Identification of *Ulva* sp. Grown in Multitrophic Aquaculture Systems

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**Abstract**

The genus *Ulva* is one of the most numerous of marine and estuarine genera. Traditionally cultivated for human consumption, since the 1990s *Ulva* was integrated into land based Integrated Multi-Trophic Aquacultures (IMTA) for biomass production and bioremediation. A proper taxonomic identification is the first critical step in implementing an algal production program. However, *Ulva* species are difficult to morphological identify due to their phenotypic plasticity. Combined molecular and morphological techniques can lead to better characterization of *Ulva* sp. This study identifies the *Ulva* sp. cultivated in earth ponds facing the Ria Formosa Lagoon (South Portugal) as well as green algae growing spontaneously in the ponds. DNA barcoding with the markers ITS (Internal Transcribed Spacer) identified six species, with *Ulva* flexuosa being the cultivated one. *Ulva* flexuosa was recorded for the first time in South Portugal. However, taxonomic questions were raised because distinct clades were found for this species using published sequences. The ‘lettuce-leaf’ morphotype observed is not attributable to any of the marine sub-species of *Ulva flexuosa*.

**Keywords:** DNA-Barcoding; ITS; Species identification; *Ulva flexuosa*

**Introduction**

The genus *Ulva* is one of the most numerous of marine and estuarine genera [1]. The cosmopolitan distribution of the genus *Ulva* makes it suitable for cultivation practically everywhere [2]. Traditionally cultivated for human consumption, since the 1990s *Ulva* was integrated into land based Integrated Multi-Trophic Aquacultures (IMTA) for biomass production and bioremediation [3]. *Ulva* spp. withstand the extreme environmental condition of earth ponds and when grown in effluent media, protein content increases (> 40%), resulting in a valuable feed for macroalgivore species with high commercial value [2-5]. The current market for these algae is limited, but could see growth considering the suitability of *Ulva* as a biomass energy resource and its application as a raw material for nutraceuticals, biomaterials and sulphated polysaccharides (*Ulvan*) [3,5-7]). Given the growing demand for algae, a proper taxonomic identification is necessary in aquaculture [8,9]. Selecting appropriate target species is the critical first step in implementing an algal production programme. Moreover, improper taxonomic identification makes comparing results difficult, inhibiting the consolidation of knowledge about production and other characteristics of cultivated species [9]. An accurate assessment of marine macroalgae is important for conservation, monitoring, and management of biological introductions and invasions [10]. Due to phenotypic plasticity, the morphological characteristics of several *Ulva* species have insufficient taxonomic value [11-13]). The combination of molecular and morphological techniques can lead to better characterization of taxa [14-18]). DNA barcoding is a taxonomic method that uses a short genetic marker in an organism’s DNA to identify it [19]. The main goal is to identify an unknown sample in terms of a pre-existing classification [20]. The Internal Transcribed Spacer region of ribosomal cistron (ITS) has been used in several studies concerning the *Ulva* species identification [16,21,22]). ITS is proving useful for identification at species level due to its multiple highly variable regions [23-25]).

The IPMA aquaculture research station in Olhão (EPPO-Estação Piloto de Piscicultura de Olhão), Portugal, cultivated *Ulva* sp. during an Integrated Multi-trophic Aquaculture (IMTA) experiment in earth ponds. The purpose of this study was to verify the taxonomic identity of the *Ulva* sp. grown and identify the green algae that grew spontaneously in the pond system.

**Materials and Methods**

The IMTA experiment was conducted at the Aquaculture Research Station in Olhão (EPPO-Estação Piloto de Piscicultura de Olhão), Portugal. The EPPO station is located in the salt marshes of Ria Formosa coastal lagoon, a mesotidal system in the south of Portugal (Figure 1a). *Ulva* spp. were collected in the main discharge channel of EPPO (Figure 1b), a portion was weighted and individually planted in 6 rafts (1 m² each), made of horizontal nets stretched between styro-foam floaters (Figure 2a).
DNA extraction

Silica dried algal biomass was prepared for the DNA extraction through homogenizing the samples by grinding with a tungsten sphere in a mixer mill (Eppendorf A-2-DWP) for 3 minutes at max speed (3,700 rpm). Seaweed DNA was extracted using the NucleoSpin® Plant II Kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) following the manufacture’s protocol.

