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Morphological and molecular characterization of *Ulva chaugulii sp. nov.*, *U. lactuca* and *U. ohnoi* (Ulvophyceae, Chlorophyta) from India

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ABSTRACT: Detailed morphological, anatomical and molecular characteristics of *Ulva* on the west coast of India revealed the presence of *U. chaugulii sp. nov. Ulva chaugulii* is characterized by tubular, compressed and fragile thalli with infundibuliform shape, a dilated apex and the presence of two pyrenoids per cell. The molecular phylogeny based on the *rbc*L gene and internal transcribed spacer rDNA sequences support the recognition of a new lineage within *Ulva*. The present study also verifies the presence of *U. ohnoi* in western India. *Ulva lactuca* and *U. ohnoi* from India were resolved as separate lineages based on *rbc*L phylogeny. The genetic divergence between *rbc*L sequences of these two species was 0.4%.

KEY WORDS: Chlorophyta, India, Molecular phylogeny, *rbc*L, *Ulva*, *Ulva chaugulii*

INTRODUCTION

Ulva Linnaeus, commonly referred to as 'sea lettuce', with 128 taxonomically accepted species, is the most species-rich genus in the family Ulvaceae (Guiry & Guiry 2015). Several species of *Ulva* are associated with notorious blooms called 'green tides' (Fletcher 1996; Blomster *et al.* 2002; Zhao *et al.* 2010). The uncertainty in taxonomic status of most taxa in this genus is again a major concern for taxonomists. This uncertainty is directly attributed to the morphological plasticity resulting in recognition of large numbers of varieties, forms and ecotypes. The plasticity was also proved at the higher taxonomic level, wherein the artificial nature of a generic character distinguishing *Ulva* and *Enteromorpha* Link (i.e. distromatic thalli in *Ulva* and tubular-monostromatic thalli in *Enteromorpha*) was established (Hayden *et al.* 2003; Shimada *et al.* 2003). Species of *Ulva* exhibit a range of morphology in thallus architecture, that is, foliose, lanceolate, linear, ovate, cuneate or tubulose. Other important taxonomical characters include cell size, shape and arrangement; thallus thickness; number of pyrenoids per cell; and morphology of holdfast and basal region (Bliding 1968; Koeman & van den Hoek 1981). However, intraspecific variations were observed for these characters in different environments and growth phases (Blomster *et al.* 1998).

Over the last two decades, remarkable progress has been made in molecular genetic techniques. The advent of molecular markers has provided new insights into organismal systematics. DNA sequence–based taxonomy has helped to resolve many challenging and difficult taxonomic issues. Recent molecular studies in *Ulva* have contributed significantly to its taxonomy, such as the merging of *Ulva* and *Enteromorpha* (Hayden *et al.* 2003), synonymization of *U. lactuca* Linnaeus and *U. fasciata* Delile (O'Kelly *et al.* 2010) and identification of green tide–forming taxa (Liu *et al.* 2010; Zhao *et al.* 2010; Guidone *et al.* 2013; Guoying *et al.* 2014). The molecular data also helped in understanding the biogeographic history, cryptic diversity and introduction of species of *Ulva* in different regions (Heesch *et al.* 2009; Hofmann *et al.* 2010; Kraft *et al.* 2010; Wolf *et al.* 2012; Kirkendale *et al.* 2013). As a consequence, recent studies recommended an integrated approach to the taxonomy of *Ulva* that used both morphological and molecular characterization (Loughnane *et al.* 2008; Hofmann *et al.* 2010; Matsumoto & Shimada 2015).

In this study, we used a polyphasic approach to *Ulva* that included morphological, ecological and molecular analyses to resolve the taxonomy from the west coast of India. The study is a part of broader goal of biodiversity assessment of economically important marine macroalgae from India. Preliminary data suggested that most species of *Ulva* identified previously (e.g. Silva *et al.* 1996) needed extensive revision. For example, several morphotypes that we identified as well-established species turned out to be *U. ohnoi* Hiraoka & Shimada, a new report from India. In India, 17 species of *Ulva* were reported (Silva *et al.* 1996), including the recently identified new species *U. paschima* F. Bast (Bast *et al.* 2014) from the west coast. Among these, *U. lactuca* was abundant. During surveys of the west coast of India, we encountered a new species of *Ulva*. This study focuses mainly on the detailed morphological, ecological and molecular characterization of the new species *U. chaugulii* M.G. Kavale & M.A. Kazi and of *U. lactuca* and *U. ohnoi* from western India.

MATERIAL AND METHODS

The samples were collected from the west coast of India. Sixteen sites were visited to survey the occurrence of *Ulva* (Fig. 1, Table S1). A total of 26 samples were handpicked at low tide and analysed. The specimens were washed with sterile filtered seawater to remove epiphytes and adhered debris. Specimens were examined for morphological and anatomical characters by using compound and phase

