

Morphological and molecular analyses of *Ulva* and *Enteromorpha* (Chlorophyta)

Satoshi Shimada¹⁾, Masanori Hiraoka²⁾ and Masahiro Notoya³⁾

¹⁾Creative Research Initiative "Sousei", Hokkaido University
Sapporo, 060-0810, Japan

E-mail : sshimada@sci.hokudai.ac.jp

²⁾Uda Marine Biological Institute, Kochi University
194, Inoshiri, Usa, Tosa, Kochi 781-1164, Japan

³⁾Faculty of Marine Science, Tokyo University of Marine Science & Technology
4-5-7 Minami,Minato-ku,Tokyo, 108-8477, Japan

Abstract *Ulva* and *Enteromorpha* are two of the best known green macroalgal genera, but recent molecular studies have revealed that both are closely related. Further, the taxonomy in this complex is confused due to a lack of stable morphological characters. In order to resolve phylogenetic relationships among the species of this complex from Japan, we have determined the nuclear encoded ITS (internal transcribed spacer) region including 5.8S gene sequences for 28 specimens. Our molecular data indicate that *Ulva* and *Enteromorpha* are not monophyletic but rather paraphyletic within a large clade. Thus, we propose to return the following six *Enteromorpha* species to *Ulva*: *E. compressa* (Linnaeus) Nees, *E. flexuosa* (Wulfen) J. Agardh, *E. intestinalis* (Linnaeus) Nees, *E. linza* (Linnaeus) J. Agardh, *E. muscoides* (Clemente) Cremades and *E. prolifera* (Müller) J. Agardh, all of which were originally described as members of *Ulva*. The ITS tree also indicates that free-floating *Ulva* samples are divided into five different lineages: *Ulva lactuca* Linnaeus, *U. pertusa* Kjellman, *U. armoricana* Dion *et al.*, *U. fasciata* Delile and *Ulva* sp. These five species are distinguished by cell morphology including the arrangement, the shape and size of cells.

Key words: Chlorophyta, *Enteromorpha*, ITS, molecular phylogeny, morphology, *Ulva*, Ulvales, Ulvophyceae

Introduction

The taxonomy of the green macroalgal genera *Ulva* and *Enteromorpha* is still problematic due to lack of stable morphological characters for their species discrimination. A number of researchers have questioned the validity of many morphological features which have been traditionally used to distinguish species of *Ulva* and *Enteromorpha*, depending on species. For example, gross morphology varies with wave action (Mshigeni and Kajumulo, 1979), branching is affected by salinity (Blomster *et al.*, 1999), thallus thickness varies through the season and is thus probably related to thallus age (Bliding, 1968; Phillips, 1988). Cell size is known to vary under different salinity (Koeman and van den Hoek, 1981), the shape of basal cells is variable with the age, the ploidy and/or the sex of individual plants (Coat *et al.*, 1998). The presence or absence of the marginal teeth is influenced

by wave action (Phillips, 1988), and the pyrenoid number is also thought to be variable under the nutritional conditions, as also for microscopic green algae (Kapraun, 1970; Tanner, 1980; Phillips, 1988; Malta *et al.*, 1999).

The green tide phenomenon may occur under conditions when extensive biomass of free-floating green algae accumulates on the shallow beaches of calm bays. Especially, free-floating *Ulva* thalli cause such green tides at several locations around the world (Fletcher, 1996; Malta *et al.*, 1999). In Japan, green tides have been observed, for example, at Hiroshima Bay (Hiroshima Pref.), Hakata Bay (Fukuoka Pref.), Uranoichi Bay (Kochi Pref.), Mikawa Bay (Aichi Pref.), Kanazawa Bay (Kanagawa Pref.) and Nemuro Bay (Hokkaido). Decayed free-floating *Ulva* thalli have created environmental problems such as killing shellfish and producing offensive smells (Kudo and Notoya, 1999).

Okamura (1921) first reported a free-floating

population of *U. pertusa* Kjellman in Japan. Arasaki (1984) provisionally reported a new species of free-floating *Ulva*, *U. leptophylla* Arasaki (invalidly published) from Japan. He thought that Japanese free-floating *Ulva* populations include *U. fasciata* Delile, *U. leptophylla* and *U. pertusa* (Arasaki, 1984). Recently, Ohno (1999) reported that free-floating *Ulva* populations found in Japanese waters included two species, *U. lactuca* Linnaeus and *U. pertusa*; however, he pointed out that a further comparative study with European species was needed for their precise identification.

