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Differentiation between some *Ulva* spp. by morphological, genetic and biochemical analyses

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Ulva is most common green seaweed in Egypt coast, it used as a source of food, feed, medicines and fertilizers in all the world. This study is the first time to investigate the morphological, genetic and biochemical variation within four *Ulva* species collected from Eastern Harbor, Alexandria. The morphology description of thallus showed highly variations according to species, but there is not enough data to make differentiation between species in the same genus since it is impacted with environmental factors and development stage of seaweeds. Genetic variations between the tested *Ulva* spp. were analyzed using random amplified polymorphic DNA (RAPD) analyses which shows that it would be possible to establish a unique fingerprint for individual seaweeds based on the combined results generated from a small collection of primers. The dendrogram showed that the most closely species are *U. lactuca* and *U. compressa*, while, *U. fasciata* was far from both *U. lactuca* and *U. compressa*. Meanwhile, *U. linzea* is showed to be a unique species. The biochemical composition (e. g. protein, carbohydrate, lipid and pigment composition) of the collected *Ulva* spp. grouped the collected *Ulva* spp. into two groups (*U. fasciata* and *U. lactuca*) and other (*U. compressa* and *U. linzea*).

Key words: morphological character; genetic; biochemical; RAPD; *Ulva* spp.

Различия между некоторыми видами рода *Ulva*, выявленные путем морфологического, генетического и биохимического анализа

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Ульва – самая распространенная зеленая водоросль, обитающая у побережья Египта. Во всем мире она используется в качестве продукта питания, применяется в медицинских целях, а также идет на удобрения и фураж. В статье приводятся результаты первого исследования морфологических, генетических и биохимических различий между четырьмя видами водорослей рода *Ulva*, образцы которых были собраны в Восточной бухте города Александрии (Египет). При исследовании морфологии таллуса были выявлены значительные видоспецифические различия, но данных для того, чтобы отделить один вид от другого в пределах одного и того же рода, недостаточно, поскольку на указанный признак влияют как экологические факторы, так и стадия развития растения. Генетические различия между исследованными видами ульвы анализировали с помощью метода случайно амплифицируемой полиморфной ДНК (RAPD). Анализ дендрограммы показал, что наиболее близки друг к другу виды *U. lactuca* и *U. compressa*, тогда как *U. fasciata* является видом, отдаленным как от *U. lactuca*, так и от *U. compressa*. В то же время было показано, что *U. linzea* является уникальным видом. Различия, выявленные между видами ульвы методом RAPD, говорят о возможности получения «фингерпринта» для индивидуальных водорослей при использовании лишь небольшого числа праймеров. На основе своего биохимического состава (белкового, углеводного, липидного и пигментного) собранные виды были разбиты на две группы: в одну вошли *U. fasciata* и *U. lactuca*, а в другую – *U. compressa* и *U. linzea*.

Ключевые слова: морфологический признак; генетический; биохимический; RAPD; ульва.

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The green algal genus *Ulva* commonly known as sea lettuce, including species previously placed in the genus *Enteromorpha* (monostromatic tubular thalli) (Hayden et al., 2003), is well known for its wide distribution in marine, freshwater and brackish environments on a global scale in the intertidal and shallow subtidal zones of rocky shores all over the world (Reed, Russell, 1979; van den Hoek et al., 1995; Shimada et al., 2007, 2008; Ichihara et al., 2009). The genus *Ulva* was first identified by Linnaeus in 1753 (Kong et al., 2011). Since many taxonomists and phycologists have been involved in the identification of *Ulva* species (Wolf et al., 2012) which are notoriously difficult to classify due to the morphological plasticity expressed by many members as well as the few reliable characters available for differentiating taxa (Heech et al., 2009; Wolf et al., 2012). It is morphology heavily influenced by environmental conditions, age of the thallus, and life style, making difficult the delineation of species by morphological features alone (Wolf et al., 2012).

