase in temperature from 20°C to

30°C increases the production of amino acid content (Reddy *et al*., 2006). *Ulva lactuca* and *U. intestinalis* from the coasts of Sunderbans found at the temperature range of 27°C-35°C (Satpati *et al*., 2012). According to Xiao *et al.*, 2016, *U. prolifera* exhibits higher growth rate and photosynthesis at 23°C as compared to low (14°C) and high (40°C) temperature range (Xiao *et al*., 2016). Steffensen *et al*., 1976, proposed that *U. lactuca* grow maximum at 25°C (Steffensen *et al*., 1976). The optimum temperature for growth of *U. scandinavica* is 10°C (Malta *et al*., 1999), whereas it is approximately 20–25°C for *U. ohnoi*, and under 10°C *U. ohnoi* shows virtually no growth (Notoya, 1999).

2.3.3 Salinity

The morphologies of *U. compressa* and *U. intestinalis* vary due to the salinity in their environment (Leskinen *et al*. 2004; Choi *et al*., 2010). High salinity influences the branch development in *U. intestinalis*. Different reports revealed the influence of salinity on the morphological development of *U. compressa* (Blomster *et al*., 1998; Tan *et al*., 1999). *Ulva intestinalis* can exhibit branching under low salinity conditions (Blomster *et al*., 1998). It is possible that the specimens identified as *U. compressa* from inner estuarine sites are actually branched form of *U. intestinalis* (Mathieson and Penniman, 1986). A combination of eutrophic conditions and low salinities may influence the presence of blade and tubular morphologies of *Ulva* taxa. Morphological variations that increase nutrient uptake efficiency provide an ecological advantage, perhaps facilitating bloom formation (Valiela *et al*., 1997). Usually *U. rigida* and *U. compressa* are found in interior estuarine sites with relatively low salinities, while *U. lactuca* and *U. pertusa* tends to occupy the open coast with relatively high salinities (Hofmann *et al*., 2010).

2.3.4 Mineral content

The optimum level of NO_3 and PO_4 for the growth of *U. lactuca* is 0.6 g/m^3. Increase in concentration of N increases the growth of *U. lactuca* thallus (Steffensen *et al*., 1976). Addition of NO_3 at high light intensity increases the growth of *U. fasciata* (Lapointe *et al*., 1981). 364, 1824, 14, 467 and 0.06 mg/100g of Na, Ca, Fe, K and P are found respectively inside *U. lactuca* (Ding *et al*., 2009; Luo *et al*., 2012). Absorption of mineral and its effect on the growth

of *Ulva* depend on the mineral content of the surrounding which varies with the geographic location (Rasyid, 2017).

2.3.5 Seasonal variation

The shape of cells is governed by the season (Cells rounded in winter) and population density (lanceolate in dense populations) (Choi *et al*., 2010). There are two reproductive periods from late August onwards, to late April. Biosynthesis of nutrient contents within *Ulva* is influenced due to seasonal variation. *Ulva pertusa* consist of higher contents of lipid, protein, amino acids and metal in the rainy season (Benjama *et al*., 2011).

2.3.6 Epiphytic diatoms

The association between extreme folding of thallus and epiphyte diatoms revealed that the epiphytes exaggerate the folding of foliose of *Ulva* (Steffensen, 1976). However, the epiphytes develop only on older thalli, which have become more bullate with age or with longer exposure to low salinity water. Epiphytic diatoms form dark green patches on the fronds of *U. lactuca* (Steffensen, 1976).

2.4 Effects of environmental factors on *Ulva* Green tides:

Tubular *Ulva* is bloom-forming green algae in the Chlorophyta that is responsible for “green tides”. Fast growing macroalgae requires high amount of nitrogen and phosphorous content as compared to slow-growing macroalgae (Valiela *et al*., 1997). Fast growing macro algae grows rapidly in the eutrophic marine environment due to its ability for faster assimilation of the nutrients (Ding *et al*., 2009; Duan *et al*., 2012a; Luo *et al*., 2012; Pedersen *et al*., 2009). Eutrophic conditions in shallow embayment results in the formation of extensive blooms of macroscopic green algae or green tides (Schramm and Nienhuis, 1997). Initially, green tides assumed to be consisting of a single species, but recent investigations have exposed that they often comprise a mixture of species (Hiraoka *et al*., 2004). *Ulva prolifera* is able to form green tides due to its high surface area to volume ratios and efficient nutrient uptake mechanisms (Rosenberg and Ramus, 1984; Taylor *et al*., 1998).

From the above-mentioned studies, it is postulated that multiple environmental factors influence the morphology of *Ulva*. Therefore, only

morphology is not reliable for species delineation. There is a requisite of molecular studies using different genome of *Ulva*.

2.5 DNA barcoding and molecular systematic studies of Nuclear, Chloroplast and Mitochondrial genome of *Ulva*

Molecular studies use a standardized DNA region for rapid and accurate species identification (G. W. Saunders and Virginia, 2005; Valentini *et al*., 2009). It can be regarded as an important tool used by taxonomists that help in the quick identification of unknown specimens, cryptic species and organisms with similar morphology (Frezal and Leblois, 2008; Hebert *et al*., 2003a). The ideal DNA barcoding marker should be variable (nearly identical among individuals of the same species but different between species), standardized and phylogenetically informative. Barcoding procedure is capable of distinguishing between unknown haplotypes of known species. It also helps in recognizing those species that are unknown to the database (Rubinoff *et al*., 2006). The goal of systematic studies is to provide insight into the history of the group of organisms and evolutionary processes that creates diversity among species. Thus, DNA taxonomy or DNA barcoding is a technique that uses DNA of organisms to delineate the species boundaries while molecular systematics deals with the evolution of species and phylogenetic relationship between them. NCBI database consists of 6229 sequences of *Ulva* species at different nuclear (18S, ITS), chloroplast (rbcL, rbcS, rps, tufA) and mitochondrial (atpB) molecular loci. In the current study, I targeted nuclear (18S, ITS), chloroplast (rbcL, tufA) and mitochondrial (atpB) regions.

The large subunit of the ribulose-bisphosphate carboxylase (rbcL) gene, located on the chloroplast genome, is the first choice for testing its utility as a DNA barcode in marine macro algae (Saunders *et al*., 2010). DNA barcode is the basis of several taxonomic and phylogenetic studies in genus *Ulva*. The universality of rbcL as a barcode marker has been negatively affected by the presence of introns in some marine macro algae because the ability to amplify and sequence large fragments with a single bidirectional read is tough (Clarkston and Saunders, 2010).

Nuclear internal transcribed spacer (ITS) suggested as a potentially usable for species identification (Hu *et al*., 2009). ITS (Internal Transcribed Spacer) refers to non-functional RNA situated between structural ribosomal RNAs (rRNAs) on a common precursor transcript. Reports have suggested that they evolve quickly and used for the intraspecific variation and biogeography in algae. Starting from 5' end polycistronic rRNA precursor transcript is arranged in the 5' external transcribed sequence (5' ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA 3'ETS form. Internal Transcribed Spacer (ITS) has shown great outcomes in studies of genetic differentiation within and among *Ulva* species (Leskinen and Pamilo, 1997). The ITS regions have been the most popular regions in the nuclear genome for evolutionary studies in a large number of taxonomic groups due to the high copy number (Gardes and Bruns, 1993; Hughes *et al*., 2006). Copy number defined as the average number of copies of a gene in a genotype of an organism. High copy number led to the high production of protein as in case of cancer cells (Cappuzzo, Federico, *et al*., 2005).

The exact number of *Ulva* species is likely to change as molecular investigations continue to reveal the identities of these algae (Kirkendale *et al*., 2013; Leliaert *et al*., 2009). The chloroplast genome of *Ulva* sp. is the smallest ulvophycean genome to be the sequenced and its size is 99,983 bp (Melton *et al*., 2015). However, this is only compared to the three complete ulvophycean genomes: *Oltmannsiellopsis viridis* (151.9 Kbps; (Pombert *et al*., 2006)), *Pseudendoclonium akinetum* (195.8 Kbps; (Pombert *et al*., 2005)), and *Bryopsis hypnoides* (153.4 Kbps; (Lü *et al*., 2011). A study from the Great Bay Estuarine System from the USA discovered the cryptic diversity of foliose *Ulva* using ITS1 sequences (Hofman *et al*., 2010). This molecular study identified four different species i.e. *U. lactuca, U. pertusa, U. rigida* and *U. pertusa* that were previously identified as *U. lactuca* based on morphology (Hofman *et al*., 2010). Another molecular study from Venice lagoon, Italy exposed the cryptic diversity of *U. californica, U. rigida, U. flexuosa, U. pertusa* and *U. compressa* using rbcL and tufA regions (Wolf *et al*., 2012).

Table 2.1: Previous reports of state-wise distribution of *Ulva* along the Indian coastal region:

Sr. no.	State	Coast	Species	Reference
1.	Gujarat	Dwarka, Okha	*U. fasciata,* *U. lactuca,* *E. compressa,* *E. intestinalis.*	(Dhargalkar and Deshmukhe, 1996; Thakur *et al*., 2008)
2.	Goa	Chapora, Anjuna, Palolem, Talpona, Polem, Baga, Reis Magos. Dona Paula, Siridao, Bogmalo, Holant, Betul	*U. clathrata,* *U. compressa,* *U. conglobata,* *U. flexuosa,* *U. intestinalis,* *U. lactuca,* *U. rigida,* *U. taeniata,* *U. paschima.*	(Bast *et al*., 2014b; Pereira and Almeida, 2014)
3.	Karnataka	Mangalore to Karwar	*E. intestinalis,* *E.clathrata,* *E. flexuosa,* *U. reticulata,* *U. fasciata,*	(Bast *et al*., 2014a; Kaladharan *et al*., 2011)

			U. lactuca, *U. rigida,* *U. paschima.*	
4.	Kerala	Vizhinjam, Varkala, Kollam, Cannanore Fort, Bekal Fort, Mulloor, Kovalam, Elathur, Thangaseri, Pudiappa.	*U. lactuca,* *U. fasciata,* *E. intestinalis,* *E. compressa,* *U. paschima.*	(Bast *et al*., 2014b; Chennubhotla *et al*., 1988)
5.	Tamil Nadu	Alantali, Manapad, Valinokkam, Kilakarai, Kanyakumari, Kudankulam	*E. compressa,* *E. intestinalis,* *U. lactuca,* *U. reticulata,* *U. fasciata.*	(Kaliaperumal *et al*., 1998; K. R. Rao *et al*., 1993; Satheesh and Wesley, 2012; Varma and Rao, 1962)
6.	Andhra Pradesh	Bhimili, Visakhapatnam Coast.	*U. fasciata,* *E. compressa.*	(Satya Rao *et al*., 2011)

7.	Orissa	Chilka Lake (Kalijal, Pathara, Satpara)	*E. flexuosa,* *E. intestinalis,* *E. compressa,* *E. usneoides,* *E. linza,* *E. clathrata,* *U. fasciata,* *U. lactuca.*	(Rath and Adhikary, 2006; Sahoo *et al*., 2003)
8.	Andaman Nicobar Island	Chidiya Tapu, North Bay, Viper Island	*E. compressa,* *E. intestinalis,* *U. lactuca,* *U. reticulata*	(Palanisamy, 2012)

9.	Diu island	Fort Area Reef	*E. clathrata,* *E. compressa,* *E. intestinalis,* *E. linza,* *U. fasciata,* *U. lactuca,* *U. reticulata.*	(Mantri and Rao, 2005)
10.	Lakshadweep	Chetlat, Kiltan, Kadmat, Amini, Bitra, Bangaram, Agatti, Androth, Kavaratti, Kalpeni, Suheli, Minicoy.	*E. clathrata,* *E. compressa,* *E. intestinalis,* *E. tubulosa,* *U. lactuca,* *U. reticulata.*	(Kaliaperumal *et. al*., 1989)
11.	Maharashtra	Ratnagiri	*U. chaugulii*	(*Kazi et al.,* 2016)

2.6 Databases focused on algal biodiversity and systematics

2.6.1 Index Nominum Algarum (INA): A card file and online reporting system maintained at the Silva Center for Phycological Documentation of the University Herbarium, University of California, and Berkeley, USA. It includes nearly 200,000 names of algae. It is a nomenclature database of algae.

2.6.2 AlgaeBase: A digital repository developed in 1996 at the University of Ireland, Galway (Guiry and Guiry, 2018). It provides information on the economically important marine algae to emphasize the sustainable use of algal resources globally. Initially, it covered marine algae of Ireland, Britain and Atlantic coast of France obtained from the 'The Species Directory of the Marine Fauna and Flora of the British Isles and Surrounding Seas'.

2.6.3 Harmful Algal Event Database (HAEDAT): A database that contains records of harmful algal events, management of harmful algae and monitoring systems globally. It works in collaboration with WoRMS, International Council for Exploration of Sea (ICES), North Pacific Marine Science Organization (PICES), and International Society for Study of Harmful Algae (ISSHA) (Hoagland *et al*., 2002).

2.6.4 Other region-specific databases: Besides these, a few other regional databases are available, which catalogue algae in specific geographic locations. For instance, the Australian Marine Algal Name Index (AMANI) provides information about taxonomy and distribution of Australian marine algae and some protists. Hawaiian Algal Database (HADB) is a database for Hawaiian Archipelago algae providing taxonomic information, photographs, micrographs and standardized DNA sequence data. AIDI (Algal Image Database of India) is an algae image database providing interactive images of various algal samples found in India (Charles *et al*., 2002; Sherwood *et al*., 2012).

2.6.5 DbIndAlgae: As of 30th June, 2018, DbIndAlgae lists around 147 different marine algal species belonging to 58 genera identified from the Indian coasts divided into three classes, viz. Phaeophyceae, Chlorophyceae and

Rhodophyceae. All the species listed under their respective classes and assigned an individual webpage. Each web page features different sections (Bhushan *et al.*, 2016).

1. Unique User ID (UUID): This is given to each sample. The three letters refer to the geographical location while the numerals represent serial numbers of the respective samples.
2. Classification: Taxonomic position of the species.
3. Distribution: Sampling sites
4. Image: Photograph of thallus taken either in the laboratory and/or at sampling site and a picture of herbarium sheet.
5. DNA sequence data: Details of markers with NCBI accession numbers if submitted/received.
6. Description: Morphological features of the algae as observed.
7. Key references: List of the latest important scholarly articles of the same alga.

2.7 Knowledge gap

Most of the previous reports available on *Ulva* from India based only on the morphology. There are a number of cryptic species reported worldwide including India. Studies have revealed morpho-species concept as impractical in this genus as members of *Ulva* shows morphological variability with respect to biotic (interacting bacteria and diatoms) and abiotic (salinity, temperature) factors. Different Indian coastal regions have versatile environmental conditions in different seasons. Varied environmental factor influences the morphology of *Ulva* in regions at the same time. Thus, taxonomic identification of the *Ulva* algae based on morphology is not reliable. Further, very few reports from India based on the phylogeography and phylogenetic study of *Ulva*. Apart from the report of *U. paschima* by Bast *et. al*. (2014a), there is no phylogeographic study of *Ulva* from Indian coasts. An extensive molecular systematic assessment of *Ulva* from Indian coasts had never done. A molecular study is required for exact taxonomic identification of *Ulva* isolates and phylogenetics for tracing out evolutionary lineages of *Ulva* from the entire coast of the Indian subcontinent.

2.8 Hypothesis

This study is observation-based exploratory biodiversity documentation endeavor. As no scientific experiments were involved; there were no prior hypothesis and statistical hypothesis testing. Nevertheless, it can be hypothesizing that Indian coastal region harbors previously uncharacterized biodiversity of genus *Ulva* and through a systematic DNA Taxonomy-assisted survey could reveal the undescribed species and new taxonomic records.

2.9 Objectives of this study

1) To study morphology and microscopic cell arrangement of *Ulva* thallus along with herbarium preparation.
2) To study taxonomy identification, isolate and sequence DNA using different loci from the collected sample of *Ulva* from different coastal areas of India.
3) To study the phylogenetic analysis of genus *Ulva* from the entire coast of India.
4) To construct the Indian algae database: DbIndAlgae.

Chapter 3: Materials and methods

3.1 Sample collection

Algal samples were collected from different locations across the coastal regions of India (Figure 3.1 and Table 3.1). Samples were collected from open ocean sites and nearby lagoons/estuaries from 2012 to 2016. All collected thallus of *Ulva* were attached to the hard surface of rocks, pebbles and stones. Multiple samples collected from each location for each species. It helped in the identification of morphological variations among one species at one sampling site. Collected specimen were transported to the laboratory under cold conditions (4-10˚C) and washed thoroughly with tap water to remove sediments and other contaminants. Samples were pressed for herbarium vouchers and processed for morphological characterization and microscopic analysis (Berges *et al*., 2001). Later, samples for molecular analyses were stored at -80˚C until further analysis.

Table 3.1: Sample collection sites

Sr. No.	Sample ID	Thallus Type	Location	Coordinates
1.	ETT-2	Foliose	Ettikulam, Kerala	12° 22′ 0.12″ N, 75° 3′ 0″ E
2.	ETT-3.1	Foliose	Ettikulam, Kerala	12° 22′ 0.12″ N, 75° 3′ 0″ E
3.	KAN-6.3	Tubular	Kannur, Kerala	11°52'57"N ,75°20'13"E
4.	KAN-6.4	Tubular	Kannur, Kerala	11°52'57" N,75°20'13"E
5.	PON-7	Tubular	Ponnani, Kerala	10° 46′ 12″ N, 75° 54′ 0″ E
6.	CAL-10	Foliose	Calicut, Kerala	11° 15′ 0″ N, 75° 46′ 12″ E
7.	MDP-13.14	Foliose	Mandapam, Tamil Nadu	9° 16′ 48″ N, 79° 7′ 12″ E
8.	MAN-14.1	Tubular	Mangalore, Karnataka	12°52'55"N, 74°50'22"E

9.	KAR-17	Tubular	Karwar, Karnataka	14° 48’ 49.00” N, 74° 07’ 46.99” E
10.	KAR-18	Tubular	Karwar, Karnataka	14° 48’ 49.00” N, 74° 07’ 46.99” E
11.	BEK-23.2	Foliose	Bekal, Kerala	12°.25’13.27”N, , 75° 1’ 23.33” E
12.	BEK-23.4	Foliose	Bekal, Kerala	12°.25’ 13.27”N, 75° 1’ 23.33” E
13.	HAV-35	Foliose	Havelock, Andaman Island	11° 57’ 59.99” N, 93° 00’ 0.00” E
14.	KAP-42.1	Tubular	Kalapathar, Andaman Island	11° 57′ 36″ N, 93° 0′ 0″ E
15.	NOB-43.2	Tubular	North bay, Andaman Island	11° 57′ 0″ N, 92° 45′ 0″ E
16.	KOL-47.2	Tubular	Kollam, Kerala	8° 52′ 48″ N,76° 36′ 0″ E
17.	DIG-48.1	Tubular	Digha, West Bengal	21° 37’ 35.82” N, 87° 30′ 26.75” E
18.	DIA-48.2	Tubular	Diamond harbour, West Bengal	21° 56′ 59″ N,89° 10′ 59.99″ E
19.	BAK-48.3	Tubular	Bakkhali, West Bengal	21° 33’ 47.76” N, 88° 15’ 33.98” E
20.	GOS-48.4	Foliose	Gosaba, West bengal	21° 56′ 59″ N,89° 10′ 59.99″ E
21.	KOL-49.1	Foliose	Kollam , Kerala	8° 52′ 48″ N, 76° 36′ 0″ E
22.	KOL-49.2	Foliose	Kollam, Kerala	8° 52′ 48″ N, 76° 36′ 0″ E
23.	KOL-49.3	Tubular	Kollam, Kerala	8° 52′ 48″ N, 76° 36′ 0″ E
24.	KOL-49.4	Foliose	Kollam, Kerala	8° 52′ 48″ N, 76° 36′ 0″ E
25.	KOV-50.1	Tubular	Kovalam, Tamil Nadu	12° 47’33”N, 80°15’10.8”E

26.	ENN-50.8	Foliose	Ennore, Tamil Nadu	13° 13′ 3″ N, 80° 19′ 17.58″ E
27.	ENN-50.16	Tubular	Ennore, Tamil Nadu	13° 13′ 3″ N, 80° 19′ 17.58″ E
28.	CHE-51.05	Foliose	Chellanam, Kerala	9° 48’ 25.96” N, 76° 16’ 38.71” E
29.	KYK-51.10	Foliose	Kanyakumari, Tamil Nadu	8° 5’ 17.9016” N, 77° 32’ 18.4272” E
30.	KYK-51.39	Foliose	Kanyakumari, Tamil Nadu	8° 5’ 17.9016” N, 77° 32’ 18.4272” E
31.	VEK-54.9	Foliose	Varkala, Kerala	8° 43’ 48.00” N, 76° 42’ 36.00” E
32.	MAL-55	Tubular	Malvan, Maharashtra	16°04′0.12″N,73°28′.1128″E
33.	MAL-57	Tubular	Malvan, Maharashtra	16°04′0.12″N,73°28′.1128″E
34.	VEN-58	Tubular	Vengurla, Maharashtra	15° 51′ 0.19″ N, 73° 37′56.21″ E
35.	VIJ-59	Tubular	Vijaydurg, Maharashtra	16° 33′ 38.52″ N, 73° 20′0.24″ E
36.	RAT-60	Tubular	Ratnagiri, Maharashtra	16°04′0.12″N, 73°28′.1128″E
37.	VEL-61	Foliose	Velneshwar, Maharashtra	17° 23′ 0″ N, 73° 12′ 0″ E
38.	OKH-70	Tubular	Okha, Gujarat	22°28′0″N, 69°4′0″E
39.	OKH-71	Foliose	Okha, Gujarat	22°28′0″N, 69°4′0″E
40.	DWA-109	Foliose	Dwarka, Gujarat	22° 13′ 48″ N, 68° 58′ 12″ E
41.	VER-127	Foliose	Veraval, Gujarat	20° 54′ 36″ N, 70° 22′ 12″ E
42.	VER-141	Tubular	Veraval, Gujarat	20° 54′ 36″ N, 70° 22′ 12″ E

43.	VER-154	Foliose	Veraval, Gujarat	20° 54′ 36″ N, 70° 22′ 12″ E
44.	BHI-164	Foliose	Bheemili, Andhra Pradesh	17° 53’ 25.08” N, 83° 27’ 21.24” E
45.	THO-175	Foliose	Thotla konda, Andhra Pradesh	17° 53’ 25.08” N, 83° 27’ 21.24” E
46.	THO-176	Foliose	Thotlakonda, Andhra Pradesh	17° 49’ 35” N, 83° 24’ 34” E
47.	MDP-241	Tubular	Mandapam, Tamil Nadu	8° 18′ 0″ N, 77° 12′ 0″ E
48.	PUL-242	Tubular	Pulicat lake, Andhra Pradesh	13°33′57″N, 80°10′29″E

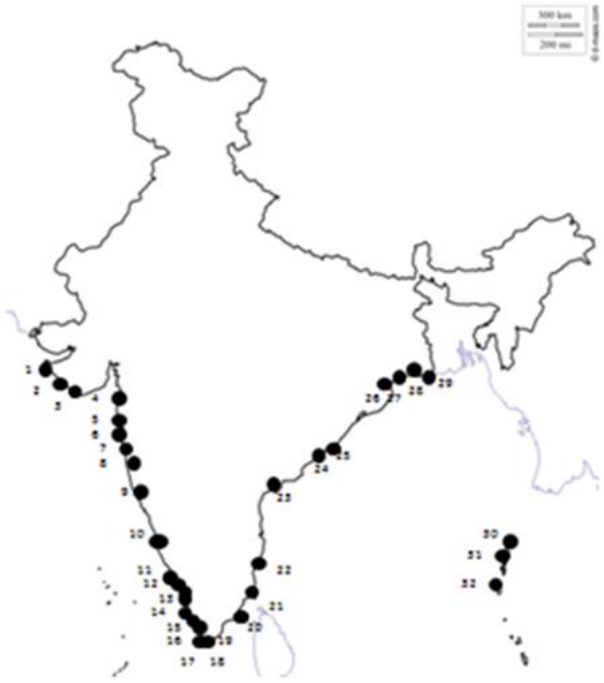


Figure 3.1: Map showing locations of algae sampling sites.

3.2 Morphological characterization

Morphological characterization of the specimen was made using an upright microscope (CX41RF, Olympus, Japan) with an attached digital camera (E450, Olympus, Japan). Public domain software ImageJ (http://rsbweb.nih.gov/ij/) was used for scale calibration and size

measurements. Monographs and books consulted for reference ID keys for species delineation of genus *Ulva*. List of referred books given below:

- Seaweeds of India (Jha *et al*., 2009)
- Seaweeds of Britain and Ireland (Guiry *et al*., 2003)
- Seaweeds of Alaska to Oregon (Gabrielson *et al*., 2006)
- Catalogue of the benthic marine algae of the Indian Ocean (Silva *et. al*., 1996)

For the taxonomic identification of genus *Ulva* based on morphological characters, described key features for each isolate was studied. List of morphological key features for each isolate analyzed include:

- Type of thallus (Foliose/Tubular)
- Branching pattern (Branched/Unbranched)
- Margin of the thallus (Smooth/Wavy)
- Denticulate (Present/Absent)
- Shape of the cell (Oval, Rounded, Rectangular, and Irregular)
- Pyrenoids (Present/Absent), Number of pyrenoids
- Chloroplast (Fully occupied/ partially occupied)
- Arrangement of cell
- Uniseriate /Multiseriate
- Zoospores
- Reticulae (Present/ Absent).

3.3 Herbarium preparation

Representative specimen from all collected isolates were pressed into herbarium vouchers and deposited in the herbarium (CAL; Central National herbarium, Botanical Survey of India, Kolkata)

3.4 DNA extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA extracted from the frozen algal specimen using HiPurA™ Algal Genomic Extraction Kit (HiMedia Laboratories Pvt. Ltd., Mumbai) following the manufacturer's protocol. Tissues from the apical part of thalli were selected to increase DNA yield. DNA re-suspended in elution buffer and quantified by absorbance at 260/280 nm using Thermo scientific Nanodrop spectrophotometer (Thermo Scientific Nanodrop 2000). Working solution of 1:10 (DNA: water) was prepared for polymerase chain reaction (PCR) in a separate tube.

Four microliters of diluted DNA were added to each 20µl reaction mix containing 2µl of 10X reaction buffer (Applied Biosystems, Foster City, CA, USA), 4µl each of 10µM primer, 2µl of 1µM dNTP mixture containing dATP, dTTP, dCTP and dGTP (Applied Biosystems, Foster City, CA, USA), 1 unit of rTaq® DNA polymerase (Applied Biosystems, Foster City, CA, USA) and double distilled water. Primers listed in Table 3.1 were used for amplifying ITS1, ITS2 (Gary W Saunders, 2005), 18S, tufA, atpB and rbcL (Zechman, 2003) regions (Imperial Life Sciences, India) (Table 3.2). Reactions contained small amount of $MgCl_2$ (Merk Specialties Pvt. Ltd. Mumbai). PCR amplifications were carried out in programmable thermal cycler (Veriti, ABI, USA) and reaction profile included an initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 1 minute, primer annealing at 50˚-54˚C for 2 minutes and primer extension at 72˚C for 2 minutes, and a final extension of 72˚C for 10 minutes. Amplified products and a standard 100 bp DNA marker were electrophoresed on 1.5% agarose gels at 100V and visualized with Ethidium Bromide in order to determine approximate length and purity.

Table 3.2: List of primers used for amplification of DNA.

Sr. No.	Name of primer	Name of Organelle	Sequence of primer
1.	tufAF	Chloroplast	GGNGCNGCNCAAATGGAYGG
	tufAR	Chloroplast	CCTTCNCGAATMGCRAAWCGC
2.	atpBF	Mitochondria	GTATGCGTGTTGCTTTAACA
	atpBR	Mitochondria	TCTTGTAGACCACCCATTTC
3.	rbcLF	Chloroplast	TAAAGCAGGTGCAGGATTTAAAGC
	rbcLR	Chloroplast	TATCAAATTCAAATTTAATTTCTTTCCAAAC
4.	18SF	Nucleus	GTCATATGCTTGTCTCAAAGATTAAGCC
	18SR	Nucleus	CCTTGTTACGACTTCTCCTTCCTCTAA
5.	ITS1	Nucleus	TCCGTAGGTGAACCTGCGG
	ITS2	Nucleus	GCTGCGTTCTTCATCGATGC

3.5 DNA sequencing template preparation and DNA sequencing

Amplified reactions were purified using ExoSAP-IT® PCR clean-up kit following manufacturer's instructions (USB Corporation, Cleveland, OH, USA). A working solution of 1:10 (DNA: water) was prepared as a sequencing template in a separate tube. PCR amplification reactions (as well as its sequencing) were carried out in duplicate for each target sequence of each isolate using the same set of primers in order to confirm the fidelity of Taq. Polymerase.

Purified PCR products were sequenced using a dideoxychain termination protocol with ABI BigDye Terminator Cycle Sequencing Ready®

Reaction Kit v3.1 (Applied Biosystems, Foster City, CA, USA) and a programmable thermal cycler (Veriti, ABI, USA). Two reactions used to amplify both strands (i.e., one with forward primer and the other with reverse primer). DNA sequencing was performed (Applied Biosystems 3730xl Genetic Analyzer, Foster City, CA, USA). The sequence data then analyzed and assembled using the licensed computer software Codon Code Aligner (Codon Code Corporation, USA). BLASTN (www.blast.ncbi.nlm.nih.gov) was used for sequence homology.

3.6 Multiple sequence alignment, phylogenetic and phylogeographic analysis

Multiple sequence alignment was carried out, which in addition to the generated sequences also included top BLASTn hits that are available in the NCBI Database. Sequences were first aligned by MUSCLE algorithm that done using MEGA, a freeware (available at www.megasoftware.net/) and alignments edited by eye. The ends of aligned sequences were trimmed to minimize the number of missing sites across taxa.

3.6.1 Phylogenetic analysis of tubular *Ulva*

The phylogenetic analysis included alignment construction, Maximum Likelihood ModelTest (Guindon and Gascuel, 2003) to find best-fitting substitution models (Tamura *et al*., 2011), phylogeny reconstruction starting tree generated by BioNJ and distance analysis as outlined in Bast (Bast, 2013).

For ITS1 regions, 31 sequences of different *Ulva* (top hits of BLASTn homology search) aligned with sequences of 19 *Ulva* isolates from India. Sequences aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Ulvaria fusca* and *Umbraulva japonica* were taken as outgroup. Substitution bias was modelled by Tamura-3-Parameter (T92) (Tamura and Nei, 1993) model with Gamma distribution, the best model in our ML ModelTest to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 14119.6.

For 18S regions, 22 sequences of different *Ulva* were aligned with the 4 isolates that amplified with 18S region. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Umbraulva japonica* and *Ulvaria*

fusca were taken as outgroup. Substitution bias was modelled by Kimura-2-Parameter (K2) (Kimura and Ohta, 1972) model and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 17808.6.

For TufA regions, 28 sequences of different *Ulva* accessions were aligned with the 4 isolates that were amplified with tufA region. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Ulvaria obscura* and *Umbraulva japonica* were taken as out-group. Substitution bias was modelled by General time reversible (GTR) model (Tamura *et al*., 2013) and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 9683.1.

For atpB regions, sequences of different *Ulva* accessions were aligned with 14 isolates that were amplified with atpB region. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Caulerpa brownii* was taken as outgroup. Substitution bias was modeled by Tamura-3-parameter (T92) model (Tamura *et al*., 2013) and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 4183.6.

For rbcL regions, 21 sequences of different *Ulva* were aligned with three isolates that amplified with rbcL region. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Caulerpa scalpelliformis* was taken as out-group. Substitution bias was modelled by Tamura-nei parameter (TN93) model (Tamura *et al*., 2013) and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 3279.2.

Heuristic searches were performed with tree bisection-reconnection, MULTREES and steepest descent options in effect. Five hundred bootstrap replicates were performed under ML criterion to estimate interior branch

support (Felsenstein, 1985). A consensus tree was constructed using the consensus tree builder within MEGA for ITS, 18S, tufA, atpB and rbcL.

3.6.2 Phylogenetic analysis of foliose *Ulva*

After BLAST, for ITS1 regions, 28 sequences of different *Ulva* aligned with 10 isolates amplified with ITS1 region. *Ulvaria fusca* and *Umbraulva japonica* were taken as an out-group. For the 18S regions, 13 sequences of different *Ulva* were aligned with the 4 isolates that were amplified with forward and reverse primers at 18S region. *Umbraulva kuaweuweu* was taken as outgroup. Sequences were aligned first by MUSCLE algorithm using computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. Substitution bias was modeled by Kimura-2-Parameter (K2) (Kimura and Ohta, 1972) model and Gamma distribution (that was the best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 23695.7 and 18245.8 respectively. Phylogenetic analysis using Maximum likelihood (ML) algorithm was conducted using MEGA with starting tree generated by BioNJ. Substitution bias was modeled by Kimura-2-Parameter (K2 model) with invariable sites.

For tufA regions, 23 sequences of different *Ulva* were aligned with the 8 isolates that were amplified with tufA region. *Ulvaria obscura* and *Umbraulva japonica* were taken as out-group. For the atpB regions, five sequences of different *Ulva* aligned with 13 isolates that amplified with the atpB region. *Caulerpa taxifolia* was taken as out-group. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. Substitution bias was modeled Tamura-3-parameter (T92) model (Tamura *et al*., 2013) and Gamma distribution (that was the best model in our test to find best fitting substitution models) (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 9766.2 and 8544.0968 respectively. Phylogenetic analysis using Maximum likelihood (ML) algorithm were conducted within MEGA starting tree generated by BioNJ. Substitution bias was model by T92 model with invariable sites.

Heuristic searches were performed with tree bisection-reconnection, MULTREES and steepest descent options in effect. Five hundred bootstrap replicates were performed under ML criterion to estimate interior branch

support. A consensus tree was constructed using the consensus tree builder within MEGA for ITS1, 18S, tufA and atpB.

3.6.3 Phylogeography analysis of *Ulva fasciata*

After BLAST, eight sequences of *Ulva fasciata* were aligned with *Ulva fasciata* isolates that were amplified with ITS1 region. *Ulva paschima* was taken as an out-group. For tufA regions, sequences of *Ulva fasciata* were aligned with these six isolates that were amplified with tufA region. *Ulva intestinalis* was taken as out-group. For the atpB regions, sequences of *Ulva fasciata* were aligned with the eight isolates that are amplified with the atpB region. *Ulva intestinalis* was taken as out-group. Sequences were aligned at first by MUSCLE algorithm inside computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. Substitution bias was modeled by Tamura-3-parameter model and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al*., 2011) with BIC (Bayesian Information Criterion) score of 2920.1, 3582.8 and 4432.6 respectively. Phylogeographic analysis using Maximum likelihood (ML) algorithm were conducted within MEGA starting tree generated by BioNJ. Substitution bias was model by Tamura-3-Parameter (T92) model with invariable sites. Heuristic searches performed with tree bisection-reconnection, MULTREES and steepest descent options in effect. Five hundred bootstrap replicates were performed under ML criterion to estimate interior branch support. A consensus tree was constructed using the consensus tree builder within MEGA for ITS1, tufA and atpB.

Chapter 4: Results

4.1 Morphological characteristics

Results of morphological features studied in the present research were as follows:

ETT-2: *Ulva reticulata* Forsskal

Sampling site:

Location: Ettikulam (Kerala); Collection date: 26-05-2012**;** Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: ETT-2; CUP Voucher ID: CUPVOUCHER-ETT-2012-UR-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ETT-2012-UR-1

Morphology:

Perforated leafy thallus and green in colour (Fig. 4.1a). Thallus was attached to the rocks with rhizoids (Fig. 4.1c). It had grown in mid littoral zone. Thallus was growing separately or some time in association with other algae, light to dark green in colour, branches aroused from the base, membranous leaf, compressed or flattened leaf, 2-3 cm broad, 10-20 cm long, distal ends of the leaves were rounded but basal region coiled like a ribbon (Fig. 4.1b). Thallus formed dense population in intertidal pools. Cells were irregular in arrangement (Fig. 4.1h), Multiseriate; 181.179±42 µm in size and irregular in shape (Fig. 4.1i). Cell wall was thick. Reticulae were present in the membranous leaves and observed in surface view (Fig. 4.1j); Thick patches of chloroplast were present inside cell (Fig. 4.1i). Margins of leaves were smooth (Fig. 4.1l). Denticulate were observed on the margin of leaves (Fig. 4.1m). Multiple pyrenoids were present in cells (Fig. 4.1k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.1d). Flagellated zoospores were appeared (Fig. 4.1e, 4.1f). Many dividing cells were also appeared (Fig. 4.1k). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763222
- 18S F &18S R: MG774432
- tufA F & tufA R: MG918115
- atpB F & atpB R: MG963795

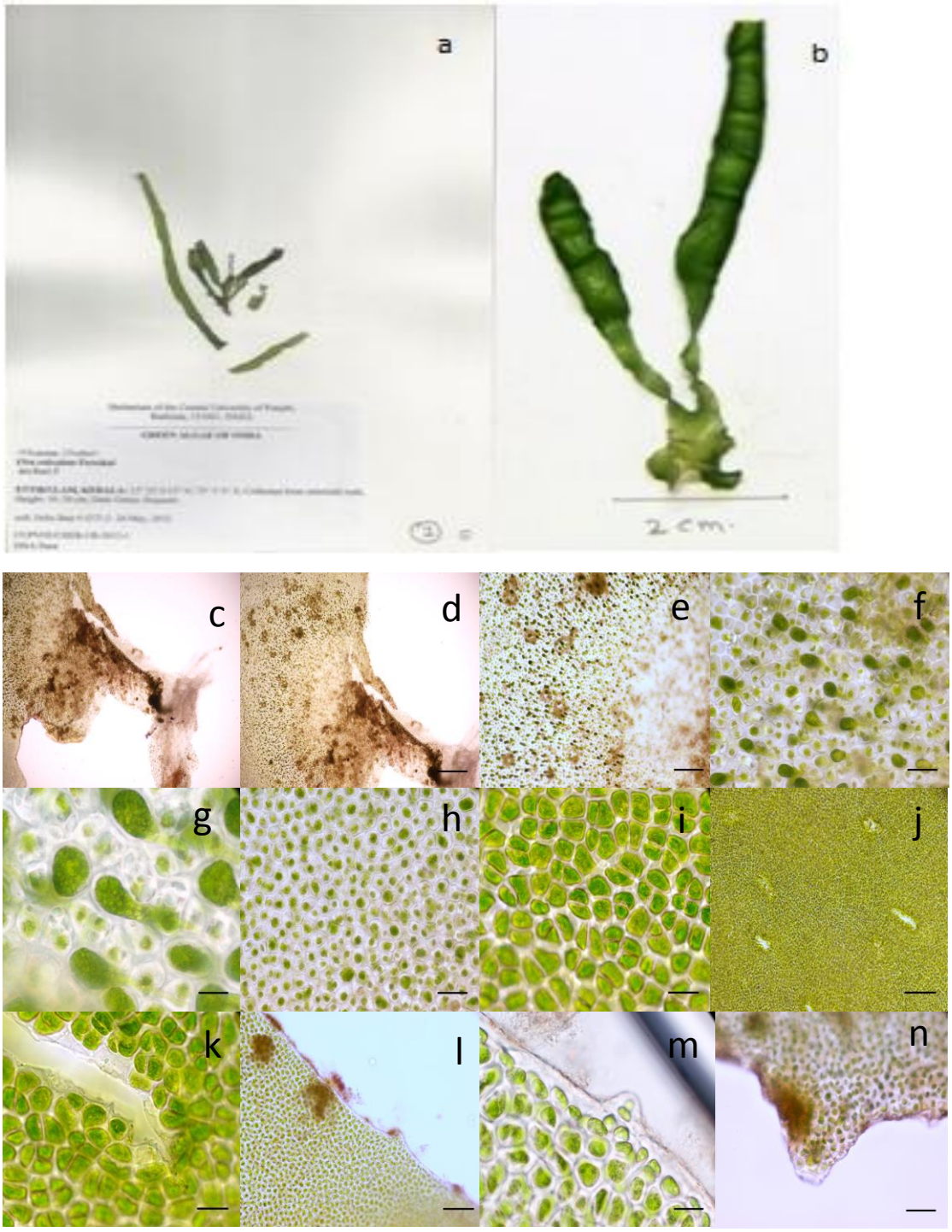


Figure 4.1: Morphology and micromorphology of *Ulva reticulata* from Ettikulam, India (ETT-2): a Herbarium, b. Morphology of thallus;

Microphotograph of thallus c, d, Rhizoids and zoospores in basal region at 4X,10X; e, f, g Presence and arrangement of flagella reproducing zoospores at 20X, 40X, 100X; g Presence of 4-7 pyrenoids at 100X; h, i Cell arrangement and chloroplast arrangement at 40X, 100X; j, k Presence of reticulae in the blade of thallus at 10X, 100X; l, m, n Margin of thallus at 10X, 40X, 100X; m Presence of denticula at margin; Dividing cells are also present; Scale bar is 200µm for d, l, j; 100µm for e; 50µm for f, h, m; 20µm for g, i, k, n.

ETT-3.1: *Ulva fasciata* Delile

Sampling site:

Location: Ettikulam, Kerala; Collection date: 26-05-2012; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: ETT-3.1; CUP Voucher: CUPVOUCHER-ETT-2012-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ETT-2012-UF-1.

