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Taxonomical Synonymy of Red Seaweed *Gracilaria foliifera* (Forsskal) Borgesen, 1932 with *Gracilaria corticata* J. Agardh, 1852 based on Multi-Local Phylogeny

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Abstract

In this study, we assessed taxonomical position of *Gracilaria corticata* J. Agardh and *Gracilaria foliifera* (Forsskal) Borgesen collected from various locations along Indian coastline by combined morphological and multi-local molecular approaches. The species were indistinguishable based on morphological features which were previously claimed by various authors as diagnosing traits for the two species.These taxa were polyphyletic in our phylograms based either on chloroplast DNA rbcL-rbcS or mitochondrial DNA cox2-3 spacer sequences. A clade consisting of *Gracilaria corticata* and *Gracilaria foliifera* was indeed monophyletic and strongly supported, resolving the conspecificity of these two taxa. Based on article 11 of ICN, the principle of taxonomic priority, the binomen *Gracilaria corticata* is valid and preferable. *Gracilaria foliifera nomen rejiciendum* is therefore a synonym of the valid binomen *Gracilaria corticata.*

1. Introduction

Gracilaria corticata J. Agardh and *Gracilaria foliifera* (Forsskal) Borgesen are tropical red seaweeds belonging to the family *Gracilariaceae* of order *Gracilariales. G. corticata* are regularly dichotomously to irregularly branched, straps usually elongate, margins smooth with a firm and cartilaginous consistency. Apices of segments are acute and sometimes proliferous. Axes are compressed, cartilaginous and constricted at the base in basal branches (Durairatnam, 1961; Iyer *et al.*, 2004). In India, this species has been reported from Tamil Nadu (Tiruchendur, Pudumadam, Cape Comorin, Idinthakarai, Covelong, Mandapam, Kilakarai, Kattapadu and Tiruchendur) (Kaliaperumal *et al.*, 1998), Goa (Vagator, Anjuna, Bogmalo, Palolem, Talpona and Polem) (Pereira and Almeida, 2014), Karnataka (Kaladharan *et al.*, 2011), South Andaman (Chidiyatapu, Northe Bay) (Palanisamy, 2012), Andaman Island (Carbyn's cove, Burmanallah) Karthick *et al.*, 2013) and Gujarat (Dwaraka coast) (Dhargalkar and Deshmukhe, 1996; Pareek *et al.*, 2010). *G. corticata* initially described as *Rhodymenia corticata* by J Agardh (Agardh, 1841). This was described on material collected from Sri Lanka (Iyer *et al.*, 2004). Kützing (1849) transferred the taxon to the genus *Sphaerococcus* (Kützing, 1849). J. Agardh (1852) transferred the species to genus *Gracilaria* (Greville, 1830; Agardh, 1852).

Gracilaria foliifera are bushy with di or polychotomously branched, irregularly and sometimes pinnately bifurcate with thin and brittle fronds and proliferous margins (James, 1987; Rao, 1972). It is an abundant species with an unusual latitudinal distribution, occurring along almost the entire Atlantic coasts of Europe, America, Mediterranean Sea, Red Sea, Indian Ocean and Malayan Archipelago. In India, this species has been reported around Tamil Nadu (Pamban, Idinthakarai, Tiruchendur, Mandapam, Rameswaram, Tuticorin and Kanyakumari), Gujarat (Gopnath and Veraval), Lakshadweep (Minicoy Island) (Kim and Humm, 1965; Rao, 1972; Desikachary *et al.*, 1998; Pareek *et al.*, 2010; Sahu and Sahoo, 2013), Goa (Mornugoa, Polem) (Pereira and Almeida, 2014) and Karnataka (Kaladharan *et al.*, 2011). The species initially described as *Fucus foliifer* by Forsskal (1775: 191); the type material (Borgesen, 1932) has brought from Mocha (AI Mukha), North Yemen (Guiry and Freamhainn, 1985). Borgesen recommend the name *G. foliifera* (Forskal) Borgesen for a species which encompassed

the entities passing under the names *G. multipartite* (Clemente) Harvey (1846: pI. XV) and *G. lacinulata* (Vahl) Howe in Britton and Millspaugh (1920: 562) and included *F. aeruginosus* Turner (1809: pI. 147) in synonymy (Borgesen, 1932). Both C. Agardh and J. Agardh investigated specimens in Forsskal's herbarium and C. Agardh (1882: 249) referred *Fucus foliifer* to a variety of *Sphaeroccocus multipartitus* (Clemente) C. Agardh (Guiry and Freamhainn, 1985).

