"Sequence-based Phylogeography and Conservation Genetics of Seaweeds from Indian Subcontinents"

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In

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BY

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Supervisor

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CERTIFICATE

I declare that the dissertation entitled “Sequence based phylogeography and conservation genetics of seaweeds from Indian subcontinents” 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

"Sequence-based Phylogeography and Conservation Genetics of Seaweeds from Indian Subcontinents"

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Phylogeography is the study of historical events which are responsible for evolution and current distribution of a species in different geographical area. However very less record is available about marine macroalgae of Indian subcontinent. This study investigated the DNA barcoding and phylogeographical distribution of marine algae from the Indian subcontinent. Different algae samples collected from various coasts of Indian subcontinent are amplified using ITS, COX and rbcL primers. In our results, we found the occurrence of green algae like *Ulva reticulata, Ulva intestinalis*, *Ulva fasciata*, *Ulva proliifera*, *Ulva ohnoi* and one sample with *Caulerpa scalpelliformis* ; Red algae, *Gracilaria foliifera*, *Gracilaria domingensis*, *Gracilaria corticata*, *Grateloupia Sp.*, *Ceramium Sp.*, *Centroceras clavulatum*, *Erythrocladia Sp.*, *Erythrocladia irregularis*, *Acanthophora Sp.*, *Dilsea socialis*, *Hypnea stelullifera*, *Sirodotia tenuissima* and *Dichotomaria Sp.*; Brown algae, *Sargassum zhangii*, *Sargassum megalocystum*, *Sargassum aquifolium* and *Turbinaria ornata* in Indian subcontinent. *Gracilaria domingenesis, Dilsea socialis, Sargassum megalosystum* were first time reported in India. On the basis of molecular studies, we found that *Ceramium* Sp. Nov., *Erythrocladia Sp. Nov., Acanthophora Sp Nov., Grateloupia Sp. Nov. and Dichotomaria Sp.* Nov. were identified as new species. *Erythrocladia irregularis* was identified as endophytic algae inside green algae *Cladophora glomerata*. Phylogenetic tree was generated to analyse the evolutionary distance between different samples. Morphological and microscopic studies were performed for each sample. This study further helps in identification and documentation of new species and cryptic species. All samples were pressed for herbarium voucher.

(POOJA RANI) (Dr. Felix Bast)

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POOJA RANI

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LIST OF ABBREVIATIONS

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.	µl	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	**Mu**ltiple **S**equence **C**omparison by **L**og-**E**xpectation

Chapter:-1

Introduction

1.1. Phylogeography:-

Phylogeography is defined 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. It is also used to find out those unique pattern or genetic regions which help in survival of organisms in a varied environment. Phylogeography combines phylogenetics and classical population genetics (Teske *et al.*, 2011). Different factors, such as migration, population bottlenecks, and biogeographical barriers, influences the distribution of the population (Avise and Wollenberg, 1997). Phylogeographic studies of widely distributed species are always an area of keen interest for evolutionary scientist. Different geographical barriers can be identified which might have led to the reproductive isolation of two populations. New species arise by establishment of reproductive barrier or isolation. Genetic differences are caused if the populations of species are geographically isolated (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 are immediately expressed in changed behaviour of gene. These may lead to the formation of multiple alleles at a given locus (Vamosi *et al.*, 2009).

Phylogeographic studies encompass the phylogenetic perspective and population genetics of a population. Phylogeography also help in defining the various mechanisms that stimulate extinction, recolonization and patterns of distribution of species. In each population there are some unique genetic patterns which are responsible for their existence but simultaneously some organisms of that population have certain alternative alleles which help in their survival in varied geographical region (Emerson *et al.*, 2011). The population is said to be continuously distributed if it has no genetic barrier. Even in presence of biogeographical barriers, size of the population and its dispersal potential throughout the geographical area are strong determinants of phylogeographic distribution of species (Manel *et al.*, 2003). Migration can be regarded as gene flow between the populations of a species.

Nevertheless, the dispersal of a population may be limited due to restricted gene flow. Transition zone between two populations have been related to historical partitions (Thorpe, 1987). Genetic drift also promotes the evolutionary process by species divergence but effect of inbreeding contributes toward increased sudden extinction rate of the population (Montecinos *et al.*, 2012). Some abiotic stresses are also believed to be major factors for diversity of species that evolve from common ancestors. Natural selection or selection pressure is the driving force for the evolution and species divergence. Natural selection favours such genes that ensure the highest level of adaptive efficiency in the new geographical location. Phylogeography highlights the influence of historical events, climatic conditions and various ecological factors which are responsible for current distribution of species (Byron, 2005). Mainly three theories are famous for modern phylogeography i.e. Cladistics thoughts, Plate tectonics and Vicariance biogeography. Various phylogeographic barriers ultimately culminate in reproductive isolation and parapatric speciation (Crandall *et al.*, 2000). Phylogeographic studies help in retracing the past as well as the present state of unequally distributed population of an organism within same or different geographical area.

1.2. Conservation genetics

Genetic methods employed for profiling, restoration and conservation of the deteriorating biodiversity are known as conservation genetics. Genetic diversity is the most important factor in conservation of species and ecosystem diversity. More the genetic variation in a species, greater is the diversity in that species. Small populations are more influenced with high genetic diversity (Latta, 2008). Few individuals in each population have specific genetic makeup that allows them to survive (Toonen *et al.*, 2011). If the genetic diversity is very low, whole population is in the brink of collapsing during unfavourable environmental conditions, irrespective of the variants surviving in the next generation that are capable of contributing to species diversity. Once gene variants are lost (gene death), they cannot be recovered. Environmental factors also play an important role for generating variability of genes in the population (Carstens and Knowles, 2007). If a population can adapt to the changing environmental conditions it has more chances to survive and grow provided it has certain alternative alleles (Manel *et al.*, 2003). Different techniques are employed for profiling conservation status in population, including RFLP, AFLP,

RAPD, SNP, Sequence analysis and fingerprinting Genetic signatures like motifs regions and conserved domains are also widely employed in conservation genetics. At molecular level, these markers have a momentous role in survival of organisms. For preserving a population, exchange of genetically variable region is required in reproductively isolated population.

In the present study, morphometric and molecular analysis of marine macro-algae collected from the coasts of Indian subcontinent was attempted with an objective to aid in its phylogeographic and conservation genetic assessment, which are entirely non-existent in the literature. Geographical location targeted in this treatise included Indian West Coast, spanning from Anjuna, Goa to Ponnani, Kerala, Indian East-Coast, spanning Ennore, Tamil Nadu to Mandapam, Tamil Nadu, and Havelock Island, Andaman and Nicobar Island.

Chapter:-2

Review of literature

2.1. Climate conditions of Indian Ocean

Lack of human, institutional and technical capabilities in some Indian Ocean countries can be held responsible for heterogeneous level of growth in understanding the biodiversity of the Indian Ocean. Habitat loss, uncontrolled developmental activities in the coastal zone, over extraction of resources and coastal pollution are serious constraints on maintenance of highly diverse biota. It is more prominent in those countries where environmental regulations are weak. Indian Ocean can be divided into two regions i.e. northern and southern regional sea. Water exchange between the Indian Ocean and Atlantic Ocean occurs at the south-western tip. The differential heating of the land mass and sea gives rise to a wind circulation that reverses direction of water current and corresponding reversal in surface circulation, twice a year. This monsoon effect has a significant bearing on climatology of the northern Indian Ocean, in turn affecting the biological productivity and agricultural economy of the regional countries (Keesing and Irvine, 2005). The Indian coastal seas receive million tons of domestic sewage and industrial waste. It has great impact on the surface salinity of the whole of Bay of BengalThe second is the seasonal reversal of currents in response to changes in monsoon seasons from southwest to northeast. This reversal in not purely wind-driven, but it occurs up to depths greater than 750 m. This reversal occurs seasonally at variable rates in nutrient rich subsurface waters of Arabian Sea and Bay of Bengal (Qasim, 1977). Among the Indian Ocean countries, India is notable for large number of oceangoing research vessels, large scientific and technical manpower, capabilities for using advanced technologies and capacity for exploring deep seas and southern parts of Indian Ocean (Wafar *et al.*, 2011).

2.2. Algae

Algae are photosynthetic plants that consist of heterogeneous complex of oxygenic photosynthesizers other than embryophyte plants. These plants grow on soil, rocks and water, marine, estuarine or limnetic. According to six kingdom classification of life on earth, algae are parts of three kingdoms; viz. Protozoa, Chromista and Plantae. Algae are highly diverse in nature and are divided into nine major lineages, which include Chlorophyta, Rhodophyta, Glaucophyta, Cryptophyta, Dinoflagellates, Heterophyta, Euglenophyta, Chlorarachniophyta and Haptophyta. The latter four groups are collectively known as Chromophyte algae; they contain

xanthophylls which give brown or yellow colour to the algae (Cavalier-Smith, 2004). According to previous studies available, approximately 200,000 species of algae are believed to be present throughout the world. Out of these only 36,000 species are already identified (Radmer, 1996). The Green algal lineage (Chlorophyta) has approximately 16,000 recognized existing species and up to 100,000 species that remain to be identified. The Red algal (Rhodophyta) lineage has approximately 5,000 recognised species but 15,000 species remain to be described (Bhattacharya and Medlin, 1998). On the basis of habitat, these algal species are broadly divided into two categories i.e. terrestrial and marine algae. Marine macroscopic algae are also known as Seaweeds. These plants live attached to rocks or other hard substratum like shells, stones or pebbles. Some Seaweeds, known in algal literature as “drift-type seaweeds”, are free-floating in marine water after maturation (De Clerck *et al.*, 2012). According to previous documentation, about 13,248 Seaweed species are recognized in world’s oceans. Seaweeds are broadly divided into three major categories i.e. Red (Rhodophyta), Green (Chlorophyta) and Brown (Phaeophyceae) which comprise of 6000 species, 18,000 species and 15,000 species, respectively. They are categorized on the basis of their colour and pigmentation. In each algae, different types of chlorophylls are recognised. Green and Red algae originated via primary endosymbiosis while Brown algae evolved secondary endosymbiosis. As a result, they are now classified under different kingdoms; Red and Green algae are described under kingdom plantae, while Brown algae under kingdom Chromista. Some marine algae grows in endophytic (inside of algae) or epiphytic symbiotic association (Felix Bast, 2014).

2.3. Marine algal species status in India

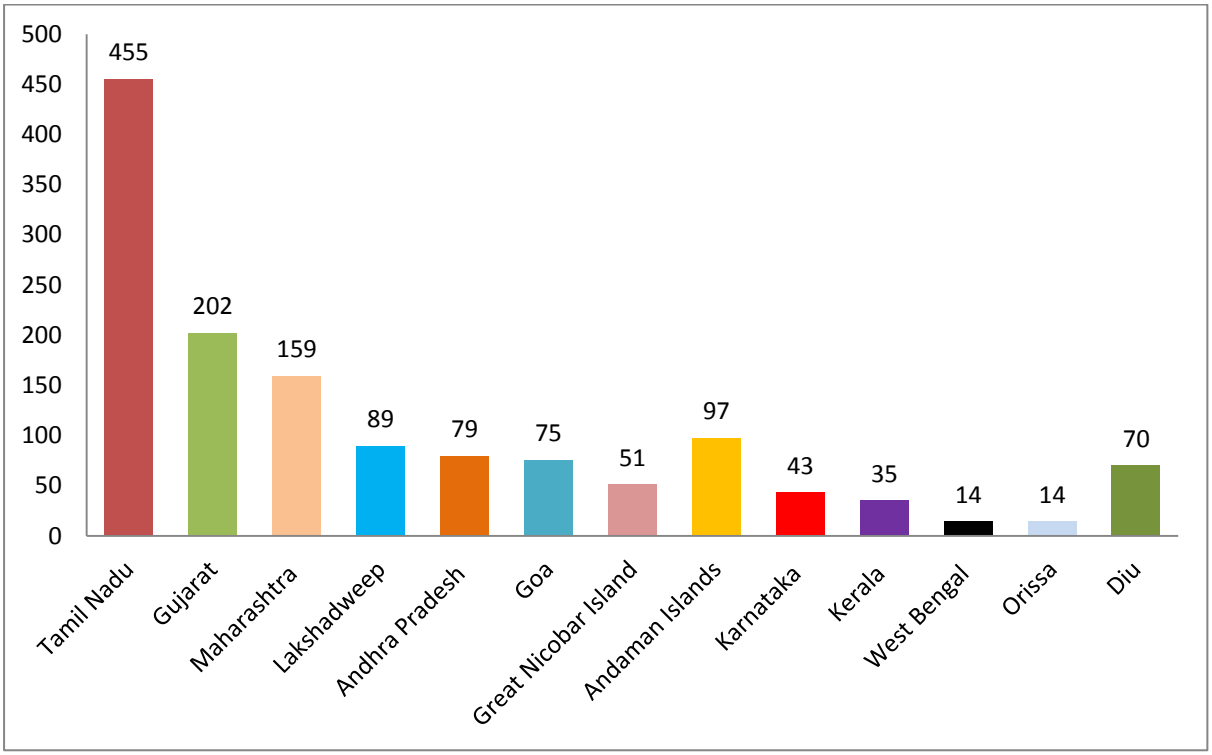

Figure 1. State wise Seaweed diversity in India (P. V. S. Rao and Mantri, 2006c)

India boasts of a coastline of more than 7,000 km (J. Rath and Adhikary, 2006b) harbouring 1153 species distributed across 217 genera, 19 families and 7 orders. Rhodophyta is the most abundant species consisting of 434 species, followed by Chlorophyta and Phaeophyta 216 and 191 species respectively. Highest diversity of species has been recorded from Tamil Nadu (455 species) and the lowest from west Bengal and Orissa (14 species) as illustrated in Table-1 (P. V. S. Rao and Mantri, 2006c; Venkataraman and Wafar, 2005). The scanty data in other maritime states like Kerala does not mean that they lack macroalgal diversity but there is a need of extensive and proper Seaweed documentation in such states.

2.3.1. Previous recorded marine algae data from Indian subcontinent

Different locations of Indian coast were explored, but there were very few records available related to identification of marine algae from Indian Ocean. Among the Chlorophyta, genus *Ulva* was maximum reported in India. Different identified *Ulva* species are *Ulva lactuca*, *Ulva fasciata*, *Ulva intestinalis*, *Ulva compressa*, *Ulva clathrata*, *Ulva flexuosa*, *Ulva rigida* and *Ulva reticulata* (Joshi and Krishnamurthy,

1972; Kaladharan *et al.*, 2011; Shivakumar and Ulhas, 2012). Dhargalkar and Deshmukhe, 1996, revealed that Red algae were dominant in Dwarka coast of Gujarat. They found 20 Red, 8 Green and 7 Brown sub-tidal marine algal species during their study (Dhargalkar and Deshmukhe, 1996). Sahoo *et al*. recorded 8 species of Chlorophyceae and 6 species of Rhodophyceae from different parts of Chilka lake of Orissa (Sahoo *et al.*, 2003). They found the abundance of genus *Ulva, Gracilaria*, *Ceramium, Grateloupia* and *Polysiphonia*. A study carried out by Rath and Adhikary (Jnanendra Rath and Adhikary, 2006a) along the entire coasts of Orissa revealed 9 species of Chlorophyta, 2 Phaeophyta and 10 Rhodophyta. During a preliminary study on marine macro algae from coastal regions of Goa total 145 species were identified which comprises of 64 Red, 41 Green and 40 Brown algae. Major growing algae belong to genus *Porphyra, Sargassum, Gracilaria, Grateloupia, Hypnea, Acanthophora, Centroceras* and *Ceramium* (Pereira and Almeida). According to a report of CMFRI in 1987, 73 Seaweeds species were recorded from Malvan coast of Maharashtra. Different species of *Caulerpa, Padina, Sargassum* and *Gelidiopsis* genus were identified at different zone of coastal regions (Rodrigues *et al.*, 2011). Their distribution varied from season to season because of reversal of water current. A study carried out by Kaladharan *et al*., 2011, investigated 78 seaweeds in Western Ghats of Karnataka coast that were described in 52 genera and 28 families. It is found that there is abundance of different species of genus *Ulva, Caulerpa, Sargassum, Dictyota, Padina, Hypnea* and *Gelidium* (Kaladharan et al., 2011). One another study was performed by Krishnamurthy and Kaladharan along the entire coast of Kerala. From Poovar to Thirumallavaram, in between the ranges of 80 km. , there were total 24 species of different alga were identified, out of which *Ulva lactuca, Sargassum wighatii, Gracilaria corticata* and *Hypnea valentine* were identified. From Thirumallavaram to Paraparagadi, 8 species were identified where U*lva lactuca, Enteromorpha compressa, Chaetomorpha antennina* and *Grateloupia filicina* were dominant. From Paraparagadi to Cannanore, 27 species were identified. It was recognised as a species rich area for algae growth along the Kerala coast. Here *Caulerpa sertularioides, Gracilaria corticata, Acanthophora specifera* and *Gelidium pusillum* were grown as dominant species. From Cannanore to Manjeshwar *Ulva lactuca, Chaetomorpha antennia* and *Gracilaria corticata* occur dominantly (Chennubhotla *et al.*, 1988). According to Kaladharan *et al*., approximately 114 species from 62 genera were identified from the coasts of 12

islands of Lakshadweep. It was found that 43 species belongs to Chlorophyceae, 14 to Phaeophyceae, 52 to Rhodophyceae and 3 to Cyanophyceae (Kaliaperumal *et al.*, 1989). According to a report of Thakur *et. al*., from Port Okha coast 62 species were identified, where 26 species of Red, 22 species of Green and 14 species of Brown algae occur (Thakur *et al.*, 2008). There were 80 algae species found from coasts of Andhra Pradesh of which 43 Rhodophytes. *Gelidium* and *Gracilaria* occur most abundantly on Visakhapatnam coasts. In another finding, total 31 marine samples were collected from entire coast of Visakhapatnam. One another report revealed 39 marine algal species from Bhimili coasts of Andhra Pradesh that is 22 km away from Visakhapatnam. According to previous records of Tamil Nadu was thought to be a hub for algal products resources. So it was always first choice for the phychologists. A survey from 1986-1991 of deep sea water from Dhanushkodi to Kanyakumari revealed a total 100 algal species of which 20 belongs to Chlorophyta, 18 belongs to Phaeophyta, 61 to Rhodophyta and 1 species to Cyanophyta. In this area maximum *Sargassum, Gracilaria* and *Hypnea* species were found (Kaliaperumal *et al.*, 1998). One another study from Kilakkarai to Dhanushkodi revealed 29 species of which 8 belonged to Brown, 8 to Green , 12 to Red and 1 to Blue-green algae (K. R. Rao *et al.*, 1993). A study from Kundakulam coasts of Tamil Nadu revealed 32 taxa of which 15 belonged to Chlorophyta, 8 of Phaeophyta and 9 of Rhodophyta. *Ulva fasciata, Ulva compressa, Acanthophora specifera, Caulerpa sertularioides* and *Sargassum wighatii* were found to be most abundant (Satheesh and Wesley, 2012). According to the study of Seaweed biologists from Diu islands, there were 70 species have been identified and 397 species still need to be identified. Most commonly occurred species are *Ulva, Caulerpa, Dictyota* and *Gracilaria (*Mantri and Rao, 2005*).* A report of CMFRI revealed 55 species from Andaman Nicobar Islands of which 16 species belongs to Green algae, 17 species to Brown and 22 species to Red algae. These islands have a domination of species like *Turbinaria, Sargassum, Padina, Gracilaria* and *Gelidium*. In another study 81 Seaweeds were recorded 26 taxa were Chlorophyceae, 19 taxa were Phaeophyceae, 32 taxa were Rhodophyceae and 4 taxa were Cyanophyceae. Both of these studies revealed that, in these islands Red algae was more diverse than other algae (Palanisamy, 2012).

2.4. Socio-economic status of marine macroalgae industries in India

Globally 7.5 to 8.0 million tons of wet Seaweed are harvested annually with an estimated market of US$ 5.5 - 6 billion per year. India has the potential of producing 77,000 tons (wet weight) of macro-algae per annum. Algae is mainly used to get raw material for the extraction of phycocolloids (alginates, agar and carrageenan) which are being used in the pharmaceutical, cosmetic and food industries. At least 145 species of macro-algae used worldwide as a food. Farming of edible Seaweeds can be a sustainable alternative to the conventional agriculture; hence there must be the proper documentation of Seaweeds.

Commercially important Seaweeds imported, exported and ~~also~~ cultivated in India for various industrial purposes. All 271 genera and 1153 species of marine algae documented till date from Indian waters. Seaweeds do not grow for the whole year (perennial) (Krishnamurthy, 2005; P. S. Rao and Mantri, 2006a), but only a fixed time duration in which they grow and mature. So fixed time duration should be recommended to harvest the standing crops by Govt. Authorities so that all species do not get destroyed to prevent damage. Some species are over used year after year for industrial purposes that ultimately result in deterioration of their natural habitat. This procedure is not employed anymore for the batch harvesting of crops and presently harvesting is done only at appropriate time and growth stage of Seaweed for its conservation (Muthuvelan *et al.*, 2001). About 5000 women of southeast India are dependent on Seaweed related activities for their livelihood. If all Seaweeds harvested at appropriate time, another 20000 people could get employment (P. S. Rao and Mantri, 2006b) **.**

2.5. Biochemical composition of marine macroalgae

The Seaweeds show high variation in nutrient contents which are related to different environmental factors like water, temperature, salinity, light and nutrients. Most of these environmental parameters change according to season. These changes in ecological conditions can inhibit or stimulate the biosynthesis of nutrients and secondary metabolites. Nutrient composition of Seaweeds varies from species to species and is affected by geographic area and temperature of water. Chemical composition of Seaweeds is poorly investigated, and most of the available information is based on traditional Japanese Seaweeds (Nisizawa *et al.*, 1987). In

some cases the mineral contents of Seaweeds is recorded even higher than land plants (Ito and Hori, 1989). Studies on biochemical constituents such as protein, carbohydrates and lipid in Brown and Green marine algae have been carried out from different locations of Indian coast. Previously, Red alga was analysed for nutrient components of species from Gujarat coastal regions. Samples collected from Mandapam have reportedly shown maximum content of mineral and chemical composition (Karthikaidevi *et al.*, 2010).

2.6. DNA barcoding and its importance in phylogeographic studies of marine macroalgae

Increasing population has encouraged farming of Seaweeds as a sustainable alternative to the traditional agriculture as they can harvest for the production of food, feed, chemicals, cosmetics and pharmaceutical products. There has been a massive loss of valuable species in the past centuries. Its adverse impact on environmental and socioeconomic status has triggered the conservation of genetic resources. In order to conserve Seaweed resources and to ensure their viable use, their proper identification, characterization and documentation is essential. For the studies related to the phylogeographic distribution of macroalgae, first a proper documentation of species' identification is required.

Macroalgal species identification is very difficult even for an experienced systematist because they possess simple morphology, anatomy, convergence, remarkable degrees of phenotypic expression in response to environmental factors, and incomplete understanding of life histories with alternation of heteromorphic generations (Saunders, 2008). In algal systematics, molecular tools like DNA barcoding are used for fast resolution and identification of species (Harper and Saunders, 2001). In morpho-metrics discrete morphology and anatomical loci are employed for identification of species that are arguably homologous in all individuals of a particular group or species (F. Bast *et al.*, 2009). It is also helpful in analysing fossil records, to check the influence of mutations on morphology of the organism, developmental changes of the organism and covariance between different ecological factors (Angielczyk and Feldman, 2013). It is also helpful for estimating quantitative-genetic parameters of the expression and can be used to identify the feature of

evolutionary importance. Ontology and phylogeny of organisms can be studied by these changes in morphology and expression.

DNA barcoding is molecular sequencing methodology which uses a standardized DNA region for rapid and accurate species identification (Saunders and Virginia, 2005; Valentini *et al.*, 2009). It can be regarded as a famous tool used by taxonomists that help in quick identification of unknown specimens and discovery of new species. It has also solved the various confusing issues related to identification of cryptic species, microscopic and other organisms with complex or similar morphology (Frezal and Leblois, 2008; P. D. N. Hebert *et al.*, 2003a). Now days it is a tool not only used by specialists, but also by the non-specialists for detection and identification of pathogenic species with environmental, medical and agronomical significance (Frezal and Leblois, 2008). The ideal DNA barcoding system should be variable, standardized and phylogenetically informative so that it will help in easy assignation of unknown species to their taxonomic groups. Based on the robust definition of species, barcoding procedure properly needs to be able to distinguish between new haplotypes of known species. It also helps in recognising those species which are unknown to the database (Rubinoff *et al.*, 2006). Species discovered by barcoding required allocation to proper genera and families. The discovery of cryptic species is exciting because they are immediately relevant to other previously known species' and genera. Barcodes from the predetermined species identifications are used to determine the mean within and between the species' genetic distances. It is also used to find out genetic variations between the species (Rubinoff *et al.*, 2006).

2.6.1. Mitochondrial genome

As evident in several reports, universal genetic markers have been used for the marine phylogeographic studies of different taxa. Cytochrome oxidase c subunit –I gene is particularly used for the mtDNA sequence data, but it has certain limitations such as being inherited in the female line and is unsuitable for the study of hybridization or reproductive isolation amongst different genetic lineages (Felsenstein, 2006). Molecular dating based on a single marker is less accurate than the dating based on the multilocus data (Teske *et al.*, 2011). In barcoding system, difference in molecular clock rates between standardized species and closely related

species could be identified by using single mitochondrial gene under specific conditions. DNA identification has useful significance in forensics, quarantine and life cycle studies. However, researchers of DNA barcoding are regularly breaking the line between identification of known species and new species (P. D. Hebert *et al.*, 2003). DNA barcoding proves to be advantageous tool in identifying species of which we know only by morphological features. It also reveals those that are un-described and identified cryptic species. So cryptic species identifications are dependent upon understanding of pre-existing species in those groups based on other sources of data (Hajibabaei *et al.*, 2006; Rubinoff *et al.*, 2006).

mtDNA is maternally inherited and usually have single copy genome. It has one fourth the effective population size of the nuclear genome and a different inheritance pattern. Several relationships derived from nuclear and mitochondrial genome, even though contradicting, should not be ignored when identifying species and clades (Rubinoff and Holland, 2005). mtDNA's more fast lineage sorting capacity can provide information about ancestral level community relationships, but some additional sources of information are also needed to understand species' level patterns (Patton and Smith, 1994). The entire role of maternal inheritance in molecular divergence is not predictable (Korpelainen, 2004)**.** Barcode is not enough to justify major conservation efforts for recognised new cryptic taxa, which are genetically diverged, but does not show differences in morphology and ecology (Will *et al.*, 2005). Barcode is no substitute for full taxonomic analysis because the combination of detailed morphological, ecological investigations with barcode results is critical for the final documentation of species' richness (Hajibabaei *et al.*, 2006). Barcoding also fails to distinguish between members of closely related species groups and morphologically highly similar species (Rubinoff *et al.*, 2006).

2.6.2. Chloroplast genome

The gene for the large subunit of the ribulose-bisphosphate carboxylase/oxygenase (*rbcL*), is located on the chloroplast genome and approximately 1430 base pairs in length. It is first choice for testing its utility as a DNA barcode in marine macroalgae as it has established among plant groups (Saunders and Kucera, 2010a). *RbcL* encodes the large subunit of ribulose-1-5-bisphosphate carboxylase/oxygenase. Ribulose-1-5-bisphosphate carboxylase

/oxygenase facilitate the primary CO_2 fixation step in the photosynthesis. The quaternary structure of the enzyme consists of eight large and eight small subunits. In Green algae and land plants, large subunits are encoded by the plastid gene *rbcL* whereas the small subunit encoded by the nuclear gene *rbcS*. *rbcL* gene most commonly used for the investigation of phylogenetic relationships in Rhodophyta. The *rbcL* barcode consists of a 599 bp region at 5' end of the gene located at bp 1-599 in the complete *Arabidopsis thaliana* plastid genome (Freshwater *et al.*, 1994)(Newmaster *et al.*, 2006). It has a conservative rate of evolution and has also formed the basis of many taxonomic and phylogenetic studies in genus *Ulva* and many other marine Green macroalgae (Hayden and Waaland, 2004). The versatility of *rbc*L as a barcode marker has been adversely affected by the presence of introns in some marine macroalgae because the ability to amplify and sequence large fragments with a single bidirectional read is very difficult. Its ability to resolve phylogenetic relationships below the family level is often poor (Clarkston and Saunders, 2010).

2.6.3. Nuclear genome

Nuclear Ribosomal DNA (nrDNA) is a selection of supplementary barcode in groups for which direct sequencing is possible (Thomas, 2009). The number of nrDNA genes within eukaryotes ranges between 40 and several thousand (Li, 1983). Each nrDNA consists of 18S,5.8S and 28SrRNA genes which are separated by ITS1 and ITS2 and are flanked by ETS (External Transcribed spacer) to form rDNA operons that exist in tandem arrays separated by NTS (non-transcribed spacer regions) (Long and Dawid, 1980). Starting from 5' end this polycistronic rRNA precursor transcript contains the 5' external transcribed sequence (5' ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and finally the 3'ETS (Stat *et al.*, 2006). Reports have suggested that they evolve quickly and can be used for the intraspecific variation and biogeography in algae (Leskinen and Pamilo, 1997).

At species' level, the nuclear internal transcribed spacer (ITS) region is the most commonly used locus for plant molecular systematic investigations (Alvarez and Wendel, 2003). The widespread use of ITS region as a DNA barcode in plant molecular systematics is due to four main reasons. ITS region include availability of various sets of universal PCR primers which can work with the large number of taxonomic groups (Hughes *et al.*, 2006), the high copy number of ITS regions makes

it easy to amplify even from small quantities of DNA (Bellemain *et al.*, 2010). ITS has moderate size usually below 700bp which makes amplification and sequencing easy (Gernandt *et al.*, 2001) and has a high degree of variation even between closely related species (Baldwin *et al.*, 1995; Feliner *et al.*, 2001). ITS frequently provide enough molecular markers suitable for evolutionary studies at the species' level (Feliner and Rossello, 2007). In fungi, it has been recently proposed as the official primary bar-coding marker (Bellemain *et al.*, 2010). Studies have suggested that ITS sequence can resolve closely related species with the same conformity as mtDNA COI-5P (Hu *et al.*, 2009).

Table:-1. List of primers worldwide used for previous algal DNA Barcoding study

Sr. No.	Target region	Locus	Primer F	Primer R	Reference
1.	Nuclear	18S(SSU)	AGTTTTAGTGTA TTTGATGATCG	TCGAAAGCTGATAGGTCAGAATC	(Oldach *et al.*, 2000)
2.	Nuclear	ITS1-5.8S-ITS2	GAGGCAATAACAGGTCTGTGATGC (ITS1)	TCCTCCGCTTATTGATATGC (ITS4)	(White et al.,1997)
3.	Nuclear	28S(LSU)	TTGAAACACGGACCAAGGAG	ACTTCGGAGGG AACCAGCTA	(Marande *et al.*, 2009)
4.	Chloroplast	rbcL	ATGTCACCACAAACAGAAACTAAAGC	TATCCATTGCTGGGAATTCAAATTTG	(Saunders and Kucera, 2010b)
5.	Chloroplast	trnL-F	CGAAATCGGTAGACGCTACG	ATTTGAACTGGTGACACGAG	(Taberlet *et al.*, 1991)
6.	Chloroplast	RUBISCO-spacer	TGTGGACCTCTACAAACAGC	CCCATAATTCCCAGTA	(Robba *et al.*, 2006)
7.	Chloroplast	tufA	TGAAACAGAAMAWCGTCATT ATGC	CCTTCNCGAATMGCRAAW CGC	(Fama *et al.*, 2002)
8.	Chloroplast	trnH-psbA	CGCGCATGGTGGATTCACAATCC	GTTATGCATGAACGTAATGCTC	(Buchheim *et al.*, 2011)
9.	Chloroplast	Matk/trnK	GGG TTGCTAACTCAATGGTAGAG	GAA CCCGGAACTHGTCGGAT	(Wicke and Quandt, 2009)
10.	Chloroplast	rpoC1	GGCAAAGAGGGAAGATTTCG	CCATAAGCATATCTTGAGTTGG	(Buchheim *et al.*, 2011)
11.	Chloroplast	rps1b	GGAAGTTTSATTTTCCCAGATG	CATTCTGGGAAAATSAAACTTCC	(Graham and Olmstead, 2000)
12.	Mitochondrial	COX2-COX3	GTACCWTCTTTDRGRRKDAAATGTGATGC	GGATCTACWAGATGRAAWGGATGTC	(Saunders, 2005)
13.	Mitochondrial	COX1	TCAACAAATCATAAAGATATTGG	ACTTCTGGATGTCCAAAAAAYCA	(Saunders, 2005)

Chapter:-3

Material and method

3.1. Sample collection

Algal samples collected from many locations across the coastal regions of India in a diving exploration performed by Felix Bast. Geographical coordinates of sample collection sites described in Table-2 and map of sampling sites was shown in Appendix-D. Collected specimens were transported to the laboratory under cold conditions (4-10˚C). Samples were washed thoroughly with tap water to remove sediments and other contaminants. It followed by morphological characterization of the specimen. Different books referred as taxonomic ID keys for identification of species. Then, a part of thallus of each sample was analysed using an upright microscope (BX53, Olympus, Japan) for the study of pattern of cell arrangement in surface view. Photographs were taken using an attached digital camera (E450, Olympus, Japan) (Berges et al., 2001). Then pressed vouchers were prepared. Public domain software ImageJ (http://rsbweb.nih.gov/ij/) used for scale calibration and size measurements. Later samples for molecular analyses stored at -80˚C till further analysis.

Table-2:-Location coordinates of sample collection sites

SR.NO	Sample ID	Thallus Colour	Location	Co-ordinates
1.	ETT-2	Green	Ettikkulam (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
2.	ETT-4	Red	Ettikulam (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
3	ETT-5	Red	Ettikkulam (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
4.	KAN-6.2	Red	Kannur (Kerala)	11°52'57"N ,75°20'13"E
5.	KAN-6.3	Green	Kannur (Kerala)	11°52'57"N ,75°20'13"E
6.	KAN-6.4	Green	Kannur (Kerala)	11°52'57"N, 75°20'13"E
7.	PON-7	Green	Ponnani (Kerala)	10° 46′ 12″ N, 75° 54′ 0″ E
8.	CAL-10	Green	Calicut (Kerala)	11° 15′ 0″ N, 75° 46′ 12″ E
9.	KER-11	Green	Elathur (Kerala)	10°35'58"N 76°4'46"E
10.	MAN-14.1	Green	Manglore (Karnataka)	12°52'55"N, 74°50'22"E

11.	BEK-15.2	Red	Bekal (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
12.	ANJ-19	Red	Anjuna (Goa)	15°35′00″N 73°44′00″E
13.	MDP-13B	Brown	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
14.	MDP-13C	Brown	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
15.	MDP-13D	Green	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
16.	MDP-13E	Red	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
17.	MDP-13F	Red	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
18.	MDP-13I	Red	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
19.	MDP-13M	Green	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
20.	MDP-13N	Brown	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
21.	MDP-13O	Brown	Mandapam (Tamil Nadu)	9° 16′ 48″ N, 79° 7′ 12″ E
22.	KOV-T-1.1	Green	Kovalam (Tamil Nadu)	8° 18′ 0″ N, 77° 12′ 0″ E
23.	ENN-T-8	Green	Ennore (Tamil Nadu)	13° 13′ 3″ N, 80° 19′ 17.58″ E
24.	BEK-23.1	Brown	Bekal (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
25.	BEK-23.4	Red	Bekal (Kerala)	12° 22′ 0.12″ N, 75° 3′ 0″ E
26.	HAV-25	Red	Havelock (Andaman islands)	11° 58′ 0″ N, 93° 0′ 0″ E
27.	HAV-26	Red	Havelock (Andaman islands)	11° 58′ 0″ N, 93° 0′ 0″ E
28.	ENN-T-7.2	Green	Ennore (Tamil Nadu)	13° 13′ 3″ N, 80° 19′ 17.58″ E
29.	POD-T-4	Green	Pondycherry (Tamil Nadu)	13° 2′ 25.55″ N, 80° 14′ 23.89″ E

3.2. DNA extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA was extracted from the frozen algal specimens using HiPurA™ Algal Genomic Extraction Kit (HiMedia Laboratories Pvt. Ltd., Mumbai) according to

manufacturer's protocol. Tissues from the apical part of thalli were selected to increase DNA yield. Some of the samples were crushed in liquid nitrogen and some with the help of silica gel. DNA was resuspended in elution buffer and quantified by absorbence at 260/280 nm using thermoscientific Nano Drop spectrophotometer (Thermo Scientific Nano Drop 2000). The DNA from both procedures was equally good. Then 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, TTP, dCTP and dGTP (Applied Biosystems, Foster City, CA, USA), 1 unit of rTaq® DNA polymerase (Applied Biosystems, Foster City, CA, USA) and sterile water. Primer used for amplifying nrDNA ITS1, ITS2, COX1, COX2, COX3 (Saunders, 2005) and *rbcL* (Zechman, 2003) regions (Imperial Life Sciences, India) are listed in Table-3. Reactions also contained 5% DMSO (Merk Specialties Pvt. Ltd. Mumbai) or $Mgcl_2$. 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 94˚C for 1 minutes, 50˚ - 54˚C for 2 minutes and 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 for 30min at 100V and visualized with Ethidium Bromide in order to determine approximate length and purity.