Morphology:

Thallus was in leafy appearance and dark green in colour (Fig. 4.2b). Thallus was branched, upto 5 cm long. Thallus was attached and exposed to rocky surface of shore. Distal ends of the leaves were appeared heart like shape (Fig. 4.2a). Microscopic studies had shown, Cells were irregular in arrangement (Fig. 4.2k), Multiseriate; 109.352±47 µm in size and irregular in shape (Fig. 4.2l). Cell wall was thick (Fig. 4.2m). Thick patches of chloroplast were present inside cell (Fig. 4.2m). Margins of leaves were wavy (Fig. 4.2i, 4.2j). Multiple pyrenoids were present in cells (Fig. 4.2m). Reproductive zoospores were observed in basal region of thallus (Fig. 4.2c, 4.2d). Long flagellated zoospores were appeared (Fig. 4.2e, 4.2f). Empty cells also were observed after release of zoospores (Fig. 4.2g). Many dividing cells were also appeared (Fig. 4.2m). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MH277343

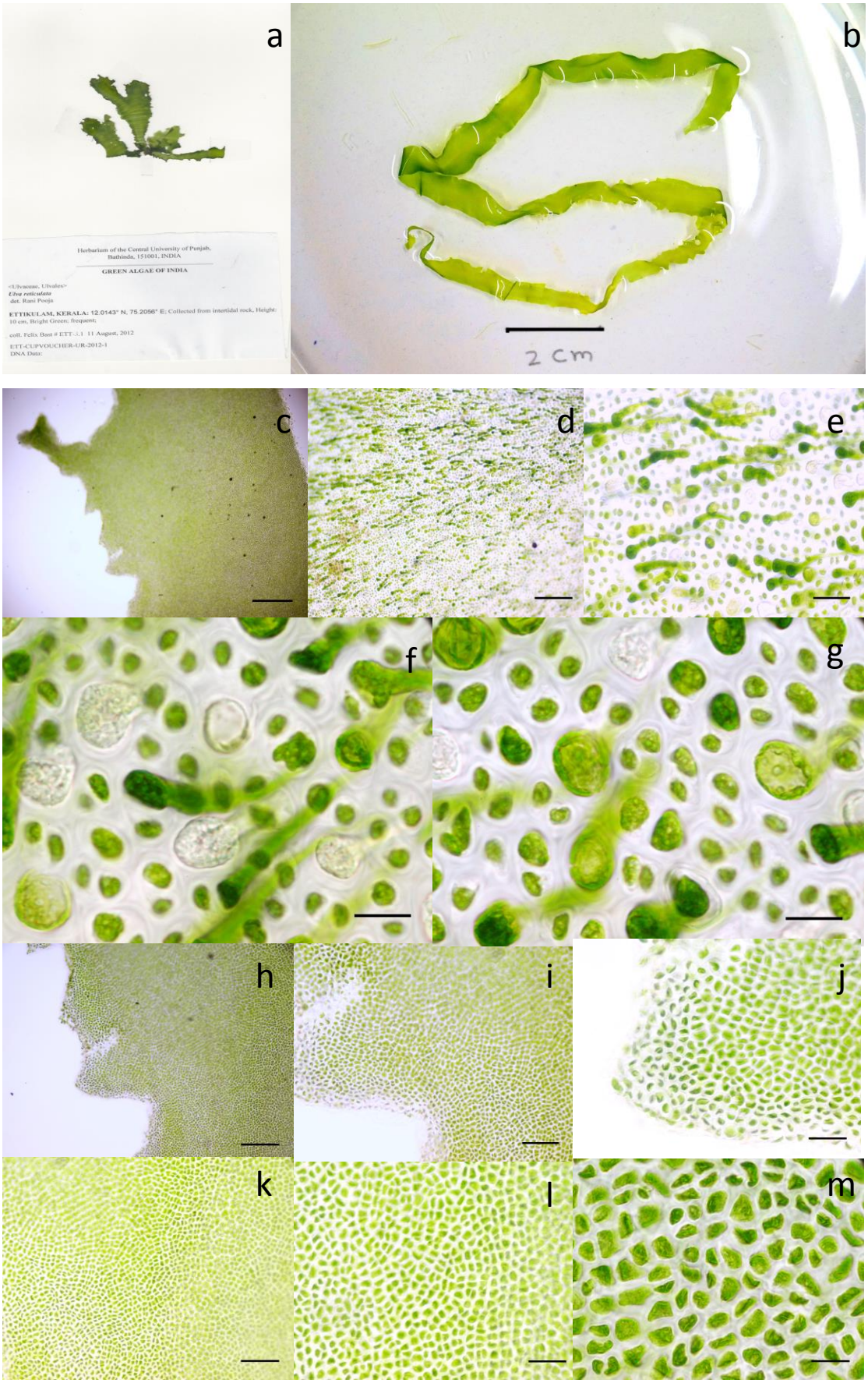

a
Herbarium of the Central University of Punjab,
Bathinda, 151001, INDIA
GREEN ALGAE OF INDIA
<Ulvaceae, Ulvales>
Ulva reticulata
det. Rani Pooja
ETTIKULAM, KERALA: 12.0143° N, 75.2056° E; Collected from intertidal rock, Height:
10 cm, Bright Green; frequent;
coll. Felix Bast # ETT-3.1 11 August, 2012
ETT-CUPVOUCHER-UR-2012-1
DNA Datz
b
2 cm
c
d
e
f
g
h
i
j
k
l
m

Figure 4.2: Morphology and Micromorphology of *Ulva fasciata* from Ettikulam, India (ETT-3.1): a Herbarium, b Morphology of thallus; c Microphotographs of basal region of thallus at 4X; d, e, f, g Presence of flagellated zoospores at 10X, 20X, 40X, 100X; h, i, j Ruffled margin at 10X, 40X, 100X; k, l, m Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X. Dividing cells are also present; Scale bar is 200µm for d, h, k; 100µm for e; 50µm for f, i, l; 20µm for g, j, m.

KAN-6.3: *Ulva intestinalis* Linnaeus

Sampling site:

Location: Kannur (Kerala); Collection date: 26-05-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KAN-6.3; CUP Voucher ID: CUPVOUCHER-KAN-2012-UI-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAN-2012-UI-1

Morphology:

Thallus was bushy and holdfast attached to the rocks or pebbles (Fig. 4.3a). Thallus was highly branched, light yellow to dark green in color and up to 5-10 cm long, attached by basal rhizoidal portion (Fig. 4.3b). Branches were tubular in appearance (Fig. 4.3c). Cells were cuboidal and elongated in shape. Cells were arranged in linear rows towards margin but comparatively irregular in middle region (Fig. 4.3i), Multiseriate; 70.605±18 µm in size and irregular in shape. Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.3j). Margins of leaves were not wavy (Fig. 4.3j). Multiple pyrenoids were present in cells (Fig.4.3k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.3e). Long flagellated zoospores were appeared (Fig. 4.3f). Many dividing cells were also appeared. Branches were tubular and monostromatic (Fig. 4.3d). Some epiphytic red algae were observed on the surface (Fig. 4.3g, 4.3h).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG768945

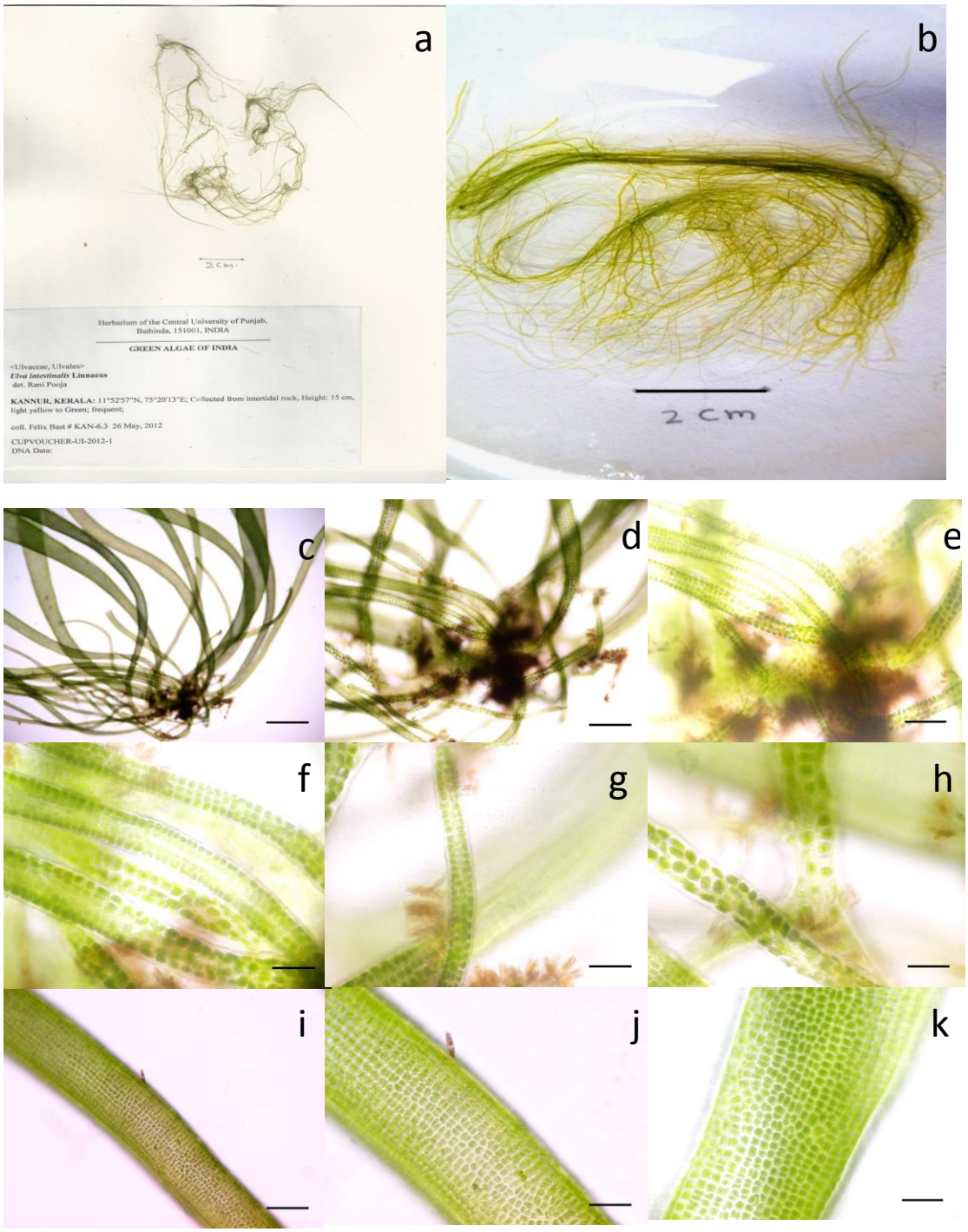


Figure 4.3: Morphology and Micromorphology of *Ulva intestinalis* from Kannur, India (KAN-6.3): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X,10X; e, f Presence and arrangement of zoospores in the basal region of tubular branches at 20X, 40X; g, h Presence of epiphytic red algae at 40X; i, j Margin of the tubular thalli, cell

arrangement at 20X, 40X; k Chloroplast arrangement and number of pyrenoids in the cell at 100X; Dividing cells are also present; Scale bar is 200µm for d; 100µm for e, i; 50µm for f, g, h, j; 20µm for k.

KAN-6.4: *Ulva sapora* Philips

Sampling site:

Location: Kannur (Kerala); Collection date: 26-05-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KAN-6.4; CUP Voucher ID: CUPVOUCHER-KAN-2012-US-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAN-2012-US-1

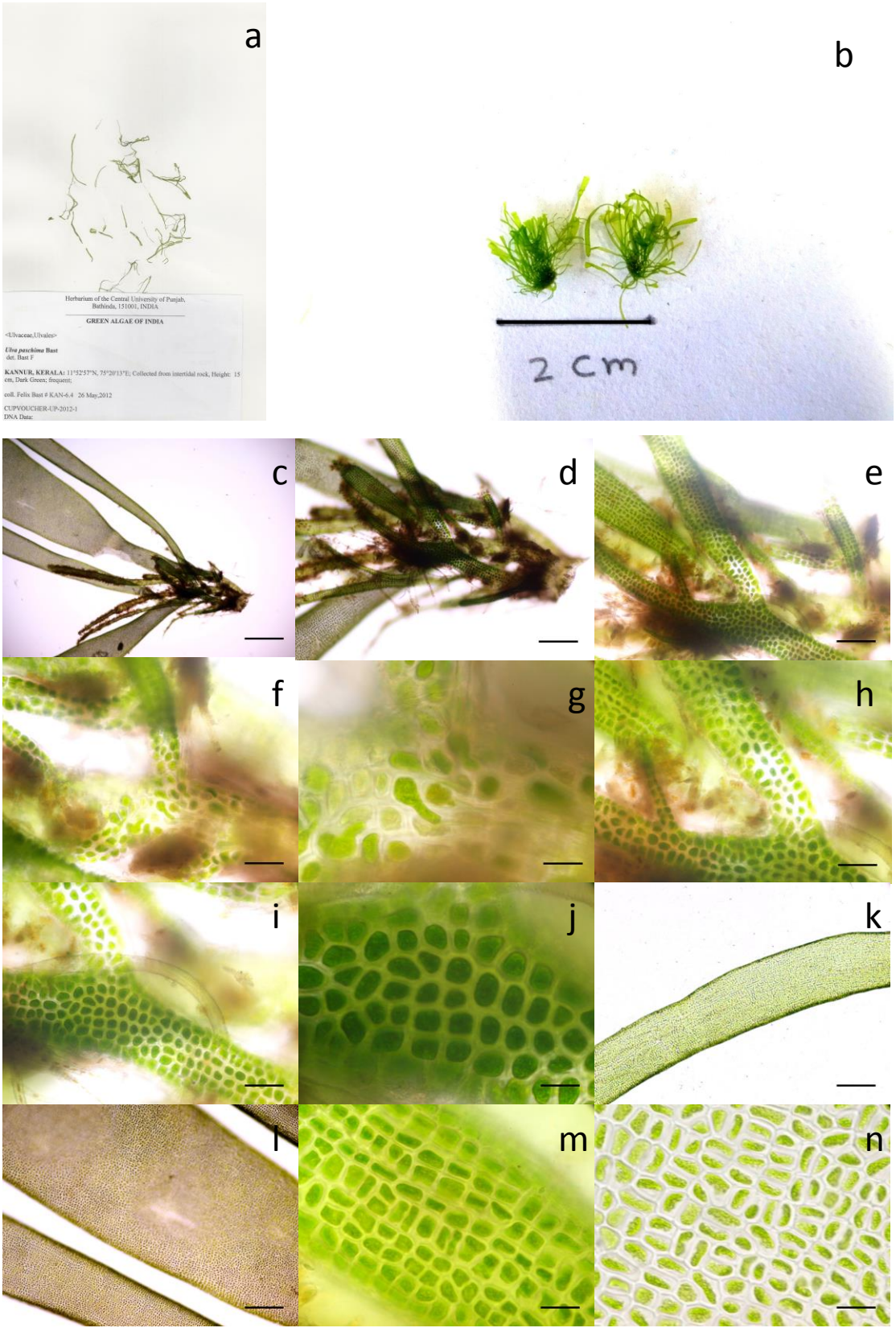
Morphology:

Thallus was tubular and contorted (Fig. 4.4a). Holdfast was attached to the rocks and pebbles. Plant was simple or branched, light yellow to dark green in colour and up to 1-2 cm long (Fig. 4.4b), attached by disc shaped structure and later become free-floating, mature specimens often inflated at intervals giving an intestine like appearance, contorted and irregularly constricted. New developing biseriate branch was observed in the thallus (Fig. 4.4d). Cells were regular in arrangement in the middle and towards margin (Fig. 4.4i). Cells were arranged in linear rows except some dividing cells (Fig. 4.4l). Horizontal and vertical cell division was observed in the cell. Cells were irregular in arrangement near the new proliferating branch point (Fig. 4.4j), Multiseriate; 76±3 µm in size and irregular in shape (Fig. 4.4h). Cells were oval or rounded in shape. Cell wall was thick (Fig. 4.4h). Thin patches of chloroplast were present inside cell (Fig. 4.4m). Margins of leaves were not wavy (Fig. 4.4k). Multiple pyrenoids were present in cells (Fig. 4.4n). Reproductive zoospores were observed in basal region of thallus (Fig. 4.4f). Long flagellated zoospores were appeared (Fig. 4.4g). Reproductive zoospores were also observed near the proliferating region of new developing branch. Thallus was monostromatic and tubular. Epiphytic red algae *Porphyridium* was present on the basal region of branches (Fig. 4.4o-4.4p).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763135



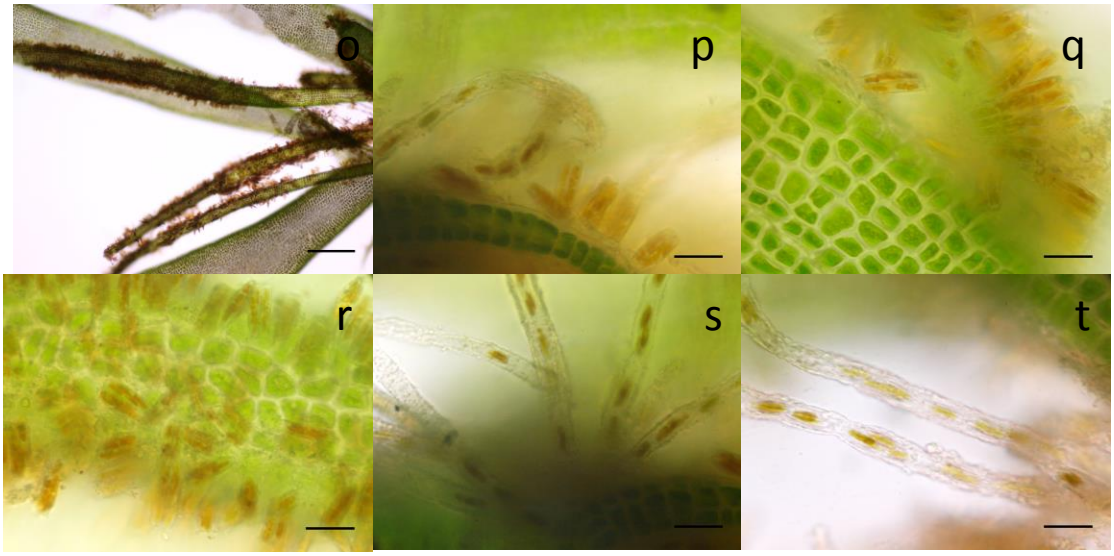


Figure 4.4: Morphology and Micromorphology of *Ulva sapora* from Kannur, India (KAN-6.4): a Herbarium, b Morphology of thallus; c, d, e Branching pattern in the basal region at 4X, 10X, 20X. f, g Presence of zoospores in the basal region at 40X, 100X; h Presence of new developing branch and arrangement of cells in the tip region at 40X; i, j Arrangement of cells in the nodal region or at the proliferation region of branch at 40X, 100X. k Marginal surface of the tubular branch at 10X; l, m Arrangement and shape of the cells at 20X, 40X. n Chloroplast arrangement and pyrenoids arrangement at 100X; Dividing cells are also present; Scale bar is 200µm for d, k; 100µm for e, l; 50µm for f, h, i, m; 20µm for g, j, n.

PON-7: *Ulva intestinalis* Linnaeus

Sampling site:

Location: Ponnani (Kerala); Collection date: 26-05-2012; collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: PON-7; CUP Voucher ID: CUPVOUCHER-PON-2012-UI-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-PON-2012-UI-1

Morphology: -

Thallus was bushy. Each branch aroused from its base, which is tubular and contorted (Fig. 4.5a). Thallus was attached to the rocks and pebbles by disc

shaped structure (Fig. 4.5c). Thallus was simple or branched, light yellow to green in color and 5-15 cm long (Fig. 4.5b), which later on become free-floating (Fig. 4.5d). Cells were regular in arrangement towards margin but slightly irregular in middle region and near the proliferating branch point (Fig. 4.5g, 4.5h). Cells were arranged in linear rows (Fig. 4.5i), multiseriate, 197.220±33 µm in size. Cells were rectangular or cuboidal in shape (Fig. 4.5l). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.5m). Margins of leaves were smooth (Fig. 4.5e, 4.5h). Multiple pyrenoids were present in cells (Fig. 4.5k). Many dividing cells were also appeared (Fig. 4.5n). Thallus was monostromatic and tubular. Distal end of the branch was slightly curved or bent (Fig. 4.5j).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG768946

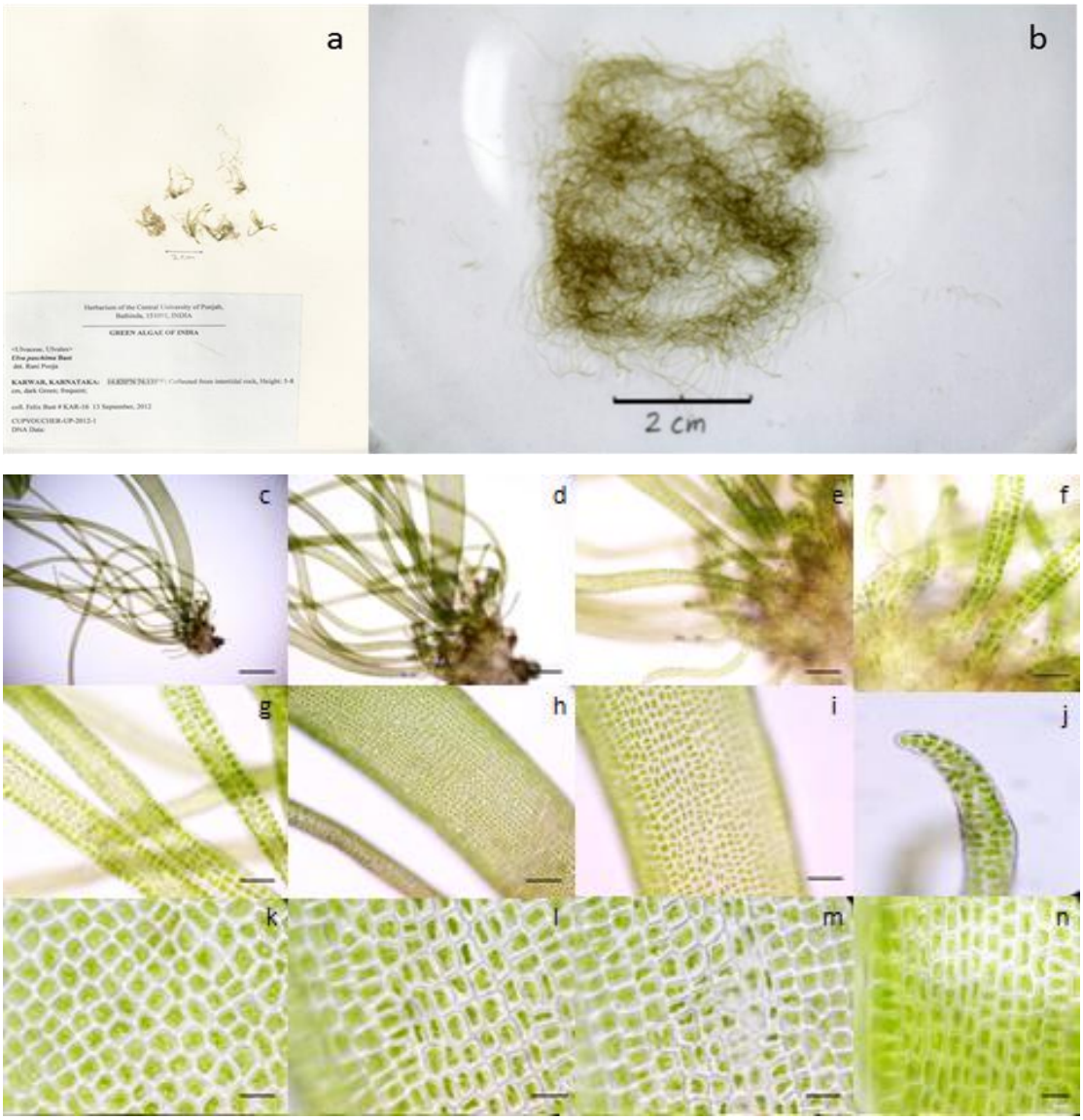


Figure 4.5: Morphology and Micromorphology of *Ulva intestinalis* from Ponnani, India (PON-7): a Herbarium, b Morphology of thallus; c Microscopic analysis of thallus at 4X; d, e, f, Presence of zoospores at basal region of thallus at 10X, 40X, 100X respectively; g, h, i Presence of cell arrangement and margin of thallus at 40X, 10X, 40X; j Tip region of branch at 100X; k, l, m, n Chloroplast arrangement, margin and pyrenoids arrangement at 100X. Dividing cells are also present; Scale bar is 200µm for d, h; 50µm for e, g, i; 20µm for f, j, k, l, m, n.

CAL-10: *Ulva reticulata* Forsskal

Sampling site:

Location: Calicut (Kerala); Collection date: 26-05-2012; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: CAL-10; CUP Voucher ID: CUPVOUCHER-CAL-2012-UR-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-CAL-2012-UR-1

Morphology: -

Perforated leafy thallus and thallus was attached to the rocks (Fig. 4.6a). It had grown in mid littoral zone. Thallus was growing separately or some time in association with other algae, light to dark green in colour, segmented leaf like structure or form separate branches (Fig. 4.6b). Leaves were flattened or compressed. Each branch was highly coiled, edges of the leaves were wavy in nature, membranous, 10-20 cm long, 2-5 cm broad, with number of lacunae; lacunae oval, circular, oblong or rectangular, divide the lamina into distinct laciniae with microscopic serrations on the edges of thallus and holes (Fig. 4.6a); Plants formed dense population in intertidal pools. Cells were irregular in arrangement (Fig. 4.6j, 4.6k), Multiseriate; 109.558±54 µm in size and rounded in shape (Fig. 4.6q). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.6r). Margins of leaves were not wavy (Fig. 4.6l, 4.6m). Multiple pyrenoids were present in cells (Fig. 4.6r). Reproductive zoospores were observed in basal region of thallus (Fig. 4.6c, 4.6d)). Long flagellated zoospores were appeared (Fig. 4.6e, 4.6f). In the middle region less number of zoospores were present (Fig. 4.6h). Few empty cells were observed after the release of zoospores (Fig. 4.6g). Many dividing cells were also appeared (Fig. 4.6p). Thallus was distromatic. Epiphytic red algae were present on the surface (Fig. 4.6n, 4.6o).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763223

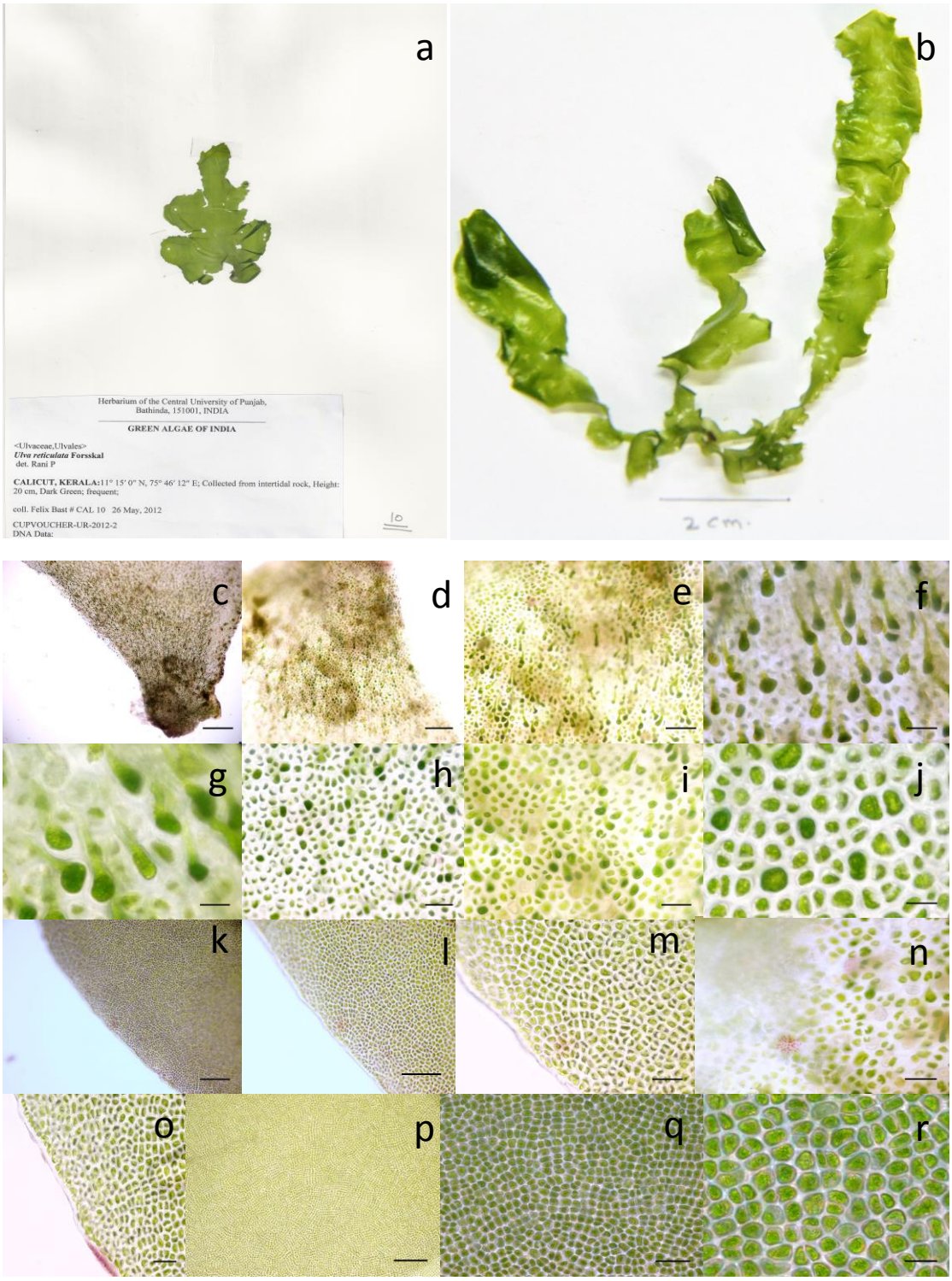


Figure 4.6: Morphology and Micromorphology of *Ulva reticulata* from Calicut, India (CAL-10): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; d, e, f Presence of flagellated zoospores at 10x, 40X, 100X; h, i, j Cell arrangement and presence of reproductive bodies at middle region at 10X, 20X, 100X; k, l, m Margin of the thallus at 10X, 20X, 40X; n, o Presence of epiphytic red algae 40X, 20X; p, q, r

Cell arrangement chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X; Dividing cells are also present; Scale bar is 200µm for d, h, k, p; 100µm for i, l, o; 50µm for e, m, n, q; 20µ f, j, r.

MDP-13.14: *Ulva fasciata* Delile

Sampling site:

Location: Mandapam (Tamil Nadu); Collection date: 19-07-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: MDP-13.14; CUP Voucher ID: CUPVOUCHER-MDP-2012-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2012-UF-1

Morphology:

Thallus was leafy (Fig. 4.7a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It occurs in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 5-40 cm long and divided into number of 1-3 cm broad lobes. Leafy thallus was irregularly lobed or sometimes divided into ligulate or linear lobes (Fig. 4.7b); Cells were irregular in arrangement (Fig. 4.7k, 4.7l), Multiseriate; 217±32 µm in size. Cells were kidney or bean shaped (Fig. 4.7o). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.7m). Margins of leaves were wavy or ruffled (Fig. 4.7h, 4.7i). Denticula was present on margin (Fig. 4.7j). Multiple pyrenoids were present in cells (Fig. 4.7n). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.7d, 4.7e). Long flagellated zoospores were appeared (Fig. 4.7c). Few empty cells were present after the release of zoospores (Fig. 4.7f, 4.7g). Many dividing cells were also appeared in surface view (Fig. 4.7m). Thallus was distromatic. Endophytic red algae were present in surface view (Fig. 4.7g).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MH277343

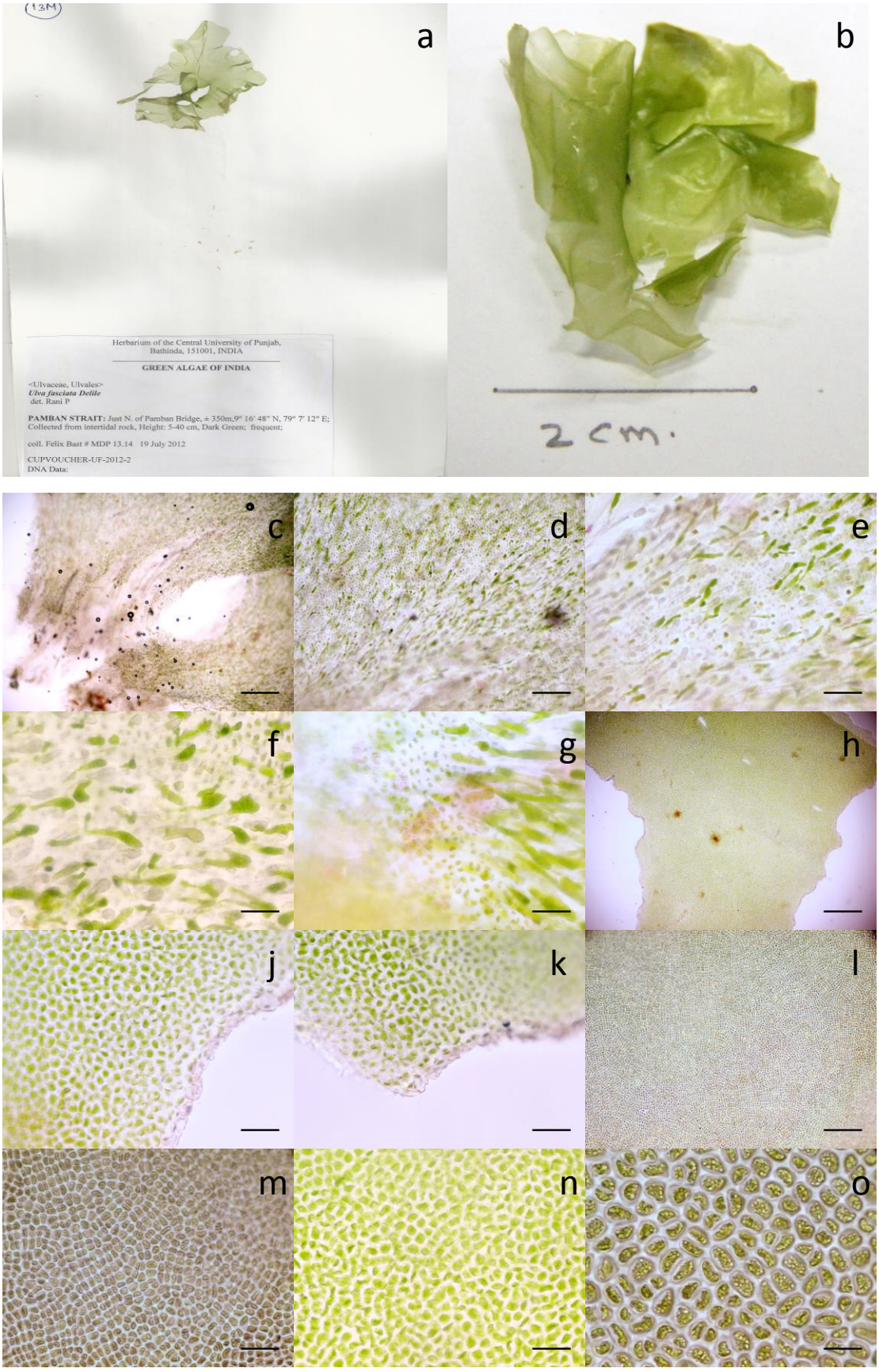


Figure 4.7: Morphology and Micromorphology of *Ulva fasciata* from Mandapam, India (MDP-13.14): a Herbarium, b Morphology of thallus; c, d

Presence and arrangement of zoospores in the basal region; e, f Presence of flagellated zoospores and empty cells after release of zoospores in the basal region at 40X, 100X; g Presence of endophytic red algae in the middle region at 20X; h, j Ruffled margin and cell arrangement in marginal region at 10X, 20X; k Denticula and cell arrangement in denticulate region at 20X; l, m Cell arrangement and shape of cells in middle region at 10X, 20X; n, o Chloroplast arrangement and number of pyrenoids in middle region at 40X, 100X; Dividing cells are also present; Scale bar is 200µm for h, l; 100µm for g, j, k, m; 50µm for e, n; 20µm for f, o.

MAN-14.1: *Ulva intestinalis* Linnaeus

Sampling site:

Location: Mangalore (Karnataka); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: MAN-14.1; CUP Voucher ID: CUPVOUCHER-MAN-2012-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MAN-2012-UF-1

Morphology:

Thallus was tubular and ribbon like coiled (Fig. 4.8a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 40 cm long and divided into number of ribbon shaped tubular or compressed thread like structure (Fig. 4.8b). Thallus was branched, uniseriate and multiseriate branches are present (Fig. 4.8c, 4.8i). Tip region of the branch was pointed (Fig. 4.8d). Surface of Thallus was smooth; thallus was free floating in water after maturation. Thallus was 2 cells thick, cells rectangular or quadratic in cross section (Fig. 4.8m). Cells were irregular in arrangement (Fig. 4.8l), multiceriate; 149.111±27 µm in size and irregular in shape (Fig. 4.8n). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.8n). Margins of leaves were smooth (Fig. 4.8l). Multiple pyrenoids were present in cells (Fig. 4.8n). Reproductive zoospores were observed in basal region of thallus (Fig. 4.8e, 4.8f). Long flagellated zoospores were appeared (Fig. 4.8g, 4.8h). Many

dividing cells were also appeared (Fig. 4.8m). Thallus was monostromatic. Epiphytic red algae were present on surface (Fig. 4.8j, 4.8k).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG918102

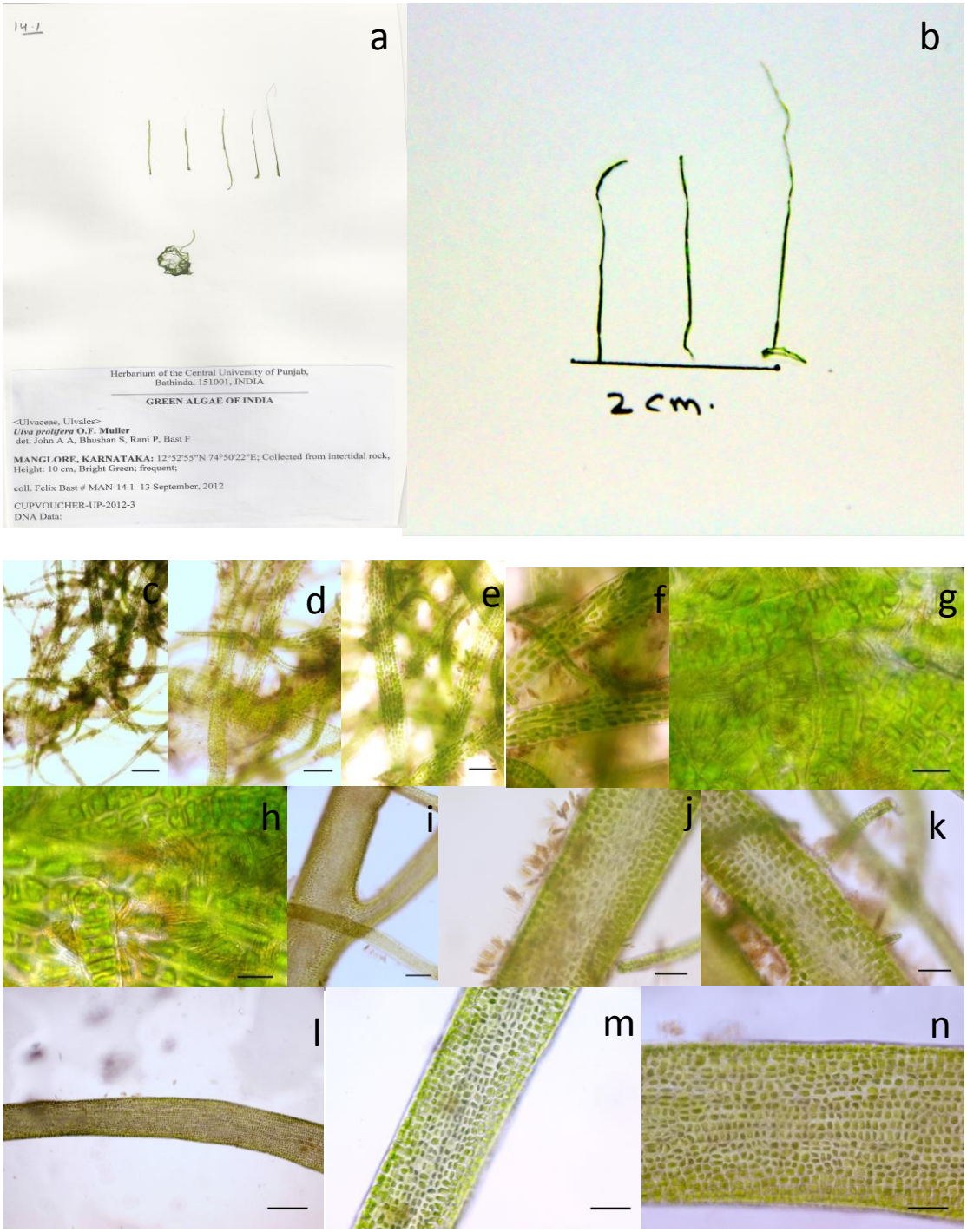


Figure 4.8: Morphology and Micromorphology of *Ulva intestinalis* from Mangalore, India (MAN-14.1): a Herbarium, b Morphology of thallus; c, d microphotographs of thallus at 10X; e, f Reproductive bodies at basal region at 10X; g, h Presence of flagellated zoospores at 100X; i Bifurcating branch at 10X; j, k Epiphytic red algae at 20X; l, m Cell arrangement and margin of thallus at 10X, 40X; n Chloroplast arrangement and pyrenoids arrangement at 100X; Dividing cells are also present; Scale bar is 200µm for c, d, e, f, i, l; 100µm for j, k; 50µm for m; 20µm for g, h, n.

MAN-14.2: *Ulva paschima* Bast

Sampling site:

Location: Mangalore (Karnataka) Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: MAN-14.2; CUP Voucher ID: CUPVOUCHER-MAN-2012-UP-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MAN-2012-UP-1

Morphology:

Thallus was bushy, tubular and ribbon like coiled (Fig. 4.9a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 10 cm long and divided into number of ribbon shaped tubular or compressed thread like structure (Fig. 4.9b). Thallus was unbranched (Fig. 4.9c, 4.9d). Some new developing branches were present at basal region (Fig. 4.9l). Tip region of the branch pointed (Fig. 4.9m). Surface of plant was smooth; thallus was free floating in water after maturation. Cells were rectangular or quadratic in surface view (Fig. 4.9i). Cells were irregular in arrangement (Fig. 4.9j), multiseriate; 157.138±23 µm in size and irregular in shape (Fig. 4.9j). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.9j). Margins of tubular filaments were smooth (Fig. 4.9i). Multiple pyrenoids were present in cells (Fig. 4.9k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.9f). Long flagellated zoospores were appeared (Fig. 4.9g, 4.9h). Many dividing cells were also appeared (Fig. 4.9n). Thallus was monostromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918118

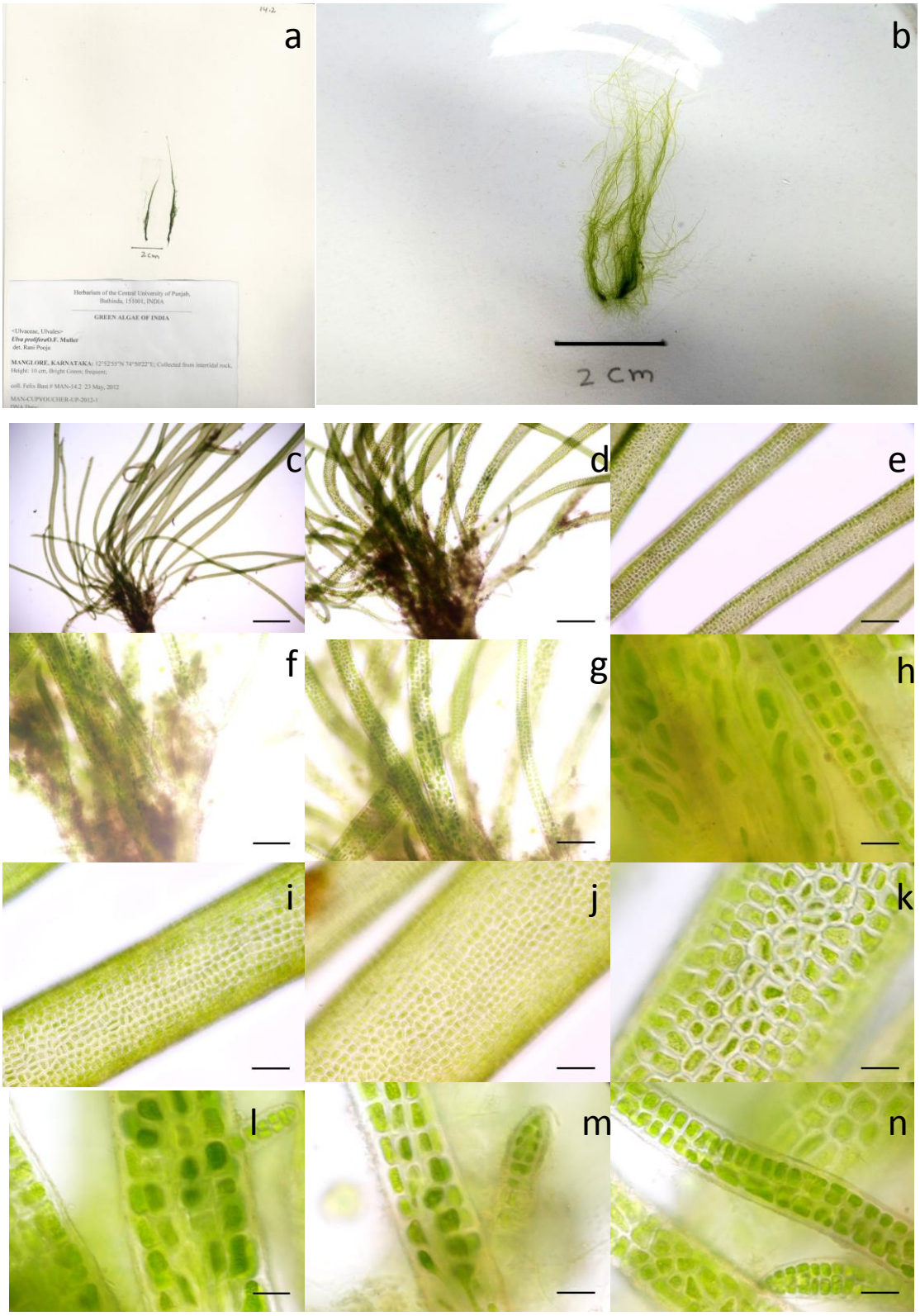


Figure 4.9: Morphology and Micromorphology of *Ulva paschima* from Mangalore, India (MAN-14.2): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e Smooth margin of thallus at 20X; f, g Presence of reproductive zoospores at 10X, 20X; h Presence of flagellated zoospores at 40X; i, j Cell shape and arrangement at 20X, 40X; k Chloroplast and pyrenoids at 100X; l, m New developing branch and tip region from basal region at 10X; n Presence of dividing cells at 100X; Scale bar is 200µm for d, f, l; 100µm for e, g, i; 50µm for h, j; 20µm for k, n.