Zanardini included *G. multiparlila, F. foliifer* and *F. laminosus* Forsskal as synonyms of *Gracilaria corticata* J. Agardh (Zanardini, 1858). The name *G. corticata* has therefore used for many years for this entity (Guiry and Freamhainn, 1985). Rao (1972) during the work on specimens collected from the Indian Ocean region observed nutritive filaments arise from the lower side of the gonimoblast tissue in both *G. foliifera* and *G. corticata* and have found difficult to separate certain growth forms. He also reported that structure and reproduction of these two species indicated a close relationship between these two algae and the differences in the thickness of thallus and branching of the fronds are the only characters by which these two plants can be distinguished (Rao, 1972). Børgesen (1938), Durairatnam (1961), Jaasund (1976), Hayee-Memon and Shameel (1996) and Iyer *et al.* (2004) expressed difficulty in differentiating *G. corticata* and *G. foliifera* from Mauritius, Sri Lanka, Tanzania, Pakistan and South Africa respectively (Børgesen, 1938; Durairatnam, 1961; Jaasund, 1976; Hayee-Memon and Shameel, 1996; Iyer *et al.*, 2004).

Solely based on morphological characters, taxonomic and species identification in *Gracilaria* is particularly challenging because to their diverse morphology, high levels of phenotypic plasticity (Lyra *et al.*, 2015). These two species of red algae remains poorly defined, minimally described and inadequately illustrated (Iyer *et al.*, 2004). Since the last decade, morphology-based classifications which have been scarce and ambiguous for the inception of species are now increasingly being complimented with more reliable molecular data (Bast *et al.*, 2014; Lyra *et al.*, 2015; Soares *et al.*, 2015; Yang and Kim, 2015). Molecular phylogenetic studies in the family Gracilariaceae are mainly based on 18S rDNA, cox2-3 spacer and rbcL-rbcS spacer (Destombe and Douglas, 1991; Goff *et al.*, 1994; Byrne *et al.*, 2002; Pareek *et al.*, 2010; Zhao *et al.*, 2013). The present study was carried out for assessing the species level taxonomic status of *G. corticata* and *G. foliifera* based on morphology and multi-marker phylogeny.

2. Materials and Methods

2.1. Morphometric examination

Samples of *Gracilaria* were collected from different localities (Table 1, Fig. 1) during diving exploration along coastal regions of India. Collection coordinates were accessed with a hand held GPS device (eTrex 30, Garmin, USA). Collected specimen were placed in plastic ziplock bags and transported to the laboratory under cold conditions (4-10°C). To remove sediments and other contaminants samples were washed thoroughly with tap water and sorted carefully. Pressed holotype vouchers were made and deposited in the Central National Herbarium, Botanical Survey of India, Calcutta (Index Herbarium Code: CAL). Samples were stored at -80°C for molecular analyses. Morphological characterization of the specimen was done using an upright microscope (BX53, Olympus, Japan) for the study of the pattern of thallus arrangement in surface view. Specimen were sectioned with steel razor blades and photomicrographs were taken with a camera mounted on bright field

Table 1: Collected sample of *Gracilaria* across coastal regions of India

Location (state) and Isolate identifier	Morphospecies	Coordinates	CAL voucher accession no.	Genebank accession no	
				Cox2-cox3 spacer	rbcL-rbcS spacer
Bekal (Kerala) – BEK-23.1	*Gracilaria corticata*	12° 22' 0.12" N, 75° 3' 0" E	CAL-CUPVOUCHER-BEK-2014-GrC-1	KX959521	KX959517
Kanyakumari (Tamil Nadu) -KAY-51.36	*Gracilaria corticata*	8° 4' 48" N, 77° 34' 12" E	CAL-CUPVOUCHER-KAY-2015- GrC-2		KX959519
Ettikulam (Kerela) - ETT-4	*Gracilaria foliifera*	12°00' 30.6" N, 75° 12' 19.9"E	CAL-CUPVOUCHER-ETT -2015- GrF-1	KX959520	KX959516
Veraval (Gujarat) - VER-111	*Gracilaria corticata*	20° 54' 8" N, 70° 22' 10" E	CAL-CUPVOUCHER-VER -2016- GrC-3		KX959518

Fig. 1: Map showing the distribution of the south Indian *Gracilaria* population studied. a. Veraval, b. Bekal, c. Ettikulam, d. Kanyakumari

microscope (CX21i, Olympus, Japan). Public domain software ImageJ was used for scale calibration and size measurements.

2.2. DNA extraction and polymerase chain reaction (PCR)

Total genomic DNA was extracted from the 250 mg frozen algal specimens using HiPurA™Algal Genomic Extraction Kit (HiMedia Laboratories Pvt. Ltd., Mumbai) according to the manufacturer's protocol. Tissues from the apical part of thalli were selected and crushed with the help of silica gel to increase DNA yield. DNA was resuspended in elution buffer and quantified by using a spectrophotometer (Thermo Scientific Nano Drop 2000). PCR amplification has accomplished under the following conditions: Two microliters of diluted DNA were added to each 10µl reaction mix containing 1 µl of 10X reaction buffer (Applied Biosystems, Foster City, CA, USA), 2 µl each of 10 µM primer, 1 µl of 1 µM dNTP mixture containing dATP, dTTP, dCTP, dGTP (Genetix Biotech Asia Pvt. Ltd, New Delhi), 0.9 µl $MgCl_2$, 0.2 mM and (Applied Biosystems, Foster City, CA, USA), 0.5 unit of rTaq® DNA polymerase (Genetix Biotech Asia Pvt. Ltd, New Delhi), 2 µl sterile water. The rbcL-rbcS spacer and cox2-3 spacers were amplified using their respective specific primers (Table 2). To visualize and determine the approximate length and of amplified products, we have electrophoresed for 30 min on 1.5% agarose gels at 100V along with a standard 100 bp DNA marker.