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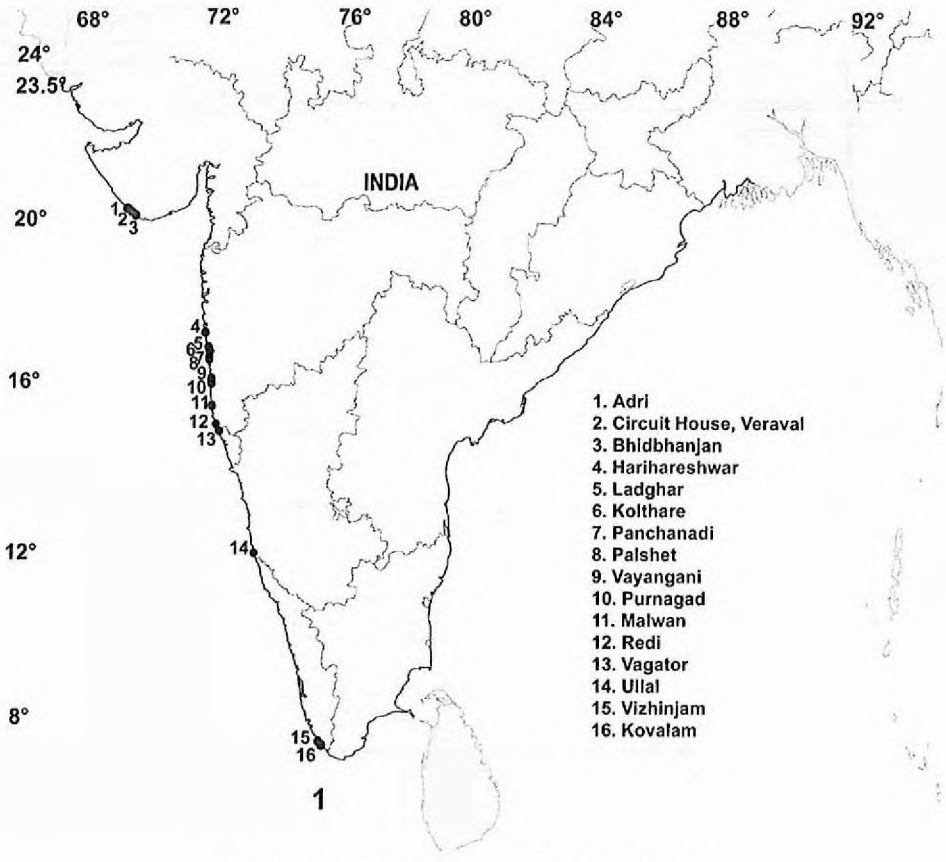


Fig. 1. Map of sampling sites for species of *Ulva* from India.

contrast microscopes (Motic BA310 Phase, Hong Kong, China). Morphometric data were based on a minimum of 30 measurements for each character. The photographic documentation and measurements were made with the software Motic Images Plus 2.0 ML (Motic, Hong Kong, China). Voucher herbarium specimens were made and submitted to the Central National Herbarium, Botanical Survey of India, Kolkata (CAL). A portion of each sample was frozen at –20°C for DNA extraction. The biomass was determined by measuring average wet weight of an identifying accompanying species from at least 10 quadrats (1 m^2).

Total genomic DNA was extracted with the Gene Elute Plant Genomic DNA miniprep kit (Sigma Aldrich, St Louis, Missouri USA) following the manufacturer's protocol. Amplification by polymerase chain reaction (PCR) was performed in a master mix of volume 25 µl containing 5 pmol of each primer, 200 µM of each dNTP, 1× assay buffer and 1.25 units of *Taq* DNA polymerase. The partial *rbc*L (large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase) gene was amplified using primer pairs RH1 (5′ATGTCACCACAAACAGAAACTAAAGC-3′) and 1385r (5′-AATTCAAATTTAATTTCTTTCC-3′) (Manhart 1994). PCR amplification was carried out for *rbc*L following Hayden *et al.* (2003). For the nuclear ribosomal internal transcribed spacer (ITS rDNA) region, the primer pair used was 18S1505 (Hayden *et al.* 2003) and ENT26SA (Blomster *et al.* 1998). The PCR amplification cycle consisted of a cycle of 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 52°C, 2 min at 72°C and one cycle of 7 min at 72°C. The PCR products were purified with a GenElute Gel Extraction Kit (Sigma Aldrich) and sent for commercial sequencing (Xcelris Labs Ltd, Ahmedabad, India). The sequences were deposited in GenBank (see Tables S2 and S3 for accession numbers).

Sequences amplified in this study were aligned with those published sequences retrieved from GenBank. The *rbc*L sequences were aligned using the ClustalW algorithm in MEGA v6 (Tamura *et al.* 2013), and the ITS rDNA sequences were aligned using MAFFT v7 (E-INS-i iterative refinement method) (Katoh *et al.* 2005). The aligned *rbc*L data set consisted of 1361 characters that included 132 parsimony informative, 1152 conserved and 209 variable sites. The aligned ITS rDNA data set consisted of 1159 characters that included 297 parsimony informative, 509 conserved and 482 variable sites. Gaps in the alignments were treated as missing data. The alignments were subjected to phylogenetic analysis by the maximum likelihood (ML) approach. The analyses were performed using the general time reversible model with gamma distributed site rates and considering certain fraction of invariable sites (I), as obtained in jModelTest v2.1.4 (Darriba *et al.* 2012) based on Akaike information criterion scores. ML analysis was carried out using raxmlGUI v1.1 (Silvestro & Michalak

2012) with 1000 bootstrap replicates. *Umbraulva olivascens* (P.J.L. Dangeard) G. Furnari, *U. japonica* (Holmes) Bae & I.K. Lee, *Gemina letterstedtoidea* V.J. Chapman, *Ulvaria obscura* var. *blyttii* (Areschoug) Bliding, *Percursaria percursa* (C. Agardh) Rosenvinge and *Ulvaria fusca* (Wittrock) Vinogradova were included as out-groups in the analyses. The pairwise distance between sequences was calculated using MEGA v6 (Tamura *et al.* 2013).

