RESEARCH ARTICLE

First Report of *Ulva sapora* (Ulvales, Chlorophyta) from Indian Subcontinent

Pooja Rani[1], Felix Bast[2]

DOI: 10.18811/ijpen.v5i01.9

Abstract

The green marine algal species *Ulva sapora (U. sapora)* had only been reported from Australia till date. In this study, we analyzed many algal specimens collected from different locations of Indian coastline and the Andaman Islands. Both morphological, as well as nuclear ITS1 based phylogenetic analysis, revealed them to be congruent with *U. sapora*. All the isolates from India clustered with the accessions from Australia forming a monophyletic clade with strong statistical support. We also generated sequence data of chloroplast-encoded CF1-ATPase beta-subunit gene (atpB) for the first time for this species. Phylogenetic assessments at this locus revealed an evolutionary affinity of *U. sapora* with *Ulva intestinalis*. As *U. sapora* is commercially important edible green seaweed and a known indicator for coastal pollution, this new record for India are expected to be significant.

Keywords: atpB, Chlorophyta, ITS1, *Ulva sapora*, Ulvales.

International Journal of Plant and Environment (2019)

Introduction

The green marine algal species *Ulva sapora* was formally described from Shelley Beach, Queensland, Australia in 2015 (Phillips *et al*., 2015). This species had been previously reported from rivers of Okinawa, Japan (Shimada *et al*., 2008) and shallow coastal waters of Hawaii (O'Kelly *et al*., 2010) under the name of *Ulva* sp. 3 and OTU6, respectively. *Ulva sapora* was reported to have been cultivated for the production of biomass, had a high growth rate in warm temperature (Lawton *et al.,* 2013), and can exist in brackish to saline water (Carl *et al*., 2014). The specific Latin epithet 'Sapora' was given to indicate the pleasant flavor of this edible algae. Amongst the species delineation characters of this species are the central axis of the thallus which is branched or unbranched, tubular with increasing breadth from a basal to the distal end. Multiple uniseriate and multiseriate branches arise from the basal region. Rectangular or polygonal cells are arranged in linear rows in the basal region, but cells unorganized towards upper thallus. The chloroplast of the cell occupies half or complete area of the cell and contain many starch granules (Phillips *et al*., 2016). Each cell consists of 2–10 pyrenoids. Zoids were reported to be biflagellate.

Genus *Ulva* has simple morphology with very few identification features (Malta *et al*., 1999). *Ulva* (Ulvophyceae, Chlorophyta) mainly consists of two morphological forms: tubular and blade-like (Tan *et al.,* 1999; Hayden *et al.,* 2003). The morphology of the genus varies between monostromatic tubular thalli and distromatic blades. A few species, like *Ulva linza,* have been described to have an intermediate morphology, with monostromatic tubular base and distromatic frond (Blomster *et al.,* 2002). Therefore, it is a major challenge to identify and delineate the species on the morphological and microscopic features of the thallus only. Twenty-seven species of *Ulva* were reported from the coasts of India till date. Earlier investigations of tubular *Ulva* growing in the coasts of Indian subcontinent reported the presence of *Ulva intestinalis, Ulva compressa* and *Ulva flexousa* (Joshi and Krishnamurthy, 1972; Chennubhotla *et al.,* 1988; Rao *et al.,* 1993; Dhargalkar and Deshmukhe, 1996; Kaliaperumal *et al.,* 1998; Sahoo *et al*., 2003; Rath and Adhikary, 2006; Kaladharan *et al.,* 2011; Pereira and Almeida, 2014). Most of the previous species identification of *Ulva* was based upon the morphology of thallus except *Ulva paschima* (Bast *et al*., 2014a) and *Ulva chaugulii* (Kazi *et al*., 2016). Tubular algae grow on generally on the rocks of coastal areas or are freely floating in the water. The growth of tubular *Ulva* was influenced by a broad range of environmental factors like water salinity, temperature variation, and sunlight (Poole and Raven, 1997; Reed and Russell, 1978). Anthropogenic activities influence the nutrient load of the littoral habitats (Smith, 2003). Excessive accumulation of inorganic nutrients promotes the growth of tubular *Ulva* in the coastal areas (Rosenberg and Ramus, 1984; Fletcher, 1996). These seaweeds form a mat like structure, or freely floating blooms known as green tides (Bonsdorff *et al.,* 1997; Alström-Rapaport *et al*., 2010). Tubular *Ulva* had been reported in marine as well as freshwater habitats (Ichihara *et al*., 2009).

In order to resolve the cryptic diversity and aid in the accurate identification of *Ulva*, we employed DNA barcoding technology in the present study. In the previous report of *U. sapora* (Phillips *et al.,* 2016), ITS1 is used for molecular systematic analysis and species delineation within the genus *Ulva*. In the present study, a total of five samples were collected from different coastal locations of India, including Andaman and Nicobar Islands. All isolates were amplified using ITS1 (Nuclear) and CF1 atpB (Chloroplast) locus.