2.3. Purification of PCR product and DNA sequencing

Using ExoSAP-IT® PCR clean-up kit positive reactions were purified following manufacturer's directions (USB Corporation, Cleveland, OH, USA). Purified PCR products were sequenced employing a dideoxy chain termination protocol including ABI Big Dye Terminator Cycle Sequencing Ready® Reaction Kit v3.1 (Applied Biosystems, Foster City, CA, USA) and a programmable thermal cycler (Veriti, ABI, USA). Reactions were then purified by vortex for 45 min with adding ex-beads and Sam solution (Applied Biosystems, Foster City, CA, USA). Purified extension products were vacuum dried and spin down. Then DNA sequencing was performed in Applied Biosystems 3730xl Genetic Analyzer (Foster City, CA, USA).

2.4. Sequence annotation and phylogenetic analysis

The quality of electropherograms was checked and manually annotated the sequences using Genieous v 8.0.5 (Biomatters, www.geneious.com). Sequences were aligned by MUSCLE algorithm using Genieous v 8.0.5 and the ends of aligned sequences were trimmed to minimize the number of missing sites across taxa. The identity of sequences was examined through BLAST available at the NCBI website (www.blast.ncbi.

Table 2: Primer used in this study for PCR Amplification and Sequencing

Primer	Sequence	Amplified region	Reference
coxF	5' GTACCWTCTTTDRGRRKDAAATGTGATGC 3'	Cox2-cox3 spacer	Zuccarello *et al.* (1999)
coxR	5' GGATCTACWAGATGRAAWGGATGTC3'		
rbcF	5'TATACTTCTACAGACACAGCTGA3'	rbcL-rbcS spacer	Pareek *et al.* (2010)
rbcR	5'ATGTCAAATAATGGTAGTCCCCA3'		

Table 3. Genbank accessions used to reconstruct phylogenetics of Gracilaria

Gracilaria species	Location	GenBank accession number	
		Cox2-cox3 intergenic spacer	RuBisCo spacer
Gracilaria chouae Zhang & B.M.Xia	South China	KC596121	
Gracilaria conferta (Schousboe ex Montagne) Montagne	North Africa	GQ292560	
Gracilaria corticata J. Agardh	South Africa		AY241148, AY241154, AY241155, AY241145
Gracilaria corticata var. *corticata* J. Agardh	India, Gujarat (Veraval)	EU937758	EU937767
Gracilaria corticata (J. Agardh) J. Agardh var. *cylindrica* U. Rao	India, Gujarat (Veraval)	EU937759	EU937768
Gracilaria debilis (Forssk.) Børgesen	India, Gujarat (Veraval)	EU937764	
Gracilaria dura (C. Agardh) J. Agardh	India, Gujarat (Okha)	EU937757	
Gracilaria fergusonii J. Agardh	India, Gujarat (Veraval)	EU937760	
Gracilaria foliifera (Forssk.) Børgesen	India, Gujarat (Veraval)	EU937761	EU937770
Gracilaria gracilis (Stackh.) M. Steentoft, L. M. Irvine et W. F. Farnham)	Portugal	GQ229504	
Gracilaria perplexa	Bare Island, Botany Bay, NSW		AY131306, AY131307, AY131308
Gracilaria salicornia (C. Agardh) E. Y. Dawson	India, Gujarat (Shivrajpur)	EU937763	
Gracilaria tenuistipitata	Southeast Asia	KC596122	
Gracilaria textorii (Suringar) De Toni	India, Gujarat (Shivrajpur)	EU937765	
Gracilaria vermiculophylla	Virginia	DQ173304	
Porphyra linearis	North Atlantic	AY316148	
Porphyra purpurea	Northwest Atlantic		DQ423788.1

nlm.nih.gov) (Altschul *et al.*, 1990). Alignment was carried out separately for all the samples with their geographical isolates available with GenBank (Table 3). The best-fitting nucleotide substitution models were tested using ML Model Test in MEGA-v6 (Tamura *et al.*, 2013). Phylogenetic analysis using Maximum likelihood (ML) was done using MEGA-v6. The evolutionary distances were computed using Tamura-3-parameter (Tamura, 1992) following the results obtained after ML Model Test in MEGA-v6. The bootstrap test results (1000 replicates) are indicated next to the branches of the phylogenetic tree (Felsenstein, 1985). Mean evolutionary diversity and Mean inter-populational evolutionary diversity (Nei and Kumar, 2000) were calculated in MEGA-v6.

3. Result

3.1. Morphological observations

Gracilaria corticata was identified from three sampling sites along coastal regions of India.