KUN-17: *Ulva paschima* Bast

Sampling site:

Location: Kundapur (Karnataka); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KUN-17; CUP Voucher ID: CUPVOUCHER-KUN-2012-UP-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KUN-2012-UP-1

Morphology:

Thallus was tubular and ribbon like coiled (Fig. 4.10a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 40 cm long and divided into number of ribbon shaped. One to two cm tubular or compressed thread like structure (Fig. 4.10b). Surface of plant was smooth; Thallus was free floating in water after maturation. Thallus was highly branched (Fig. 4.10c). Numbers of uniseriate or multiseriate newly developed branches were observed from the primary branch of thallus (Fig. 4.10h). Secondary branches were growing from primary branch in alternative fashion (Fig. 4.10i, 4.10j). Tip of the branch was conical (Fig. 4.10h). Cells were cuboidal or oval in shape and regular in arrangement in the tubular region (Fig. 4.10m), Cells were arranged in linear rows (Fig. 4.10k). Multiseriate; Cells were irregular in shape and arrangement near the proliferating region (Fig. 4.10i), 212.422±26 µm in size (Fig. 4.10). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.10m). Margins of leaves were smooth (Fig. 4.10l). Multiple

pyrenoids were present in cells (Fig. 4.10n). Reproductive zoospores were observed in basal region of main branch of thallus (Fig. 4.10e). Long flagellated zoospores were appeared (Fig. 4.10f, 4.10g). Many dividing cells were also appeared (Fig. 4.10n). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG918105

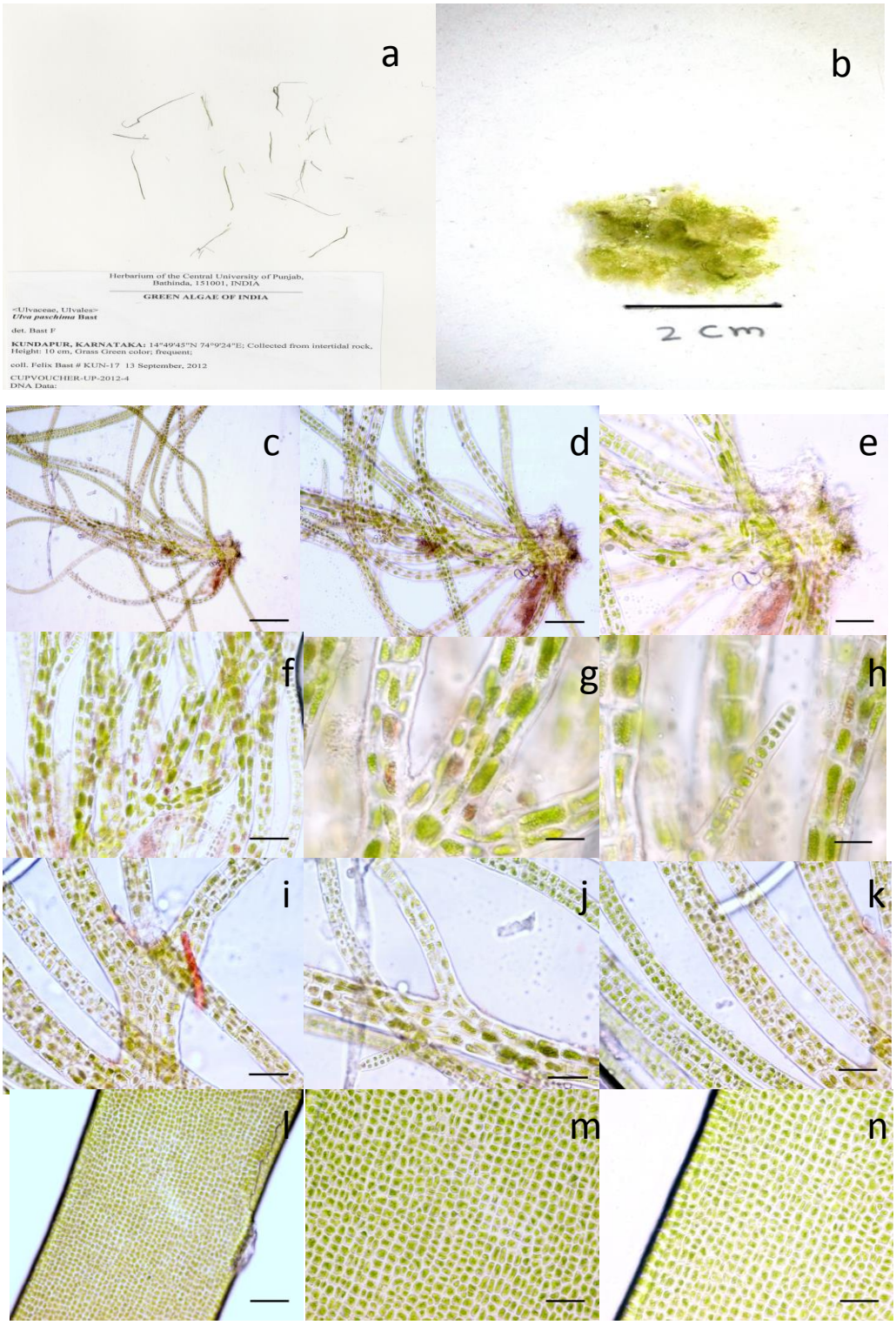


Figure 4.10: Morphology and Micromorphology of *Ulva paschima* from Kundapur, India (KUN-17): a Herbarium, b Morphology of thallus; c, d microphotographs of basal region of thallus at 4X, 10X; e, f Branching pattern of thallus at 10X; g, h Arrangement of reproductive zoospores at 100X; j Tip region of a branch at 40X; i, k, Presence of alternatively growing uniseriate and

multiseriate secondary branches at 40X; l, m Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 20X, 40X; n Presence of dividing cells at 40X; Scale bar is 200µm for d, e, f; 100µm for l; 50µm for i, j, k, m, n; 20µm for g, h.

KAR-18: *Ulva paschima* Bast

Sampling site:

Location: Karwar (Karnataka); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KAR-18; CUP Voucher ID: CUPVOUCHER-KAR-2012-UP-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAR-2012-UP-1

Morphology:

Thallus was tubular and ribbon like coiled (Fig. 4.11a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 40 cm long and divided into number of ribbon shaped, tubular or compressed thread like structure (Fig. 4.11b). Surface of plant was smooth; plants were free floating in water after maturation. Thallus was highly branched (Fig. 4.11c, 4.11d). Numbers of uniseriate or multiseriate newly developed branches were observed from the primary branch of thallus (Fig. 4.11e, 4.11f). Secondary branches were growing from primary branch in alternative fashion (Fig. 4.11g). Tip of the branch was slightly curved or bent (Fig. 4.11j). Cells were cuboidal or oval in shape and regular in arrangement in the tubular region (Fig. 4.11m), Cells were arranged in linear rows (Fig. 4.11l). Multiseriate; Cells were irregular in shape and arrangement near the proliferating region, 119.452±32 µm in size (Fig. 4.11k). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.11m). Margins of tubular filament were smooth or wavy (Fig. 4.11j). Multiple pyrenoids were present in cells (Fig. 4.11k). Reproductive zoospores were observed in basal region of main branch of thallus (Fig. 4.11g). Long flagellated zoospores were appeared (Fig. 4.11h). Many dividing cells were also appeared (Fig. 4.11m). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG918101

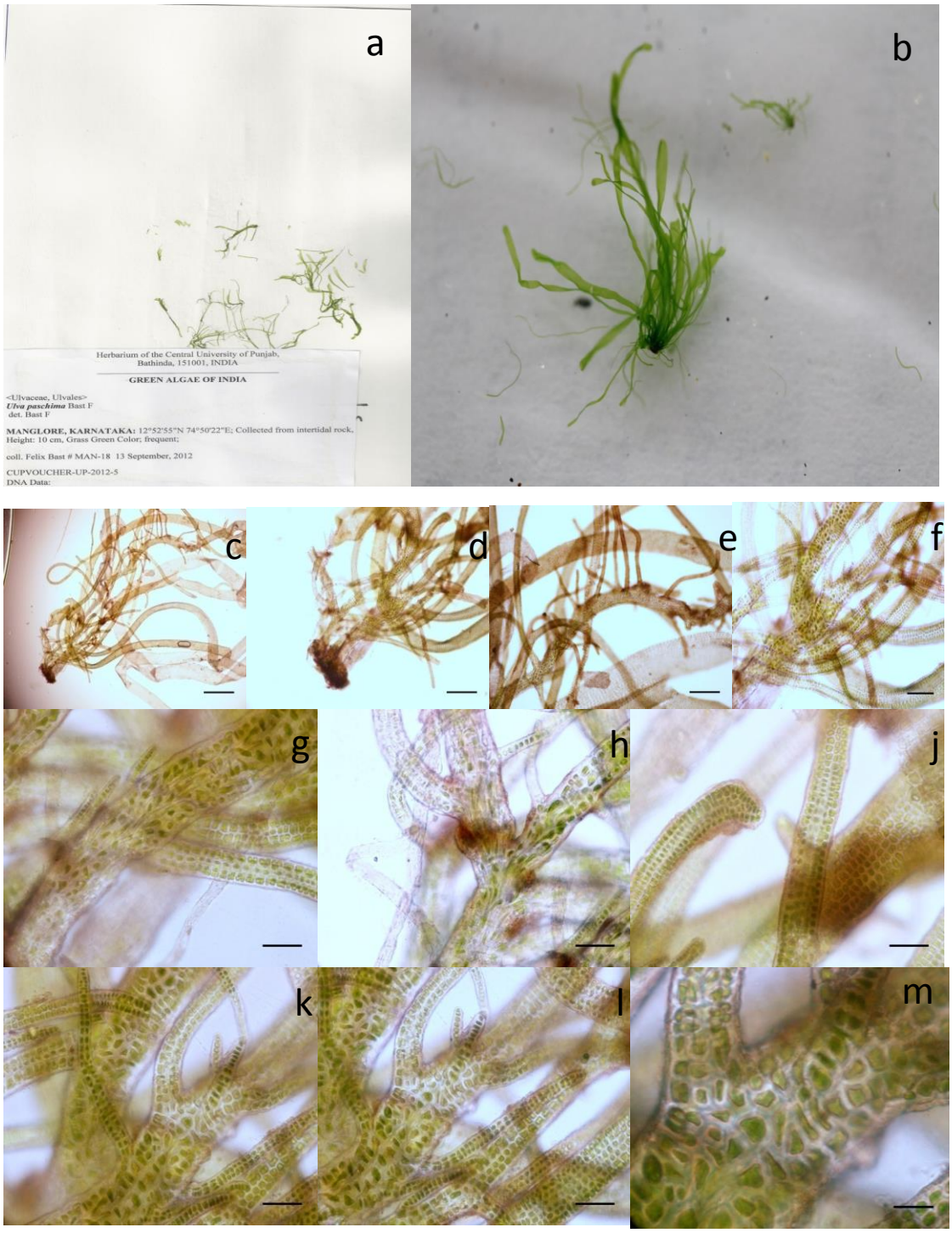


Figure 4.11: Morphology and Micromorphology of *Ulva paschima* from Karwar, India (KAR-18): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Branching pattern of thallus at 10X; g, h Arrangement of reproductive zoospores at 40X; i, j Tip

region of a branch at 40X; k, l Presence of alternatively growing uniseriate and multiseriate secondary branches at 40X; m Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 100X; Dividing cells are also present; Scale bar is 200µm for d, e, f; 50µm for g, h, i, j, k, l; 20µm for m.

BEK-23.2: *Ulva reticulata* Forsskal

Sampling site:

Location: Bekal (Kerala); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: BEK-23.2; CUP Voucher ID: CUPVOUCHER-BEK-2012-UR-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BEK-2012-UR-1

Morphology:

Thallus was leafy and lobed (Fig. 4.12a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 20-40 cm long (Fig. 4.12b). Surface of plant was smooth; lobes of leaves were torned off and start free floating in water after maturation. Thallus was 2 cells thick, cells oval or kidney shaped in surface view (Fig. 4.12j). Cells were irregular in arrangement (Fig. 4.12i), multiseriate; 199.462±30.841 µm in size and irregular in shape (Fig. 4.12j). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.12k). Margins of leaves were wavy (Fig. 4.12c). Multiple pyrenoids were present in cells (Fig. 4.12k). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.12d, 4.12f). Long flagellated zoospores were appeared (Fig. 4.12e). Few empty cells were present after the release of zoospores (Fig. 4.12g, 4.12h). Many dividing cells were also appeared (Fig. 4.12j). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG963793

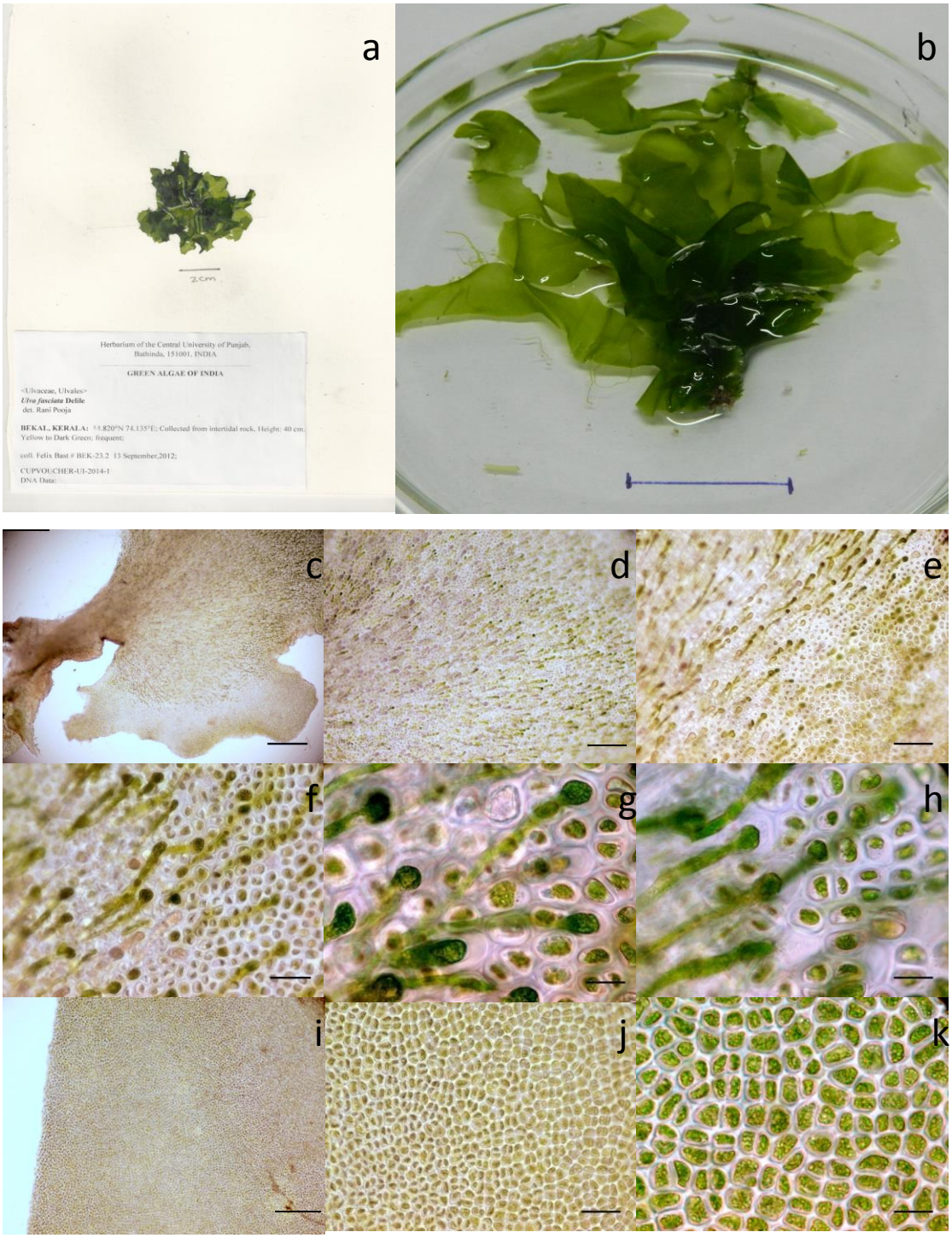


Figure 4.12: Morphology and Micromorphology of *Ulva reticulata* from Bekal, India (BEK-23.2): a Herbarium, b Morphology of thallus; c Microphotograph of basal region of thallus at 4X; d, e Reproductive zoospores at basal region of thallus at 10X, 20X; f, g Flagellated zoospores at middle region at 20X, 100X; g, h Empty cells at middle region after release of zoospores at 100X; i Arrangement of cells at 10X; j Shape and division of cell at 20X; k Chloroplast and pyrenoids in the cell at 40X; Dividing cells are also

present; Scale bar is 200µm for d, i; 100µm for e, f, j; 50µm for k; 20µm for g, h.

BEK-23.4: *Ulva fasciata* Delile

Sampling site:

Location: Bekal (Kerala); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: BEK-23.4; CUP Voucher ID: CUPVOUCHER-BEK-2012-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BEK-2012-UF-1

Morphology:

Thallus was leafy and lobed (Fig. 4.13a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 20-40 cm long (Fig. 4.13b). Surface of plant was smooth; lobes of leaves were torned off and start free floating in water after maturation. Thallus was two cell thick; cells were irregular in shape in surface view (Fig. 4.13i). Cells were irregular in arrangement (Fig. 4.13j), multiseriate; 203.364±45 µm in size. Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.13j). Margins of leaves were wavy (Fig. 4.13c, 4.13d). Multiple pyrenoids were present in cells (Fig. 4.13k). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.13f). Long flagellated zoospores were appeared (Fig. 4.13g). Few empty cells were present after the release of zoospores (Fig. 4.13h). Many dividing cells were also appeared (Fig.4.13e). Thallus was distromatic. Endophytic red algae also found in the thallus (Fig.4.13i).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG963794

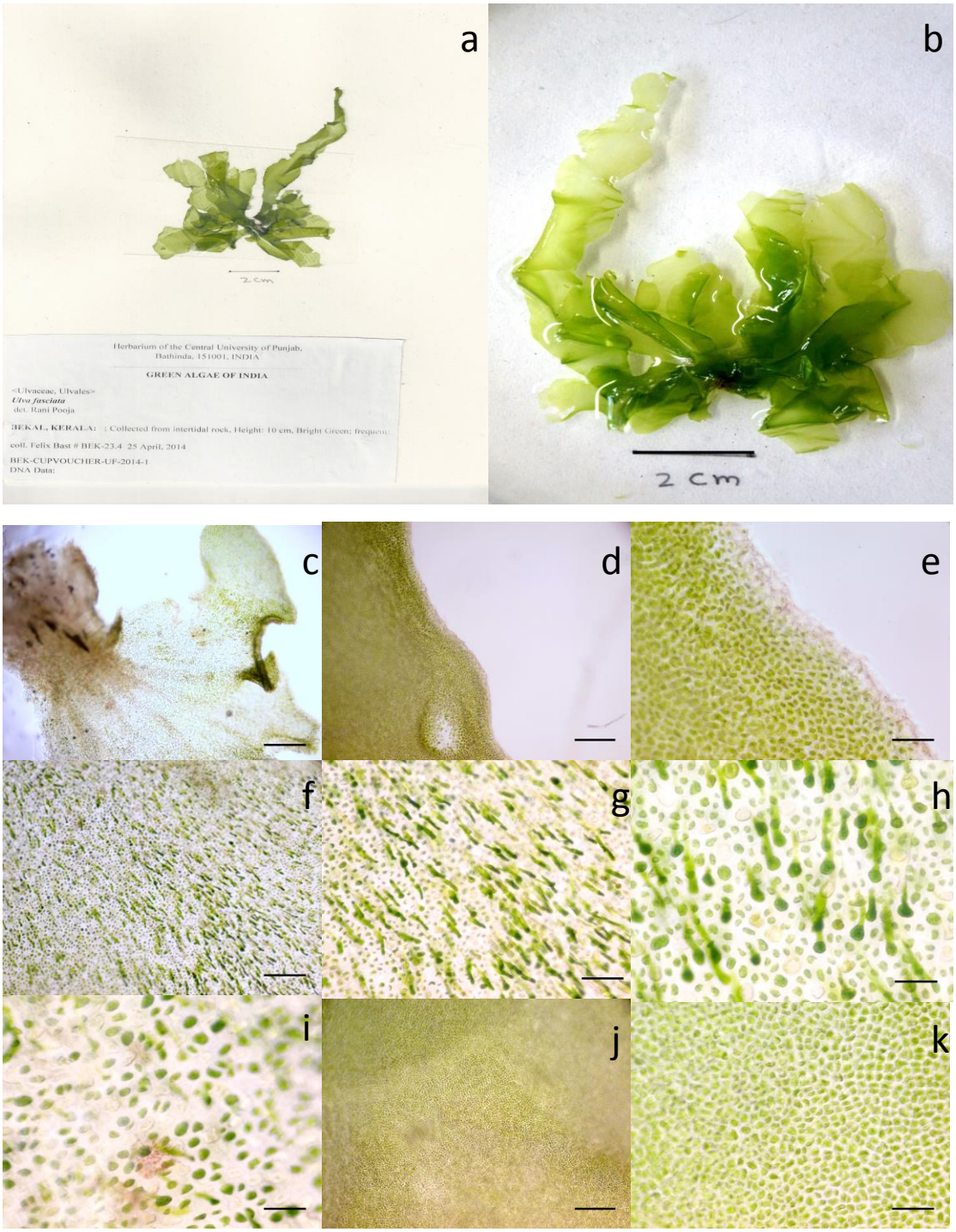


Figure 4.13: Morphology and Micromorphology of *Ulva fasciata* from Bekal, India (BEK-23.4): a Herbarium, b Morphology of thallus; c Microphotographs of basal region of thallus at 4X; d, e Cell arrangement and margin of thallus at 10X, 40X; f, g Presence and distribution of zoospores in the basal region at 20X, 40X; h, i Cell arrangement and zoospores in the middle region of thallus at 100X; i Endophytic red algae at 100X; j, k Cell arrangement

and shape of the cell at 10X, 40X; Dividing cells are also present; Scale bar is 200µm for d, j; 100µm for f; 50µm for e, g, k; 20µm for h, i.

HAV-35: *Ulva fasciata* Delile

Sampling site:

Location: Havelock (Andaman Island); Collection date: 13-09-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: HAV-35; CUP Voucher ID: CUPVOUCHER-HAV-2012-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-HAV-2012-UF-1

Morphology:

Thallus was leafy and lobed (Fig. 4.14a). Holdfast was attached to rocks or any other hard substrata in intertidal area (Fig. 4.14c). It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 20-40 cm long (Fig. 4.14b). Surface of thallus was smooth; lobes of leaves torned off and start free floating in water after maturation. Thallus was 2 cells thick, cells oval or kidney shaped in surface view (Fig. 4.14k). Cells were regular in arrangement (Fig. 4.14i), multiseriate; 167.103±13 µm in size and irregular in shape (Fig. 4.14j). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.14k). Margins of leaves were wavy (Fig. 4.14b). Multiple pyrenoids were present in cells (Fig. 4.14h). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.14d, 4.14e). Long flagellated zoospores were appeared (Fig. 4.14e, 4.14f). Few empty cells were present after the release of zoospores (Fig. 4.14g). Many dividing cells were also appeared (Fig. 4.14j). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- 18S F &18S R: MG774430
- tufA F & tufA R: MG918116
- atpB F & atpB R: MG963797

Figure 4.14: Morphology and Micromorphology of *Ulva fasciata* from Havelock, India (HAV-35): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Presence of zoospores in the basal and middle region of thallus at 20X, 40X; g, h Cell arrangement and presence of empty cells in the middle region at 40X, 100X; i, j Shape of the cell and arrangement of the cells in middle region at 10X, 20X; k

Shape of chloroplast and number of pyrenoids in the distal end at 40X; Dividing cells are also present; Scale bar is 200µm for d, i; 100µm for e, j; 50µm for f, g, k; 20µm for h.

KAP-42.1: *Ulva sapora* Philip

Sampling site:

Location: Kalapathar (Andaman Island); Collection date: 13-01-2014; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KAP-42.1; CUP Voucher ID: CUPVOUCHER-KAP-2014-US-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAP-2014-US-1

Morphology:

Thallus was tubular and ribbon like coiled (Fig. 4.15a). Holdfast was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 40 cm long and divided into number of ribbon shaped. 1-.2 cm tubular or compressed thread like structure (Fig. 4.15b). Surface of plant was smooth; plants were free floating in water after maturation. Thallus was unbranched (Fig. 4.15c). Cells were cuboidal or rectangular and regular in arrangement in the tubular region (Fig. 4.15d), Cells were arranged in linear rows (Fig. 4.15e), Multiseriate, 34±1 µm in size (Fig. 4.15e). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.15f). Margins of leaves were smooth (Fig. 4.15c). Multiple pyrenoids were present in cells (Fig. 4.15f). Many dividing cells were also appeared (Fig. 4.15f). Thallus was monostromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763136

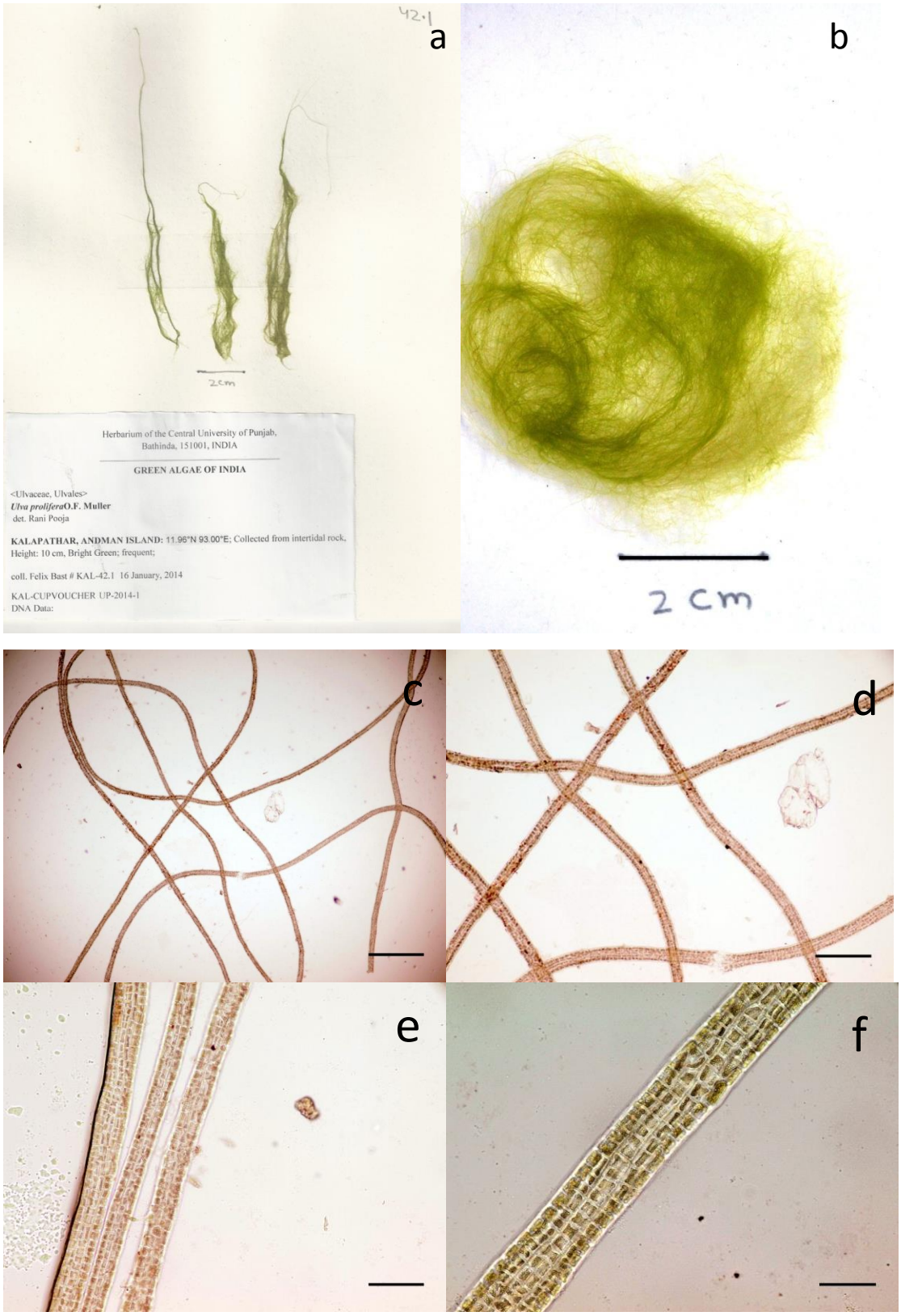


Figure 4.15: Morphology and Micromorphology of *Ulva sapora* from Kalapathar, India (KAP-42.1): a Herbarium, b Morphology of thallus; c Microphotographs and margin of thallus at 4X; d Cell arrangement of thallus at 10X; e Shape of cell at 20X; f Shape of chloroplast and number of pyrenoids in

the cell at 40X. Dividing cells are also present; Scale bar is 200µm for d; 100µm for e; 50µm for f.

NOB-43.2: *Ulva sapora* Philip

Sampling site:

Location: North Bay (Andaman Island); Collection date: 13-01-2014; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: NOB-43.2; CUP Voucher ID: CUPVOUCHER-NOB-2014-US-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-NOB-2014-US-1

Morphology:

Thallus was bushy and tubular (Fig. 4.16a). Holdfast was attached to the rocks or any other hard substrata with the help of disc shaped structure in intertidal area (Fig. 4.16c). It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 20 cm long and tubular or compressed thread like structure (Fig. 4.16b). Surface of thallus was smooth; plants. Thallus was highly branched (Fig. 4.16d). Numbers of uniseriate or multiseriate newly developed branches were observed from the primary branch of thallus (Fig. 4.16d). Secondary branches were growing from primary branch in alternative fashion (Fig.4.16d). Tip of the branch was pointed (Fig. 4.16c). Cells were cuboidal or oval in shape and regular in arrangement in the tubular region (Fig. 4.16f), Cells were arranged in linear rows (Fig. 4.16e). Multiseriate; Cells were irregular in shape and arrangement near the proliferating region (Fig. 4.16d), 58±3 µm in size (Fig. 4.16g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.16g). Margins of leaves were smooth (Fig. 4.16f). Multiple pyrenoids were present in cells (Fig. 4.16h). Reproductive zoospores were observed in basal region of main branch of thallus (Fig. 4.16c). Long flagellated zoospores were appeared (Fig. 4.16c). Many dividing cells were also appeared (Fig. 4.16h). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763137

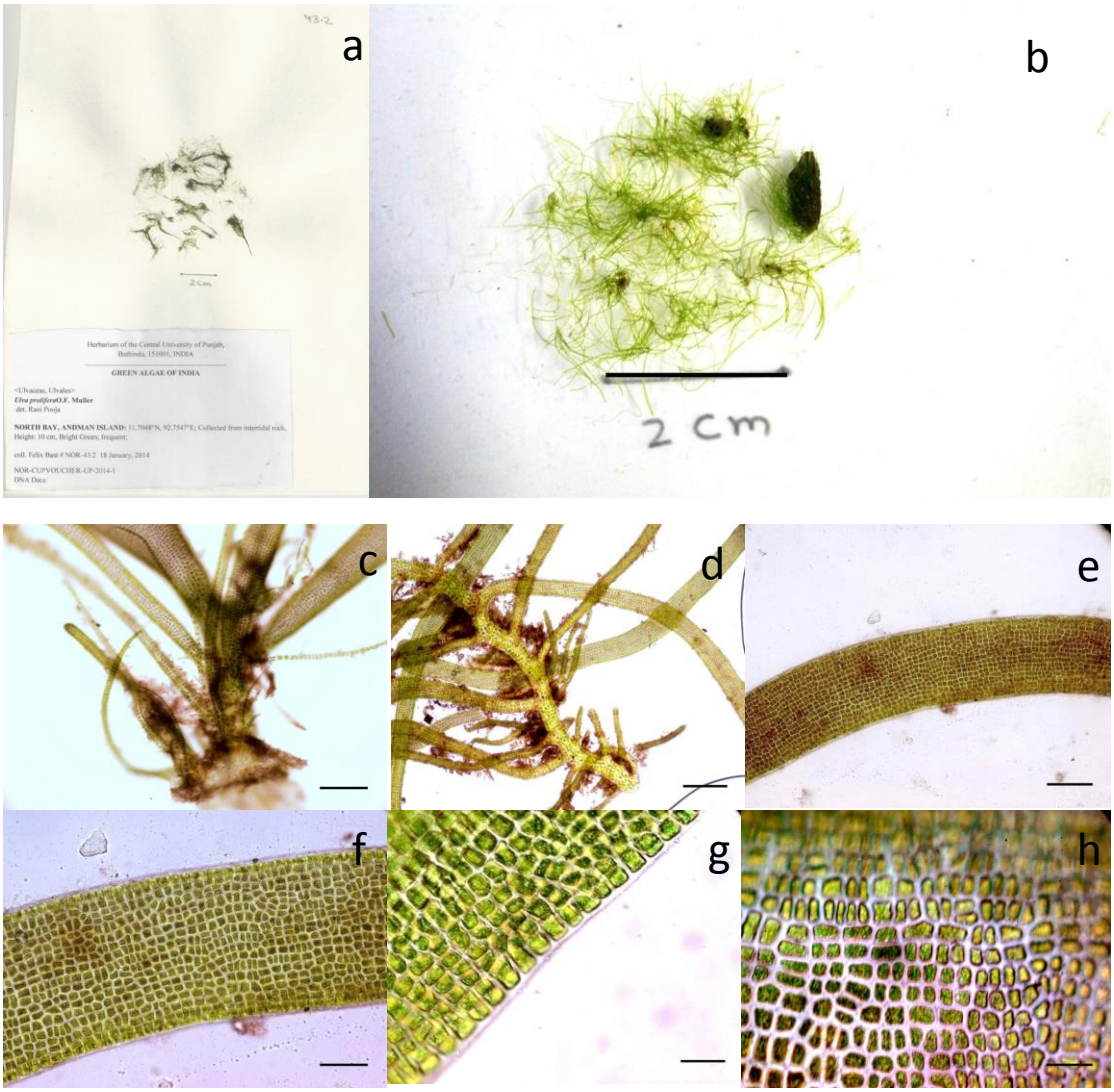


Figure 4.16: Morphology and Micromorphology of *Ulva sapora* from North bay, India (NOB-43.2): a Herbarium, b Morphology of thallus; c, d Microphotographs and presence of reproductive zoospores in the basal region of thallus at 4X, 10X respectively; e Margin of the filament at 20X; f Shape of cell and arrangement of cells in the filament at 40X; g Shape of chloroplast inside at 100X; h Number of pyrenoids and presence of dividing cells at 100X; Scale bar is 200µm for d; 100µm for e; 50µm for f; 20µm for g, h.

KOL-47.2- *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Kollam, Kerala; Collection date: 21-07-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KOL-47.2; CUP Voucher: CUPVOUCHER-KOL-2014-UX-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOL-2014-UX-1.

Morphology:

Plant was bushy in appearance and dark green in colour (Fig. 4.17a). Thallus was branched and 1-2 cm long, tubular and hollow (Fig. 4.17b, 4.17c). Thallus was attached to the surface and exposed to rocky shores. Sometimes also, present in pebbles and freely floating in water. Cells were arranged in linear series, elongated in shape (Fig. 4.17e). Chloroplast was present in the form of patches (Fig. 4.17g). Cells were cuboidal or rectangular and regular in arrangement (Fig. 4.17g), multiseriate; 99.03±10 µm in size (Fig. 4.17g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.17g). Margins of leaves were plain (Fig. 4.17f). Multiple pyrenoids were present in cells (Fig. 4.17k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.17i). Long flagellated zoospores were appeared (Fig. 4.17j). Many Dividing cells were also appeared (Fig. 4.17h). Thallus was tubular and highly branched. Bifurcating branch was observed and conical of biseriate branch was observed (Fig. 4.17h). Tubular branches were formed hollow opening in the branch at distal end (Fig. 4.17e).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763140

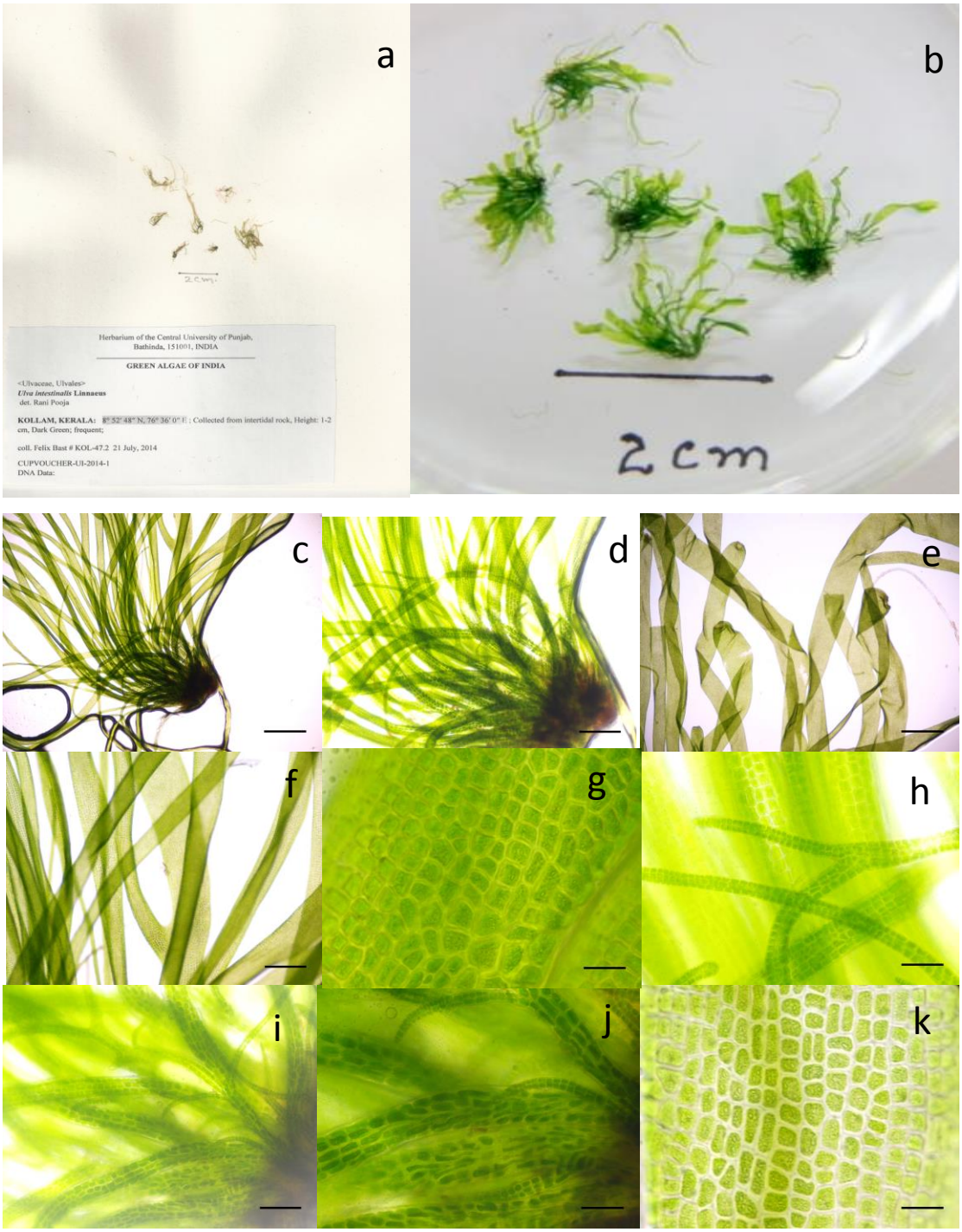


Figure 4.17: Morphology and Micromorphology of *Ulva shanxiensis* from Kollam, India (KOL-47.2): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e Distal ends of tubular thalli at 20X; f Margin of tubular branch at 20X; g Cell arrangement and shape of the cell at 100X; h Bifurcating branch at distal end and arrangement of cells at the end at 40X; i, j Presence and arrangement of flagellated zoospores in the basal region at 20X,40X; k Chloroplast arrangement and pyrenoids

arrangement inside the cell at 100X; Dividing cells are also present; Scale bar is 200µm for d; 100µm for e, f, i; 50µm for h, j; 20µm for g, k.

DIG-48.1-*Ulva intestinalis* Linnaeus

Sampling site:

Location: Digha (West Bengal); Collection date: 25-04-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: DIG-48.1; CUP Voucher: CUPVOUCHER-DIG-2014-UI-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-DIG-2014-UI-1.

Morphology:

Plant was bushy in appearance and dark green in colour (Fig. 4.18a). Thallus was branched and 10-20 cm long, tubular and hollow (Fig. 4.18b). Thallus was attached to the surface and exposed to rocky shores. Disc shaped structure was observed in basal region that help for attachment to the substratum (Fig. 4.18c). Sometimes also, present in pebbles and freely floating in water. Filaments of the thallus were highly branched. Many newly developing uniseriate branches were observed near the basal region (Fig. 4.18d, 4.18f). Some epiphytic red algae were also present near the basal region (Fig. 4.18e). Cells were arranged in linear series, elongated in shape (Fig. 4.18k). Chloroplast was present in the form of patches. Cells were regular in arrangement (Fig. 4.18l), Multiseriate; 185.028±20 µm in size and irregular in shape (Fig. 4.18l). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.18l). Margins of leaves were smooth (Fig. 4.18j). Multiple pyrenoids were present in cells (Fig. 4.18m). Reproductive zoospores were observed in basal region of thallus (Fig. 4.18g, 4.18h). Long flagellated zoospores were appeared (Fig. 4.18i). Many dividing cells were also appeared (Fig. 4.18m). Horizontal and vertical dividing cells were observed. Thallus was tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- 18S F &18S R: MG774434
- atpB F & atpB R: MG918106

Figure 4.18: Morphology and Micromorphology of *Ulva intestinalis* from Digha, India (DIG-48.1): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Branching pattern of thallus at 10X; g, h, i Presence of reproductive zoospores at basal region of thallus at 40X; j, k Cell arrangement and margin of thallus at 10X, 20X; l, m Chloroplast arrangement and pyrenoids arrangement at 40X, 100X. Dividing

cells are also present; Scale bar is 200µm for d, e, f, j; 100µm for k; 50µm for g, h, i, l; 20µm for m.