RESULTS

Morphological characterization

Species identifications based on morphological characters (attachment, shape and size of blade, nature of margin, cell shape and size in surface view and cross section, type of chloroplast and number of pyrenoids per cell) were made using descriptions given by Krishnamurthy (2000). *Ulva fasciata* and *U. lactuca* were initially identified. However, after molecular analysis, most of the specimens were recognized as *U. ohnoi* (previously identified as *U. lactuca*). In the present study, specimens of *U. fasciata* were treated as *U. lactuca* following O'Kelly *et al.* (2010). The detailed morphological study was conducted to analyze the intra- and interspecific variations in *U. lactuca* and *U. ohnoi* (Tables S2 and S3).

Ulva chaugulii M.G. Kavale & M.A. Kazi *sp. nov.*
Figs 2–13

DESCRIPTION: Thalli tubular, light green, fragile and compressed, up to 4.0 cm (2.93 ± 0.93, mean ± *s*) long, 0.6 cm (0.48 ± 0.11) broad. Thalli simple or branched at base, with a cylindrical hollow stipe up to 0.5 cm (0.37 ± 0.11) long. Apex of thalli obtuse and dilated. Frond margins smooth or irregularly constricted. Cells in surface view polygonal 14.9–18.7 µm (16.7 ± 1.4) in greatest dimension. Chloroplasts parietal with two pyrenoids. In transverse section, cells longer than wide with blunt corners and rectangular, 16.5–24.0 µm (19.5 ± 2.8) high, 9.6–20.3 µm (14.7 ± 4.3) wide. Cells aligned in longitudinal series through the thallus. Frond consisted of two layers adhering with each other except in the margin. Monostromatic layer 37.1–63.3 µm (50.7 ± 9.2) thick.

HOLOTYPE: CAL/ALG./029, collected 6 September 2014, deposited in Central National Herbarium, Botanical Survey of India, Kolkata.

ISOTYPE: CAL/ALG.030 and CAL/ALG./031, collected 6 September 2014, deposited in Central National Herbarium, Botanical Survey of India, Kolkata.

ETYMOLOGY: This species is named in honour of Prof. B.B. Chaugule in appreciation of his immense contribution to phycology in India.

GENBANK ACCESSION: KP710829–KP710833 represent the *rbc*L sequences, and KT429218–KT429219 are the ITS rDNA sequences.

TYPE LOCALITY: Vayangani (16°55.52′N, 73°17.01′E), Maharashtra, India.

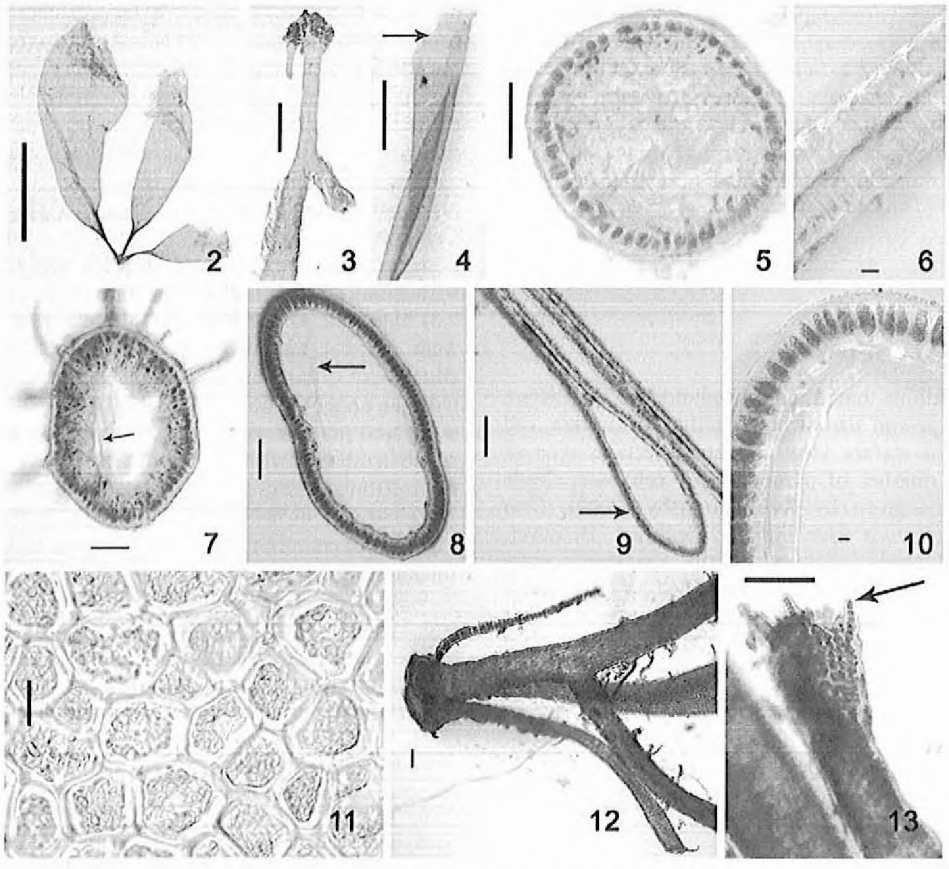
Thalli of *U. chaugulii* were light green, tubular, compressed and fragile, up to 4.0 cm (2.93 ± 0.93) long and 0.6 cm (0.48 ± 0.11) broad, simple or branched at base and conspicuously septate. The stipe was cylindrical and hollow, up to 0.5 cm (0.37 ± 0.11) long. There were frequent proliferations on the stipe. Proliferations were elongated into horn-like projections. Blades expanded above the stalk and became flat and infundibuliform; margins smooth, apex dilated, sometimes constricted at intervals. Plants were attached by basal discs, occasionally several arising from a common disc. Cells in surface view were polygonal, regularly arranged in longitudinal rows in greater part of the thallus. Cell size was 14.9–18.7 µm (16.7 ± 1.4) at their greatest dimensions. In transverse section, cells were longer than wide with blunt corners and rectangular, 16.5–24.0 µm (19.5 ± 2.8) high and 9.6–20.3 µm (14.7 ± 4.3) wide. Chloroplasts were parietal with two pyrenoids. In transverse section, stipes were cylindrical, 308–432 µm (357 ± 56) in diameter, with the upper portion traversed by trabeculae. At the end of stipe and at the edge of blade expansion, thalli were monostromatic with a hollow ring-like structure. The monostromatic ring gradually became distromatic except at marginal regions in the middle to upper parts of the thallus. The two layers rarely adhered all the way to the margin. The thickness of the monostromatic layer was 37.1–63.3 µm (50.7 ± 9.2); whereas, the combined thickness of the two layers was 51.0–107.1 µm (80.0 ± 23.0). Cell mucilage was 1.1–2.7 µm (2.0 ± 0.6); whereas, thickness of mucilage ranged from 2.2–4.4 µm (3.3 ± 0.9). Rhizoids were 36.7–112.9 µm (76.0 ± 31.3) long.