3.1.1. Gracilaria corticata J. Agardh, 1852

Identification and Taxonomy: Isolates (BEK-23.1, KAY-51.36 and VER-111) were identified as *Gracilaria corticata.* Holotype: *Gracilaria corticata* J. Agardh. This Species belongs to Order Gracilariales of Family Gracilariaceae in Class Florideophyceae.

Morphology: Thallus is hemispherical bushy in appearance, compressed, thick and cartilaginous in nature. Discoid holdfast attached to a rocky substrate. Reddish brown in colour, 7-12 cm in height, frond membranous (Fig. 2A). Dichotomously or poly dichotomously usually branched in the single plane of the blade. Both smooth and proliferous blade surface and margin. Stipe expanded gradually into a blade that was upto 0.3-1 mm thick and about 0.6-1.5 cm broad (Fig. 2B, 2C). Abrupt cortex to medulla transition with 4-5 rounded to slightly flattened medullary cells (Fig. 2D) and cortex is found 1-2 pigmented cell layers (Fig. 2E). The medullary cells are larger towards the center, 0.11-0.37 x 0.28-0.58 mm (Fig. 2F). Cruiatete trasporangia

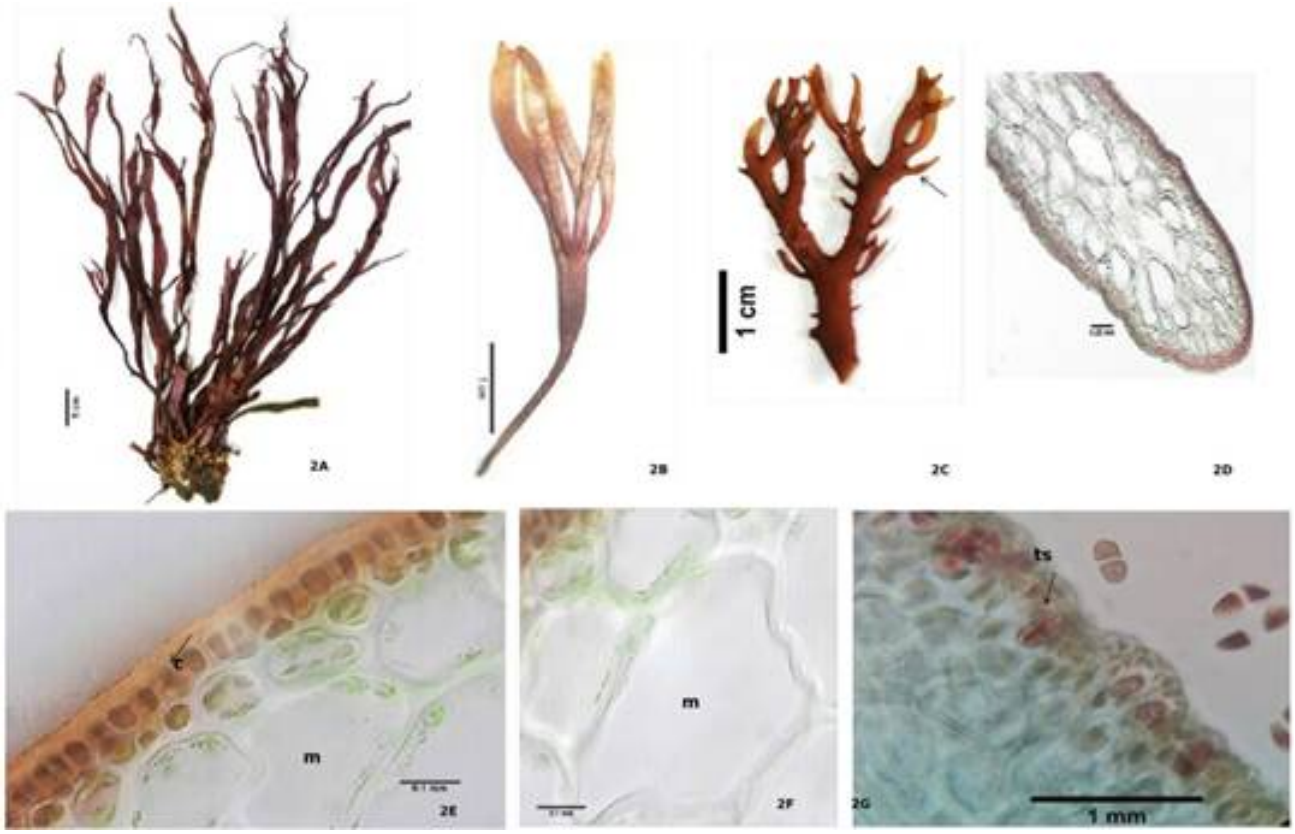

Fig. 2: *Gracilaria corticata* (A) Hemispherical bushy thick thallus with discoid holdfast attached to a rocky substrate. (B) Detail compressed thallus, usually branching in the plane of the blade. (C) Blade with proliferous margin. (D) Transverse section of thallus showing cortex (c) and medullar layers (m). (E) Detail of cortical region, composed of 1-2 cortical cell layers. (F) Feature rounded to slightly flattened medullary cells. (G) Cruciate trasporangia

embedded in a transverse section of tetrasporophyte cortex (Fig. 2G).