DIA-48.2 *Ulva uniseriata* sp.nov.

Sampling site:

Location: Diamond harbor, West Bengal; Collection date: 25-04-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: DIA-48.2; CUP Voucher: CUPVOUCHER-DIA-2014-UU-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-DIA-2014-UU-1.

Morphology:

Plant was tubular and light green in colour. Thallus was unbranched, flattened, uncoiled and 3-7cm long (Fig. 4.19a). Thallus was attached to the rock surface. Cells were regular in arrangement, having cell-to-cell connection, 3-5µm in size, and rectangular in shape (Fig. 4.19b). Chloroplast was present in the form of thick patches (Fig. 4.19c). Cells were linear in arrangement (Fig. 4.19d), uniseriate; 154.169 ± 9µm in size and regular in shape (Fig. 4.19d). Cell wall was thick.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: KX668899

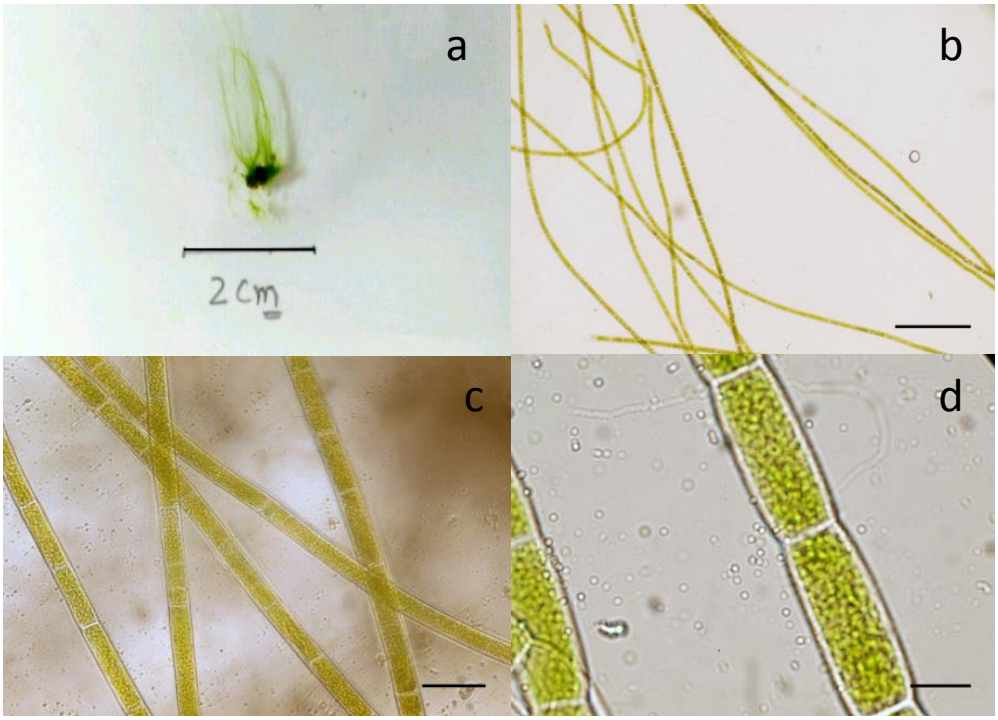


Figure 4.19: Morphology and Micromorphology of *Ulva uniseriata* sp. Nov. from Diamond Harbor, India: a Morphology of thallus; b Microphotographs of thallus at 10X; c Cell arrangement and margin of thallus at 40X; d Chloroplast arrangement at 100X. Scale bar is 200µm for b; 50µm for c; 20µm for d.

BAK-48.3: *Ulva intestinalis* Linnaeus

Sampling site:

Location: Bakkhali (West Bengal); Collection date: 25-04-2014; collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: BAK-48.3; CUP Voucher: CUPVOUCHER-BAK-2014-UI-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BAK-2014-UI-1.

Morphology:

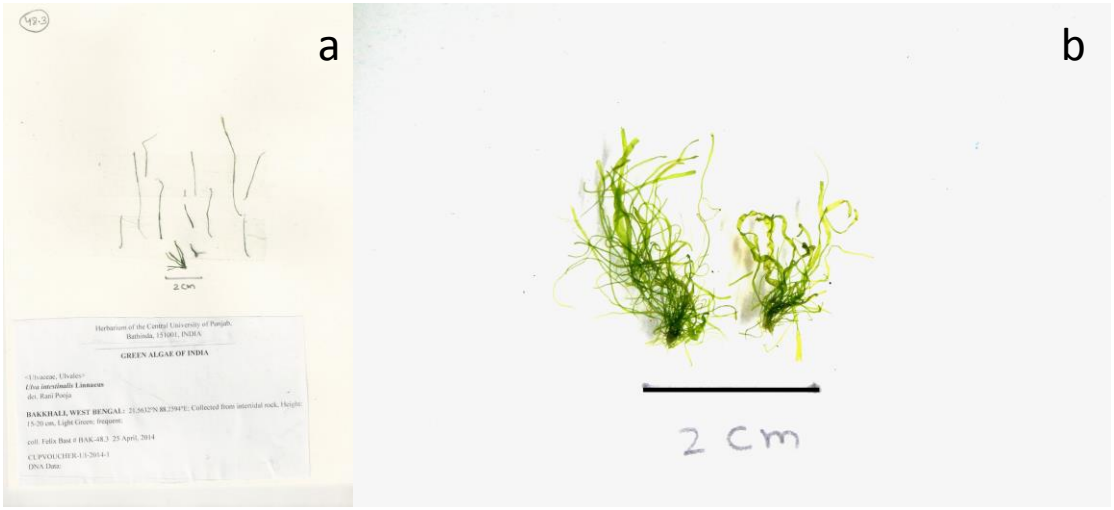
Plant was bushy in appearance and light to dark green in colour (Fig. 4.20a). Thallus was highly branched, 15-20 cm long with tubular filaments (Fig. 4.20b). Filaments were formed alternatively growing secondary branches (Fig. 4.20d). Thallus was attached to the surface and exposed to the rocky shore (Fig. 4.20c). Sometimes also, present in pebbles and freely floating. Cells were irregular in arrangement at distal end (Fig. 4.20n), but arranged in linear rows near the basal region, irregular in shape (Fig. 4.20h), Multiseriate; 141.461±53

µm in size and irregular in shape (Fig. 4.20n). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.20m). Margins of leaves were smooth (Fig. 4.20l). Multiple pyrenoids were present in cells (Fig. 4.20n). Reproductive zoospores were observed in basal region of thallus (Fig. 4.20e). Long flagellated zoospores were appeared (Fig. 4.20i, 4.20j). Many dividing cells were also appeared (Fig. 4.20k). Thallus was monostromatic, tubular. Secondary filaments were formed conical structure near the tip region (Fig. 4.20f, 4.20g). Main branch was less broad as compared to distal end (Fig.4.20l).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG918107



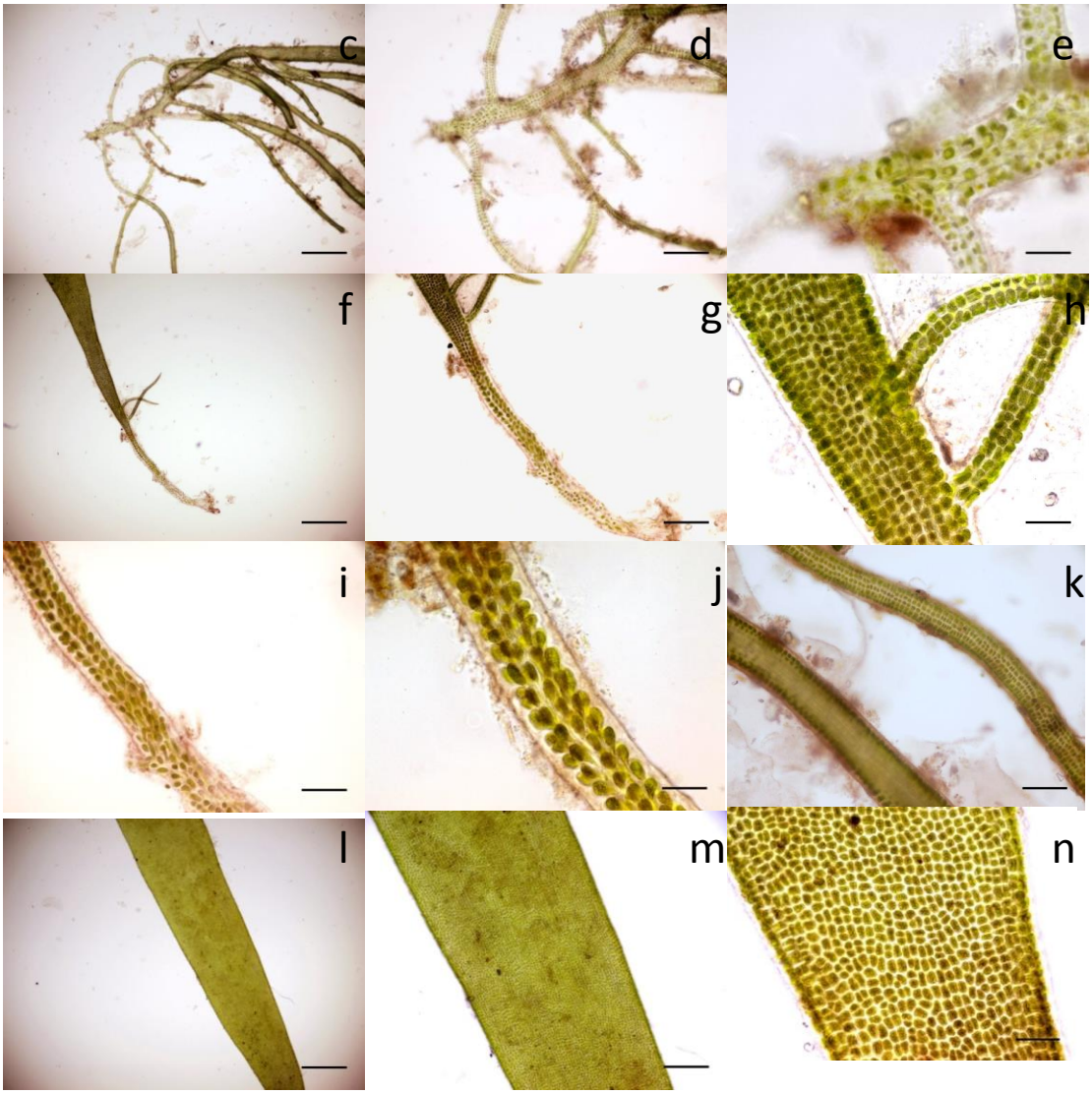


Figure 4.20:Morphology and Micromorphology of *Ulva intestinalis* from Bakkhali, India (BAK-48.3): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; f, g, h Presence of alternatively growing secondary branches at 10X, 20X, 40X; e, i, j Zoospores at basal region of the branch at 100X, 40X, 100X; k, l Cell arrangement and margin of thallus at 10X, 20X; m, n indicates chloroplast arrangement and pyrenoids arrangement at 40X, 100X; Dividing cells are also present; Scale bar is 200µm for d, f, k; 100µm for g, l; 50µm for h, i, m; 20µm for e, j, n.

GOS-48.4-*Ulva linza* Linza

Sampling site:

Location: Gosaba, West Bengal; Collection date: 25-04-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: GOS-48.4; CUP Voucher: CUPVOUCHER-GOS-2014-UL-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-GOS-2014-UL-1.

Morphology:

Thallus was leafy in appearance and light green in colour (Fig. 4.21a). Thallus was branches, 1-5 cm long with broad leaf. Leaves had lobed edges (Fig. 4.21b). Thallus was attached to the surface with a disc shaped structure and exposed to the rocky shore (Fig. 4.21d). Sometimes also, present in pebbles and freely floating. Cells were irregular in arrangement, rounded, oval or irregular in shape; multiseriate (Fig. 4.21e); 167.182±51µm in size. Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.21h). Margins of leaves were smooth (Fig. 4.21f). Multiple pyrenoids were present in cells (Fig. 4.21i). Reproductive zoospores were observed in basal region of thallus (Fig. 4.21d). Long flagellated zoospores were appeared. Many dividing cells were also appeared (Fig. 4.21g). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918123

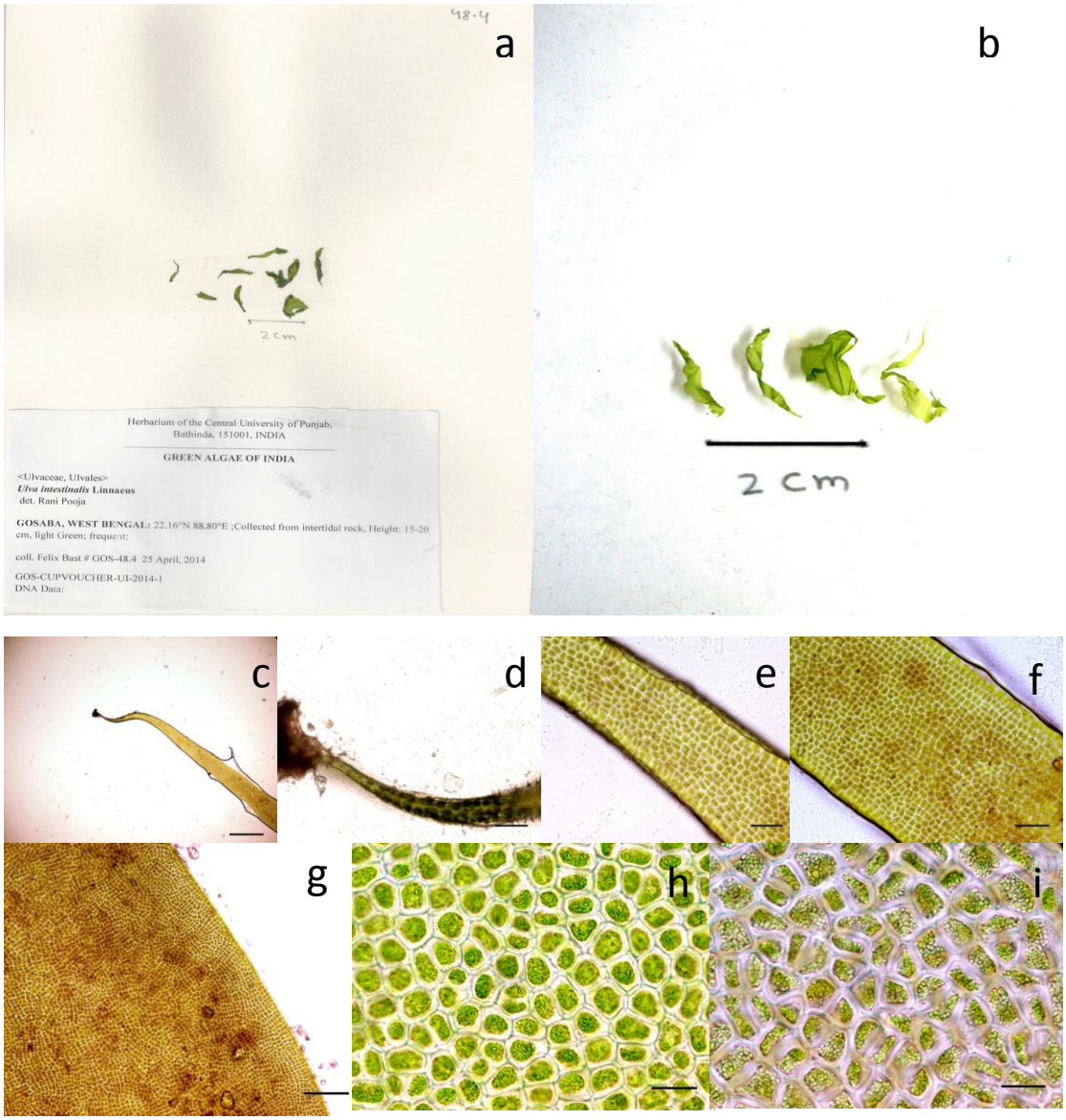


Figure 4.21: Morphology and Micromorphology of *Ulva linza* from Gosaba, India (GOS-48.4): a Herbarium, b Morphology of thallus; c Microscopic analysis of basal region of thallus at 10X; d Reproductive zoospores at basal region of thallus at 20X; e, f Cell arrangement and margin of thallus at 20X, 40X; g, h Chloroplast arrangement and pyrenoids arrangement at 10X, 40X in the middle region of thallus. i Chloroplast arrangement and pyrenoids arrangement at distal end at 100X; Dividing cells are also present; Scale bar is 200µm for c, g; 100µm for d, e; 50µm for f, h; 20µm for i.

KOL-49.1 *Ulva reticulata* Forsskal

Sampling site:

Location: Kollam, Kerala; Collection date: 04-08-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KOL-49.1; CUP Voucher: CUPVOUCHER-KOL-2014-UR-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOL-2014-UR-1

Morphology:

Thallus was leafy in appearance and dark green in colour (Fig. 4.22a). Thallus was branched, 5-7cm long, distal ends form heart shape structure (Fig. 4.22b). Thallus was attached to the rocky surface of shore. Chloroplast was scattered. Cells were irregular in arrangement (Fig. 4.22o), Multiseriate; 199.462±30 µm in size and rounded or oval in shape (Fig. 4.22p). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.22p). Margins of leaves were wavy (Fig. 4.22j, 4.22k). Denticulate were present on the margin (Fig. 4.22l, 4.22m). Cell arrangement in the denticulate region was irregular (Fig. 4.22n). Multiple pyrenoids were present in cells (Fig. 4.22p). Reproductive zoospores were observed in basal region of thallus (Fig. 4.22d, 4.22e). Long flagellated zoospores were appeared (Fig. 4.22h). In the middle region, comparatively less number of zoospores were present (Fig. 4.22f). Some empty cells were also observed in the middle region after the release of the zoospores (Fig. 4.22i). Many dividing cells were also appeared (Fig. 4.22g). Thallus was distromatic. Epiphytic red algae *Erythrocladia* was present on the surface of leaf blade (Fig. 4.22q, 4.22r).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763224

Figure 4.22: Morphology and Micromorphology of *Ulva reticulata* from Kollam, India (KOL-49.1): a Herbarium, b Morphology of thallus; c Microphotograph of basal region of thallus at 4X; d, e Reproductive zoospores at basal region of thallus at 10X, 20X; f, g Arrangement of zoospores and dividing cells at middle region of thallus at 40X, 100X; h, i Presence of the fully

matured flagellated zoospores and empty cell after release of zoospores in the middle region at 100X. j, k Reticulae in the blade and denticulate at the margin at 10X, 20X. l, m, n Denticula and cell arrangement of denticula at marginal region 20X, 40X, 100X; o, p Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 20X, 100X; q, r Epiphytic red algae *Erythrocladia* at the foliose leaf surface at 100X; Dividing cells are also present; Scale bar is 200µm for d, j; 100µm for e, k, l, o; 50µm for f, m; 20µm for g, h, i, n, p, q, r.

KOL-49.2-*Ulva fasciata* Delile

Sampling site:

Location: Kollam, Kerala; Collection date: 04-08-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KOL-49.2; CUP Voucher: CUPVOUCHER-KOL-2014-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOL-2014-UF-1.

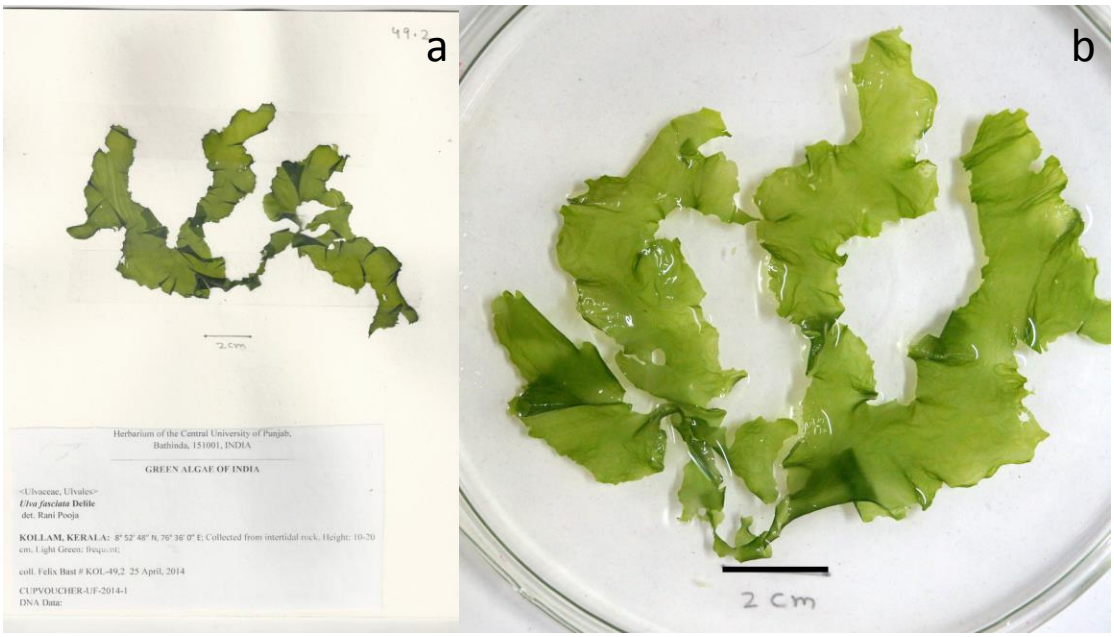
Morphology:

Thallus was leafy in appearance and light green in colour (Fig. 4.23a). Thallus was branched, with lobed ends, 10-20 cm long (Fig. 4.23b). Thallus was attached to the rocky surface. Branches formed lacunae. Leaves were formed zigzag shape in arrangement (Fig. 4.23b). Margins of the leaf were crumbled and wavy (Fig. 4.23c, 4.23d). Cells were irregular in arrangement (Fig. 4.23q, 4.23r), Multiseriate; 158.023±42 µm in size and rounded, oval, bean or irregular in shape (Fig. 4.23r). At the margin of the leaf surface, some disc shaped or arc shaped structure were present (Fig. 4.23e, 4.23f). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.23r). Margins of leaves were wavy. Multiple pyrenoids were present in cells (Fig. 4.23s). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.23k, 4.23l, 4.23n). Some empty cells were also observed in middle region after the release of zoospores (Fig. 4.23p). Long flagellated zoospores were appeared (Fig. 4.23m, 4.23n). Many dividing cells were also appeared (Fig. 4.23h). Horizontal and vertical division were observed. Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763218
- 18S F &18S R: MG774429
- tufA F & tufA R: MG918117
- atpB F & atpB R: MG963796



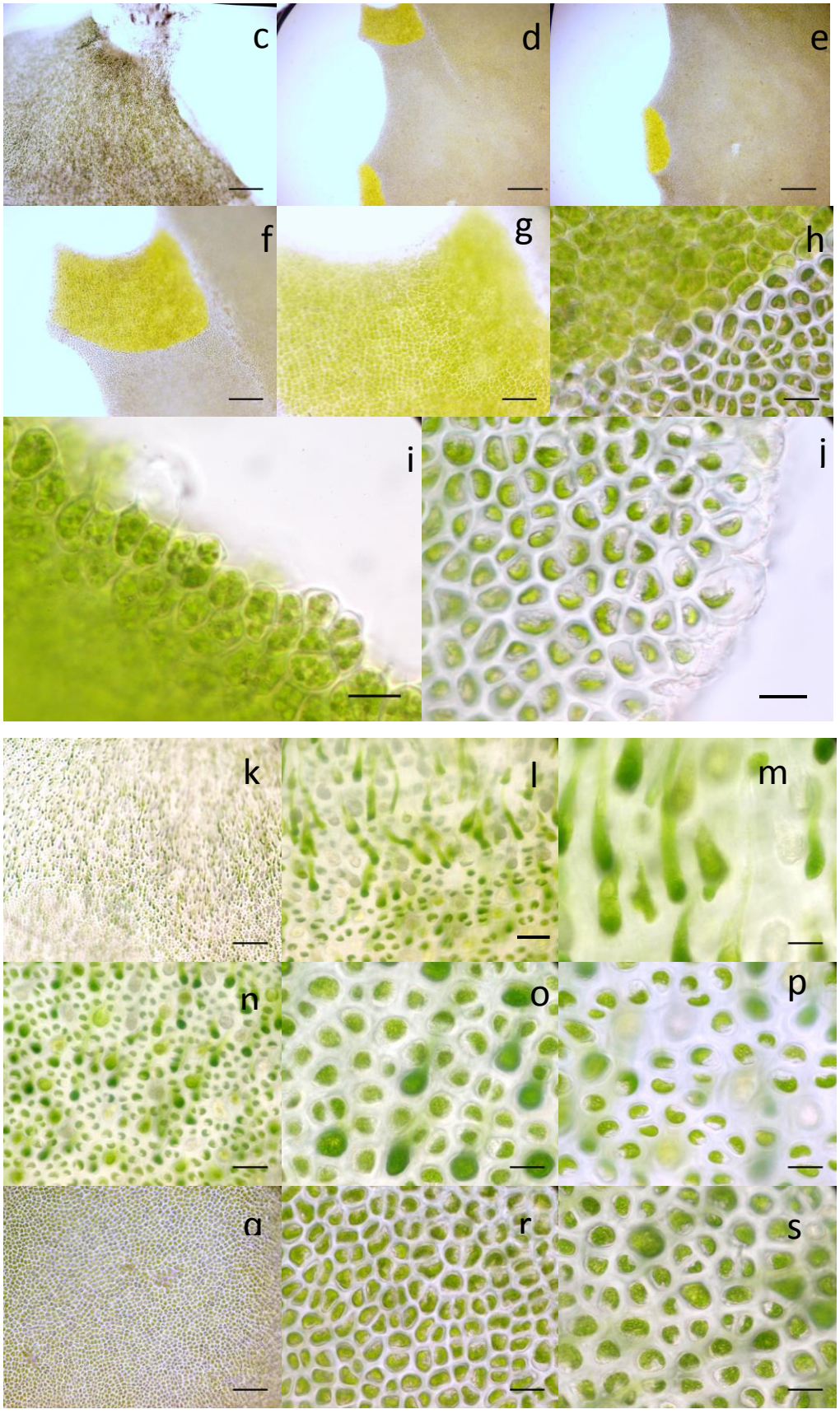

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Figure 4.23: Morphology and Micromorphology of *Ulva reticulata* from Kollam, India: a Herbarium, b Morphology of thallus; c Microphotograph of thallus at 10X; d, e, f, g Cell arrangement and margin of thallus at 10X, 20X, 40X; h, i Zoospores, j Shape of cell at 100X; k, l, m, n Reproductive bodies at basal region at 10X, 40X respectively; o, p Empty cells at 100X; q, r, s Chloroplast arrangement and pyrenoids arrangement at 100X. Dividing cells are also present; Scale bar is 200µm for d, e, k, q; 100µm for f; 50µm for g, l, n; 20µm for h, I, j, m, o, p, r, s.

KOL-49.3 *Ulva shanxiensis* L.Chen, J. Feng & S. Xie

Sampling site:

Location: Kollam, Kerala; Collection date: 04-08-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KOL-49.3; CUP Voucher: CUPVOUCHER-KOL-2014-UX-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOL-2014-UX-1.

Morphology:

Thallus was bushy in appearance and dark green in colour (Fig. 4.24a). Thallus was branched, tubular and 2 cm long (Fig. 4.24b). Thallus was attached with disc shaped structure to the substratum and exposed to rocky shore. Sometimes grow in pebbles or freely floating in water. Cells were arranged in linear series, 2-3µm in size and rectangular or cuboidal in shape (Fig. 4.24g). Chloroplast was present in the form of thick patches (Fig. 4.24h). Cells were regular in arrangement (Fig. 4.24i), Multiseriate; 86.215±3 µm in size. Cell wall was thick. Margin of the filament was smooth (Fig. 4.24d, 4.24e). Multiple pyrenoids were present in cells (Fig. 4.24i). Reproductive zoospores were observed in basal region of thallus (Fig. 4.24j). Long flagellated zoospores were appeared (Fig. 4.24k, 4.24l). Zoospores were also observed in middle region near the dividing cells. Many dividing cells were also appeared (Fig. 4.24f). Horizontal and vertical division was observed. Thallus was monostromatic and tubular (Fig. 4.24c).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763141
- 18S F &18S R: MG774433

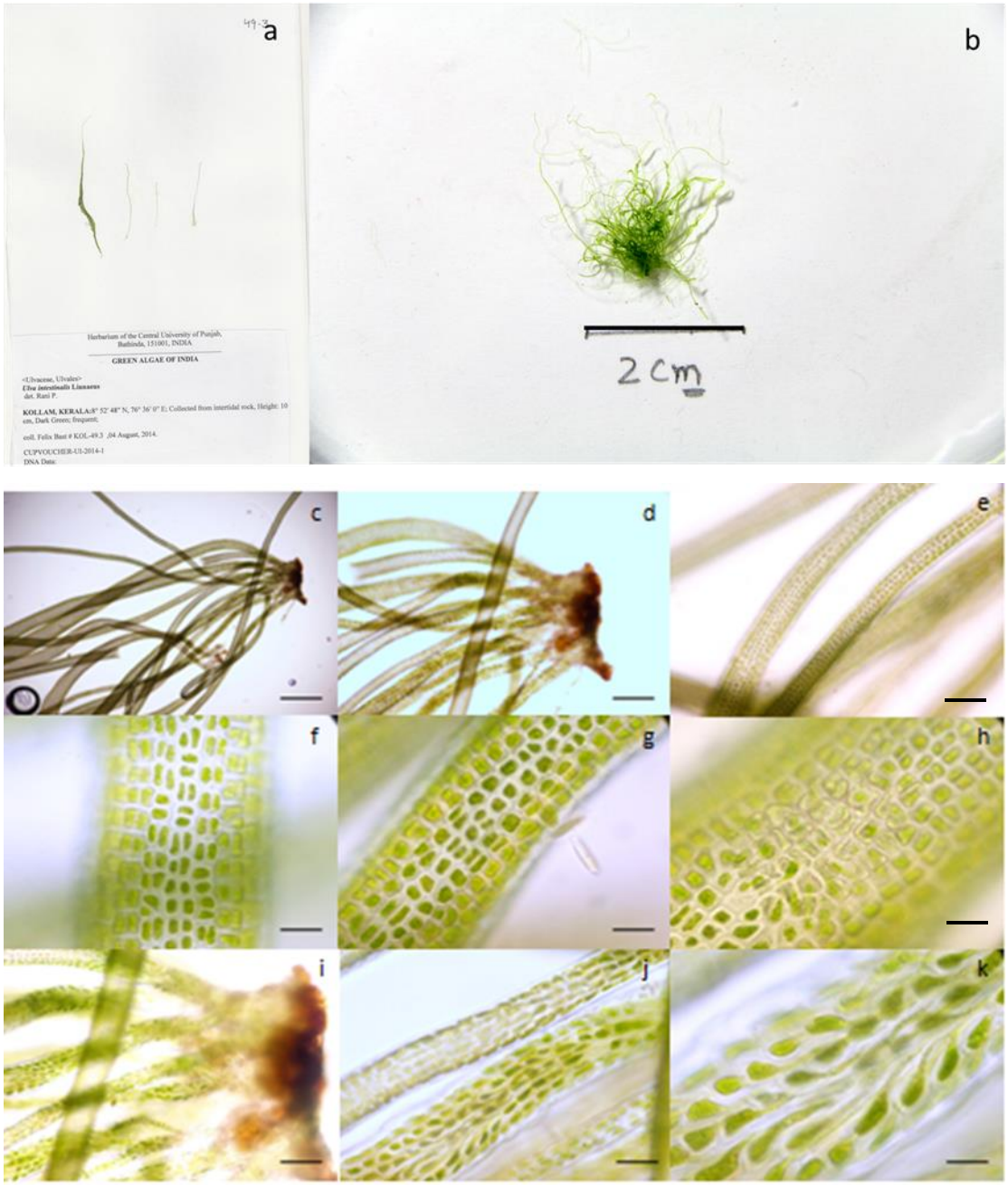

Figure 4.24: Morphology and Micromorphology of *Ulva shanxiensis* from Kollam, India (KOL-49.3): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f, g Cell arrangement, presence of dividing cells and margin of thallus at 20X, 40X; h Chloroplast arrangement and pyrenoids arrangement at 100X. i, j Reproductive bodies at

basal region at 20X, 40X respectively; k Reproductive zoospores at 100X; Scale bar is 200µm for d; 100µm for e, f, i; 50µm for g, j; 20µm for h, k.

KOL-49.4 *Ulva reticulata* Forsskal

Sampling site:

Location: Kollam, Kerala; Collection date: 04-08-2014; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KOL-49.4; CUP Voucher: CUPVOUCHER-KOL-2014-UR-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOL-2014-UR-1

Morphology:

Thallus was leafy and green in colour (Fig. 4.25a). Thallus was unbranched, 5-7 cm long, compressed leaf like appearance with lobed ends (Fig. 4.25b). Thallus was attached to the substratum and exposed to rocky shore. Cell were irregular in arrangement (Fig. 4.25i, 4.25j), Multiseriate; 201.160.723±29 µm in size and oval, rounded or irregular in shape (Fig. 4.25f, 4.25k). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.25l). Margins of leaves were wavy (Fig. 4.25g). Multiple pyrenoids were present in cells (Fig. 4.25m, 4.25h). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.25c). Long flagellated zoospores were appeared (Fig. 4.25d, 4.25e). Many dividing cells were also appeared (Fig. 4.25h). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763225

Figure 4.25: Morphology and Micromorphology of *Ulva reticulata* from Kollam, India (KOL-49.4): a Herbarium, b Morphology of thallus; c Microphotographs of basal region of thallus at 10X; d, e Reproductive bodies at basal region at 10X, 40X respectively; f, g, h Wavy margin and arrangement of cells at marginal region at 20X, 40X, 100X; i, j, k, l Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 20X, 40X, 100X respectively; Dividing cells are also present; Scale bar is 200µm for c, d, i; 100µm for f, j; 50µm for e, g, k; 20µm for h, l.

KOV-50.1- *Ulva prolifera* O.F. Muller

Sampling site:

Location: Kovalam (Tamil Nadu); Collection date: 11-07-2011; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: KOV-50.1; CUP Voucher ID: CUPVOUCHER-KOV-2011-UP-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOV-2011-UP-1

Morphology:

Thallus was green to dark green in colour (Fig. 4.26a). Thallus was bushy in appearance, highly branched, tubular, 15-20 cm long (Fig. 4.26b). Filaments were soft, coiled and surface is smooth. Cells were regular in arrangement, arranged in linear rows (Fig. 4.26l), Multiseriate; 160.464±47 µm in size and rectangular in shape (Fig. 4.26m). Alternative branching pattern was present in the thallus (Fig. 4.26h). Bifurcating branches were observed in thallus (Fig. 4.26j). Tip of the branch was conical or rounded in cell arrangement (Fig. 4.26k). Arrangement of cells near the proliferating region was irregular (Fig. 4.26i). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.26n). Margins of leaves were smooth (Fig. 4.26l). Multiple pyrenoids were present in cells (Fig. 4.26n). Reproductive zoospores were observed in basal region of thallus (Fig. 4.26d, 4.26e). Long flagellated zoospores were appeared (Fig. 4.26f, 4.26g). Many dividing cells were also appeared (Fig. 4.26i). Thallus was monostromatic and tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG768947
- atpB F & atpB R: MG918103

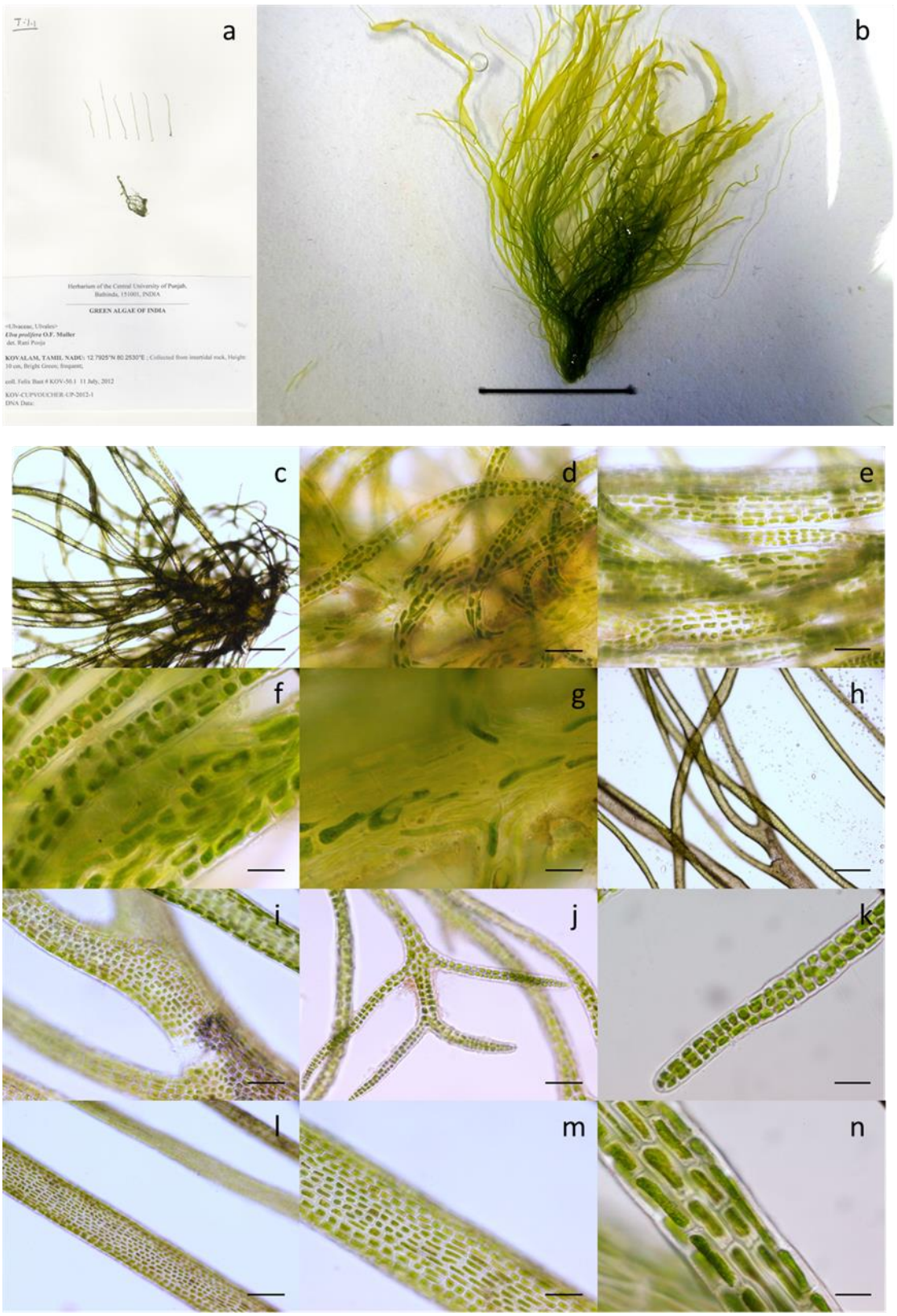

Figure 4.26: Morphology and Micromorphology of *Ulva prolifera* from Kovalam, India (KOV-50.1): a Herbarium, b Morphology of thallus; c, d Microphotographs of highly alternatively branched basal region of thallus at 10X, 20X; e, f, g Reproductive zoospores at basal region at 20X, 40X, 100X; h,

i, j Branching pattern and arrangement of cells at proliferated region at 10X, 40X; k Arrangement of cells at the tip region of filament at 100X; l, m Cell arrangement and margin of thallus at 10X, 40X; n Chloroplast arrangement and pyrenoids arrangement at 100X; Dividing cells are also present; Scale bar is 200µm for c, i, l; 100µm for d, e; 50µm for f, j, m; 20µm for g, k, n.

ENN-50.8: *Ulva ohnoi* M. Hiraoka & S. Shimada

Sampling site:

Location: Ennore (Tamil Nadu); Collection date: 20-07-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: ENN-50.8; CUP Voucher ID: CUPVOUCHER-ENN-2012-UO-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ENN-2012-UO-1

Morphology:

Thallus was light green in colour and growing attached to the rocks in marine water (Fig. 4.27a). Thallus was nearly 2-3 cm wide but thallus was 20-30 cm long (Fig. 4.27b). Thallus was highly coiled like a ribbon and thicker in upper and middle regions. Tiny serrations were present on the leaf surface. Cells were irregular in arrangement (Fig. 4.27f), Multiseriate; 110.075±29 µm in size and irregular in shape (Fig. 4.27g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.27h). Margins of leaves were wavy or ruffled (Fig. 4.27i). Multiple pyrenoids were present in cells (Fig. 4.27h). Reproductive zoospores were observed more in basal region of thallus as compared to thallus (Fig. 4.27c). Long flagellated zoospores were appeared (Fig. 4.27d, 4.27e). Many dividing cells were also appeared (Fig. 4.27k). Thallus was distromatic. Epiphytic and endophytic red algae were present in the leaf blade (Fig. 4.27j, 4.27k).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763227

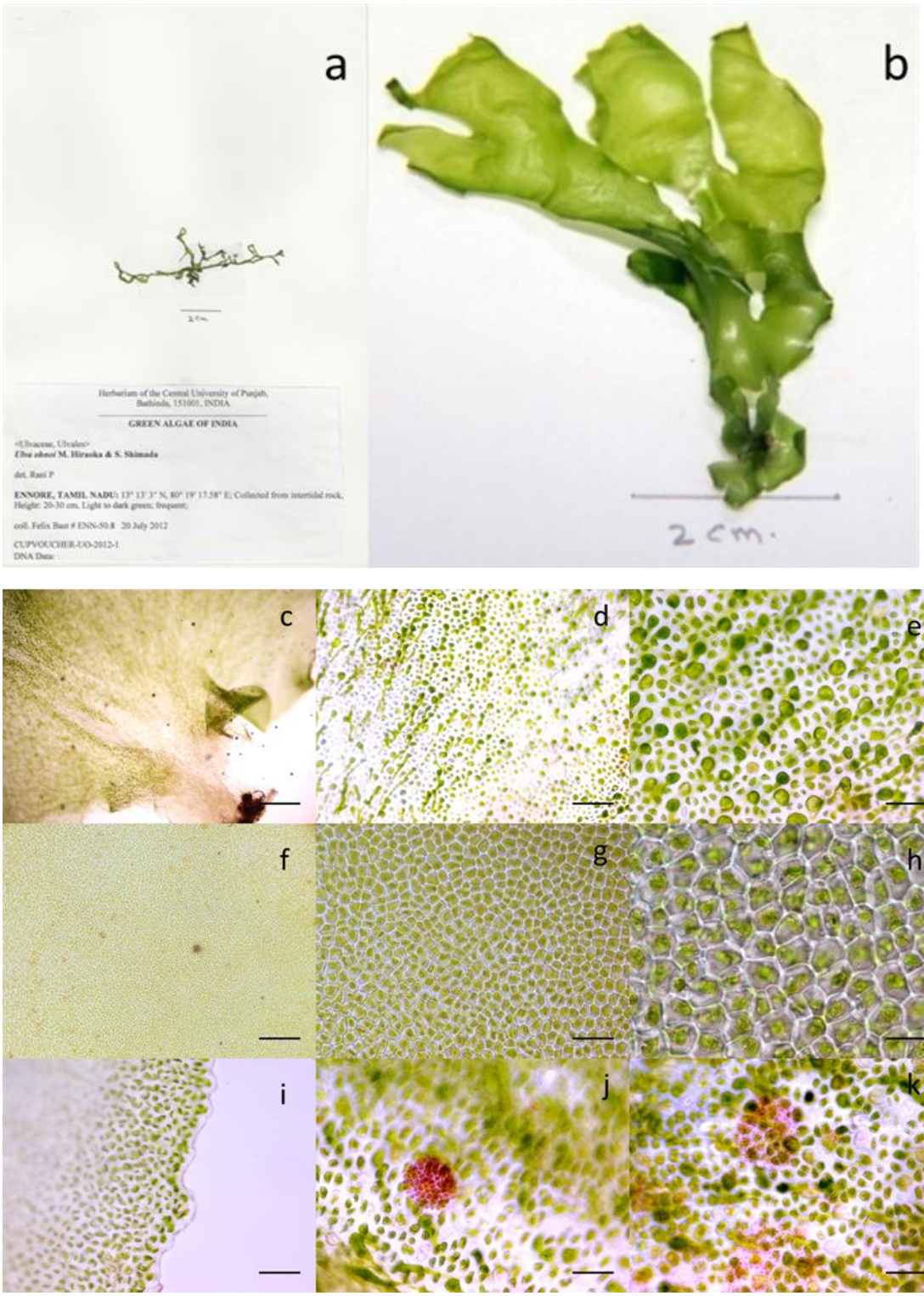

Figure 4.27: Morphology and Micromorphology of *Ulva ohnoi* from Ennore, India (ENN-50.8): a Herbarium, b Morphology of thallus; c Microphotographs of basal region of thallus at 10X; d, e Reproductive zoospores at the basal region of thallus at 20X, 40X. f, g, h Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X. i Ruffled margin of thallus at 40X; j, k Epiphytic as well as endophytic red algae

at 40X; Dividing cells are also present; Scale bar is 200µm for c, f; 100µm for d; 50µm for e, g, i, j, k; 20µm for h.