Ulva lactuca Linnaeus

Plants were 4–100 cm high (26.29 ± 13.21), 1–40 cm (9.48 ± 7.36) wide, dark green to pale green and attached by a circular disc, with or without a small stipe (Figs 14–19). The thalli were deeply divided with lanceolate lobes, broadly expanded or with less divided, lanceolate to irregular lobes (Fig. S1). The blade margin was smooth, undulate to ruffled and more or less spinulous. Spines were 2.0–69.3 µm (20.4 ± 12.4) in height. Broadly expanded thalli had numerous perforations; whereas, deeply lobed, lanceolate thalli had few perforations. Thalli were 43.2–105.5 µm (72.4 ± 10.6), 62.3–192.9 µm (106.0 ± 30.0) and 85.0–375.5 µm (173.7 ± 50.9) thick in apical, middle and basal regions, respectively. Vegetative cells in surface view were round to polygonal and ranged from 8.8 to 30.7 µm (17.3 ± 2.3) in its greatest dimension. The chloroplasts almost filled the cells and had one to three pyrenoids.

Ulva ohnoi Hiraoka & Shimada

Plants were orbicular, lanceolate or irregularly expanded, wider than long, 1–12 cm (4.20 ± 2.80) in height and 2–35 cm (6.71 ± 5.19) in width and attached by circular disc with small stipe (Figs 20–25). These plants were pale green to dark green in colour, easily separated in two layers, larger thalli with numerous perforations. Tufts of thalli developed from a common disc. The thallus margin was ruffled, with microscopic spines ranging from 4.5 to 44.3 µm (15.9 ± 7.5). Thalli were 33.2–69.7 µm (48.7 ± 7.6), 41.3–82.2 µm (58.0 ± 7.6) and 45.0–182.8 µm (83.2 ± 32.1) in apical, central and basal regions, respectively. Cells in surface view were polygonal and completely filled by a chloroplast with one to three pyrenoids. In transverse section, vegetative cells were rectangular with



Figs 2–13. *Ulva chaugulii*. Fig. 2, scale bar = 1 cm; Figs 3, 4, scale bar = 1 mm; Figs 5, 7–9, 12, 13, scale bar = 100 μm; Figs 6, 10, 11, scale bar = 10 μm.

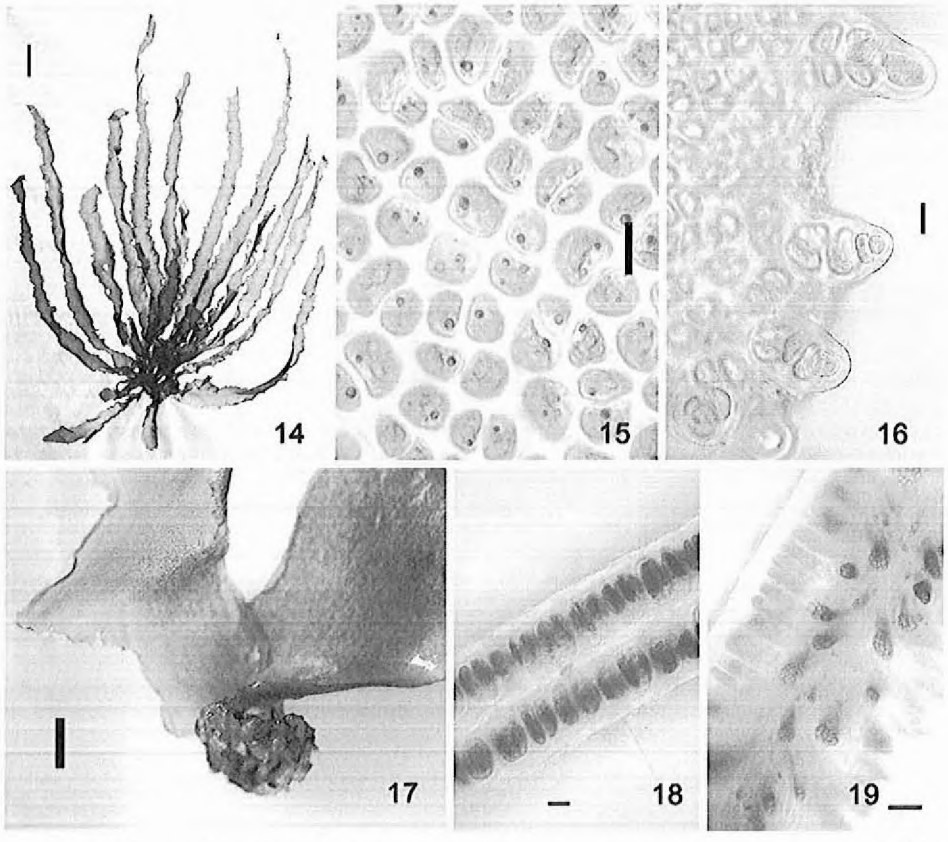
Fig. 2. Holotype (CAL/ALG./029).
Fig. 3. Stipitate thallus.
Fig. 4. Dilated apex of thallus (arrow).
Fig. 5. Transverse section of basal portion of stipe.
Fig. 6. Transverse section of thallus showing two layers adhering to each other.
Fig. 7. Transverse section of middle portion of stipe, arrow indicates trabeculae.
Fig. 8. Transverse section of upper portion of stipe, arrow indicates trabeculae.
Fig. 9. Transverse section of lamina showing middle (arrow) and marginal portion.
Fig. 10. Monostomatic layer of thallus.
Fig. 11. Surface view of thallus.
Fig. 12. Thallus showing branching and cell arrangement.
Fig. 13. Proliferations on branches (arrow).