3.1.2. Gracilaria foliifera (Forsskal) Borgesen, 1932

Identification and taxonomy: Isolate (ETT-4) was identified as *Gracilaria foliifera.* Holotype: *Gracilaria foliifera* (Forsskal) Borgesen. This Species belongs to Order Gracilariales of Family Gracilariaceae in Class Florideophyceae.

Morphology: Thallus is hemispherical bushy in appearance, compressed thin fronds than *Gracilaria corticata*. Reddish brown in colour, 6-14 cm in height, dichotomously or sub-dichotomously branched, frond membranous, branches tapering towards the apices, oblanceolate with acuminate tips, proliferations often marginal. Stipe expanded gradually into a blade that was up to 0.2-1 mm thick and about 0.4-0.9 cm broad (Fig. 3A). Discoid holdfast attached to a rocky substrate (Fig. 3B). Branching usually in the plane of the blade (Fig. 3C). Cystocarpic blade with proliferous margin (Fig. 3D). The medullary cells are composed 4-5 rounded to slightly flattened larger towards the center, 0.06-0.26 x 0.18-0.53 mm (Fig. 4A). Cortex to medulla transition is abrupt with 1-2 pigmented cortical cell layers (Fig. 4B). Tetrasporangium in undivided and two-celled stage surrounded by narrowly elongated and inwardly curved vegetative cells (Fig. 4C) and also cruiatete trasporangia showing in transverse section of tetrasporophyte cortex (Fig. 4E). Hemispherical cystocarp showed a non-rostrate pattern with constriction at the base (Fig. 4D). Contain terminal chain of ovoid and subspherical shape carpospores showing stellate central body below the pericarp (Fig. 4F). The pericarp is 0.12-0.15 mm thick, consist of a 10-12 layer of anti clinically and radially arranged cell (Fig. 4G). Contain irregularly arranged irregularly shape cell (Fig. 4H).

Fig. 3: *Gracilaria foliifera*. (A) Hemispherical bushy female gametophyte thallus. (B) Showing discoid holdfast attached to a rocky substrate. (C) Detail compressed thallus, usually branching in the plane of the blade. (D) Detail of female gametophyte thallus showing proliferation along the margins and cystocarp scattered over the surface of female the thallus.

3.2. Phylogenetic analysis

The rbcL-rbcS spacer data file included 15 sequence and 847 sites in total. The analysis of different isolates yielded 226 (26.6 %) conserved sites and 143 (16.8 %) variable sites, of which 17 (2%) were potentially parsimony informative. The data set yielded unequal frequency of base (A 35.7 %; T 35.3%; C 14.7%; G 14.3 %) and a transition-to-transversion ratio of 7 with almost equal purine-purine transitions (50.2%) and pyrimidine-pyrimidine transitions (49.4%). The mean evolutionary diversity for the 11 nucleotide sequences of *Gracilaria corticata* and *Gracilaria foliifera* with 244 positions in the dataset was 0.006. The mean inter-populational evolutionary diversity of 15 nucleotide sequences along with 243 positions in the dataset was 0.023. *G. corticata* from South Africa and both (*G. corticata* and *G. foliifera*) from India forms a monophyletic subclade with 98% bootstrap value. *G. perplexa* occupies the basal position of the large (*G. corticata* and *G. foliifera*) clade. The large subclade included *G. foliifera* from India (Ettikulam and Veraval) and *G. corticata* from India (Bekal, Kanyakumari and Veraval) with 94% bootstrap value. This clade also included other varieties *Gracilaria corticata* var. *corticata*, and *Gracilaria corticata* var. *cylindrica* from Veraval (India) (Fig. 5).

The cox2-cox3 spacer data file included 16 sequence and 426 sites in total. The analysis of different isolates yielded 209 (49%) conserved sites and 190 (44.6%) variable sites, of which 105 (24.6%) were