The quality of the DNA was verified by running 5 µl of the DNA extraction (with 1 µl Gel-Red and 2 µl of loading buffer (5 X Green Go-Taq Flexi Buffer)) of six randomly selected samples on a 0.8 % agarose gel.

DNA amplification and sequencing

The nuclear primers ITS1 5'-TCCGTAAGGTAACCTGTCCG-3' and ITS4 3'-CGTATAGTTATCCCTCCT-5' were used to amplify nuclear rDNA (Ribosomal DNA) fragment [27]. This fragment contains the Internal Transcribed Spacer 1 (ITS1), the 5.8 S gene, and the Internal Transcribed Spacer 2 (ITS2) [27]. Each reaction consisted of 23.95 µl H2O Milli-Q, 4 µl of 5 X Buffer, 1.6 µl 25 mM Mg, 1.25 µl 2 mM of each dNTP, 2 µl 1.0 µM of each primer, 0.2 µl 5 U/µl Go-Taq, 5.0 µL of diluted (1:100 H2O Milli-Q) genomic DNA extract.

PCR amplification was run a Applied Biosystems 2720 Thermal Cycler (Applied Biosystems™, Foster City, CA) and the profile of the reaction consisted of an initial denature at 95°C for 5 min followed by 35 cycles of 95°C for 30s, 55°C for 30s min and 72°C for 1 min, and a final extension at 72°C for 10 min.

The 54 PCR products were visually checked on a stained electrophoreses gel (2 % agarose). PCR products consisting of a single band with the right size were sequenced. DNA sequencing was performed on an ABI 3130 × 1 capillary sequencer (Applied Biosystems - CCMAR, Portugal) using the forward primers that were used for PCR.

Molecular analysis

The generated sequences were trimmed and aligned manually using Geneious R7.1.9 [28]. Subsequently identification was based on their DNA sequences by comparing them with sequences present in Genbank [29]. This operation was performed using Nucleotide BLAST web interface [30].

Phylogenetic analyses - alignment

DNA sequence alignment was created using the best quality sequence of each Ulva recognized in this study and from respective sequences chosen from BLAST results. Additional sequences for phylogenetic calculation were downloaded from Genbank selecting from other species used in previous papers [11,16,18,22] (Annex, Table 1).

Initial alignment of the nucleotide sets was obtained using Geneious R7.1.9 [28]. Subsequently, the sequences were trimmed to a standard length and the identical sequences removed. The final alignment contained 33 taxa (32 in group taxa plus one outgroup (Ulvaria obscura)), of which five sequences from this study. The alignment was realigned with MAFFT v. 7.310 online applications using Q-INS-I algorithm (with default parameters) [31]. The lasts adjustments of the resulting alignments were carried out using Geneious.

Phylogenetic analyses - construction of phylogenetic tree

The phylogenetic analyses were performed using the Maximum-Likelihood (ML) and Bayesian Inference (BI) methods [32]. The ML tree was obtained using the PhyML online program [33] and the BI tree was constructed using MrBayes present in Geneious R7.1.9. The program jModelTest version 2.1.10 [34] was used to find the model of sequence evolution that best fit the dataset. ML and Bayesian trees were built using the Generalized Time Reversible (GTR) substitution model with discrete gamma distribution in four categories. One thousand bootstrap replications were performed for both methods using default setting to compare relative support of branches.
The phylogenetic analyses, nucleotide homology (%) and sequence divergence (bp) estimates were based on 520 bp, including gaps (Annex, Table 2).

Analysis of morphology and anatomy

Morphology of thalli was assessed for fresh algae using a Nikon SMZ 1000 Stereo microscope whereas for anatomy a Nikon H550S Microscope (© 2017 Nikon Instruments Europe B.V) was used. All photos were captured and prepared using Nis-Elements Software (© 2017 Nikon Instruments Europe B.V).