ENN-50.16: *Ulva prolifera* O.F. Muller

Sampling site:

Location: Ennore (Tamil Nadu); Collection date: 20-07-2012; Collected by: Felix Bast.

Voucher ID:

Frozen Voucher ID: ENN-50.16; CUP Voucher ID: CUPVOUCHER-ENN-2012-UP-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ENN-2012-UP-1

Morphology:

Thallus was green to dark green in colour, thallus with tubular branches that had numerous slender branchlets (Fig. 4.28a), up to 10 cm long, attached by means of disc-like holdfast (Fig. 4.28b); fronds tubular, profusely branched with numerous slender branches (Fig. 4.28c); cells in surface view were polygonal to sub-rectangular, always arranged in linear series in certain parts of the thallus (Fig. 4.28g). Thallus was highly branched. Secondary branches were growing in alternatively fashion on the surface of main branch (Fig. 4.28d). Tip of the secondary branch was rounded in shape (Fig. 4.28c). Secondary branches were thin towards the basal region but become wider towards the distal end (Fig. 4.28d). Cells were regular in arrangement (Fig. 4.28h), Multiseriate; 208.511±29 µm in size and irregular in shape. Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.28j). Margins of leaves were smooth (Fig. 4.28h). Multiple pyrenoids were present in cells (Fig. 4.28k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.28e). Long flagellated zoospores were appeared (Fig. 4.28f). Many dividing cells were also appeared (Fig. 4.28i) Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG768948
- atpB F & atpB R: MG918104

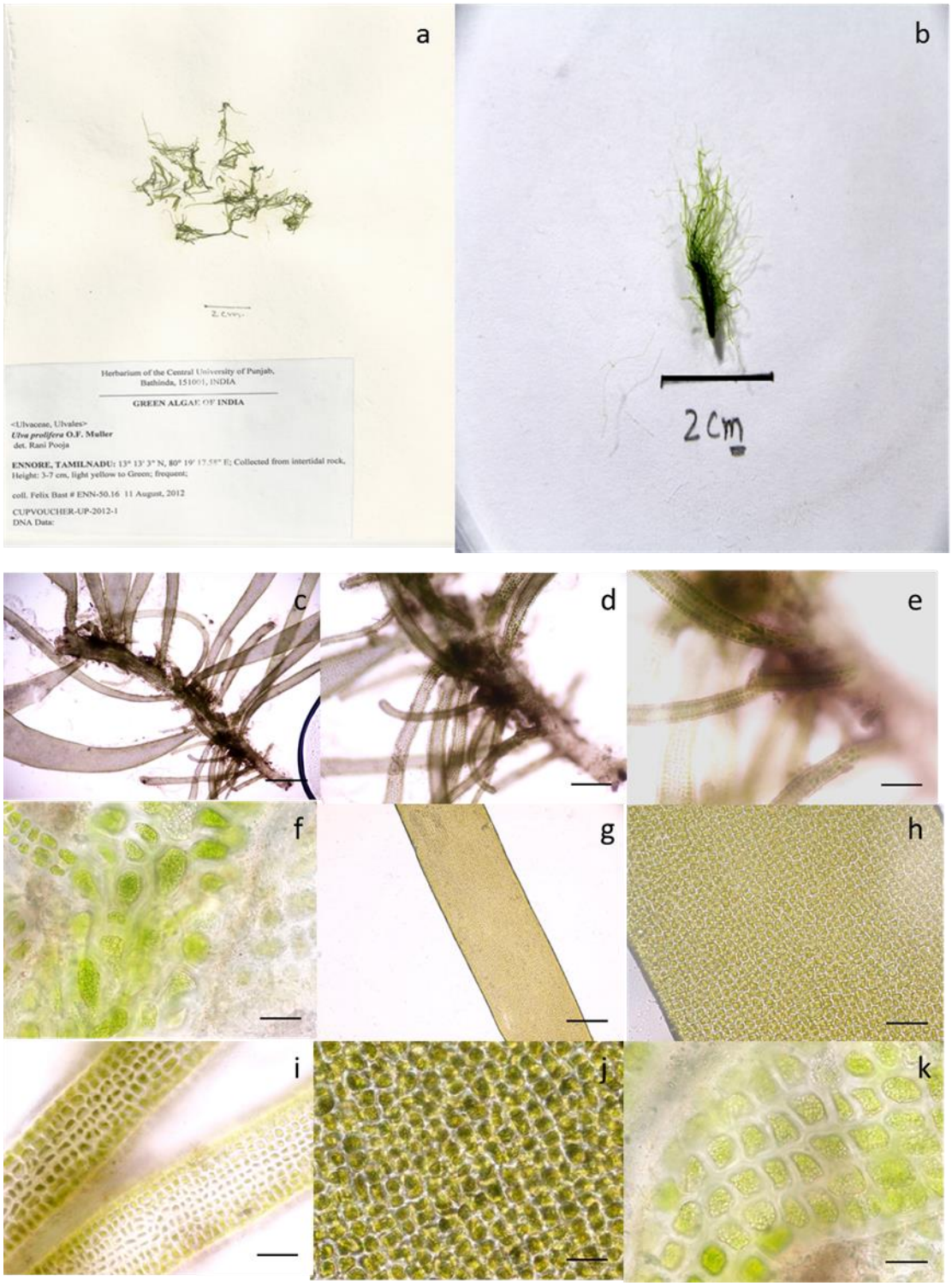

Figure 4.28: Morphology and Micromorphology of *Ulva prolifera* from Kollam, India (ENN-50.16): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Distribution of flagellated zoospores at the basal region of thallus at 20X, 100X; g , h Margin and cell arrangement in the marginal region of thallus at 10X, 20X; i, j Shape of the cell and arrangement of the cell in the middle region of the thallus at 40X; k

Chloroplast arrangement and pyrenoids arrangement in the cell at 100X; Dividing cells are also present; Scale bar is 200µm for d, g; 100µm for e, h; 50µm for i, j; 20µm for f, k.

CHE-51.05 *Ulva fasciata* Delile

Sampling site:

Location: Chellanam, Kerala; Collection date: 16-01-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: CHE-51.05; CUP Voucher: CUPVOUCHER-CHE-2015-UF-1**;** Central National Herbarium Voucher ID: CAL- CUPVOUCHER-CHE-2015-UF-1

Morphology:

Thallus was in leafy in appearance and dark green in colour (Fig. 4.29a). Thallus was branched, perforated leafy, 5-7 cm and heart shaped distal end (Fig. 4.29b). Thallus was attached to the rocky surface of shore. Cells were irregular in arrangement (Fig. 4.29i), Multiseriate; 14.832±26 µm in size and irregular in shape (Fig. 4.29j). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.29k). Margins of leaves were ruffled and cells were irregularly arranged in marginal region (Fig. 4.29k, 4.29n). Multiple pyrenoids were present in cells (Fig. 4.29k). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.29c, 4.29d, 4.29g). Few empty cells were also observed in middle region after the release of zoospores (Fig. 4.29h). Long flagellated zoospores were appeared (Fig. 4.29e, 4.29f). Many dividing cells were also appeared (Fig.4.29g). Thallus was distromatic (Fig. 4.29m).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763219
- atpB F & atpB R: MG963798

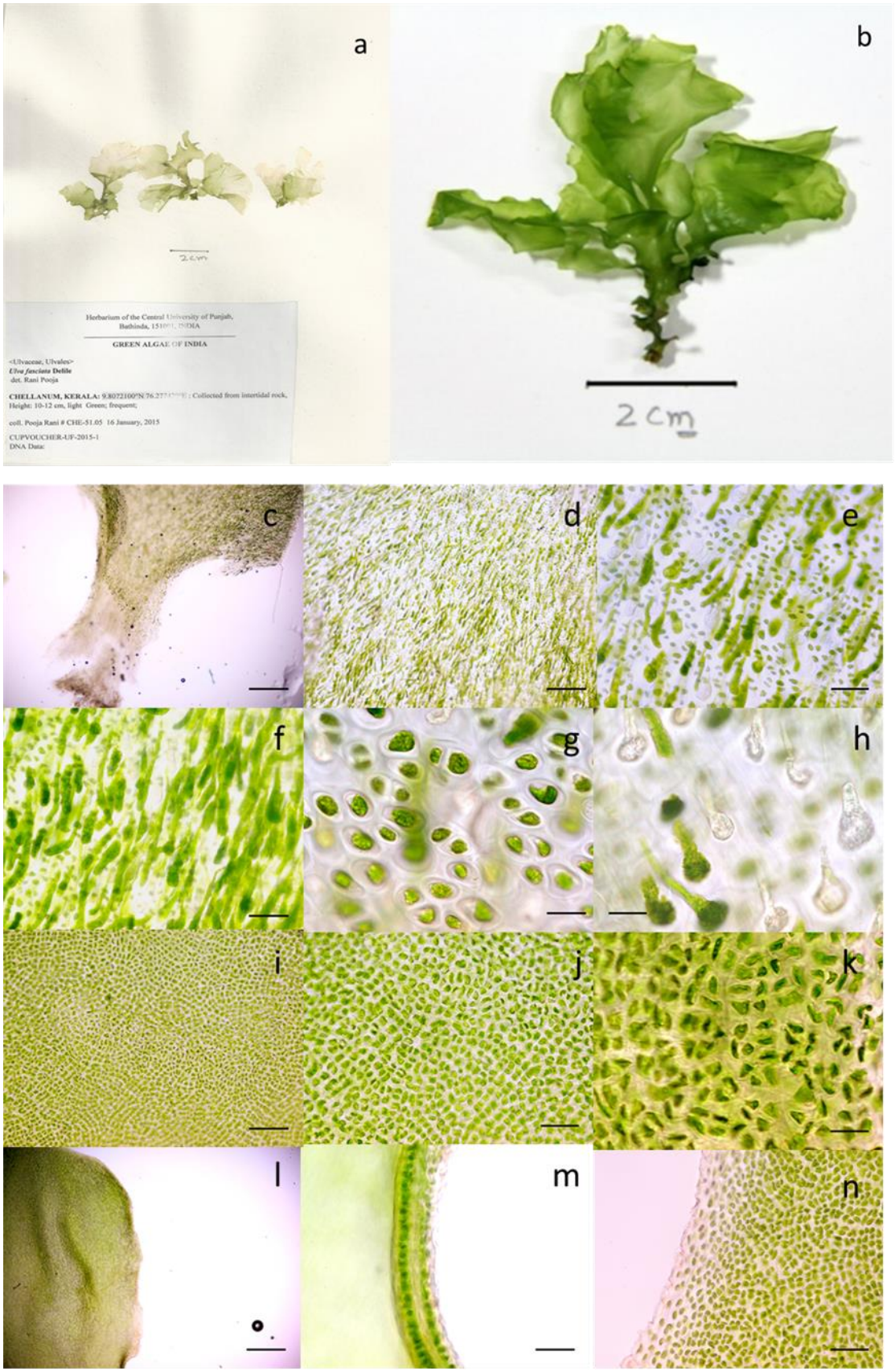

Figure 4.29: Morphology and Micromorphology of *Ulva fasciata* from Chellanum, India (CHE-51.05): a Herbarium, b Morphology of thallus; c

Microphotographs of basal region of thallus at 4X; d, e, f flagellated reproductive zoospores in the basal region at 10X, 20X, 40X. g Reproductive zoospores and dividing cells in the middle region of foliose blade at 100X. h flagellated zoospores and some empty cells after release of zoospores at 100X; i, j, k Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X; l Ruffled margin at 10X; m indicates distromatic foliose blade at 100X; n Arrangement of cells at margin of thallus at 40X; Dividing cells are also present; Scale bar is 200µm for d, i, l; 100µm for e; 50µm for f, j, n; 20µm for g, h, k, m.

KYK-51.10 *Ulva fasciata* Delile

Sampling site:

Location: Kanyakumari, Tamil Nadu; Collection date: 16-01-2015; collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KYK-51.10; CUP Voucher: CUPVOUCHER-KYK-2015-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KYK-2015-UF-1

Morphology:

Thallus was leafy in appearance and green in colour (Fig. 4.30a). Thallus was highly branched, 3-5 cm long with broad leaf. Leaves were having lobed edges, form heart shaped structure (Fig. 4.30b). Margins of the leaves were slightly wrinkled (Fig. 4.30c). Thallus was attached to the surface and exposed to the rocky shore. Sometime torned leaves were freely floating in the water. Cells were irregular in arrangement, and oval in shape. Chloroplast was completely covered the cytosol (Fig. 4.30e). Cells were irregular in arrangement (Fig. 4.30i), Multiseriate; 127.269±48 µm in size and rounded or oval in shape (Fig. 4.30e). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.30e). Parietal chloroplast was present in the marginal cells (Fig.4.30l). Margins of leaves were smooth (Fig. 4.30k). Denticulate were present on the margin of leaves (Fig. 4.30k). Multiple pyrenoids were present in cells (Fig. 4.30j). Reproductive zoospores were observed in basal region of thallus (Fig. 4.30f). Short flagellated zoospores were present (Fig. 4.30g). Empty cells were also observed after the release of zoospores (Fig. 4.30h). Many dividing cells

were also appeared (Fig. 4.30j). Thallus was distromatic. Some endophytic and epiphytic red algae were observed in the leafy thallus (Fig. 4.30m, 4.30n).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918119

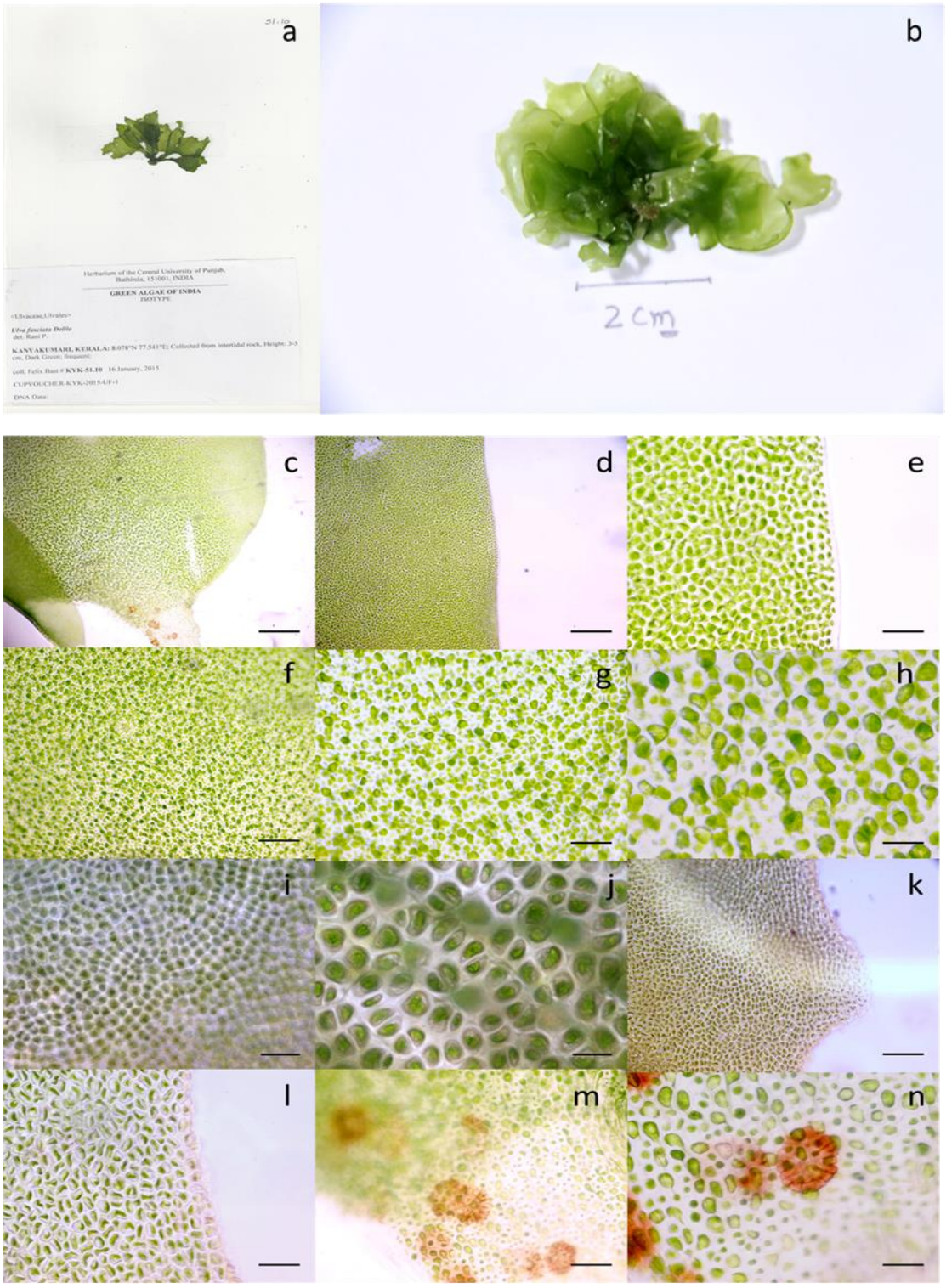

Figure 4.30: Morphology and Micromorphology of *Ulva fasciata* from Kanyakumari, India (KYK-51.10): a Herbarium, b Morphology of thallus; c Microphotographs of thallus at 4X; d Margin of the thallus and cell arrangement in the margin at 10X; f, g, h Zoospores in the basal region of thallus at 10X, 20X, 40X; i Arrangement of cell in the middle region at 20X; j Arrangement of chloroplast and number of pyrenoids in the middle region at 100X; k Denticula in marginal region and cell arrangement in denticulate region at 20X; l Cell arrangement and chloroplast arrangement in marginal region at 40X; m, n Endophytic algae at 40X, 100X; Dividing cells are also present; Scale bar is 200µm for d, f; 100µm for g, i, k; 50µm for h, l, m; 20µm for j, n.

KYK-51.39 *Ulva fasciata* Delile

Sampling site:

Location: Kanyakumari, Tamil Nadu; Collection date: 16-01-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: KYK-51.39; CUP Voucher: CUPVOUCHER-KYK-2015-UF-2; Central National Herbarium Voucher ID: CAL- CUPVOUCHER-KYK-2015-UF-2.

Morphology:

Thallus was leafy in appearance and dark green in colour (Fig. 4.31a). Thallus was highly branched, 2-4 cm long with broad leaf. Leaves were having lobed edges (Fig. 4.31b). Thallus was attached to the surface and exposed to the rocky shore. Cells were irregular in arrangement (Fig. 4.31h), Multiseriate; 162.295±35 µm in size and rounded or oval in shape (Fig. 4.31h). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.31i). Margins of leaves were wavy and denticulate were present on the surface (Fig. 4.31f, 4.31g). Multiple pyrenoids were present in cells (Fig. 4.31i). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.31c). Long flagellated zoospores were appeared (Fig. 4.31d, 4.31e). Many dividing cells were also appeared (Fig. 4.31g). Vertical and horizontal division was observed in the cell. Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918120
- atpB F & atpB R: MG963799

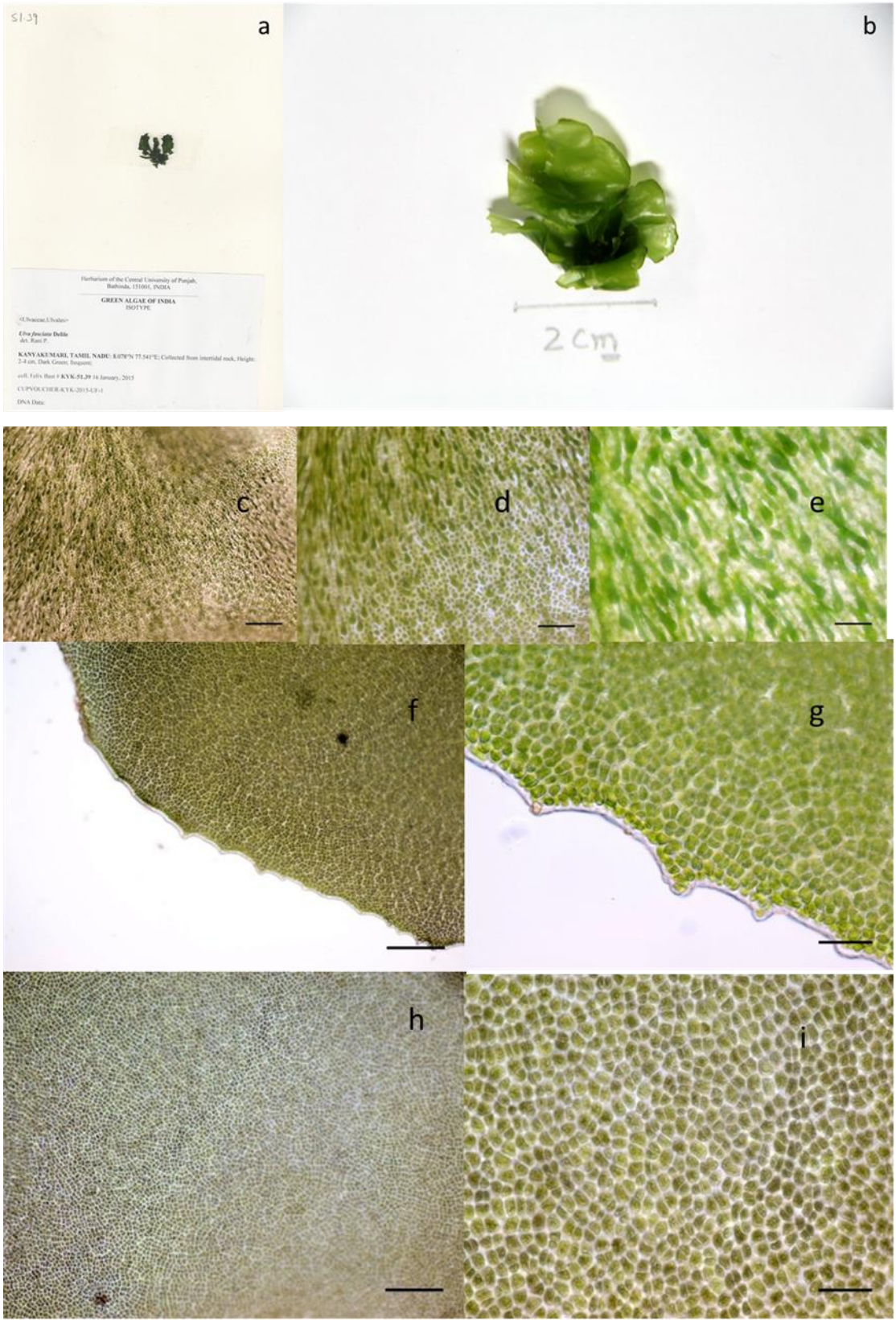

Figure 4.31: Morphology and Micromorphology of *Ulva fasciata* from Kanyakumari, India (KYK-51.39): a Herbarium, b Morphology of thallus; c, d, e Zoospores in the basal region of thallus by microscopic analysis at 10X, 20X, 40X; f, g Denticulate at the margin of thallus and arrangement of cells in denticula at 20X, 40X; h, i Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X. i Dividing cells present; Scale bar is 200µm for c, h; 100µm for d, f; 50µm for e, g, i.

VEK-54.9 *Ulva fasciata* Delile

Sampling site:

Location: Varkala, Kerala; Collection date: 14-01-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: VEK-54.9**;** CUP Voucher: CUPVOUCHER-VEK-2015-UF-1; Central National Herbarium Voucher ID: CAL- CUPVOUCHER-VEK-2015-UF-1

Morphology:

Thallus was leafy in appearance and yellowish green in colour (Fig. 4.32a). Thallus was highly branched, 5-10 cm long with broad leaf. Leaves with lobed edges were formed heart shaped structure (Fig. 4.32b). Thallus was attached to the surface and exposed to the rocky shore. Sometime torned leaves were freely floating in the water. Cells were irregular in arrangement (Fig. 4.32c), Multiseriate; 156.271±37.518 µm in size and polygonal in shape (Fig. 4.32d). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.32g). Margins of leaves were wavy (Fig. 4.32e). New developing leafy bud was present on the margin (Fig. 4.32h). Some scale as structures were also observed on the margin (Fig. 4.32i). Multiple pyrenoids were present in cells (Fig. 4.32f). Many dividing cells were also appeared (Fig. 4.32e). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918121
- atpB F & atpB R: MG963800

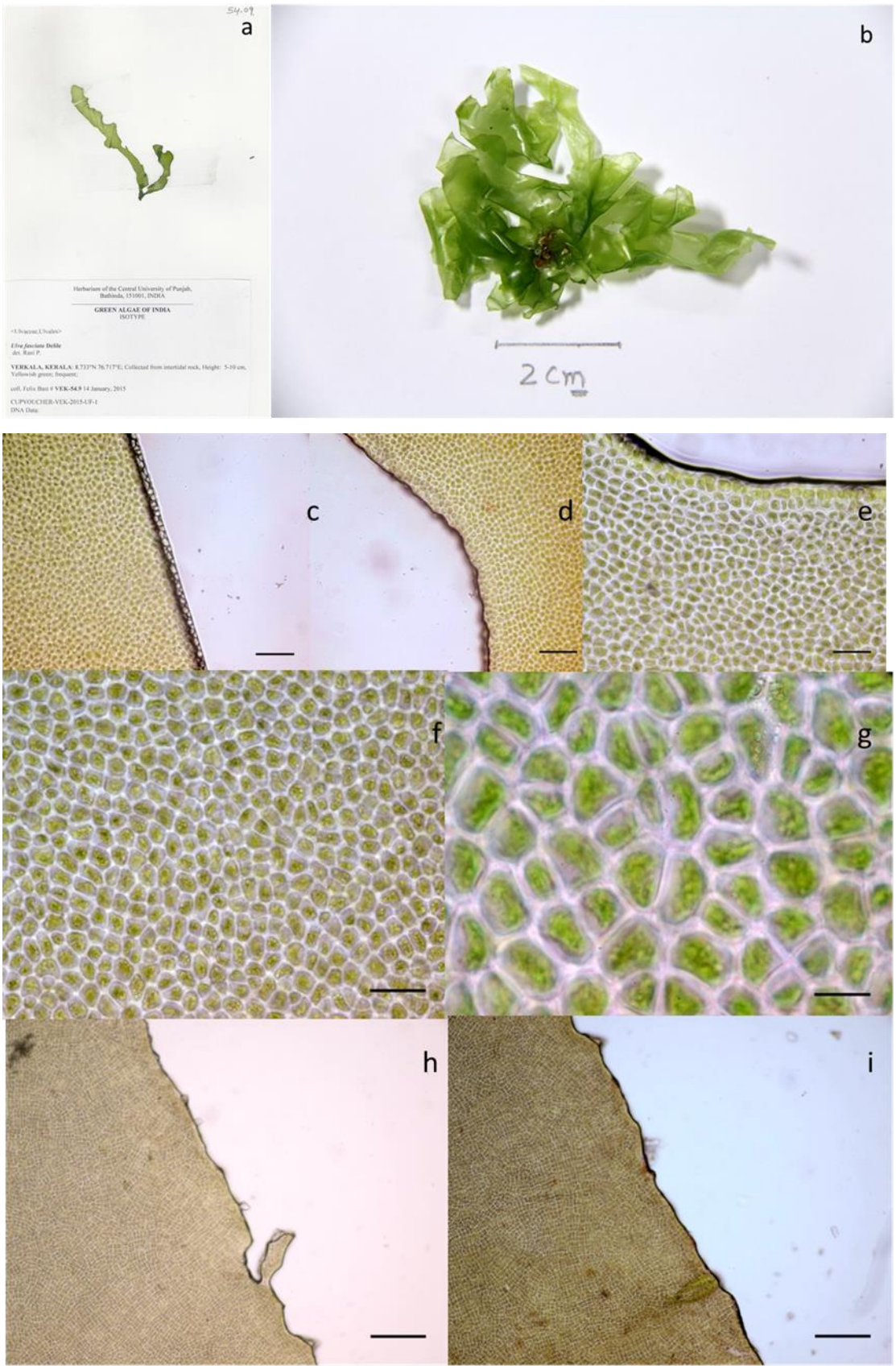

Figure 4.32: Morphology and Micromorphology of *Ulva fasciata* from Varkala, India (VEK-54.9): a Herbarium, b Morphology of thallus; c, d, e Ruffled or wavy margin and arrangement of cells in marginal end at 20X, 40X; f, g Cell

arrangement, chloroplast arrangement and pyrenoids arrangement at 40X, 100X. h, i New developing buds or filament like branch for growth of foliose at 10X; Dividing cells are also present; Scale bar is 200µm for h, i; 100µm for c, d; 50µm for e, f; 20µm for g.

MAL-55: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Malvan, Maharashtra; Collection date: 23-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: MAL-55; CUP Voucher: CUPVOUCHER-MAL-2015-UX-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MAL-2015-UX-1

Morphology:

Thallus was bushy in appearance and yellowish green algae (Fig. 4.33a). Branches were filament like, 5-10 cm long (Fig. 4.33b). Thallus was attached to the surface and exposed to the rocky shore. Mature filaments were torned off and freely floating in water. Long filaments were highly coiled. Cells were rectangular in shape, regular in arrangement, arranged in the form of linear rows. Marginal cells were cuboidal (Fig. 4.33f), Multiseriate; 119.01±7µm in size. Thallus was highly branched; secondary branches were growing in alternative fashion (Fig. 4.33c). Cells near the proliferating branch region were irregular in shape and arrangement (Fig. 4.33f). Scale like structure was present at the nodal region (Fig. 4.33d). Tip of the branch was pointed or rounded (Fig. 4.33e). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.33h). Margins of leaves were smooth (Fig. 4.33g). Multiple pyrenoids were present in cells (Fig. 4.33h). Reproductive zoospores were observed in basal region and nodal region of main branch of thallus (Fig. 4.33f). Long flagellated zoospores were appeared (Fig. 4.33g). Many Dividing cells were also appeared (Fig. 4.33h). Horizontally and vertically dividing cells were present. Thallus was monostromatic and tubular (Fig. 4.33c).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763142
- atpB F & atpB R: MG918108

Figure 4.33: Morphology and Micromorphology of *Ulva shanxiensis* from Malvan, India (MAL-55): a Herbarium, b Morphology of thallus; c Microscopic analysis of thallus at 4X; d Alternative branching pattern of thallus and tip region of filament at 10X; e, f Flagellated reproductive zoospores near the nodal region of main axis at 20X, 40X; g indicates presence of scale like structure at nodal region at 40X; h indicates cell arrangement, margin of thallus, chloroplast arrangement and pyrenoids arrangement at 40X; Dividing cells are also present; Scale bar is 200µm for d; 100µm for e; 50µm for f, g, h.

MAL-57: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Malvan, Maharashtra; Collection date: 23-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: MAL-57**;** CUP Voucher: CUPVOUCHER-MAL-2015-UX-2; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MAL-2015-UX-2

Morphology:

Thallus was bushy in appearance and yellowish green algae (Fig. 4.34a). Branches were filamentous, tubular or compressed, 5-10 cm long (Fig. 4.34b). Thallus was attached to the surface by disc shaped structure to the substratum and exposed to the rocky shore. Mature filaments were torned off and freely floating in water. Long filaments were highly coiled. Thallus was highly branched. Branches were growing in alternative fashion around the main branch (Fig. 4.34c, 4.34d). Uniseriate or biseriate developing branches were observed (Fig. 4.34e, 4.34f). Tip region of the branch was rounded (Fig. 4.34f, 4.34j). Uniseriate branches were start dividing vertically after few horizontal divisions i.e. 8-10 cells in length (Fig. 4.34d, 4.34j). Cells were regular in arrangement towards marginal region, arranged in the form of linear rows, rectangular; marginal cells were cuboidal (Fig. 4.34k). Chloroplast was present in the form of patches (Fig. 4.34n), 172.184±13 µm in size. Cells were polygonal or irregular in shape near the proliferating region and irregular in arrangement (Fig. 4.34m), Multiseriate. Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.34n). Margins of leaves were smooth (Fig.4.34l). Multiple pyrenoids were present in cells (Fig. 4.34n). Reproductive zoospores were observed in basal region and near the nodal region of thallus (Fig. 4.34g). Long flagellated zoospores were appeared (Fig. 4.34h, 4.34i). Many dividing cells were also appeared (Fig. 4.34m). Thallus was monostromatic and tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763143
- atpB F & atpB R: MG918109

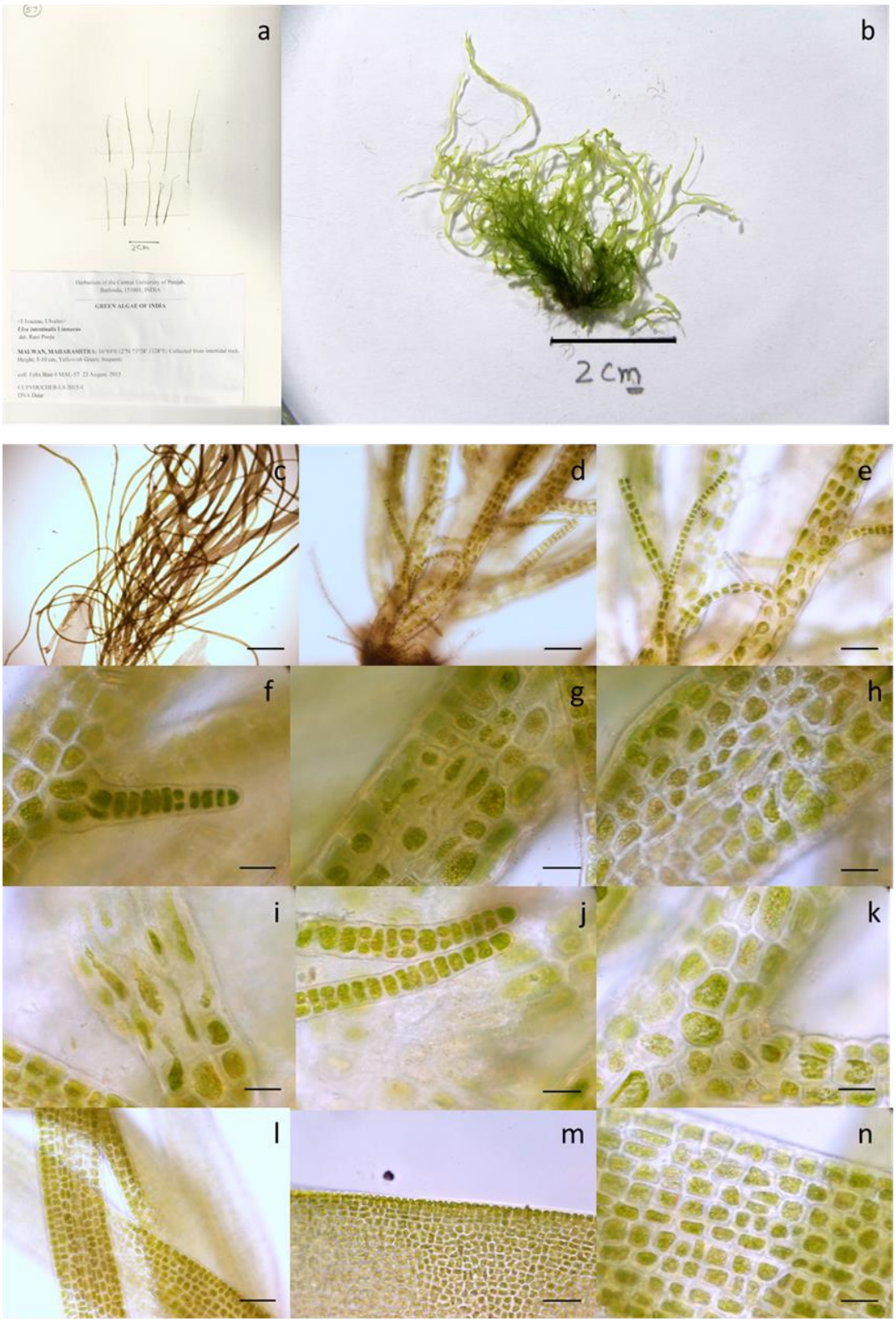

Figure 4.34: Morphology and Micromorphology of *Ulva shanxiensis* from Malvan, India (MAL-57): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus and alternative branching pattern

at 4X,10X; e, f Uniseriate and biseriate new developing branches at 40X, 100X; g, h, i Flagellated reproductive zoospores at the basal region of branches at 100X; j Tip region of filament at 100X; k Arrangement of adaxial region of branch at 100X; l, m, n Cell arrangement, margin of thallus, chloroplast arrangement and pyrenoids arrangement at 20X, 40X, 100X. Dividing cells are also present; Scale bar is 200µm for d; 100µm for l; 50µm for e, m; 20µm for f, g, h, i, j, k, n .

VEN-58: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Vengurla, Maharashtra; Collection date: 24-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: VEN-58; CUP Voucher: CUPVOUCHER-VEN-2015-UX-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-VEN-2015-UX-1.

Morphology:

Thallus was bushy, tubular and ribbon like coiled (Fig. 4.35a). Thallus was attached to rocks or any other hard substrata in intertidal area. It had grown in mid littoral zone and tide pools. Thallus was yellow to dark green in colour, up to 20 cm long and divided into number of ribbon shaped. One to two cm tubular or compressed thread like structure (Fig. 4.35b). Thallus was highly branched. Branches were growing in alternative fashion (Fig. 4.35f). Many uniseriate or multiseriate branches were developed from main branch (Fig. 4.35c, 4.35d). Tip of the branches were rounded in shape (Fig. 4.35i). Main branch was broader than secondary branch (Fig. 4.35f, 4.35g). Surface of thallus was smooth; thallus was free floating in water after maturation. Cells were regular in arrangement in younger branches and arranged in linear rows (Fig. 4.35k), Multiseriate; 63.09±15 µm in size and cuboidal or rectangular in shape (Fig. 4.35k). In mature branches or at distal end of branch, cells were irregular in shape and arrangement (Fig. 4.35l). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.35n). Margins of leaves were smooth (Fig. 4.35m). Multiple pyrenoids were present in cells (Fig. 4.35n). Reproductive

zoospores were observed in basal region of main branch of thallus (Fig. 4.35e, 4.35h). Long flagellated zoospores were appeared (Fig. 4.35n). Many dividing cells were also appeared (Fig. 4.35l). Thallus was monostromatic and tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763144
- 18S F &18S R: MH071442

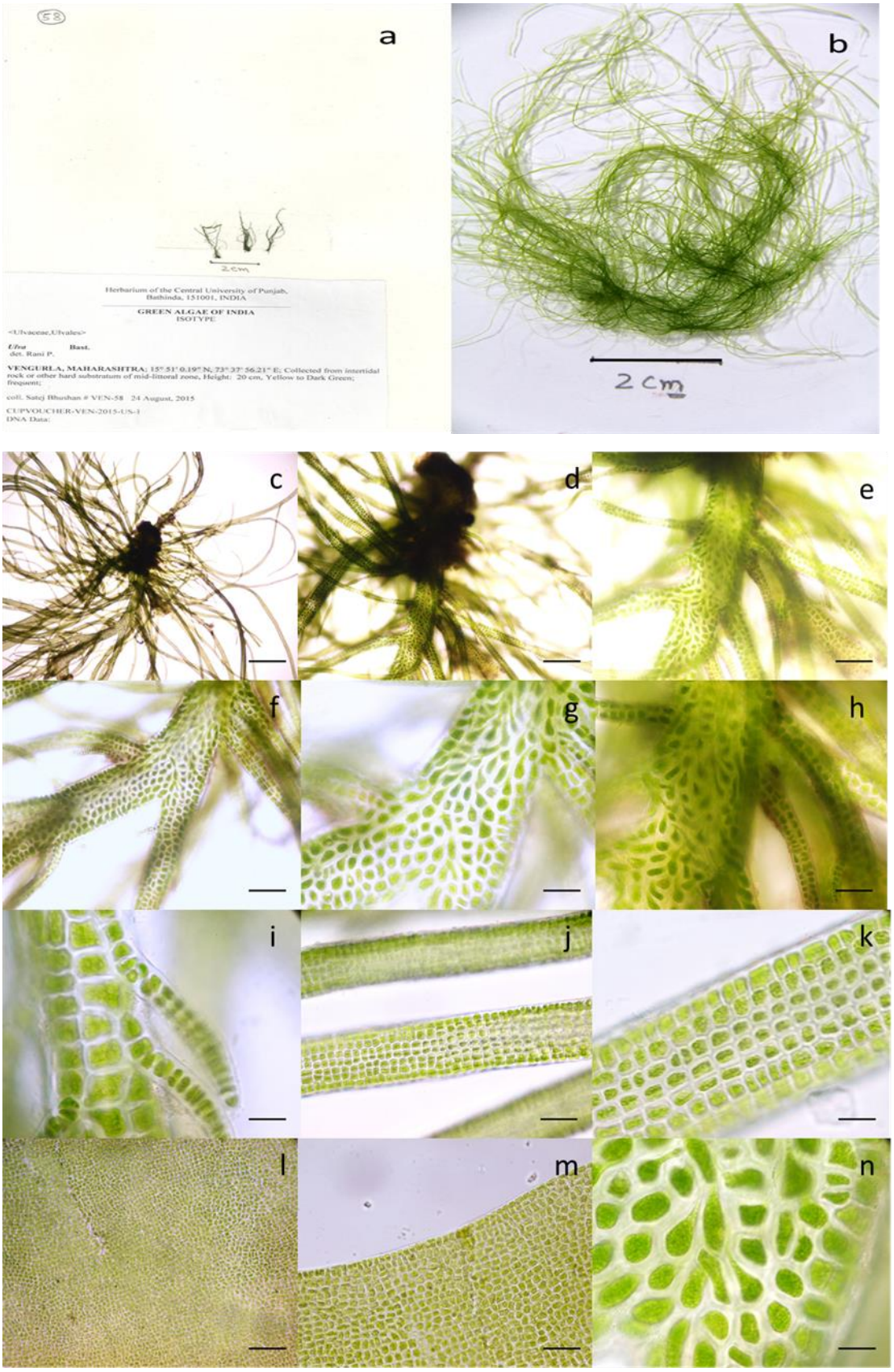

Figure 4.35: Morphology and Micromorphology of *Ulva shanxiensis* from Vengurla, India (VEN-58): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Alternative

branching pattern of thallus at 20X; g, h Reproductive flagellated zoospores at basal region of thallus at 40X; i New uniseriate branch and tip region of filament at 100X; j, k, l Cell arrangement and margin of thallus at 20X, 40X, 10X; m, n Chloroplast arrangement and pyrenoids arrangement at 20X, 100X. Dividing cells are also present; Scale bar is 200µm for d, l; 100µm for e, f, j, m; 50µm for g, h, k; 20µm for i, n.