Recent molecular studies of the *Ulva* and *Enteromorpha* complex show that they can provide clues to the phylogeny of this complex, and that the sequences of the nuclear encoded internal transcribed spacer (ITS) including 5.8S gene have been used for species identification (Tan *et al.*, 1999; Blomster, 2000). Molecular studies also can evaluate which morphological characters can be used for species discrimination (Blomster *et al.*, 1998, 1999; Coat *et al.*, 1998; Malta *et al.*, 1999). However, the Japanese *Ulva* and *Enteromorpha* have not been included in these molecular studies.

To clarify the generic status of *Ulva* and *Enteromorpha*, and to elucidate the species composition of free-floating *Ulva* in Japan, we determined sequences of the ITS region including the 5.8S gene of *Ulva* from several countries, including Japanese free-floating *Ulva*.

Materials and Methods

Sampling, DNA extraction and sequencing

Thalli of *Ulva* were collected from various localities of Japan, Philippines and some European countries (Table 1). Free-floating thalli are indicated as "free-floating" in Table 1. Unialgal cultures were established using the punching method (Hiraoka and Enomoto 1998). Total DNA was extracted from these unialgal cultures or directly from field-collected plants. Voucher specimens are deposited in the Herbarium of Graduate School of Science, Hokkaido University, Sapporo (SAP) (Table 1).

Methods for total DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of the nuclear encoded ITS were as described by Shimada *et al.* (1999). We used 2 pairs of primers: ITS1 (5'-TTTGTACACACCGCCG-3')-ITS2 (5'-GAATTCTGCAATTCA-

CA-3') and ITS3 (5'-CTCTCAACAACGGAT-ATCT-3')-ITS4 (5'-TCCTCCGCTTATTGAT-ATGC-3') (Coat *et al.*, 1998, Malta *et al.*, 1999). Based on the direct sequencing of the PCR-amplified fragments, we could not determine the ITS1 sequence of #1 *U. fasciata* and #17 *U. reticulata* Forsskål, due to overlap two different signals. Thus, we determined their ITS1 sequences using sub-cloning methods using the TOPO TA Cloning Kit (Invitrogen Corp., La Jolla, California) following the manufacturer's instructions, and plasmid DNA from each recombinant colony was used for the template of direct colony PCR. Ten clones obtained from each sample were sequenced as mentioned above.

Phylogenetic analysis

We have sequenced the ITS region from 28 samples, representing 15 species of Asian and European *Ulva* (Table 1). In addition, 13 ITS sequences were downloaded from GenBank and included in the alignment (Table 1). Representative ITS sequences were selected from multiple sequences within individuals of #1 and #17, and included in the analysis. The ITS sequences (629 bp) were aligned by eye with regard to their secondary structure using the mFOLD program (Zuker, 1989). The alignment is available from the first author upon request. *Percursaria percursa* (C. Agardh) Rosenvinge and *Ulvaria fusca* Ruprecht were designated as the outgroups in the present ITS analyses, because these two species represent a sister group to a monophyletic group composed of *Ulva* and *Enteromorpha* in the *rbcL* gene tree including sequences of different order, *Codium fragile* (Suringar) Hariot (M67453) and *Bryopsis maxima* Okamura (X55877) (not shown).

The maximum parsimony (MP) method was used to construct phylogenetic trees. Regions (52 bp-74 bp, 119 bp-140 bp and 432 bp-448 bp of 629 base pairs of the alignment obtained) that had any possible ambiguity in the ITS alignment were excluded. Gaps in the ITS alignment were treated as missing data. MP analysis of ITS sequences was performed PAUP 4.0b10 (Swofford, 2002). All sites were treated as unordered and equally weighted. Heuristic search option with random addition of sequences (10 replicates) and tree-bisection-reconnection branch swapping algorithm (TBR) were used

Table 1. List of species used in molecular phylogenetic analysis and GenBank accession number.