Results

Molecular analysis

Of the 54 samples used for molecular analysis 24 had the required high quality for analyses. The molecular analysis of the macroalgae collected from the EPPO ponds established that the Ulva cultivated in the rafts during the IMTA experiment was Ulva flexuosa (Wulfen, 1803). In addition, 5 other Ulva and 2 Cladophora species were identified from the pond system (Annex, Table 3).

The Ulva genus was well represented and consisted of: Ulva flexuosa (Wulfen, 1803, xii,1), Ulva clathrata ((Roth) C. Agardh, 1811: 23), Ulva intestinalis (Linnaeus, 1753: 1163), Ulva sapora\(^1\) [35], Ulva torta ((Mertens) Trevisan, 1842: 480) and Ulva prolifera (O.F.Müller, 1778: 7). Ulva sapora sequence obtained had a bad quality (5.5%) and was omitted from the phylogenetic analysis.

Phylogenetic trees

The phylogenetic analyses performed with the ML (Maximum Likelihood) and BI (Bayesian Inference) methods gave comparable tree topologies with the Ulva species coming from the ponds forming four distinct groups (Figures 3 & 4). These four groups, well supported in both the ML and BI trees, consist of: Two monophyletic (C,D) groups, one polyphyletic (A) group and in group B) U. torta is paraphyletic with respect to U. clathrata. However, the internal nodes are well supported only in the BI tree, with Bayesian Inference Posterior probability (BP) between 56 % and 86 %.

Group A showed that Ulva flexuosa present in the pond system formed a monophyletic clade with Ulva flexuosa from Hokkaido, Japan, with a nucleotide homology of 99.47 % (2 bp difference) (Table 1). According to this phylogram, either U. flexuosa are closely related to monophyletic group of Ulva californica (internal node value of 69 %) and the nucleotide homology showed between two species (≈ 97 %) supported a high similarity between these taxa. The Ulva torta identified showed a low similarity with other European Ulva californica subspecies with nucleotide homology < 87.9 % (Table 2).

Also the groups C, B and D were well supported and showed that all Ulva species sampled were closely related with the species from the North Pacific (nucleotide homology between ≈ 99 % to ≈ 96 %) (Annex, Table 2).

Morphological observations

The gross morphological characteristics (Annex, Table 3) presented a marked homogeneity among the varied species collected, underlining the importance of genetic analysis to identify the different Ulva species. The filamentous, herbaceous-like shape was the most common and, with a few exceptions of turf forms (Ulva sapora and one Ulva clathrata), Ulva flexuosa was the only species present with 3 different dominant morphotypes:

a. The lettuce-leaf (Figure 5a-b).

b. Narrow and broad gregarious thalli (Figure 5c).

c. Filamentous, herbaceous-like shape (Figure 6a-c).

The lettuce-like Ulva flexuosa was the cultivated one. The specimens had a less rigid structure (thin and papery in texture) than those collected in the drainage channel. Moreover, they lost any anchoring structure present in the wild type. Their thalli had medium to light green, broader than long, flat, irregular contoured with undulated margins and was unbranched (Figure 5a). Under the microscope the...
central part of lettuce-like’s thallus showed a disordered cell arrangement with 2-4 pyrenoids per cell. Cells were irregularly arranged, polygonal, usually with rounded corners (Figure 5b). Principally measurements are shown in table 3.

Figure 4: Bayesian tree of ITS sequences. Bayesian Probabilities (%), BP, are given on the branches. Posterior probabilities < 50 % have been omitted. Sequences are labelled with taxon name and GenBank accession number of ITS sequence (Annex, Table 1). The tree is rooted using Ulvaria obscura. A, B, C and D refer to class containing Ulva collected from EPPO ponds. In bold is stressed the Ulva flexuosa identified in this study.

Table 2: Nucleotide homology (in percentage) of ITS region sequences between Ulva flexuosa grown within the ponds and European Ulva flexuosa subsp. In red is stressed the nucleotide homology of Ulva flexuosa grown in the ponds.

<table>
<thead>
<tr>
<th></th>
<th>U. flexuosa T114ITS</th>
<th>U. flexuosa subsp. flexuosa HM447564</th>
<th>U. flexuosa subsp. paradoxa HM447561</th>
<th>U. flexuosa subsp. pilifera HM447579</th>
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<td>–</td>
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<td>91.71</td>
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<tr>
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<td>85.75</td>
<td>90.52</td>
<td>85.53</td>
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Table 3: Size of Ulva flexuosa cells with wide leaf thalli.