Until 2003, leafy forms and tubular forms were generally attributed to two separate genera, *Ulva* and *Enteromorpha*, respectively. However, intermediate forms between *Ulva* and *Enteromorpha* had been observed in some circumstances and there was evidence that under certain environmental conditions some species could switch from a morphotype to the other (Tan et al., 1999). Bliding (1968) recognized eight *Ulva* species in Europe and used mainly microscopic characters: cell size and shape in the blade and basal area (both in surface view and in section), thallus thickness, cell arrangement, chloroplast position and number of pyrenoids. Several authors have questioned the validity of morphological characteristics for identification purposes, because of the large variation observed between individuals at different sites and between individuals at the same site in different seasons (Mshigeni, Kajamulo, 1979; Tanner, 1986). Seaweeds have an interesting chemical composition that makes their commercial exploitation attractive to produce functional or health promoting food. *Ulva* is a good source for food, development of novel drugs and functional foods, pharmaceutical and agricultural applications (Costa et al., 2010; Wijesekara et al., 2011).

Recent studies based on molecular data carried out in different parts of the world invariably have shown a higher species diversity than suggested by morphology, revealing cryptic species and introduced taxa not previously detected (Loughnane et al., 2008; O'Kelly et al., 2010). The molecular data produced in the last 10 years have led to a much better characterization of species concepts and circumscriptions (Hayden et al., 2003). Recent improvements in DNA extraction procedures have made it possible to obtain partial sequences from type specimens, and in some cases this has led to unexpected results. For example, it has been shown that the molecular identity of the type specimen of *U. lactuca* does not match that of specimens that have since been assigned to this name, and corresponds to the entity named *U. fasciata* (O'Kelly et al., 2010).

Genetic variation between *Ulva* species are assessed by the presence or absence of each product. Random Amplified Polymorphic DNA (RAPD) technique based on the Polymerase Chain Reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers (Bardakci, 2001). Polymorphisms can occur due to base substitutions at the primer binding sites or to differences in the regions between the sites. RAPD markers have been used for species identification, analysis of population structure, analysis of genetic impacts of environmental stress and analysis of genetic diversity (Williams et al., 1993).

The aim of the present study was to provide information on the morphology, genetic and biochemical characters of most common *Ulva* spp. in Eastern Harbor of Alexandria.

Materials and methods

Collection and identification of *Ulva* species. Four species of *Ulva* were handpicked collected in spring 2014 at depth of 0.2 m or less from rocky of Eastern Harbor water, Alexandria (longitudes 29°53′–29°54′ E and latitudes 31°12′–31°13′ N) which are excellent domains for seaweed attachment (Fig. 1). All samples were brought to the laboratory in plastic bags containing seawater to prevent evaporation. In laboratory, seaweeds were cleaned from epiphytes and rock debris and given a quick fresh water rinse to remove surface salts. On the same of collection; some of fresh samples were processed as herbarium specimens and deposited in the Taxonomy Museum Marine Environmental Division (National Institute of Oceanography and Fisheries, Kayet Bay, Alexandria, 21556, Egypt); other of them were preserved in 5 % formalin seawater for morphology study and the other cleaned seaweeds were then air dried in the shade at room temperature (25–30 °C) on absorbent paper for estimation of moisture content. Then, they were pulverized in a cereal grinder for 5 min and sieved, using a 100 mesh sieve, to obtain a fine and homogeneous powder that was stored in hermetic sealed plastic bags and stored at –20 °C until for genetic and biochemical analysis. All seaweeds were identified taxonomically following the methods of Taylor (1960), Abbott and Hollenberg (1976), Aleem (1993), Jha et al. (2009). The names of the species were used according to Guiry, Guiry (2011) and were confirmed using algaebase website.

Morphological studies. Macroscopic observations on the algae included the thallus colour, length, texture and, if attached morphotypes were found, the holdfast was screened. The following microscopic characteristics were recorded: cell sizes of at least ten randomly chosen cells, thallus thickness, shape and arrangement of the cells in surface view, shape and arrangement of the chloroplast in surface view (cap-like appearance or not) and number and distribution of pyrenoids.

Genetic studies. *DNA extraction.* About 500 mg of the freeze algae (–20 °C) was ground in a pre-cooled mortar and pestle by adding liquid nitrogen. DNA was isolated from samples by CTAB method (Doyle, Doyle, 1987).