Species	Sample number	Collection (date and voucher specimen number or reference)	Accession number ITS
<i>Percursaria percursa</i>			AY016305
<i>Ulvaria fusca</i>	# C136	Oshoro, Hokkaido, Japan (4 Jun. 2001; SAP095049)	AB097637
<i>Umbraulva japonica</i>	# C14	Shimoda, Shizuoka Prefecture, Japan (13 Mar. 2000; SAP095050)	AB097638
<i>Umbraulva japonica</i>	# C87	Tateyama, ?hiba Prefecture, Japan (14 Sep. 2000; SAP095051)	AB097639
<i>Umbraulva amamiensis</i>	# C100	Kaifu, Tokushima Prefecture, Japan (1 Jun. 2000; SAP095052)	AB097640
<i>Umbraulva olivascens</i>		Carna, County, Galway, Ireland (Tan et al. 1999)	AJ234322
<i>Enteromorpha compressa</i>		Portaferry, Strangford Lough, Northern Ireland (Tan et al. 1999)	AJ234302
<i>Enteromorpha intestinalis</i>	# C161	Karlskrona, Sweden (3 Oct. 2001; SAP095055)	AB097643
<i>Enteromorpha cylindracea</i>		Scotland (Blomster 2000)	AJ234308
<i>Enteromorpha flexuosa</i>	# C47	Oshoro, Hokkaido, Japan (25 Jun. 2000; SAP095056)	AB097644
<i>Enteromorpha prolifera</i>		Ythan, Estuary, Aberdeenshire, Scotland (Tan et al. 1999)	AJ234304
<i>Enteromorpha procera</i>		Portaferry, Strangford Lough, Northern Ireland (Blomster et al. 2000)	AF185939
<i>Enteromorpha linza</i>	# E4	Yoshino Riv., Tokushima Prefecture, Japan (12 Mar. 2000; SAP095061)	AB097649
<i>Enteromorpha muscoides</i>		Backstrand, Tramore, Ireland (Tan et al. 1999)	AJ234307
<i>Ulva arasakii</i>	# 6	Shizugawa, Miyagi Prefecture, Japan (11 May 1999; SAP095062)	AB097650
<i>Ulva californica</i>		Otter, Crest, Oregon, U.S.A. (Tan et al. 1999)	AJ234315
<i>Ulva lactuca</i>		Sweden (Tan et al. 1999)	AJ234311
<i>Ulva lactuca</i> free-floating	# C207	Nemuro, Hokkaido, Japan (19 Apr. 2002; SAP095063)	AB097651
<i>Ulva lactuca</i>	# C208	Nemuro, Hokkaido, Japan (19 Apr. 2002; SAP095064)	AB097652
<i>Ulva pertusa</i>	# 12	Usa, Kochi Prefecture, Japan (1 May 1997; SAP095065)	AB097653
<i>Ulva pertusa</i> free-floating	# 25	Hakata, Fukuoka Prefecture, Japan (28 Sep. 1999; SAP095066)	AB097654
<i>Ulva pertusa</i>	# C46	Oshoro, Hokkaido, Japan (25 Jun. 2000; SAP095067)	AB097655
<i>Ulva pertusa</i> free-floating	# 35	Miyajima, Hiroshima Prefecture, Japan (28 Aug. 1999; SAP095068)	AB097656
<i>Ulva pertusa</i> free-floating	# 36	Yokohama, Kanagawa Prefecture, Japan (14 Jun. 1998; SAP095069)	AB097657
<i>Ulva pertusa</i> free-floating	# 38	Mikawa, Aichi Prefecture, Japan (17 Jun. 1998; SAP095070)	AB097658
<i>Ulva pseudocurvata</i>		Ythan, Estuary, Aberdeenshire, Scotland (Tan et al. 1999)	AJ234312
<i>Ulva fenestrata</i>		North Boardman St. Park, Oregon, U.S.A. (Tan et al. 1999)	AJ234316
<i>Ulva taeniata</i>		Seal Rock, Oregon, U.S.A. (Tan et al. 1999)	AJ234320
<i>Ulva scandinavica</i>	# 19	Wadden Sea, Netherlands (6 Oct. 1998; SAP095071)	AB097659
<i>Ulva rigida</i>		Skara Brae, Orkney, Scotland (Tan et al. 1999)	AJ234319
<i>Ulva armoricana</i>	# C105	Brittany, France (11 Oct. 1998; SAP095072)	AB097660
<i>Ulva armoricana</i> free-floating	# 33	Miyajima, Hiroshima Prefecture, Japan (10 Jul. 1999; SAP095073)	AB097661
<i>Ulva armoricana</i> free-floating	# 37	Yokohama, Kanagawa Prefecture, Japan (14 Jun. 1998; SAP095074)	AB097662
<i>Ulva fasciata</i>	# 1	Usa, Kochi Prefecture, Japan (11 May 1999; SAP095075)	AB097663
<i>Ulva fasciata</i> free-floating	# 11	Miyajima, Hiroshima Prefecture, Japan (1 Jul. 1999; SAP095076)	AB097664
<i>Ulva reticulata</i>	# 17	Cebu Island, Philippines (16 Apr. 1998; SAP095077)	AB097665
<i>Ulva spinulosa</i>	# 54	Fubenhama, Kochi, Japan (29 Apr. 1999; SAP095078)	AB097666
<i>Ulva</i> sp.	# 21	Naha, Okinawa Prefecture, Japan (10 Dec. 1998)	not applicable
<i>Ulva</i> sp. free-floating	# 22	Usa, Kochi Prefecture, Japan (19 Sep. 1997)	not applicable
<i>Ulva</i> sp. free-floating	# 23	Hakata, Fukuoka Prefecture, Japan (14 Sep. 1999)	not applicable
<i>Ulva</i> sp. free-floating	# C178	Yokohama, Kawagawa Prefecture, Japan (18 Nov. 2001)	not applicable