blunt corners and ranged from 14.3 to 45.2 μm (23.4 ± 6.9) in height and from 8.8 to 19.9 μm (13.9 ± 1.2) in width.

Distribution and ecology

Ulva chaugulii was found in the upper littoral zone in patches during the monsoon season from July to September. The highest biomass recorded was 20 g m^{-2} with a percent cover of 1.9 ± 0.5%. *Ulva lactuca*, *Pyropia acanthophora* (E.C. Oliveira & Coll) M.C. Oliveira, D. Milstein & E.C. Oliveira and *Pyropia vietnamensis* (Tak. Tanaka & P.H. Ho) J.E. Sutherland & Monotilla were also observed associated with *U. chaugulii*. The air temperature was 25–29°C, and the seawater had pH 8.5 and salinity of 10 practical salinity units.

Ulva lactuca was distributed along the entire west coast of India. The plants grew luxuriantly on the entire intertidal region of rocky shore attached to the substratum. The rocky substratum consisted mainly of basaltic wave-cut platforms with overhanging cliffs, numerous huge boulders and water pools. The growth of plants started early in the monsoon season, and plants were found until the middle of winter (July–December). The associated genera with *U. lactuca* at collection sites in the states of Maharashtra, Goa, Karnataka and Kerala were *Pyropia*, *Chaetomorpha* and *Grateloupia*; whereas, in Gujarat state, *Gracilaria*, *Sargassum*, *Caulerpa*, *Stoechospermum*, *Padina*, *Centroceros*, *Jania*, *Dictyota*, *Spongomorpha*, *Halimeda*, *Spatoglossum*, *Boodlea* and so on were observed.



Figs 14–19. Representative specimen of *Ulva lactuca*. Fig. 14, scale bar = 2 cm; Fig. 15, 16, 18, scale bar = 10 μm; Fig. 17, scale bar = 2 mm; Fig. 19, scale bar = 20 μm

Fig. 14. Habit.
Fig. 15. Surface view showing pyrenoids.
Fig. 16. Surface view showing marginal spines.
Fig. 17. Basal portion showing rhizoidal disc.
Fig. 18. Transverse section of thallus.
Fig. 19. Transverse section of thallus showing rhizoids.

The biomass of *U. lactuca* ranged from 0.5 to 4.0 kg m^{-2}, with average percent cover of 64.0 ± 24.1%.

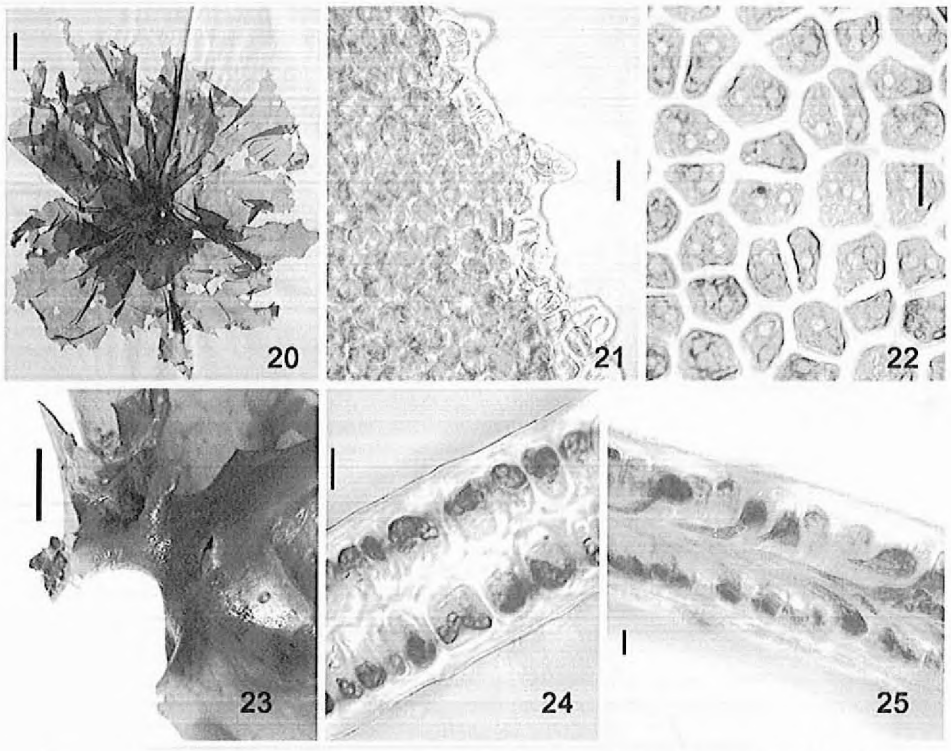
Ulva ohnoi was encountered mostly on northwestern coasts (states of Maharashtra and Gujarat). Growth initiated at the end of the monsoon and continued until the middle of summer (September–April). In Maharashtra, the associated flora found with *U. ohnoi* were species of *Porphyra*, *Sargassum*, *Padina*, *Dictyota*, *Gracilaria*, *Caulerpa*, *Chaetomorpha*, *Spongomorpha* and *Jania*; whereas, in Gujarat, all above-mentioned species except *Pyropia* were observed. The biomass estimated was comparatively lower than *U. lactuca* and varied from 0.5 to 1.5 kg m^{-2}, with average percent cover of 41.2 ±19.9%.