RAPD-PCR. Amplification of genomic DNA was performed in 25 µl reaction mixture containing 9.5 µl water, 0.5 µl of RAPD primer, 12.5 µl PCR master mix and 2.5 µl DNA. RAPD-PCR was performed by 14 different RAPD primers (Tab. 1) after checking the DNAs by agarose gel electrophoresis. PCR bands were checked again by agarose gel electrophoresis after PCR analysis. Amplification done with 5 cycles; denaturation at 94 °C for 1 minute, annealing at 40 ºC for 30 seconds and extension at 72 °C for 1 minute, 40 cycles; denaturation at 94 °C for 45 seconds, annealing at 60 °C for 1 minute and extension at 72 °C for 45 seconds, final extension at 72 °C for 11 minutes. The amplified prod-

Fig. 1. Location of collected seaweeds.

uct kept in 4 °C until gel electrophoresis. 12 µl of each PCR product was electrophoresed on 1.2 % agarose gel in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 80 V for 1 hour. The gel was stained with ethidium bromide and photographed.

Biochemical studies. Biochemical studies including ash content which was estimated by ashing the ground dried samples overnight in a muffle furnace at 525 °C, the protein fraction (% of DW) was calculated from the elemental N determination using the nitrogen-protein conversion factor of 6.25 according to AOAC (1995), total carbohydrate was estimated by following the phenol-sulphuric acid method of Dubois et al. (1956), using glucose as standard, lipids were extracted with a chloroform-methanol mixture (2:1 v/v) then were dried over anhydrous sodium sulphate, after which the solvent was removed by heating at 80 °C under vacuum (AOAC, 2000) and the pigment contents e. g. chlorophyll *a* and *b* were extracted in 90 % acetone, estimated spectrophotometrically according to the method of Jeffrey, Humphrey (1975) and carotenoids were estimated in the same extract of chlorophyll at 480 nm by Ridley method (1977).

Statistical analysis. Results are expressed as mean ± standard deviation (SD) from three replicates. Data obtained were analyzed statistically using SAS (6.12) to determine the degree of significance using one way analysis of variance (ANOVA) at probability level $p \leq 0.05$, followed by the Community Analysis Package (CAP) 4.0. The relationships between the *Ulva* spp. were determined by cluster analysis (Richard, Peter, 2007).

Results and discussion

Morphological and taxonomical description of *Ulva* species

Algal collection were identified as presented in the literatures, checked for synonyms and latest accepted names, referred to its systematic groups and described. The collected species were identified as *Ulva compressa*, *U. fasciata, U. lactuca* and *U. linzea* **(**Phylum: Chlorophyta; Class: Chlorophyceae; Order: Ulvales; Family: Ulvaceae). There was significant variation in morphological characteristics of *Ulva* spp.

Table1. The sequence of the RAPD primers used in the study

Primer number	Nucleotide sequence (5′– 3′)
1	TGCCGAGCTG
2	AATCGGGCTG
3	TCTGTGCTGG
4	TTCCGAACCC
5	CAGGCCCTTC
6	GGTCCCTGAC
7	TCGGCGATAG
8	CAGCACCCAC
9	CAAACGTCGG
10	GTTGCGATCC
11 (OPB-10)	CTGCTGGGAC
12 (OPB-12)	CCTTGACGCA
13 (OPC-07)	GTCCCGACGA
14 (OPW-03)	GTCCGGAGTG

Genus (1): ***Ulva compressa*** **(Linnaeus) Grev**

Synonyms: *Enteromorpha compressa* (Linnaeus) Nees, *E. intestinalis* (L.) Nees, subsp. *compressa* De Silva and Burrows.

Morphological description: Thalli green color, up to 20 cm, 1–2.5 mm broad membranaceous**,** flat, narrow bladed form, tapering upward, waving margin, branching at the base, attached at first by a short cylindrical stipe (Fig. 2, *a*).

Surface view: Surface cells minute, polygonal, irregular arrangement, cell contents chloroplast with pyrenoids 1–2 per cell (Fig. 2, *b*).

Remarks: It is present in peaches and act as habitats for different polycheates sp.

Genus (2): ***Ulva fasciata*** **Delile**

Synonyms: *Ulva lactuca* f. *fasciata* (Delile) Hering, *Phycoseris fasciata* (Delile) Montagne.