[1,2]Department of Plant Sciences, Central University of Punjab, Bathinda, Punjab, India

Corresponding Author: Felix Bast, Department of Plant Sciences, Central University of Punjab, Bathinda, Punjab, India, Phone: +919872152694, e-mail: felix.bast@cup.ac.in

How to cite this article: Rani, P., & Bast, F. (2019). First Report of Ulva sapora (Ulvales, Chlorophyta) from Indian Subcontinent. Int J of Plant and Environ, 5(1):50-56.

Source of support: DST Inspire Faculty Award, awarded to FB

Conflict of interest: None

Submitted: 31/03/2018 **Accepted:** 12/07/2018 **Published:** 01/01/2019

Materials and Methods

Sample Collection

Samples of tubular green algae were collected from five different locations of the Indian coast (Fig. 1). The description of the location, sampling site, and coordinates were summarized in Table 1. Multiple specimens were collected randomly for each

taxon from each locality to study the range of morphology. Collected specimens were transported to the laboratory in zip-lock polythene bags under cold conditions (4–10°C). After washing the thalli in tap water to remove sediments and other contaminants, morphological characterization of the specimens was carried out using an upright microscope (CX41RF, Olympus, Japan) with an attached digital camera (E450, Olympus, Japan). Public domain software ImageJ (http://rsbweb.nih.gov/ij/) was used for scale calibration and size measurements. Pressed vouchers were prepared and deposited in the herbarium of the Central University of Punjab, Bathinda (Table 1).

DNA extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA was extracted from the apical thalli of the samples using a HiPurA Algal Genomic Extraction Kit (HiMedia Laboratories Pvt. Ltd., Mumbai). The quality of DNA was checked on 0.8% agarose gel and the quantity of DNA was tested with a spectrophotometer (Thermo Scientific Nano Drop 2000). The isolated DNA was subjected to PCR amplification using the primers described in Table 2. A DNA working solution of 25ng/µL was prepared for polymerase chain reaction (PCR) in a separate tube. The 20 µl PCR reaction mix contained 2 µL reaction buffer with 15 mM $MgCl_2$ (Applied Biosystems, India), 4 µL each of 10 mM primer, 2 µl of 1 mM dNTPs (Genetix Biotech Asia Pvt. Ltd, New Delhi), 0.6 unit of rTaq DNA polymerase (Genetix Biotech Asia Pvt. Ltd, New Delhi), 4 µl of template DNA and distilled water. The four universal primers (Table 2) used for amplifying the ITS1 (Internal Transcribed Spacer) regions and atpB (ATPase beta subunit) gene region. 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 minute, 45°-52°C (52°C for ITS1 region and 45°C for atpB) for 2 minutes and 72°C for 2 minutes, and a final extension of 72°C for 10 minutes.

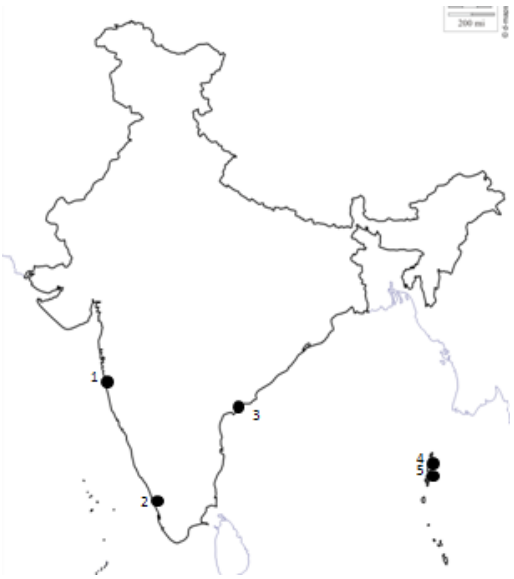

Fig. 1: Sampling locations: 1 (Ratnagiri), 2 (Kannur), 3 (Thotlakonda), 4 (Kalapather) and 5 (North Bay)

Table 1: Sampling location, Geographical coordinates, Herbarium voucher, Description of habitat, Sequence length, Accession number and Date of collection of *Ulva* thallus

S. No.	Name of algae	Location	Coordinates	Voucher herbarium	Habitat	Accession number (Seq. length)		Collection date
						ITS1	atpB	
1.	*Ulva sapora*	Kannur, Kerala	11°52'57"N, 75°20'13"E	CUPVOUCHER-KAN-2014-US-2	Attached, exposed rocky shore, pebbles or freely floating.	MG763135 (582 bp)	Nil	26 May 2012
2.	*Ulva sapora*	Kalapathar, Andaman Island	11° 57′ 36″N, 93° 0′ 0″ E	CUPVOUCHER-KAP-2014-US-1	Attached, exposed to rocky shores, pebbles or freely floating.	MG763136 (250 bp)	Nil	16 January 2014
3.	*Ulva sapora*	North Bay, Andaman Island	11° 57′ 0″N, 92° 45′ 0″ E	CUPVOUCHER-NOB-2014-US-1	Attached, exposed to rocky shores, pebbles or freely floating.	MG763137 (598 bp)	Nil	16 January 2014
4.	*Ulva sapora*	Ratnagiri, Maharashtra	16°04′0.12″N 73°28′.1128″E	CUPVOUCHER-RAT-2015-US-1	Attached, exposed rocky shores, found in midlittoral zone.	MG763138 (599 bp)	MG918111 (218 bp)	23 Jul., 2015
5.	*Ulva sapora*	Thotlakonda, Andhra Pradesh	17° 49′ 35″N, 83° 24′ 34″E	CUPVOUCHER-THO-2015-US-1	Attached to rocky surface, found in intertidal zone.	MG763139 (352 bp)	MG918113 (221 bp)	17 Dec., 2015