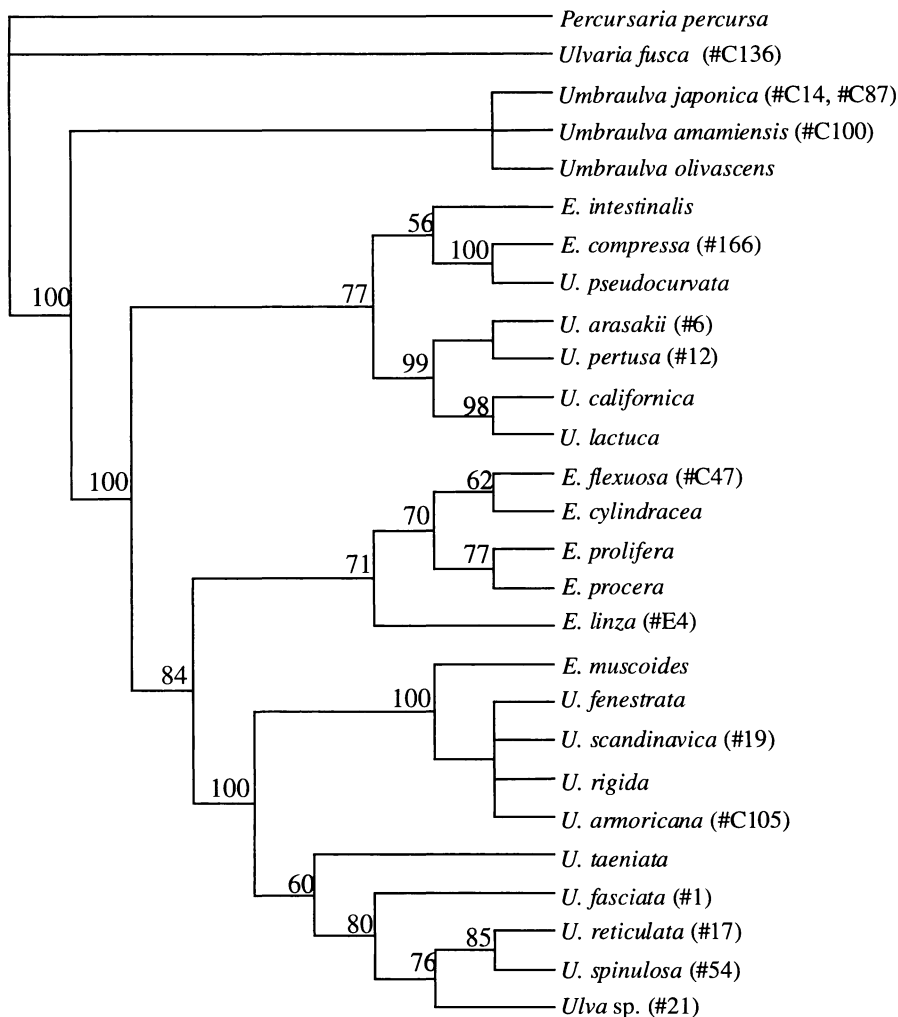


Fig. 1. Strict consensus tree of 12 most parsimonious trees constructed from an analysis of ITS sequences (including the 5.8S gene) from *Ulva* (*U.*) and *Enteromorpha* (*E.*). The tree was rooted with *Percursaria percura*. The numbers under the branches represent full heuristic bootstrap values (1000 replicates) greater than 50%. The sample numbers correspond to those in Table 1.

for tree searching. Bootstrap analysis based on 1000 re-samplings (simple addition) of the data set (Felsenstein, 1985) was calculated to evaluate statistical reliability.