Table-3.Sequences of primer used for amplification of genomic DNA in present study.

Sr.no.	**Primer name**	**Targeted organelle's genome**	**Nucleotide sequence of primers**
1.	ITS1	Nuclear	GAGGCAATAACAGGTCTGTGATGC
2.	ITS2	Nuclear	GCTGCGTTCTTCATCGATGC
3.	ITS3	Nuclear	GCATCGATGAAGAACGCAGC
4.	ITS4	Nuclear	TCCTCCGCTTATTGATATGC
5.	COX1gaz F	Mitochondria	TCAACAAATCATAAAGATATTGG
6.	COX1gaz R	Mitochondria	ACTTCTGGATGTCCAAAAAAYCA
7.	COX F	Mitochondria	GTACCWTCTTTDRGRRKDAAATGTGATGC

8.	COX R	Mitochondria	GGATCTACWAGATGRAAWGGATGTC
9.	*rbcL* F	Chloroplast	ATGTCACCACAAACAGAAACTAAAGC
10.	*rbcL* R	Chloroplast	TATCCATTGCTGGGAATTCAAATTTG

3.3. DNA sequencing template preparation and DNA sequencing

Reactions that got amplified 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 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 fidelity of Taq polymerase. Also, gradient PCR was done to ascertain the proper annealing temperature for the different DNA samples.

Purified PCR products were sequenced using a dideoxy chain 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 were used to amplify both strands (i.e., one with forward primer and the other with reverse primer). In order to eliminate unincorporated dye terminators, SDS (0.2% final concentration) was added to the cycle sequencing reaction products and heat treated at 98˚C for 5 minutes, followed by 25˚C for 10 minutes. Reactions were then purified by Centri-Sep® spin column (Applied Biosystems, Foster City, CA, USA). Purified extension products were vacuum dried and over drying was avoided. Then DNA sequencing was performed (Applied Biosystems 3730xl Genetic Analyzer, Foster City, CA, USA).

The sequence data was then analysed and assembled using licensed computer software CodonCodeAligner (CodonCode Corporation, USA). Then BLASTN (www.blast.ncbi.nlm.nih.gov) was used for sequence homology.

3.4. Multiple alignment and phylogenetic analysis

Multiple sequence alignment was carried out using all the sequences generated by DNA sequencing from all the identified samples. Sequences were first aligned by MUSCLE algorithm that was done using MEGA, a freeware (available at www.megasoftware.net/) and alignments were edited by eye. The ends of aligned sequences were trimmed to minimize the number of missing sites across taxa.

The best-fitting nucleotide substitution models were tested using ML ModelTest in MEGA. Then, Pairwise distances between sequences of all the samples were calculated using nucleotide substitution test model in MEGA. Positions containing gaps and missing data were eliminated only in pairwise sequence comparison. Phylogenetic analysis using Maximum likelihood (ML) was conducted using MEGA software (Ronquist and Huelsenbeck, 2003). For all sample sets, analyses were run with four Markov chains for 106 generations with a tree saved every 100th generation. First 1000 trees were discarded as burn-in. Different Consensus trees were then constructed using MEGA for Red, Green and Brown algae. Analysis by maximum likelihood (ML) algorithm was conducted using PhyML plug-in v2.4.5 (Guindon and Gascuel, 2003) inside MEGA. Substitution bias was modelled by the Tamura-3-parameter, Jukes-Cantor 69 model (Jukes et al., 1969), Hasegawa-Kishino-Yano model (Hasegawa et al., 1985), General time reversible model etc. for different sample sets in accordance with the results obtained after ML Model Test in MEGA. A total of 1000 bootstrap replicates were performed under ML criterion to estimate interior branch support (Felix Bast, 2013; Felsenstein, 1985).Phylogenetic diversity (PD) of each was measured using cm scale.

Simpson's diversity index was used to calculate species diversity i.e.

$$\textbf{Simpson's index= SI= } \sum \mathbf{n(n-1)/N(N-1)}$$

$$\textbf{Simpson's diversity index= D=1-SI}$$

where n= number of individuals of a species

N= Total number of species in community.

Shannon Wiener index was used to calculate species evenness.

$$\mathbf{H' = -\sum Pi/\mathit{n}Pi}$$

where Pi= the proportion of individuals of species i.

Chapter:-4

RESULTS AND DISCUSSION

4.1. Results

There were total twenty nine samples, out of which thirteen Green, twelve Red and four Brown algae found. After sequencing, Total 23 contigs were formed out of 48 sequence pairs from 29 samples using different primers. Other sequences did not form contigs, but their single strand shows sequence homology to the specific algae. Details of all samples i.e. sequence, homology percentage, identity and collection site of sample after BLASTN explained one by one. Some results were very surprising. Morphologically they were identified as Green algae but after DNA sequencing they show similarity with Red algae. So there were some endophytic Red algae present in those samples. Some samples first time reported in India. Few samples identified as new species. Morphological and microscopic pattern of cells was also studied using micro and macro-photographs.

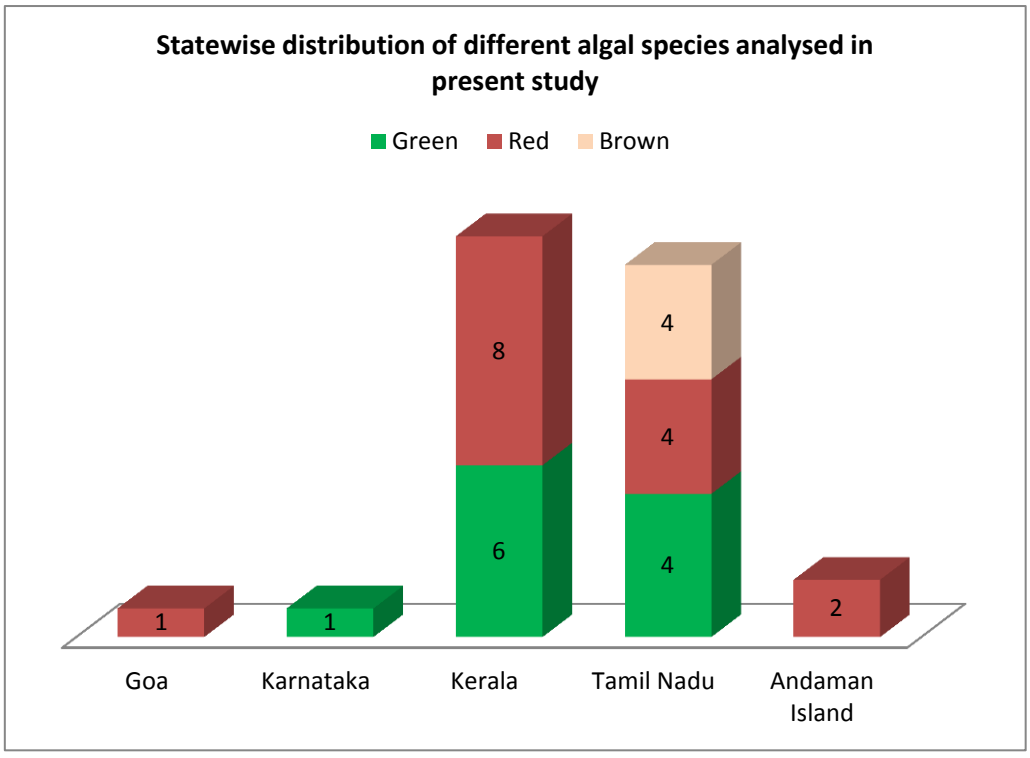

Figure 2. State wise distribution of different algal species analysed in present study

1. Name: *Ulva reticulata* Forsskal

Location: Ettikulam (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: ETT-2

CUP Voucher ID: CUPVOUCHER-ETT-2014-UR-1

Central National Hebarium Voucher ID: CAL-CUPVOUCHER-ETT-2014-UR-1

Classification:-

Class: Cholorophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Perforated leafy thallus and it attached to rocks. It is generally occurs in mid littoral zone or seawater streams. Plants 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, 2-3cm broad, 10-20 cm long, distal ends of the leaves are rounded but basal region is coiled like a ribbon; thallus 2 cells thick, cells elongated. Plants form dense population in intertidal pools.

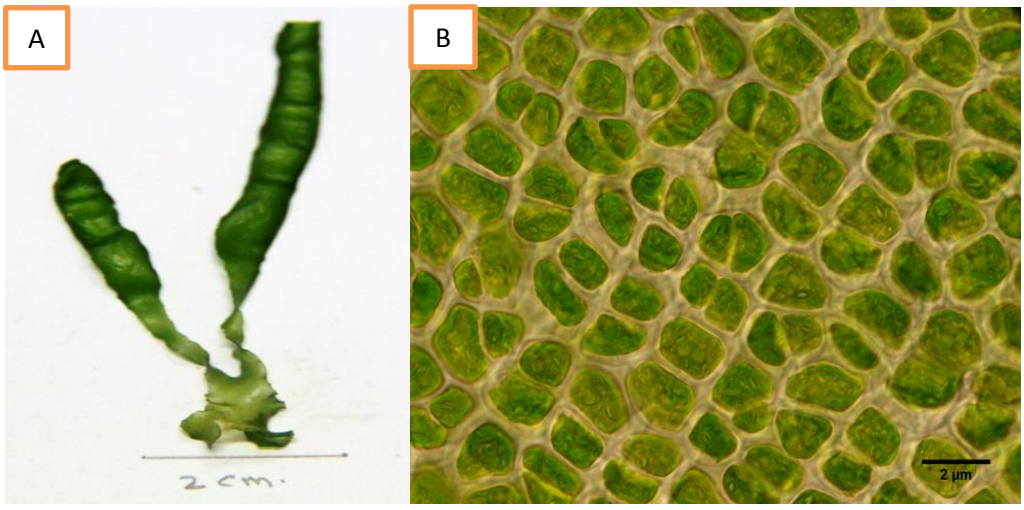

Figure 3. **A**. Photograph showing the complete thalli of the processed algal sample; **B**. Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva reticulata*

- Query cover-97%
- %identity-98%

>ETT-2-ITS1 & ITS2 (Ettikulam) Kerala

ATAAGTGGTGTGGTGTTTATGATATTAAAAACACAACAGAACAAGAAAACACCCCTTTGACACGAACTTAA
AAAATAAAATTTACTACCTCTTTAACCCCCAGCAGGTATGGTTTAAATAATAGGGGCATTTAGAGTATAGG
GGGGTGTAGGTAGCTTTTGAAGGAGTACGCACCGGAAATCATTTTTTTAGAAACCAACAAGGTCTAAGGT
TAATACACACGGGCTATTAAGATTTCTTTAGGTAAGCCAAAAAAAAAAAAAAGAAAAAAAAAAACTAGTTC
AATTTGTTGGAATCCAGATAGGGATTTTTTTTTTTTTTTTTAACCAGCAAAAAAAAATTTCAGTGTAAATGTT
TAGGTACAGGGGGGGGTCCCCGTGGGGTTTTGTGGGAGGGGGGGTTGGCTCCCCCCCCCCCCCCCCCG
GGGGGCCGGAAAGGGGGATCAAAACTCGCGCCGGGCCGGGTGTCCTTTGAGGGATTATGGTTTCCGTAG
GTGAACCTGCGGAGGGATCATTGAAACCGATCAATCCACTCACAGAGCACCAGCGGGCCCCGGCCCGCCG
TAGTTTGGATCCGCCGGTCATCCTGGCCCCCCCCGGGGGGGGCGGGCGGCCGGCTGGAGCCTCAACCCA
GCCAACCCTCTGCCCTGAAGCAGCTTCGCATGGGGACACCCCTGCGACGTTAACCAGAGACAACTCTCAAC
AACGGATATCTTGGCTCTCGCATCGATGAAAAACGCACAAAACCACATCATAGAAACAATTACTGGGAACT
ATGGGGACTACCTTTTATTCGCCATCATCCGGCCCCCCAAGGGGCGCGGGGGGGCGGAGAAAAAAACAC
CACCATACACAAACAAACCGCGGGGGCGCACCCGCGCATTCACCTACCCACTCCACGGGGGGACATTGGG
CACCTCCACCCTAGAAAATAGCACTCACCCTCTTTTTTTTTTTTCAGTC

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2. Name: *Ulva intestinalis* Linnaeus

Location: Kannur (Kerala); 11°52'57"N, 75°20'13"E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: KAN-6.3

CUP Voucher ID: CUPVOUCHER-KAN-2014-UI-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAN-2014-UI-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Thallus is bushy and Substratum attached to the rocks or pebbles. Plant is highly branched, light yellow to dark green in colour and up to 5-10 cm long, attached by basal rhizoidal portion. Branches have feather like appearance. Cells are irregular in shape and arrangement.

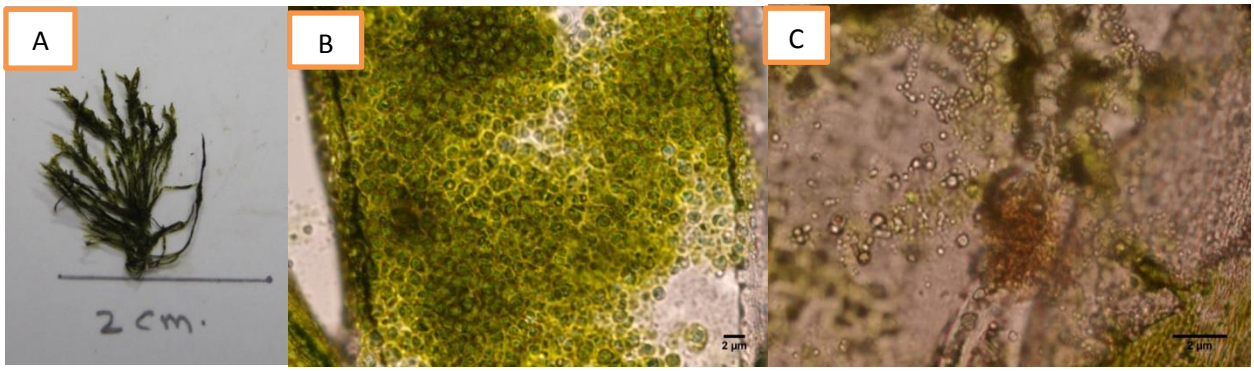

Figure 4. A. Photograph showing the complete thalli of the processed algal sample. **B** and **C**. Micro-photographs of a part of thallus at 40X and at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: Ulva intestinalis

- Query cover-99%
- %identity-99%

>Kan-6.3-ITS1 & ITS2 (Kannur) Kerala

TTGCTGCGTTCTTCATCGATGCGAGAGCCAAGATATCCGTTGTTGAGAGTTGTCTCTGTTACTG
TCGCTCGGGGTGTCCCCTTGCGAAGCTGCTTCAGGGCAGAAGGGTTCAGATGGGTTGGGGAT
CCGGGACCGGCCTCCCCCGAGGGGGCGACCGGCGGACCCTTAGAATACGGCGGGCCCGCCCC
AAAACCCCCCGGGCGCCCCCGAGAGGGGACGGCACCGGGCTGGGGGCGGACCCACAGGTGCT
CTGTGGTTGGATTGATCGGTTTCAATGATCCCTCCGCAGGTTCACCTACGGAA

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3. Name: *Ulva intestinalis* Linnaeus

Location: Kannur (Kerala); 11°52'57"N, 75°20'13"E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: KAN-6.4

CUP Voucher ID: CUPVOUCHER-KAN-2014-UI-2

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAN-2014-UI-2

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Thallus is tubular and contorted. Substratum attached to the rocks and pebbles. Plant simple or branched, light yellow to green in colour and up to 15 cm long, attached by basal rhizoidal portion and later become free-floating, mature specimens often inflated at intervals giving an intestine like appearance, contorted and irregularly constricted.

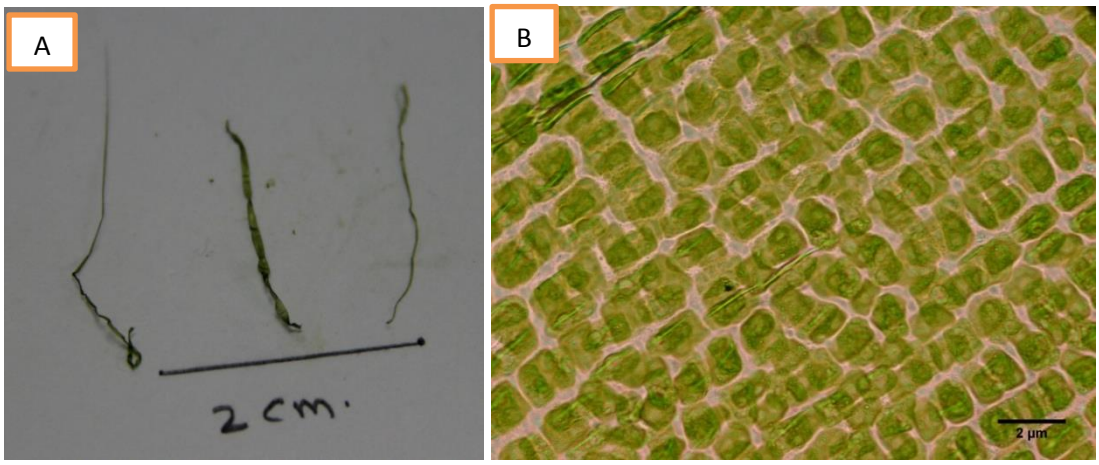

Figure 5. A. Photograph showing the complete thalli of the processed algal sample; **B**. Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva intestinalis*

- Query cover-38%
- %identity-99%

>Kan-6.4-ITS1 & ITS2 (Kannur) Kerala

TCAAAGAAAGAAGTATTAAAATTACGATCAGTTAAAAGCATTGTAATTGCTCCCGCTAAAACCGGAACTGC
TAGTAACAATAGAAATGCTGTTACAAAGATAGATCAAACAAATAAAGGTATTCTATACATACTTTGACCTG
GATTACGCATATTTAGGATTGTAGAAATAAAATTAATTGCTCCTAAAATAGATGATGCTCCTGATAAATGA
AGGCTAAAAATTGCTAAGTCTACGGCACCGCCTGAGTGACTTTGTATTGAACTTAAAGGAGGATAAACAG
TTCATCCAGTCCCTACCCCTACTTCTACAATTGCAGAA

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4. Name: *Ulva intestinalis* Linnaeus

Location: Ponnani (Kerala); 10° 46′ 12″ N, 75° 54′ 0″ E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: PON-7

CUP Voucher ID: CUPVOUCHER-PON-2014-UI-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-PON-2014-UI-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Thallus is bushy but each branch aroused from base is tubular and contorted. Substratum attached to the rocks and pebbles. Plant simple or branched, light yellow to green in colour and 5-15 cm long, attached by basal rhizoidal portion and later become free-floating. Each branch is segmented and each segment is connected to other by cell-cell junctions.

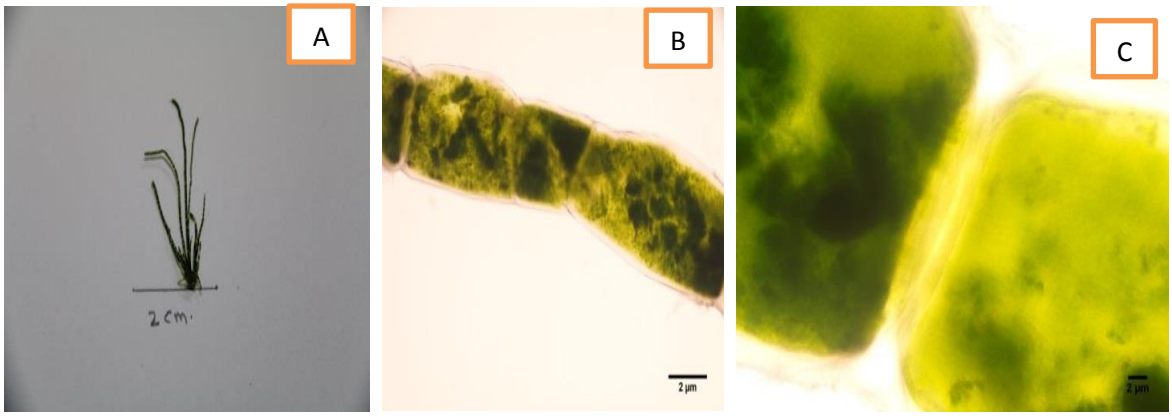

Figure 6. A. Photograph showing the complete thalli of the processed algal sample; **B** and **C.** Micro-photographs of a part of thallus at 10X and at 40X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva intestinalis*

Query cover: 19%

%identity-89%

>PON-7-ITS1& ITS2 (Ponnani) Kerala

GGGTGGCTCGCGAGGGGAAAGGAACATCGTGGGTCGCTAAGCCGGGGCCGTCCCTCCGGGGGGCCGGA
TGTTGGGGGGGCCCGCCGTATGTAAGGTCGCCGGTCGCCTCTCGGGGGAGGCGGTCCCGGATCCCCAAC
CCATCTGAACCTTCTGCCCTGAAGCAGCTTCGCAAGGGGACACCCCGAGCGACAGTAACAGAGACAACTC
TCAACAACGGATATCTTGGCTCTCGCATCGACAGAACGCAGCCCGCAGGAATCGCCCCCGTCAGCGGGGG
GGCGGACTGTGGAGGGGAGGCAGTGTTGAGAGATAAAAACACTGTAGGCGCGTGGGGCCAGTTGGTTG
TGCAGAGAATATCTTCAGAGAAATTACACATTTCACAATGGCACCAACCGGCCCCGAGAACAATTCGCGTA
TAGAAAAGATAGCTGAAGCCCCTCCCACCCCCGGGGCCCCAGCCCACTTTTTTTTTACCCCCCGGCCGGAG
AGTTGTTCCCCCTTACTTATTTTTTCACATACCGGGCTTCTCCCGTCCGGTTAAAAAAACCCACACACAAAAT
TGGGGGGGGAACCCCCCCCCCCCCAAAAAAAAAAAAAAAATTTTTAATATTTTTTTGGTTGTGGGGGGGG
GGTTGTTTTTTTTATAAAAAAAAAAAACAGGAGCCGGTCCCCCACTTTTTTTGTAAAATATTCTTTATTCTGT
TCCCGGTTCAAACCCTCCCTCCAATAAACTCCGTTGTTCGGGGAGGCGGCTCCCCCTTTTTTTTTCTCAATAA
AAAAACAGAAACACCCCCCGTCTCCCCCCCCCCTTTTTTCCGCGAAAAAGTATTATTTGTGAGAAAAAACCC
CCCGGCGCGCCTCACACACCACCCTATCATAAAAAAGCTTTTTAAAAGGAAGCTCTTCTTTTTTTTTTTGTCC
TCGACTCTCGCTCAGCGGCGGCGCGAAAAGAAAAAACATCTCATAAATGACTCTATCACAACGACAACCAT
ACTACTTCTGTAGTGTATTTTTTTTTTTTTATCCGGCGCAGCGGTCGCTGCAGAT

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5. Name: *Ulva reticulata* Forsskal

Location: Calicut (Kerala); 11° 15′ 0″ N, 75° 46′ 12″ E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: CAL-10

CUP Voucher ID: CUPVOUCHER-CAL-2014-UR-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-CAL-2014-UR-1

Classification:-

Class: Cholorophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Perforated leafy thallus and thallus grows attached to rocks. It generally occurs in mid littoral zone or seawater streams. Plants growing separately or some time in association with other algae, light to dark green in colour, segmented leaf like structure or form separate branches. Leaves are flattened or compressed. Each branch is highly coiled, edges of the leaves are 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; thallus 2 cells thick, cells elongated. Plants form dense population in intertidal pools.

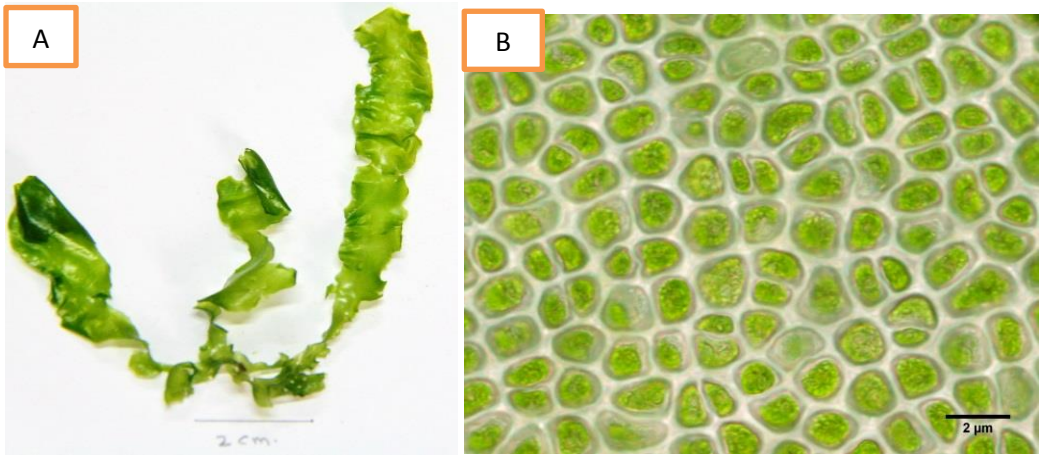

Figure 7. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva reticulata*

- Query cover: 98%
- %identity: 99%

>CAL-10-ITS1 & ITS2 (Calicut) Kerala

ATAAGGGCATTTCCGGGATTTTGGGGGAGAATATAATTTCTTCGAAAGGAATAAGCCACCGCAA
TCGTTATTTTAGAAAAAACAAAGGTCTAATGTTTAATTATAGGAAGGGGTATTACGATTTTTTTG
GTTAACCCAATTAAATAATGTTTAGGAAAAGAGTGGTCCAATCTGATGGGGGTCGAAAACGGT
TTTCCCCGTTTTTCGTTTTTCTTCGGGGACAAGCCCCCATCTGTATTTTAGTTTTAATTTACGGGGT
GGGTACTAATCTTGTTTCAGGGGAGGGGTGGTGCCGGCGTTTAATCGAGCAACCACAGGGGTTT
CACCGGACACTTGCTGTGACTCCATAATTCCGTAGGTGAACCTGCGGAGGGATCATTGAAACCG
ATCAATCCACTCACAGAGCACCAGCGGGCCCCGGCCCGCCGTAGTTTGGATCCGCCGGTCATCCT
GGCCCCCCCCGGGGGCGGGCGGCCGGCTGGAGCCTCAACCCAGCCAACCCTCTGCCCTGAAACA
GCTTCGCATGGGGACACCCCTGCGACGTTAACCAGAGACAACTCTCAACAACGGATATCTTGGC
TCTCGCATCGATGAAGAACGCAGCC

---

6. Name: *Ulva fasciata* Delile

Location: Manglore (Karnataka); 12°52'55"N, 74°50'22"E

Collection date: 13-09-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MAN-14.1

CUP Voucher ID: CUPVOUCHER-MAN-2014-UF-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MAN-2014-UF-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Thallus is tubular and ribbon like coiled. Substratum attached to rocks or any other hard substrata in intertidal area. It occurs in mid littoral zone and tide pools. Plants 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. Surface of plant is smooth; plants are free floating in nature after maturation. Thallus 2 cells thick, cells rectangular or quadratic in cross section.

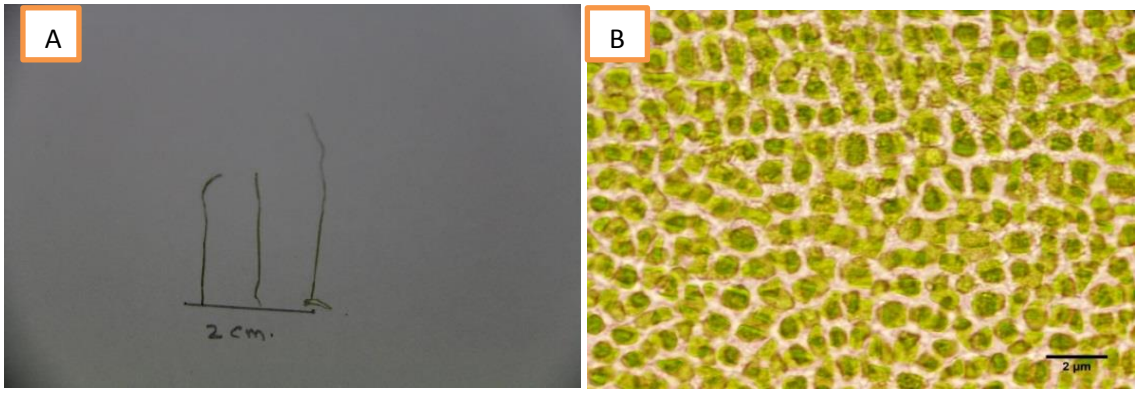

Figure 8. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva fasciata*

- Query cover: 98%
- %identity: 95%

>MAN-14.1-ITS1 & ITS2 (Manglore) Karnataka

GTTGGCCGGGAAAGTCCCAAATATAGGGGCCCCCCACAGGCAAATTCCGAATAGCACCGCAGTA
TGCTCAGTTCATCGATCCTTCCGTAGGTGAACCTGCGGAGGGATCATTGAAACCGATCAAACCAA
CCACAGAGCACCTGCGGGCGCTCACTCGGGCGTTCTCGCCCGGGTGGGGCGGCCCGCCGTCGTA
TCTCGGGACCCGGTCCTCCGGGCCCCGCCCAAACCCATCCAACCCTTCTGCCCTGAAGCAGCTT
CGCACGGGGACACCCCGCGCGACAGTAACAGAGACAACTCTCAACAACGGATATCTTGGCTC
TCGCATCGATGAAGAACGCAGCCATGCGGATTTCTTGGCTCTGCCTGGTGAACAAGGGGCATTT
CCCGGGGGGGGGGCCCCCTTTTTGTGGGAC

---

7. Name: *Ulva fasciata* Delile

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13M

CUP Voucher ID: CUPVOUCHER-MDP-2014-UF-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-UF-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:

ID Features:

Thallus is leafy. Substratum is attached to rocks or any other hard substrata in intertidal area. It occurs in mid littoral zone and tide pools. Plants yellow to dark green in colour, up to5-40 cm long and divided into number of ribbon shaped 1-3 cm broad lobes. Leafy thallus is irregularly lobed or sometimes divided into ligulate or linear lobes; thallus 2 cells thick, cells rectangular or quadratic in cross section. Cells are slightly curved or sickle shaped.

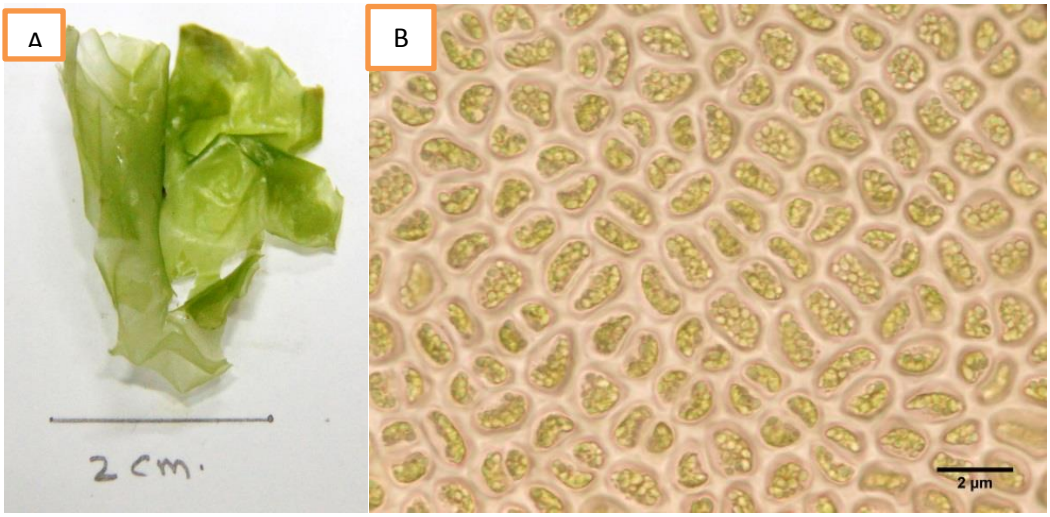

Figure 9. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1: *Ulva fasciata*

- Query cover: 96%
- %identity: 98%

>MDP-13M-ITS1 & ITS2 (Mandapam) Tamil Nadu

GGGTTTCCCCTGGAAAGGGTTTGAGGGAGGGGTTGGGGTTAGATCCCCCCCGCCCCCCCCCCC
GGGGGGGTGGAGTGTTCCGGGGGATCCCAAAATAGGGCCCCCGAGGCCCCCGTGTTTTCTTAGGGATTA
AAGGATCAGCTGCGTTCTTTCATCGATGCGAGAGCCAAGATATCCGTTGTTGAGAGTTGTCTCTGGTTAAC
GTCGCAGGGGTGTCCCCGTGCGAAGCTGCTTCAGGGCAGAGGGTTTGCTGGGTTGAGGCTCCAGCCGGG
CCCGCCCCCCGGGTGGGGGGGCGAGGGTCCGGCGGATCCAAACTACGGCGGGCCGGGGCCCGCTGGTG
CTCTGTGAGTGGATTGATCGGTTTCAATGATCCCTCCGCAGTTCACCTACGGAAACCGATAATCCCTAGAG
CACGCGGGCCGGCCGCTATTTTGGATCCCCGACTCGCCACTGGGGGGGCGCTGAGCTCAC

---

8. Name*: Ulva prolifera* O.F. Muller

Location: Kovalam (Tamil Nadu); 8° 18′ 0″ N, 77° 12′ 0″ E

Collection date: 11-07-2011

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: KOV-T-1.1

CUP Voucher ID: CUPVOUCHER-KOV-2014-UP-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KOV-2014-UP-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Plants are green to dark green in colour, thallus with leaf like structure, leaves have lacunae and form net like structure, leaves are 5-8cm broad, 15-20 cm long, branches are divided at distal ends in a lobed like structure which form heart shaped structure, base is broad, coiled which attached by means of disk-like holdfast; fronds flattened, profusely branched with numerous slender branches; membranous and very soft tissue, cells in surface view oval to sub-rectangular, irregularly arranged in surface view of the thallus.