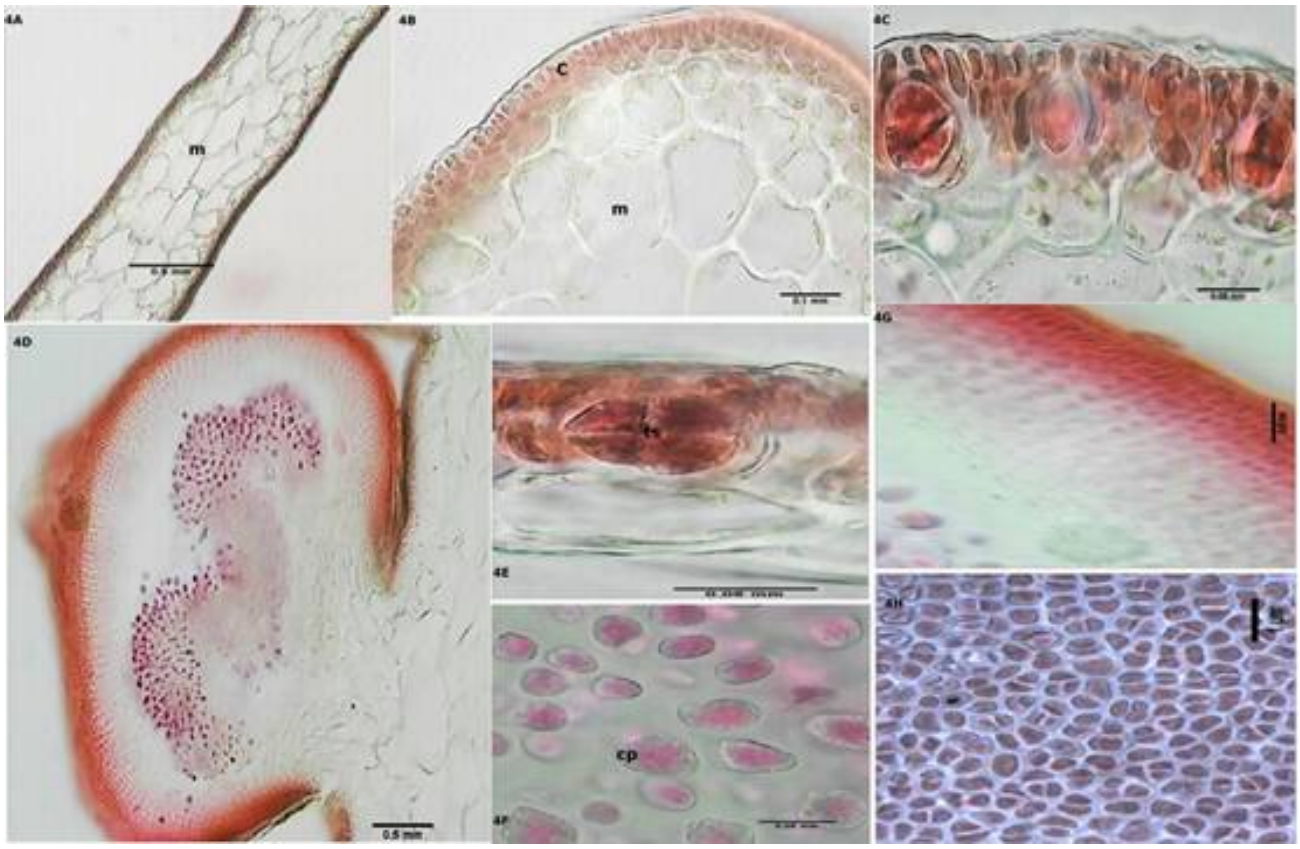

Fig. 4: *Gracilaria foliifera*. (A) Transverse section of thallus showing cortex (c) and medullar layers (m). (B) Detail of cortical region, composed of 1-2 cortical cell layers. Note the gradual transition between cortex and medulla. (C) Tetrasporangium in undivided and two-celled stage surrounded by narrowly elongated and inwardly curved vegetative cells. (D) Cross-section of cystocarp showing a non-rostrate pattern with constriction at the base. (E) Cruciate tetrasporangia is showing in transverse section of tetrasporophyte cortex. (F) Detail of subspherical shape carpospores showing the stellate central body. (G) Part of the pericarp of cystocarp in section. (H) Showing irregular arranged irregularly shape cell

potentially parsimony informative. The data set yielded an unequal frequency of base (A 35.2%; T 39.9%; C 10.6%; G 14.3%) and a transition-to-transversion ratio of 28 with a 53.6% bias toward pyrimidine-pyrimidine transitions. The mean evolutionary diversity for the five nucleotide sequences of *Gracilaria corticata* and *Gracilaria foliifera* with 319 positions in the dataset was 0.018. The mean inter-populational evolutionary diversity of 15 nucleotide sequences along with 271 positions in the dataset was 0.098. *G. foliifera* from India (Ettikulam and Veraval) along and *G. corticata* from India (Bekal) with other varieties *Gracilaria corticata* var. *corticata*, and *Gracilaria corticata* var. *cylindrica* from Veraval (India) forms a monophyletic subclade with 100% bootstrap value (Fig. 6).

4. Discussion

There has been a substantial morphological plasticity among the species of *Gracilaria corticata* and *Gracilaria foliifera* leading to ambiguity in the identification of the two based on the external appearance of the thallus, holdfast, branching pattern, axes diameter, cortex and medullary cell thickness, and position of tetrasporangia (Table 4). According to earlier reports, the only characters that discriminate *G. corticata* and *G. foliifera* are the thallus thickness and branching of the frond (Rao, 1972). In our study, the thickness of the thallus was found to be varying whereas the branching pattern was found to be more or less dichotomously branched and it varied according to the life stage of the plant. In our study, two species

Table 4: Morphological character commonly used to delimit species of *G. corticata* and *G. foliifera*