Morphological description: Thallus yellow to dark green in colour, thin, up to 40 cm long, 8–12 cm wide at base, tapering upward to less than 1.5 cm wide at tip and deeply divided into a number of lobes, 1–3 cm broad lobes; sheet like, blades irregularly linear with slightly irregular margins with occasional coarse microscopic marginal teeth or spine projections (Fig. 3, *a*).

Surface view: Cells in surface view polygonal, palisade like with two cells thick and irregularly arranged. Cells uninucleate, chloroplast plate-like filling outer part of cell and having two pyrenoids (Fig. 3, *b*).

Remarks: It is the most common species in collected season and place. It is good habitats for polycheats and zooplankton spp.

Genus (3): ***U. lactuca*** **Linnaeus**

Synonyms: *U. lactuca efolia* Gray, *U. fenestrate* Postels and Ruprecht, *U. stipitata* Areschoug, *U. crassa* Kjellman.

Morphological description: Thalli bright green, much broader (15–20 cm) than long (10–15), flat, rounded, foliose, leafy, soft, broad, membranous with undulated margins and

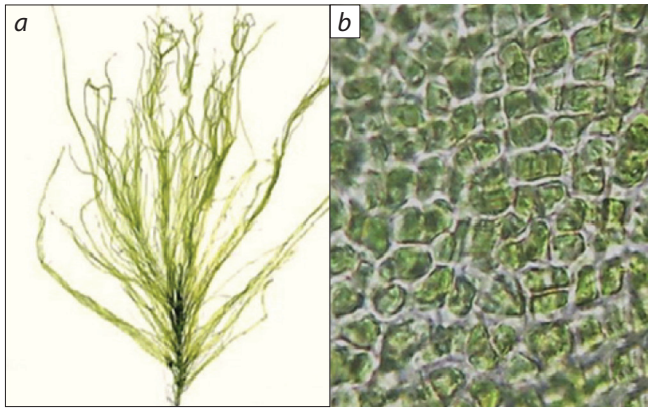

Fig. 2. (*a*) *U. compressa* and (*b*) surface view of cells of blade.

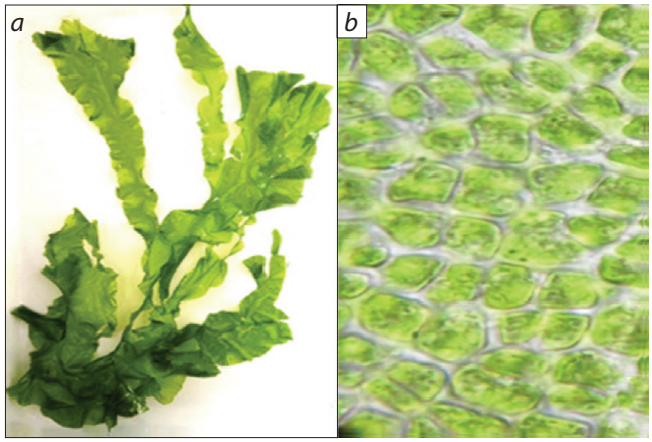

Fig. 3. (*a*) *U. fasciata* and (*b*) surface view of cells of blade.

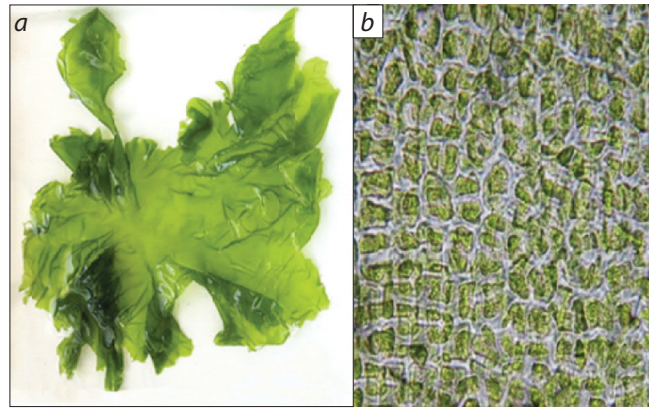

Fig. 4. (*a*) *U. lactuca* and (*b*) surface view of cells of blade.