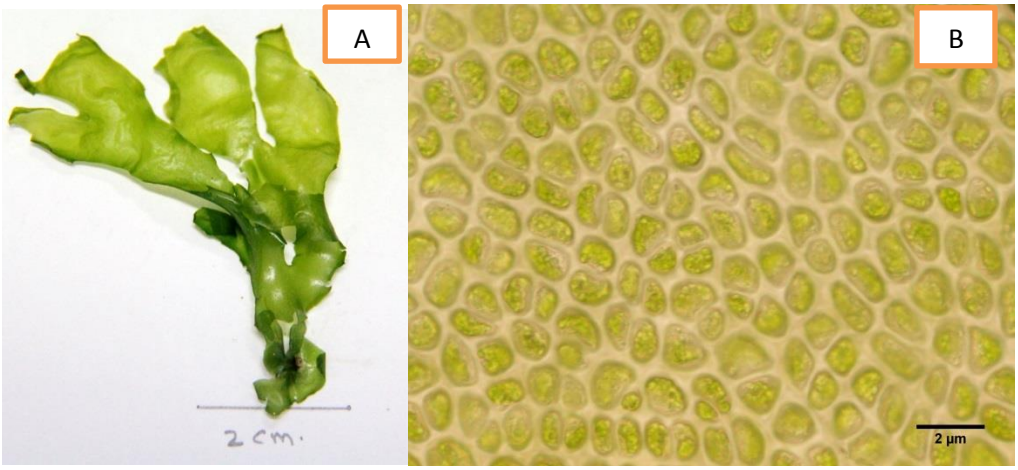

Fig.10. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva prolifera*

- Query cover: 53%
- %identity: 95%

>KOV-T-1.1-ITS1 & ITS2 (Kovalam) Tamil Nadu

TTTCAAACAAAATCATAAAGATATTGGTACGTTATATTTAATATTTGGGGCTTTTTCCCGGTGTTTTAGGTA
CAGCGATGTCTGTTCTTATAAGGTTGCAGCTTGCAAGCCCGGGCAATATGTTTTTGGGGGGGAATTATCAG
TTATATAATGTTATTGTTACGGCTCATGCTTTTTTGATGATTTTCTTTATGGTTATGCCTGTTCTTATAGGGG
GTTTTGGTAATTGGTTTGTTCCTTTAATGATAGGTGCTCCAGATATGGCGTTTCCTCGTATGAATAATATAA
GCTTTTGGTTGTTACCGCCGTCTTTAATACTTCTTTTAGCATCCTCATTAGTGGAGGCTGGAGCCGGTACAG
GTTGGACCGTTTATCCACCGTTAAGTGGTATCCAGGCGCATTCGGGCCCATCAGTGGATTTAGCTATCTTTA
GTTTACATTTATCTGGTGCTGCTTCTATTTTGGGTGCCATTAATTTCATAACAACCATTTTCAATATGCGTGC
TCCGGGTTGGGTATGCACCGTTTACCTTTATTTGTTTGGTCTGTTTTGATAACAGCATTTTTACTTTTATTATC
TCTTCCGGTTTTGGCTGGAGGCATTACTATGCTTTTAACGGACCGTAATTTTAATACTACATTTTTGATCCG

Based on COX1gaz F & R: *Erythrocladia*

- Query cover: 56%
- %identity: 93%

>KOV-T-1.1-COX1gaz F & R (Kovalam) Tamil Nadu

GTATTATTCGTATGGAGTTAGCACACCCGGGTTCACAAATTTTATCTGGTAATTATCAATTATACAATGTAT
TAATTACTAGCCATGCCTTCTTAATGATTTTTTTCTTTGTAATGCCCATTATGATTGGTGGATTTGGTAATTG
GTTTGTTCCCATCATGATTGGAGCTCCTGACATGGCTTTTCCTAGGTAAATAACATATCATTTTGGTTATTAC
CACCAGCTCTATTATATTATTACTTTTCACTTGTTAATTGGAGAAGACTGGTTGGACATGTTCCCCTCTATGT
GTTCAAATTACTCGGAGACTTTAATTTGTATTTTTTCTCAATTCAGGACTTTCATCTAGGAAATAAATCAAAT
ACAATTAAAATGAAATCCAGTCAAGGGTCCCCAATCCTTTTTTTTTTTTGTCATTTTAATACCCCCTTTAATCT
GTTACGTCCCGAATTGAGGACGAACAAAGTTCTAAAAACCAATTTTACCCTTTTTTTTTTCCAAAGGGGGG
GGAATCCTTTATTCACCCCTTTTTTTTTTT

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9. Name: *Ulva ohnoi* M. Hiraoka & S. Shimada

Location: Ennore (Tamil Nadu); 13° 13′ 3″ N, 80° 19′ 17.58″ E

Collection date: 20-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: ENN-T-7.2

CUP Voucher ID: CUPVOUCHER-ENN-2014-UO-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ENN-2014-UO-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Thallus is light green in colour and grows attached to the rocks in marine water. Plant is nearly 2-3 cm wide but thallus is 20-30 cm long. Thallus is highly coiled like a ribbon and thicker in upper and middle regions. Tiny serrations are present on the leaf surface. Cells are irregular in shape and irregularly arranged.

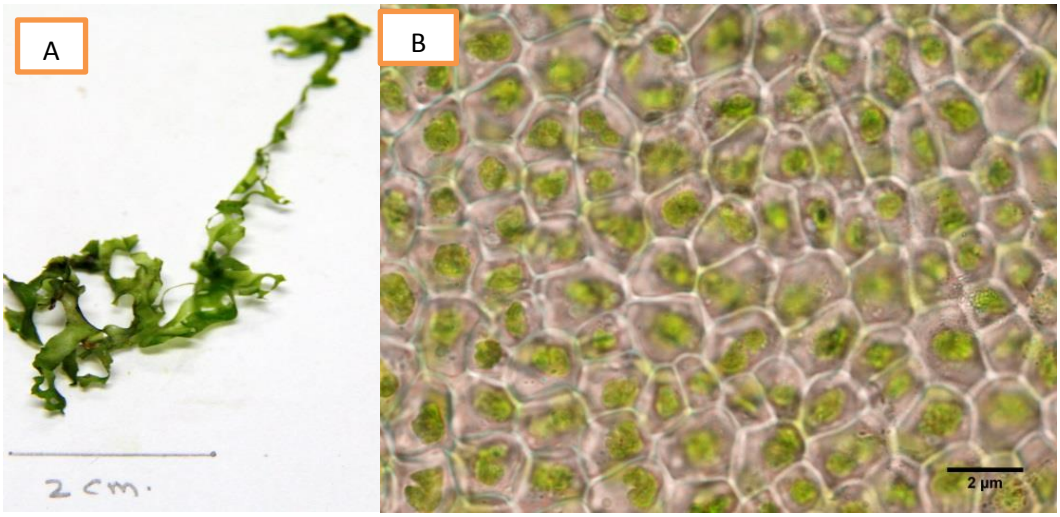

Figure 11. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva ohnoi*

- Query cover: 38%
- %identity: 98 %

>T-7.2-ITS1 &ITS2-Tamil Nadu

GTTGGACGCGTTAATCACACTGACAGTAGCACCAGCGGGCCCCGGCCCGCCGTAGTTTGGATCCGCCGGA
CCCTCTCCCCCCCACCCGGGGGGCGGGCCCGGCTGGATCCTCAACCCAGCAAACCCTCTGCCCTGAAGCA
GCTTCGCACGGGGACACCCCTGCGACGTTAACCAGAGACAACTCTCAACAACGGATATCTTGGCTCTCGCA
TCGTGAATAACGCAGCTAGATCTCTTGGCTCCCGTATCCAACAAAAACGCCACATCTCGCATCGATGAAGA
ACGCAGCATGTCGCAGCTGGTGTGGGGGTGTGTAGTATGATAAATACTTTTGTTAGGCTCGTTGTTTGAG
GTTGTTATAGCTAGCCCCCTTTTCTTTAAACAAAAAAAAAAAACAAAATAGTTGGGAGTACTGCAACCCCCCC
AAAAATTTTTTTATTTTTTTTTCCCCCCCTTTTTTAAAAAAAAATTTGGCCTTACTTAAATAACACAAAAATTT
TTAAAAAGGCAAAAAAATAGTTAAAAATTTTTTTTTTATTTATGAAATTTTTTA

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10. Name: *Ulva prolifera* O.F. Muller

Location: Ennore (Tamil Nadu); 13° 13′ 3″ N, 80° 19′ 17.58″ E

Collection date: 20-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: ENN-T-8

CUP Voucher ID: CUPVOUCHER-ENN-2014-UP-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ENN-2014-UP-1

Classification:-

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Morphology:-

ID Features:

Plants are green to dark green in colour, thallus with tubular branches that have numerous slender branchlets, up to 10 cm long, attached by means of disk-like holdfast; fronds tubular, profusely branched with numerous slender branches; cells in surface view polygonal to sub-rectangular, always arranged in linear series in certain parts of the thallus.

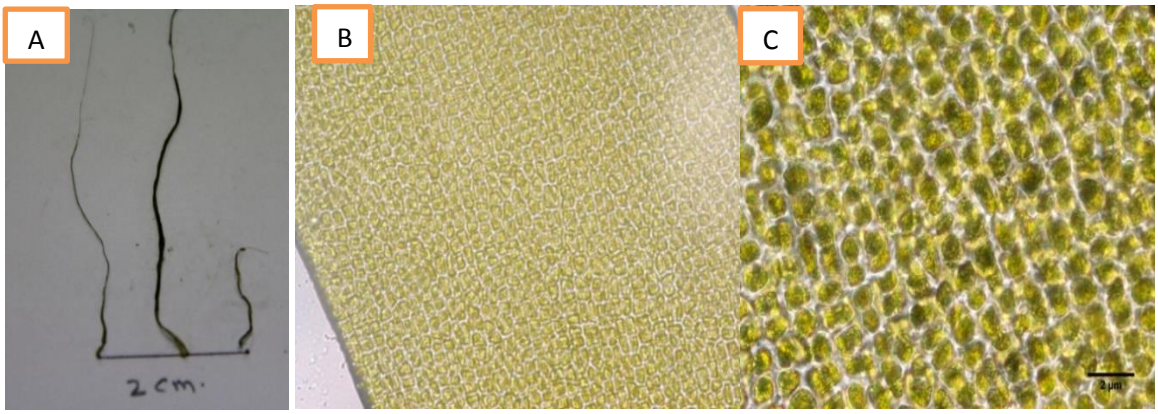

Figure 12. A. Photograph showing the complete thalli of the processed algal sample; **B** and **C.** Micro-photograph of a part of thallus at 40X and at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Ulva prolifera*

- Query cover: 41%
- %identity: 93 %

>ENN-T-8-ITS1 &ITS2 (Ennore) Tamil Nadu

AATTAATTAACTGTGCGGAGAGCGCCGCCATTTACGGAGCACATGAAAAAAGTTGGAAGG
GGTTCAACAAGGTGTTTTAGGAGCAATGGAGTTATACAGAGTCCTAGGGTAGAAGTATGT
AATCGCCGCGGCCCCCAGGTTCCGACGGACCTAAAGCGATCCCCACCCCCCCCCCCCAGG
AAGTACCCGCAGTCTACGTATGTTTCCGTAGGGAACTGGGGAGGGATCATTGAAACCGAT
CAAACCAATCACAGAGCACTTGCGGGCGCTCACCTCTCCCCCTCGTGGGGGGGGGGTGGG
GCAGTCTGCCGTTTACAGGTGCCGCCGGTGCGGGAAGCCCCTCGCGGGGCCCCCGGCCGG
GCCGGACCCTTTAACCCATTGAACCCTCTGCCCTGAAGCAGCTTCGTATGGGGACACCCC
TGCGATAGTAA-CTGAAACAACT-CTCAACAACG--GATATCTTGGCTCTCGCATCGATA
AAAAACGCACCCACCAACACTTCATTGGTTTGGGGGGCCCCCCCCCCCCCGGGGGGGGG
GGGGGGGGATTCCCCCTTAAAGTGCCCCGTGGGAAACCATTTGACCCCCCCCCGCCTTCA
CTTTACCCTTCAAATTACTTCTATGGCCCCTTGAGAAACCCCCCCCTTAACCGAAATTTG
ACCCCCTTAAAAACCACCAAAACT

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11. Name: *Caulerpa scalpelliformis* (R. Brown ex Turner) C. Agardh

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13D

CUP Voucher ID: CUPVOUCHER-MDP-2014-CS-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-CS-1

Classification:-

Class: Bryopsidophyceae

Order: Bryopsidales

Family: Caulerpaceae

Morphology:

ID Features:

Thallus ramiform and creeping, attached to intertidal rocks in lower and mid littoral zone. Plants bright green in colour, siphonous with rhizomes and erect foliar assimilators; foliar branches up to 20 cm long, 2-3 cm broad, simple with forked or lobed margins, the upper ends of marginal lobes broadly rounded , slightly curved and denticulate.

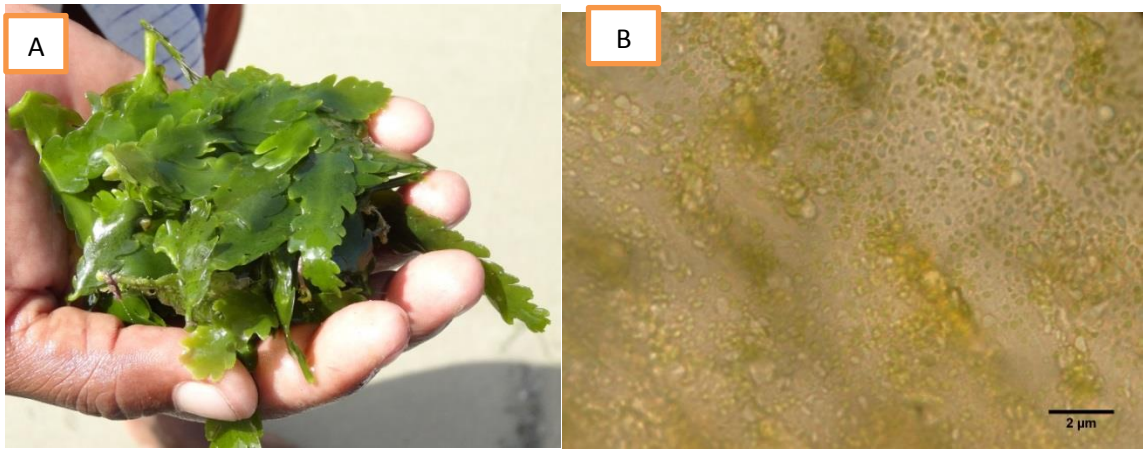

Figure 13. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS1 & ITS2: *Caulerpa scalpelliformis*

- Query cover: 55 %
- %identity: 97%

>MDP-13D-ITS1 & ITS2 (Mandapam) Tamil Nadu

GCGTCACAGTCACTCACATTCACATACACGCTGAAGGAAAGTGCTCGCTACCCCAGACCCATCCACTTACA
GAGAATGTCCGTCATCTCCGTACCTCAGTGGTATCGGTAAGTGACGAGCCATTCACTGTCGACAGATTTGC
ACAAGTTTTTCGAACTGCGACCCCTGGCATCGTCGGAGACTGCCACATGGACCCGATGCATTGCAGGAGA
ATCAATGACTCGAACTAAATCATGACAGTCGACAACTGCTTTCCGGATTCCGCTACGTACTTCCATCGACAC
TGGAAACCCGAGCCGTTCCAGCTATCGCATGCTTTATCTGGTCAAGCATTGTTGTGATTACAAACTATATG
GTGTTGTATCAACAACGACAGTTTCTTAACTACTTAATCACATAGATCTGGTGTAGTGTGGGAGGCGAATT
CGCCCAAAAATWATCTCTTCCGTAGGTGATCCTGCGGAAGGATCATTATCGGCAAATTCACTACTACACTA
TACCAGTCTATGTGTTAAAGAGTTATGAACTGCGTTGTTGTTAAACACATATGTTTATATTGTAACAATGTT
GTCTCTAATAAAGCATGCGTAGTGGACGGCTCGGTTCTCGTGTCGATGAAGGACGTAGCGAAATACGAAA
GGTGTTGCGAATTGCAAGATTTCGTGAGTCAATGAATATCCGAATGCATTGTCGTCCCTTCGCAGCTTCCG
GGTTGCAAGGGGCGCGTCGGCAACATCGGCTAATTGTCGACCTTCATAGTCTCTTTTGCAGTCTCTCACGT
GAGGTTGCTGGATTCTATGAAGCAGTGGTTCAGTGAGCAAGCATTCTCCTACGTTATACGTGACTGTGTGC
TTGTGACGACTCCTTCTCACTATCGACTACTGACTGAGATTAACGACAGTATTGTGACTTGCATACTCTATG
TGCTTGTCCTATTGCAATCAATCGCTGAGATTGAAGTGCATGGAAAACTGTGCTCGACTGTAGTCTTTGTTG
CTGAATGAGGTTCTATTCGTCGATCTAAAAAATATTTAATAAT

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12. Name: *Gracilaria folifera* (Forsskal) BØrgensen

Location: Ettikulam (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: ETT-4

CUP Voucher ID: CUPVOUCHER-ETT-2014-GF-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ETT-2014-GF-1

Classification:-

Class: Florideophyceae

Order: Gracilariales

Family: Gracilariaceae

Morphology:-

ID Features:

Thallus is leathery and bushy form. It grows on intertidal rocks or calcareous substratum in lower mid littoral zone. Plants brownish to yellowish red in colour, up to 16 cm in height, flat or compressed dichotomously or sub-dichotomously branched, attached by discoid holdfast; frond membranous, proliferous and branches tapering towards the apices, laciniate with acuminate tips, proliferations often marginal.

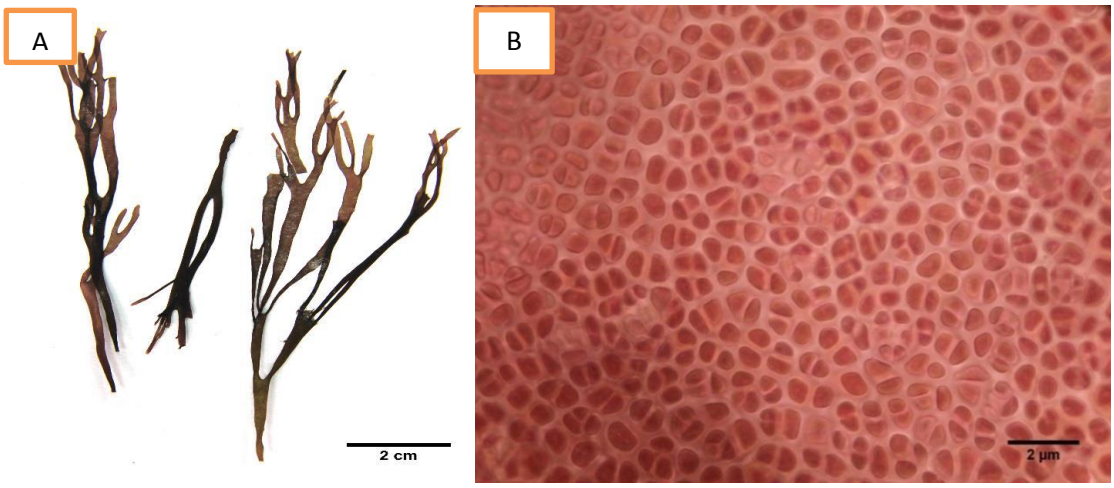

Figure 14. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX2 & COX3: *Gracilaria folifera*

- Query cover-88%
- %identity-97%

>ETT-4-COX2 & COX3 (Ettikulam) Kerala

TGTACCTTCTTTAGGAGGAAAATGTGATGCTATTCCGGGAAGATTGAATCAAACATCAATTTTTGTTAAAA
GAGAAGGAATTTTTTATGGTCAATGTAGTGAAATATGTGGTATAAACCATGGATTTATGCCTATTGTAATT
GAGGTAGTAAAATTACCTAGTTATATTTCTTGAATTTCTAATAAGCTGAACGAATAAATTGATGCGACTTTC
TTTTTCACAGATTATCGTATTACTTTTAGTTTTTTTTATACTTTTTTCGATGAATTCTAAAATTTGAAAAAAAT
TAATTGGATTTTTAAACCAATTTTTAAAAAATTT TTTA AAATAAAATTATATTTA
CTTATCACAAATATCAAAGTCTATACAGAGACATCCTTTCCATCTAAGTAGA

Note:- This sample was first time identified from coastal region of Ettikulam Kerala.

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13. Name: *Gracilaria domingensis* (Kutzing) Sonder Ex Dickie

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: MDP-13I

CUP Voucher ID: CUPVOUCHER-MDP-2014-GD-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-GD-1

Classification:-

Class: Florideophyceae

Order: Gracilariales

Family: Gracilariaceae

Morphology:-

ID Features:

Thallus is in bushy form. It attached to the rocks in marine water. Plant is pinkish red in colour. Main axes are compressed and secondary branches are tubular. Small scale like structure arises from the main axes. Reproductive organs attached to the flattened surface.

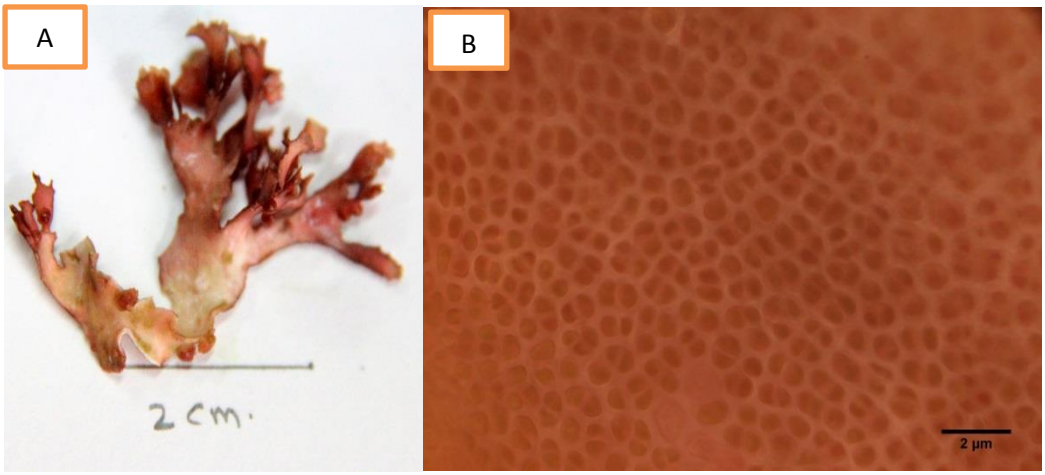

Figure 15. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F: *Erythrocladia*

- Query cover: 86%
- %identity: 99 %

>MDP-13F-COX1gaz F (Mandapam) Tamil Nadu

CCGTTAACTTCAGGCTTCTTCCAGCTTGTACGCAGAGTGTTTTAGGTGGTTGTATGTCAATGTTAATCCGAA
TGGAGTTAGCTCAACCAGGAAACCAATTATTGTTAGGAAATCATCAAATTTATAATGTTTTAATAACAGCAC
ATGCGTTTTTGATGATTTTTTTTATGGTAATGCCTGTAATGATAGGAGGGTTTGGGAATTGATTAGTACCTA
TTATGATTGGAAGCCCTGATATGGCTTTCCCGCGTTTAAATAACATATCATTTTGATTACTACCACCGTCACT
TTGTTTACTTTTAGCGTCAGCGGTTGTTGAAGTTGGTGTTGGGACGGGATGGACAGTTTACCCTCCATTAA
GTTCAATTCAAAGCCATTCAGGAGGTGCTGTAGACCTTGCAATATTTAGTTTGCATATATCGGGAGCGTCA
TCGATTTAGGTGCATTAATTTTATTTTACGATTTAATATGCGTATCCTGGACAAGTATGTATCGTATGCTTTT
TTGTTGGTCATTTTTATACAGCATTTTATTATATAGCTGTCCAGTTTAGGAGGGCTATACAATGCTTTACTGA
CGTAATTTAATCAGCGTTTTGATCCGGCGGGGAGAAACCTATTAAACAACTCTTTTGTTTTTGACTCCAAAA
TA

Based on COX1gaz R: *Gracilaria domingensis*

- Query cover: 87%
- %identity: 87%

>MDP-13F-COX1gaz R (Mandapam) Tamil Nadu

TTGGGCTGTAGCTTCGTTGCTCTGTTTGACCAATATACTTTATGATTTGTTGACCGGTAGATCTAAAATTAC
TGTCTGATAGCAACATTGTGATCGCTCCTGCAAGTACAGGTACTGCTAACAGTAATAAAACGGCCGTAATT
AAAATTGACCAAACAAAAAGAGGCATCTTGTGGTATCCTTGACCTGGATTTCTCATATTTAAAATTGTAACT
ATGAAATTTACTGCCCCTAAGATTGAGCTAGCTCCTGCAATGTGAAGAGATAAAATACATAACTCAATAGC
TCCTCCCGAGTGACTTTGAACAGCACTAAGAGGTGGATACACTGTCCAACCAGTTCCTGCTCCAAATTCAA
CAAGTGATGAAGTTAATAATAAAATAAGAGCTGGTGGTAATAACCAAAATGATATATTATTTAACCTAGGA
AAAGCCATGTCAGGAGCTCCAATCATGATGGGAACAAACCAATTACCAAATCCACCAATCATAATGGGCAT
TACAAAGAAAAAAATCATTAAGAAAGGCATGGCTAGTAATTAATACATTGTATAATTGATAATTACCAGAT
AAAATTTGTGAACCCGGGTGTGCTAACTCCATACGAATAATTACACTTAGAATCGTACCTAGCACTCCAGA
GAAGGCTCCAAAAAATAAGGTATAAAGTACCAATATTCTTTTATGAATTTGTTTGAAAAGA

Note:- This sample was first time reported from any coastal region of India.

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14. Name: *Gracilaria corticata* (J. Agardh)

Location: Bekal (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 13-09-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: BEK-23.1

CUP Voucher ID: CUPVOUCHER-BEK-2014-GC-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BEK-2014-GC-1

Classification:-

Class: Florideophyceae

Order: Gracilariales

Family: Gracilariaceae

Morphology:-

ID Features:

Thallus is in bushy appearance and grows on intertidal rocks or calcareous substratum in lower mid littoral zone. Plants dark red to yellowish red in colour, 9-17 cm in height, rigid, cartilaginous, flattened, dichotomously branched with narrow segments usually 2-4 mm wide, tips of segments acute, sometimes with proliferation. Thallus consists of 1-2 layered cortex and medulla of large cells at the centre.

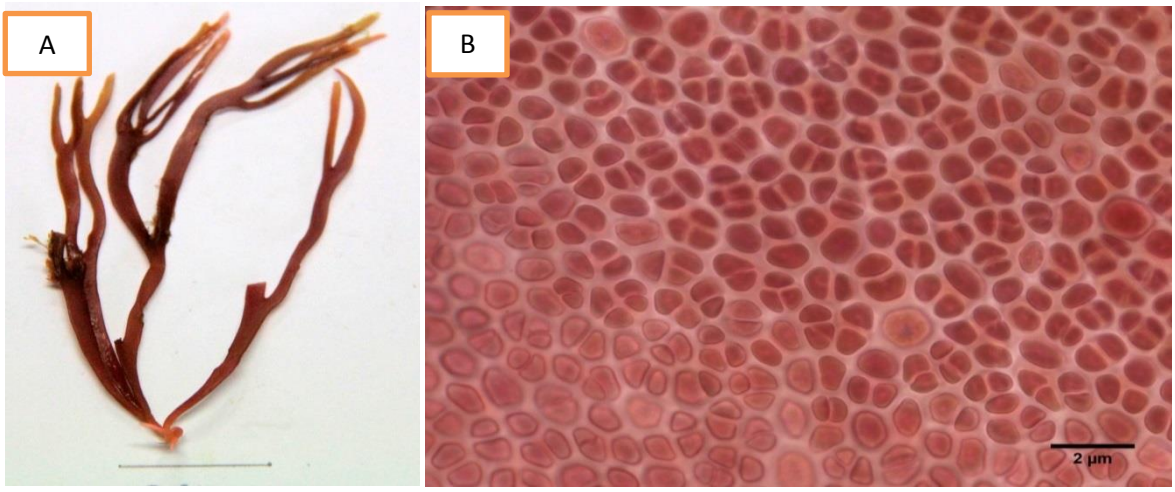

Figure 16. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX F & R: *Gracilaria corticata*

- Query cover: 57%
- %identity: 96%

>BEK-23.1-COX F & R (Bekal) Kerala

TTAGAATGGGAGGTAGTGAAAATATGGAAAAAATCGACTGGCGGGTGGGAGAGAGAACTCAACGAATG
AGGTCGAGGAGGGCTTTTTATAAAAAAACATGTCCGGTGTGACATGTAAAAAGAGGATCTTCTTTAGTCG
AAAAGGATCTTTCGGAAATAACCGATTCAATGGCGGAAAGAAGGCTTGCTTTGGATCTACAAGATGGAAT
GGATGTCTCTGTATAGACTTTGATATTTGTGATAAGTAAATCATAATTTTATTTTAAAAAATTTTTTAAAAAT
TGGTTTAAAAATCCAATTAATTTTTTTCAAATTTTAGAATTCATCGAAAAAAGTATAAAAAAAACTAAAAGT
AATACGATAATCTGTGAAAAAGAAAGTCGCATCAATTTATTCGTTCAGCTTATTAGAAATTCAAGAAATAT
AACTAGGTAATTTTACTACCTCAATTACAATAGGCATAAATCCATGGTTTATACCACATATTTCACTACATTG
ACCATAAAAAATTCCTTCTCTTTTAACAAAAATTGATGTTTGATTCAATCTTCCCGGAATAGCATCACATTTC
CCCCCTAAAGATGGTACA

NOTE:- This sample was first time reported from coastal region of Kerala.

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15. Name: *Grateloupia Sp. Nov.* (Bast & Rani)

Location: Ettikulam (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: ETT-5

CUP Voucher ID: CUPVOUCHER-ETT-2014-GA-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ETT-2014-GA-1

Classification:-

Class: Florideophyceae

Order: Halymeniales

Family: Halimeniaceae

Morphology:-

ID Features:

Thallus is of bushy appearance and attached to the rocks in marine water. Plant is brownish red in colour and very small in size. It is highly branched; main axes are compressed but secondary branches are tubular in structure. Cells are rectangular or oval in shape and irregularly arranged.

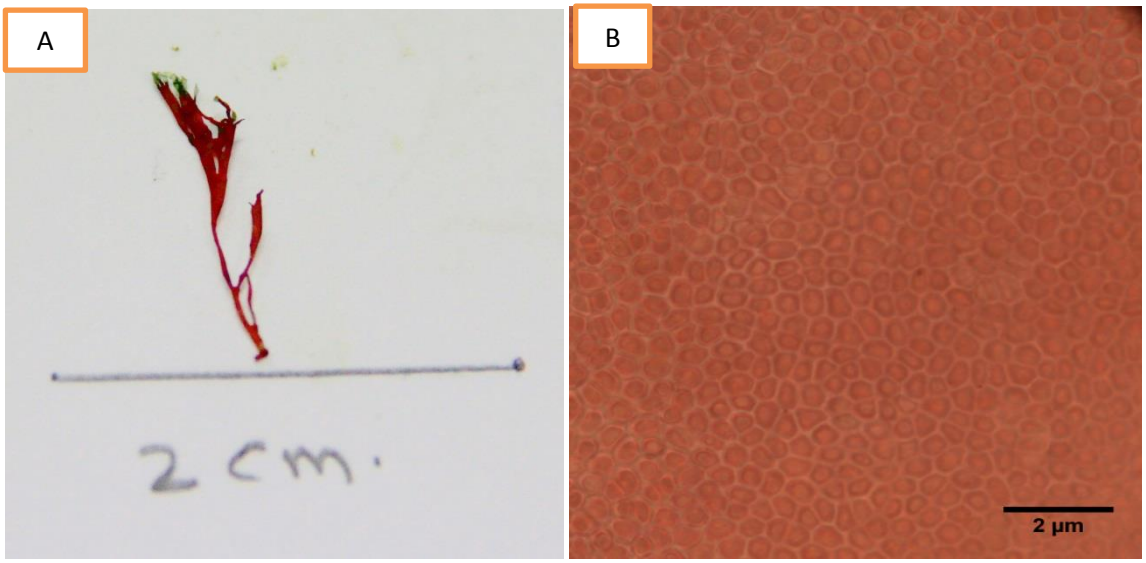

Figure 17. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Grateloupia angusta*

- Query cover: 97 %
- %identity: 90%

>ETT-5-COX1gaz F & R (Ettikulam) Kerala

TTATGTCTTCGAAGTCACAAAAAAAACTAAATAGATGTTGATATAAAACAGGATCTCCTCCACCTGCTGGG
TCAAAGAAAGAAGTATTAAAATTACGATCAGTTAAAAGCATTGTAATTGCTCCCGCTAAAACCGGAACTGC
TAGTAACAATAGAAATGCTGTTACAAAGATAGATCAAACAAATAAAGGTATTCTATACATACTTTGACCTG
GATTACGCATATTTAGGATTGTAGAAATAAAATTAATTGCTCCTAAAATAGATGATGCTCCTGATAAATGA
AGGCTAAAAATTGCTAAGTCTACGGCACCGCCTGAGTGACTTTGTATTGAACTTAAAGGAGGATAAACAG
TTCATCCAGTCCCTACCCCTACTTCTACAATTGCAGAAGCTAAAAGTAAACAAAGAGATGGAGGAAGTAAT
CAAAAAGAAATATTGTTTAATCGAGGGAATGCCATATCTGGGCTTCCAATCATTATAGGTACTAATCAATT
ACCAAAACCTCCGATCATTACAGGCATAACCATGAAGAAAATCATTAAAAATGCATGTGCAGTAATTAAAA
CATTGTAAACTTGATGATTACCTAATAATAAATGATTACCAGGTTGTGCTAATTCCATACGAATTAACATAG
ACATACAACCACCCAAAACTCCTGAAAATGCTCCAAAAATTAAGTAAAGCGTTCCAATAT

Based on COX F & R: *Grateloupia angusta*

- Query cover: 50%
- %identity: 89%

>ETT-5-COXF & R (Ettikulam) Kerala

TTCTACTAAACAAAACACAAAAGAAATATAGTTGGTACTGTTGCTATTTTTTATTTGGGGGCCTCTTTTTTG
GGGGTAGTTAGGGGGGGGTTTCTTATGTGTATTTTTGCGTGGTGGGGATATGCGCGCCCCCCGGGGGCA
CACATTTTCTTTGGGGGAATCACCAGAAAGTTTAGGATGTCTTTAAATCACAGCACGCGTTATTTAAAGGTT
TTTTCTTTTAAGATAACCCCCTGTTATGTTGGGGGCATTGGAAATGGGATTGTTTCCCTTATGTTTTGGGCG
ATCCCCACATAGGCGCTTTTCCCGGGAGTTAAAAACAAATATTACCTTGGGGTTGCCCCCCTGTGTACTATT
CTCTTCCTCTGGGGTGAAATGTGATGCAATTCCTGGACGTTTAAATCAAACTTCATTATTTATAAAAAGAGA
AGGATTATACTATGGGCAATGTAGTGAAATTTGTGGAATTAACCATGGTTTTATGCCTATTGTTGTTGAAG
CAGTTTCACTACCAAATTATATTACTTGAATTTCTAATAAGTTAAGTGAATAATTTTATGAGAATTTCTGCAA
CGCAATTAATTCTACTAATTATTTTATTTTTATTACTTTTTAATTCAAACTCACGAATAGTTAAAAATTTTCGT
GAGTTGGTTAAACAATTCTTTAAACAAATATCAAAATAGTAATTGTAAAAATGACTTTTTTAACTCAAATTT
CAAAGTCTGTACAAAGACATCCAT

Note:- This sample was first time reported from coastal region of Ettikulam, Kerala.