Character	*Gracilaria corticata*	*Gracilara foliifera*
Maximum thallus height (cm)	7-12	6-14
Colour of mature plant	Reddish brown	Reddish brown
Structure of holdfast	Discoid	Discoid
Axes	Compressed	Compressed and comparatively thin
Axes diameter (mm)	3.8 mm	4 mm
Branching pattern	Dichotomously or poly dichotomously, bushy appearance	Dichotomously or sub-dichotomously, bushy appearance
Blade surface	Both smooth and Proliferous	Both smooth and Proliferous
Cortex thickness	1-2 cells thick	1-2 cells thick
Medullary cell (µm)	Up to 210 µm	Up to 150 µm
Tetrasporangia	Embedded in cortex	Embedded in cortex

distinguished only by the thickness of thallus, with *G. corticata* being thicker. Thus the isolates: BEK-23.1, KAY-51.36, VER-111 were identified as *G. corticata* and isolate ETT-4 as *G. foliifera*. There has always been an ambiguity in the identification of the two species based on morphological studies (Zanardini, 1858; Durairatnam, 1961; Rao, 1972; Jaasund, 1976; Hayee-Memon and Shameel, 1996). Rao (1972) reported that the *G. foliifera* reported from Indian Ocean region can be treated as a variety of *G corticata* if exhaustive details of the species from Atlantic coast of Europe and America are available (Rao, 1972).

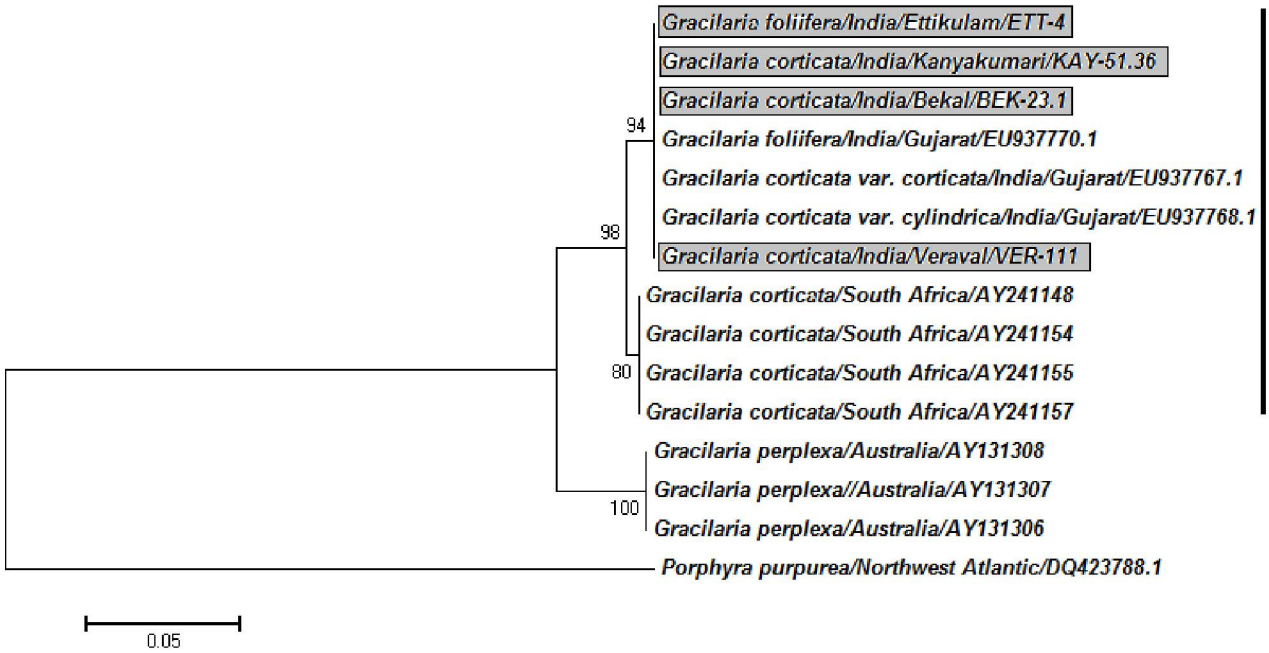

Fig. 5: Maximum Likelihood phylogenetic tree of rbcL-rbcS spacer sequence data and Tamura 3-parameter corrected distances of *Gracilaria corticata*, *Gracilaria foliifera* and other accessions from Genbank. Bootstrap value (1000 replicates) manifested near the node. The tree with the highest log likelihood (-606.2733) has shown. The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data has eliminated. Scale bar is the unit of average nucleotide substitutions per site.