Results and Discussion

Phylogeny of the *Ulva* and *Enteromorpha*

The strict consensus tree of ITS sequences is presented in Fig. 1 (12 equally parsimonious trees, 531 steps, CI = 0.659, RI = 0.783, RC = 0.516, HI = 0.341). All species of *Ulva* and *Enteromorpha* examined were shown to constitute a large monophyletic clade with 100% bootstrap value in Mp analysis (Fig. 1). However, the

genera *Ulva* and *Enteromorpha* did not constitute respective monophyletic clades. The generitype of *Ulva*, *U. lactuca*, was clustered with *U. arasaki* Chihara, *U. californica* Wille and *U. pertusa* with 99% bootstrap value (*U. lactuca* clade). The generitype of *Enteromorpha*, *E. intestinalis* (Linnaeus) Nees, was clustered with *E. compressa* (Linnaeus) Nees and *U. pseudocurvata* Koeman et van den Hoek with 56% bootstrap value, and these two species were clustered with *U. lactuca* clade with 77% bootstrap value in MP analysis. Five other species of *Enteromorpha*, *E. cylindracea* [provisional name given by Blomster (2000)], *E. flexuosa*, *E.*

linza (Linnaeus) J. Agardh, *E. procera* Ahlner and *E. prolifera* (Müller) J. Agardh were shown to be monophyletic with 71% bootstrap value (Fig. 1). *Enteromorpha muscoides* Dangeard was clustered with several *Ulva* species, *U. armoricana* Dion *et al.*, *U. fasciata*, *U. fenestrata* Postels et Ruprecht, *U. reticulata*, *U. rigida* C. Agardh, *U. scandinavica* Bliding, *U. spinulosa* Okamura et Segawa, *U. taeniata* (Setchell) Setchell et Gardner and *Ulva* sp. with 100% bootstrap value in MP analysis.

Only a single morphological feature has distinguished *Ulva* and *Enteromorpha*: *Ulva* has a distromatic flat blade, whereas *Enteromorpha* has a monostromatic tubular thallus (van den Hoek *et al.* 1995). However, previous molecular studies using ITS sequences indicate that the distinction between these two genera is no longer valid (Tan *et al.*, 1999; Malta *et al.*, 1999; Blomster, 2000). Our phylogenetic tree of ITS sequences including Japanese species shows that *Ulva* and *Enteromorpha* are not monophyletic, respectively, within a large monophyletic *Ulva-Enteromorpha* clade. Although the generitypes of *Ulva* and *Enteromorpha* belong to separate clades within the large *Ulva-Enteromorpha* complex clade, the majority of these subclades are a mixture of both genera (Fig. 1). This result indicates that all species of the *Ulva-Enteromorpha* complex clade should be assigned to a single genus. This view is also supported by the presence of some species with intermediate morphology, such as *U. curvata* (Kützinger) De Toni having a central cavity in the basal region (Koeman and van den Hoek, 1981) and *E. linza* having a distromatic flat blade except for marginal and basal regions which show a monostromatic tubular structure (Bliding, 1963). Furthermore, Gayral (1959) notes that *U. linearis* Dangeard produces tubular thalli in one generation and distromatic in another. As the genus *Ulva* (Linnaeus, 1753) has nomenclatural priority over *Enteromorpha* (Link in Nees, 1820), the species of *Enteromorpha* should be transferred to *Ulva*.

The genus *Umbraulva*, although closely related to *Ulva* and *Enteromorpha*, can be distinguished by the possession of the photosynthetic pigments siphonaxanthin, which is absent in *Ulva* and *Enteromorpha* (Bae and Lee, 2001). The pattern of thallus development distinguishes *Ulvaria* and *Chloropelta* Tanner (thallus split in early development) from *Ulva* and *Enteromorpha*

pha (thallus not split) (Tanner, 1980). The pattern of germination of the erect thallus can be used to distinguish *Kornmannia* Bliding and *Blidingia* Kylin (developed from discoid growth) and *Ulva* and *Enteromorpha* (directly developed) (Hori, 1994). The fact that *Ulva* and *Enteromorpha* share these characters also provide further support that they are congeneric.