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16. Name*: Ceramium Sp. Nov.* (Bast & Rani)

Location: Kannur (Kerala); 11°52'57"N, 75°20'13"E

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: KAN-6.2

CUP Voucher ID: CUPVOUCHER-KAN-2014-CS-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-KAN-2014-CS-1

Classification:-

Class: Florideophyceae

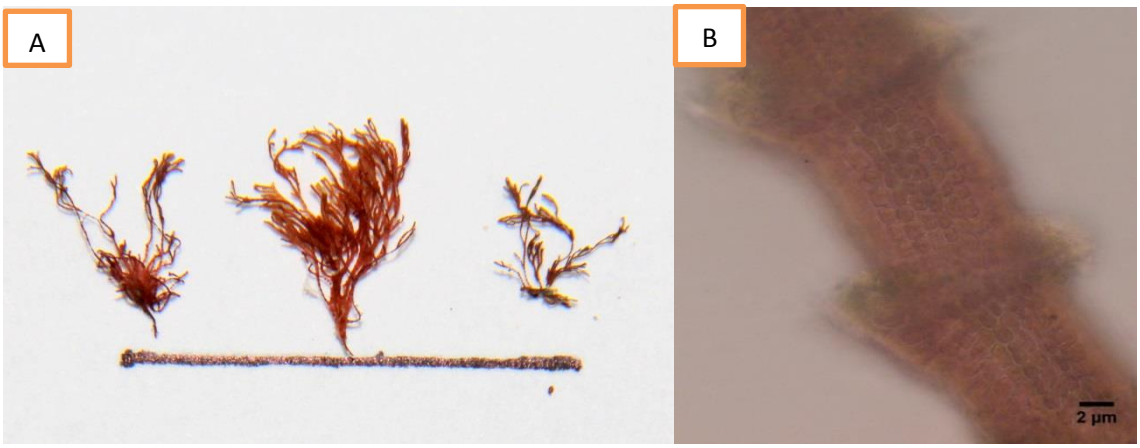
Order: Ceramiales

Family: Ceramiaceae

Morphology:-

ID Features:

Thallus is in bushy form and grows on rocks with the help of rhizoids arising from basal nodes of the plant in marine water. Plant is red in colour and repeatedly branched soft tissue; branches are dichotomous, trichotomous or tetrachotomous branching pattern. Reproductive organs are grown on basal nodes; between axes and base of branch. Nodes and internodes are constricted.



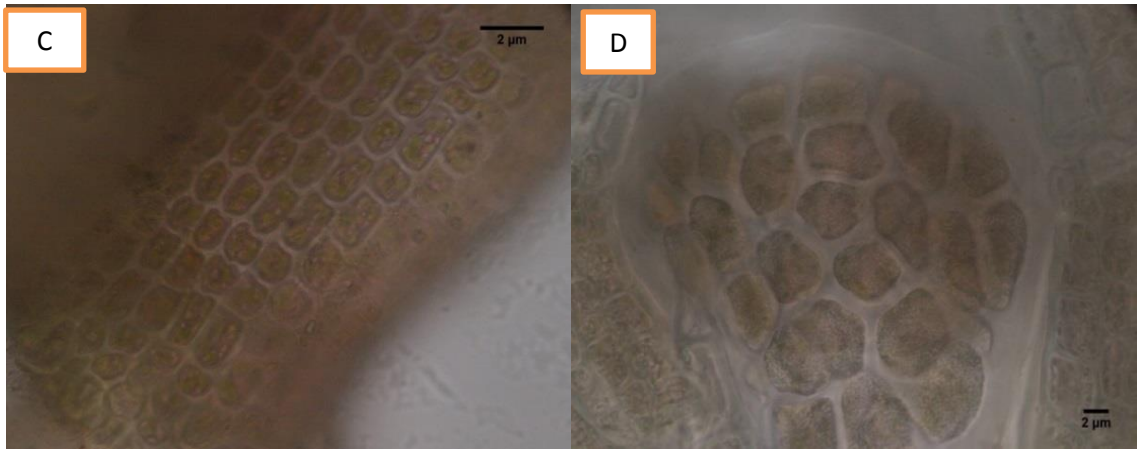


Figure 18. A. Photograph showing the complete thalli of the processed algal sample; **B and C.** Micro-photographs of a part of thallus at 40X and at 100X; **D**. Microphotograph of reproductive organ at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: (*Ceramium sp.)*

- Query cover-98%,
- %identity-85%

>KAN-6.2 –COX1gaz F & R (Kannur) Kerala

AAAATCAAAATAAATGTTGATATAAAATAGGATCTCCTCCTCCTGAAGGATCAAAAAATGCAGTATTAAAA
TTTCGATCTGTTAGTAACATTGTAATAGCTCCAGCTAAAACTGGTACTGCAAGAAGTAACAAAACTGCTGT
AACAAAAATAGACCATACAAAAAGTGGGATACGGTACATACTTTGTCCTGGATTTCGCATATTTAAAATTG
TTGATATAAAATTTACCGCTCCTAATATTGAAGAAGCTCCTGAAATATGCAAGCTAAAAATAGCTAGATCA
ACTGCTGCACCAGAATGGCTTTGAATAGAACTTAAAGGTGGGTAAACAGTTCATCCTGTACCAACTCCTAC
TTCCACAATAGCTGAAATTAAAAGTAAACATAGTGACGGGGGTAACAATCAAAATGAAATATTGTTTAAAC
GAGGAAAAGCCATATCTGGACTTCCAATCATAATTGGAACTAATCAGTTTCCGAATCCACCTATCATAACTG
GCATTACCATGAAGAAAATCATTAAAAAAGCATGTGCTGTAATTAAAACATTATAAACTTGGTGGTTTCCT
AATAATAAATGATTTCCAGGTTGCGCTAACTCCATTCGAATAAGCATAGACATACATCCCCCTAATACTCCT
GAAAATGCTCCAAAAATTAAATATAAAGATCCGATAT

Based on RbcL F & R: (*Ceramium kondoi*)

- Query cover-87%
- %identity-92%

>KAN-6.2-RbcL F & R (Kannur) Kerala

AGTAAAATATAAAGTTACTTGTAAAATTAGCAAATAAATATAATTAAAGGTTTAATAATTACTTAACCACCA
TAAGGAGTATACAATAGTGAGATTAACACAAGGAACTTTTTCCTTCTTACCTGATTTAACAGACGAACAAA
TTACAAAACAAATTGAATACGCAATCTCAAATAACTGGGCGATCAATATTGAGTATACTGAAGATCCACAT
CCAAGAAACAATTATTGGGAACTATGGGGACTA

Based on COXF & COXR: (*Ceramium pacificum*)

- Query cover-99%
- %identity-78%

>KAN-6.2-COX F &COXR (Kannur) Kerala

GGGGGAAATGTGATGCCGTTCCTGGAAGGCTTAACCAAACATCATTATTCGTTAAAAGAGAAGGGATTTA
TTACGGGCAGTGTAGTGAAATTTGTGGAGTAAATCATGGTTTTATGCCTATTGCTGTTGAAGCAGTAGATT
TAAATCGTTATGTTTCATGAATTTCAAATAAATTAAGTGAGTAATGAGATTTTCATTTCCTCAACTAGTTTTT
TTTATAACTAGTTTTTTTTTACTTTTTTATCCGCATTACATAAAAAATTTACTTAAACATATTAAAAACTTTCT
AAGTAAATTAATTTAGAT-ACATTATTCAAATCAAATGA-CTAATATAACTAA
AAATTATAAATTATATCAAAGACATCCTTT

Note:- **On the basis of query cover and % identity, this sample is thought to be a new species of genus *Ceramium***.

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17. Name: *Centroceras clavulatum* (C. Agardh) Montagne

Location: Bekal (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 13-09-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: BEK-15.2

CUP Voucher ID: CUPVOUCHER-BEK-2014-CC-1

Central National Herbaium Voucher ID: CAL-CUPVOUCHER-BEK-2014-CC-1

Classification:-

Class: Florideophyceae

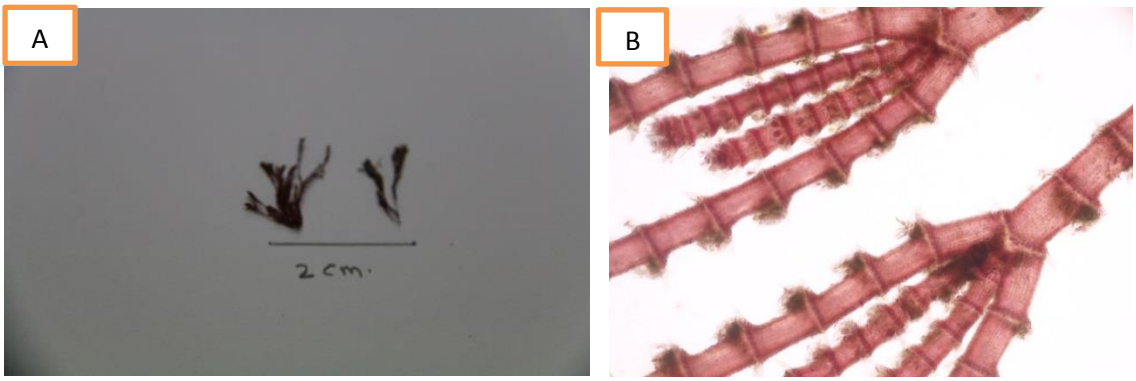
Order: Ceramiales

Family: Ceramiaceae

Morphology:-

ID Features:

Thallus is filamentous and in bushy form. It grows on intertidal rocks or calcareous stones in lower mid littoral zone. Plants dark red in colour, 5-8 cm tall, erect, filamentous and rigid; filaments regularly tetrachotomously branched; filaments with nodes and fully corticated internodes, 500µm long, 120-180 µm broad; ultimate branches forcipate, slightly incurved; cortical cells quadrate or rectangular arranged in longitudinal rows, nodes with a ring of 1-3 celled spines.



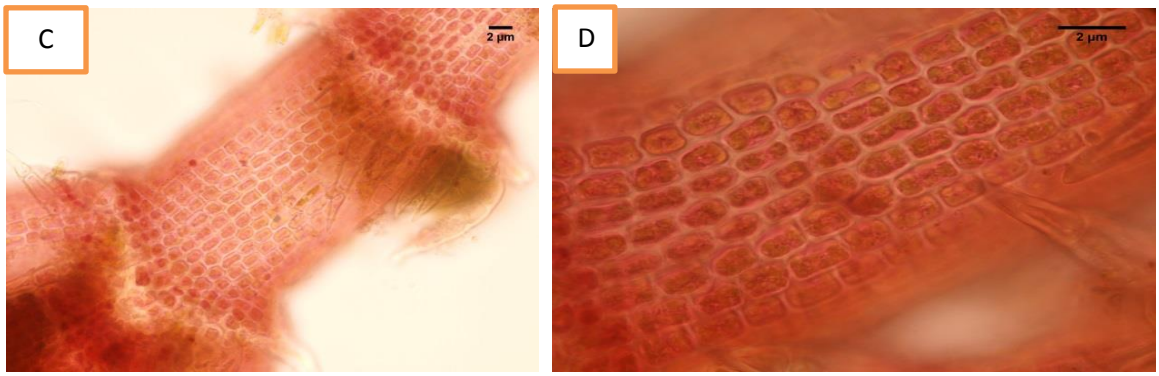


Figure 19. A. Photograph showing the complete thalli of the processed algal sample; **B, C** and **D.** Micro-photographs of a part of thallus at 10X, 40X and at 100X.

DNA Sequence data:-

Based on *rbcL* F & R: *Centroceras clavulatum*

- Query cover: 89%
- %identity: 96 %

>BEK-15.2-*rbcl* F & R (Bekal) Kerala

TCTTCAGTATATTCAATATTGATTGCCCAATTATTTTTAATTGCGTATTCAATTTGTTTTTTAATTTGTTCGTC
AGTTAAATCAGGTAGAAACGAAAAAGTTCCTTGTGTTAATCTCACTATTTTATACTCCTTATGGTAGTTAAG
CAATTATTAAAGCTTCAATTATTTTATCCATCTTAATTGTAAGAAAAATGAATAAGTGACTATTTTATAGTAC
TATTAAACGTTAGCTGTTGGAGTTTCTACGAAATCAGCTGTGTCTGTAGAAGTATAAAAAGTAGTCACTTA
TTCATTTGGCTTACATTGGATGGATAAAATAATTGAAGCTTTAATAATTGCTTAACTACCAGAAGGAGTATA
AAATAGTGAGATTAACACAAGGAACTTTTTCGTTTCTACCTGATTCAACTGACGAACAAATTAAAAAACAA

---

18. Name: *Centroceras clavulatum* (C. Agardh) Montagne

Location: Anjuna (Goa); 15°35′00″N 73°44′00″E

Collection date: 13-09-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: ANJ-19

CUP Voucher ID: CUPVOUCHER-ANJ-2014-CC-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ANJ-2014-CC-1

Classification:-

Class: Florideophyceae

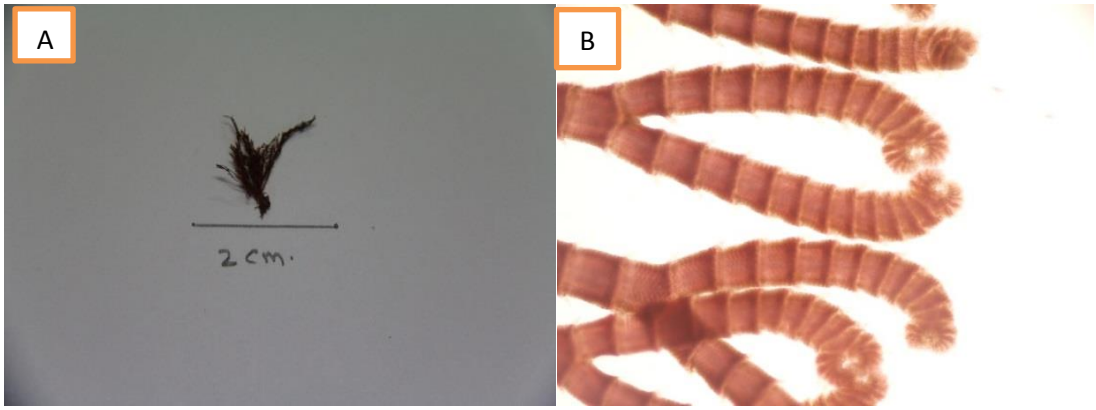
Order: Ceramiales

Family: Ceramiaceae

Morphology:-

ID Features:

Thallus is filamentous and in bushy form. It grows on intertidal rocks or calcareous stones in lower mid littoral zone. Plants dark red in colour, 5-8 cm tall, erect, filamentous and rigid; filaments regularly tetrachotomously branched; filaments with nodes and fully corticated internodes, 500µm long, 120-180 µm broad; ultimate branches forcipate, slightly incurved; cortical cells quadrate or rectangular arranged in longitudinal rows, nodes with a ring of 1-3 celled spines.



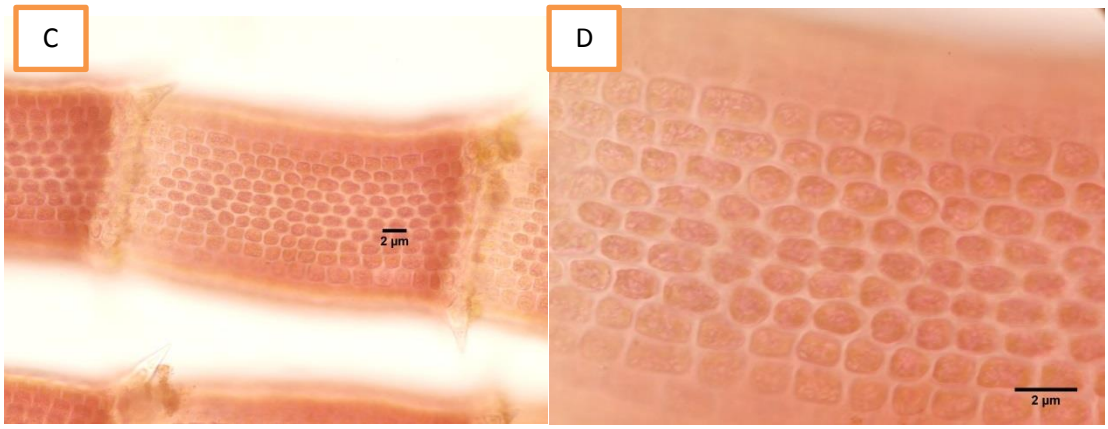


Figure 20. A. Photograph showing the complete thalli of the processed algal sample; **B, C and D.** Micro-photographs of a part of thallus at 10X, 40X and 100X.

DNA Sequence data:-

Based on *rbcL* F & R: *Centroceras clavulatum*

- Query cover: 97 %
- %identity: 98%

>ANJ-19-*rbcl* F & R (Anjuna) Goa

AGAAAACAACAAGGGTCCAAAAATTATTAATTAAAAAAAGAGCTTCTTTTCGTTATTTCTATAGGGGGCGC
CCAAATTAAAATAGAAGGCGTCAAAATGTTTTTTAATTTGTTCGTCAGTTTAATCAGGTAGAAACGAAAAA
GTTCCTTGTGTTAATCTCACTATTTTATACTCCTCCTGGTAGTTAAGCAATTATTAAAGCTTCAATTATTTTAT
CCATCTCAAGTGTAAGAAAAATGAATAAGTGACTAGTTATTTATACTTCTACAGACACAGCTGATTTCGTAG
AAACTCCAACAGCTAACGTTTAATAGTACTATAAAATAGTCACTTATTCATTTTTCTTACAATTAAGATGGAT
AAAATAATTGAAGCTTTAATAATTGCTTAACTACCATAAGGAGTATAAAATAGTGAGATTAACACAAGGAA
CTTTTTCGTTTCTACCTGATTTAACTGACGAACAAATTAAAAAACAAATTGAATACGCAATTAAAAATAATT
GGGCAATCAATATTGAATATACTGAAGATCCACATCCAAGAAATAACTATTGGGAATTATGGGGACTACCA
TTATTTGACATATA

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19. Name: *Erythrocladia Sp. Nov.* (Bast & Rani)

Location: (Elathur) Kerala

Collection date: 26-05-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: KER-11

CUP Voucher ID: CUPVOUCHER-ELA-2014-ER-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-ELA-2014-ER-1

Classification:-

Class: Compsopogonophyceae

Order: Erythropeltidales

Family: Erythrotrichiaceae

Morphology:-

ID Features:

Erythrocladia was detected as endophytic algae inside the intercellular spaces of *Cladophora glomerata*. It is seen inside as red small flower like form. All cells are irregularly arranged but form of oval shaped structure.

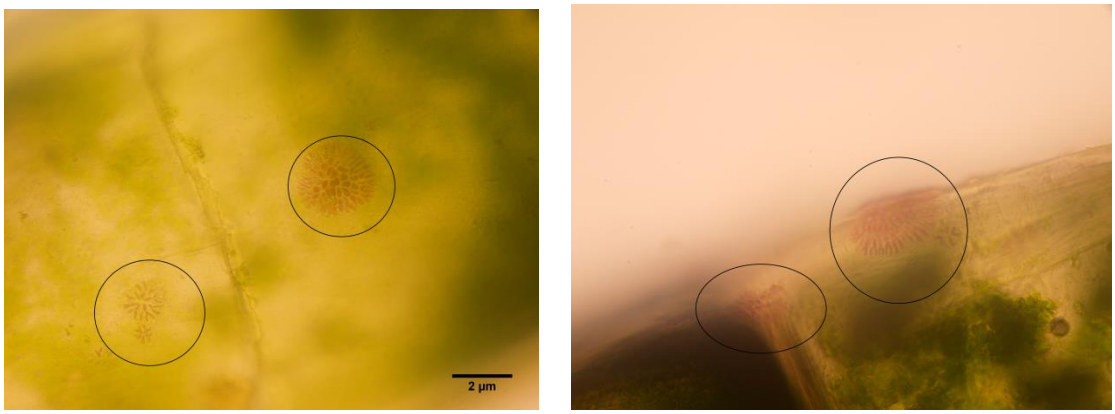

Figure 21. A and **B.** Micro-photographs of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz R: *Erythrocladia*

- Query cover: 77%
- %identity: 93%

>11-Kerala-COX1gazR

GGACGGTCTGAAAAAGACGGCAGTTGTCGCAGTTCGACTGCTGGGTCAAAGAAAGAAGTATTAAAATTAC
GATCAGTTAAAAGCATTGTAATTGCTCCCGCTAAAACCGGAACTGCTAGTAACAATAGAAATGCTGTTACA
AAGATAGATCAAACAAATAAAGGTATTCTATACATACTTTGACCTGGATTACGCATATTTAGGATTGTAGA
AATAAAATTAATTGCTCCTAAAATAGATGATGCTCCTGATAAATGAAGGCTAAAAATTGCTAAGTCTACGG
CACCGCCTGAGTGACTTTGTATTGAACTTAAAGGAGGATAAACAGTTCATCCAGTCCCTACCCCTACTTCTA
CAATTGCAGAAGCTAAAAGTAAAACAAAGAGATGGAGGAAGTAATCAAAAAAAAAATATTTGTTTTAATCT
AGGGAATGCCATATCTGGGCTTTCCAATCATTATAGGTACTAATATCAATTACCAAAAACCTTCCGATCATT
ACAGGTATTATCATAGAAAAAAAAATC

NOTE:- This sample was identified as a new species of genus *Erythrocladia*.

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20. Name: *Erythrocladia irregularis* Rosenvinge

Location: Pondicherry ; 13° 2′ 25.55″ N, 80° 14′ 23.89″ E

Collection date: 20-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: POD-T-4

CUP Voucher ID: CUPVOUCHER-POD-2014-ER-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-POD-2014-ER-1

Classification:-

Class: Compsopogonophyceae

Order: Erythropeltidales

Family: Erythrotrichiaceae

Morphology:-

ID Features:

Thallus is green in colour but *Erythrocladia* is present as an endophytic alga inside the green thallus. It form red colour, oval shaped flower like appearance.

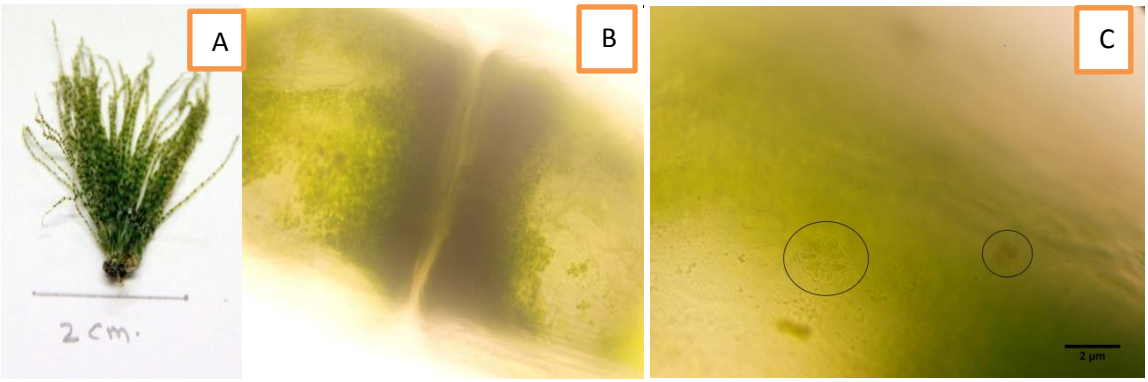

Figure 22. A. Photograph showing the complete thalli of the processed algal sample; **B** and **C.** Micro-photograph of a part of thallus at 40X and 100X.

DNA Sequence data:-

Based on COX 1gaz F & R: *Erythrocladia*

- Query cover: 88 %
- %identity: 98%

>POD-T-4-COX1gaz F & R (Pondicherry)

GTTAAAATTACGGTCTGTTAGCAACATTGTGATCGCTCCTGCAAGTACAGGTACTGCTAACAGTAATAAAA
CGGCCGTAATTAAAATTGACCAAACAAAAAGAGGCATCTTGTGGTATCCTTGACCTGGATTTCTCATATTTA
AAATTGTAACTATGAAATTTACTGCCCCTAAGATTGAGCTAGCTCCTGCAATGTGAAGAGATAAAATACAT
AACTCAATAGCTCCTCCCGAGTGACTTTGAACAGCACTAAGAGGTGGATACACTGTCCAACCAGTTCCTGC
TCCAAATTCAACAAGTGATGAAGCTAATAATAAAATAAGAGCTGGTGGTAATAACCAAAATGATATGTTAT
TTAACCTAGGAAAAGCCATGTCAGGAGCTCCAATCATGATGGGAACAAACCAATTACCAAATCCACCAATC
ATAATGGGCATTACAAAGAAAAAAAATCATTAATAATGCTTGGCTAGTAATTAATACATTGTATAATTGAT
AATTACCAGATAAAATTTGTGAACCCGGGTGTGCTAACTCCATACGAATAATTACACTTAGAATCGTACCCT
AGTCACTCCA

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21. Name: *Acanthophora Sp. Nov.* (Bast & Rani)

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13E

CUP Voucher ID: CUPVOUCHER-MDP-2014-AS-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-AS-1

Classification:-

Class: Florideophyceae

Order: Ceramiales

Family: Rhodomelaceae

Morphology:-

ID Features:

Thallus is remiform and bushy type. It grows on intertidal rocks or coralline stones in mid littoral zone. Plants dark red to brownish red in colour, up to 20 cm tall, bushy , erect, cylindrical and attached to substratum by irregularly lobed discs; main axes with spines, branches irregular or alternate, scarce, branchlets spirally disposed, ultimate short branchlets covered with short spines. Cells are oval, elongated and rectangular in shape.

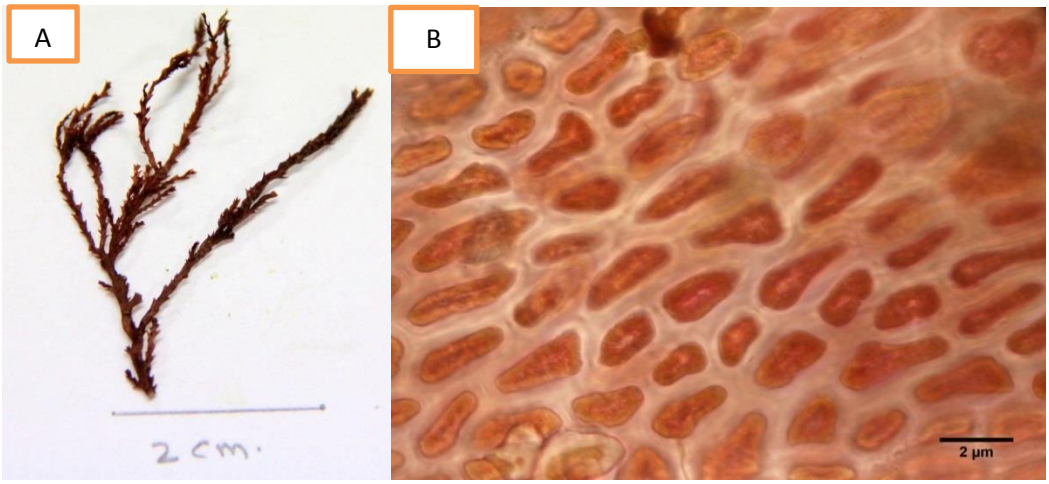

Figure 23. A. Photograph showing the complete thalli of the processed algal sample**; B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz R: *Acanthophora Sp. Nov.*

- Query cover: 97 %
- %identity: 92%

>MDP-13E-COX1gaz R (Mandapam) Tamil Nadu

GGCTGGATCAAAAAAGAGGTATTAAAATTCCTATCAGTTAATAACATAGTTATAGCTCCTGCTAATACTGG
AACTGCCAATAATAATAAAAATGCTGTAACAAAAATTGATCAAACAAATAAAGGGATTCTATAAAATGTTT
GTCCAGGATTACGCATATTTAATATTGTAGAAATAAAATTAATTGCACCTAAAATAGATGAAGCTCCTGAT
AAATGTAAACTAAATATTGCTAAATCAACAGAAGCACCAGAATGACTCTGTATAGAACTCAAAGGTGGAT
AAACCGTCCAACCTGTACCTGTACCTACTTCTACTATAGATGACAATAATAATAAACATAATGAAGGAGGT
AATAATCAAAAAGAAATATTATTTAATCTAGGAAATGCCATATCAGGACTTCCAATCATAATTGGTACAAAT
CAATTACCAAATCCTCCTATCATTACCGGCATGACCATAAAAAATATCATTAAAAATGCATGAGCTGTAATA
AGAACATTATAAATTTGATGATTACCTAAAAGTAATTGATTCCCAGGTTGAGCTAATTCCATACGAATTAAC
ATTGACATACAACCCCCCAATACTCCAGAAAAAGATCCAAAAATTAAATATAAAGTACCAATATCTTTA

NOTE:- This sample was identified as new species of genus *Acanthophora*.

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22. Name: *Dilsea socialis* (Postels & Ruprecht) Perestenko

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen voucher ID: MDP-13F

CUP Voucher ID: CUPVOUCHER-MDP-2014-DS-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-DS-1

Classification:-

Class: Florideophyceae

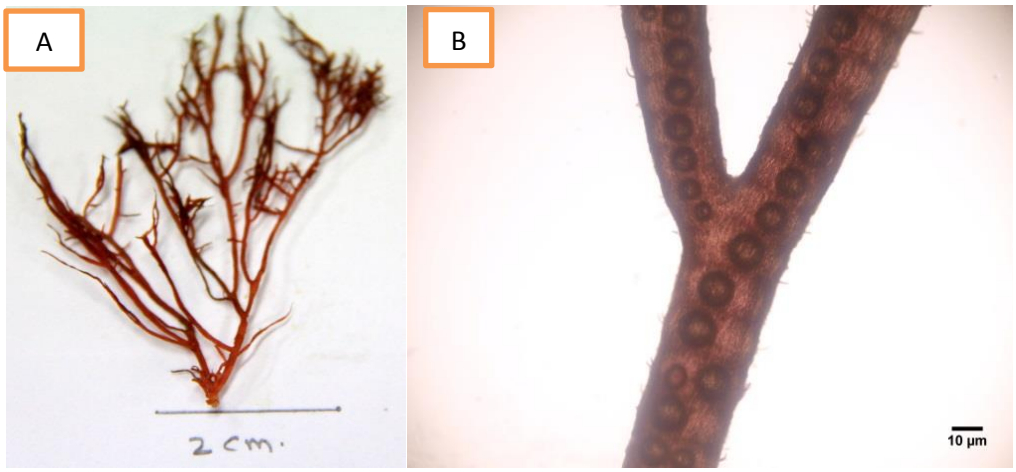
Order: Gigartinales

Family: Dilceaceae

Morphology:-

ID Features:

Thallus is bushy and thallus attached to the substratum of rocks or calcareous shell. Plant is highly branched in structure. Plant is red in colour and 10-20 cm long. Main axes are long and have smooth surface. Secondary branches are comparative short and scale like structure at surface. Primary branches are dichotomously branched but secondary branches are trichotomous or tetrachotomous. Cells are elongated, nucleated and densely packed.



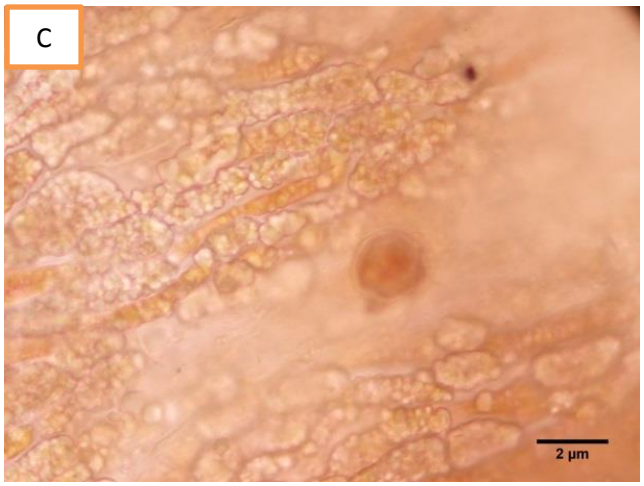


Figure 24. A. Photograph showing the complete thalli of the processed algal sample. **B and C.** Micro-photographs of a part of thallus at 10X and 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Dilsea socialis*

- Query cover: 99%
- %identity: 86%

>MDP-13F-COX1gaz F & R (Mandapam) Tamil Nadu

TTCAACAAAATCATAAAGATATTGGTACTCTTTATTTAATTTTTGGAGCGTTTTCAGGAATTTTGGGTGGTT
GTATGTCAATGCTTATTCGCATGGAATTAGCGCAACCTGGAAATCATTTATTAATGGGTAATTATCAAGTTT
ACAACGTTTTAATAACAGCGCATGCATTTTTAATGATTTTTTTTATGGTTATGCCTGTAATGATTGGTGGGT
TTGGAAATTGATTTGTACCAATTATGATTGGTAGTCCTGATATGGCATTTCCACGTTTAAATAATATATCTTT
TTGATTATTACCACCATCTTTATGTTTACTTCTTAGTTCAGCTATAGTTGAAATTGGTGTTGGAACTGGATG
GACAGTGTATCCGCCATTAAGTTCAATTCAAAGCCATTCAGGAGCTGCAGTAGATTTAGCTATTTTTAGTTT
ACATTTATCAGGAGCTTCTTCTATTCTAGGTGCAATTAACTTTATTTCAACAATTTTAAATATGCGTAATCCA
GGGCAAAGTATGTACAGAATTCCTTTATTTGTATGATCCATTTTTGTTACTGCTTTTTTATTACTATTAGCTG
TACCAGTTTTAGCAGGTGCGATTACTATGTTGTTAACTGATCGTAWWTTCAACACTTCATTTTTTGATCCA
GCAGGTGGTGGTGATCCAATTCTATATCAACATTTATTCTGATTTTTTGGACATCCCAGAAG

Note:- This sample was first time reported from India. No any previous record from family *Dilceacae.*

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23. Name: *Hypnea stelullifera* (J. Agardh) Yamagishi & Masuda

Location: Bekal (Kerala); 12° 22′ 0.12″ N, 75° 3′ 0″ E

Collection date: 13-09-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: BEK-23.4

CUP Voucher ID: CUPVOUCHER-BEK-2014-HS-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-BEK-2014-HS-1

Classification:-

Class: Florideophyceae

Order: Gigartinales

Family: Cystocloniaceae

Morphology:-

ID Features:

Thallus is of bushy appearance with spinous ramuli which grows on intertidal rocks in mid littoral zone. Plant greenish to pinkish red in colour with main axes arising from the basal part, alternately branched, branches caespitose, densely covered with spinous ramuli throughout the length, less densely covered towards the apex; spines given out all round, held horizontally in lower parts of branches and patent towards the tip, mostly simple, tapering from base to the acuminate tip, alternately forked.