<table>
<thead>
<tr>
<th>U. flexuosa</th>
<th>Length of cells (µm)</th>
<th>Width of cells(µm)</th>
<th>ø of pyrenoids</th>
<th>Nº of pyrenoids (in one cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>8.04</td>
<td>5.61</td>
<td>1.84</td>
<td>3.5</td>
</tr>
<tr>
<td>Min.</td>
<td>5.19</td>
<td>1.99</td>
<td>0.97</td>
<td>1</td>
</tr>
<tr>
<td>Max.</td>
<td>11.27</td>
<td>5.87</td>
<td>2.91</td>
<td>4</td>
</tr>
<tr>
<td>SD*</td>
<td>1.2</td>
<td>1.08</td>
<td>0.42</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*SD= Standard Deviation

Figure 5: a) Lettuce-shape Ulva flexuosa; b) polygonal cells with pyrenoids (black rows); c) Gregarious thalli with discoidal base (red circle). Scale bar a) and c) 1cm. Scale bar for b) is 10µm.

The two remaining morphotypes belong to Ulva flexuosa grown within the ponds or attached to the framework. The first of these was characterized by a narrow and broad gregarious thallus attached to substrate by means of a small discoid base and like the cultivated morphology was unbranched, flat with a thin texture and, started from a narrow base, widening towards the top. The Ulva flexuosa on the framework had a filamentous herbaceous shape and it often presented thalli polyform, slender, tubular compressed or laminar, widening at the top. Observations under the stereoscope revealed the presence of some branches at the base and a stipe that could be hollow. The thalli were fixed by means of a basal disc reinforced by numerous robust rhizoidal filaments. It is worth mentioning the presence of a fourth
morphotype, with lanceolate thallus, although it is represented by a single specimen collected around the 13-pond’s perimeter.

**Discussion**

The identification of *Ulva* spp. present in the EPPO ponds revealed a heterogeneous community, consisting of six taxa of which two were never reported in the Ria Formosa area. *Ulva flexuosa* and *Ulva torta*.

*Ulva flexuosa* was identified as the species cultivated and its lettuce-leaf morphotype is not attributable to any of the subspecies of this marine species.

The ITS allowed to differentiate *Ulva* taxa among our samples. The huge morphological plasticity of the kind probably would otherwise have led to associate the different phenotypes found with a species already recorded in the Ria Formosa. The presence of multiple bands in PCR products has already been reported in the past [24,37]. Therefore, ITS is commonly associated with rbcL (plastid rubisco large subunit) marker to increase the successes of identification [1,11,15,22,38].

**Ulva flexuosa**

*U. flexuosa* was originally described by Wulfen from the Adriatic Sea in the 1803. Currently, *Ulva flexuosa* species includes 4 subspecies and one variety: *U. flexuosa* ssp. *flexuosa* (Wulfen 1803: xxii, 1), *U. flexuosa* ssp. *paradoxa* (C. Agardh) M.J. Wynne [39], *U. flexuosa* var. *linguiformis* [13], *U. flexuosa* ssp. *biflagellata* (Bliding) A. Sfriso & D. Curiel [40] and *U. flexuosa* ssp. *pilifera* (Kützing) M.J. Wynne [39,41,42]. Among the three morphotype here reported the lettuce-leaf observed is not referable to any of the marine subspecies belonging to *Ulva flexuosa*. However, previous studies recorded a similar phenotype for the freshwater *Ulva flexuosa* ssp. *pilifera* [16,42]). Perhaps this morphotype may be explained considering algae grown in IMTA systems tend to develop leaves larger than wild types [43]. The remaining two morphologies have a taxonomic response. The filamentous one, based on the polymorphism of the thallus and the presence of a tubular stigma, could be associated to *Ulva flexuosa* ssp. *flexuosa* [13]. The gregarious thallus, instead, was similar to the *Ulva flexuosa* morphotype described by Wolf et al. [17] in the Venice lagoon, *Ulva flexuosa* from Busan and Pohang, Korea [44] and with the *U. flexuosa* ssp. *pilifera* identified in a recent study in the Polish freshwater [42]. However, genetic identity discarded the hypothesis of three distinct subspecies confirming instead the enormous plasticity of *Ulva genus*. There are several factors that can explain this phenomenon. *Ulva flexuosa* can ‘switch’ its thallus morphotype from tubular to foliose along their life and it is more frequent in culture due to stresses unique to artificial systems [22,45]. The place where the thalli develop (e.g. bottom or water surface) and environmental factors such as salinity and temperature can also affect morphological plasticity [25,42]). In our case, the fact of having collected seaweed in November after a week of intense rain may have favoured the finding of different morphotypes due to lowering of the temperature and salinity. Furthermore, in the past the role of bacterial community on morphology variation of *Ulva* genus has been shown [46,47]). The capacity of *Crassostrea gigas* to remove large amounts of bacteria [48] could perhaps have provoked a change in their community promoting change in *Ulva flexuosa* phenotype. All these possibilities need further studies.