We referred to the *Enteromorpha* species in Algal database (Guiry and Dhonncha, 2002) and the private taxonomic cards made by Dr. T. Yoshida, Emeritus Professor of Hokkaido University. Of the 136 species to have been described as *Enteromorpha* to date, 53 species have been transferred to *Ulva*, *Capsosiphon*, *Ulvoopsis*, *Blidingia* or reduced to synonyms of other species of *Enteromorpha*, and thus 83 species remain in *Enteromorpha*. We included only eight *Enteromorpha* species in this molecular phylogenetic analysis. There is a strong possibility that hitherto circumscribed *Enteromorpha* may include some species that should be transferred to other genera, not only the genus *Ulva* but also *Capsosiphon* or *Blidingia*. Thus, we have transferred *Enteromorpha* species to *Ulva* for which phylogenetic positions have already been determined by molecular phylogenetic analyses. *Enteromorpha compressa* (Linnaeus) Nees [basionym: *Ulva compressa* Linnaeus (1753)], *Enteromorpha flexuosa* (Wulfen) J. Agardh [basionym: *Ulva flexuosa* Wulfen (1803)], *Enteromorpha intestinalis* (Linnaeus) Nees [basionym: *Ulva intestinalis* Linnaeus (1753)], *Enteromorpha linza* (Linnaeus) J. Agardh [basionym: *Ulva linza* Linnaeus (1753)], *Enteromorpha muscoides* (Clemente) Cremades [basionym: *Ulva muscoides* Clemente (1807)] and *Enteromorpha prolifera* (Müller) J. Agardh [basionym: *Ulva prolifera* Müller (1778)] were originally described as species of *Ulva* so that no proposals are necessary. We have left *E. procera* and *E. cylindracea* in the genus *Enteromorpha*: *E. procera* is probably a synonym of *E. prolifera* and *E. cylindracea* is a provisional name by Blomster (2000).

Free-floating *Ulva*

The strict consensus tree of ITS sequences including the samples of free-floating *Ulva* is presented in Fig. 2 (105 equally parsimonious trees, 609 steps, CI = 0.677, RI = 0.817, RC = 0.553, HI = 0.323). The eleven samples of free-