VIJ-59: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Vijaydurg, Maharashtra; Collection date: 25-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: VIJ-59; CUP Voucher: CUPVOUCHER-VIJ-2015-UX-1**;** Central National Herbarium Voucher ID: CAL- CUPVOUCHER-VIJ-2015-UX-1

Morphology:

Thallus was bushy in appearance and highly branched. Thallus was tubular and filamentous (Fig. 4.36a). Filaments were highly coiled. Secondary branches were growing in alternative fashion around the main axis (Fig. 4.36c, 4.36d). Tip of the filaments was rounded in shape (Fig. 4.36d). Cells were regular in arrangement in young branches (Fig. 4.36h), Multiseriate; 173.771±18 µm in size and rectangular in shape (Fig. 4.36i). Cells were polygonal in shape at distal ends and irregular in arrangement (Fig. 4.36g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.36h). Margins of leaves were smooth (Fig. 4.36e). Multiple pyrenoids were present in cells (Fig.4.36j). Reproductive zoospores were observed in basal region of thallus (Fig. 4.36f). Long flagellated zoospores were appeared (Fig. 4.36f). Many dividing cells were also appeared (Fig. 4.36i). Thallus was monostromatic and tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG768949
- atpB F & atpB R: MG918110

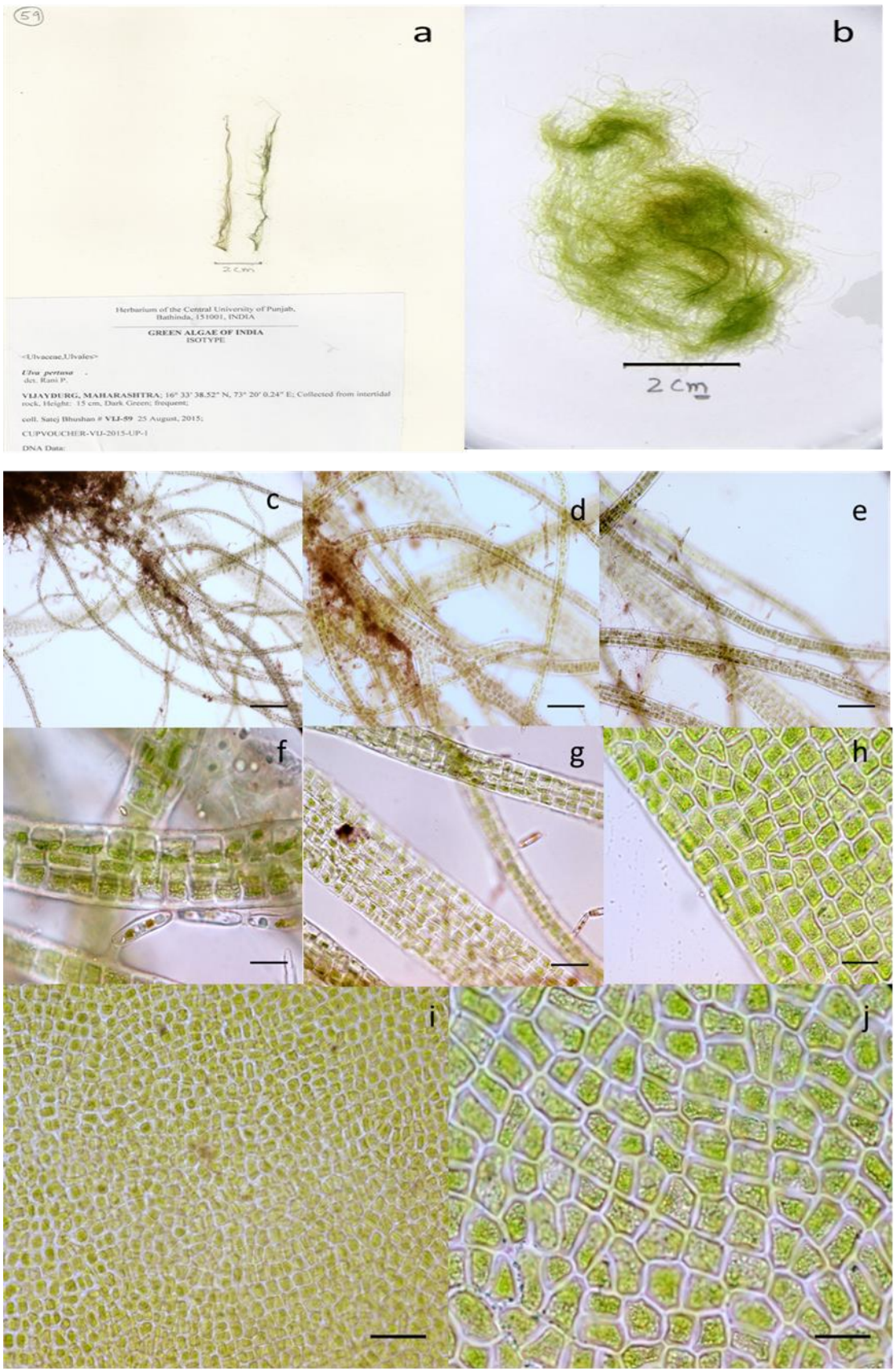

Figure 4.36: Morphology and Micromorphology of *Ulva shanxiensis* from Vijaydurg, India (VIJ-59): a Herbarium, b Morphology of thallus; c, d

Microphotographs of basal region of thallus and branching pattern at 4X, 10X; e, f Reproductive zoospores at 10X, 40X. g, h Margin of thallus and cell arrangement in marginal region at 20X, 40X; i, j Chloroplast arrangement and pyrenoids arrangement near distal end at 20X, 100X. k flagellated zoospores at 100X; Dividing cells are also present; Scale bar is 200µm for d, e; 100µm for g, i; 50µm for f, h; 20µm for j, k.

RAT-60: *Ulva sapora* Philip

Sampling site:

Location: Ratnagiri, Maharashtra; Collection date: 23-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: RAT-60; CUP Voucher: CUPVOUCHER-RAT-2015-US-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-RAT-2015-US-1

Morphology:

Thallus was bushy in appearance and yellowish green algae (Fig. 4.37a). Thallus was highly branched and branches were growing in alternative fashion (Fig. 4.37c, 4.37d). Branches were filamentous and tubular, 5-10 cm long (Fig. 4.37b). Thallus was attached to the surface by disc shaped structure and exposed to the rocky shore. Mature filaments were torned off and freely floating in water. Long filaments were highly coiled. Cells were regular in arrangement, arranged in linear rows (Fig. 4.37h), Multiseriate; 30±1 µm in size and cuboidal in shape (Fig. 4.37d). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.37h). Margins of leaves were smooth (Fig. 4.37g). Multiple pyrenoids were present in cells (Fig. 4.37h). Reproductive zoospores were observed in basal region of the thallus and from the nodal region of newly developing region (Fig. 4.37e). Long flagellated zoospores were appeared (Fig. 4.37f). Many dividing cells were also appeared (Fig. 4.37g). Thallus was monostromatic and tubular.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763138
- atpB F & atpB R: MG918111

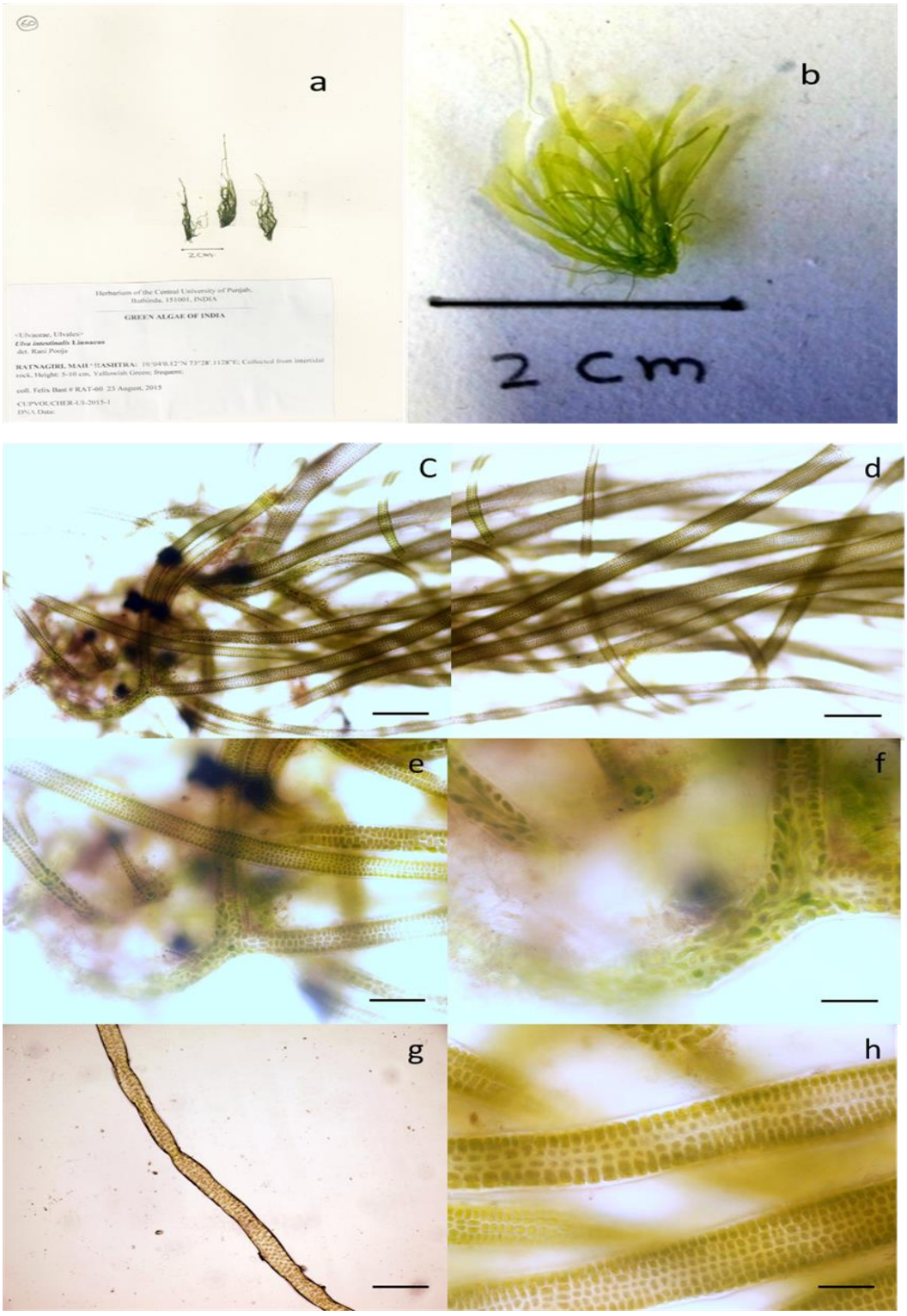

Figure 4.37: Morphology and Micromorphology of *Ulva sapora* from Ratnagiri, India (RAT-60): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus and branching pattern at 10X; e, f

flagellated reproductive zoospores in basal region at 20X, 40X; g, h Cell arrangement, margin of thallus, chloroplast arrangement and pyrenoids arrangement at 10X, 40X; Dividing cells are also present; Scale bar is 200µm for c, d, g; 100µm for e; 50µm for f, h.

VEL-61: *Ulva reticulata* Forsskal

Sampling site:

Location: Velneshwar, Maharashtra; Collection date: 27-07-2015; Collected by: Satej Bhushan.

Voucher ID:

Frozen voucher ID: VEL-61; CUP Voucher: CUPVOUCHER-VEL-2015-UR-1; Central National Herbarium Voucher ID: CAL- CUPVOUCHER-VEL-2015-UR-1

Morphology:

Thallus was leafy in appearance and dark green in colour (Fig. 4.38a). Thallus was circular shaped, star like appearance and highly branched. Thallus was 5-10 cm broad, freely floating in water (Fig. 4.38b). Cells were irregular in arrangement (Fig. 4.38f), Multiseriate; 143.819±47µm in size and polygonal in shape (Fig. 4.38g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.38h). Margins of leaves were wavy; cells were irregularly arranged in marginal region (Fig. 4.38f). New developing bud was start appearing at marginal region (Fig. 4.38d). Cells were irregular in arrangement in developing bud (Fig. 4.38e). Scale as structures were also present at margin of leafy blade (Fig. 4.38c). Multiple pyrenoids were present in cells (Fig. 4.38k). Many dividing cells were also appeared (Fig. 4.38j). Horizontally and vertically dividing cells were observed in leafy thallus (Fig. 4.38i). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- 18S F &18S R: MG774431

- atpB F & atpB R: MG963801

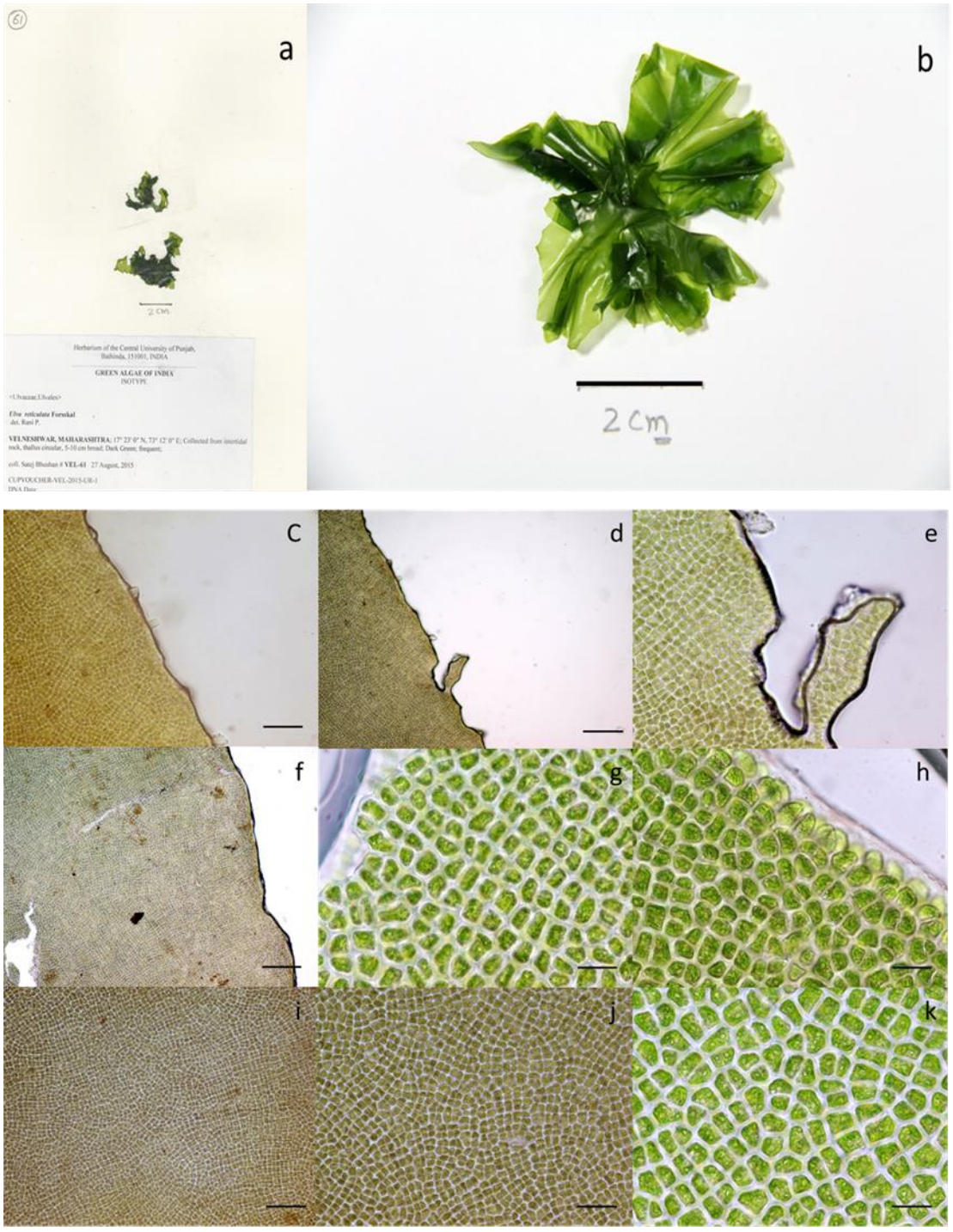

Figure 4.38: Morphology and Micromorphology of *Ulva reticulata* from Velneshwar, India (VEL-61): a Herbarium, b Morphology of thallus; c, d Scale and new developing bud in microphotographs of thallus at 10X; e Cell arrangement of new developing bud from basal blade at 20X; f Reticulate in blade at 10X; g, h Cell arrangement in margin of thallus at 100X; i, j, k Cell arrangement, chloroplast arrangement and pyrenoids arrangement in middle

region at 10X, 20X, 100X; Dividing cells are also present; Scale bar is 200µm for c, d, f, i; 100µm for e, j; 20µm for g, h, k.

OKH-70: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Okha, Gujarat; Collection date: 23-11-2015; Collected by: Pooja Rani.

Voucher ID:

Frozen voucher ID: OKH-70; CUP Voucher: CUPVOUCHER-OKH-2015-UX-1**;** Central National Herbarium Voucher ID: CAL-CUPVOUCHER-OKH-2015-UX-1

Morphology:

Thallus was bushy in appearance. Thallus was attached to the rocks or stones in the intertidal areas. Thallus was highly branched (Fig. 4.39a). Uniseriate and multiseriate newly developing secondary branches were growing around the main axis of the thallus (Fig. 4.39d, 4.39e). Thallus was yellowish green in color and 2-5 cm in length (Fig. 4.39b). Branches were flattened but unbranched. Each branch was slightly coiled like ribbon (Fig. 4.39c). After maturation, branches were freely floating in water. Surface of the thallus was smooth**.** Cells were regular in arrangement in basal region (Fig. 4.39f), Multiseriate; 149.228±2 µm in size and rectangular or cuboidal in shape (Fig. 4.39g). Cells were polygonal or irregular in shape in the distal end of the thallus and irregular in arrangement (Fig. 4.39h). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.39i). Margins of leaves were smooth (Fig. 4.39h). Multiple pyrenoids were present in cells (Fig. 4.39g, 4.39k). Reproductive zoospores were observed in basal region of thallus (Fig. 4.39f). Few empty cells were also present after the release of matured zoospores (Fig. 4.39i). Long flagellated zoospores were appeared (Fig. 4.39g). Many dividing cells were also appeared (Fig. 4.39j). Thallus was distromatic and compressed.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763145
- 18S F &18S R: MG774435

- atpB F & atpB R: MG918112

Figure 4.39: Morphology and Micromorphology of *Ulva shanxiensis* from Okha, India (OKH-70): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e Alternatively developing new secondary branches at 10X; f, g Flagellated reproductive zoospores at basal region at 40X, 100X; h, i Margin and cell arrangement in marginal region of thallus at 40X,100X; j, k Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 20X, 100X; Dividing cells are also

present; Scale bar is 200µm for d, e; 100µm for j; 50µm for f, h; 20µm for g, i, k.

OKH-71: *Ulva reticulata* Forsskal

Sampling site:

Location: Okha, Gujarat; Collection date: 23-11-2015; Collected by: Pooja Rani.

Voucher ID:

Frozen voucher ID: OKH-71**;** CUP Voucher: CUPVOUCHER-OKH-2015-UR-1**;** Central National Herbarium Voucher ID: CAL-CUPVOUCHER-OKH-2015-UR-1**.**

Morphology:

Thallus was leafy in appearance and leaves divided in multiple lobes (Fig. 4.40a). Thallus was dark green in colour and 5-7 cm in length (Fig. 4.40b). Thallus was attached to the surface of the rocks and stones and exposed to the water. It was found in the intertidal region. 5-8 leaves were present in the thallus. Margins of the leaves were wavy. After maturation, leaves were torned off and start growing as a new thallus. Surface of the leaves were very smooth**.** Cells were irregular in arrangement (Fig. 4.40i), Multiseriate; 104.853±42 µm in size and oval or rounded in shape (Fig. 4.40k). Cell wall was thick. Thin patches of chloroplast were present inside cell (Fig. 4.40k). Margins of leaves were wavy (Fig. 4.40h). Denticula were also present on the margin of leaf blade (Fig. 4.40l, 4.40m). Multiple pyrenoids were present in cells (Fig. 4.40n). Reproductive zoospores were observed more in basal region of the thallus as compared to middle region (Fig. 4.40c,4.40d). Long flagellated zoospores were appeared (Fig. 4.40e, 4.40f). Many dividing cells were also appeared (Fig. 4.40i). Thallus was distromatic (Fig.4.40h). Endophytic and epiphytic red algae *Erythrocladia* was also present on the surface of foliose blade (Fig. 4.40o-4.40r).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763226
- atpB F & atpB R: MG963802


a
Herbarium of the Central University of Punjab.
Bathinda, 151001, INDIA
GREEN ALGAE OF INDIA
ISOTYPE
<Ulvaceae,Ulvales>
Ulva reticulata Forsskal.
det. Rani P.
OKHA, GUJARAT; 22°28'0"N, 69°4'0"E; Collected from intertidal rock, Height: 5-7 cm, Dark Green; frequent;
coll. Pooja Rani # OKH-71 23 November, 2015
CUPVOUCHER-OKH-2015-UR-1
DNA Data:
b
2cm
c
d
e
f
g
h
i
j
k
l
m
n

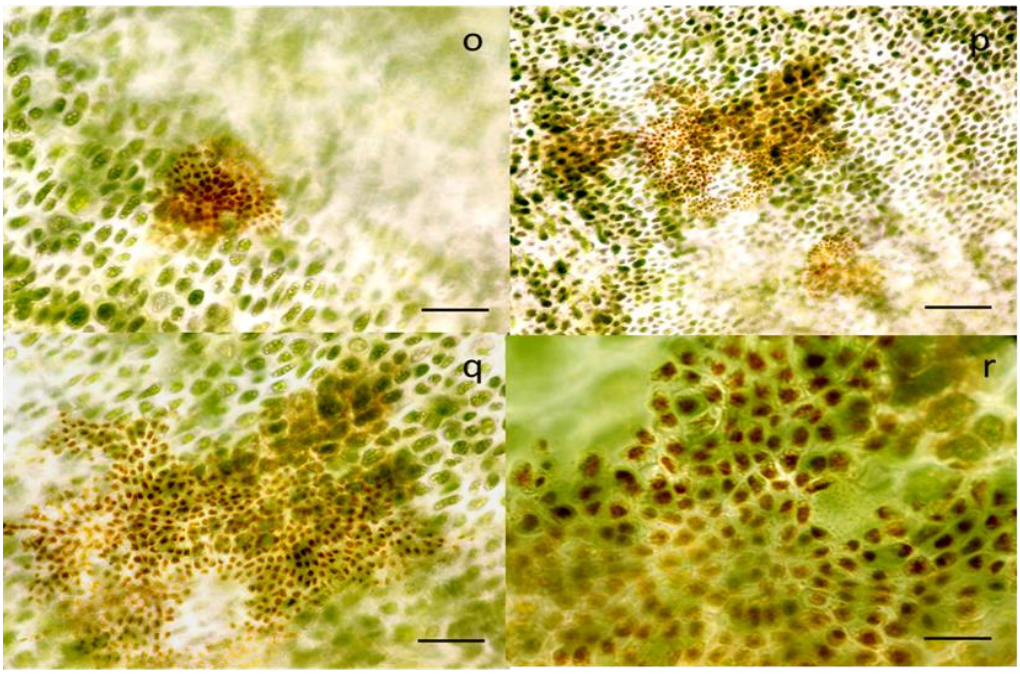

Figure 4.40: Morphology and Micromorphology of *Ulva reticulata* from Okha, India (OKH-71): a Herbarium, b Morphology of thallus; c Microphotographs of thallus at 4X; d, e Reproductive zoospores in basal region at 20X, 40X; f, g Distribution of zoospores in middle region at 40X; h Distromatic cell arrangement of foliose at 10X; i, j, k Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X. l, m, n Denticula in the margin of thallus and cell arrangement of denticula at 10X, 40X, 100X; o, p, q Epiphytic red algae *Erythrocladia* at 40X, 20X, 40X; r Cell arrangement of *Erythrocladia* at 100X; Dividing cells are also present; Scale bar is 200µm for h, i, l; 100µm for d, p; 50µm for e, f, g, j, o, q; 20µm for k, n, r.

DWA-109: *Ulva fasciata* Delile

Sampling site:

Location: Dwarka, Gujarat; Collection date: 22-11-2015; Collected by: Pooja Rani.

Voucher ID:

Frozen voucher ID: DWA-109; CUP Voucher: CUPVOUCHER-DWA-2015-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-DWA-2015-UF-1

Morphology:

Thallus was leafy and roots attached to the rocks or stone. Thallus was dark green in colour and 4-5 cm in length (Fig. 4.41a). Leaves were lobed with wavy margins (Fig. 4.41b). It had grown in the intertidal region and matured leaves torned off from the thallus. Leaves were smooth but lacunae were present. Cells were irregular in arrangement (Fig. 4.41h), Multiseriate; 153.356±62 µm in size and oval or rounded in shape (Fig. 4.41i). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.41j). Margins of leaves were wavy (Fig. 4.41g). Denticulate were present on the margin of the foliose blade (Fig. 4.41h). Multiple pyrenoids were present in cells (Fig. 4.41k). Reproductive zoospores were observed more in basal region as compared to middle region of thallus (Fig. 4.41c,4.41d). Few empty cells were also observed after the release of zoospores (Fig. 4.41f). Long flagellated zoospores were appeared (Fig. 4.41e). Many dividing cells were also appeared (Fig. 4.41i). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763221

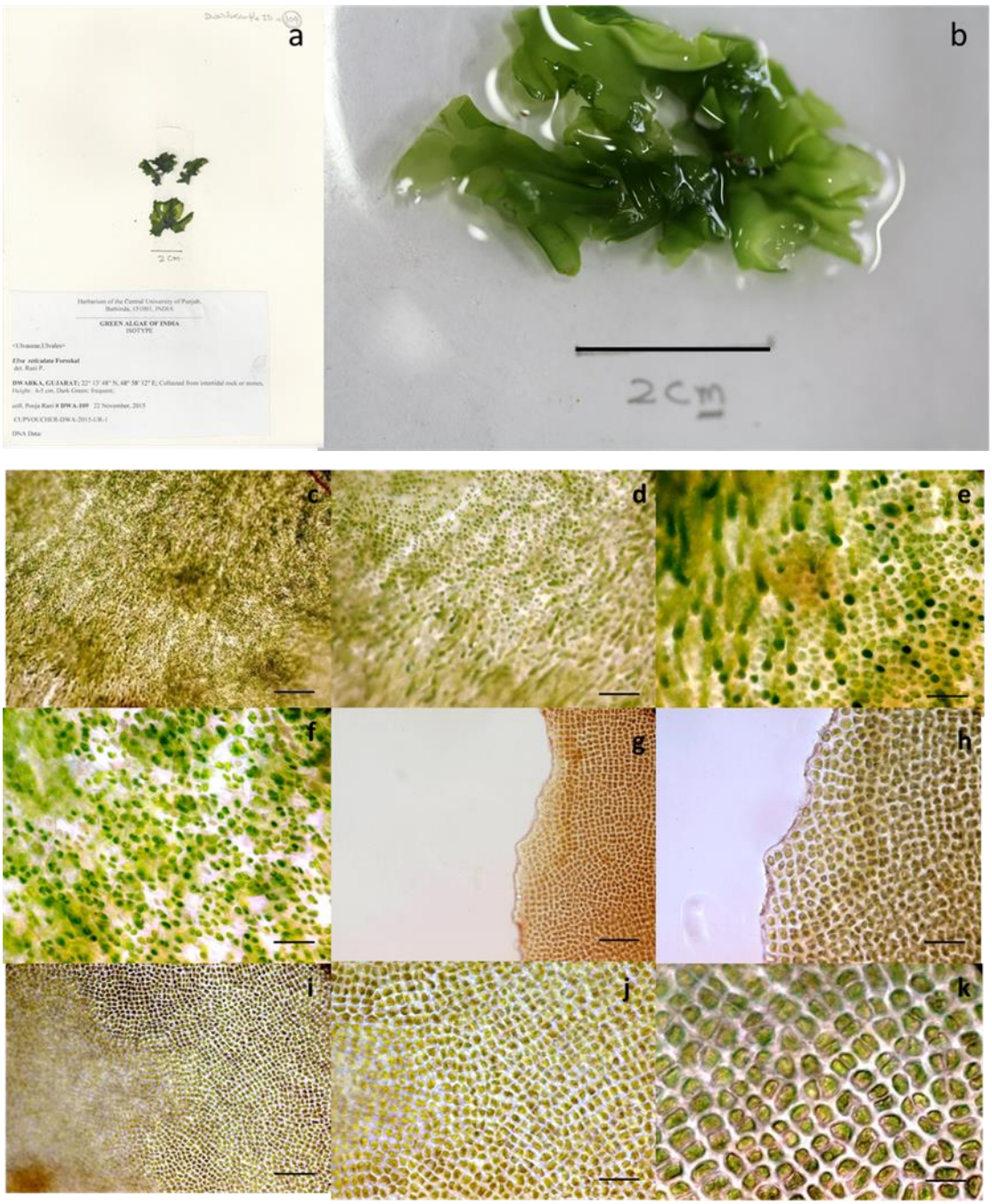

Figure 4.41: Morphology and Micromorphology of *Ulva fasciata* from Dwarka, India (DWA-109): a Herbarium, b Morphology of thallus; c, d, e Flagellated reproductive zoospores in basal region of thallus at 10X, 20X, 40X; f Empty cells after releasing the zoospores at 40X; g, h Denticulate in the margin and cell arrangement in denticulate at 20X, 40X; i, j, k Cell arrangement, chloroplast arrangement and pyrenoids arrangement at 10X, 40X, 100X. Dividing cells are also present; Scale bar is 200µm for c, i; 100µm for d, g; 50µm for e, f, h, j; 20µm for k.

VER-127: *Ulva taeniata* Setchell

Sampling site:

Location: Veraval, Gujarat; Collection date: 20-11-2015; Collected by: Pooja Rani.

Voucher ID:

Frozen voucher ID: VER-127; CUP Voucher: CUPVOUCHER-VER-2015-UT-1**;** Central National Herbarium Voucher ID: CAL-CUPVOUCHER-VER-2015-UT-1

Morphology:

Thallus was leafy and roots attached to the rocks or stone. Thallus was dark green in colour and 2-3 cm in length (Fig. 4.42a). Leaves were lobed with wavy margins (Fig. 4.42b). It had grown in the intertidal region in the mid littoral zone. After maturation, leaves were torned off from the thallus. Leaves were smooth. Cells were irregular in arrangement (Fig. 4.42g), multiseriate; 201.124±34 µm in size and irregular in shape (Fig. 4.42h). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.42i). Margins of leaves were wavy (Fig. 4.42c, 4.42d). Denticulate were present on the surface of leaf (Fig. 4.42e, 4.42f). Multiple pyrenoids were present in cells (Fig. 4.42m). Reproductive zoospores were observed more in basal region of thallus as compared to middle (Fig. 4.42j, 4.42k). Long flagellated zoospores were appeared (Fig. 4.42l, 4.42m). Many dividing cells were also appeared (Fig. 4.42i). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG963803

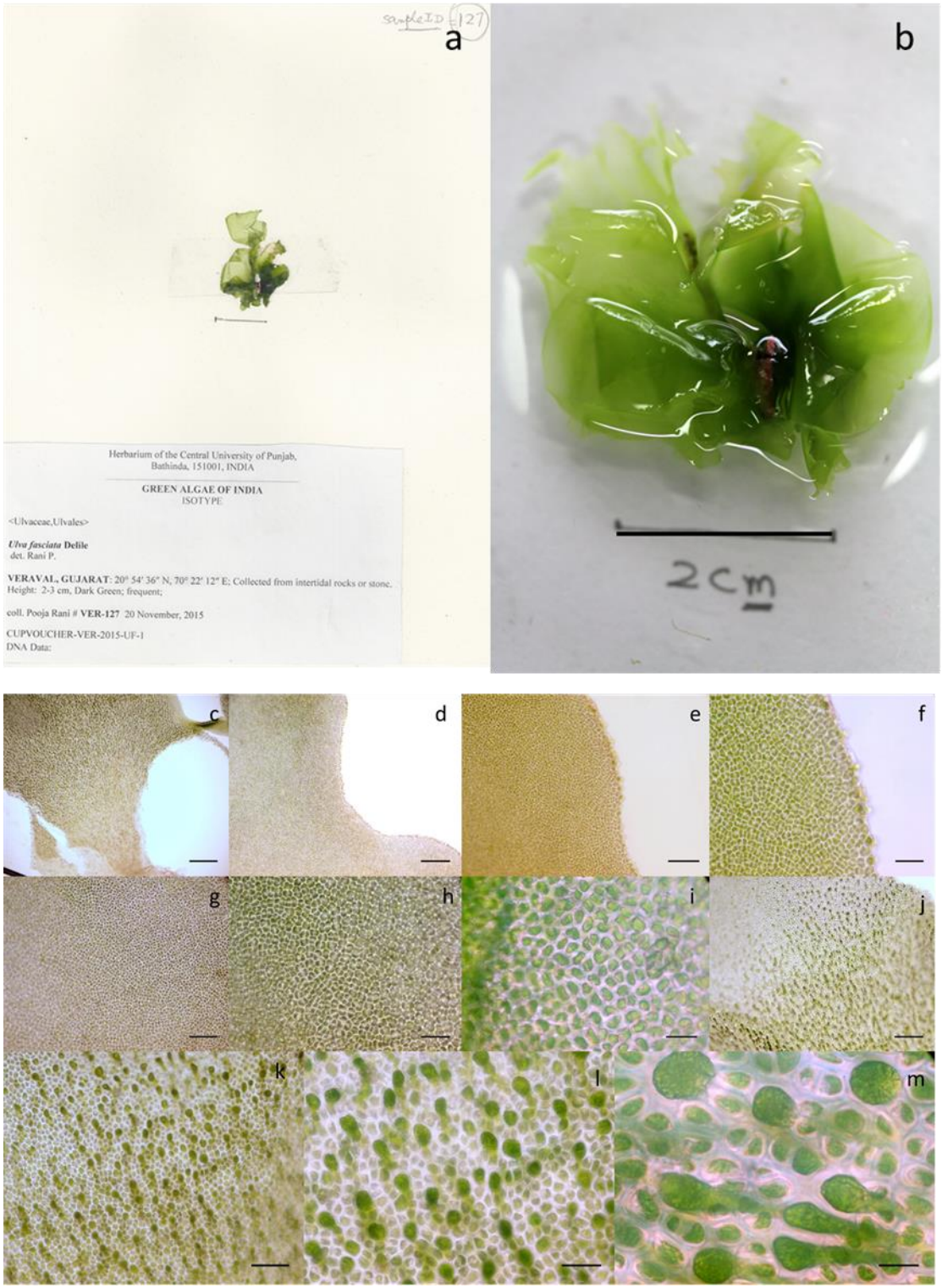

Figure 4.42: Morphology and Micromorphology of *Ulva taeniata* from Veraval, India (VER-127): a Herbarium, b Morphology of thallus; c Microphotograph of thallus at 10X; c, d Margin of thallus at 10X, 40X; e, f Margin of thallus at 10X, 20X; g, h Cell arrangement at 10X, 20X; i Shape of cell and presence of chloroplast inside the cell at 40X; j, k Reproductive bodies on basal region and middle region at 20X, 40X; l, m Flagellated zoospores at

40X, 100X; Dividing cells are also present; Scale bar is 200µm for c, e, g; 100µm for f, h, j; 50µm for d, i, k, l; 20µm for m.

VER-141: *Ulva fasciata* Delile

Sampling site:

Location: Veraval, Gujarat; Collection date: 20-11-2015; Collected by: Pooja Rani.

Voucher ID:

Frozen voucher ID**:** VER-141**;** CUP Voucher: CUPVOUCHER-VER-2015-UF-1**;** Central National Herbarium Voucher ID: CAL-CUPVOUCHER-VER-2015-UF-1

Morphology:

Thallus was leafy and roots attached to the rocks or stone (Fig. 4.43c). Thallus was dark green in colour and 2-3 cm in length (Fig. 4.43a). Leaves were lobed and margins of the leaves are wavy (Fig. 4.43b). It had grown in the intertidal region in the mid littoral zone. After maturation, leaves were torned off from the thallus. Leaves were smooth. Cells were irregular in arrangement (Fig. 4.43i), Multiseriate; 179.513±41 µm in size and irregular in shape (Fig. 4.43j). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.43d). Margins of leaves were wavy (Fig. 4.43d). Denticulate were present on margin of leaf (Fig. 4.43e). Multiple pyrenoid were present in cells (Fig. 4.43k). Reproductive zoospores were observed more in basal region of thallus as compared to middle region (Fig. 4.43f, 4.43g). Long flagellated zoospores were appeared (Fig. 4.43h). Many dividing cells were also appeared (Fig. 4.43i). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MH282868

Figure 4.43: Morphology and Micromorphology of *Ulva fasciata* from Veraval, India (VER-141): a Herbarium, b Morphology of thallus; c Microphotograph of thallus at 10X; d Chloroplast and margin of thallus at 40X; e Denticulate at 40X; f, g Flagellated reproductive zoospores at basal and middle region at 20X, 40X; h flagellated zoospores at 40X; i, j Arrangement and

shape of cell at 10X, 20X; k Pyrenoids inside the cell at 40X; Dividing cells are also present; Scale bar is 200µm for c, i; 100µm for f, j; 50µm for d, e, g, h, k.

BHI-164: *Ulva fasciata* Delile

Sampling site:

Location: Bheemili, Andhra Pradesh; Collection date: 16-12-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: BHI-164; CUP Voucher: CUPVOUCHER-BHI-2015-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BHI-2015-UF-1

Morphology:

Thallus was leafy and appeared star shaped (Fig. 4.44a). Leafy thallus was attached to the surface but most of the time freely floating in water. Thallus was dark green in color. Centre of the thallus was smooth but margins were lobed and highly wavy or coiled (Fig. 4.44b). Lacunae were present in the leaves. Surface of the leaves were smooth. Thallus was 4-5 cm in length (Fig. 4.44b). Cells were irregular in arrangement (Fig. 4.44f), Multiseriate; 113.298±45 µm in size and irregular in shape (Fig. 4.44g). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.44i). Margins of leaves were wavy (Fig. 4.44j). Denticulate were present on the margin of the leaves (Fig. 4.44j, 4.44k). Multiple pyrenoids were present in cells (Fig. 4.44h). Reproductive zoospores were observed in basal region of thallus (Fig. 4.44a, 4.44b). Small patches of zoospores were also present in the middle region of leafy surface (Fig. 4.44b). Long flagellated zoospores were appeared (Fig. 4.44c). Many dividing cells also appeared (Fig. 4.44d, 4.44e). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- atpB F & atpB R: MG963804

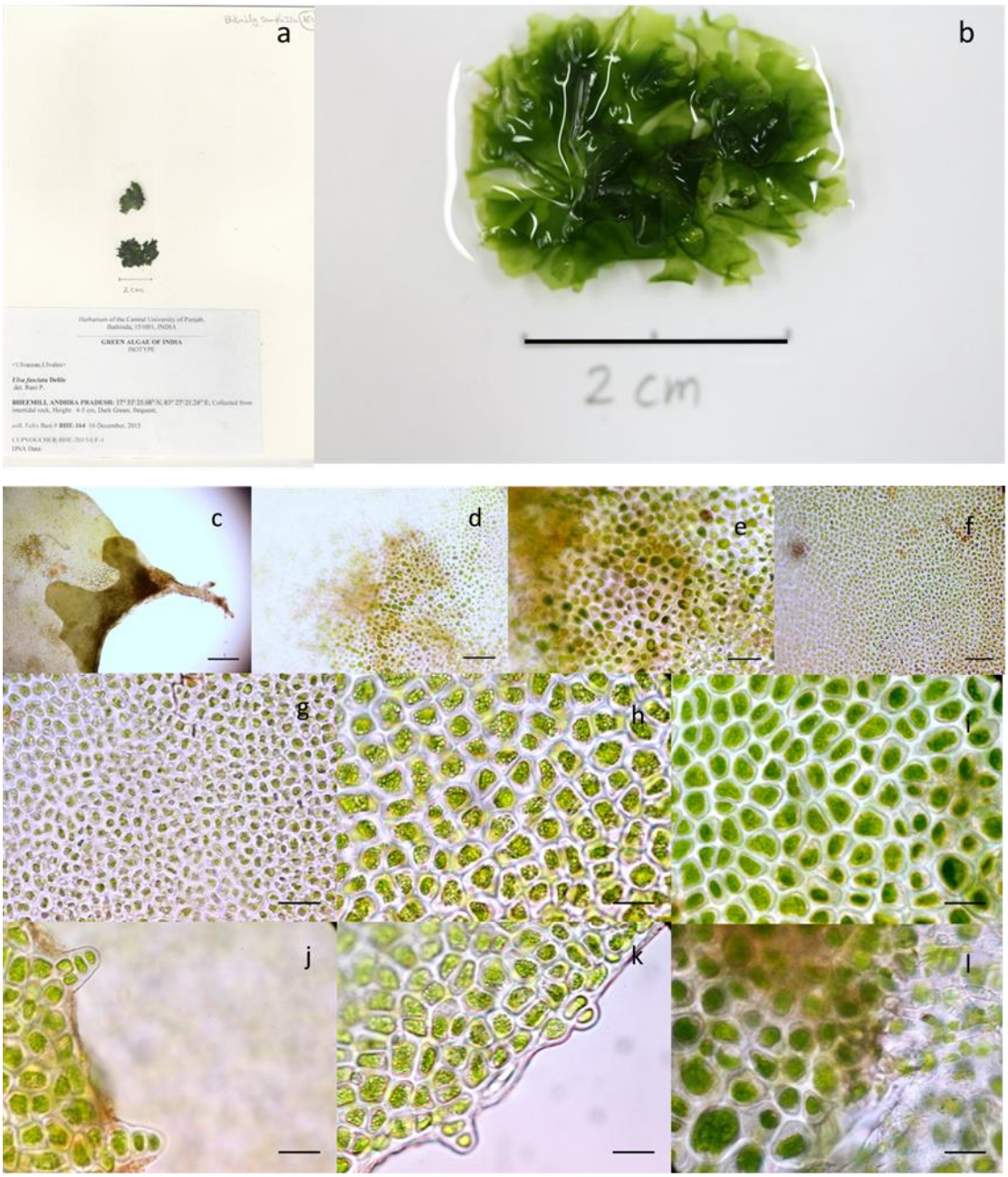

Figure 4.44: Morphology and Micromorphology of *Ulva fasciata* from Bheemili, India (BHI-164): a Herbarium, b Morphology of thallus; c Microphotographs of basal region of thallus at 4X; d, e Reproductive zoospores in basal region and middle region in the form of patches at 20X, 40X; f, g, h, i Cell arrangement, chloroplast arrangement, pyrenoids arrangement and matured dividing cells at 10X, 40X, 100X. j, k Denticula in margin of the foliose and cell arrangement in denticula at 100X; l Flagellated zoospores at 100X; Dividing cells are also present; Scale bar is 200µm for f; 100µm for d; 50µm for e, g; 20µm for h, i, j, k, l.