Table 2: List of primers for PCR amplification.

S. No.	Target region	Name of primer	Sequence of primer	Reference
1.	ITS1 (Nuclear)	ITS1	TCCGTAGGTGAACCTGCGG	Saunders and Kucera (2010)
		ITS2	GCTGCGTTCTTCATCGATGC	
2.	atpB (Chloroplast)	atpB Forward	GTATGCGTGTTGCTTTAACA	Saunders and Kucera (2010)
		atpB Reverse	TCTTGTAGACCACCCATTTC	

DNA Purification and DNA Sequencing

Amplicons were purified using ExoSAP-IT PCR clean-up kit following the manufacturer's instructions (USB Corporation, Cleveland, OH, USA). A working solution of 1:10 (DNA: water) was prepared as a sequencing template. 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 as quality control. Purified PCR products were subjected to bidirectional Sanger sequencing 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), as per Bast *et al*. (2014b).

Sequence Annotations and Phylogenetic Analysis

Raw DNA sequences were assembled using Codon Code Aligner version V.0.6.2. (CodonCode Corporation, USA). The assembled sequences were deposited in Gen Bank database. Accession numbers are listed in Table 1. All sequences were analyzed for sequence similarity search using NCBI-BLASTn. Top 35 results of BLAST were aligned with total 5 isolates that were amplified with ITS1 primer. Sequences were aligned at first by MUSCLE algorithm inside the computer program MEGA (www.megasoftware.net/). Ends were trimmed to refine the alignment. *Ulvaria fusca* and *Umbraulva japonica* were taken as out-group. Substitution bias was modeled by Tamura-3-Parameter (T92) (Tamura and Nei, 1993) model and Gamma distribution (that was the best model in our test to find best fitting substitution models (Tamura *et al.,* 2011) with BIC (Bayesian Information Criterion) score of 12213.9. Pairwise distance between sequences was calculated using the T92 model within MEGA. The analysis involved 42 sequences. Phylogenetic analysis using Maximum likelihood (ML) algorithm was conducted within MEGA starting tree generated by BioNJ. Substitution bias was modeled by T3P model with invariable sites. Heuristic searches were performed with tree bisection-reconnection, MULTREES and steepest descent options in effect. Five hundred bootstrap replicates were performed under the ML criterion to estimate interior branch support. A consensus tree was constructed using the consensus tree builder within MEGA. The ML analysis was carried out using 726 bp aligned characters for ITS1. The final ITS1 alignment contained 382 variable, 329 conserved sites, and 229 parsimony-informative sites. The nucleotide frequency was A=20.13%, T/U=17.01%, C=34.05%, G=28.80%. The estimated rate of transition/transversion bias was 1.03 and evolutionary rate difference among sites is 0.3483. All the five isolates amplified with ITS1 primer had sequence length 250-599 bp and showed 98-100% identity. Mean intra-populational evolutionary distance between the *U. sapora* from India and Australia based on ITS1 was 0.018.

For atpB locus, three top BLASTn hits of *Ulva* accessions in Genbank were aligned with the generated sequences of our two isolates that are amplified with atpB primer. *Caulerpa taxifolia* is taken as out-group. Final atpB alignment had 221 characters. Substitution bias was modeled by Tamura-3-parameter (T92) model (Tamura *et al.,* 2013) and Gamma distribution that was the best model in our test to find best fitting substitution models (Tamura *et al.,* 2011) with BIC (Bayesian Information Criterion) score of 3605.8. Pairwise distances between sequences were calculated using the T92 model within MEGA. The analysis involved 6 sequences. Phylogenetic analysis using Maximum likelihood (ML) algorithm was conducted in MEGA, with starting tree generated by BioNJ. Substitution bias was modeled by T3P model with invariable sites. Heuristic searches were performed with tree bisection-reconnection, MULTREES and steepest descent options in effect. 500 bootstrap replicates were performed under the ML criterion to estimate interior branch support. A consensus tree was constructed using the consensus tree builder within MEGA. The ML alignment contained 72 variable sites, 150 conserved sites, and 17parsimony-informative sites. The nucleotide frequency was A = 32.95%, T/U = 30.77%, C = 17.12%, G = 19.16%. The estimated rate of transition/transversion bias was 1.34 and evolutionary rate difference among sites is 2.9977. All data including cell area measurements, DNA sequence alignment in FASTA format, and results of Model Test, pair-wise distances, tree and original electropherograms of DNA sequences are available from authors upon request.