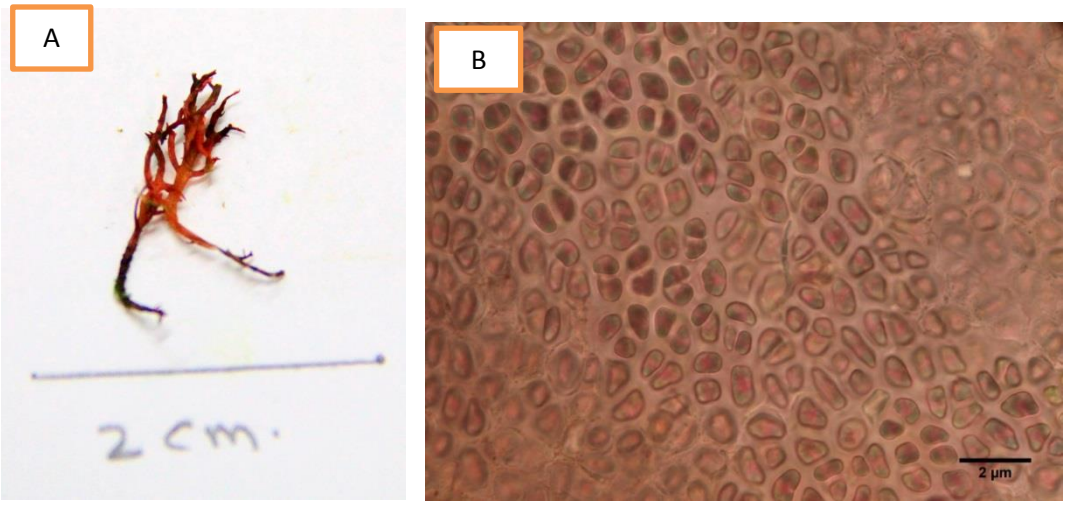

Figure 25. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX F & R: *Hypnea stelullifera*

- Query cover: 89%
- %identity: 86%

>BEK-23.4-COX1gazF & R (Bekal) Kerala

TCACAAAAAATCGAATAACAAGATGTTGATATCAAACGGGATCTACCTCCCACCGCAGGCATCAAAACAAT
GATGTATTAAAATTTCGATCCGTTAGAAGCATTGTGATCGCACCTGCTAAAACAGGTACTGCTAGTAATAA
TAAGAATGCTGTACTAAGATCGACCACACGAATAACGGTATTCTATACTTGCTTTGTCCCGGACTTCTCATG
TTTAAAATAGTTGMAATAAAGTTTACGGCTCCTAATATTGAAGAAGCTCCTGAAATGTGTAAACTCAATAT
TGCTAAATCTACKGCTCCTCCTGAATGACTTTGTATAGAGCTTAAAGGTGGATAAACTGTTCAACCTGTACC
TACTCCTACTTCCACTAAAGCTGAAATAATAATAAACATAACGAAGGTGGAAGTAACCAAAAAGAAATATT
ATTTAACGAGGGAATGCCATATCAGGASTWCCTATCATTATAGGTACCAATCATTACCAAATCCTCCTATCA
TAACAGGCATTACCATAAAGAAAATCATTAGAAACGCATGTGCTGTTATTAATACATTATAAACTTGGTGA
TTACCTAGATAGTAACTGATTACTGGTTGGGCTAATTCCATACGAATCAACATCAGACATGATCCCCTAAAC
CCCGAAAAGCTCCAAAATAAATATACGTACATATCTAGTTTTTTTTAAA

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24. Name: *Sirodotia tenuissima* (F.S. Collins) Skuja EC L.H. Flint

Location: Havelock (Andaman); 11° 58′ 0″ N, 93° 0′ 0″ E

Collection date: 15-01-2014

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: HAV-25

CUP Voucher ID: CUPVOUCHER-HAV-2014-ST-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-HAV-2014-ST-1

Classification:-

Class: Florideophyceae

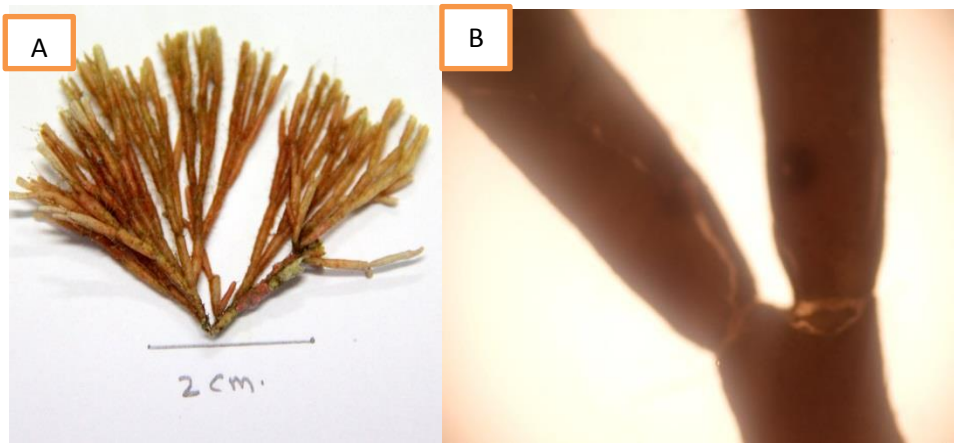
Order: Batrachospermales

Family: Batrachospermaceae

Morphology:-

ID Features:

Thallus is of bushy in appearance and attached to the rocks in marine water. Plant is highly branched and light brown in colour. Plant is 5-10 cm long. Branches are tubular in structure and dichotomously branched. Cells are pentagonal in shape and tightly packed.



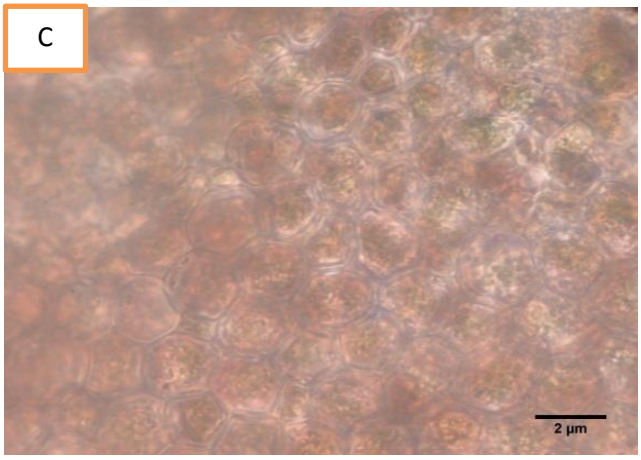


Figure 26. A. Photograph showing the complete thalli of the processed algal sample**; B and C.** Micro-photographs of a part of thallus at 10X and 100X.

DNA Sequence data:-

Based on ITS1 & ITS2*: Sirodotia tenuissima*

- Query cover: 10 %
- %identity: 87%

>HAV-25-ITS1 & ITS2 (Havelock) Andaman

TGAGCGGATGGGGGGTACTCCAGGGAAAAAATCGCCTGCGGGGCGGGGGGAGGGACCGTCACTTTTTA
AGTCAAGCGAGGGCTTTTTCAAAAATGCCTGAATGTAAAAAAAAAAAAAAAAAAGGCCTCTTTATGTCGG
GAGTCCAATATTCTTCCGGATCATCAGTTATGCCGAGAAAGACCCCTTACTATATGTTACTTTTTCTTTCCTT
CAAAAAGAGGGCAACAACCTTTTTAACTTGACCCAAAAAAAAAAAGGAAACGTGGGTGCTGCGTTCTTCA
TCGATTGCTGCGTTCTATGATCGATGCGGGAGCCAAGATATCCTCCGTCACGAGTTTTTTCTTGGTTTCGTA
AAGAATTCCTTTTGGTTGTAGTTTGCATTAGGTAGTTGAAAGGTTGAAAGAAAAAAAATCGTAGAGAAAA
AGGAAAGCAGGTTACCTTCCCTATTCTCTCGCCAAAAAAAGGAAGCGCAGGAGGCTTTCATTTTTTTGTGA
TGAGCAACCGAAWTGTAATTTCTCCCAGTGCCCCCGGAGGGGATGTTAAACGAAACTCTCCAAACCCCCC
CGCAGGACCTTCATGAATTCTGCATGATATTTGGACCCAACGGGCCCTTGGGACTCCATTCGTTCCCCCAA
AAAAAGCAACCCCCGCCTTCTTTTTGGGAAAAAAGGGGAGGAACCCCCTTTTTTCTCCAATTTTTTTTTCCC
CCCCCGGGAACACAAAAAAGATTTTTAAAACAAAAAAACGGGCGGGTTTTTTGCCCCCTTAAAAAAACCAA

Note:- This sample was first time reported from Andaman Island.

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25. Name: *Dichotomaria Sp. Nov.* (Bast & Rani)

Location: Havelock (Andaman); 11° 58′ 0″ N, 93° 0′ 0″ E

Collection date: 15-01-2014

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: HAV-26

CUP Voucher ID: CUPVOUCHER-HAV-2014-DM-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-HAV-2014-DM-1

Classification:-

Class: Florideophyceae

Order: Nemaliales

Family: Galaxauraceae

Morphology:-

ID Features:

Thallus is of bushy in appearance and grows on rocks in marine water. Plant is dark pink or red in colour. Plant is 15-40 cm long. It is highly branched structure, mainly dichotomous, hollow tubular branches, and regular ring like pattern at the surface of branches. Distal ends of the branches having mouth like opening. Transverse section of branches consists of white slimy material. Cells are irregular in shape and arrangement.

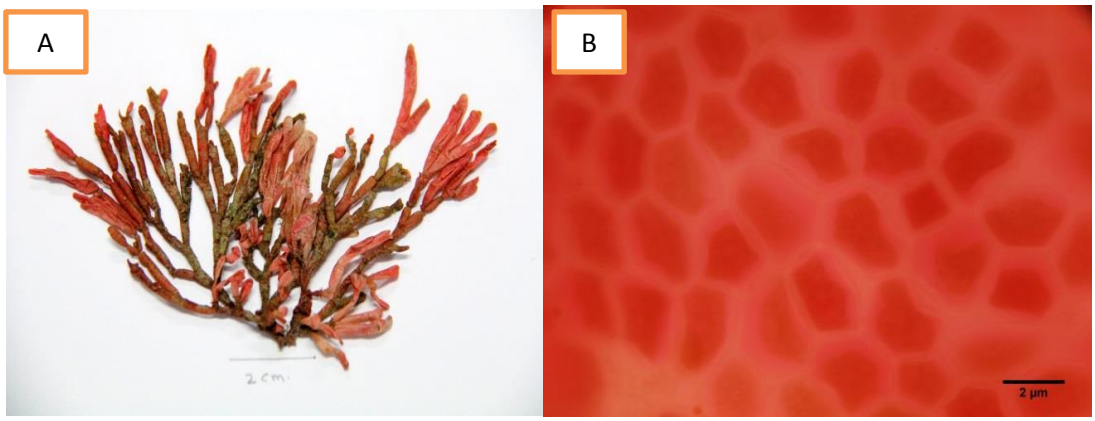

Figure 27. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Dichotomaria Sp. Nov.*

- Query cover: 89 %
- %identity: 93%

>HAV-26-COX1gaz F & R (Havelock) Andaman

GGTCGAAAATGATGTATTAAAATTTCGATCTGTTAAAAGCATTGTAATTGCTCCTGCTAAAACTGGAACTG
CTAAAAGTAATAAGAAAGCTGTAACAAAAATAGATCAAACAAATAAAGGCATTCTATACATACTTTGTCCC
GGATTACGCATATTTAATATTGTGGAAATAAAATTTACTGCACCTAAAATTGATGAAGCTCCTGATAAATGT
AAACTAAATATTGCTAAATCTACAGAACCTCCTGAATGACTTTGAATTGAACTTAAAGGAGGATAAACTGT
TCAACCAGTTCCTGCACCTACTTCTACTAAAGATGAAACTAAAAGTAAACACAAAGAAGGAGGTAATAATC
AAAAACTTATATTGTTTAATCTCGGAAAAGCCATATCAGGACTACCTATCATGATAGGAACTAACCAATTAC
CAAAACCTCCTATCATAATTGGCATAACCATAAAAAAAATCATTAAAAATGCATGTGCAGTTATTAAAACAT
TATAAGTTTGATGATTTCCTGCTAATAAATGATTTCCTGGTTGTGCTAACTCCATTCTTATCAATAATGACAT
ACATCCTCCTAAAACTCCTGAAAAAGCACCAAAAACTAAATATAAAGTC

NOTE:- This sample was identified as a new species of genus *Dichotomaria*.

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26. Name: *Sargassum zhangii* C.K.Tsang & Lu Baoren

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13B

CUP Voucher ID: CUPVOUCHER-MDP-2014-SZ-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-SZ-1

Classification:-

Class: Phaeophyceae

Order: Fucales

Family: Sargassaceae

Morphology:-

ID Features:

Sampled algal thalli were brown, terete, 20-30 cm in length and had numerous globular, hollow vesicles towards the apical parts .Thalli were attached to the substrata via discoid holdfast. Primary axis was cylindrical, spinous and was about 2mm in diameter. Secondary branches were arising from leaf axils of primary branches. Compressed and lanceolate leaves were seen arising from both primary and secondary branches with their pattern being alternative and size around 2 cm in length and 6-9 mm in breadth. Leaves were undulated, had numerous conspicuous and raised glandular dots (Cryptostomata) throughout the surface and had round tips. Older leaves had distinct midrib.

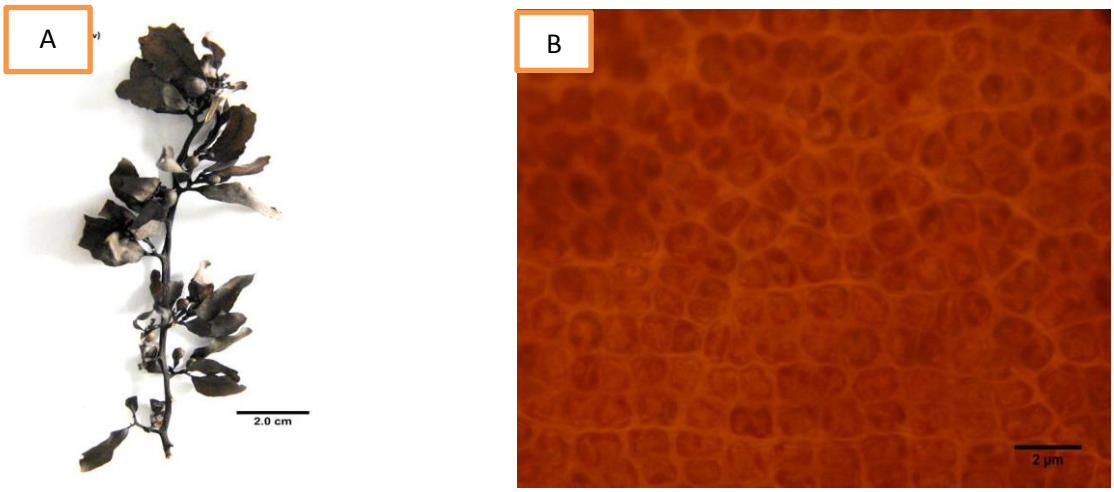

Figure 28. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Sargassum zhangii*

- Query cover: 90%
- %identity: 97 %

>MDP-13B-COX1gaz F & R-(Mandapam) Tamil Nadu

TTTCAAACAAAATCATAAAGATATTGGTACGTTATATTTAATATTTGGGGCTTTTTCCCGGTGTTTTAGGTA
CAGCGATGTCTGTTCTTATAAGGTTGCAGCTTGCAAGCCCGGGCAATATGTTTTTGGGGGGGAATTATCAG
TTATATAATGTTATTGTTACGGCTCATGCTTTTTTGATGATTTTCTTTATGGTTATGCCTGTTCTTATAGGGG
GTTTTGGTAATTGGTTTGTTCCTTTAATGATAGGTGCTCCAGATATGGCGTTTCCTCGTATGAATAATATAA
GCTTTTGGTTGTTACCGCCGTCTTTAATACTTCTTTTAGCATCCTCATTAGTGGAGGCTGGAGCCGGTACAG
GTTGGACCGTTTATCCACCGTTAAGTGGTATCCAGGCGCATTCGGGCCCATCAGTGGATTTAGCTATCTTTA
GTTTACATTTATCTGGTGCTGCTTCTATTTTGGGTGCCATTAATTTCATAACAACCATTTTCAATATGCGTGC
TCCGGGTATGGGTATGCACCGTTTACCTTTATTTGTTTGGTCTGTTTTGATAACAGCATTTTTACTTTTATTA
TCTCTTCCGGTTTTGGCTGGAGGCATTACTATGCTTTTAACGGACCGTAATTTTAATACTACATTTTTGATCC
G

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27. Name: *Sargassum megalocystum* Tseng & Lu

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13N

CUP Voucher ID: CUPVOUCHER-MDP-2014-SM-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-SM-1

Classification:-

Class: Phaeophyceae

Order: Fucales

Family: Sargassaceae

Morphology:-

ID Features:

Sampled algal thalli were brown, terete, 20-30 cm in length and had numerous globular, hollow vesicles towards the apical parts .Thalli were attached to the substrata via discoid holdfast. Primary axis was cylindrical, spinous and was about 2mm in diameter. Secondary branches were arising from leaf axils of primary branches. Compressed and lanceolate leaves were seen arising from both primary and secondary branches with their pattern being alternative and size around 2 cm in length and 6-9 mm in breadth. Leaves were undulated, had numerous conspicuous and raised glandular dots (cryptostomata) throughout the surface and had round tips. Older leaves had distinct midrib.

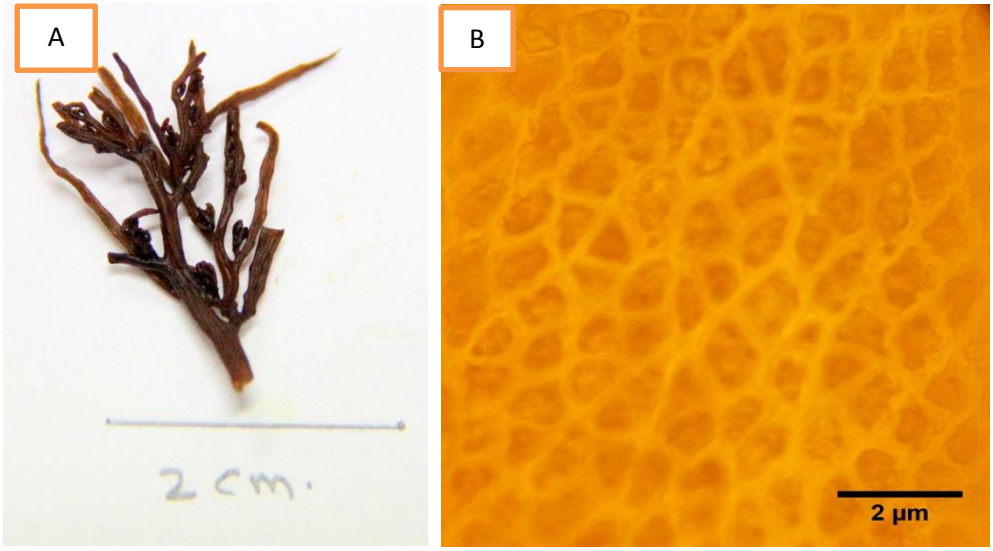

Figure 29. A. Photograph showing the complete thalli of the processed algal sample; **B**. Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on ITS2: *Sargassum megalocystum*

- Query cover: 99%
- %identity: 99 %

>MDP-13N-ITS2 (Mandapam) Tamil Nadu

CCGAGCCTCAAGCCGCCCGCGCACTCCCCGGCGACCCGTCGTGTCCCCACCCCGCGATAGGGGCGCCGTC
AACAACGGATCCTCCGGAGGACGCAAGGTAAACTTGCCTCCAGCGATGCCTCATGGTCACCCGCGCACAT
CCCTGGCGACTCGTCGGTGTCCCCGCCCCGTGAGAGGGGGCGCGGTCAATAACGGATCTTCCGGAGGAC
GCAAGGTGGACCGGTCTACAGCGCCGCAAAGACAATAGAAGCCTGGACAATCGGTAGCTCTCTAGGCTTT
GGTGGACTCAGGGGACGAGCAGGCAGCTTGTACAAAAATATACACACCACACCACCGCTCCCCGGAAACA
CTCAGATTTCCGC

Based on COX1gaz F & R: *Sargassum megalocystum*

- Query cover: 66%
- %identity: 91%

>MDP-13N-COX1gazF & R (Mandapam) Tamil Nadu

CCAGATAAATGTAAACTGAAAATGGCTAAATCCACTGATGGTCCCGAATGAGCTTGGATACAACTTAAAG
GTGGATAAACAGTCCAACCTGTACCGGCCCCAACCTCCACTAATGAAGATGCCAATAGAAGTATTAAAGAT
GGCGGTAACAACCAAAAGCTTATATTATTCATACGAGGAAACGCCATATCTGGACCACCTATCATTAAAGG
AACAAACCAATTACCAAACCCCCCTATCATAACAGGCATAACCATAAAAAAAATCATTAAAAAAGCATGA

Note:- *Sargassum megalocystum* was first time reported from India.

28. Name: *Sargassum aquifolium* (Turner) C. Agardh

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13O

CUP Voucher ID: CUPVOUCHER-MDP-2014-SA-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-SA-1

Classification:-

Class: Phaeophyceae

Order: Fucales

Family: Sargassaceae

Morphology:-

ID Features:

Sampled algal thalli were brown, terete, 20-30 cm in length and had numerous globular, hollow vesicles towards the apical parts .Thalli were attached to the substrata via discoid holdfast. Primary axis was cylindrical, spinous and was about 2mm in diameter. Secondary branches were arising from leaf axils of primary branches. Compressed and lanceolate leaves were seen arising from both primary and secondary branches with their pattern being alternative and size around 2 cm in length and 6-9 mm in breadth. Leaves were undulated, had numerous conspicuous and raised glandular dots (cryptostomata) throughout the surface and had round tips. Older leaves had distinct midrib.

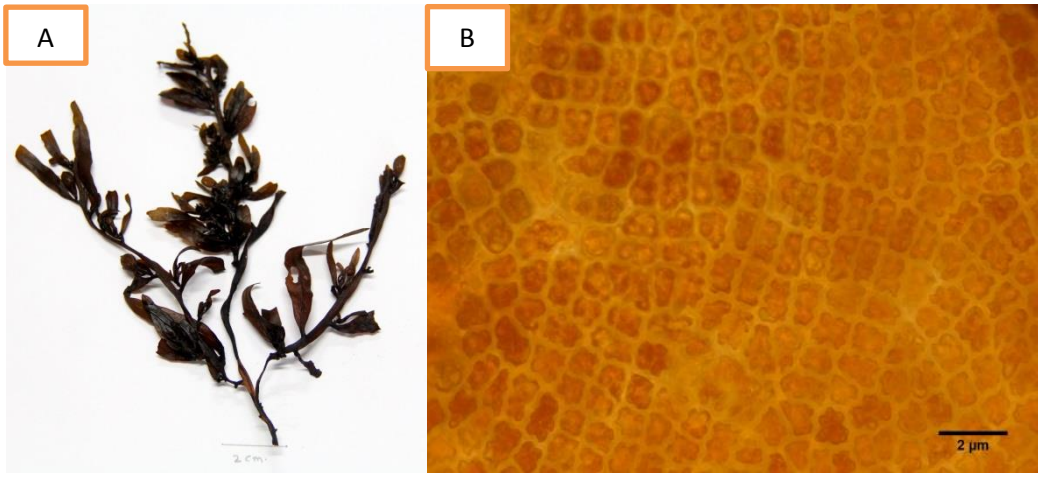

Figure 30. A. Photograph showing the complete thalli of the processed algal sample; **B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Sargassum aquifolium*

- Query cover: 81%
- %identity: 99%

>MDP-13O-COX1gazF & R (Mandapam) Tamil Nadu

TTTCAACAAATCATAAAGATATTGGTACTTTATATTTAAATATTTGGGGCTTTTTCTGGTGTTTTAGGCACAG
CGATGTCTGTTCTTATAAGGTTGCAGCTTT-CAAGTCCCGGCAATACGTTTTTGGGG
GGGAATTATCAGTTGTATAACGTTATTGTTACGGCTCATGCCTTTTTAATGATTTTCTTTATGGTTATGCCTG
TTCTTATAGGGGGGTTTGGTAATTGGTTTGTTCCTTTAATGATAGGTGCTCCAGATATGGCGTTTCCTCGTA
TGAATAATATAAGCTTTTGGTTGTTACCGCCATCTTTAATACTTCTTTTGGCATCCTCACTAGTGGAGGCTG
GAGCCGGTACAGGTTGGACTGTTTATCCACCTTTAAGTGGTATCCAGGCGCATTCGGGACCATCAGTGGAT
TTAGCTATCTTTAGTTTACATTTATCTGGTGCTGCCTCTATTTTAGGTGCTATTAATTTCATAACAACCATTTT
TAATATGCGTGCTCCGGGTATGGGTATGCACCGTTTACCTCTATTTGTTTGGTCTGTTTTAATAACAGCATT
TTTACTTTTATTATCTCTTCCGGTTTTGGCCGGGGGTATTACTATGCTTTTAAGCGGATCGCAATTTTAATAC
TACATGTGTG

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29. Name: *Turbinaria ornata* (Turner) J. Agardh

Location: Mandapam (Tamil Nadu); 9° 16′ 48″ N, 79° 7′ 12″ E

Collection date: 19-07-2012

Collected by: Felix Bast

Identified by: Pooja Rani

Frozen Voucher ID: MDP-13C

CUP Voucher ID: CUPVOUCHER-MDP-2014-TO-1

Central National Herbarium Voucher ID: CAL-CUPVOUCHER-MDP-2014-TO-1

Classification:-

Class: Phaeophyceae

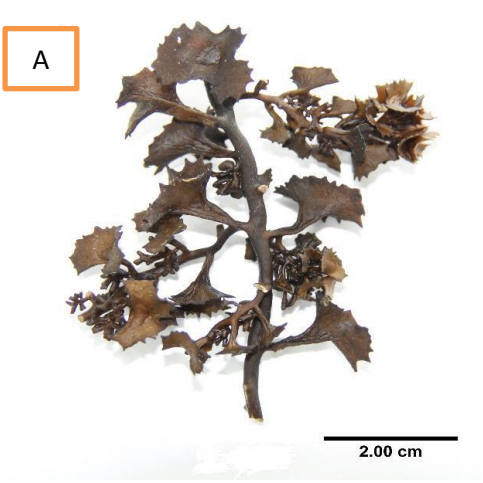
Order: Fucales

Family: Sargassaceae

Morphology:-

ID Features:

Thallus is ramified with obconical leaves. This algae generally grow attached to the rocks in infra littoral fringe and sub littoral zone. Plants dark brown in colour, up to 50 cm tall, bushy, axes arising from dichotomously branched holdfast; main axes erect and cylindrical and irregularly branched; leaves closely arranged, turbinate to obconical, coarse, 0.5-1.5 cm long, 10-15 mm broad at the distal ends; distal ends of the leaves triangular, sub-concave with double row of spines on the surface with terete stalks; vesicles immerged in the leaves; arising on the stalks of the upper leaves.



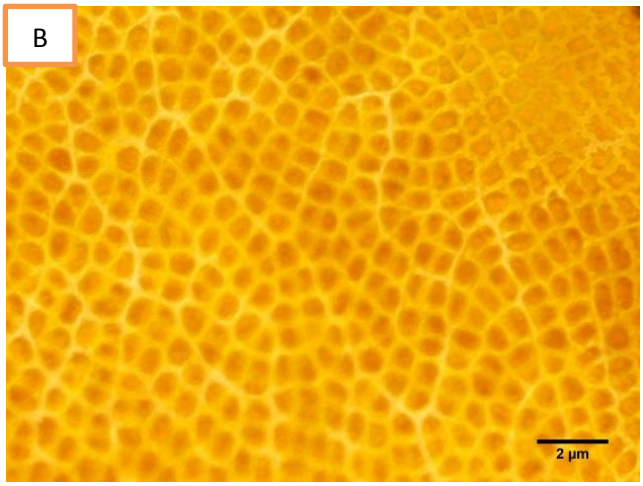


Figure 31. A. Photograph showing the complete thalli of the processed algal sample**; B.** Micro-photograph of a part of thallus at 100X.

DNA Sequence data:-

Based on COX1gaz F & R: *Turbinaria ornata*

- Query cover: 51%
- %identity: 98%

>MDP-13C-COX1gaz F & R (Mandapam) Tamil Nadu

TTTTTTAAAAAAAAAAAAACAAGATATTGGTACGCTATATTTTTATTTTTTGGAGCTTTTTCGGGAGTTTTG
GGAGGGTTGTATGTGTATGTTAATTCGTATGGAATTAGCACAACCTGGTAATCATTTATTATTAGGTAATC
ATCAAGTTTACAATGTTTTAATTACAGCACACGCATTTTTAATGATTTTCTTCATGGTAATGCCTGTAATGAT
AGGAGGATTTGGTAATTGATTAGTACCTATAATGATTGGAAGCCCAGATATGGCATTCCCTCGATTAAACA
ATATTTCTTTTTGATTACTTCCTCCATCTTTATTTTTATACTTCTACAGACACAGCTGATTTTGTTGAAGTAGC
AACTGAAAGTCCATAAAGTTATTCTGTTCTACAGTTTAATTTTTATTATAAAATCTAAAAGAATATATTAGC
GAGCTTTAATCTTTTTAATACTTTACATTAAAATAAAAAACATAAAAAGTTTGGTAGTTAACTAAAAATAAA
ACTAAAATATTTACATTAGATAAAAGAATTGAAGAGTAATGAGACTTACACAAGGATGTTTTTCATTTTTAC
CAGATTTAAGTGACGAGCAAATTAAAAATCAAGTTGCTTATGCTATTTCAAAAGGTTGGGCCGTTAGTGTA
GAATGGACTGATGATCCTCACCCCCGCAATGCGTATTGGGAATTATGGGGACTACCATTATTTGACGTCAT
AAA

Phylogeographic analysis of Green algae:-

BLASTN revealed that, among the Green algae (Chlorophyta) samples 10 sequences was showing homology to different species of *Ulva* i.e. (ETT-2) *Ulva reticulata*, (KAN-6.3) *Ulva intestinalis*, (KAN-6.4) *Ulva intestinalis*, (PON-7) *Ulva intestinalis*, (CAL-10) *Ulva reticulata*, (MAN-14.1) *Ulva fasciata*, (MAN-13M) *Ulva fasciata*, (KOV-T-1.1) *Ulva prolifera*, (ENN-T-8) *Ulva prolifera*, (TAM-T-7.2) *Ulva ohnoi* and one sample with (MAN-13D) *Caulerpa scalpelliformis*. One sequence of *Chara australis* was taken as out-group. Sequence downloaded from NCBI. For Phylogeographic analysis of Green algae from Indian subcontinent, sequences were first aligned by MUSCLE algorithm in MEGA (Appendix-E). Best fitting nucleotide substitution models were tested using ML-Model test in MEGA. The model with lowest Bayesian information criterion (BIC) score was K2+G (Kimura-2-parameter+gamma distribution) i.e. with BIC score 18584 using 23 parameters (Table.4). Numbers near nodes represent posterior probabilities of the phylogenetic tree (Fig.32). Pairwise distances between sequences were calculated using Kimura-2-parameter model in MEGA. Pairwise distance ranged between 0.063 and 2.038. Distance matrix (Table.5.) based on Green algae revealed that CAL-10-*Ulva reticulata* from Calicut had shown least distance with another sample, ETT-2-*Ulva reticulata* from Ettikulam i.e. 0.063. Both samples belong to same species and form same clade in the phylogenetic tree. On the other hand, green algae samples CAL-10-*Ulva reticulata* from Calicut and T-7.2-*Ulva ohnoi* from Tamil Nadu had least distance, and they form same clade. Phylogenetic distance of these two samples was more than *Ulva reticulata* of Ettikulam, but it has longer branch length than CAL-10 sample. Most of the samples belonged to genus *Ulva* only one sample had been showing similarity with *Caulerpa scalpelliformis*. So it formed a very long and separate branch in the tree.

Table 4: Maximum Likelihood estimation of goodness of fit of 24 different nucleotide substitution models of Green algae using MEGA software.

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	(+*I*)	(+*G*)	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(
K2+G	23	18584.991	18425.803	-9189.828	n/a	3.60	1.17	0.250	0.250	0.250	0.250	0.057	0.057	0.135	0.057	0.135	0.057	0.057	0.135	0.(
T92+G	24	18592.816	18426.714	-9189.277	n/a	3.60	1.17	0.251	0.251	0.249	0.249	0.058	0.057	0.135	0.058	0.135	0.057	0.058	0.135	0.(
K2+G+I	24	18593.918	18427.816	-9189.828	0.00	3.60	1.17	0.250	0.250	0.250	0.250	0.057	0.057	0.135	0.057	0.135	0.057	0.057	0.135	0.(
T92+G+I	25	18601.744	18428.727	-9189.277	0.00	3.60	1.17	0.251	0.251	0.249	0.249	0.058	0.057	0.135	0.058	0.135	0.057	0.058	0.135	0.(
HKY+G	26	18609.921	18429.991	-9188.902	n/a	3.61	1.17	0.248	0.253	0.254	0.244	0.058	0.058	0.132	0.057	0.137	0.056	0.057	0.137	0.(
TN93+G	27	18618.817	18431.974	-9188.886	n/a	3.59	1.18	0.248	0.253	0.254	0.244	0.058	0.058	0.131	0.057	0.139	0.056	0.057	0.138	0.(
HKY+G+I	27	18618.848	18432.005	-9188.902	0.00	3.61	1.17	0.248	0.253	0.254	0.244	0.058	0.058	0.132	0.057	0.137	0.056	0.057	0.137	0.(
TN93+G+I	28	18627.745	18433.989	-9188.886	0.00	3.59	1.18	0.248	0.253	0.254	0.244	0.058	0.058	0.131	0.057	0.139	0.056	0.057	0.138	0.(
K2	22	18628.427	18476.155	-9216.010	n/a	n/a	1.35	0.250	0.250	0.250	0.250	0.053	0.053	0.143	0.053	0.143	0.053	0.053	0.143	0.(
K2+I	23	18629.225	18470.037	-9211.945	0.01	n/a	1.35	0.250	0.250	0.250	0.250	0.053	0.053	0.143	0.053	0.143	0.053	0.053	0.143	0.(
T92	23	18636.297	18477.110	-9215.481	n/a	n/a	1.35	0.251	0.251	0.249	0.249	0.053	0.053	0.143	0.053	0.143	0.053	0.053	0.144	0.(
T92+I	24	18637.086	18470.983	-9211.412	0.01	n/a	1.35	0.251	0.251	0.249	0.249	0.053	0.053	0.143	0.053	0.143	0.053	0.053	0.144	0.(
GTR+G	30	18642.828	18435.249	-9187.500	n/a	3.61	1.17	0.248	0.253	0.254	0.244	0.061	0.057	0.130	0.059	0.138	0.064	0.056	0.138	0.(
GTR+G+I	31	18651.756	18437.265	-9187.500	0.00	3.61	1.17	0.248	0.253	0.254	0.244	0.061	0.057	0.130	0.059	0.138	0.064	0.056	0.138	0.(
HKY	25	18653.295	18480.278	-9215.053	n/a	n/a	1.35	0.248	0.253	0.254	0.244	0.054	0.054	0.140	0.053	0.146	0.052	0.053	0.145	0.(
HKY+I	26	18654.096	18474.166	-9210.989	0.01	n/a	1.35	0.248	0.253	0.254	0.244	0.054	0.054	0.140	0.053	0.146	0.052	0.053	0.145	0.(
GTR	29	18667.778	18467.110	-9204.439	n/a	n/a	1.04	0.248	0.253	0.254	0.244	0.065	0.060	0.125	0.063	0.129	0.067	0.059	0.128	0.(
TN93	26	18667.810	18487.880	-9217.846	n/a	n/a	1.33	0.248	0.253	0.254	0.244	0.054	0.055	0.122	0.053	0.163	0.052	0.053	0.162	0.(
TN93+I	27	18668.311	18481.467	-9213.633	0.01	n/a	1.33	0.248	0.253	0.254	0.244	0.054	0.055	0.122	0.053	0.163	0.052	0.053	0.162	0.(
GTR+I	30	18670.724	18463.144	-9201.448	0.01	n/a	1.05	0.248	0.253	0.254	0.244	0.064	0.060	0.125	0.063	0.129	0.066	0.059	0.129	0.(
JC+G	22	18708.625	18556.353	-9256.109	n/a	4.66	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+G+I	23	18717.552	18558.365	-9256.109	0.00	4.66	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC	21	18723.570	18578.214	-9268.045	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+I	22	18727.095	18574.823	-9265.344	0.01	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(

NOTE.-- Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+*G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+*I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well. They are followed by nucleotide frequencies (*f*) and rates of base substitutions (*r*) for each nucleotide pair. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 12 nucleotide sequences. There were a total of 1429 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [2].

Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

1. Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

2. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.

Table.5. Pairwise distance matrix using K2+G Model for ITS1 sequences from Green algae

		A	B	C	D	E	F	G	H	I	J	K	L
A	ETT-2-*U.reticulata* (Kerala)												
B	Kan-6.3-*U.intestinalis* (Kerala)	0.886											
C	Kan-6.4-*U.intestinalis* (Kerala)	1.538	1.273										
D	PON-7-*U.intestinalis* (Kerala)	1.224	1.030	1.145									
E	CAL-10-*U.reticulata* (Kerala)	0.063	0.968	1.646	1.257								
F	MAN-14.1-*U.fasciata* (Karnataka)	0.254	0.924	2.019	1.000	0.254							
G	MDP-13M-*U.fasciata* (Tamil Nadu)	0.960	0.387	0.681	1.008	0.914	1.052						
H	KOV-T-1.1-*U.prolifera* (Tamil Nadu)	1.224	1.070	1.490	1.427	1.224	1.065	1.432					
I	T-7.2-*U.Ohnoi* (Tamil Nadu)	0.152	0.839	1.577	0.999	0.164	0.208	0.865	1.267				
J	ENN-T-8-*U.prolifera* (Tamil Nadu)	0.336	1.145	1.426	1.052	0.319	0.305	1.086	1.630	0.372			
K	MDP-13D-*C.Scalpelliformis* (Tamil Nadu)	1.683	2.038	1.469	1.239	1.485	1.976	1.401	1.400	1.468	1.946		
L	AF033652.1|*Chara australis*	1.061	1.350	0.936	1.518	1.114	1.046	1.585	1.427	1.028	1.132	1.910	

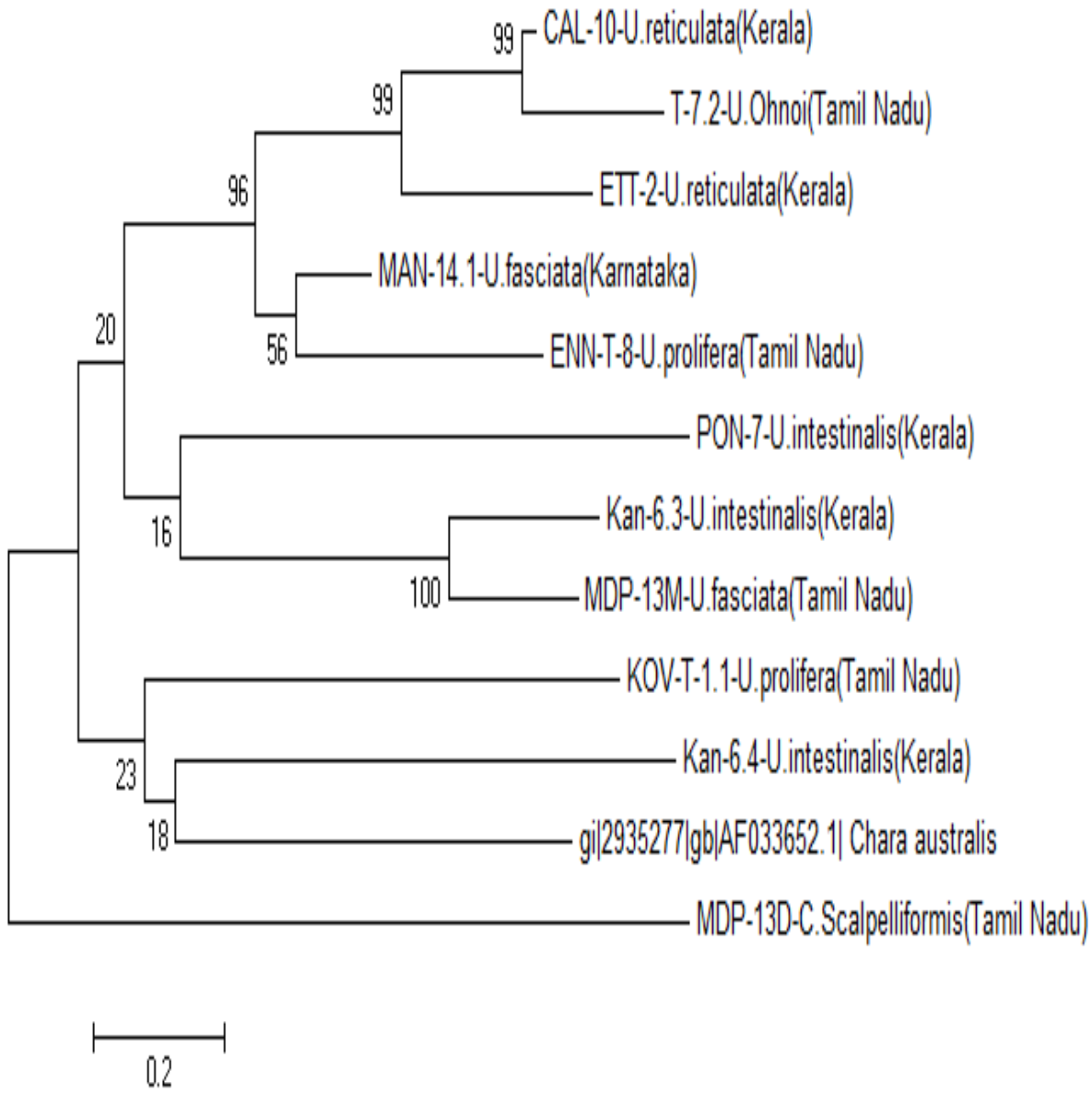


Figure.32. Maximum likelihood (ML) phylogram based on ITS1 sequences using K2+G model of molecular evolution in MEGA phylogenetic framework. Numbers near nodes represent ML bootstrap proportions exceeding 50. *LnL* = −9189.823. Scale bar is on the unit of average nucleotide substitutions per site.

Phylogeographic analysis of Red algae:-

Fourteen Red algae (Rhodophyta), samples, showed homology to (ETT-4) *Gracilaria folifera*, (MAN-13I) *Gracilaria domingensis*, (BEK-23.1) *Gracilaria corticata*, (ETT-5) *Grateloupia Sp. Nov.*, (KAN-6.2) *Ceramium Sp. Nov.*, (BEK-15.2) *Centroceras clavulatum*, (ANJ-19) *Centroceras clavulatum*, (KER-11) *Erythrocladia Sp. Nov.*, (POD-T-4) *Erythrocladia irregularis*, (MAN-13E) *Acanthophora Sp. Nov.*, (MAN-13F) *Dilsea socialis*, (BEK-23.4) *Hypnea stelullifera*, (HAV-25) *Sirodotia tenuissima* and (HAV-26) *Dichotomaria Sp. Nov.*. Samples ID KER-11 and POD-T-4 were showing some surprising results as their morphology shown similarity with Green algae but BLASTN analysis of these COX1 sequences was showing homology with *Erythrocladia.* Microscopic images of these samples had shown the presence of Red coloured *Erythrocladia* under high magnification (Fig.21 and Fig.22). These findings had shown the presence of endophytic *Erythrocladia* in *Cladophora glomerata* with morphology comparable to previous reports. All samples belonged to different species except BEK-15.2 and ANJ-19 (Fig.19 and Fig.20) which were showing similarity with *Centroceras clavulatum*. (MAN-13I) *Gracilaria domingensis* (Fig.15) was the first report from India. (MAN-13F) *Dilsea socialis* (Fig. 24) belonged to family *Dilceaceae*, was reported first time in India. There was not any previous record of this family from India. *Gracilaria folifera* and *Grateloupia Sp. Nov.* (Fig.14 and Fig.17)) were first time recorded from coastal regions of Ettikulam, Kerala, India. *Gracilaria corticata* was the first time reported from coasts of Bekal, Kerala. *Sirodotia tenuissima* (Fig.26) was the first report from Andaman Islands. Most of the samples were amplified using mitochondrial (COX1, COX2, COX3) and chloroplast (RbcL) regions. So different trees constructs for each region, as all samples amplified from different genetic region. For Phylogeographic analysis of Red algae from Indian subcontinent, those samples that were amplified by COX1gaz F&R, sequences were first aligned by MUSCLE algorithm in MEGA (Appendix-F). Best fitting nucleotide substitution model were tested using ML-Model test in MEGA. The model with lowest Bayesian information criterion (BIC) score was GTR+G (General time reversible +Gamma distribution) i.e. with BIC score 7511.235 using 22 parameters (Table.6). Numbers near nodes represent posterior probabilities of phylogenetic tree (Fig.33). Pairwise distances between sequences were calculated by using General time reversible model in MEGA (Table.7). Pairwise distances

ranged between 0.014 and 1.025. When phylogenetic tree was constructed by using MEGA software for phylogeographic analysis, all samples were placed in different nodes. *Dilsea socialis* formed a separate branch in the tree (Fig.33) so it may be very distantly related to all other identified species. For Phylogeographic analysis of Red algae from Indian subcontinent, those samples that were amplified by COX F&R, sequences were first aligned by MUSCLE algorithm in MEGA (Appendix-G). Best fitting nucleotide substitution model were tested using ML-Model test in MEGA. The model with lowest Bayesian information criterion (BIC) score was T92 (Tamura-3-parameter) i.e. with BIC score 6416.827 using 9 parameters (Table.8). Numbers near nodes represent posterior probabilities of phylogenetic tree (Fig.34). Pairwise distances between sequences were calculated by using Tamura-3-parameter model in MEGA (Table.9). Pairwise distances ranged between 0.342 and 0.938. When phylogenetic tree was constructed by using MEGA software for phylogeographic analysis, all samples were placed in different nodes. *Gracilaria folifera* and *Gracilaria corticata* formed same clade (Fig.34), so these two species may be more closely related than other identified species. (HAV-25) *Sirodotia tenuissima* was amplified using ITS. It did not include in tree construction because there was not sufficient data of Red algae for tree generation. There were only three samples that were amplified by *rbcL,* so it was not sufficient data for tree generation. One sample was *Ceramium sp.* and other two samples were *Centroceras clavulatum.* Both samples of *Centroceras clavulatum* i.e. from Bekal and Anjuna, aligned for pairwise alignment (Appendix-I). Sequences of both the samples had high similarity. But there were a number of positions where mutations take place. So according to the previous review of the literature it was thought that even a small distance play an important role in causing mutations in genetic material. Distance between Bekal and Anjuna beach is 378 km. So these two locations must have some environmental differences which were enough to caused mutations in these two samples. Thallus of *Ceramium* species was showing morphological similarity with *Centroceras clavulatum*. Initially on the basis of morphological studies, it was thought that these are similar species, but molecular and microscopic studies revealed that they belonged to a different genus.

Table 6: Maximum Likelihood estimation of goodness of fit of 24 different nucleotide substitution models of Red algae using COX1gaz region of mitochondrial DNA in MEGA software.

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	*(+I)*	*(+G)*	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(CG
GTR+G	22	7511.235	7367.378	-3661.591	n/a	1.01	0.82	0.375	0.291	0.174	0.160	0.139	0.016	0.086	0.179	0.084	0.021	0.035	0.141	0.029
T92+G	16	7513.532	7408.872	-3688.383	n/a	0.79	0.87	0.333	0.333	0.167	0.167	0.084	0.042	0.083	0.084	0.083	0.042	0.084	0.165	0.042
GTR+G+I	23	7519.783	7369.396	-3661.591	0.00	1.01	0.82	0.375	0.291	0.174	0.160	0.139	0.016	0.086	0.179	0.084	0.021	0.035	0.141	0.029
T92+G+I	17	7522.080	7410.885	-3688.383	0.00	0.79	0.87	0.333	0.333	0.167	0.167	0.084	0.042	0.083	0.084	0.083	0.042	0.084	0.165	0.042
GTR+I	22	7528.055	7384.198	-3670.001	0.18	n/a	0.80	0.375	0.291	0.174	0.160	0.138	0.018	0.085	0.177	0.084	0.023	0.038	0.140	0.028
T92+I	16	7541.138	7436.477	-3702.185	0.18	n/a	0.80	0.333	0.333	0.167	0.167	0.088	0.044	0.079	0.088	0.079	0.044	0.088	0.157	0.044
HKY+G	18	7549.921	7432.192	-3698.029	n/a	0.76	0.89	0.375	0.291	0.174	0.160	0.073	0.044	0.080	0.094	0.087	0.040	0.094	0.146	0.040
GTR	21	7552.926	7415.600	-3686.710	n/a	n/a	0.78	0.375	0.291	0.174	0.160	0.134	0.021	0.080	0.172	0.084	0.024	0.045	0.141	0.031
TN93+G	19	7558.275	7434.013	-3697.932	n/a	0.76	0.89	0.375	0.291	0.174	0.160	0.073	0.044	0.083	0.094	0.083	0.040	0.094	0.139	0.040
HKY+G+I	19	7558.469	7434.207	-3698.029	0.00	0.76	0.89	0.375	0.291	0.174	0.160	0.073	0.044	0.080	0.094	0.087	0.040	0.094	0.146	0.040
T92	15	7563.628	7465.503	-3717.705	n/a	n/a	0.76	0.333	0.333	0.167	0.167	0.090	0.045	0.077	0.090	0.077	0.045	0.090	0.153	0.045
TN93+G+I	20	7566.823	7436.028	-3697.932	0.00	0.76	0.89	0.375	0.291	0.174	0.160	0.073	0.044	0.083	0.094	0.083	0.040	0.094	0.139	0.040
HKY+I	18	7572.866	7455.137	-3709.502	0.18	n/a	0.81	0.375	0.291	0.174	0.160	0.076	0.046	0.076	0.098	0.083	0.042	0.098	0.139	0.042
TN93+I	19	7580.863	7456.600	-3709.226	0.18	n/a	0.81	0.375	0.291	0.174	0.160	0.076	0.046	0.080	0.098	0.078	0.042	0.098	0.131	0.042
HKY	17	7600.003	7488.807	-3727.344	n/a	n/a	0.77	0.375	0.291	0.174	0.160	0.078	0.047	0.074	0.100	0.081	0.043	0.100	0.135	0.043
TN93	18	7608.393	7490.663	-3727.265	n/a	n/a	0.77	0.375	0.291	0.174	0.160	0.078	0.047	0.076	0.100	0.079	0.043	0.100	0.131	0.043
K2+G	15	7760.812	7662.686	-3816.296	n/a	1.68	0.75	0.250	0.250	0.250	0.250	0.071	0.071	0.107	0.071	0.107	0.071	0.071	0.107	0.071
JC+G	14	7767.672	7676.083	-3824.001	n/a	1.66	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K2+I	15	7768.781	7670.656	-3820.281	0.15	n/a	0.74	0.250	0.250	0.250	0.250	0.072	0.072	0.106	0.072	0.106	0.072	0.072	0.106	0.072
K2+G+I	16	7769.360	7664.699	-3816.296	0.00	1.68	0.75	0.250	0.250	0.250	0.250	0.071	0.071	0.107	0.071	0.107	0.071	0.071	0.107	0.071
JC+I	14	7775.358	7683.769	-3827.844	0.16	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
JC+G+I	15	7776.220	7678.095	-3824.001	0.00	1.66	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K2	14	7778.939	7687.350	-3829.634	n/a	n/a	0.73	0.250	0.250	0.250	0.250	0.072	0.072	0.105	0.072	0.105	0.072	0.072	0.105	0.072
JC	13	7785.856	7700.804	-3837.366	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083

NOTE.-- Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (*+G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (*+I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well. They are followed by nucleotide frequencies (*f*) and rates of base substitutions (*r*) for each nucleotide pair. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 8 nucleotide sequences. There were a total of 872 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [2].

Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

1. Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

2. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.

Table.7. Pairwise distance matrix using GTR+G model for COX1 sequences from Red algae.

		A.	B.	C.	D.	E.	F.	G.	H
A.	MDP-13F-*Gracilaria domingensis* (Mandapam)								
B.	ETT-5-*Grateloupia Sp. Nov.*(Ettikulam)	0.387							
C.	KAN-6.2 *Ceramium Sp. Nov*. (Kannur)	0.321	0.234						
D.	KER-11-*Erythrocladia Sp. Nov*. (Elathur)	0.401	0.021	0.254					
E.	POD-T-4-*Erythrocladia irregularis* (Pondicherry)	0.014	0.375	0.318	0.389				
F.	MDP-13E-*Acanthophora Sp. Nov.* (Mandapam)	0.347	0.212	0.231	0.222	0.347			
G.	MDP-13F-*Dilsea socialis* (Mandapam)	1.025	0.887	0.809	0.905	0.985	0.960		
H.	HAV-26-*Dichotomaria Sp. Nov.* (Havelock)	0.329	0.173	0.215	0.192	0.318	0.168	0.859	

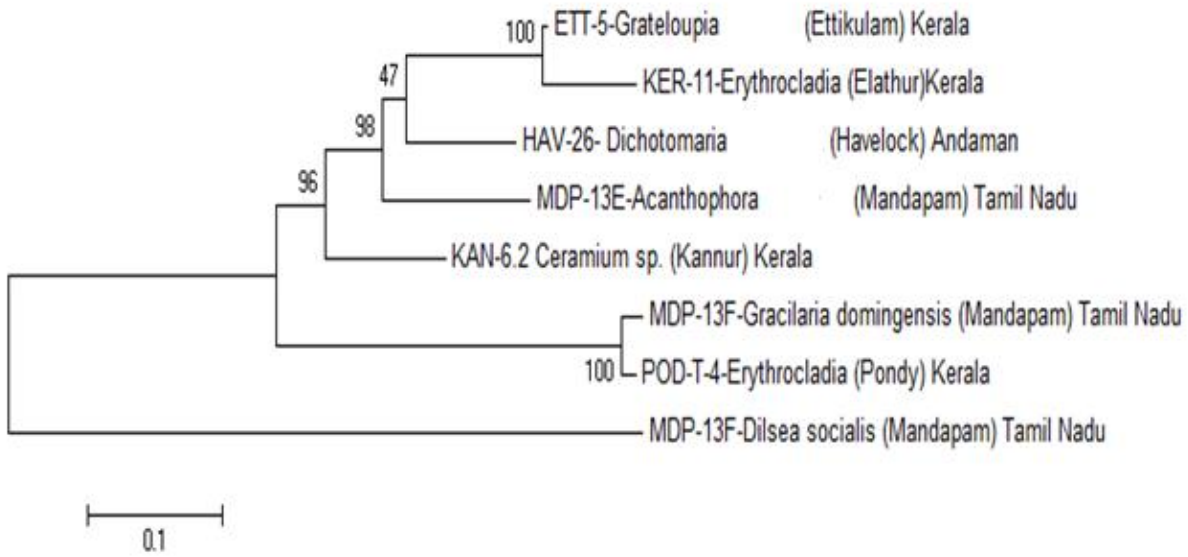


Figure.33. Maximum likelihood (ML) phylogram based on COX1 sequences using GTR+G model of molecular evolution MEGA phylogenetic framework. Numbers near nodes represent ML bootstrap proportions exceeding 50. *Ln*L = −3661.591. Scale bar is on the unit of average nucleotide substitutions per site.

Table 8: Maximum Likelihood estimation of goodness of fit of 24 different nucleotide substitution models of Red algae using COX2-COX3 region of mitochondrial DNA in MEGA software.

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	(+*I*)	(+*G*)	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(
T92	9	6416.827	6363.497	-3172.716	n/a	n/a	0.97	0.342	0.342	0.158	0.158	0.081	0.037	0.083	0.081	0.083	0.037	0.081	0.181	0.(
T92+I	10	6419.242	6359.994	-3169.957	0.08	n/a	1.02	0.342	0.342	0.158	0.158	0.079	0.036	0.085	0.079	0.085	0.036	0.079	0.185	0.(
T92+G	10	6420.923	6361.675	-3170.798	n/a	3.68	1.04	0.342	0.342	0.158	0.158	0.078	0.036	0.086	0.078	0.086	0.036	0.078	0.187	0.(
T92+G+I	11	6427.196	6362.031	-3169.968	0.08	200.00	1.02	0.342	0.342	0.158	0.158	0.079	0.036	0.085	0.079	0.085	0.036	0.079	0.185	0.(
HKY	11	6436.411	6371.246	-3174.575	n/a	n/a	0.98	0.344	0.339	0.151	0.165	0.080	0.035	0.088	0.081	0.080	0.039	0.081	0.180	0.(
HKY+I	12	6438.777	6367.697	-3171.792	0.08	n/a	1.03	0.344	0.339	0.151	0.165	0.078	0.035	0.090	0.079	0.082	0.038	0.079	0.184	0.(
TN93	12	6439.525	6368.445	-3172.166	n/a	n/a	0.99	0.344	0.339	0.151	0.165	0.079	0.035	0.073	0.080	0.096	0.038	0.080	0.215	0.(
HKY+G	12	6440.476	6369.396	-3172.642	n/a	3.67	1.05	0.344	0.339	0.151	0.165	0.077	0.034	0.091	0.078	0.083	0.037	0.078	0.186	0.(
TN93+I	13	6443.326	6366.332	-3170.100	0.07	n/a	1.03	0.344	0.339	0.151	0.165	0.077	0.034	0.075	0.079	0.096	0.038	0.079	0.216	0.(
TN93+G	13	6444.107	6367.113	-3170.491	n/a	3.96	1.06	0.344	0.339	0.151	0.165	0.076	0.034	0.074	0.077	0.100	0.037	0.077	0.224	0.(
HKY+G+I	13	6446.732	6369.737	-3171.803	0.08	200.00	1.03	0.344	0.339	0.151	0.165	0.078	0.035	0.090	0.079	0.082	0.038	0.079	0.184	0.(
TN93+G+I	14	6451.265	6368.359	-3170.104	0.07	200.00	1.03	0.344	0.339	0.151	0.165	0.077	0.034	0.075	0.079	0.096	0.038	0.079	0.217	0.(
GTR	15	6460.232	6371.414	-3170.620	n/a	n/a	1.00	0.344	0.339	0.151	0.165	0.087	0.035	0.074	0.088	0.098	0.041	0.081	0.219	0.(
GTR+I	16	6462.907	6368.180	-3167.992	0.08	n/a	1.04	0.344	0.339	0.151	0.165	0.088	0.034	0.078	0.089	0.099	0.040	0.078	0.222	0.(
GTR+G	16	6464.274	6369.547	-3168.675	n/a	3.55	1.07	0.344	0.339	0.151	0.165	0.085	0.035	0.076	0.087	0.102	0.039	0.080	0.229	0.(
GTR+G+I	17	6470.856	6370.220	-3168.000	0.08	200.00	1.04	0.344	0.339	0.151	0.165	0.088	0.034	0.078	0.089	0.099	0.040	0.079	0.222	0.(
K2	8	6592.427	6545.017	-3264.483	n/a	n/a	0.83	0.250	0.250	0.250	0.250	0.068	0.068	0.114	0.068	0.114	0.068	0.068	0.114	0.(
K2+I	9	6595.562	6542.233	-3262.084	0.08	n/a	0.86	0.250	0.250	0.250	0.250	0.067	0.067	0.115	0.067	0.115	0.067	0.067	0.115	0.(
K2+G	9	6598.952	6545.622	-3263.779	n/a	6.69	0.85	0.250	0.250	0.250	0.250	0.067	0.067	0.115	0.067	0.115	0.067	0.067	0.115	0.(
K2+G+I	10	6603.580	6544.332	-3262.126	0.08	200.00	0.86	0.250	0.250	0.250	0.250	0.067	0.067	0.115	0.067	0.115	0.067	0.067	0.115	0.(
JC	7	6603.681	6562.192	-3274.076	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+I	8	6606.870	6559.460	-3271.704	0.08	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+G	8	6610.288	6562.879	-3273.413	n/a	6.86	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+G+I	9	6614.890	6561.560	-3271.748	0.08	200.00	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(

NOTE.-- Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+*G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+*I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well. They are followed by nucleotide frequencies (*f*) and rates of base substitutions (*r*) for each nucleotide pair. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 5 nucleotide sequences. There were a total of 1021 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [2].

Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

1. Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

2. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.

Table.9. Pairwise distance matrix using T92 model for COX2-COX3 sequences from Red algae.

		A	B	C	D	E
A.	ETT-4-*Gracilaria foliifera* (Ettikulam)					
B.	BEK-23.1-*Gracilaria corticata* (Bekal)	0.686				
C.	BEK-23.4-*Hypnea stelullifera* (Bekal)	0.741	0.901			
D.	ETT-5-*Grateloupia angusta* (Ettikulam)	0.342	0.746	0.938		
E.	KAN-6.2-*Ceramium pacificum* (Kannur)	0.397	0.741	0.791	0.358	

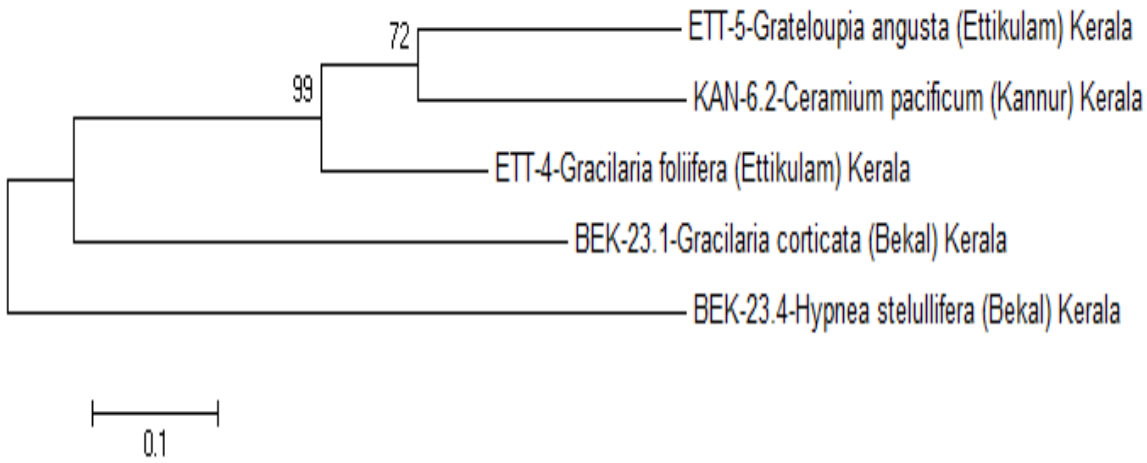


Figure.34. Maximum likelihood (ML) phylogram based on COX2-COX3 intergenic spacer sequences using T92 Model of molecular evolution in MEGA phylogenetic framework. Numbers near nodes represent ML bootstrap proportions exceeding 50.*Ln*L = −3172.716. Scale bar is on the unit of average nucleotide substitutions per site

Phylogeographic analysis of Brown algae:-

Four Brown algae (Phaeophyceae), samples, showed homology to (MAN-13B) *Sargassum zhangii*, (MAN-13N) *Sargassum megalocystum*,(MAN-13O) *Sargassum aquifolium* and (MAN-13C) *Turbinaria ornata*. Out of four samples of Brown algae, three samples were identified as *Sargassum* species. MAN-13N identified as *Sargassum megalocystum*, which was the first report from India. It previously identified from China. For phylogeographic analysis of Brown algae from Indian subcontinent, sequences were first aligned by MUSCLE algorithm in MEGA. Best fitting nucleotide substitution model were tested using ML-Model test in MEGA. The model with lowest Bayesian information criterion (BIC) score was T92 (Tamura-3-parameter) i.e. with BIC score 1848.386 using 9 parameters (Table.10). Numbers near nodes represent posterior probabilities (Fig.35). Pairwise distances between sequences were calculated by using HKY model in MEGA (Table.11). Phylogenetic tree was constructed using MEGA software for phylogeographic analysis. MDP-13B *Sargassum zhangii* and MDP-13O-*Sargassum aquifolium* were showing least phylogenetic distance i.e. 0.030. So they were closely related than *Sargassum megalocystum*. *Turbinaria ornata* shows the maximum distance with *Sargassum zhangi* i.e. 0.423. So it was placed in the different branch.

Table 10: Maximum Likelihood estimation of goodness of fit of 24 different nucleotide substitution models of Brown algae using MEGA

Table. Maximum Likelihood fits of 24 different nucleotide substitution models

Model	Parameters	BIC	AICc	*lnL*	(+*I*)	(+*G*)	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(C
HKY	9	3766.438	3714.851	-1848.386	n/a	n/a	1.28	0.249	0.393	0.155	0.203	0.081	0.032	0.119	0.051	0.091	0.042	0.051	0.231	0.(
T92	7	3766.608	3726.472	-1856.211	n/a	n/a	1.25	0.321	0.321	0.179	0.179	0.068	0.038	0.103	0.068	0.103	0.038	0.068	0.185	0.(
T92+G	8	3768.321	3722.459	-1853.198	n/a	0.63	1.87	0.321	0.321	0.179	0.179	0.053	0.029	0.120	0.053	0.120	0.029	0.053	0.215	0.(
HKY+G	10	3768.617	3711.307	-1845.605	n/a	0.69	1.89	0.249	0.393	0.155	0.203	0.063	0.025	0.137	0.040	0.105	0.033	0.040	0.266	0.(
T92+I	8	3768.809	3722.946	-1853.442	0.38	n/a	1.90	0.321	0.321	0.179	0.179	0.052	0.029	0.121	0.052	0.121	0.029	0.052	0.216	0.(
HKY+I	10	3768.938	3711.628	-1845.766	0.36	n/a	1.92	0.249	0.393	0.155	0.203	0.063	0.025	0.138	0.040	0.106	0.032	0.040	0.268	0.(
TN93	10	3774.178	3716.867	-1848.386	n/a	n/a	1.28	0.249	0.393	0.155	0.203	0.081	0.032	0.119	0.051	0.092	0.042	0.051	0.232	0.(
TN93+I	11	3774.834	3711.802	-1844.843	0.38	n/a	2.31	0.249	0.393	0.155	0.203	0.055	0.022	0.108	0.035	0.135	0.028	0.035	0.343	0.(
TN93+G	11	3776.051	3713.020	-1845.452	n/a	0.67	1.94	0.249	0.393	0.155	0.203	0.062	0.025	0.126	0.039	0.114	0.032	0.039	0.288	0.(
T92+G+I	9	3776.062	3724.475	-1853.198	0.00	0.63	1.87	0.321	0.321	0.179	0.179	0.053	0.029	0.120	0.053	0.120	0.029	0.053	0.215	0.(
HKY+G+I	11	3776.360	3713.328	-1845.607	0.06	0.81	1.90	0.249	0.393	0.155	0.203	0.063	0.025	0.138	0.040	0.105	0.033	0.040	0.267	0.(
TN93+G+I	12	3782.575	3713.824	-1844.844	0.38	200.00	2.35	0.249	0.393	0.155	0.203	0.054	0.022	0.108	0.035	0.136	0.028	0.035	0.345	0.(
GTR	13	3791.477	3717.008	-1845.424	n/a	n/a	1.27	0.249	0.393	0.155	0.203	0.094	0.047	0.117	0.059	0.091	0.029	0.075	0.232	0.(
GTR+I	14	3793.835	3713.649	-1842.733	0.36	n/a	2.24	0.249	0.393	0.155	0.203	0.040	0.054	0.100	0.025	0.135	0.024	0.086	0.342	0.(
GTR+G	14	3795.509	3715.324	-1843.570	n/a	0.85	1.76	0.249	0.393	0.155	0.203	0.064	0.049	0.119	0.041	0.110	0.026	0.079	0.280	0.(
GTR+G+I	15	3801.573	3715.673	-1842.731	0.36	200.00	2.30	0.249	0.393	0.155	0.203	0.038	0.054	0.100	0.024	0.137	0.024	0.086	0.346	0.(
K2	6	3867.832	3833.425	-1910.694	n/a	n/a	1.20	0.250	0.250	0.250	0.250	0.057	0.057	0.137	0.057	0.137	0.057	0.057	0.137	0.(
K2+G	7	3868.414	3828.278	-1907.114	n/a	0.55	1.81	0.250	0.250	0.250	0.250	0.045	0.045	0.161	0.045	0.161	0.045	0.045	0.161	0.(
K2+I	7	3875.573	3835.437	-1910.694	0.00	n/a	1.20	0.250	0.250	0.250	0.250	0.057	0.057	0.137	0.057	0.137	0.057	0.057	0.137	0.(
K2+G+I	8	3875.575	3829.713	-1906.825	0.43	200.00	2.07	0.250	0.250	0.250	0.250	0.041	0.041	0.168	0.041	0.168	0.041	0.041	0.168	0.(
JC	5	3892.538	3863.860	-1926.917	n/a	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+I	6	3897.927	3863.520	-1925.742	0.29	n/a	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+G	6	3897.998	3863.591	-1925.777	n/a	1.25	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(
JC+G+I	7	3905.738	3865.602	-1925.777	0.02	1.35	0.50	0.250	0.250	0.250	0.250	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.(

NOTE.-- Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (*lnL*), and the number of parameters (including branch lengths) are also presented [1]. Non-uniformity of evolutionary rates among sites may be modeled by using a discrete Gamma distribution (+*G*) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+*I*). Whenever applicable, estimates of gamma shape parameter and/or the estimated fraction of invariant sites are shown. Assumed or estimated values of transition/transversion bias (*R*) are shown for each model, as well. They are followed by nucleotide frequencies (*f*) and rates of base substitutions (*r*) for each nucleotide pair. Relative values of instantaneous *r* should be considered when evaluating them. For simplicity, sum of *r* values is made equal to 1 for each model. For estimating ML values, a tree topology was automatically computed. The analysis involved 4 nucleotide sequences. There were a total of 752 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [2].

Abbreviations: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

1. Nei M. and Kumar S. (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

2. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.

Table.11. Pairwise distance matrix using HKY model for COX1 sequences from Brown algae.