THO-175: *Ulva sapora* Philip

Sampling site:

Location: Thotla Konda, Andhra Pradesh; Collection date: 17-12-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: THO-175; CUP Voucher: CUPVOUCHER-THO-2015-US-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-THO-2015-US-1

Morphology:

Thallus was bushy, leafy and unbranched (Fig. 4.45a). Leaves were compressed, thin towards base and broader towards distal ends. Thallus was 2 cm in length (Fig. 4.45b). Thallus was green in color. Roots of the thallus was attached to the surface of rocks and leaves exposed to the water. Thallus had grown in intertidal region and exposed to the rocky shore. After maturation, leaves torned off and start freely floating in water. They were growing in the midlittoral zone**.** Thallus was highly branched and alternatively growing new branches were observed in basal region (Fig. 4.45c). Uniseriate and multiseriate branches were observed (Fig. 4.45d). Tip of the branch was rounded in shape (Fig. 4.45k). Compressed branches were thin at basal region but become broader at distal ends (Fig. 4.45l). Cells were irregular in arrangement in middle region of tubular filament (Fig. 4.45g), Multiseriate; 84±9 µm in size and irregular in shape (Fig. 4.45h). Cells were cuboidal or rectangular in the marginal region and arranged in linear rows (Fig. 4.45i). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.45j). Margins of leaves were smooth (Fig. 4.45e). Multiple pyrenoids were present in cells (Fig. 4.45q). Reproductive zoospores were observed in basal region of thallus (Fig. 4.45o, 4.45p). Long flagellated zoospores were appeared (Fig. 4.45q, 4.45r). Many dividing cells were also appeared (Fig. 4.45g). Horizontal and vertical division was observed in the tubular branches. Zoospores were also present at the internodal region of the filament. Thallus was monostromatic and tubular (Fig. 4.45f).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763139
- atpB F & atpB R: MG918113

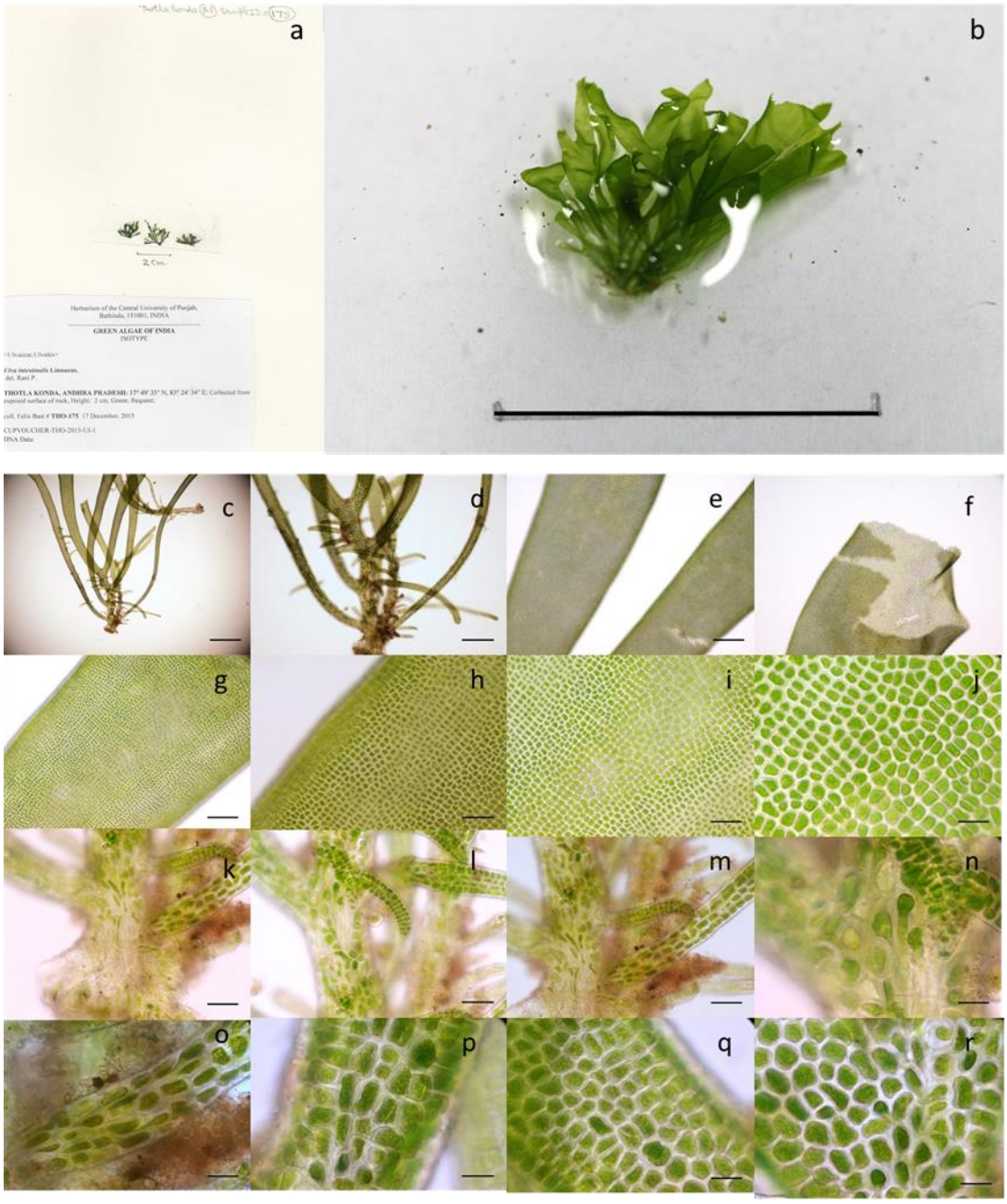

Figure 4.45: Morphology and Micromorphology of *Ulva sapora* from Thotlakonda, India (THO-175): a Herbarium, b Morphology of thallus; c, d

Microphotographs of basal region of thallus at 4X, 10X; e, f Tubular branches at 20X; g, h, i Cell arrangement and margin of thallus at 20X, 40X; j Matured dividing cells at 100X; k, l New developing branches from basal region at 40X; m, n Reproductive zoospores at basal region of thallus at 100X; o, p Zoospores at intermodal region at 100X; q, r Chloroplast arrangement and pyrenoids arrangement at 100X; Dividing cells are also present; Scale bar is 200µm for d; 100µm for e, g; 50µm for h, i, k, l, m; 20µm for j, n, o, p, q, r.

THO-176: *Ulva fasciata* Delile

Sampling site:

Location: Thotla Konda, Andhra Pradesh; Collection date: 17-12-2015; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: THO-176; CUP Voucher: CUPVOUCHER-THO-2015-UF-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-THO-2015-UF-1.

Morphology:

Thallus was leafy and heart shaped. Lobed leaves were and margins were wavy (Fig. 4.46a). Thallus was 3-5 cm in length and dark green in colour (Fig. 4.46b). Surface of the leaves were smooth. Thallus was attached to the surface of rocks and mature leaves torned off, freely float in water. Thallus had grown in intertidal region**.** Cells were irregular in arrangement (Fig. 4.46j), multiseriate; 148.411±38 µm in size and polygonal in shape (Fig. 4.46e). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.46f). Margins of leaves were smooth (Fig. 4.46j). Multiple pyrenoids were present in cells (Fig. 4.46i). Reproductive zoospores were observed more in basal region as compare to middle region of thallus (Fig. 4.46c, 4.46d). Long flagellated zoospores were appeared (Fig. 4.46i). Many dividing cells were also appeared (Fig. 4.46g). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- tufA F & tufA R: MG918122
- atpB F & atpB R: MG963805

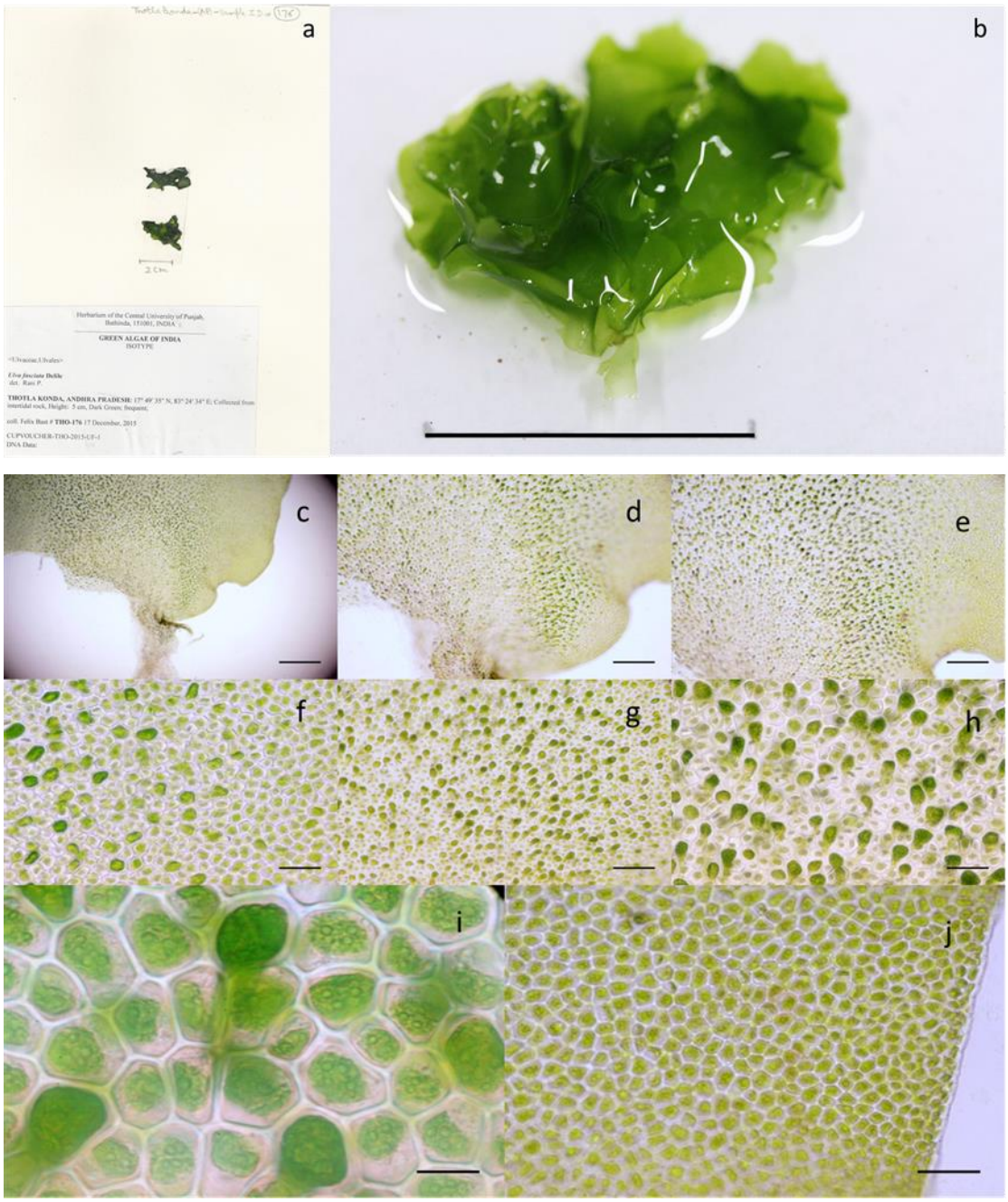

Figure 4.46: Morphology and Micromorphology of *Ulva fasciata* from Thotlakonda, India (THO-176): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Distribution if reproductive zoospores and cell arrangement in middle region of thallus at 10X, 40X; g, h Reproductive zoospores in basal region of thallus at 20X, 40X; i Chloroplast arrangement and pyrenoids arrangement at 100X. j Cell arrangement and margin of thallus at 40X; Dividing cells are also present; Scale bar is 200µm for d, e; 100µm for g; 50µm for f, h, j; 20µm for i.

MDP-241: *Ulva shanxiensis* L. Chen, J. Feng & S. Xie

Sampling site:

Location: Mandapam, Tamil Nadu; Collection date: January, 2016; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: MDP-241; CUP Voucher: CUPVOUCHER-MDP-2016-UX-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2016-UX-1

Morphology:

Thallus was bushy in appearance and unbranched. Branches were leafy and compressed (Fig. 4.47a). Thallus was pale yellow in color and slightly coiled. Thallus was 5-7 cm in length (Fig. 4.47b). Thallus was attached to the surface of rocks and mature leaves freely float in the water. Surface of the leaves were smooth. Cells were linear in arrangement (Fig. 4.47i), multiseriate; 130.705±14 µm in size and irregular in shape (Fig. 4.47j, 4.47k). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.47l). Margins of leaves were not wavy (Fig. 4.47k). Multiple pyrenoids were present in cells (Fig. 4.47m). Reproductive zoospores were observed in basal region of thallus (Fig. 4.47f). Long flagellated structures were appeared (Fig. 4.47g, 4.47h). Many dividing cells were also appeared (Fig. 4.47i). Thallus was distromatic.

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: MG763146
- 18S F &18S R: MG774436
- tufA F & tufA R: MH105040
- atpB F & atpB R: MG918114

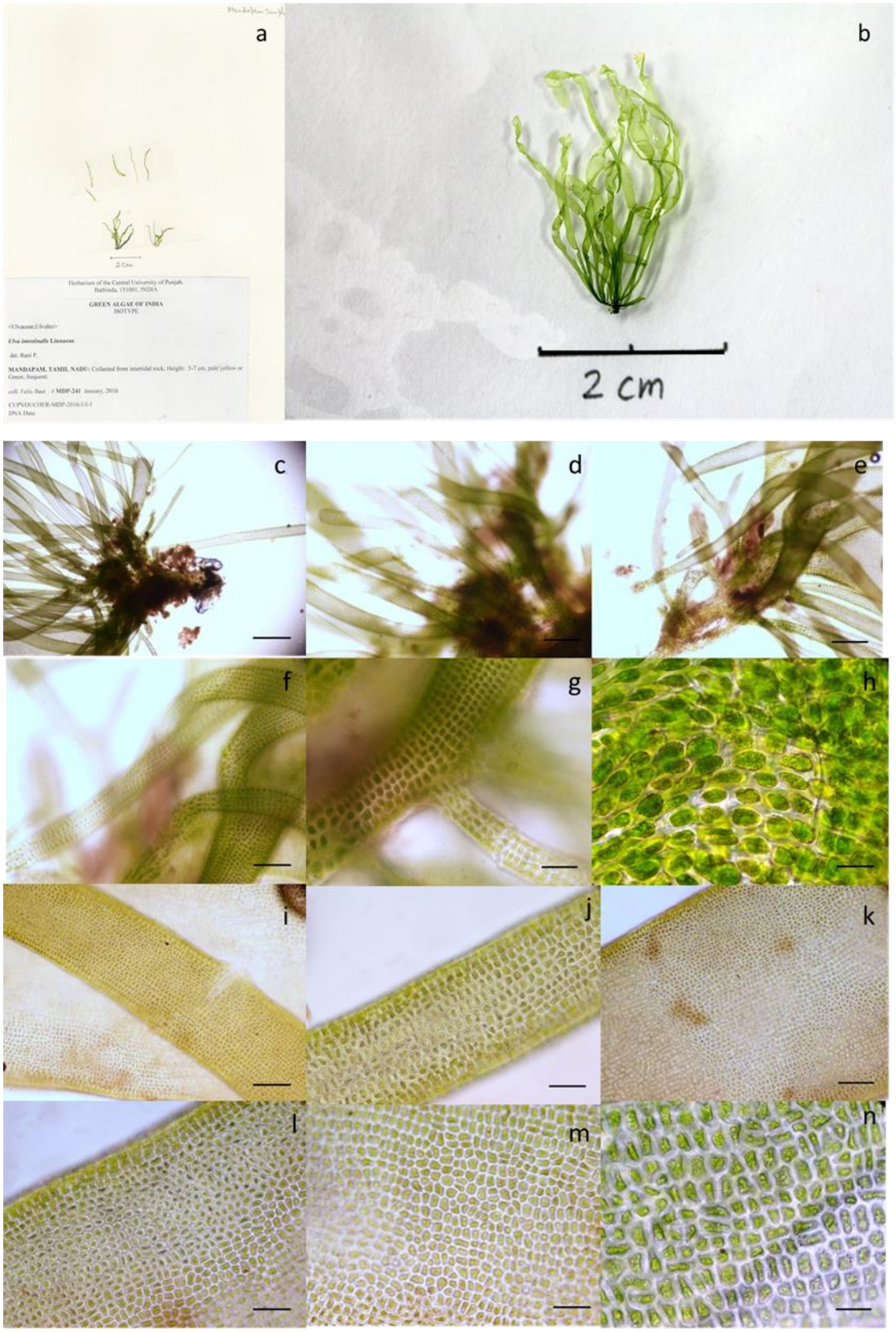

Figure 4.47: Morphology and Micromorphology of *Ulva shanxiensis* from Mandapam, India (MDP-241): a Herbarium, b Morphology of thallus; c, d Microphotographs of basal region of thallus at 4X, 10X; e, f Branching pattern

of thallus at 20X, 40X; g, h Flagellated reproductive zoospores at 40X, 100X; i, j Margin of basal branches thallus at 10X, 40X; k, l Cell arrangement in marginal region of distal ends at 10X, 40X; m, n Chloroplast arrangement and pyrenoids arrangement at 40X,100X; Dividing cells are also present; Scale bar is 200µm for d, i, k; 100µm for e; 50µm for f, g, j, l, m; 20µm for m, n.

PUL-252: *Ulva uniseriata* Bast

Sampling site:

Location: Pulicat lake, Andhra Pradesh; Collection date: 3-01-2016; Collected by: Felix Bast.

Voucher ID:

Frozen voucher ID: PUL-252; CUP Voucher: CUPVOUCHER-MDP-2016-UU-1; Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2016-UU-1

Morphology:

Thallus was tubular and light green in colour (Fig. 4.48a). Thallus was 3-7cm long, unbranched, flattened and uncoiled (Fig. 4.48a). Thallus was attached to the rock surface. Cells were regular in arrangement, having cell-to-cell connection and rectangular in shape (Fig. 4.48b). Chloroplast was present in the form of thick patches (Fig. 4.48c). Cells were linear in arrangement (Fig. 4.48b), uniseriate; 151.560 ± 12µm in size and irregular in shape (Fig. 4.48c). Cell wall was thick. Thick patches of chloroplast were present inside cell (Fig. 4.48d). Margins of leaves were not wavy (Fig. 4.48d).

Molecular data:

Accession number of DNA Sequence data based on Locus:

- ITS1 & ITS2: KX668900

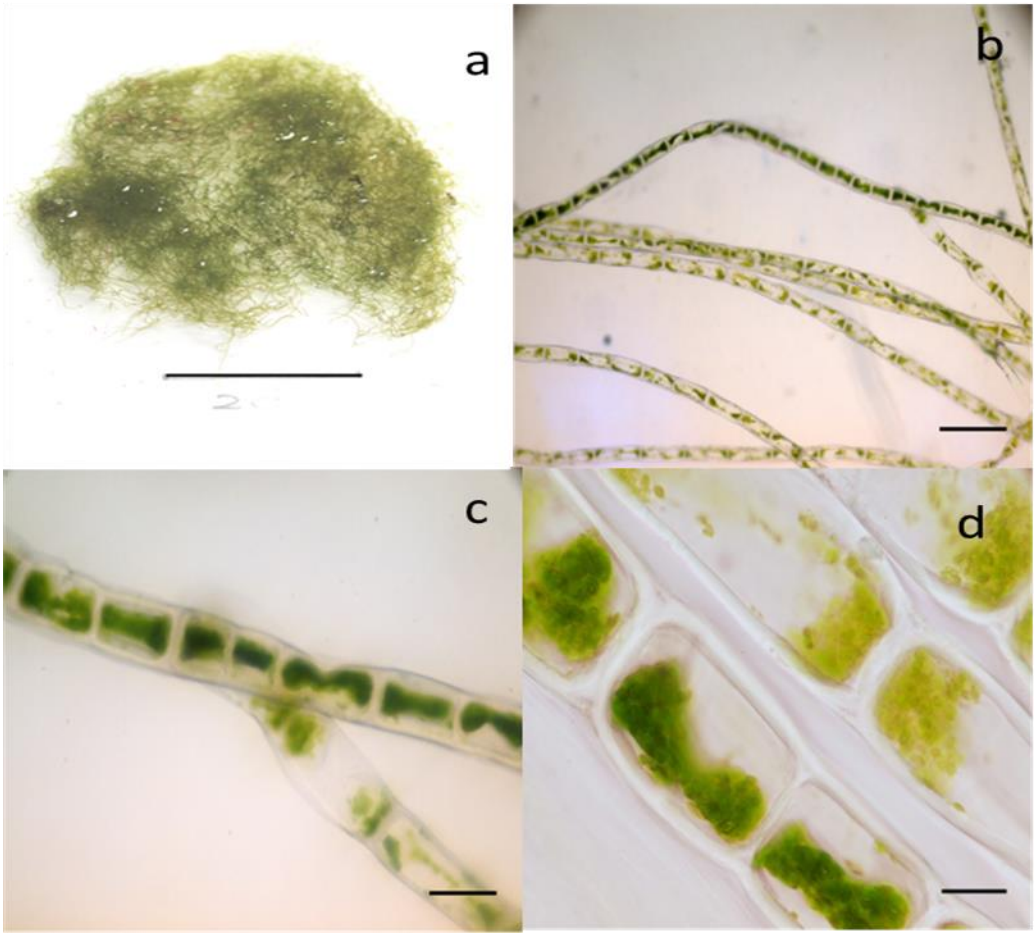

Figure 4.48: Morphology and Micromorphology of *Ulva uniseriata* sp. Nov. from Pulicat Lake, India (PUL-252): a Morphology of thallus; b Microscopic analysis and branching pattern of thallus at 20X; c Cell arrangement at 40X; d Chloroplast arrangement at 100X; Scale bar is 100µm for b; 50µm for c; 20µm for d.

4.2 Phylogenetic analysis

4.2.1 Phylogenetic analysis of tubular *Ulva*:

As only *U. shanxiensis* among tubular *Ulva* samples were amplified at 18S locus, only accessions of this species were included in this tree (Fig. 4.49). *Ulva shanxiensis* accessions formed a monophyletic clade with an accession from China, affirming conspecificity. No other tubular *Ulva* accessions formed a monophyletic clade in the phylogram.

In case of tufA locus, only *U. shanxiensis* among tubular *Ulva* samples were amplified. Only accessions of this species were included in this tree (Fig.

4.50). *Ulva shanxiensis* accessions (KOL-49.3, VEN-58, OKH-70, and MDP-241) formed a monophyletic clade with an accession from China (KJ617036.1) (*Chen et al.,* 2015) reported from inland waters of China, affirming conspecificity. No other tubular *Ulva* accessions formed a monophyletic clade in the phylogram.

Similarly, with ITS1 (Fig. 4.51) sequences of *Ulva sapora*, NOB-43.2, KAN-6.4 and RAT-60 formed a separate clade with *Ulva sapora* from Australia (KT374009.1) (Philips et al., 2016). *Ulva uniseriata* formed a distinct monophyletic clade. However, *U. shanxiensis*, *U. intestinalis, U. sapora* and *U. paschima* formed a paraphyletic clade.

In atpB phylogram, *U. shanxiensis*, *U. sapora* and *U. paschima* formed a monophyletic clade. However, *Ulva prolifera* and *Ulva intestinalis* were paraphyletic (Fig.4.52).

As only *U. shanxiensis* among tubular *Ulva* samples were amplified at rbcL locus, only accessions of this species were included in this tree (Fig. 4. 53). All *U. shanxiensis* formed a monophyletic clade (Fig. 4.53). No other tubular *Ulva* accessions formed a monophyletic clade in the phylogram.

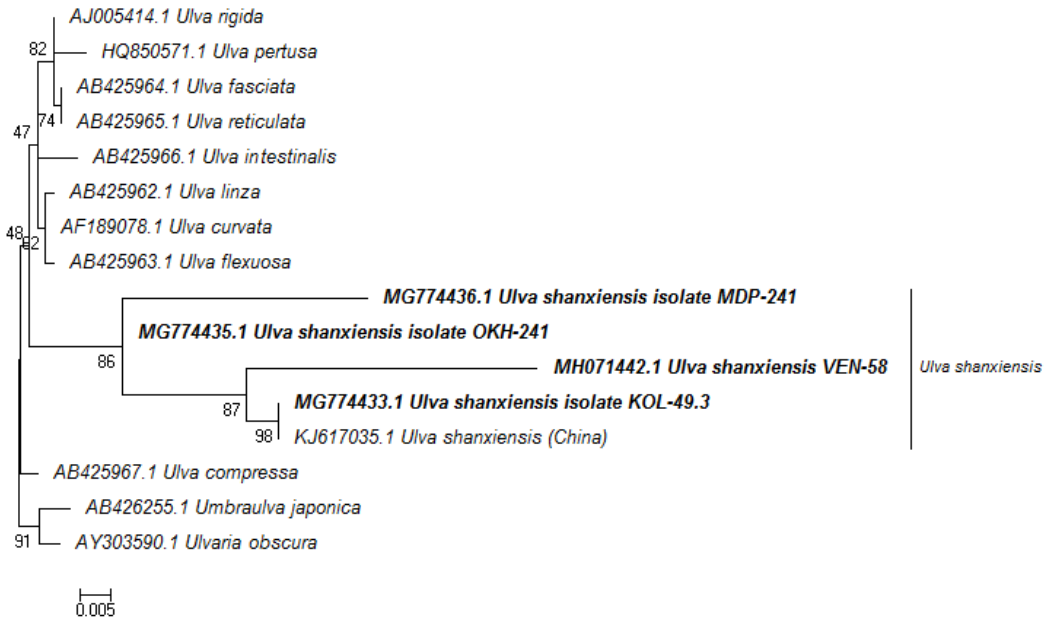

Figure 4.49: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in 18S dataset. Analysis was performed using

Maximum Likelihood phylogenetic reconstruction method (LnL=--7393.5) with Kimura-2-parameter and Gamma distribution model of molecular evolution (K2+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Umbraulva japonica* and *Ulvaria obscura* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

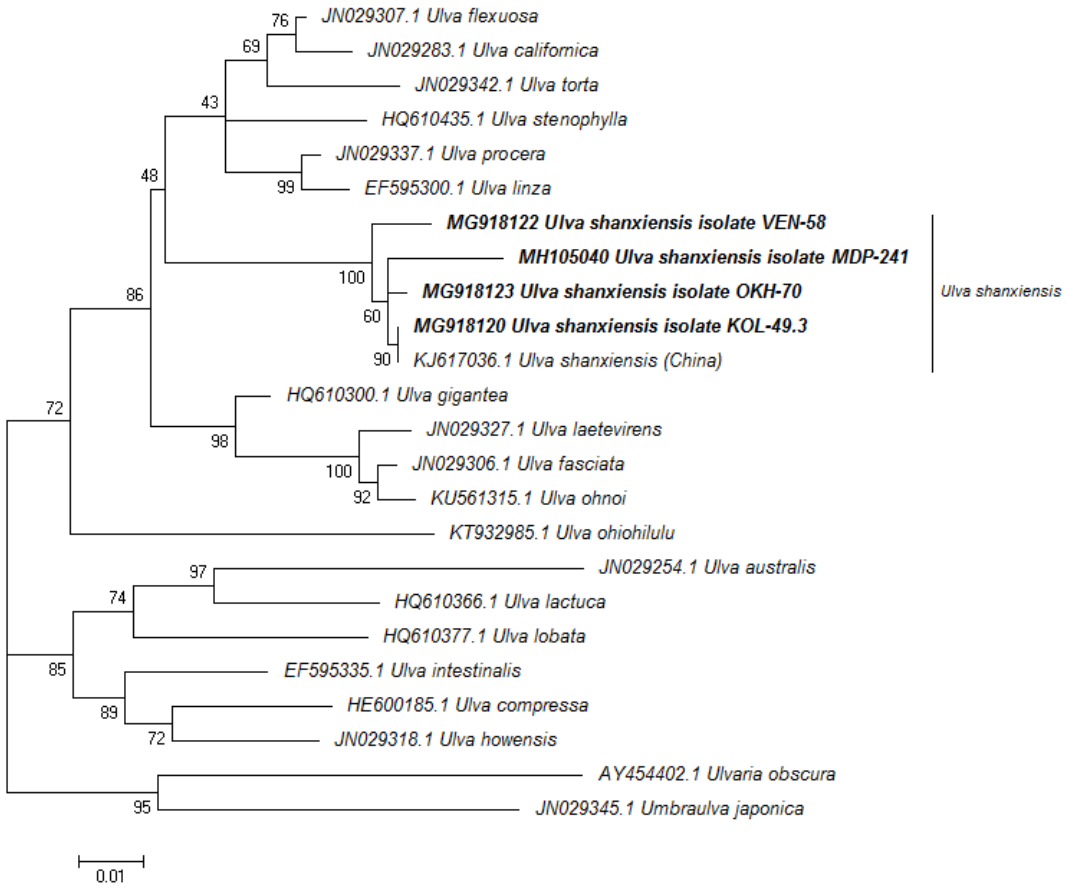

Figure 4.50: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in tufA dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--4452.5) with Tamura-3-parameter (T92) and Gamma distribution model of molecular evolution. Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulvaria obscura* and *Umbraulva japonica* as an outgroup. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

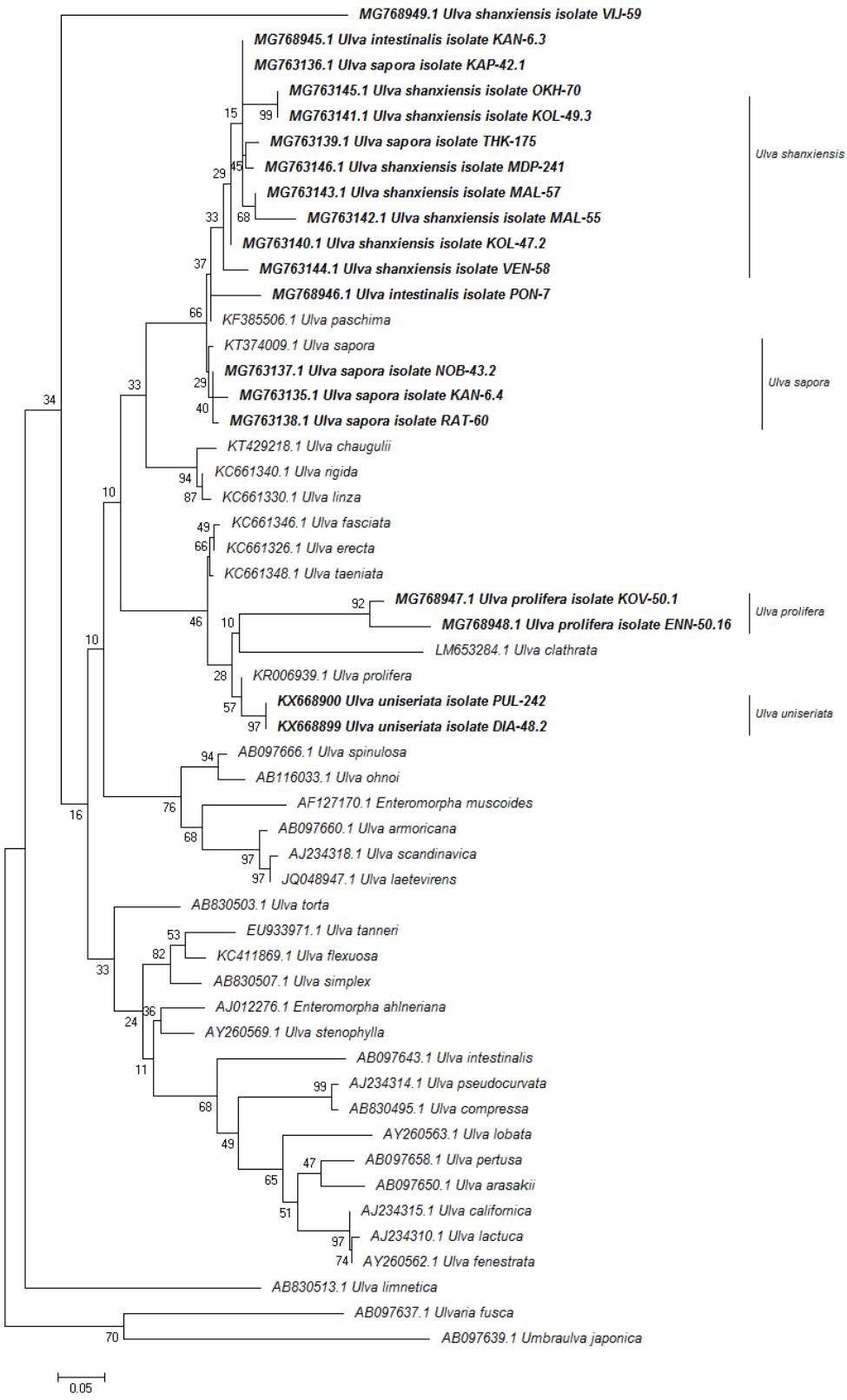

Figure 4.51: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in ITS1 dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--6521.4) with

Tamura-3-parameter and Gamma distribution model of molecular evolution (T92). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulvaria fusca* and *Umbraulva japonica* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

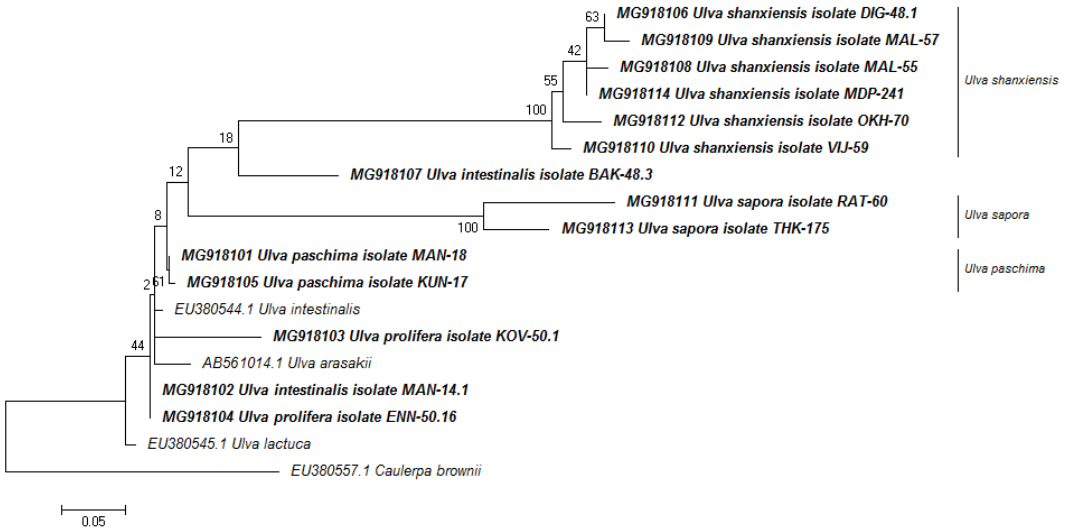

Figure 4.52: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in atpB dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=-1935.7) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Caulerpa brownii* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site

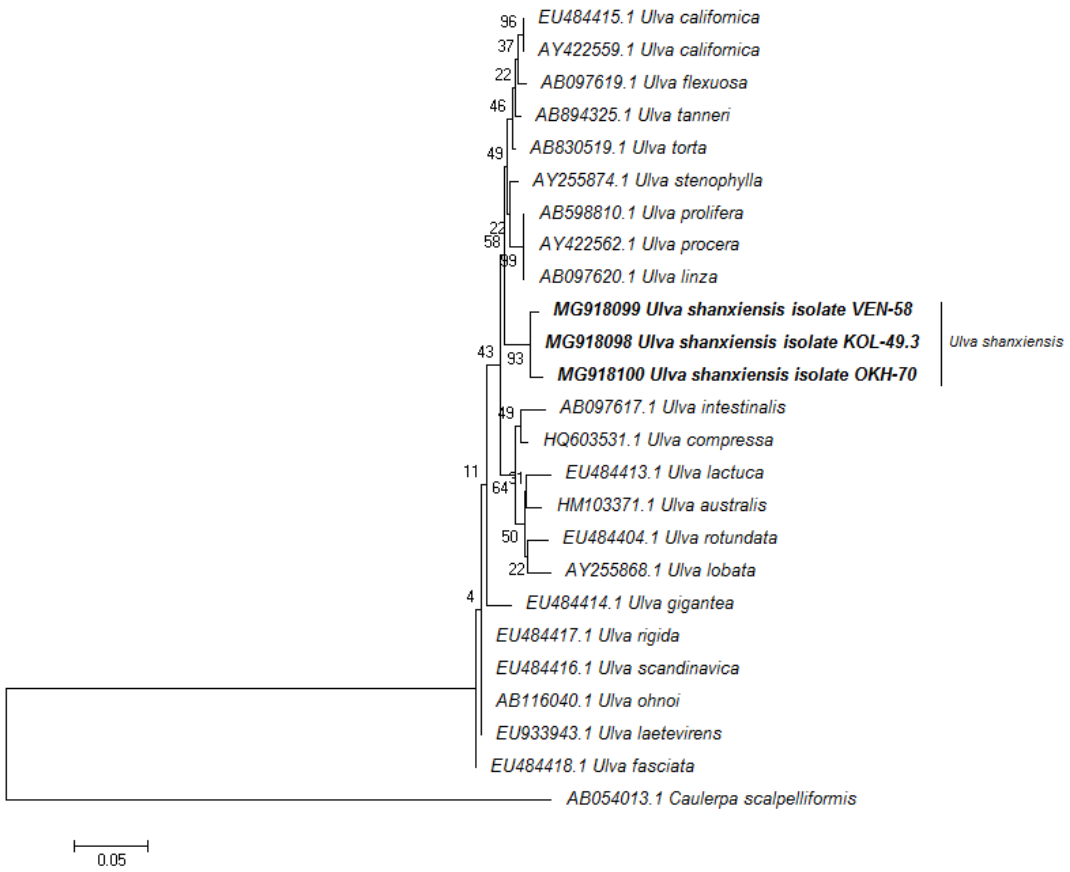

Figure 4.53: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in RbcL dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (Lnl=-1873.8) with Tamura-nei parameter and Gamma distribution model of molecular evolution (TN93). Numbers near nodes represents bootstrap support (500 replicates). This phylogram is rooted with *Caulerpa taxifolia* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitution per site.

4.2.2 Phylogenetic analysis of foliose *Ulva*:

All foliose *Ulva* samples amplified with ITS1 primers were assembled in three different clades (Fig. 4.54). Sample ID CAL-10 (*U. reticulata*), ETT-2 (*U. reticulata*), KOL-49.1 (*U. reticulata*), KOL-49.2 (*U. fasciata*), KOL-49.4 (*U. reticulata*) and OKH-71 (*U. reticulata*) formed one clade. ENN-50.8 (*U. ohnoi*) occupied the basal position to the clade consisting of *U. fasciata* and *U. reticulata*. CHE-51.05 (*U. fasciata*), VER-54.9 (*U. fasciata*) and DWA-109 (*U. fasciata*) formed monophyletic clade with *U. fasciata*.

In the 18S tree (Fig. 4.55), two of our "*reticulata*" accessions formed a clade; however, this species was not monophyletic in the tree. Our two accessions of *U. fasciata* occupied a basal position in a clade encompassing several other foliose *Ulva* species.

In tufA tree (Fig. 4.56), all 8 samples assembled in 3 different clades. Except *Ulva californica*, all the taxa showed non-monophyly in this tree. *Ulva intestinalis* occupied a basal position. While '*fasciata*' showed phylogenetic affinity with '*prolifera*', *'linza'* clustered with '*compressa*' and '*fasciata*' with '*reticulata*'.

All atpB sequences of Indian isolate formed 4 different clades (Fig. 4.57). HAV-35 (*U. fasciata*), VER-127(*U. taeniata*), VAL-61 (*U. reticulata*) and OKH-71 (*U. reticulata*) formed one clade. BEK-23.2 (*U. reticulata*), KOL-49.2 (*U. fasciata*), CHE-51.05 (*U. fasciata*), BHI-164 (*U. fasciata*) and THO-176 (*U. fasciata*) formed one clade. BEK-23.4 (*U. fasciata*), ETT-2 (*U. reticulata*) and VEK-54.9 (*U. fasciata*) form one clade. KYK-51.39 (*U. fasciata*) was basal to all ingroup accessions.