Results

Morphological Examination

The collected specimens KAN-6.4 (Kannur, Kerala), KAP-42.1 (Kalapather, Andaman Island), NOB-43.2 (North Bay, Andaman Island), RAT-60 (Ratnagiri, Maharashtra) and THK-175 (Thotlakonda, Andhra Pradesh) (Fig. 2, Table 1) had several distinct morphological features. The thallus was fragile, filamentous or compressed, unbranched or branched and alternatively growing secondary branches around the main axis (Figs 1B to 5B). The fronds were 2 to 40 cm in length (Figs 1A to 5A). On comparing morphological features, KAN-6.4, NOB-43.2, RAT-60, and THK-175 were branched while KAP-42.1 was unbranched (Fig. 2A). Secondary branches were mostly present in the basal region only. The margin of each branch was smooth and broader towards distal ends as compared to the basal region. Filaments were broader at distal ends. Cells were rectangular or irregular in shape in surface view (Figs 1E to 5E) and arranged in the linear rows in basal region (Figs 1D to 5D). Cells become unordered towards distal ends. Tip of the apices is rounded in the secondary branch (Figs 1B to 5B). Uniseriate or multiseriate branches were present in the basal region. Multiple zoospores are present in the basal region of the thallus (Figs 1C to 5C). Thick patches of chloroplast occupied the complete cell areas (Figs 1F to 5F).3-10 Pyrenoids were present in each cell. Thalli had disc-shaped holdfasts. All of these morphological features were congruent with the described characters of *Ulva sapora* from Australia (Phillips *et al.,* 2016). Therefore, the specimens KAN-6.4 (Kannur, Kerala), KAP-42.1 (Kalapather, Andaman Island), NOB-43.2 (North Bay, Andaman Island), RAT-60 (Ratnagiri, Maharashtra) and THK-175 (Thotlakonda, Andhra Pradesh) were identified as *Ulva sapora*.

Molecular and Phylogenetic Analysis

Sample ID NOB-43.2 (MG763137), KAN-6.4 (MG763135), KAP-42.1 (MG763136), RAT-60 (MG763138), and THK-175 (MG763139) from North Bay, Kannur, Kalapather, Ratnagiri and Thotlakonda respectively, were identified as *U. sapora* by BLASTn homology search with recently described *U. sapora* (KT374010) from Australia (Phillips *et al.,* 2016). On the ML tree (Fig. 3), all *U. sapora* from India formed a distinct monophyletic clade with accessions of *U. sapora* from Australia. This clade was supported by high bootstrap value (i.e., 77). The pair-wise distances between *U. sapora* isolates from India and Australia ranged between 0.000–0.065%.

In contrast to ITS1, the sequence of the atpB gene showed maximum similarity with *U. intestinalis*. However, there were no accessions of *U. sapora* at atpB region in NCBI prior to this study. For phylogeny inference, we used the isolates RAT-60 (MG918111) and THK-175 (MG918113). In the phylogenetic assessment of atpB gene, sequences of *U. sapora* isolates from Indian coastline formed a separate clade (Fig. 4). This clade of *U. sapora* was supported by