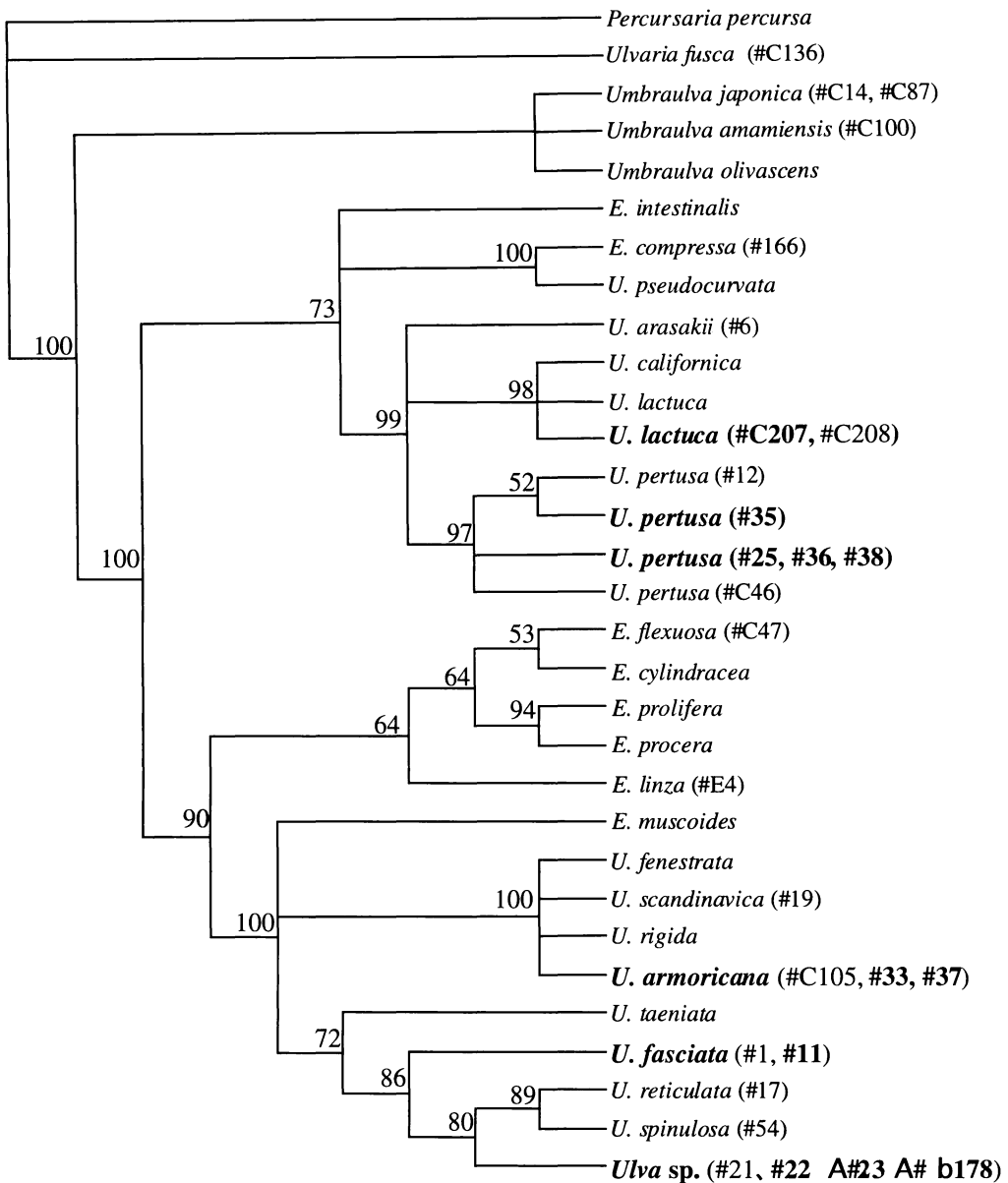


Fig. 2. Strict consensus tree of 105 most parsimonious trees constructed from an analysis of ITS sequences (including the 5.8S gene) from *Ulva* (*U.*) and *Enteromorpha* (*E.*), including free-floating *Ulva*. The tree was rooted with *Percursaria percura*. The numbers under the branches represent full heuristic bootstrap values (1000 replicates) greater than 50%. The sample numbers correspond to those in Table 1. Boldfaces of the species and sample number indicate free-floating *Ulva*.

floating *Ulva* collected from six localities, were divided into five phylogenetic lineages. They were clustered with attached samples of an *Ulva lactuca-californica* complex, *U. pertusa*, *U. armoricana*, *U. fasciata* and *Ulva* sp., respectively (Fig. 2).

Our attached and free-floating materials

clustered with the *U. lactuca-californica* complex are more than 50 cm in length and 30 cm in width, and possess morphological similarities with previous reports of *U. lactuca* (Bliding, 1968; Koeman and van den Hoek, 1981), i.e. in having regularly arranged rectangular small cells with one to two pyrenoids (Fig. 3). In con-