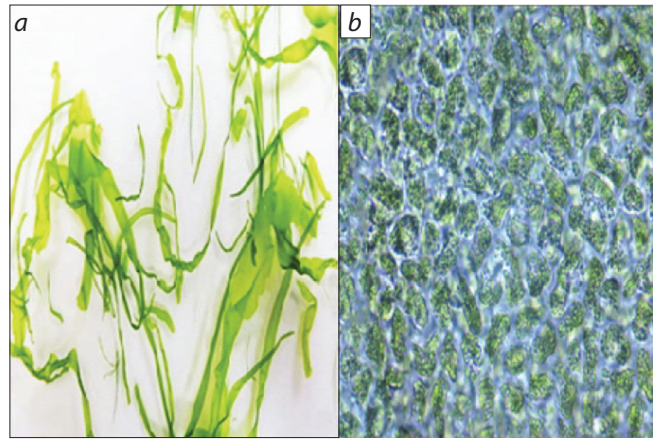

Fig. 5. (*a*) *U. linzea* and (*b*) surface view of cells of blade.

normally wider at the top than at the base resembling a lettuce leaf (Fig. 4, *a*).

Surface view: Under microscope examination; surface cells have various shapes and sizes with no regular arrangement with rounded angles, chloroplast usually fills the outer end of the cell with one or more pyrenoids. Transverse section of frond shows the two layers of cells (Fig. 4, *b*).

Remark: The natural blades of *U. lactuca* and *U. fasciata* were more rigid than other tested *Ulva* spp. This characteristic was attributed to the number of strata of cells intransversal section.

Genus (4): ***Ulva linzea* Linnaeus**

Synonyms: *Enteromorpha linza* (Linnaeus) J. Agardh, *U. procera* (K. Ahlner) Hayden.

Morphological description: Thalli green in color, compressed, linear lanceolate, unbranched, flattened, up to 30 cm long and less than 1.0 cm broad in basal part which are tapered from the base towards the apex to become spine like, with wave margin (Fig. 5, *a*).

Surface view: Microscope examination showed that the cells in surface view generally polygonal without regular arrangement, interspace between cell is wide and no hollow parts in blades. Cell is taller than broad, uninucleate, chloroplast with the large number of pyrenoids (Fig. 5, *b*).

As shown from previously morphological examination, there are significantly different in macroscopic description. On the other hand, there are some similarity in microscopic examination so these methods are not suitable for differentiation between *Ulva* spp. There are little data related to our study, the morphological and anatomical structure (thickness of marginal, mid, and basal regions; cell height in transverse section and cell size in surface section) of *U. rigida* significantly affected by thallus age (Phillips, 1988), seasons and locations (Dural, Demir, 2001). Cell size is known to vary under different environmental conditions such as salinity (Koeman, van den Hoek, 1981), temperature and light availability (Israel et al., 1995) and for these reasons does not seem to be a good characteristic for identification. Pyrenoid number and distribution useful for identification (Koeman, van den Hoek, 1981) but the pyrenoids can be very hard or even impossible to observe if the cells contain many starch grains (Malta et al., 1999). Pettett (2009) stated that the ability of *Ulva* to switch morphological characteristics under different environmental conditions makes *Ulva* taxonomy difficult so it should be used more advanced methods like genetic information. Genetic information reassigned the genus *Enteromorpha* to the genus *Ulva* (Hayden et al., 2003).

Genetic variation

Data in Table 2 showed a total of amplified fragments 31, 38, 38 and 31 over all the 14 random primers using the ran-

Table 2. Amplified, absent, polymorphic and common bands over all random primers *Ulva* species

Species	No of amplified bands	No of absent bands	No of polymorphic bands	% of common bands	% of polymorphic bands
U. compressa	38	13	28	26.32	73.68
U. fasciata	31	20	21	32.25	67.74
U. lactuca	38	13	28	26.32	73.68
U. linzea	31	20	21	32.25	67.74