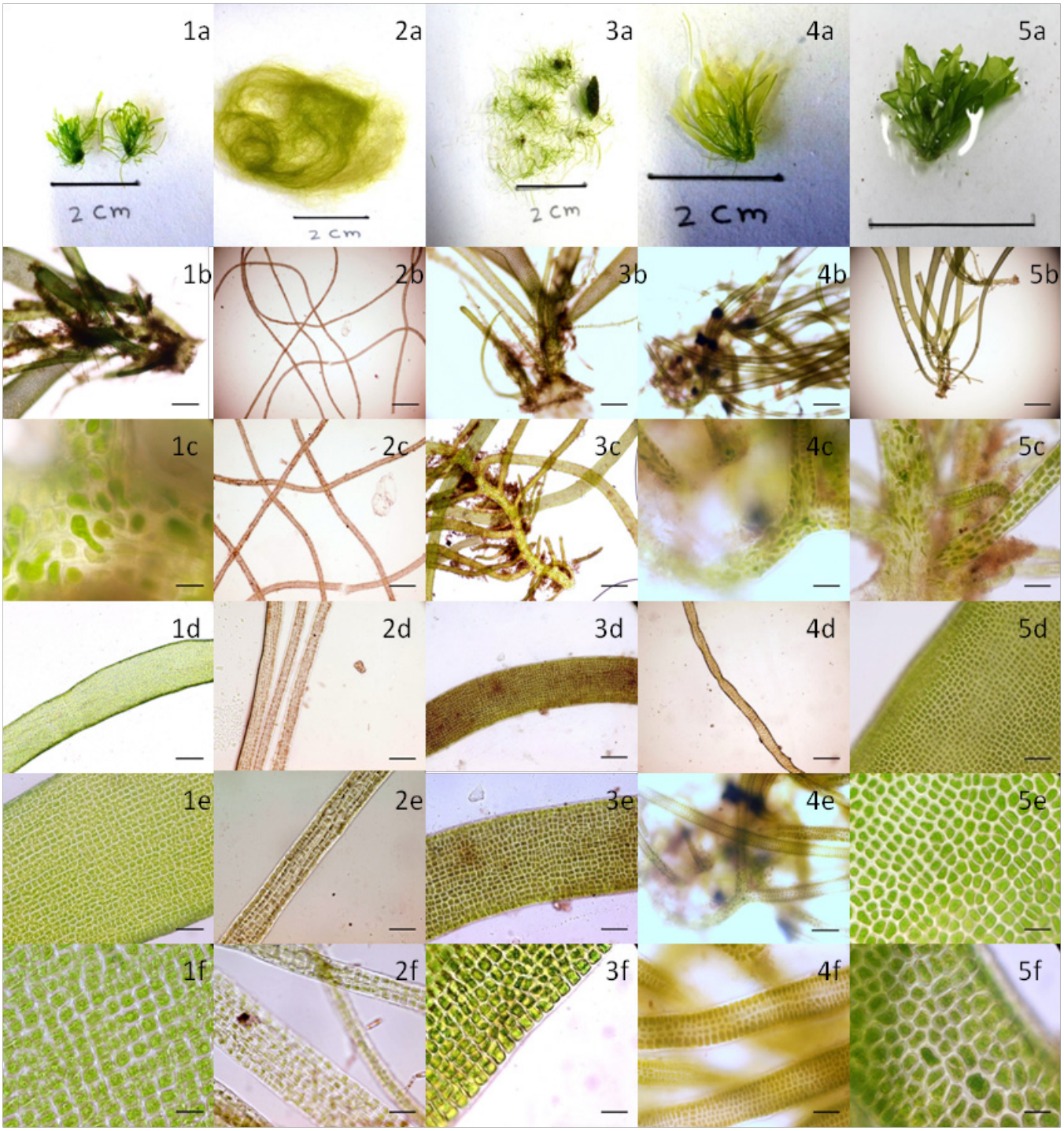

Fig. 2: Morphology of *Ulva* isolates from India. KAN-6.4 (1A to 1F), KAP-42.1 (2a-2f), NOB-43.2 (3a-3f), RAT-60 (4a-4f) and THK-175 (5a-5f).:1a-5a indicate morphology of thallus; 1b-5b indicate basal branching pattern; 1c-5c indicate presence of zoospores; 1d-5d indicate margin of filament and cell arrangement;1e-5e indicate shape of cell and 1f-5f indicate number of pyrenoids. Scale bar are 2cm for 1a-5a at 4X; 20 µmfor 1b-5b at 10X; 10 µM for 1c-5c at 40X; 20 µm for 1d-5d at 10X; 10 µm for 1e-5e at 40X; 2µm for 1f-5f at 100X

high bootstrap value (99%). The pair-wise distance between Indian isolates of *U. sapora* was 0.020%. The pair-wise distance between *U. sapora* and other species of *Ulva* ranged between 0.046-0.086%. Based on morphological and DNA sequence data, the taxonomical identity of our collected isolates was confirmed to be *U. sapora*.

Discussion

Record of this species for Indian coastline. All Indian and Australian isolates together formed a strongly supported monophyletic clade in our ITS1 phylogram indicating the conspecificity. Indian isolates of *U. sapora* had similar morphological characters (Basal uniseriate and multiseriate branching, cells arranged in linear

rows) with Australian isolates as described by Phillips *et al*. (2016). The morphological characters and the molecular data corroborate the identity of these samples to be *U. sapora*. The morphological variations were found in some samples of *U. sapora*. It caused the problem in the species identification of *Ulva* when using the morphological identification keys provided by Phillips *et al*. (2016). However, our samples of *U. sapora* were 1 to 40 cm long that was outside the range of description of *U. sapora*. This difference could be due to ecological or biogeographic constraints. There were also differences in the branching pattern. Most of our samples were branched around the base except KAP-42.1. Several studies have shown that branching is not a stable character in tubular *Ulva* species. Branching could be induced to un-branched thallus under varied salinity conditions. Cell size, organization and the number of pyrenoids also fluctuate in response to environmental conditions. Results of ITS1 sequence data, when combined with earlier findings from molecular and morphological studies of *U. sapora* from Australia, provide strong evidence that all Indian isolates were *U. sapora*. Monophyly of these taxa was also affirmed in this study. *U. sapora* formed a sister clade with *U. chaugulii, U. rigida*, and *U. linza*, revealing evolutionary affinity of these taxons (Table 3).

Due to lack of adequate coverage and taxa representation at Genbank, our atpB phylogram did not resolve the fine evolutionary legacy. *U. intestinalis* was found to be its closest match, yet these species have a number of differences.