		A	B	C	D
A.	MDP-13B-*Sargassum zhangii* (Mandapam) Tamil Nadu				
B.	MDP-13O-*Sargassum aquifolium* (Mandapam) Tamil Nadu	0.030			
C.	MDP-13C-*Turbinaria ornata* (Mandapam) Tamil Nadu	0.423	0.409		
D.	MDP-13N-*Sargassum Megalocystum* (Mandapam) Tamil Nadu	0.077	0.061	0.413	

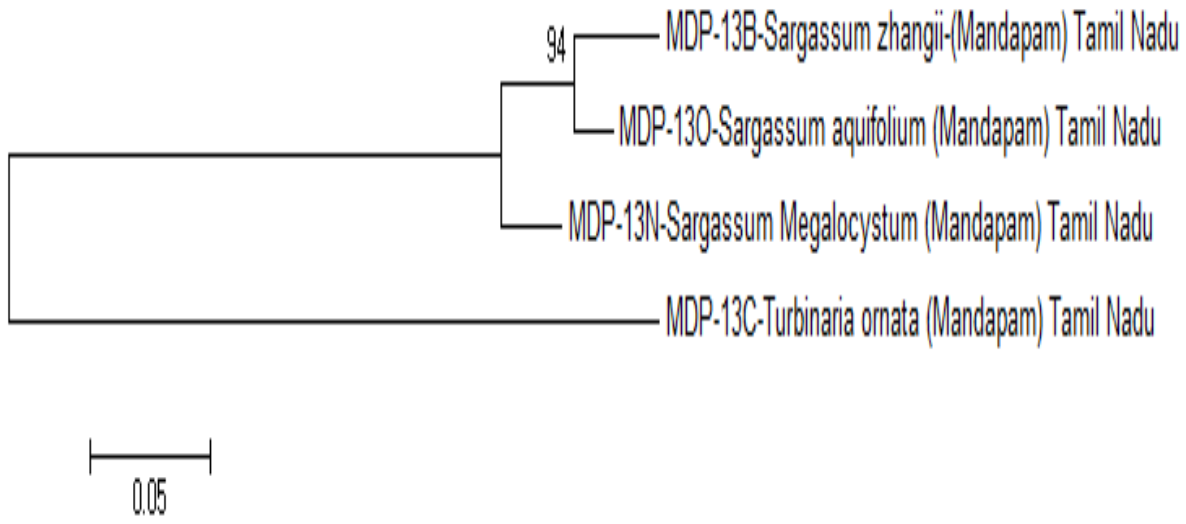


Figure.35. Maximum likelihood (ML) phylogram based on COX1 sequences using HKY Model in MEGA phylogenetic framework. Numbers near nodes represent ML bootstrap proportions exceeding 50. *Ln*L = -1848.386. Scale bar is on the unit of average nucleotide substitutions per site.

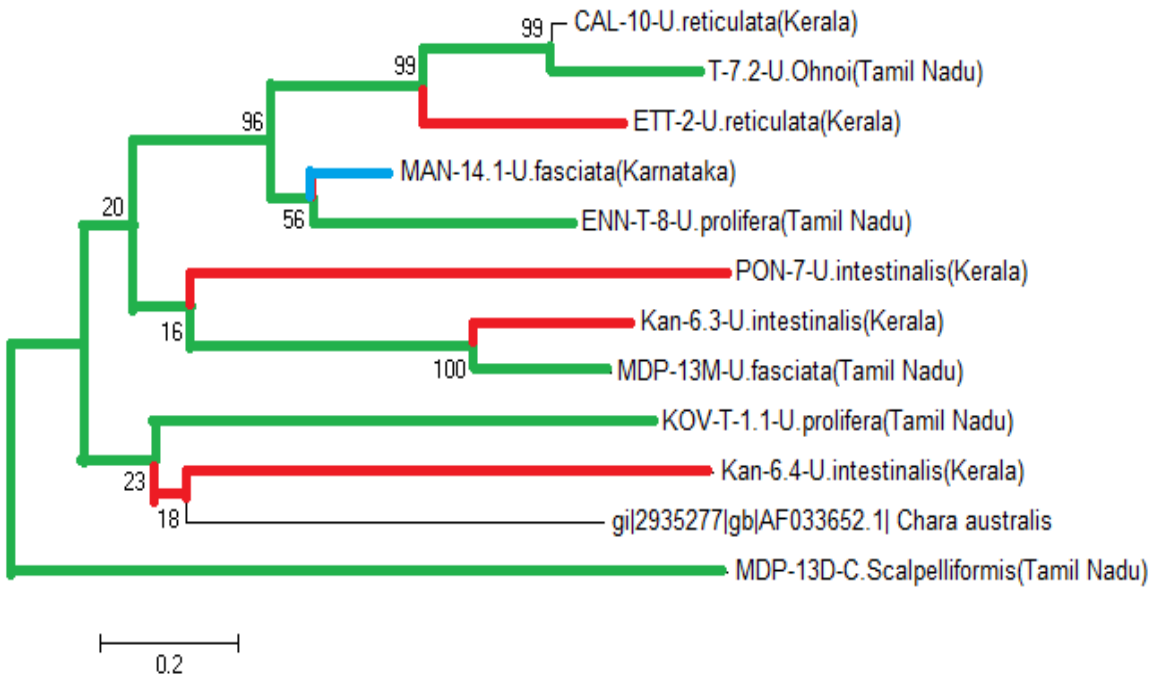


Figure 36. Phylogenetic diversity (PD) in Green algae samples collected from Indian subcontinent

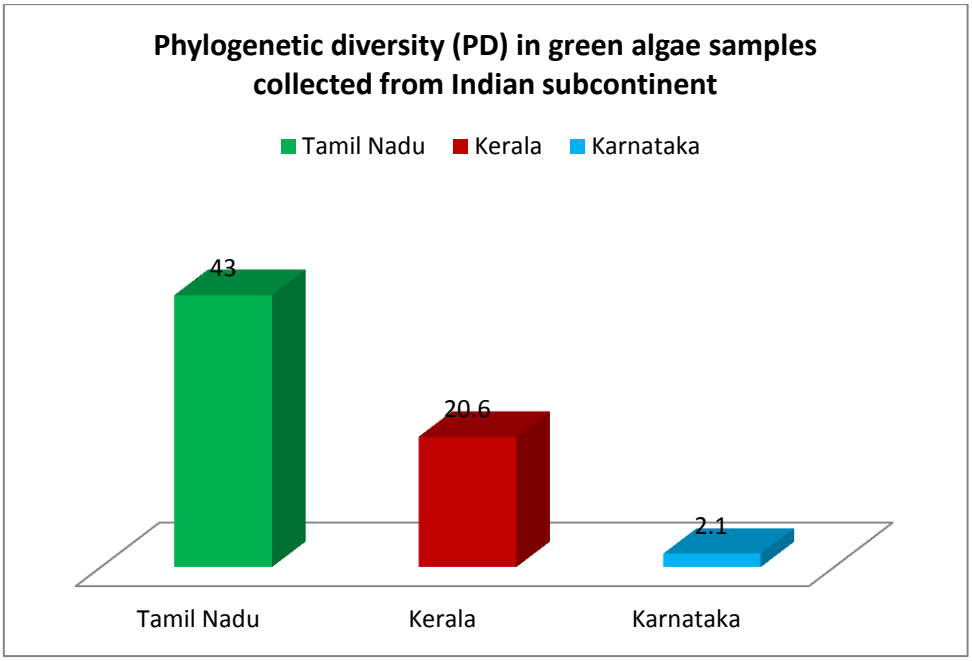


Figure 37. Comparative analysis of Phylogenetic Diversity (PD) of Green algae from different states of Indian subcontinent

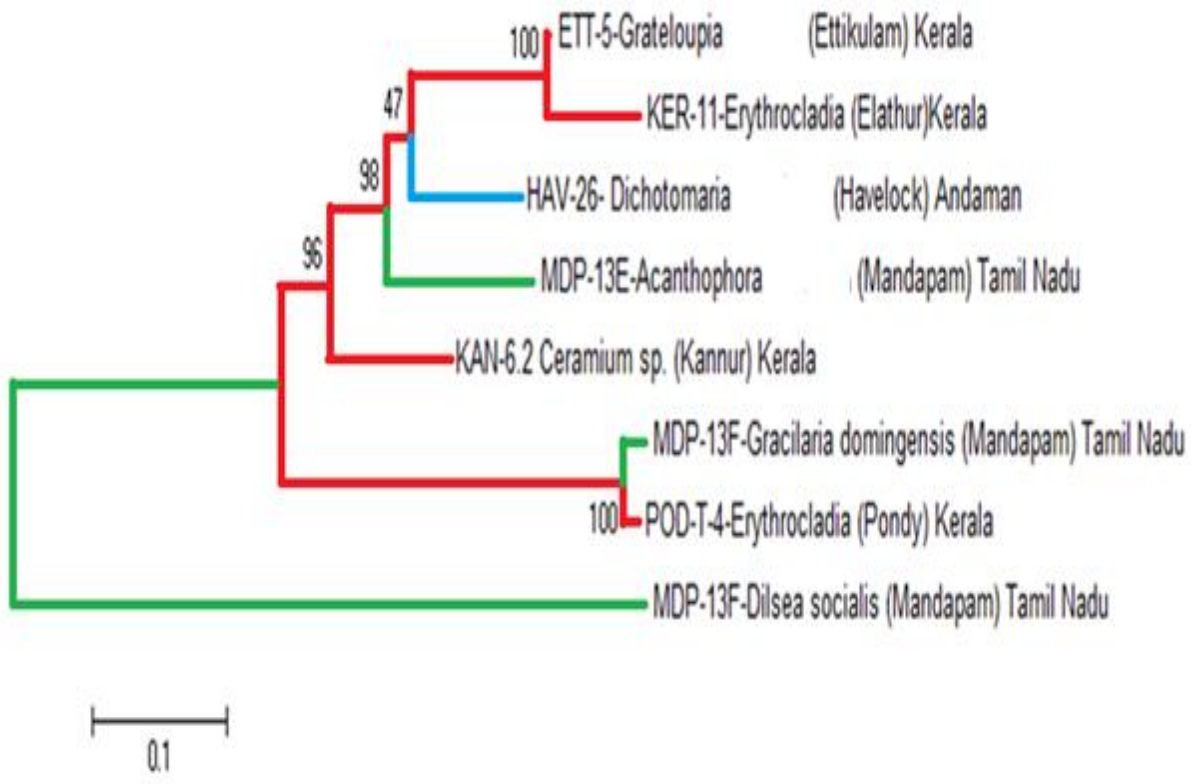


Figure 38. Phylogenetic diversity (PD) in Red algae samples collected from Indian subcontinent

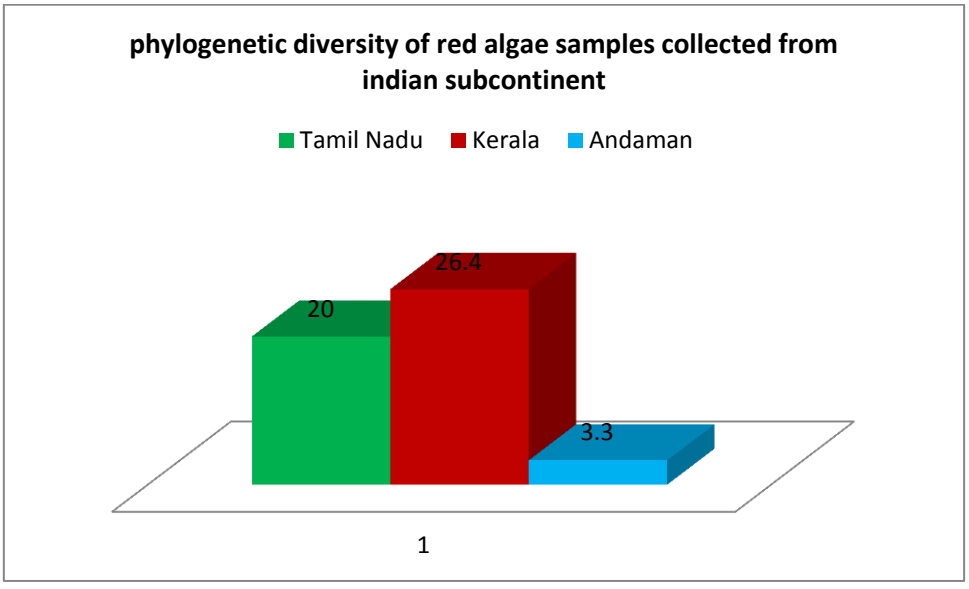


Figure 39. Comparative analysis of Phylogenetic Diversity (PD) of Red algae different states of Indian subcontinent

Simpson's diversity Index of Green Algae:-

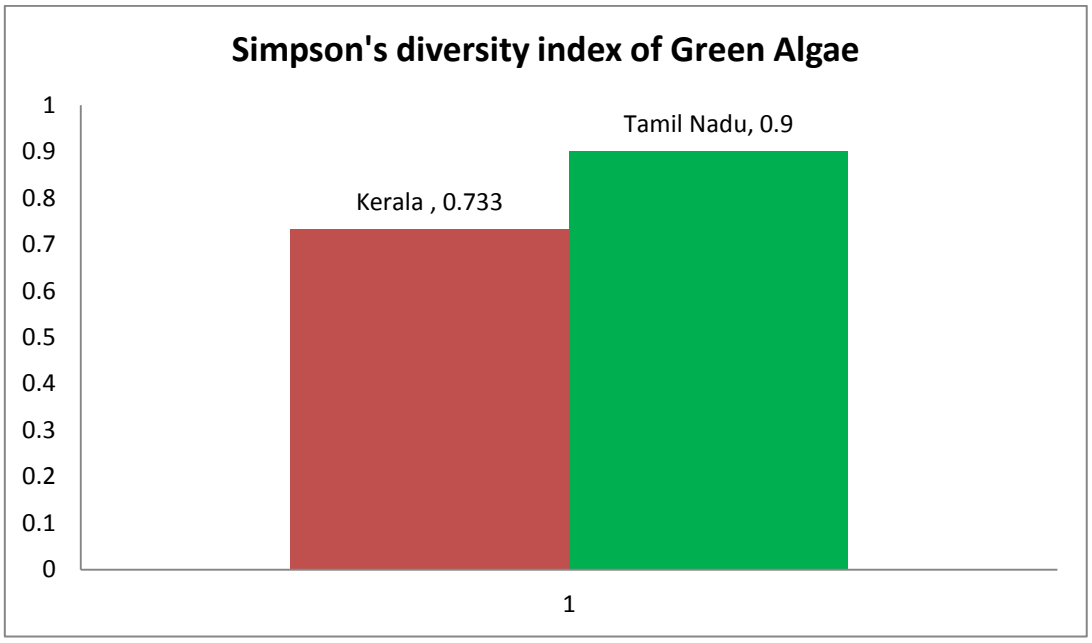


Figure 40. Diversity Index of Tamil Nadu samples are more as compared to Kerala.

Shannon Wiener Index of Green Algae:-

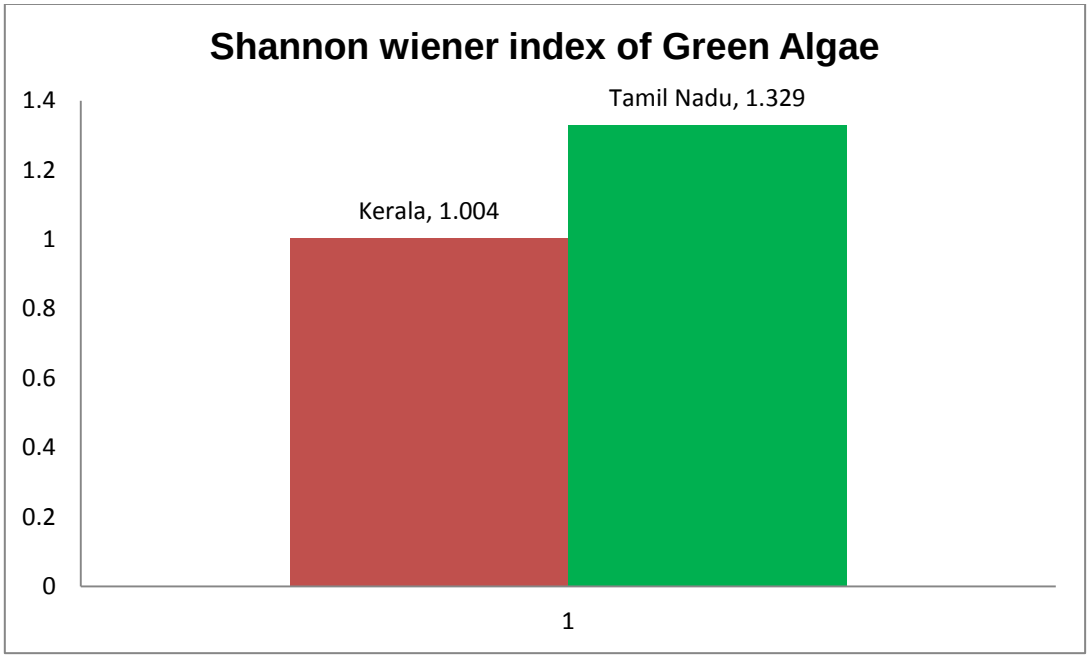


Figure 41. Shannon Wiener index of Tamil Nadu samples are more as compared to Kerala**.**

Phylogenetic diversity was calculated by measuring branch length using cm scale. In the case of Green algae, it was revealed that Tamil Nadu samples had longer branch length as compared to Kerala and Karnataka. So on plotting a bar graph it was showing higher bar length as compared to other. So Green algae were phylogenetically more diverse at Tamil Nadu coast. In the case of Red algae Kerala samples were showing longer branch length as compared to Tamil Nadu and Andaman Island, so Red algae isolates from Kerala coasts was showing more diversity. Species' diversity was calculated by using Simpson's diversity index. According to our data, it analysed that only Green alga data was suitable for species' diversity analysis. Red algae and Brown algae were not having enough primary data to perform such analysis. Based on Green algae it found that Tamil Nadu coasts had more species diversity as compared to Kerala. Shannon Wiener index was used to calculate species' evenness. Tamil Nadu coasts were showing more species evenness as compared to Kerala. So we can conclude that Tamil Nadu coast was richer in occurrence of Green algae as compared to Kerala coasts.

Summary:-

Marine macroalgae or Seaweeds are macroscopic, multicellular, benthic non vascular marine algae with simple morphology and anatomy. It has remarkable degrees of phenotypic plasticity in response to environmental factors and incomplete understanding of life histories with alternation of heteromorphic generations. Even for the experienced systematist it is very difficult to identify many macroalgae species. Modern algal systematists exploit genetic tools for molecular assisted alpha taxonomy and DNA barcoding is one such molecular tool that relies on the use of a standardized DNA region as a tag for rapid and accurate species identification. In this study marine Green, Red and Brown macroalgae thalli attached to intertidal and sub-tidal rocks were collected from Indian subcontinent. Photography of each sample was carried out to morphological and microscopic analysis. Molecular analysis performed by DNA extraction, PCR amplification of nr ITS, COX1, COX2, COX3, *rbcL* region, purification and DNA sequencing. The DNA sequences from the samples captured as a colour coded electropherograms and were assembled using computer program CodonCodeAligner (CodoneCode Corporation, USA). These Sequences analysed on BLASTN which revealed that, eleven sequences showed homology to Green algae, fourteen sequences showed homology to Red algae and four sequences to Brown algae. Maximum likelihood phylogenetic trees were constructed using Kimura-2-parameter model of molecular evolution and Gamma distribution model (K2+G) in case of Green algae using ITS genomic region of DNA. In the case of Red algae, General time reversible + Gamma distribution (GTR+G) model was used for tree construction that was amplified from COX1gaz region. Those samples that were amplified from COX2-COX3 region, they used Tamura-3-parameter (T92) model for tree construction. In the case of Brown algae, phylogenetic tree was constructed by using Tamura-3-parameter (T92) model for tree construction. *Erythrocladia* identified as endophytic algae inside *Cladophora glomerata*. *Gracilaria domingensis, Dilsea socialis* and *Sargassum Megalocystum* were first time reported in India. *Gracilaria folifera* and *Grateloupia angusta* were first time recorded from coastal regions of Ettikulam, Kerala, India. *Gracilaria corticata* first was the first time reported from coasts of Bekal, Kerala. *Sirodotia tenuissima* was the first time reported from Andaman Island. Based on molecular studies, an

isolate of genus *Ceramium* sp., *Erythrocladia, Acanthophora, Grateloupia* and *Dichotomaria* was identified as new species. Phylogenetic diversity of identified Green and Red algae samples was calculated using cm scale. Tamil Nadu shows more phylogenetic diversity in case of Green algae as compared to Kerala and Karnataka. Kerala shows more phylogenetic diversity in case of Red algae as compared to Tamil Nadu and Andaman islands. Diversity Index of Tamil Nadu coasts was more as compared to Kerala. Shannon Wiener Index of Tamil Nadu samples were more as compared to Kerala. So from the above data it concluded that Tamil Nadu coast has more species richness and diversity of algae species.

Chapter:-5

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Appendices:-

Appendix:-A.Protocol for DNA extraction

1. To the ground method immediately add 3 ml of STE buffer and mix it thoroughly.
2. Add the sample to the HiShredder placed in a 2.0 ml collection tube and centrifuge for 2 minutes at a maximum speed (~13,000 rpm). Discard the flow-through fraction without disturbing the cell pellet.
3. To each pellet, add 200µl of CTAB Extraction buffer, mix thoroughly and pool the contents of both tubes into a single tube.
4. If RNA free genomic DNA is required, add 20µl of RNase A solution, mix and incubate for 10 minutes at room temperature (15-20˚C).
5. Incubate the samples at 65˚C for 30 minutes.
6. Add 400 µl of chloroform: Isoamyl alcohol (24:1). Mix the samples gently by inversion for 10 minutes. Centrifuge for 10 minutes at a maximum speed (~ 13000 rpm).
7. Following centrifugation, mixture separates into three phases: lower organic phase, an interphase containing debris and upper aqueous phase containing DNA.
8. Transfer the top aqueous layer into a fresh tube. Add 2 volumes of ethanol and 0.1% of 3M Sodium acetate,pH 5.2; mix gently.
9. Incubate the sample at-20˚c for 10 minutes and centrifuge at maximum speed (˷13,000 rpm). Discard the supernatant.
10. Resuspend the pallet in 500µl of 70% ethanol and centrifuge for 10 minutes at a maximum speed (˷13,000 rpm). Discard the supernatant.
11. Air dried the pallet to remove the traces of ethanol.
12. Dissolve the pallet gently in 200µl of Elution Buffer (ET) by pipetting.

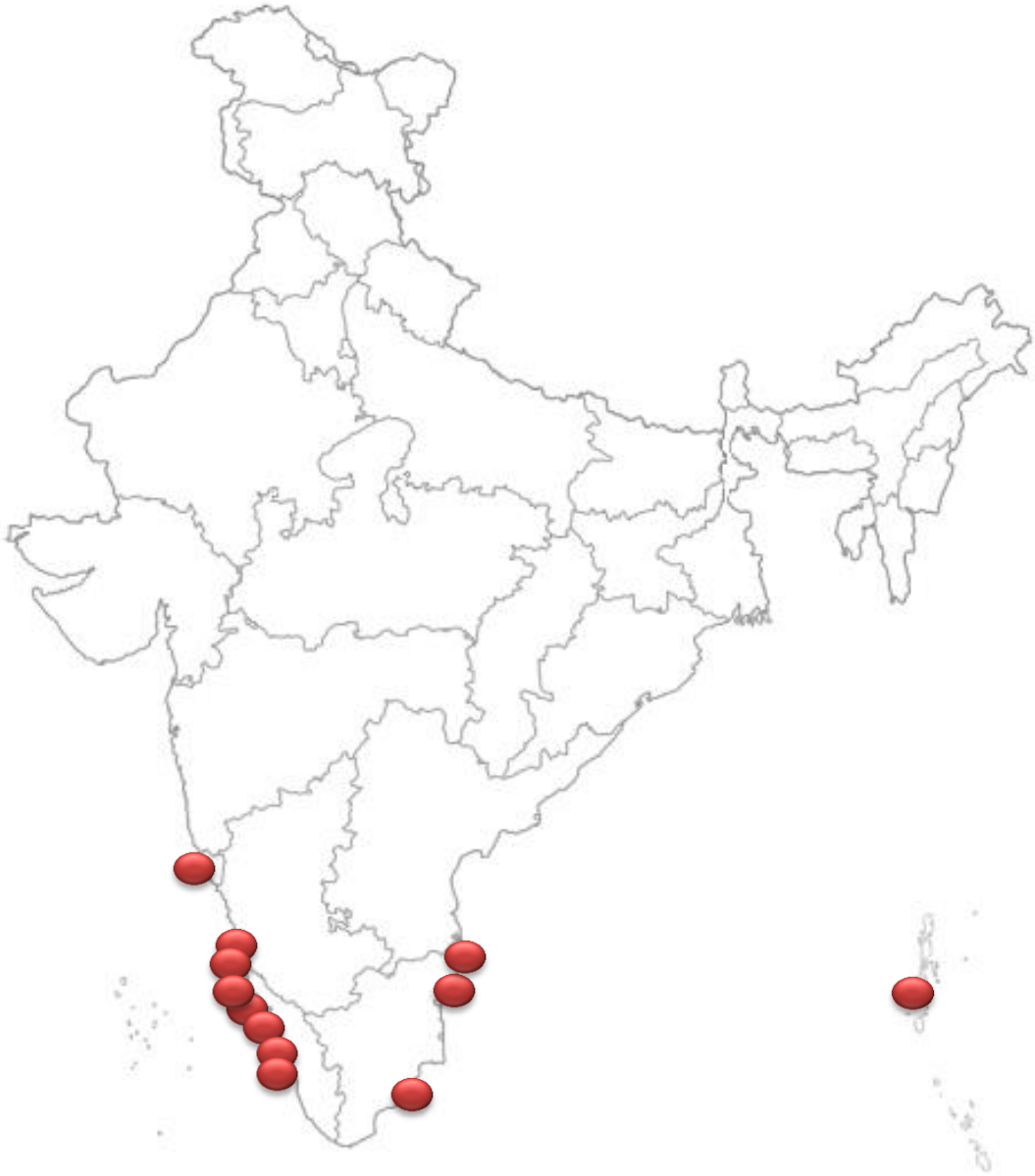
Appendix :-B. PCR reagents and their volumes for amplifying ITS1 , cox1, Cox2-3 and rbcL spacer regions

PCR reagents	Volume in µl (for 20µl)
10x buffer	2 µl
dNTP's	2 µl
Forward Primer	4 µl
Reverse Primer	4 µl
Taq. polymerase enzyme	0.2 µl
Distilled water	2 µl
$Mgcl_2$	1.8 µl
DNA sample	4 µl

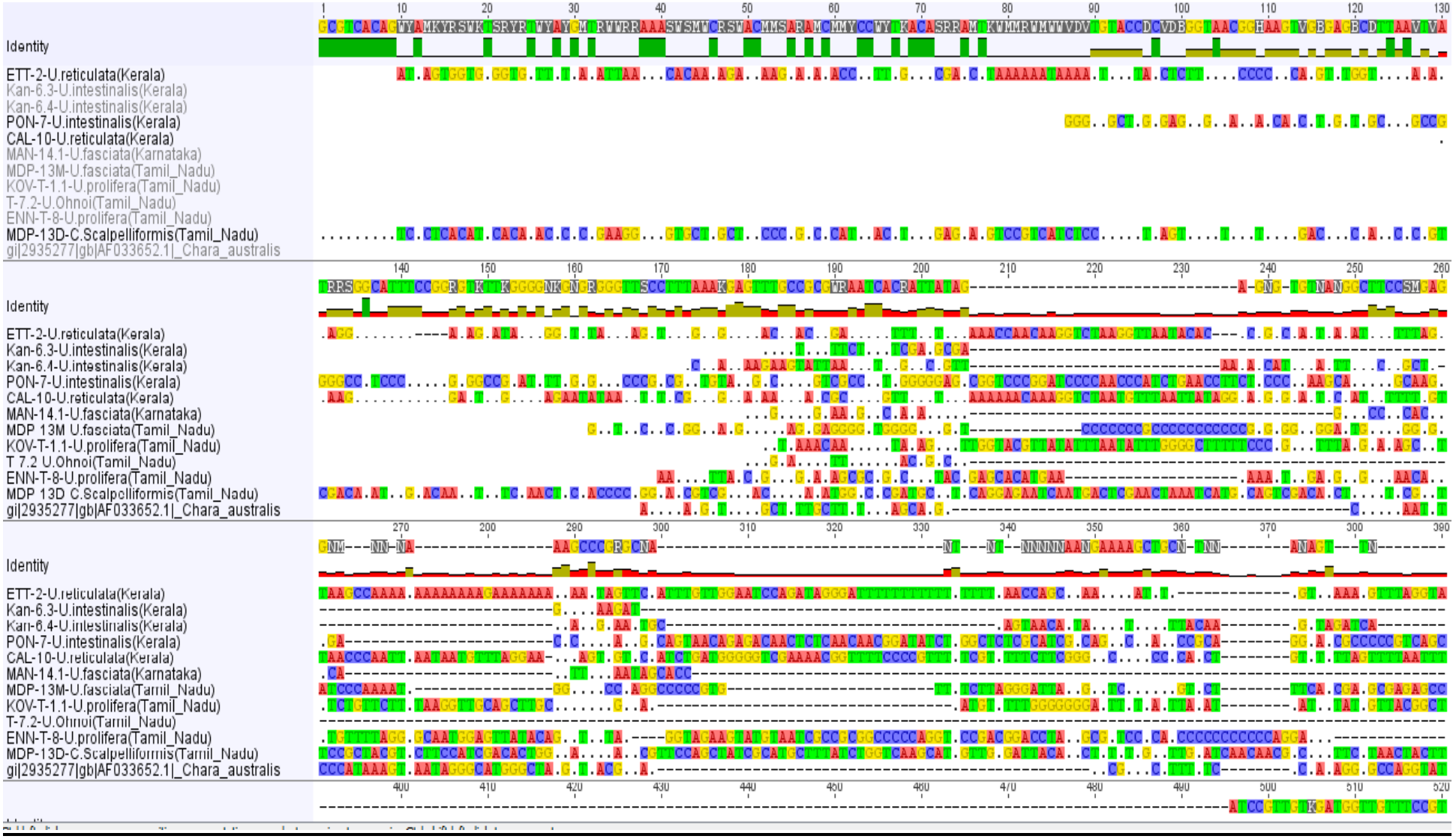
Appendix:-C. Reagents and volumes used for sequencing reaction

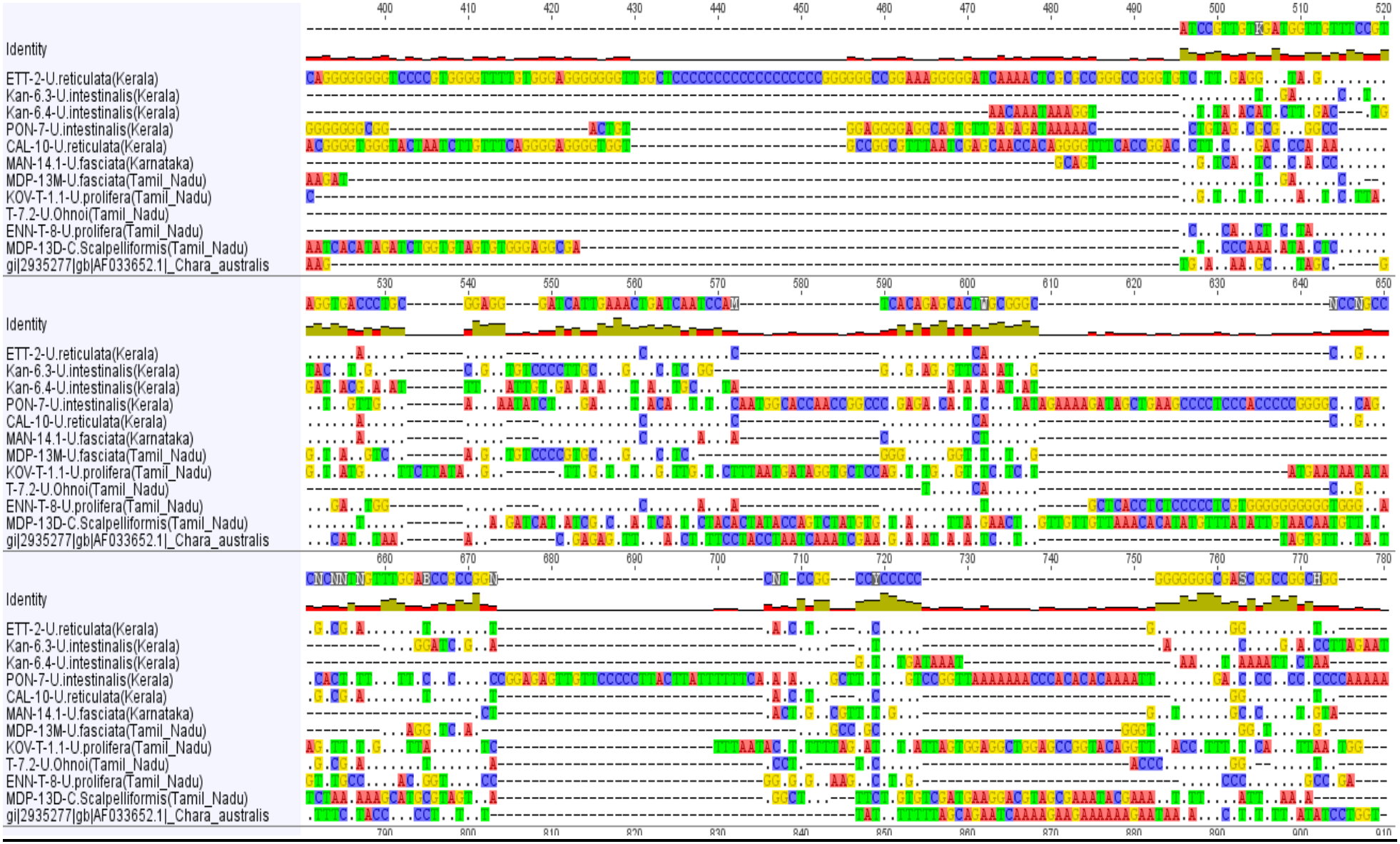
Sequencing reagents	**Volume (10 µl)**
Big dye 3.1 V	1 µl
Big dye buffer	2 µl
Primer	2 µl
$d.H_2O$	3 µl
DNA template	2 µl