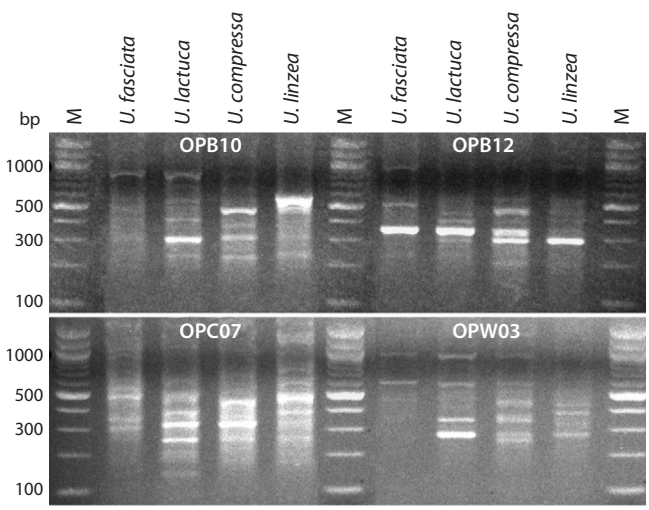

Fig. 6. RAPD polymorphism in four *Ulva* species.
Primer No. 11 (5′-CTGCTGGGAC), Primer No. 12 (5′-CCTTGACGCA), Primer No. 13 (5′-GTCCCGACGA) and Primer No. 14 (5′-GTCCGGAGTG).

dom amplified polymorphic DNA technique for *U. fasciata*, *U. lactuca*, *U. compressa* and *U. linzea*, respectively. The ratio of the common fragments was 32.25 % for each of *U. fasciata* and *U. linzea* and 26.32 % for each of *U. lactuca* and *U. compressa*.

It is worthy to mention that each of the genomic DNA of *U. lactuca* and *U. compressa* had thirty-eight (38) amplified fragments over all used primers of them twenty-eight (28) polymorphic fragments with a ratio of 73.68 % for each of the two species.

Meanwhile, each of the genomic DNA of *U. fasciata* and *U. linzea* had thirty-one (31) amplified fragments as present bands of them twenty-one (21) polymorphic fragments with a ratio of 67.74 % for each of the two species.

Data in Table 3, revealed that the genetic similarity between *U. lactuca* and *U. compressa* had the score of 0.79, meanwhile it had the score of 0.55 between *U. fasciata* and *U. linzea*. These results clearly indicated that the species *U. lactuca* is the closest one to species *U. compressa* and the species *U. linzea* was the most divergent from species *U. fasciata.* Moreover, the phylogenetic relationship indicated by the dendrogram in Fig. 6 and confirmed the aforementioned results obtained from Table 3.

The dendrogram (Fig. 7) showed that the most closely species are *U. lactuca* and *U. compressa* (Guidone et al., 2013). However, *U. fasciata* (Pasad et al., 2009) is showed to be far from both *U. lactuca* and *U. compressa*. Meanwhile, *U. linzea* showed to be a unique species, suggesting that it is quite different than the other three species at the level of RAPD-PCR primers used in this study. Moreover, these clustering dendrogram suggests that using more primers could be beneficial to confirm these results.

Biochemical characteristics of *Ulva* spp.

The biochemical composition (moisture content, ash, protein, carbohydrate and lipid) of *Ulva* spp. is illustrated in Table 4. No significant differences were noticed in moisture content in all tested *Ulva* spp. which ranged between 82.142 to 85.545 % DW. Our results are in agreement with Moustafa and Saeed (2014) who stated that the moisture content of macroalgae within the range 83.67–86.98 % DW.

Ash value was highest in *U. fasciata* (22.987 % DW) followed by *U. lactuca* (21.345 % DW) then *U. linzea* (20.897 % DW) and *U. compressa* (20.135 % DW). The present study showed no significant differences in the ash content between *Ulva* spp. and ranged between 20.135 to 22.987 % DW. Our results are consistent with the observations reported by Wong and Cheung (2001) who stated that the ash content of *U. lactuca* range between 21.3–24.6 % DW and 25.4 % DW for *U. fasciata* (McDermid, Stuercke, 2003). Khairy, El-Shafay (2013) recorded that the ash content of *U. lactuca* which range between 17.6–23.4 % DW in spring which the same season of collected samples.