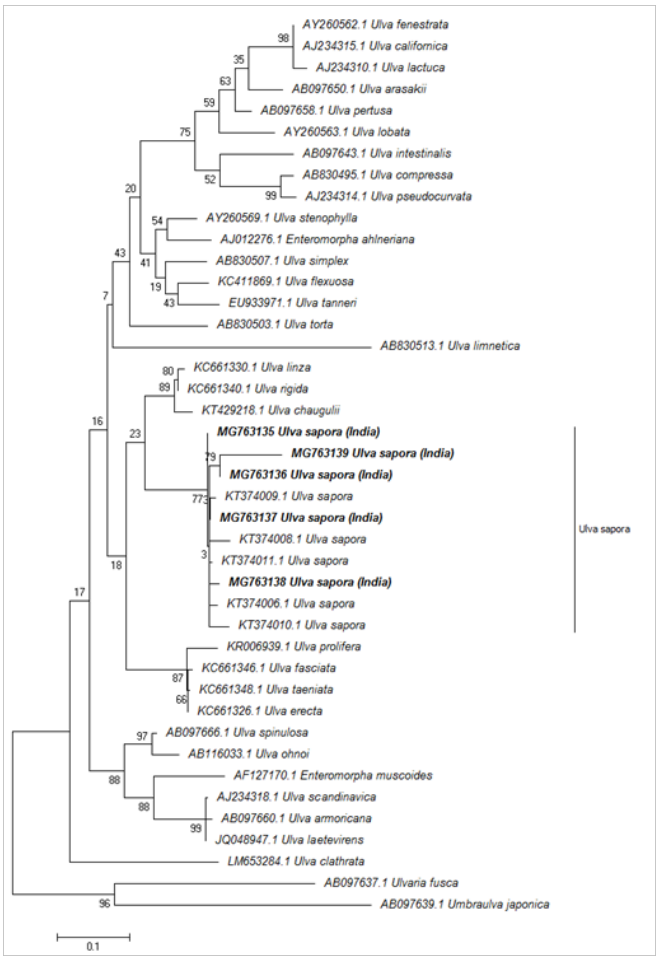

Fig. 3: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in ITS1 dataset. Analysis was performed using Maximum Likelihood phylogenetic reconstruction method (LnL=--5686.3408) with Tamura-3-parameter and Gamma distribution model of molecular evolution (T92). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted with *Ulvaria fusca* and *Umbraulva japonica* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitutions per site

Ecological and Economic Relevance

Ulva sapora is edible algae, and this new record of this seaweed in Indian Coasts opens up avenues for the utilization of this important seaweed resource. The algae is in high demand in East Asian countries (Japan, Korea, and Taiwan). Commercial cultivation of this alga, if optimized, could be a potential revenue generator for the economically poorer remote coastal communities through exports. The finding of this important marine alga also has ecological ramification. Green seaweeds of the genus *Ulva* are known primary producers of the intertidal ecosystem. Several past research has highlighted the high degree of susceptibility of this alga towards anthropogenic activities and pollution (Wan *et al*., 2017). This alga could, therefore, function as an indicator organism for the coastal pollution. It is noteworthy that all the five locations from where we have collected the isolates of this alga are relatively free from anthropogenic activities and pollution. In addition, ensuring healthy natural communities of this alga in our coastal regions would lead to an overall healthy coastal ecosystem and potentially contribute to the sustainable production of fishery resources.

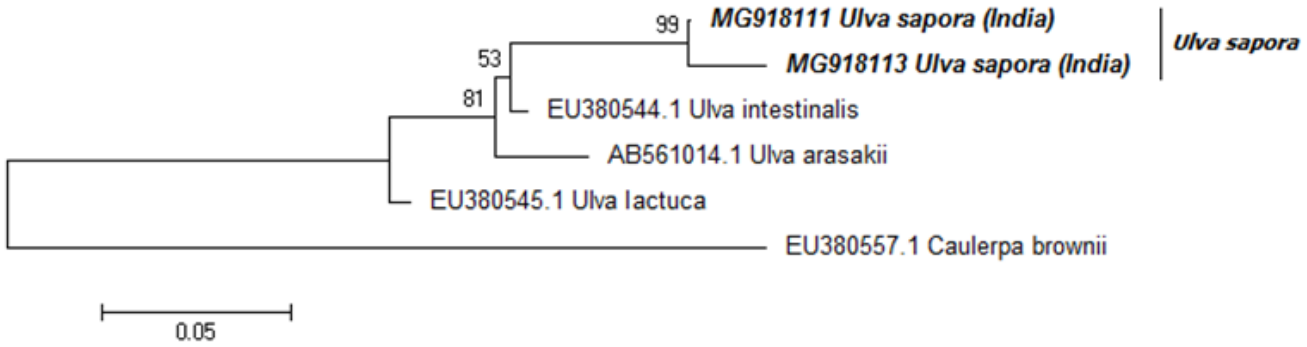

Fig. 4: Phylogenetic position of tubular *Ulva* isolates from India among other *Ulva* accessions in atpB dataset. The analysis was performed using Maximum Likelihood phylogenetic reconstruction method (Lnl=-1758.978) with Tamura-3-Parameter and gamma distribution model of molecular evolution (T92). Numbers near nodes represent bootstrap support (500 replicates). This phylogram is rooted in *Caulerpa brownii* as an out-group. Scale bar given on bottom is in the units of average nucleotide substitution per site

Table 3: Morphological and microscopic characteristics of *Ulva* thallus.