Appendix:-D. **Map of India with sampling sites**



Appendix:-E- Multiple sequence alignment of Green algae using ITS1 & ITS2

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Kan-6.3-U.intestinalis(Kerala)
Kan-6.4-U.intestinalis(Kerala)
PON-7-U.intestinalis(Kerala)
CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
MDP-13D-C.Scalpelliformis(Tamil_Nadu)
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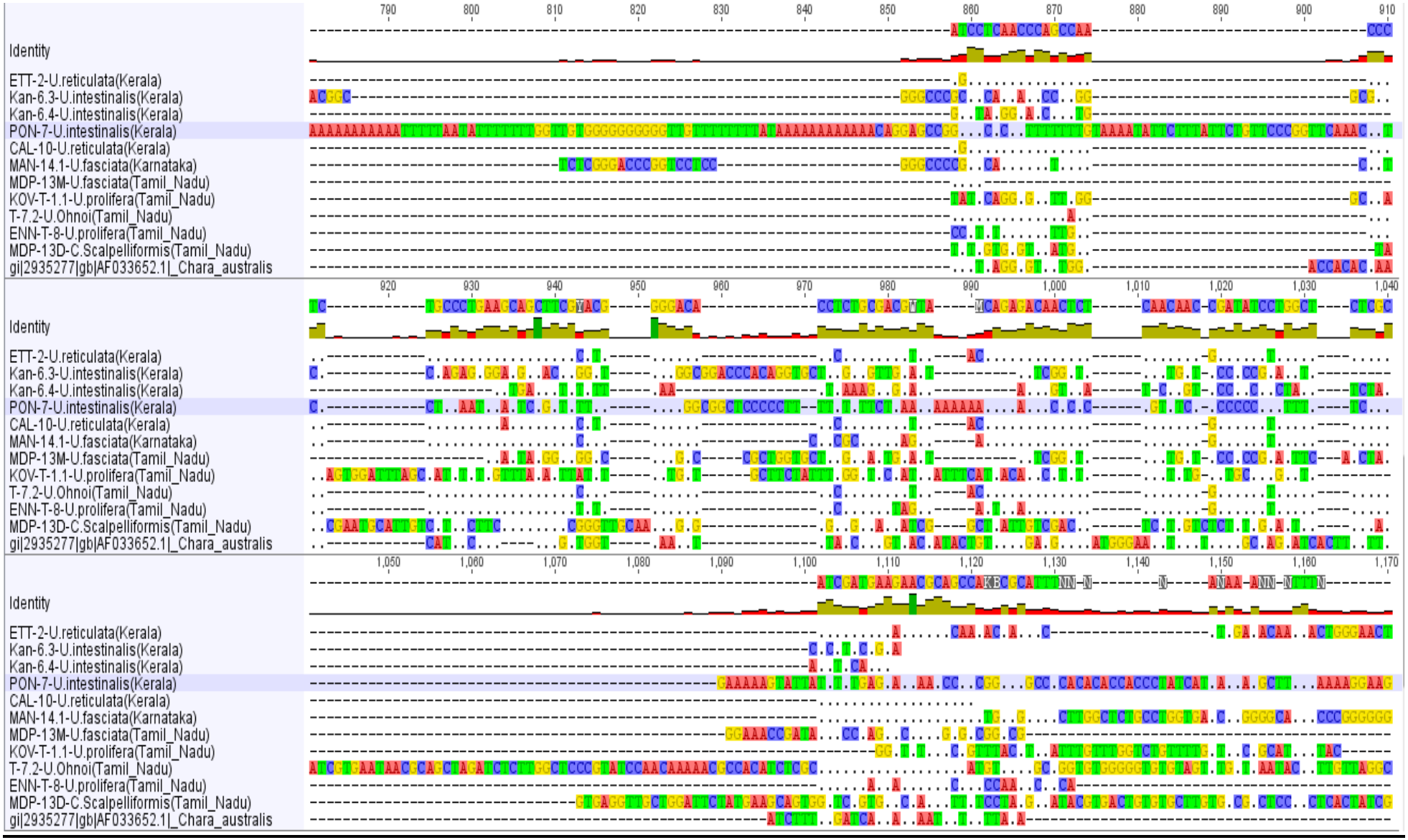
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Kan-6.4-U.intestinalis(Kerala)
PON-7-U.intestinalis(Kerala)
CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
MDP-13D-C.Scalpelliformis(Tamil_Nadu)
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Identity
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Kan-6.4-U.intestinalis(Kerala)
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CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
MDP-13D-C.Scalpelliformis(Tamil_Nadu)
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Identity
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Kan-6.3-U.intestinalis(Kerala)
Kan-6.4-U.intestinalis(Kerala)
PON-7-U.intestinalis(Kerala)
CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
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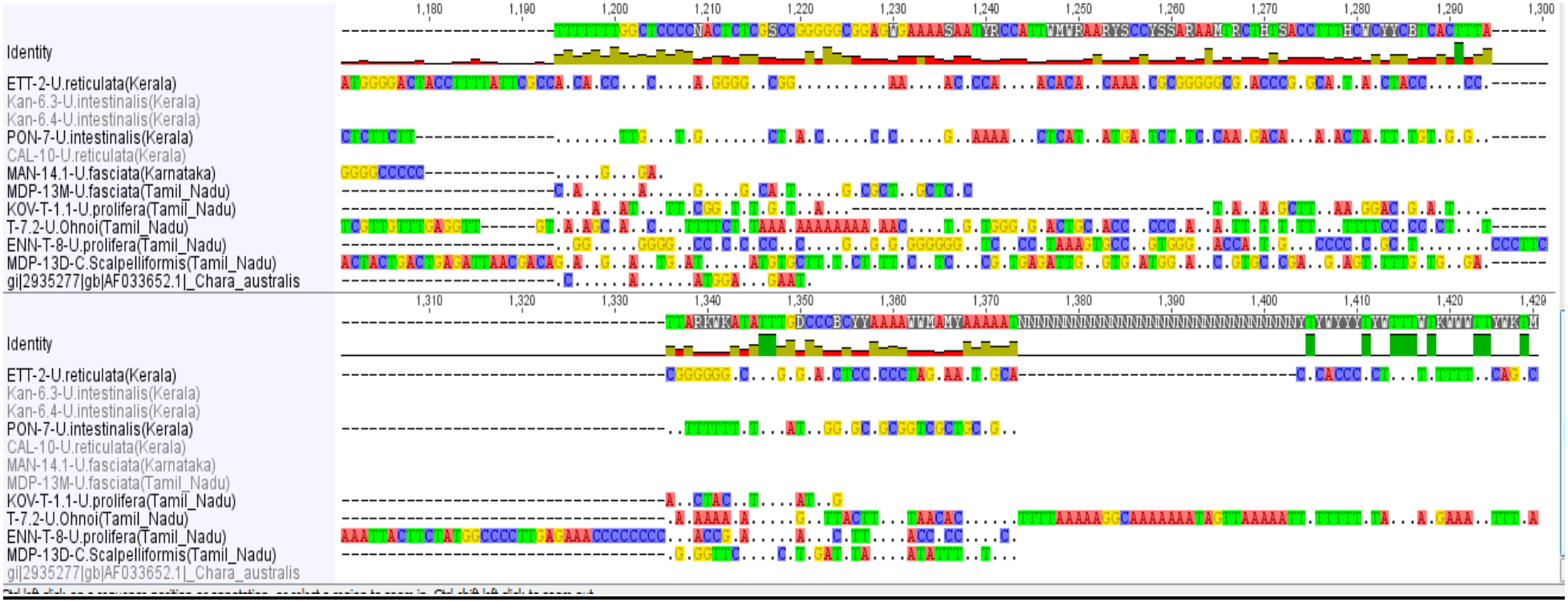
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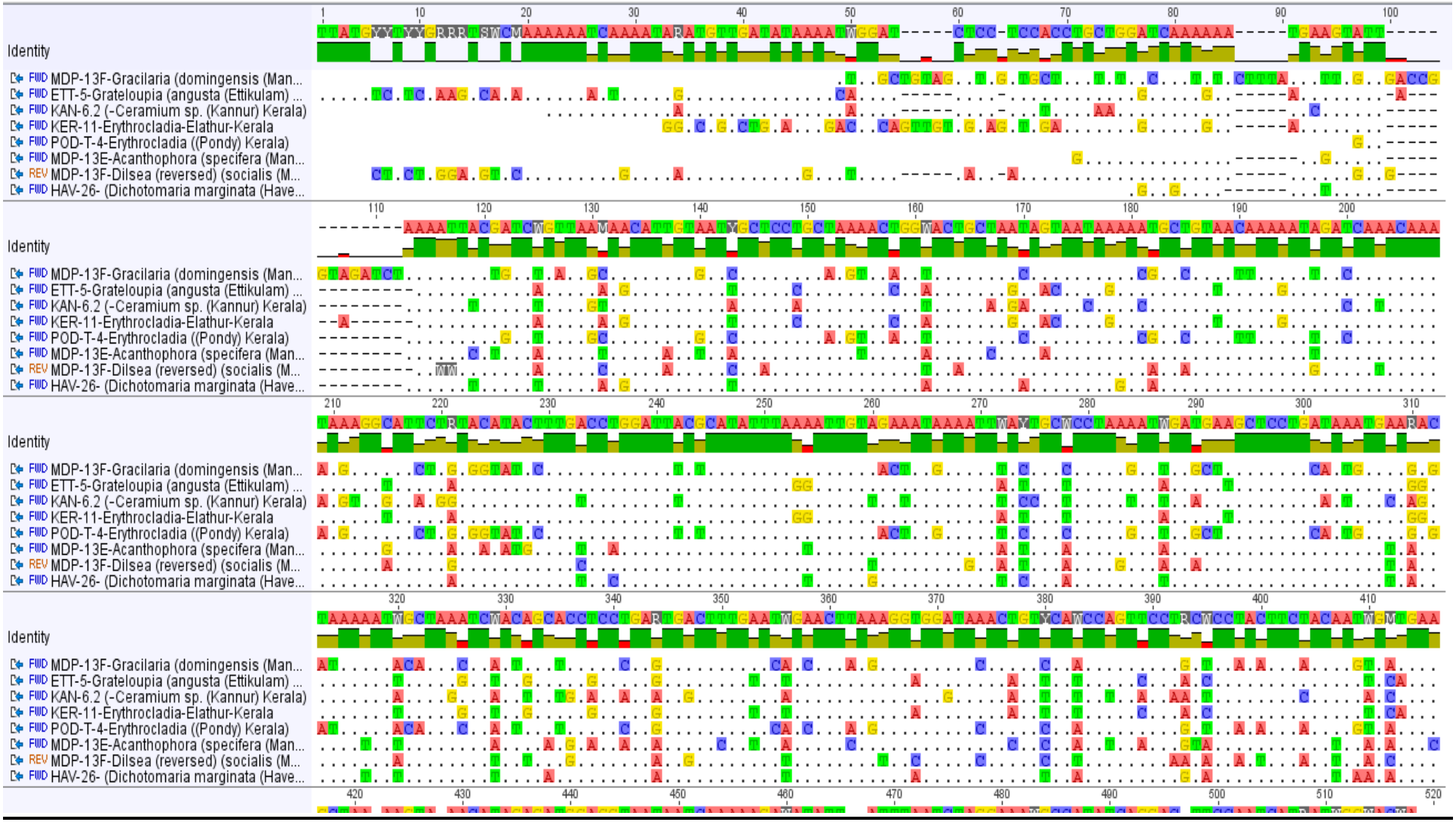
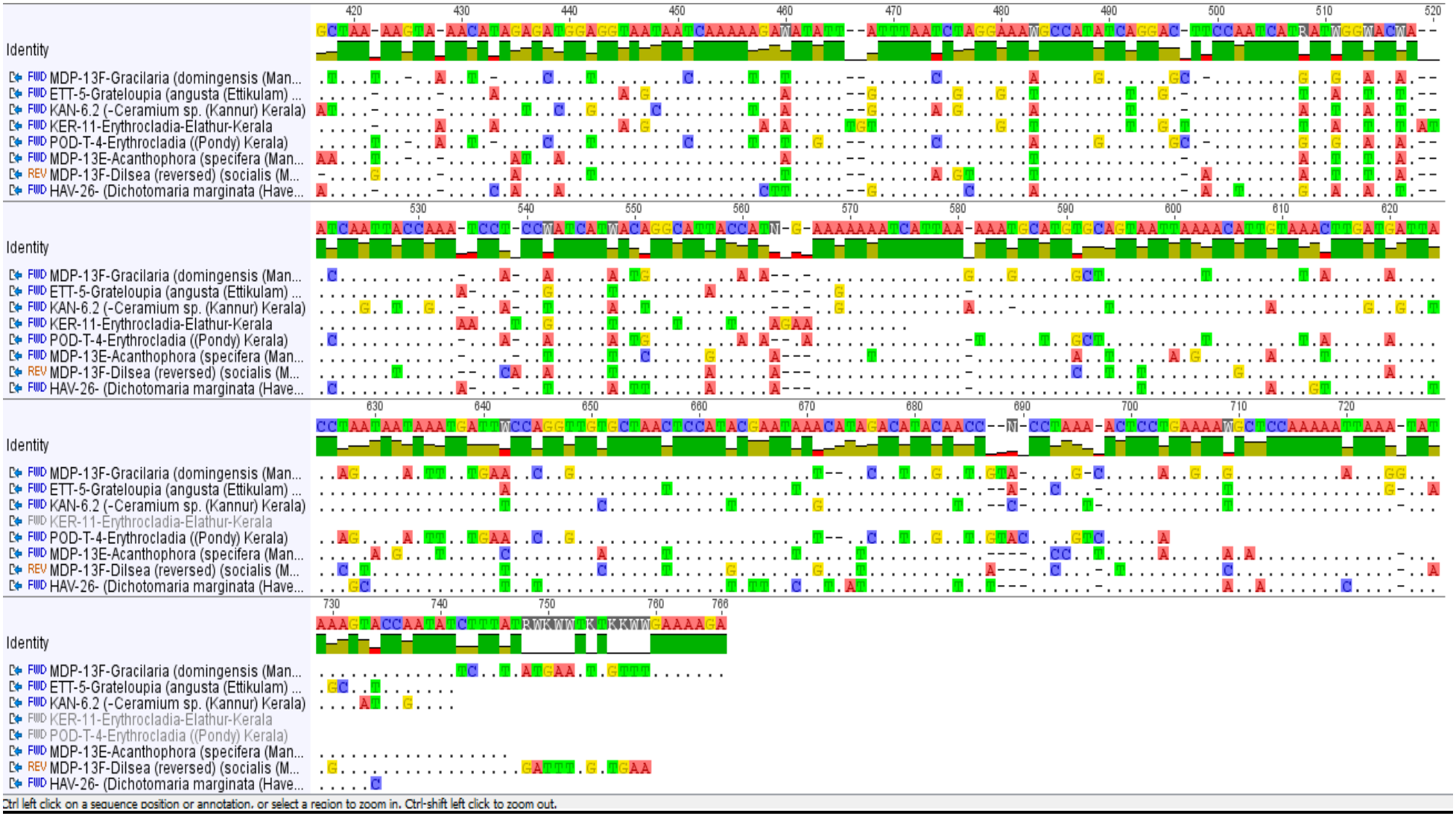
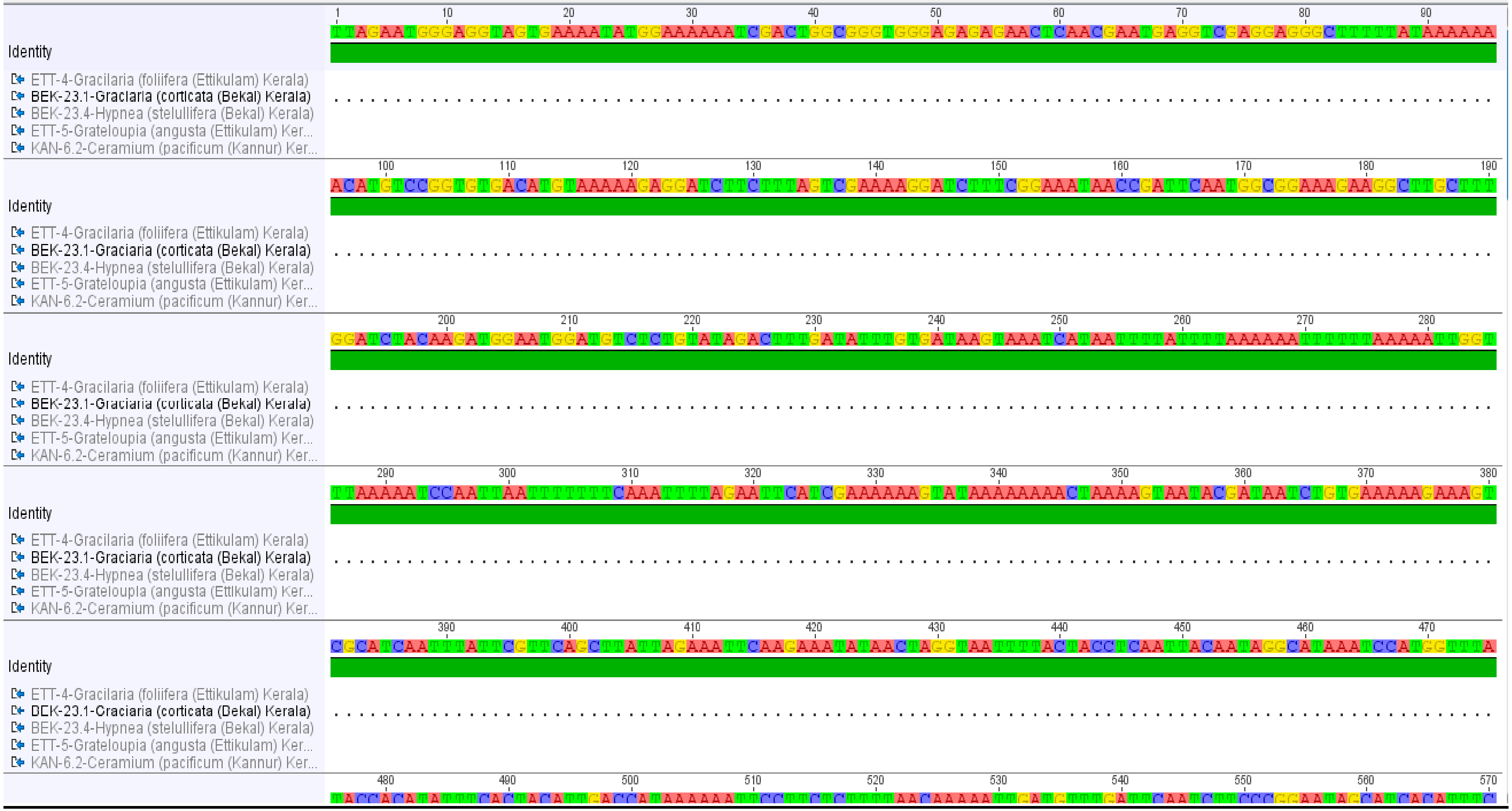
Identity
ETT-2-U.reticulata(Kerala)
Kan-6.3-U.intestinalis(Kerala)
Kan-6.4-U.intestinalis(Kerala)
PON-7-U.intestinalis(Kerala)
CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
MDP-13D-C.Scalpelliformis(Tamil_Nadu)
gi|2935277|gb|AF033652.1|_Chara_australis
1,310
1,320
1,330
1,340
1,350
1,360
1,370
1,380
1,390
1,400
1,410
1,420
1,429
Identity
ETT-2-U.reticulata(Kerala)
Kan-6.3-U.intestinalis(Kerala)
Kan-6.4-U.intestinalis(Kerala)
PON-7-U.intestinalis(Kerala)
CAL-10-U.reticulata(Kerala)
MAN-14.1-U.fasciata(Karnataka)
MDP-13M-U.fasciata(Tamil_Nadu)
KOV-T-1.1-U.prolifera(Tamil_Nadu)
T-7.2-U.Ohnoi(Tamil_Nadu)
ENN-T-8-U.prolifera(Tamil_Nadu)
MDP-13D-C.Scalpelliformis(Tamil_Nadu)
gi|2935277|gb|AF033652.1|_Chara_australis

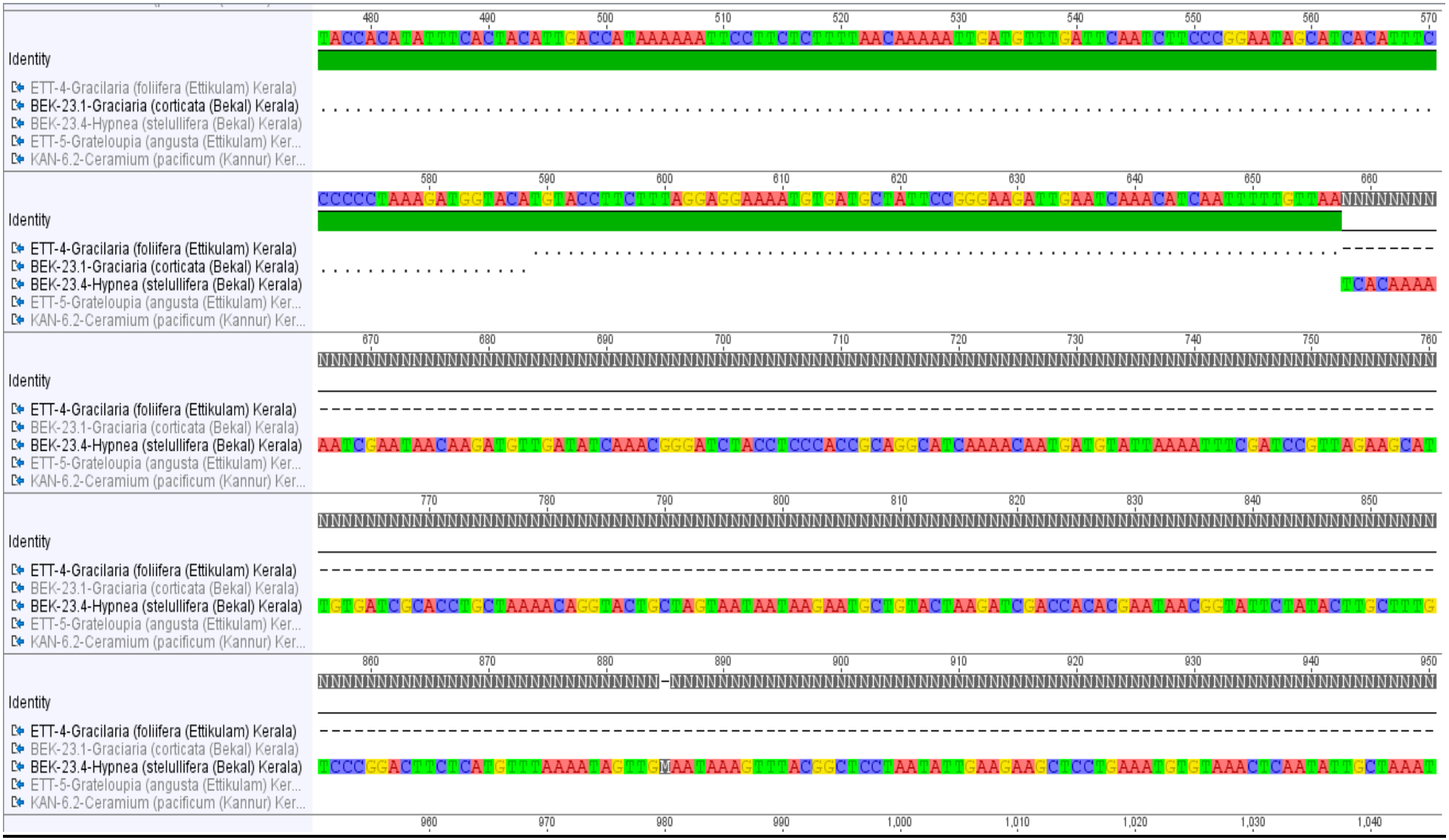
Appendix:-F. Multiple sequence alignment of Red algae using cox1gaz

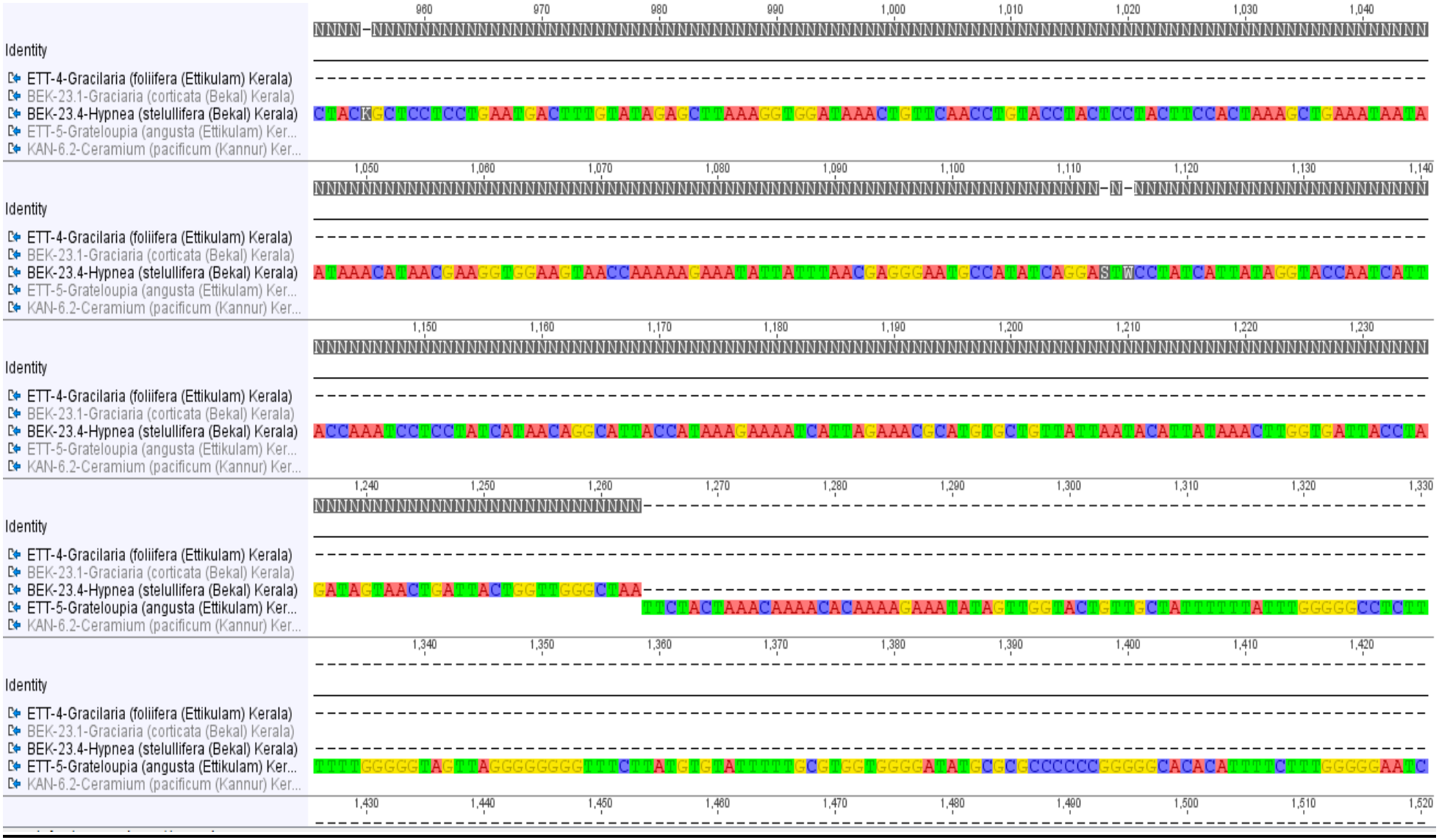


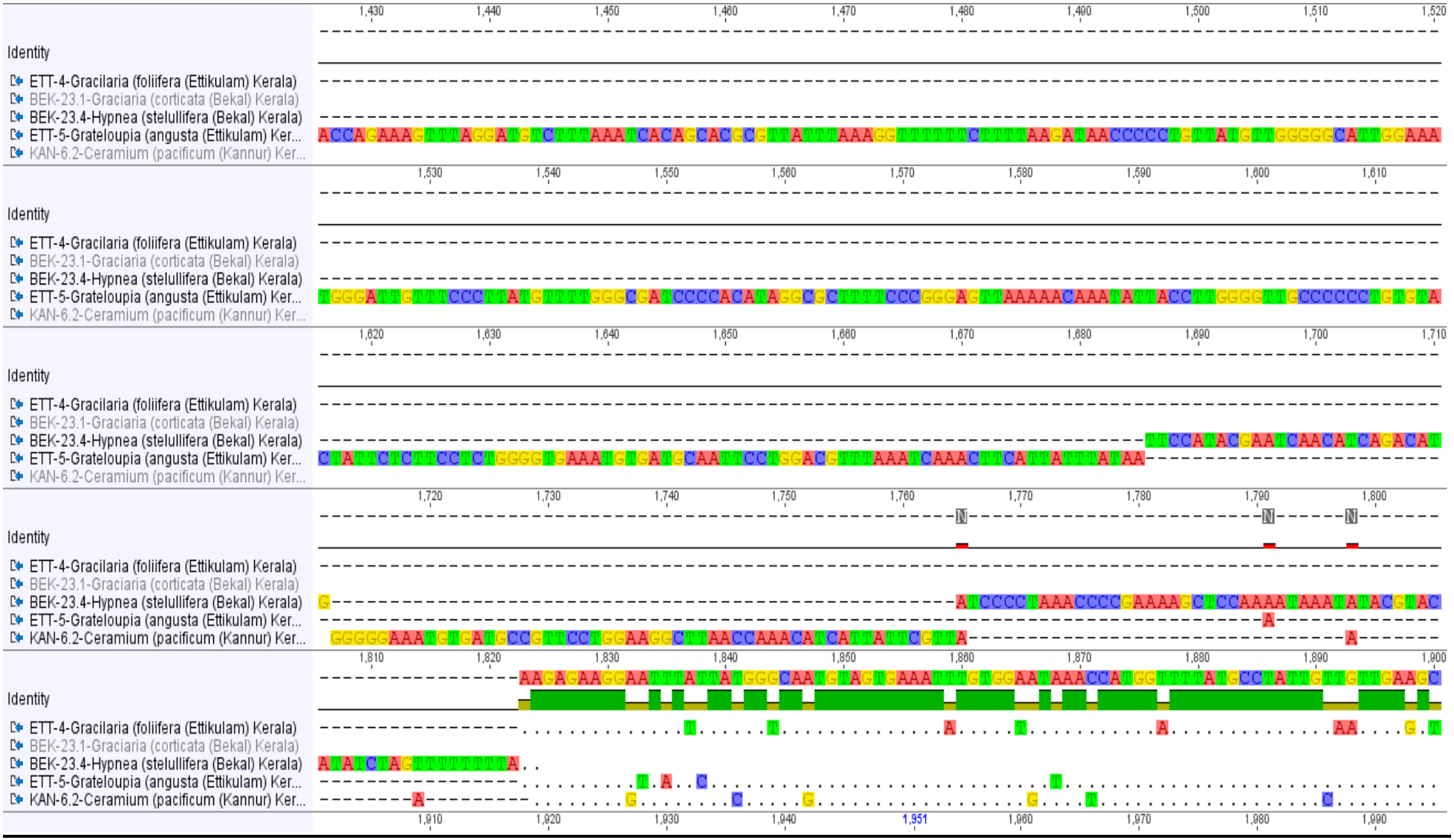

Identity
FWD MDP-13F-Gracilaria (domingensis (Man...
FWD ETT-5-Grateloupia (angusta (Ettikulam) ...
FWD KAN-6.2 (-Ceramium sp. (Kannur) Kerala)
FWD KER-11-Erythrocladia-Elathur-Kerala
FWD POD-T-4-Erythrocladia ((Pondy) Kerala)
FWD MDP-13E-Acanthophora (specifera (Man...
REV MDP-13F-Dilsea (reversed) (socialis (M...
FWD HAV-26- (Dichotomaria marginata (Have...
Ctrl left click on a sequence position or annotation, or select a region to zoom in. Ctrl-shift left click to zoom out.

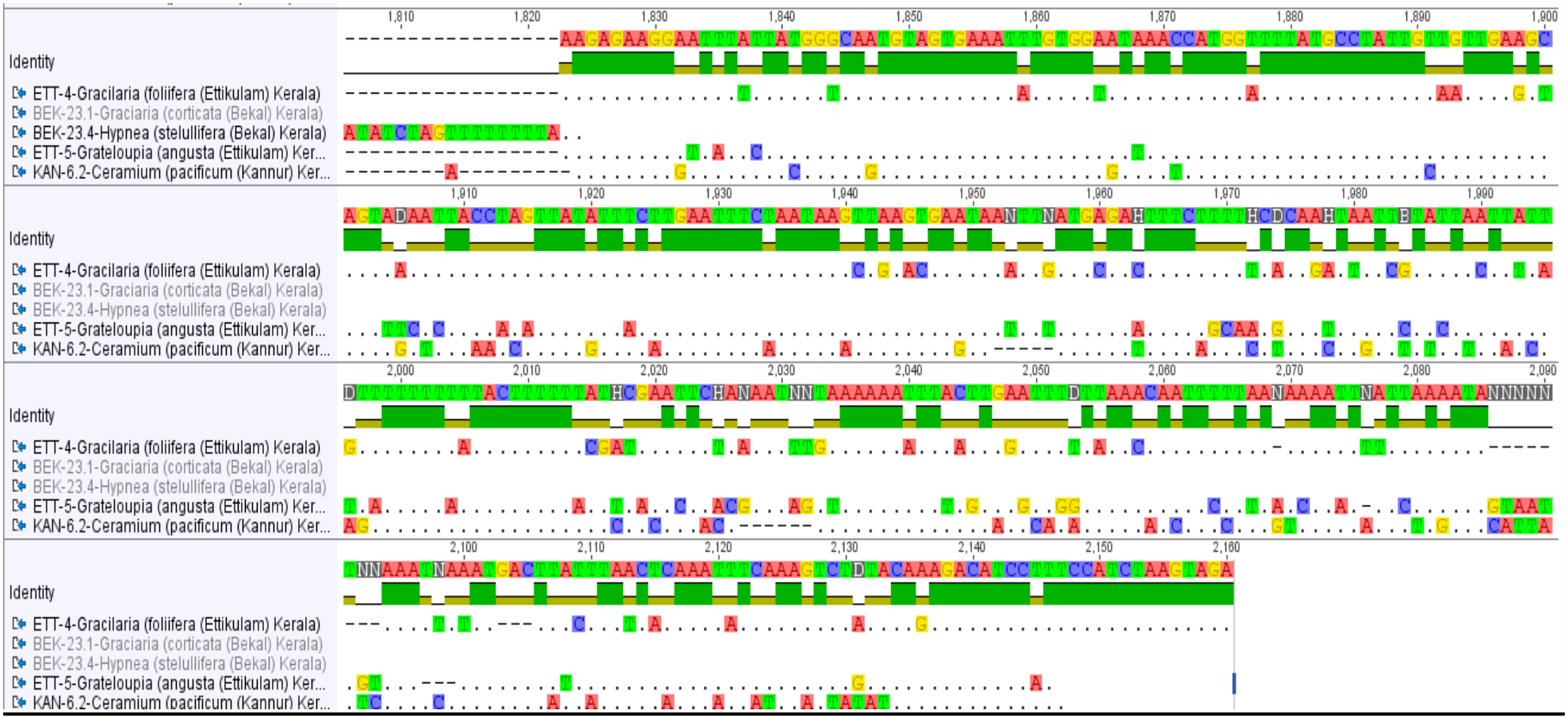
Appendix:-G. Multiple sequence alignment of red algae using COX F & R




Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...


960 970 980 990 1,000 1,010 1,020 1,030 1,040
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,050 1,060 1,070 1,080 1,090 1,100 1,110 1,120 1,130 1,140
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,150 1,160 1,170 1,180 1,190 1,200 1,210 1,220 1,230
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,240 1,250 1,260 1,270 1,280 1,290 1,300 1,310 1,320 1,330
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,340 1,350 1,360 1,370 1,380 1,390 1,400 1,410 1,420
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,430 1,440 1,450 1,460 1,470 1,480 1,490 1,500 1,510 1,520


1,430
1,440
1,450
1,460
1,470
1,480
1,490
1,500
1,510
1,520
Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...
1,530
1,540
1,550
1,560
1,570
1,580
1,590
1,600
1,610
1,620
1,630
1,640
1,650
1,660
1,670
1,680
1,690
1,700
1,710
1,720
1,730
1,740
1,750
1,760
1,770
1,780
1,790
1,800
1,810
1,820
1,830
1,840
1,850
1,860
1,870
1,880
1,890
1,900
1,910
1,920
1,930
1,940
1,951
1,960
1,970
1,980
1,990


Identity
ETT-4-Gracilaria (foliifera (Ettikulam) Kerala)
BEK-23.1-Graciaria (corticata (Bekal) Kerala)
BEK-23.4-Hypnea (stelullifera (Bekal) Kerala)
ETT-5-Grateloupia (angusta (Ettikulam) Ker...
KAN-6.2-Ceramium (pacificum (Kannur) Ker...

Appendix:-H. Multiple sequence alignment of Brown algae using COX1gaz F & R

<u>Appendix:-I.</u>Pairwise sequence alignment of Red algae *Centroceras clavulatum* using *rbcL* region from different locations.