Historically this species has been recorded in neighbouring countries along the coastal zone between Tanger (Morocco) and Melilla (Spain) [49] and in the Cadiz Bay [50]. Furthermore, *U. flexuosa* has been included in the list of macroalgae of the North coast of Portugal, along Minho, Douro Litoral, and Beira Litoral regions [51] and in Corunna harbour, Spain [52].

The *Ulva flexuosa* T11t4 sequence turned out to be almost identical (2 bp of difference) to that reported by Shimada in Hokkaido, Japan [11] forming a well-defined clade in both phylogenetic trees. This observation may suggest the origin of these macroalgae could be the North Pacific and other studies suggest a common origin between the *Ulva flexuosa* of South Europe and the Pacific. An investigation about cryptic species with morphologies identical or similar, although genetically different [17] and new species in the North Adriatic reported an *Ulva flexuosa* quite identical to one reported in British Columbia (Canada) [17]. Moreover, a Greek *Ulva flexuosa* var. *linguiformis* was closer related with a Japanese specimen [11,13,41]).

The *Ulva flexuosa* specimens from the EPPO ponds and South Europe did not match genetically with *Ulva flexuosa* subspecies from North Europe [16,22]), as was already detected by Marès and Shimada [16,23]. Marès et al. [16] proposed to indicate *U. flexuosa* as indigenous species of the inland waters of the Europe proposing a different nomenclature for the Asian, however, no mention was made about marine *Ulva flexuosa*.

**Other taxa**

Not only *Ulva flexuosa* was recorded for the first time in the Ria Formosa lagoon, also *Ulva torta* was first reported whereas *Ulva intestinals*, *Ulva prolifera* and *Ulva clathrata* have been already mentioned in some studies that took place in the lagoon [53,54]. Historically, all these taxa, with sometimes the exception of *Ulva torta*, have shown a similar geographical distribution, jointly with *U. flexuosa*, in Portugal and neighboring countries [49-52]. Moreover, in the port of Corunna they occupied the same environment [52]. Nevertheless, among the studies listed above only Alsuwyani et al. [54] provided a molecular identification by means of molecular techniques. This can lead to some doubts about the real distribution of this species.

**Conclusion**

The presence of *Ulva flexuosa* in the South Portugal broadens its geographic distribution in the country. The use of the molecular marker ITS was successful on macroalgae cultivated but there was low amplification success. For this reason subsequent investigations of green macroalgae would require the use of markers with a higher success rate such as tufA or associating rbcL (plastid rubisco large subunit) with the use of ITS [24]. Two studies in Poland and the USA leaded to the hypothesis that macroalgae previously identified as subspecies of *Ulva flexuosa* may be young species undergoing separation due to isolation and adaptation to different habitats [42,55]). Hence the recommendation to investigate into the phylogenetic relationships between *U. flexuosa* subspecies using more sensitive and specific molecular markers (e.g. ISSR (Inter-Simple Sequence Repeat), or SCAR (Sequence Characterized Amplified Region)) [42,56]). The genetic data collected in this experiment may lead to conclude that the origin of the macroalgae present in EPPO ponds could be the North Pacific. However, the scale of the present study does not allow to state which is the actual distribution area of the *Ulva* spp. identified and their
status of native or introduced species. The presence of several species of Ulva suggests they withstand the ponds environment and proposes Ulva spp. as excellent candidates for the IMTA land-based systems.