S. No.	*Name of algae*	*Sample id*	*Thallus type/ colour/ branching/type of secondary branch*	*Thallus size/ shape*	*Cell shape/ cell arrangement*	*Cell size µm^2/ Chloroplast type/ No. of pyrenoids*	*Reproductive zoospores/ position*
1.	*Ulva sapora*	KAN-6.4	Tubular, contorted/ light yellow to dark green/ highly branched/ multiseriate/ distal end broader	1-2 cm/ bushy	Rectangular or cuboidal/irregular	76±3.2/Thick patches/multiple	Multiple/ flagellated/ at basal and proliferating branch point
2.	*Ulva sapora*	KAP-42.1	Tubular, coiled/yellow to dark green/unbranched/ multiseriate	35-40 cm/ tubular	Rectangular/ regular, linear rows	34±1.92/Thick patches/multiple	Not observed
3.	*Ulva sapora*	NOB-43.2	Tubular or compressed/ alternative branched/ multiseriate/ distal end broader	15-20 cm/tubular or compressed	Rectangular or irregular/linear rows/ tip apices rounded	58± 3.501/Thick patches/multiple	Multiple/ flagellated/on main axis
4.	*Ulva sapora*	RAT-60	Tubular/yellowish green/ bifurcating at basal region, branched/ multiseriate/ distal end broader	5-10 cm/bushy	Cuboidal/regular/ linear rows	30±1.16/ Thick patches/multiple	Multiple/ flagellated/ at bifurcating branch point
5.	*Ulva sapora*	THK-175	Tubular at the base, compressed at the distal end/alternatively branched/ uniseriate or multiseriate	2 cm/bushy	Cuboidal, rectangular, pentagonal/linear rows/irregular at middle region	84±9.7/Thick patches/ multiple	Multiple/ flagellated / branching areas

Conclusion

In the present study, *U. sapora*is reported for the first time from the Indian coast. All *U. sapora* samples show significant morphological and molecular resemblance with isolates from Australia. In the phylogenetic analyses, Indian isolates formed a monophyletic clade with Australian isolates. Sequence data at the AtpB region for *U. sapora* was generated for the first time in this study, so as a genus-level phylogeny based on this locus. As *Ulva sapora* is commercially important edible green seaweed and a known indicator for coastal pollution, this new record for India are expected to be significant.

Acknowledgment

Authors thank Dr. Pankaj Bhardwaj for supporting the sequencing process. We are thankful to the Central University of Punjab, Bathinda, for providing infrastructure for this work. This study was supported by the grant-in-aid from DST INSPIRE Award, Government of India awarded to FB (IFA11-LSPA-02).

References

1. Alström-Rapaport, C., Leskinen, E. and Pamilo, P. 2010. Seasonal variation in the mode of reproduction of Ulva intestinalis in a brackish water environment. Aquatic Botany 93(4):244-249.
2. Bast, F., John, A.A. and Bhushan, S. 2014a. Strong endemism of bloom-forming tubular Ulva in indian west coast, with description of Ulva paschima sp. Nov. (Ulvales, Chlorophyta). PloS One 9:e109295.
3. Bast, F., Rani, P. and Meena, D. 2014b. Chloroplast DNA phylogeography of holy basil (Ocimum tenuiflorum) in Indian subcontinent. The Scientific World Journal 10:e847482.
4. Blomster, J., Bäck, S., Fewer, D.P., Kiirikki, M., Lehvo, A., Maggs, C.A. and Stanhope, M.J. 2002. Novel morphology in Enteromorpha (Ulvophyceae) forming green tides. American Journal of Botany 89(11):1756-1763.
5. Bonsdorff, E., Blomqvist, E., Mattila, J. and Norkko, A. 1997. Coastal eutrophication: Causes, consequences and perspectives in the Archipelago areas of the northern Baltic Sea. Estuarine, Coastal and Shelf Science 44:63-72.
6. Carl, C., de Nys, R. and Paul, N.A. 2014. The seeding and cultivation of a tropical species of filamentous Ulva for algal biomass production. PloS One 9(6):e98700.
7 Chennubhotla, V., Ramachandrudu, B., Kaladharan, P. and Dharmaraja, S. 1988. Seaweed resources of Kerala coast. Bulletin of the Department of Aquatic Biology and Fisheries 7:69-74.
8. Dhargalkar, V. and Deshmukhe, G. 1996. Subtidal marine algae of the Dwaraka coast (Gujarat). Indian Journal of Marine Sciences 25:297-301.
9. Fletcher, R. 1996. The occurrence of "green tides"—a review. Marine Benthic Vegetation 123:7-43.
10. Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C., Stanhope, M.J. and Waaland, J.R. 2003. Linnaeus was right all along: Ulva and Enteromorpha are not distinct genera. European Journal of Phycology 38(3):277-294.
11. Ichihara, K., Arai, S. and Shimada, S. 2009. cDNA cloning of a lectin-like gene preferentially expressed in freshwater from the macroalga Ulva limnetica (Ulvales, Chlorophyta). Phycological Research 57(2):104-110.
12. Joshi, H. and Krishnamurthy V. 1972. The species of Enteromorpha from India. Botanical Journal of the Linnean Society 65(1):119-128.
13. Kaladharan, P., Zacharia, P. and Vijayakumaran, K. 2011. Coastal and marine floral biodiversity along the Karnataka coast. Journal of the Marine Biological Association of India 53(1):121-129.
14. Kaliaperumal, N., Chennubhotla, V., Kalimuthu, S., Ramalingam, J., Pillai, S.K., Muniyandi, K., Rao, K.R., Rao, P., Thomas, P. and Zaidi, S. 1998. Seaweed resources and distribution in deep waters from Dhanushkodi to Kanyakumari, Tamil Nadu. Seaweed Research and Utilisation 20(1&2):141-151.
15. Kazi, M.A., Kavale, M.G. and Singh, V.V. 2016. Morphological and molecular characterization of Ulva chaugulii sp. Nov., U. lactuca and U. ohnoi (Ulvophyceae, Chlorophyta) from India. Phycologia 55(1):45-54.
16. Lawton, R.J., Mata, L., de Nys, R. and Paul, N.A. 2013. Algal bioremediation of waste waters from land-based aquaculture using Ulva: Selecting target species and strains. PloS one 8(10):e77344.
17. Malta, E.J., Draisma, S.G. and Kamermans, P. 1999. Free-floating Ulva in the southwest Netherlands: Species or morphotypes? A