The protein content of *Ulva* spp. had significant difference among *Ulva* species. The protein content of *U. compressa* (22.056 % DW) was higher than other species while the protein content of *U. fasciata* (13.091 % DW) was the lowest one. This observation is similar to that observed Dhargalkar et al. (1980) who detected that protein content varied among different genera and also in different species of the same genus, Fleurence (1999) reported that the protein content of *Ulva* spp. range of 10–26 %, and Kokilam, Vasuki (2014) stated that *Ulva* sp. had protein content within the range of 10–20 % (DW).

The level of carbohydrate was very high compared to protein and lipid. There are significant variations in carbohydrate between *Ulva* species. The carbohydrate content of *U. fasciata* was richer than other *Ulva* spp. (46.670 % DW), while the lowest carbohydrate content was recorded in *U. linzea* (33.221 % DW). These results were in conformity with Khairy and El-Shafay (2013) who recorded that the carbohydrate content of *U. lactuca* ranged from 42.1 to 46.5 % DW and Moustafa and Saeed (2014) detected that the carbohydrate content in *Ulva* sp. ranged between 49.21 to 51.37 % DW in spring.

In comparison to protein and carbohydrate, lipid exhibited no significant different and low proportion in *Ulva* spp. The

Table 3. Genetic similarity matrix among four *Ulva* spp. based on polymorphic RAPD band

Species	*U. compressa*	*U. fasciata*	*U. lactuca*	*U. linzea*
U. compressa	1	0.64	0.79	0.64
U. fasciata	0.64	1	0.67	0.55
U. lactuca	0.79	0.67	1	0.67
U. linzea	0.64	0.55	0.67	1

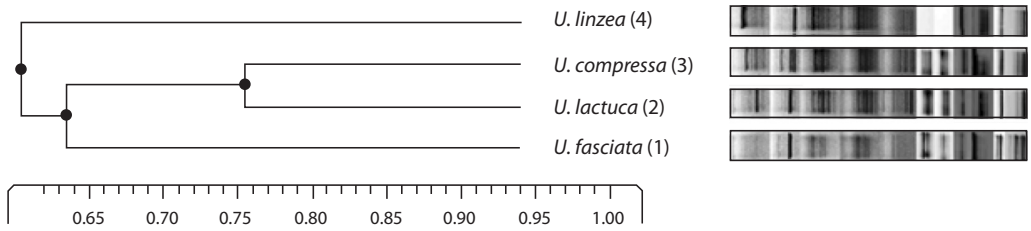

Fig. 7. RAPD cluster analysis of four *Ulva* species depending upon simple matching coefficient and using the UPGMA clustering method.

Table 4. The mean (± SD) contents of moisture, ash, protein, carbohydrate and lipid (% dry weight) in the different *Ulva* spp.

% DW	Moisture	Ash	Protein	Carbohydrate	Lipid
U. compressa	83.027 ± 1.12	20.135 ± 0.21	22.056 ± 1.01	29.572 ± 2.22	2.646 ± 0.66
U. fasciata	83.358 ± 1.03	22.987 ± 0.89	13.091 ± 0.89	46.670 ± 2.25	4.395 ± 0.85
U. lactuca	85.545 ± 0.87	21.345 ± 0.71	16.416 ± 0.08	39.733 ± 1.23	3.875 ± 0.13
U. linzea	82.142 ± 0.65	20.897 ± 0.14	15.728 ± 0.45	33.221 ± 0.34	3.476 ± 0.03
F-value	128.774	131.252	803.971	48.981	10.168
P-value	0.0001	0.0001	0.0001	0.0001	0.0001

highest concentration of lipid was recorded in *U. fasciata* and the lowest concentration was observed in *U. compressa*. These results were in consistence with those of Dawes (1998) who stated that seaweeds exhibit low lipid contents. McDermid, Stuercke (2003) stated that the most macroalgae contained lipid less than 4 % DW.