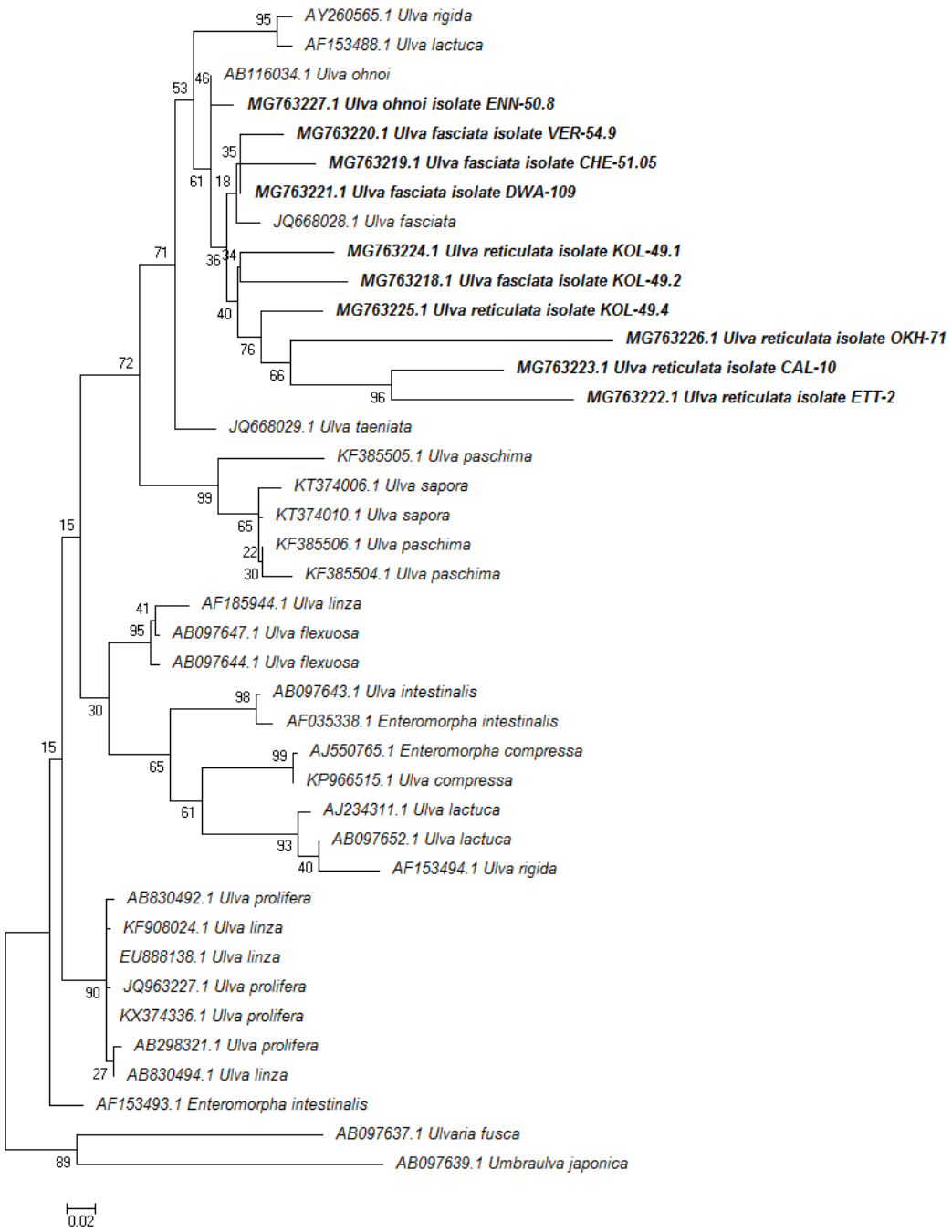

Figure 4.54: Phylogenetic position of foliose *Ulva* isolates from India among other *Ulva* accessions in ITS1 dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--11349.6) with Kimura-2-parameter and Gamma distribution model of molecular evolution (K2+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Umbraulva japonica* and *Ulva*ria *fusca* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

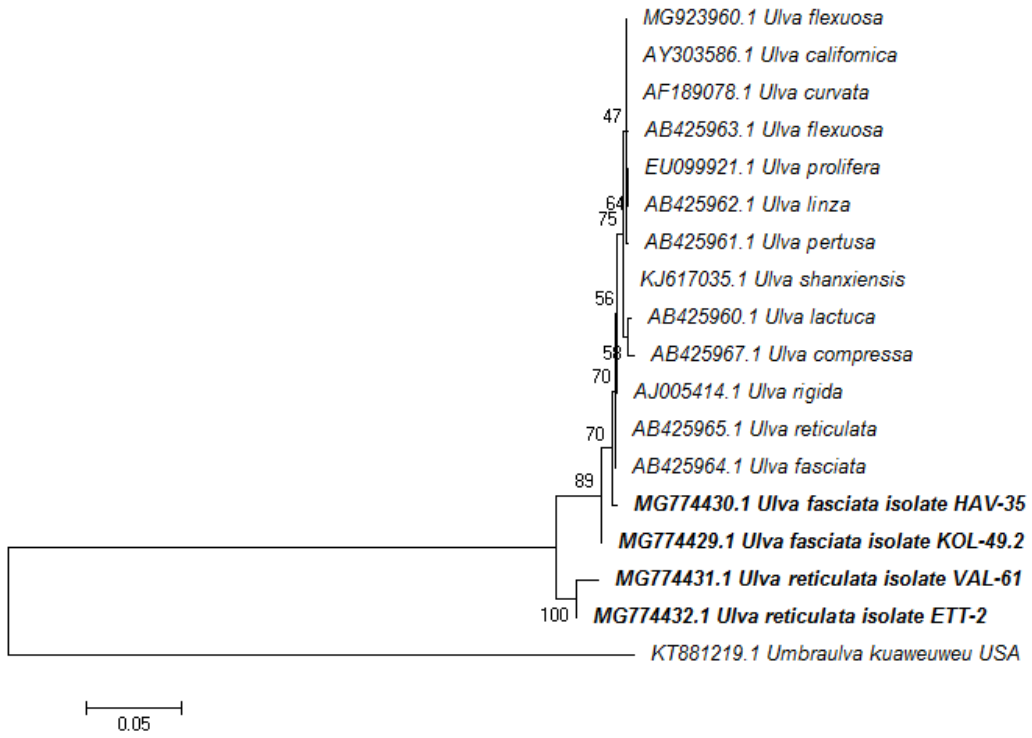

Figure 4.55: Phylogenetic position of foliose *Ulva* isolates from India among other *Ulva* accessions in 18S dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--8740.1) with Kimura-2-parameter and Gamma distribution model of molecular evolution (K2+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Umbraulva kuaweuweu* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

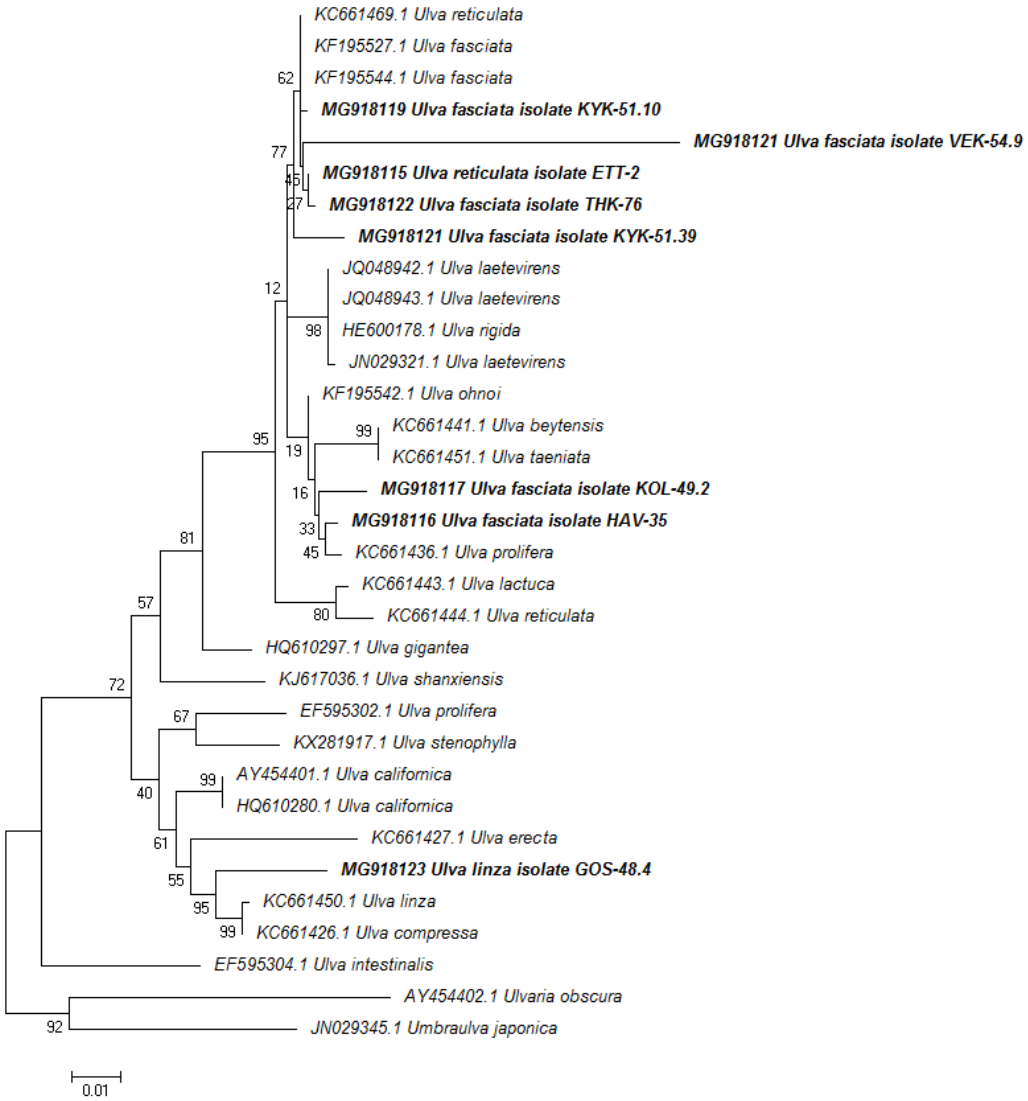

Figure 4.56: Phylogenetic position of foliose *Ulva* isolates from India among other *Ulva* accessions in TufA dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--4323.6) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Umbraulva japonica* and *Ulva*ria *obscura* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

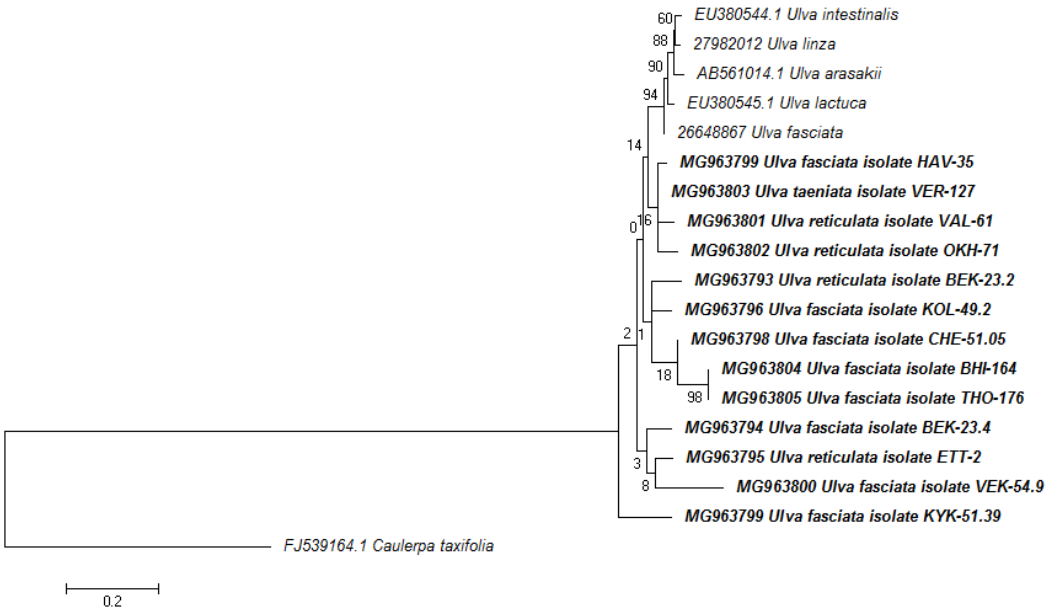

Figure 4.57: Phylogenetic position of foliose *Ulva* isolates from India among other *Ulva* accessions in atpB dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--4092.1) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Caulerpa taxifolia* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

4.3 Phylogeography of *Ulva*

4.3.1 Phylogeographic analysis of foliose *Ulva fasciata:*

The final analysis of atpB contained 156 variable sites, 396 conserved sites and 63 parsimony-informative sites. The nucleotide frequency value was A=31.34%, T/U=32.72%, C=18.37% and G=17.57%. On construction of the phylogenetic tree using atpB, all isolates from different locations of India formed one well-supported clade (Fig. 4.58), suggestive of phylogeographic signal. Inside this clade, two accessions from Andhra Pradesh formed a well-supported clade. No obvious east-west bifurcation was visible in the phylogram.

On construction of the phylogenetic tree using ITS1, all isolates from India were paraphyletic (Fig. 4.59). Total 589 sites were observed for final analysis. No obvious phylogeographic pattern was discernable in the tree, as clades consisted of accessions from mixed coasts.

For TufA, a total 741 sites were included in the final analysis. The compared sequences contained 91 variable sites, 650 conserved sites and 16 parsimony-informative sites. The nucleotide frequency with A=36.20%, T/U=30.04%, C=14.09% and G=19.67% values. On construction of phylogenetic tufA tree (Fig. 4.60), total 4 major clades were formed. No obvious phylogeographic pattern was discernable in the tree, as clades consisted of accessions from mixed coasts.

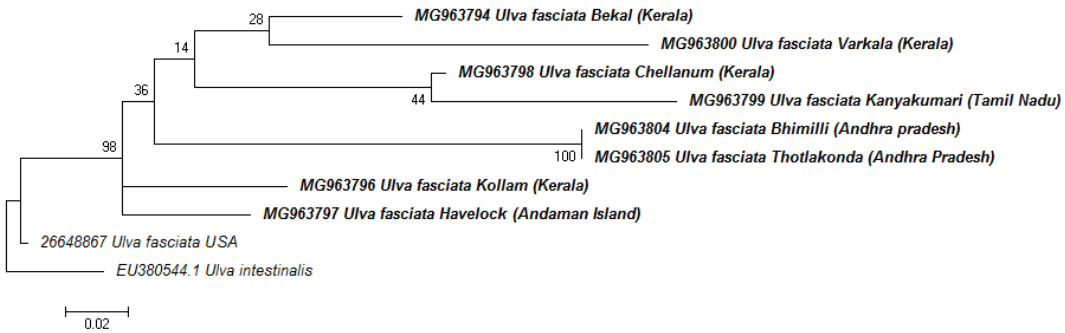

Figure 4.58: Phylogeography of foliose *Ulva fasciata* isolates from India among other *Ulva* accessions in atpB dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=-2132.7703) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulva intestinalis* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

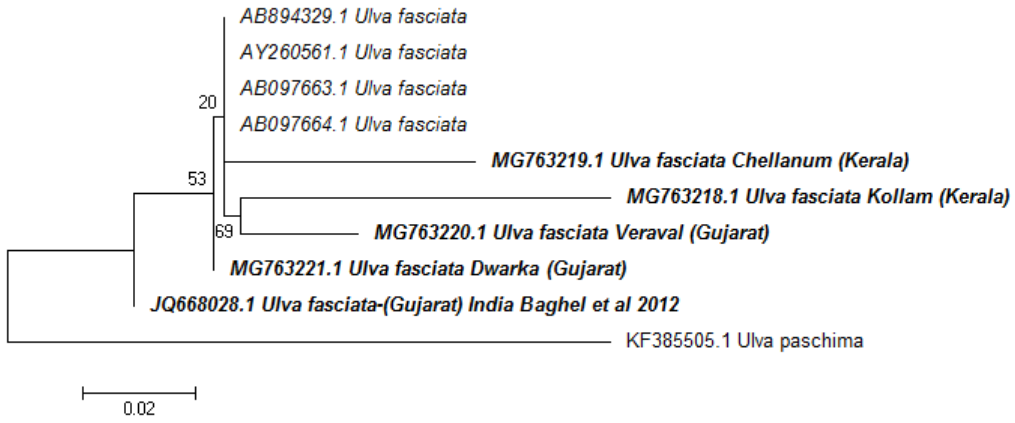

Figure 4.59: Phylogeography of foliose *Ulva fasciata* isolates from India among other *Ulva* accessions in ITS1 dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=-1374.6494) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulva paschima* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

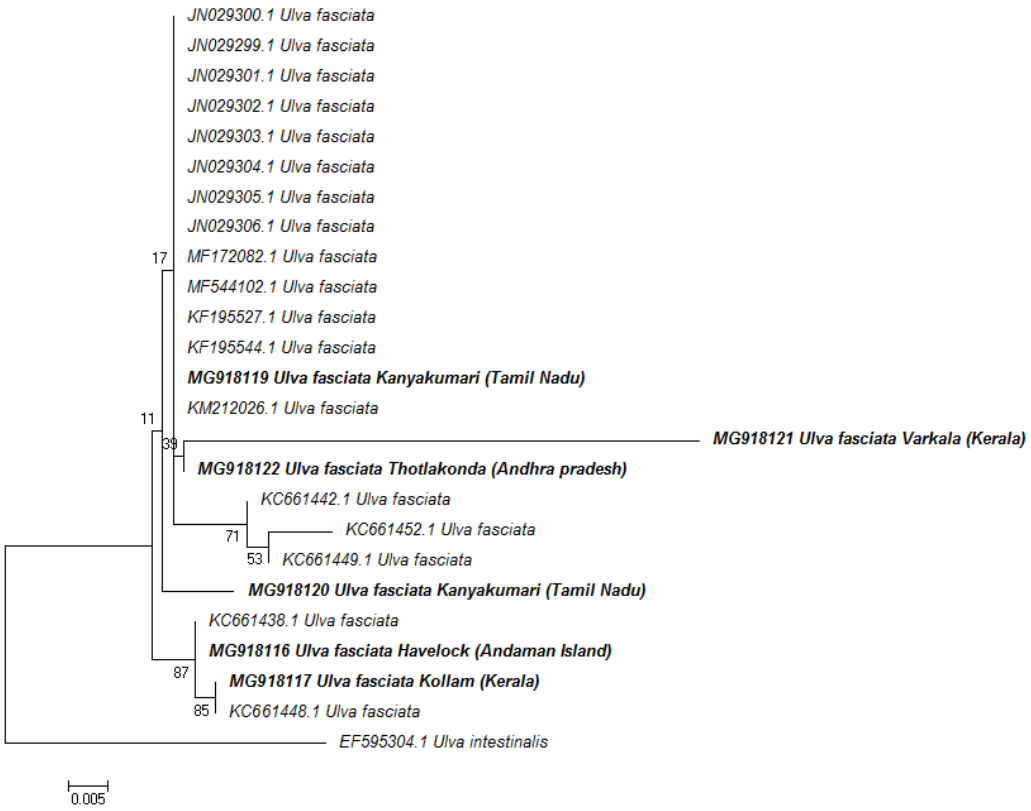

Figure 4.60: Phylogeography of foliose *Ulva fasciata* isolates from India among other *Ulva* accessions in tufA dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=-1546.2) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92+G). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulva intestinalis* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site.

Chapter 5: Discussion

Ulva (Ulvales, Chlorophyta) is difficult to identify up-to species level due to simple morphology and few diagnostic characteristics. The primary morphological features used for taxonomic identification of species include, cell size, cell shape, and structure of chloroplast, number of pyrenoids and alignment of cells in surface view. These features may be phenotypically variable. Genus *Ulva* exhibit mainly foliose blade or hollow tube-like structure. Both of these are characteristics of the genus *Ulva*. Due to the unreliability of only morphological features, molecular analysis is also required for exact taxonomic identification of species using nuclear, chloroplast and mitochondrial genomic regions. Even molecular analyses misidentify the specimens that compared against sequences already posted in the database without validation of morphological features. Molecular data analyses along with morphological features are used to resolve taxonomic issues within the genus. It may lead to estimate an accurate biodiversity of macro-algal communities.

In the present study, 48 samples were collected from 32 locations on the east and west coast of the Indian subcontinent. Samples were collected from the rocky surface of the intertidal region or sub-littoral zone. Few freely floating algal samples were also collected. Morphologically two types of *Ulva* species are reported like the foliose blade or thread-like. However, using microscopic analysis for characterizing the morphological features along with molecular studies revealed 11 different species from the coasts of the Indian subcontinent. *Ulva uniseriata* is reported as a new species in this study. *Ulva shanxiensis* and *U. sapora* are reported as a first report from any Indian coasts. *Ulva shanxiensis* is reported for the first time from any marine habitat. *Sahlingia subintegra* and *Erythrocladia* were reported epiphytic on green algae *Ulva,* for the first time. *Sahlingia subintegra* were first time reported from any Indian coast in this study. All 48 samples were also pressed for herbarium vouchers along with morphological studies of the thallus. Microscopic studies were performed to analyze the size and shape of the cell, arrangement of the cell, number of pyrenoids, branching pattern, location of reproductive zoids, denticula, the margin of the leaf, the position of chloroplast and the number of files of cells.

All samples were amplified using different primers from nuclear, chloroplast and mitochondrial regions for taxonomic identification of the collected species.

Objective 1: To study morphology and microscopic arrangement of *Ulva* thallus along with herbarium preparation.

5.1 Morphology of *Ulva*

5.1.1 Morphology of *U. intestinalis*

The specimens KAN-6.3 (Kannur, Kerala), PON-7 (Ponnani, Kerala), MAN-14.1(Mangalore, Karnataka), DIG-48.1 (Digha, West Bengal) and BAK-48.3 (Bakkhali, West Bengal) were identified as *U. intestinalis* collected from intertidal zones of different coasts. This group showed considerable variation in morphology, with *U. intestinalis* showing unique features. The fronds were tubular or ribbon-like and 2-40 cm in length. The sample had branches on at the base; the thalli were highly branched with primary and secondary branches along the entire axis. The apices were uniseriate upto few cells and multiseriated in few cases. Cells were cuboidal and ordered in the arrangement in basal region, but disordered in distal ends. Size of the cell was 70.605±18.332 to 197.220±33.234 µm. Cell arrangement was found disordered near the developing new branch. Reproductive zoospores were present near the basal region and multiple pyrenoids per cell were observed.

5.1.2 Morphology of *U. prolifera*

The specimens MDP-50.1 (Mandapam, Tamil Nadu) and ENN-50.16 (Ennore, Tamil Nadu) were identified as *U. prolifera* collected from the intertidal regions of the coasts. This group showed different morphology and *U. prolifera* had its unique features. The fronds were tubular and 10-20 cm in length, sample had bifurcating branches or proliferating branches near the basal region. The thalli were highly branched with primary and secondary branches. Basal distal end of the branches was broader than the basal region. Tip region of the apices were round or conical. Bifurcating branch was multiseriate but some newly proliferating branches were uniseriate up-to few cell divisions. Proliferating branches were alternatively or spirally arranged around the main branch. Cells of the thalli were rectangular, polygonal or elongated in shape. Size of the cell was 160.464±47.682 to 208.511±29.132 µm. Cells were arranged in linear rows

but become irregular in shape and arrangement near the proliferating branch point. Low or densely packed chloroplast was present in cells with multiple pyrenoids. Reproductive zoospores were near the basal region and proliferating branch point region.

5.1.3 Morphology of *U. shanxiensis*

The specimens, KOL-47.2, KOL-49.3 (Kollam, Kerala), MAL-55, MAL-57 (Malvan, Maharashtra), VEN-58 (Vengurla, Maharashtra), VIJ-59 (Vijaydurg, Maharashtra), OKH-70 (Okha, Gujarat) and MDP-241 (Mandapam, Tamil Nadu) were identified as *U. shanxiensis*. This group had shown distinguished and unique features than *U. prolifera* and *U. intestinalis*. The thalli were abundantly spinally branched. The filaments were generally unbranched but multiple spinal branches were present near the basal region. The fronds were tubular and 1-20 cm in length. Spinal branches were single tiers with the rounded tip. Cells were different as compare to *U. prolifera* and *U. intestinalis* and were cuboidal or polygonal in shape, arranged in linear rows. Size of cells was in 63.09±15.74 to 173.771±18.75 µm. Multiple pyrenoids were present. Flagellated reproductive zoospores were present in the basal region.

5.1.4 Morphology of *U. sapora*

The specimens KAN-6.4 (Kannur, Kerala), KAP-42.1 (Kalapathar, Andaman Island), NOB-43.2 (North Bay, Andaman Island), RAT-60 (Ratnagiri, Maharashtra), and THK-175 (Thotlakonda, Tamil Nadu) were identified as *U. sapora*. This group had shown unique features totally different from other species. The thallus was filamentous or compressed, branched and alternatively growing secondary branches around the main axis. The fronds were 1-40 cm in length. Filaments were broader at distal ends. Cells were rectangular or irregular in shape and arranged in linear rows. Sizes of cells were in 30±1.16 to 84±9.7 µm. Tip of the apices were rounded in the secondary branch. Uniseriate or multiseriate branches were start grown in the basal region. Thick patches of chloroplast with multiple pyrenoids were present. Multiple flagellated zoospores were present at the basal region of the thallus.

5.1.5 Morphology of *U. paschima*

The specimens MAN-14.2 (Mangalore, Karnataka), KAR-17 (Kundapur, Karnataka) and MAN-18 (Mangalore, Karnataka) were identified as *U. paschima*. The thallus was tubular and contorted, 15-30 cm long and bushy. Thallus was highly branched, multiseriate, monostromatic with smooth margin many secondary branches were arising from the basal region. Bifurcating type branches were also present. Tip region of the secondary branch was rounded and slightly bent. Cells were cuboidal, oval or irregular in shape. Size of cells was 119.452±3 to 212.422±26 µm. Cells were arranged in linear rows regularly but irregular near bifurcating branch point. Very thin patches of the chloroplast were present with multiple pyrenoids. Multiple flagellated zoospores were present near basal region and near branch point area.

5.1.6 Morphology of *U. linza*

The specimens GOS-48.4 (Gosaba, West Bengal) identified as *U. linza*. The thallus was tubular, partially foliose, and 1-2 cm in length. Monostromatic basal region but distal end was distromatic. Blade was with lobed and wavy margin. The basal region was tubular and distal end was broad leaf like. The cells measured 167.182±51 µm in size and were oval and irregular in arrangement. Cells were. The chloroplast was restricted to one end with numerous pyrenoids present in cells. Multiple flagellated zoospores were present in basal region.

5.1.7 Morphology of *U. fasciata*

ETT-3.1 (Ettikulam, Kerala), MDP-13.14 (Mandapam, Tamil Nadu), BEK-23.4 (Bekal, Kerala), HAV-35 (Havelock, Andaman Island), KOL-49.2 (Kollam, Kerala), CHE-51.05 (Chellanam, Kerala), KYK-51.10 (Kanyakumari, Tamil Nadu), KYK-51.39 (Kanyakumari, Tamil Nadu), VEK-54.9 (Varkala, Andhra Pradesh), DWA-109 (Dwarka, Gujarat), VER-141 (Veraval, Gujarat), BHI-164 (Bheemili, Andhra Pradesh), and THO-176 (Thotlakonda, Tamil Nadu) were identified as *U. fasciata*. The thallus was a single large flat lobed or cleft blade 3-40 cm long. The thallus was pale to dark green in colour. The thallus was attached by a small holdfast. Blade was moderately to highly lobed or clefted, flat or spirally twisted but without ruffled margins. Blades 2 cell thick with cells of some thallus. Size of cells was 109.352±47.746 to 203.364±45.158 µm. Most

of the blades with cells were as tall as wide at the margins and throughout the thallus. Shape of cells was in surface view irregularly square to rectangular, with 1-3 pyrenoids.

5.1.8 Morphology of *U. reticulata*

ETT-2 (Ettikulam, Kerala), CAL-10 (Calicut, Kerala), BEK-23.2 (Bekal, Kerala), KOL-49.1 (Kollam, Kerala), KOL-49.4 (Kollam, Kerala), VAL-61 (Veleneshwer, Maharashtra) and OKH-71 (Okha, Gujarat) were identified as *U. reticulata*. The leafy thallus was branched and green in color. The thallus was attached to rocks with rhizoids. It was grown in mid-littoral zone. Thallus was growing separately or some time in association with other algae, light to dark green in color, branches aroused from the base, membranous leaf, compressed or flattened leaf and 5-20 cm long, distal ends of the leaves rounded but the basal region coiled like a ribbon; Thallus form the dense population in intertidal pools. Cells were irregular in arrangement, Multiseriate; 181.179±42 to 199.462±30 µm in size and irregular in shape. The cell wall was thick. Reticulae was present in the membranous leaves and observed in surface view; Thick patches of the chloroplast were present inside cell. Margins of leaves were smooth. Denticulate were observed on the margin of leaves. Multiple pyrenoids were present in cells. Reproductive zoospores were observed in the basal region of the thallus. The long flagellated structure was appeared in asexual reproduction. Many dividing cells had also appeared. The thallus was distromatic.

5.1.9 Morphology of *U. taeniata*

The specimen VER-127 (Veraval, Gujarat) was identified as *U. taeniata. Ulva taeniata* the thalli had lobes differentiating from a discoid base. Densely ruffled and commonly spirally twisted with dentate margins as the criteria was to identify and define these species. The thallus was branched foliose, dark green in colour. Distal ends of the blade formed heart-shaped structure. Membranous leaves had ruffled or wavy margin and denticulate were present at the margin. The thallus was distromatic, 2-3 cm long, with lacunae at the basal region. Blades were arranged in the form of whorls in basal region. Thallus was attached to the substratum or rocky surface with a disc-shaped

holdfast. Cells were irregular in shape and arrangement. Size of the cells was 201.124±34 µm. Thick patches of the chloroplast were present that occupied the complete cell area. Multiple pyrenoids were present. Flagellated zoospores were present in the basal region.

5.1.10 Morphology of *U. ohnoi*

The specimen ENN-50.8 (Ennore, Tamil Nadu) was identified as *U. ohnoi*. Thalli was showing orbicular or ovoid-branched thalli with blades of different shapes at the mid or upper end. The blade was very fragile and delicate. The Thallus was 20-30 cm long. Tiny serrations were present on blade surface and having ruffled or wavy margin. The thallus was attached to the surface with disc-shaped holdfast. Cells were rounded in the basal region but pentagonal in distal ends. Cells were110.075±29 µm in size. Cells were irregularly arranged throughout the thallus. Multiple pyrenoids were present. Zoospores were present more in the basal region as compared to the middle region. Matured flagellated zoospores were released from the cell and leaving behind empty cells.

5.1.11 Morphology of *U. uniseriata sp. Nov*.

The specimen DIA-48.2 (Diamond Harbor, West Bengal) and PUL-252 (Pulicat lake, Andhra Pradesh were identified as *U. uniseriata sp. nov*. The plant was tubular and light green in colour. The thallus was unbranched, flattened, uncoiled and 3-7cm long. The thallus was attached to the rock surface. Cells were regular in the arrangement, having the cell-to-cell connection, 3-5µm in size, and rectangular. The chloroplast was present in the form of thick patches. Cells were linear in arrangement, uniseriate; 151.560 ± 12 to 154.169 ± 9 µm in size and regular in shape. The cell wall was thick.

Description of *U. uniseriata sp. nov*.

Description

Thallus saxicolous, uniseriate, filamentous, free-living, grass green in color; 3-15 cm in length; unbranched, compressed; tufts of thallus attached via rhizoids; cells quadrilateral to elongated, ends rounded; parietal chloroplast with multiple

pyrenoids per cell. Primary diagnosis was the phylogenetic affiliation of OTUs with distinct monophyletic ITS clade “uniseriata”.

Type locality

Near Boat Jetty, Diamond Harbour, West Bengal, India, 21° 56′ 59″ N 89° 10′ 59.99″.

Holotype

Collected from Diamond Harbour, West Bengal, India; Collected on 25-05-2014; Collected by Felix Bast; Deposited in the Central National Herbarium, Botanical Survey of India, Calcutta (CAL) under voucher ID# CAL-CUPVOUCHER-DIA-2014-US-1. DNA sequence of ITS1 regions of complete holotype deposited at Gen Bank under accessions #

Isotype

Collected from Diamond Harbour, West Bengal, India; Collected on 25-05-2014; Collected by Felix Bast; Deposited in Herbarium, Central University of Punjab (CUP) under voucher No.: CUPVOUCHER-DIA-2014-US-1. Frozen voucher maintained at Centre for Plant Sciences, Central University of Punjab under voucher No.: CUPVOUCHER-DIA-2014-US-1.

Etymology

Specific epithet refers to the uniseriate morphology of thallus.

From the morphological and microscopic analysis, it was found that cells were rectangular or cuboidal in shape in tubular thalli. Cells become irregular in shape and arrangement near bifurcating region. In case of foliose thallus, cells were mostly oval, rounded or polygonal in shape but never rectangular or cuboidal in shape. Therefore, it observed that morphology of the *Ulva* was also influenced by shape of cell. All isolates of one species from different geographical location were exhibited morphological plasticity. Therefore, only morphological characteristics were not sufficient for exact taxonomic identification of various *Ulva* species. Molecular analysis of all isolates were performed using different organelle gene.

5.2 Epiphytic algae on genus *Ulva*

During morphological and microscopic analyses, various epiphytic algae also reported on the thallus of *Ulva* thallus. Various types of epiphytic red algae reported on the thallus of tubular and foliose *Ulva*. In this study, *Erythrocladia irregularis*, *Sahlingia subintegra* and *Porphyridum* red algae reported epiphytic on green algae *Ulva*. All these algae first time reported epiphytic on genus *Ulva*. *Sahlingia subintegra* was a first report from Asia.

5.3 Herbarium preparation

Herbarium vouchers were prepared for all 48 isolates in duplicate. One set of all herbarium vouchers submitted to the Herbarium of Central University of Punjab, Bathinda. Another set of herbarium voucher submitted to National Herbarium Centre for Calcutta, India. Both set of herbarium vouchers will help in future studies and can use as a reference data.

Objective 2: To study taxonomy, isolate and sequence DNA using different loci from the collected sample of *Ulva* from different coastal areas of India.

5.4 Taxonomy identification using molecular analysis: For the taxonomic identification of species, DNA was isolated using hipura algal genomic kit. Isolated DNA was processed for PCR amplification reactions using different organelle primers. Sequencing of PCR products was performed using sequence analyzer. All sequences were analyzed for quality by using Codon-code aligner and Geneious software. Good quality forward and reverse sequences were used for contigs generation. These contigs were used for identification of the species using NCBI BLASTn. Different primers (ITS1, 18S, atpB, tufA and rbcL) were used to obtain a total of 87 sequences from different species. All sequences were submitted to GenBank. Details of accession number of all sequences are listed in Table 4.1.

Table 4.1: List of accession number of sequences submitted to GenBank for different amplified loci.

Sr. no.	Isolate ID	Location	Species identified	ITS1	18S	TufA	AtpB	rbcL
1.	ETT-2	Ettikulam, Kerala	*Ulva reticulata*	MG763222	MG774432	MG918115	MG963795	
2.	ETT-3.1	Ettikulam, Kerala	*Ulva fasciata*	MH277343				
3.	KAN-6.3	Kannur, Kerala	*Ulva intestinalis*	MG768945				
4.	KAN-6.4	Kannur, Kerala	*Ulva sapora*	MG763135				
5.	PON-7	Ponnani, Kerala	*Ulva intestinalis*	MG768946				
6.	CAL-10	Calicut, Kerala	*Ulva reticulata*	MG763223				
7.	MDP-13.14	Mandapam, Tamil Nadu	*Ulva fasciata*	MH277344				
8.	MAN-14.1	Mangalore, Karnataka	*Ulva intestinalis*				MG918102	
9.	MAN-14.2	Mangalore, Karnataka	*Ulva paschima*	MG918118				
10.	KUN-17	Kundapur, Karnataka	*Ulva paschima*				MG918105	
11.	MAN-18	Mangalore, Karnataka	*Ulva paschima*				MG918101	
12.	BEK-23.2	Bekal, Kerala	*Ulva reticulata*				MG963793	
13.	BEK-23.4	Bekal, Kerala	*Ulva fasciata*				MG963794	
14.	HAV-35	Havelock, Andaman Island	*Ulva fasciata*		MG774430	MG918116	MG963797	
15.	KAP-42.1	Kalapathar, Andaman Island	*Ulva sapora*	MG763136				
16.	NOB-43.2	North bay, Andaman Island	*Ulva sapora*	MG763137				
17.	KOL-47.2	Kollam, Kerala	*Ulva shanxiensis*	MG763140				
18.	DIG-48.1	Digha, West Bengal	*Ulva intestinalis*		MG774434		MG918106	

19.	DIG-48.2	Diamond Harbour, West Bengal	*Ulva uniseriata*	KX668899				
20.	BAK-48.3	Bakhali, Andhra Pradesh	*Ulva intestinalis*				MG918107	
21.	GOS-48.4	Gosaba, West Bengal	*Ulva linza*			MG918123		
22.	KOL-49.1	Kollam, Kerala	*Ulva reticulata*	MG763224				
23.	KOL-49.2	Kollam, Kerala	*Ulva fasciata*	MG763218	MG774429	MG918117	MG963796	
24.	KOL-49.3	Kollam, Kerala	*Ulva shanxiensis*	MG763141	MG774433			MG918098
25.	KOL-49.4	Kollam, Kerala	*Ulva reticulata*	MG763225				
26.	KOV-50.1	Kovalam, Kerala	*Ulva prolifera*	MG768947			MG918103	
27.	ENN-50.8	Ennore, Tamil Nadu	*Ulva ohnoi*	MG763227				
28.	ENN-50.16	Ennore, Tamil Nadu	*Ulva prolifera*	MG768948			MG918104	
29.	CHE-51.05	Chellanam, Kerala	*Ulva fasciata*	MG763219			MG963798	
30.	KYK-51.10	Kanyakumari, Tamil Nadu	*Ulva fasciata*			MG918119		
31.	KYK-51.39	Kanyakumari, Tamil Nadu	*Ulva fasciata*			MG918120	MG963799	
32.	VEK-54.9	Varkala, Andhra Pradesh	*Ulva fasciata*			MG918121	MG963800	
33.	MAL-55	Malvan, Maharashtra	*Ulva shanxiensis*	MG763142			MG918108	
34.	MAL-57	Malvan, Maharashtra	*Ulva shanxiensis*	MG763143			MG918109	
35.	VEN-58	Vengurla, Maharashtra	*Ulva shanxiensis*	MG763144	MH071442			MG918099
36.	VIJ-59	Vijaydurg, Maharashtra	*Ulva shanxiensis*	MG768949			MG918110	

37.	RAT-60	Ratnagiri, Maharashtra	*Ulva sapora*	MG763138			MG918111	
38.	VAL-61	Velneshwar, Maharashtra	*Ulva reticulata*		MG774431		MG963801	
39.	OKH-70	Okha, Maharashtra	*Ulva shanxiensis*	MG763145	MG774435		MG918112	MG918100
40.	OKH-71	Okha, Gujarat	*Ulva reticulata*	MG763226			MG963802	
41.	DWA-109	Dwarka, Gujarat	*Ulva fasciata*	MG763221				
42.	VER-127	Veraval, Gujarat	*Ulva taeniata*				MG963803	
43.	VER-141	Veraval, Gujarat	*Ulva fasciata*	MG763220		MH282868		
44.	BHI-164	Bheemili, Andhra Pradesh	*Ulva fasciata*				MG963804	
45.	THK-175	Thotlakonda, Tamil Nadu	*Ulva sapora*	MG763139			MG918113	
46.	THO-176	Thotlakonda, Tamil Nadu	*Ulva fasciata*			MG918122	MG963805	
47.	MDP-241	Mandapam, Tamil Nadu	*Ulva shanxiensis*	MG763146	MG774436	MH105040	MG918114	
48.	PUL-242	Pulicat lake, Andhra Pradesh	*Ulva uniseriata*	KX668900				

Objective 3: To study the phylogenetic analysis of genus *Ulva* from entire coast of India.

All isolates were studied for morphological and microscopic characteristics as illustrated in objective 1. Molecular analyses were performed using different loci of successfully amplified isolates, and were submitted to GenBank as described in objective 2. Most of the isolates were amplified with ITS1 primer. Phylogenetic analyses using Maximum Likelihood method were performed to aid in species delineation in the genus *Ulva*.

5.5 Phylogenetic analysis of tubular *Ulva*

As this study included five loci, viz. nuclear (ITS, 18S), mitochondrial (atpB) and chloroplast (tufA and, rbcL) regions, this forms the most comprehensive phylogenetic assessment of *Ulva* species fom India attempted till date. The study revealed existence of 26 tubular *Ulva* in Indian coasts. The study not only revealed intricate phylogenetic structures of various species within genus *Ulva* at various loci, but also revealed existence of two new records in Indian coasts; *U. shanxiensis* and *U. sapora*. Compared with foliose *Ulva*, tubular *Ulva* remains less described as there are morphological plasticity among various tubular *Ulva* species that confuse taxonomists who attempt to identify using only morphological characteristics. Additionally, the study also revealed a new species of uniseriate filamentous *Ulva* species, *Ulva uniseriata sp. nov*., based on morphological and phylogenetic synapomorphy.

5.6 Phylogenetic analysis of foliose *Ulva*

Many taxonomists found that identification of foliose *Ulva* based on morphology was very difficult due to phenotypic plasticity. This forms the first comprehensive attempt to classify foliose *Ulva* based on multi-locus phylogeny. The study revealed existence of 22 tubular *Ulva* in Indian coasts. The study revealed existence of five foliose *Ulva* species in the coastal regions; viz., *U. fasciata*, *U. reticulata*, *U. ohnoi*, *U. linza* and *U. taeniata.* All of these species had been previously reported from India; therefore, there were no new records that this

study revealed. Comparing with tubular *Ulva*, foliose *Ulva* remains well characterized. As this study generated four phylograms, intricate patterns of with-in genus evolutionary legacies were revealed for the first time. This will aid in understanding the evolution of this genus.

5.7 Phylogeography of *Ulva fasciata*:

This study attempted to analyse phylogeography of *U. fasciata*, a foliose *Ulva* species across Indian coasts. No obvious east-west bifurcation was visible in any of the phylograms generated at three of the selected loci; viz., atpB, ITS1 and TufA. Out of these three, atpB showed some phylogeographic signal, as two accessions from Andhra Pradesh formed a monophyletic clade. This is suggestive of the advantage of this loci as a choice for future phylogeographic attempts.

Objective 4: To construct the Indian algae database: DbIndAlgae.

5.8 DbIndAlgae

All *Ulva* accessions of the newly created database, DbIndAlgae was curated as part of this study. In total 87 numbers of new entries of *Ulva* from Indian coasts were made and annotated with all information, including scanned herbarium vouchers, morphological and microscopic photographs, *in-situ* photographs, geographical location and GenBank accessions of newly generated DNA sequence data.

Conclusion and Future Perspectives

Conclusion

In the present study, total 48 isolates of various *Ulva* species from 32 different locations were studied. A new uniseriate species of genus *Ulva*, *U. uniseriata sp. nov*. was reported in this study from Diamond Harbor, West Bengal and Pulicat Lake, Andhra Pradesh for the first time. *Ulva sapora* was reported for the first time from Indian coast. All *U. sapora* samples had shown significant morphological and molecular resemblance with isolates from Australia. In the phylogenetic analysis too, isolates from Indian coasts formed a monophyletic clade with Australian isolates. Sequence data at the AtpB region for *U. sapora* was generated for the first time in this study, so as a genus-level phylogeny based on this locus. This study is the first phylogenetic and phylogeographic report of foliose algae of genus *Ulva*. In the current study, total 22 specimens of foliose *Ulva* belonging to 4 species were collected from various locations of Indian coasts. In addition, a total 26 specimens of tubular *Ulva* belonging to 7 species were also collected from various locations of Indian coasts. Morphological and molecular analyses were performed. Herbarium sheets were prepared for future references of all isolates. This study reported the presence of *U. shanxiensis* for the first time outside China. Morphological characteristics had shown significant similarity to previously reported *U. shanxiensis*. Molecular and phylogenetic analyses revealed its affinity towards *U. shanxiensis* accession from China. It was also the first report of *U. shanxiensis* from any Indian coast. So, this species should not be recognized as endemic to China. *Ulva shanxiensis* has never been reported from marine habitats anywhere in the world before this study. *Ulva fasciata* was reported from 13 and *U. reticulata* from 7 different locations while *U. linza*, *U. taeniata* and *U. ohnoi*, each from only one location. The study revealed that *U. fasciata*, *U. reticulata*, *U. taeniata* and *U. ohnoi* showed evolutionary affinity towards each other, and *Ulva linza* showed more affinity towards members of the tubular *Ulva*. Cryptic species of *U. fasciata* and *U. reticulata* were reported from Kollam, Kerala and Kanyakumari, Tamil Nadu. *Ulva fasciata* and *U. reticulata* were reported from

different locations and showed morphological variations perhaps owing to varied abiotic geographical conditions like seawater salinity, sunlight, temperature or biotic factors like different bacteria and diatoms controlling the morphology of foliose *Ulva*. Total 87 sequences were generated for different isolates using ITS1, 18S, TufA, atpB and rbcL primers.

Limitations of study

- Repeated sampling could have been done from all places to analyze the seasonal variation.
- All isolates could have been amplified and sequenced from multiple loci. In this study most of the accessions were recalcitrant such that they got amplified with only some of the selected loci. If all loci worked with all accessions, the inferences would have been more complete and meaningful.
- Ultra-structure of isolates would have helped to clarify phylogenetic patterns.
- Life cycle information would have substantially improved the study as well as inferences.

Summary

This study reported total 11 different species of genus *Ulva*. Five *U. intestinalis* (West Bengal, Kerala and Karnataka), two *U. prolifera* (Tamil Nadu), two *U. paschima* (Karnataka), five *U. sapora* (Andhra Pradesh, Maharashtra, Andaman Island), eight *U. shanxiensis* (Tamil Nadu, Gujarat, Maharashtra, Kerala), two *U. uniseriata* (West Bengal, Andhra Pradesh), twelve *U. fasciata* (Gujarat, Kerala, Andhra Pradesh, Tamil Nadu), nine *U. reticulata* (Kerala, Gujarat, Andhra Pradesh), one *U. linza* (West Bengal), one *U. ohnoi* (Tamil Nadu) and one *U. taeniata* (Gujarat). *Ulva uniseriata sp. nov*. was reported as a new species and it is clearly phylogenetically evolved as a separate entity. *Ulva shanxiensis* was reported for the first time from marine habitat in this study. Earlier, *U. shanxiensis*

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Significance of the study

1. This is a first DNA Barcoding and phylogenetic study of genus *Ulva* from Indian coast. Therefore, generated data woud be a valuable addition to the biodiversity documentation of this important *Ulva* species in India.
2. The study discovered a new species of marine and estuarine green alga, a valuable addition to the attempts of biodiversity characterization of this species.
3. The study revealed two new taxonomic records of *Ulva* species in Indian coasts.
4. The study also revealed several new epi and endophytc red algal symbionts on various *Ulva* species.
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