morphological, molecular and ecological comparison. European Journal of Phycology 34(05):443-454.
18. O'Kelly, C.J., Kurihara, A., Shipley, T.C. and Sherwood, A.R. 2010. Molecular assessment of *Ulva* sp. (Ulvophyceae, Chlorophyta) in the Hawaiian Islands. Journal of Phycology 46(4):728-735.
19. Pereira, N. and Almeida, M. 2014. A preliminary checklist of marine algae from the coast of Goa. Indian Journal of Geo-Marine Sciences 43:655-665.
20. Phillips, J.A., Lawton, R.J., Denys, R., Paul, N.A. and Carl, C. 2016. Ulva sapora sp. Nov., an abundant tubular species of Ulva (Ulvales) from the tropical pacific ocean. Phycologia 55(1):55-64.
21. Poole, L. and Raven, J. 1997. The biology of Enteromorpha. Progress in Phycological Research 12:1-148.
22. Rao, K.R., Rao, P., Thomas, P., Zaidi, S., Subbaramaiah, K., Kaliaperumal, N., Kalimuthu, S., Muniyandi, K., Ramalingam, J. and Najmuddin, N. 1993. Seaweed resources of Tamil Nadu coast, Sector-iv Kilakkarai-Rameswaram Island (Dhanushkodi). Seaweed Research and Utilisation 16(1&2):103-110.
23. Rath, J. and Adhikary, S.P. 2006. Marine macro-algae of Orissa, East coast of India. Algae-Inchon 21(1):49.
24. Reed, R. and Russell, G. 1978. Salinity fluctuations and their influence on "Bottle brush" morphogenesis in Enteromorpha intestinalis (L.) link. British Phycological Journal 13(2):149-153.
25. Rosenberg, G. and Ramus, J. 1984. Uptake of inorganic nitrogen and seaweed surface area: Volume ratios. Aquatic Botany 19(1-2):65-72.
26. Sahoo, D., Sahu, N. and Sahoo, D. 2003. A critical survey of seaweed diversity of Chilika lake, India. Algae-Inchon 18(1):1-12.
27. Saunders, G.W. and Kucera, H. 2010. An evaluation of rbcL, tufA, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogamie Algologie 31(4):487-528.
28. Shimada, S., Yokoyama, N., Arai, S. and Hiraoka, M. 2008. Phylogeography of the genus Ulva (Ulvophyceae, Chlorophyta), with special reference to the Japanese freshwater and brackish taxa. Journal of Applied Phycology 20(5):979-989.
29. Smith, V.H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research 10(2):126-139.
30. Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3):512-526.
31. 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(10):2731-2739.
32. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12):2725-2729.
33. Tan, I.H., Blomster, J., Hansen, G., Leskinen, E., Maggs, C.A., Mann, D.G., Sluiman, H.J. and Stanhope, M.J. 1999. Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, Ulva and Enteromorpha. Molecular Biology and Evolution 16(8):1011-1018.
34. Wan, A.H., Wilkes, R.J., Heesch, S., Bermejo, R., Johnson, M.P. and Morrison, L. (2017). Assessment and characterisation of Ireland's green tides (Ulva species). PloS one 12(1):e0169049.