Molecular characterization

The *rbc*L phylogenetic data set consisted of 69 gene sequences, including 25 generated in the present study. In ML phylogenetic analysis (Fig. 26), *U. chaugulii* did not cluster with any published *rbc*L sequences of *Ulva*. The interspecific sequence divergence between *U. chaugulii* and other species of *Ulva* ranged from 2.3% to 4.0%. All specimens of *U. lactuca* were clearly monophyletic and clustered with *U. fasciata* (AY255872) and *U. lactuca* (GU138294). The specimens of *U. ohnoi* showed a monophyletic association with *U. ohnoi* (AB116040 and GU138284). The genetic divergence between sequences of these two clades was 0.4%. The ITS rDNA phylogenetic data set consisted of 49 gene sequences. In the ML tree (Fig. 27), *U. chaugulii* formed a sister lineage to *U. paschima* F. Bast. The interspecific sequence divergence between *U. chaugulii* and other species of *Ulva* ranged from 11.4% to 35.1%.

DISCUSSION

Species of *Ulva* Linnaeus [including *Enteromorpha* (Hayden *et al.* 2003)] have been regularly reported and studied from India due to their widespread occurrence. The most recent detailed taxonomic account of the genus was by Krishnamurthy (2000), based on the morphological characters. Here we describe a new species of *Ulva* in addition to characterizing *U. lactuca* and *U. ohnoi* from western India.



Figs 20–25. Representative specimen of *U. ohnoi*. Fig. 20, scale bar = 2 cm; Fig. 21, scale bar = 20 μm; Figs 22, 24, 25, scale bar = 10 μm; Fig. 23, scale bar = 2 mm.
Fig. 20. Habit.
Fig. 21. Surface view showing marginal spines.
Fig. 22. Surface view showing pyrenoids.
Fig. 23. Basal portion showing rhizoidal disc.
Fig. 24. Transverse section of thallus.
Fig. 25. Transverse section of thallus showing rhizoids.

Ulva chaugulii can be distinguished from the other species of *Ulva* based mainly on the smaller size and shape of the plant (infundibuliform), the long stipe and dilated apex and the presence of two pyrenoids per cell. *Ulva chaugulii* showed strong resemblance to *U. linza* Linnaeus with respect to dimensions of cells, thickness of thalli and the loosely adherent two layers of cells, except at the thallus margin. *Ulva linza* is simple, unbranched and up to 45 cm in height and 6 cm in width, with a single pyrenoid per cell.

It also showed similarities with other species of *Ulva*, including *U. lingulata* J.G. Agardh, *U. clathrata* (Roth) Greville, *U. flexuosa* Wulfen, *U. flexuosa* (Wulfen) subsp. *paradoxa* Bliding, *U. gujaratensis* Kale, *U. clathratioides* L.G. Kraft, Kraft & R.F. Waller and *U. proliferoides* L.G. Kraft, Kraft & R.F. Waller, but all these species are branched throughout the thallus. *Ulva prolifera* O. Muller and *U. compressa* Linnaeus have single pyrenoids per cell. The cells of *U. intestinalis* Linnaeus and *U. ovata* Thivy &Visalakshmi *ex* Joshi & Krishnamurthy are irregularly arranged throughout the thallus. *Ulva brisbanensis* L.G. Kraft, Kraft & R.F. Waller and *U. meridionalis* Horimoto & Shimada lack a stipe. A newly identified species, *U. sapora* J.A. Phillips, R.J. Lawton & C. Carl from Australia, has 2–10 pyrenoids and filiform thalli (Phillips *et al.* 2016). Molecular data also confirmed the distinction of *U. chaugulii* from the above-mentioned species. The estimated sequence divergence for *rbc*L and ITS rDNA sequence was well within interspecific range reported in earlier studies (Hayden & Waaland 2002; Shimada *et al.* 2003; Ichihara *et al.* 2009). This warrants its recognition as a new species in the genus *Ulva*.