As shown in Figure 8, the pigment contents were significantly different between *Ulva* spp. The highest chlorophyll *a* was registered in *U. linzea* (1.662 mg g^{-1} FW) followed by *U. compressa* (1.522 mg g^{-1} FW), then *U. fasciata* (1.154 mg g^{-1} FW), and *U. lactuca* (0.667 mg g^{-1} FW). Chlorophyll *b* was observed to be the richest *U. linzea* (1.090 mg g^{-1} FW), while the lowest chlorophyll *b* was noted in *U. lactuca* (0.224 mg g^{-1} FW). The greater total chlorophyll value was observed in *U. linzea* (2.792 mg g^{-1} FW) followed by *U. compressa* (2.578 mg g^{-1} FW). The minimum value was noted in *U. lactuca* (1.023 mg g^{-1} FW). On the other hand, the maximum carotenoids was noted in *U. compressa* (0.507 mg g^{-1} FW) followed by *U. linzea* (0.321 mg g^{-1} FW). The lowest ratio was recorded in *U. lactuca* (0.24 mg g^{-1} FW). Similar findings and trends were also reported by Dere et al. (2003) who detected that the pigment contents varied significantly with respect to the algal taxa, stations and depth. Moustafa, Saeed (2014) detected the total pigment of *Ulva* spp. ranged between 2.52–5.22 mg/g DW.

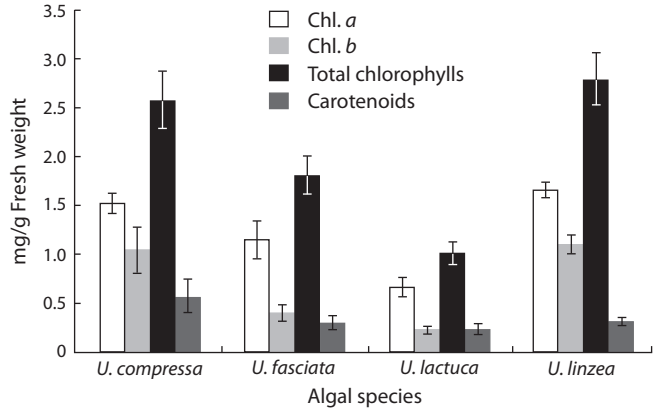

Fig. 8. Variation in photosynthetic content of the collected *Ulva* spp. (mg/g FW).

The reliable taxonomic identification of *Ulva* species is unlikely to be obtained on the basis of morphology alone (Malta et al., 1999). However, the traditional generic names represent a useful morphological distinction between the two-thallus types and, for the purposes of this research; the genera will be referred to as separate for the sake of clarity and continuity with previous authors.

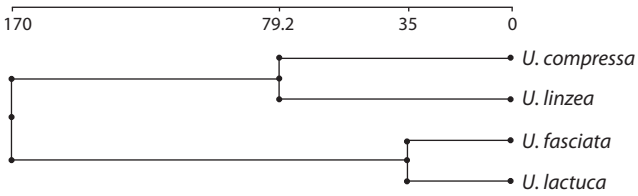

Fig. 9. The relationships of four *Ulva* species based on variation in biochemical composition expressed as Euclidean distance ward tree using CAP software.

The CAP program was used to measure the relationships between biochemical composition of *Ulva* spp. based on similarity estimates using ward tree building methods that indicate the species in this tree are divided into two major groups (*U. compressa* and *U. linzea*) and (*U. fasciata* and *U. lactuca*) which are closely related to each other (Fig. 9), respectively. Biochemical analysis of the collected *Ulva* spp. confirmed there are similarity between *U. lactuca* and *U. fasciata* and on the other hand, between *U. linzea* and *U. compressa*.

The reliable taxonomic identification of *Ulva* species is unlikely to be obtained on the basis of morphology alone (Malta et al., 1999).

Conclusion

The present investigation represented initial steps in creating a reliable database on morphological, genetic and biochemical variation between *Ulva* spp. collected from Eastern Harbor, Alexandria, Egypt. Taxonomical study of seaweeds should be not depend on morphological description and biochemical composition only because were not enough for differentiation between seaweeds especially *Ulva* species. These characters more affected by age, seasonal changes so it should be used genetic methods which are more specialization and accuracy, while RAPD-PCR is useful in differentiation between *Ulva* species. It may also be useful in detecting relationship between species within a genus. Future work in the seaweeds differentiation and classification should be performed by modern genetic technology to study the variation between different species.

Conflict of interest

The authors declare no conflict of interest.

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