Acknowledgements

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References


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57. Zhang Q (2015) Porphyra aquaculture rafts is the major source of float- ing green algae in the yellow sea: Evidence of intraspecific genetic analysis on Ulva prolifera. National Center for Biotecnology Information, Maryland, USA.

## ANNEX

<table>
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<td>La Jolla, CA, USA</td>
<td>Hayden et al. [45]</td>
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**Table 1:** Sources of taxa used to create the phylogenetic trees.

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<td>Ulva clathrata</td>
<td>Yellow Sea (China)</td>
<td>HQ197901</td>
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<td>B</td>
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<td>EPPO pond</td>
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<td>Ulva clathrata</td>
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<td>HQ197901</td>
<td>99.49</td>
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<td>Ulva torta</td>
<td>Fukui (Japan)</td>
<td>AB330503</td>
<td>97.71</td>
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<td></td>
<td>Ulva torta</td>
<td>Clovelly, NSW (Australia)</td>
<td>KF195491</td>
<td>95.69</td>
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<td>Ulva torta T16t2</td>
<td>EPPO pond</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Ulva clathrata</td>
<td>Yellow Sea, (China)</td>
<td>HQ197901</td>
<td>95.17</td>
<td>19</td>
</tr>
<tr>
<td>C</td>
<td>Ulva prolifera</td>
<td>EPPO pond</td>
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<td></td>
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<tr>
<td></td>
<td>Ulva prolifera</td>
<td>Yellow Sea (China)</td>
<td>KT802960</td>
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<td>D</td>
<td>Ulva intestinalis</td>
<td>EPPO pond</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Ulva saporae</td>
<td>Shelly Beach, Caloundra (Australia)</td>
<td>KT374006</td>
<td>96.48</td>
<td>14</td>
</tr>
</tbody>
</table>

*Distance between sequences (base-pair)

**Table 2:** Nucleotide homology (%) of ITS region sequences of the EPPO samples and other Ulva specimens available in GenBank, that grouped in the ITS phylogenetic tree. *Distance between sequences (base-pair).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
<th>Morphological assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-t3</td>
<td>Ulva flexuosa (Wulfen,1803)</td>
<td>Filamentous, herbaceous shape</td>
</tr>
<tr>
<td>11-t4</td>
<td>Ulva flexuosa (Wulfen,1803)</td>
<td>Filamentous, herbaceous shape</td>
</tr>
<tr>
<td>11-f2</td>
<td>Ulva flexuosa (Wulfen,1803)</td>
<td>Lettuce-leaf, flat, rounded undulate margins.</td>
</tr>
<tr>
<td>16-t6</td>
<td>Ulva sapora (Phillips et al. [35])*</td>
<td>Turf form, Thin-short filamentous</td>
</tr>
<tr>
<td>12-t2</td>
<td>Cladophora albida ((Nees) Kutzing, 1843)</td>
<td>Dark green, musk form</td>
</tr>
<tr>
<td>12-t3</td>
<td>Cladophora vagabunda ((Linnaeus) Hoek, 1963)</td>
<td>Narrow liner flat leaf</td>
</tr>
<tr>
<td>12-t5</td>
<td>Ulva flexuosa (Wulfen,1803)</td>
<td>Filamentous, herbaceous shape</td>
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<tr>
<td>Ulva prolifera (O.F.Müller, 1778)</td>
<td>Filamentous, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Filamentous, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Linear compress thalli, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Lettuce-Leaf, flat, rounded edges, undulate margin</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Lanceolate Leaf.</td>
<td></td>
</tr>
<tr>
<td>Ulva clathrata ((Roth) C.Agardh, 1811)</td>
<td>Filamentous, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Filamentous, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Lettuce-leaf present some perforation</td>
<td></td>
</tr>
<tr>
<td>Ulva intestinalis (Linnaeus, 1753)</td>
<td>Tubular, herbaceous shape</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Narrow and broad gregarious thalli, small discoid base</td>
<td></td>
</tr>
<tr>
<td>Ulva flexuosa (Wulfen, 1803)</td>
<td>Linear compress thalli, round on top.</td>
<td></td>
</tr>
<tr>
<td>Ulva clathrata ((Roth) C.Agardh, 1811)</td>
<td>Turf form, Thin-short filamentas</td>
<td></td>
</tr>
</tbody>
</table>