Ulva fasciata is now considered as junior synonym of *U. lactuca* (O'Kelly 2010). However, in recent studies, they were still treated as distinct species (Guidone *et al.* 2013; Kirkendale *et al.* 2013) on the grounds that no formal revision has been made. In the present study, we identified samples of *U. lactuca* and *U. fasciata* following the morphological keys of Krishnamurthy (2000). In molecular analysis of these specimens, sequences of *U. fasciata* clustered with the specimen designated as *U. lactuca* by O'Kelly *et al.* (2010). Therefore, in the present study, specimens of *U. fasciata* from India were also treated as *U. lactuca*. Most of the morphological characters of collected specimens were relatively constant and consistent with the description of *U. fasciata* by Krishnamurthy (2000), except for the presence of marginal teeth and greater thallus thickness. In addition, at some sites (e.g. Redi and Kovalam), lamina were palmately lobed. The

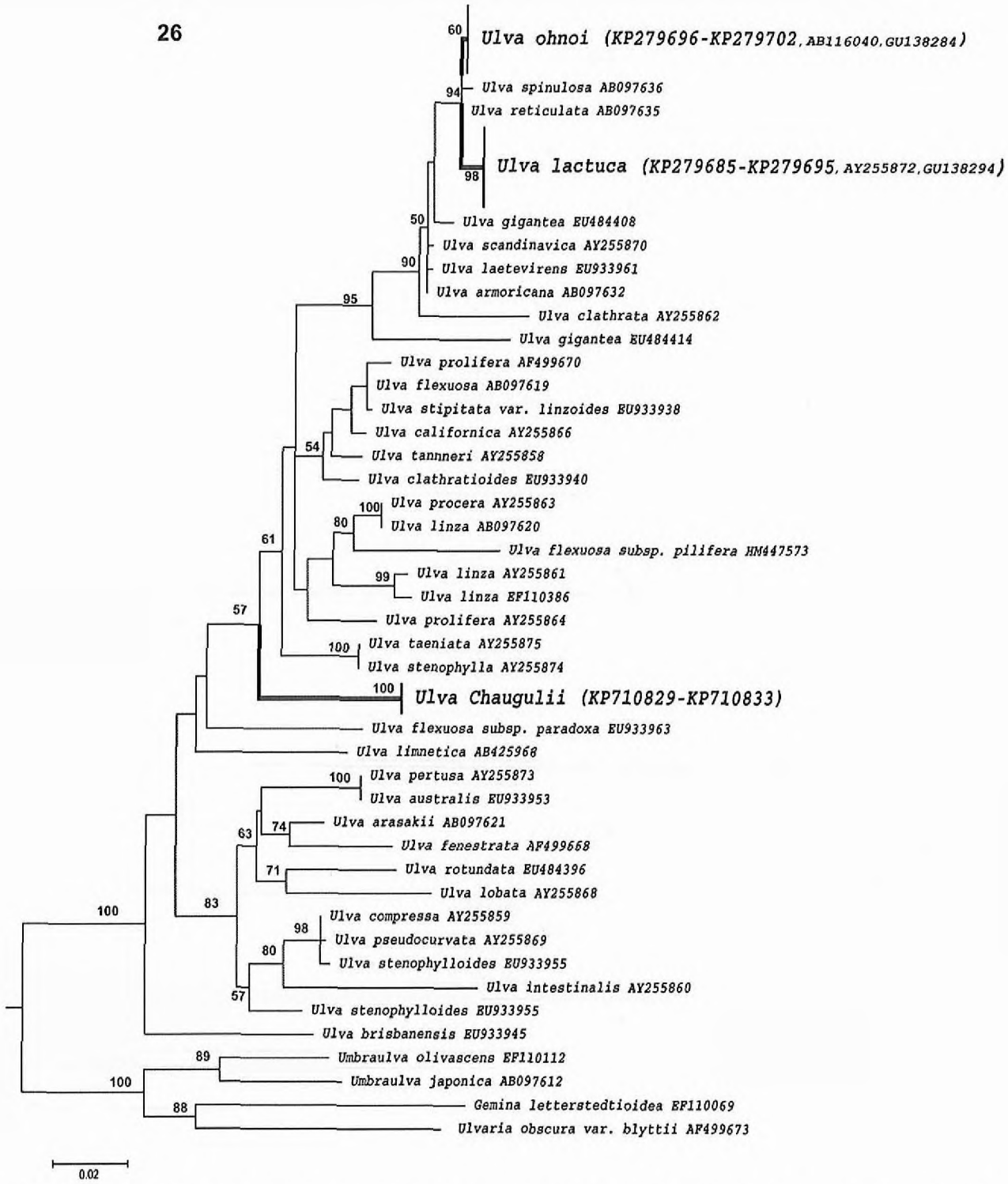


Fig. 26. ML phylogenetic tree based on *rbc*L sequence data. Bootstrap values for ML analysis are shown at nodes. Samples sequenced in this study are shown in bold.