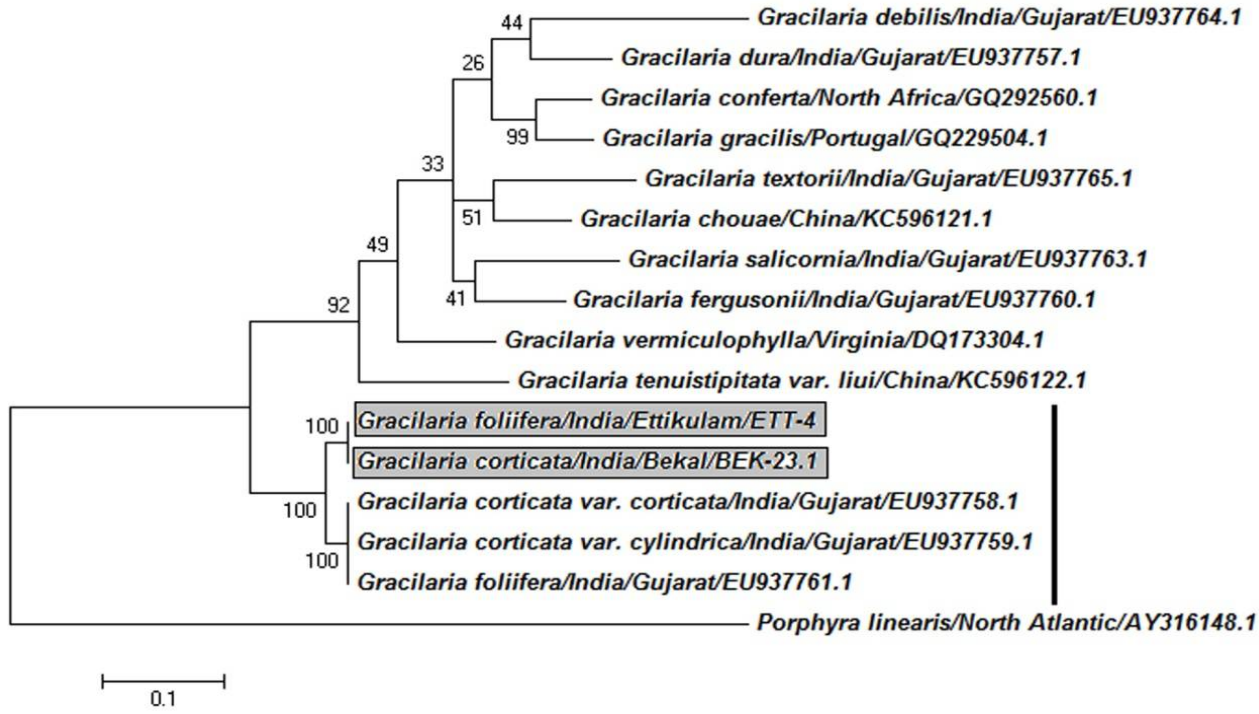

Fig. 6: Maximum Likelihood phylogenetic tree of cox2-cox3 sequence data and Tamura 3-parameter corrected distances of *Gracilaria corticata, Gracilaria foliifera* and other accessions available from Genbank. Bootstrap value (1000 replicates) manifested near the node. The rate variation model permitted for some sites to be evolutionarily invariable (*+I*, 37.6857% sites). The tree with the highest log likelihood (-1576.3085) has shown. The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data has eliminated. Scale bar is the unit of average nucleotide substitutions per site.

Such challenge could be solved with the support of molecular data. The rbcL-rbcS and cox2-3 intergenic spacer sequences of *G. corticata* and *G. foliifera* obtained were found to be identical with *G. corticata* and *G. foliifera* reported from Gujarat, India (rbcL-rbcS: *Gracilaria corticata* var. *corticata*, EU937767; *Gracilaria corticata* var. *cylindrica*, EU937768 and *Gracilaria foliifera*, EU937770; cox2-3: *Gracilaria corticata* var. *corticata*, EU937758; *Gracilaria corticata* var. *cylindrica*, EU937759 and *Gracilaria foliifera*, EU937761) and thus formed a same clade in the phylogenetic tree (Fig. 5-6). *Gracilaria corticata* and *Gracilaria foliifera* isolate from India together formed a strongly supported subclade with strong bootstrap support based on the rbcL-rbcS and cox2-3 intergenic spacer, but they are not monophyletic. The majority of these subclades are a mixture of both *G. corticata* and *G. foliifera* with high bootstrap support in trees from both analyses. Mean intra-populational evolutionary diversity between the *G. corticata* and *G. foliifera* based on the rbcL-rbcS spacer (0.006), and cox2-3 intergenic spacer (0.018) were minimal than mean inter-populational evolutionary diversity. Thus, the divergence between *G. corticata* and *G. foliifera* is also within range of conspecific.

These results, combined with earlier findings from molecular (Iyer *et al.*, 2005; Pareek *et al.*, 2010) and morphological studies (Zanardini, 1858; Durairatnam, 1961; Rao, 1972; Jaasund, 1976; Hayee-Memon and Shameel, 1996), provide strong evidence that *G. corticata* and *G. foliifera* are not distinct evolutionary entities and should not be recognized as separate species. Consequently, we postulate that *G. corticata* and *G. foliifera* represent to be conspecific and that the morphological forms represent, with phenotypic rather than genotypic variation. As *Gracilaria corticata* J. Agardh (1852) has nomenclatural priority over *Gracilaria foliifera* (Forsskal) Borgesen (1932), therefore we propose that *Gracilaria foliifera* should be included in the synonymy under *Gracilaria corticata*.

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