*This name is currently regarded as a synonym of Ulva tepida (Masakiyo and S.Shimada, 2014) (Algaedatabased).*

Table 3: Ulva taxa identified with short morphological description.
Journal of Anesthesia & Clinical Care
Journal of Addiction & Addictive Disorders
Advances in Microbiology Research
Advances in Industrial Biotechnology
Journal of Agronomy & Agricultural Science
Journal of AIDS Clinical Research & STDs
Journal of Alcoholism, Drug Abuse & Substance Dependence
Journal of Allergy Disorders & Therapy
Journal of Alternative, Complementary & Integrative Medicine
Journal of Alzheimer’s & Neurodegenerative Diseases
Journal of Angiology & Vascular Surgery
Journal of Animal Research & Veterinary Science
Archives of Zoological Studies
Archives of Urology
Journal of Atmospheric & Earth-Sciences
Journal of Aquaculture & Fisheries
Journal of Biotech Research & Biochemistry
Journal of Brain & Neuroscience Research
Journal of Cancer Biology & Treatment
Journal of Cardiology: Study & Research
Journal of Cell Biology & Cell Metabolism
Journal of Clinical Dermatology & Therapy
Journal of Clinical Immunology & Immunotherapy
Journal of Clinical Studies & Medical Case Reports
Journal of Community Medicine & Public Health Care
Current Trends: Medical & Biological Engineering
Journal of Cytology & Tissue Biology
Journal of Dentistry: Oral Health & Cosmesis
Journal of Diabetes & Metabolic Disorders
Journal of Dairy Research & Technology
Journal of Emergency Medicine Trauma & Surgical Care
Journal of Environmental Science: Current Research
Journal of Food Science & Nutrition
Journal of Forensic, Legal & Investigative Sciences
Journal of Gastroenterology & Hepatology Research
Journal of Gerontology & Geriatric Medicine
Journal of Genetics & Genomic Sciences
Journal of Hematology, Blood Transfusion & Disorders
Journal of Human Endocrinology
Journal of Hospice & Palliative Medical Care
Journal of Internal Medicine & Primary Healthcare
Journal of Infectious & Non Infectious Diseases
Journal of Light & Laser: Current Trends
Journal of Modern Chemical Sciences
Journal of Medicine: Study & Research
Journal of Nanotechnology: Nanomedicine & Nanobiotechnology
Journal of Neonatology & Clinical Pediatrics
Journal of Nephrology & Renal Therapy
Journal of Non Invasive Vascular Investigation
Journal of Nuclear Medicine, Radiology & Radiation Therapy
Journal of Obesity & Weight Loss
Journal of Orthopedic Research & Physiotherapy
Journal of Otolaryngology, Head & Neck Surgery
Journal of Pathology Clinical & Medical Research
Journal of Pharmacology, Pharmaceutics & Pharmacovigilance
Journal of Physical Medicine, Rehabilitation & Disabilities
Journal of Plant Science: Current Research
Journal of Psychiatry, Depression & Anxiety
Journal of Pulmonary Medicine & Respiratory Research
Journal of Practical & Professional Nursing
Journal of Reproductive Medicine, Gynaecology & Obstetrics
Journal of Surgery: Current Trends & Innovations
Journal of Toxicology: Current Research
Journal of Translational Science and Research
Trends in Anatomy & Physiology
Journal of Vaccines Research & Vaccination
Journal of Virology & Antivirals
Archives of Surgery and Surgical Education
Sports Medicine and Injury Care Journal
International Journal of Case Reports and Therapeutic Studies
Journal of Ecology Research and Conservation Biology