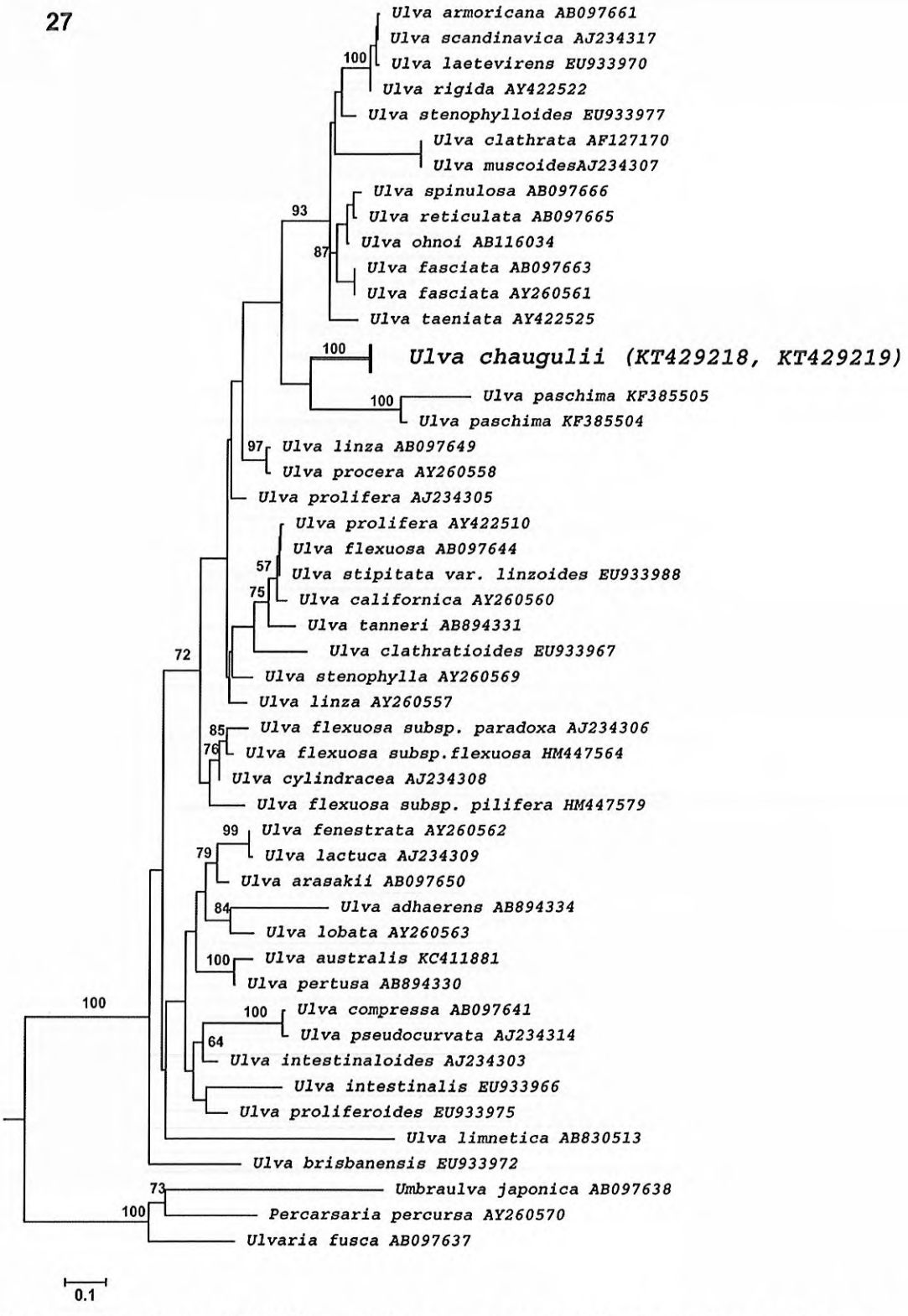


Fig. 27. ML phylogenetic tree based on ITS rDNA sequence data. Bootstrap values for ML analysis are shown at nodes. Samples sequenced from this study are shown in bold.

presence of spines was not reported for this species by earlier workers. All of our specimens had a more or less dentate margin. Detailed field and culture studies are required to find the factors governing these morphological variations.

The morphological and anatomical observations for the specimens collected as *U. lactuca* corroborated the description given by Krishnamurthy (2000). However, a short stipe and microscopic marginal teeth were observed. These plants were fragile and easily torn to separate into two distinct layers. The molecular analysis using the *rbcL* gene showed that thalli, identified morphologically as *U. lactuca*, instead belonged to the *U. ohnoi* clade rather than the *U. lactuca* clade. The position of taxa in the *rbc*L phylogenetic tree was congruent with data presented by Hiraoka *et al.* (2004). The *rbc*L sequences were also identical to the sequences of Hawaiian specimens reported by O'Kelly *et al.* (2010). Later, comparison of morphological characters with *U. ohnoi* reported by Hiraoka *et al.* (2004) also supported the identity of Indian specimens of *U. lactuca* as *U. ohnoi*. The only difference was plant size; Indian *U. ohnoi* ranged from 1 to 12 cm; whereas, Hiraoka *et al.* (2004) reported thalli from Japan to be 20–30 cm. No intraspecific variations were observed in *rbc*L sequences. The intraspecific variation in morphological characters was observed only in plant size.

Our results also showed some morphological characters that can be utilized for preliminary routine identification of specimens. For example, *U. ohnoi* had mostly orbicular shape, and the two layers of lamina separated easily. In *U. lactuca*, the thallus was broadly expanded, lanceolate, ribbon-like and more or less deeply divided. These two species also differed in thallus thickness. The thallus of *U. ohnoi* was thinner compared to *U. lactuca*. However, to confirm species identity, it is essential to subject the specimens to molecular characterization.

In conclusion, the present study substantiates the occurrence of three species of *Ulva* from the west coast of India: *U. chaugulii sp. nov.*, *U. lactuca* and *U. ohnoi*. The present work has implications for future studies related to life cycles, morphogenesis and cultivation and further biochemical and molecular characterization of these taxa.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/15-11.1.s1.

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