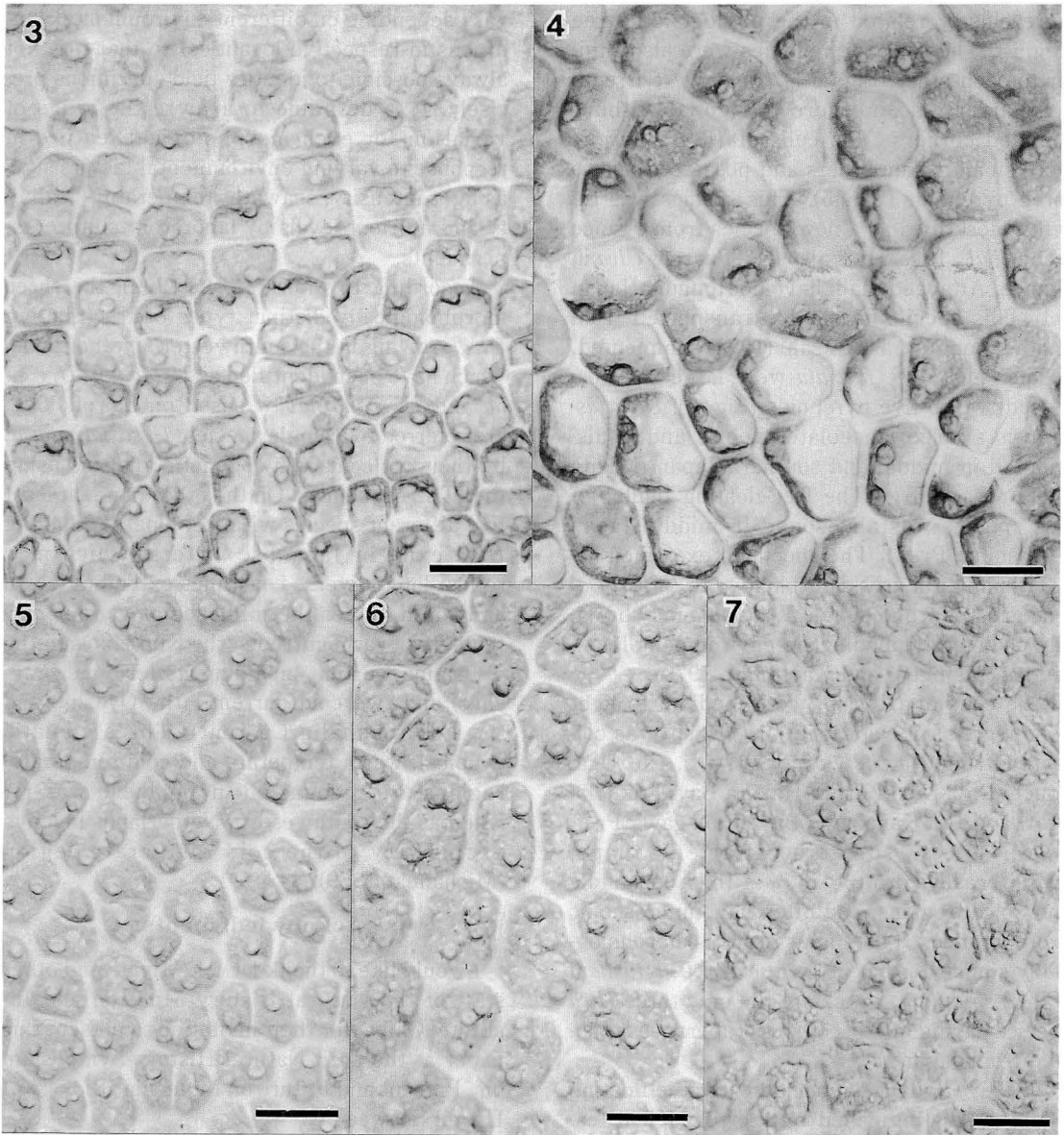


Fig. 3–7. Surface views of 2-week-old cultured thalli of five types of free-floating *Ulva* grown under the same condition: in PES medium (Provasoli, 1968) at 20°C, 16 : 8 h L : D (light : dark) on a rotary shaker at 40 rpm (Taiyo R-II, Taiyo Corp., Tokyo) with the photon flux of 15–25 $\mu\text{Em}^{-2}\text{s}^{-1}$ for 2 weeks.

Fig. 3. *Ulva lactuca* (#207). Fig. 4. *Ulva pertusa* (#35). Fig. 5. *Ulva armoricana* (#33). Fig. 6. *Ulva fasciata* (#11). Fig. 7. *Ulva* sp. (#22).

trast, blades of *U. californica* are small, mostly less than 2 cm (Abbott and Hollenberg, 1976). Thus, we identified this *Ulva* as *U. lactuca*. As Bliding (1968) mentioned that *U. lactuca* had a northerly distribution in Europe, this species grows in a northern, cold region (Nemuro) in Japan.

Ulva pertusa is the most common species in

Japan, and can be characterized by possessing larger and rounded cells and restricted chloroplasts to one side of each cell (Fig. 4) (Yoshida, 1998). Free-floating *U. pertusa* is also widely distributed around Japan (Fukuoka Pref., Hiroshima Pref., Aichi Pref. and Kanagawa Pref.).

Ulva armoricana, *Ulva fasciata* and *Ulva* sp.

were closely related in the phylogenetic tree (Fig. 2). The cell morphologies were also similar to each other (Figs 5-7). They were irregularly arranged and were polygonal without rounded corners. The cells of *U. armoricana* were smaller than others and possessed one to three pyrenoids (Fig. 5). This species was recently described as a new species from France (Dion *et al.*, 1998). It is also free-floating *Ulva* in France. Attached thalli have not found in Japan yet. This species might be transported from another country, as Arasaki (1984) commented.

The cells of *U. fasciata* were large and possessed two to five pyrenoids (Fig. 6). *Ulva fasciata* is a large lanceolate species and is distributed in middle and southern Japan (Migita and Fujita, 1987). The free-floating thallus of this species was also found in middle Japan (Hiroshima Pref.). The specimen examined (# 11) is similar in gross morphology to *U. pertusa*. The gross morphology of *U. fasciata* may be easily changed depending on environmental conditions (Mshigeni and Kajumulo, 1979).

The cells of *Ulva* sp. were medium to light yellow green in color (Fig. 7). *Ulva* sp. was collected from Naha, Okinawa Pref., the Uranoichi Bay, Kochi Pref., Hakata Bay, Fukuoka Pref. and the Kanazawa Bay, Kanagawa Pref. Thalli of *Ulva* sp. were variable in shape, fragile, easily torn, 30-55 μ m thick in middle and apical regions, and often have microscopic protuberances. The thallus tissue is morphologically similar to that of *U. fasciata*, *U. reticulata* and *U. spinulosa*, which are the only three *Ulva* species having microscopic protuberances reported from Japan.

The five Japanese species with free-floating thalli are distinguished by morphological features including the arrangement of cells, the shape and size of the cells and the position of chloroplast (Figs 3-7), features which are congruent with previous observations of their attached thalli (Bliding, 1968; Koeman and van den Hoek, 1981; Coat *et al.*, 1998; Yoshida, 1998). This result indicates that each species might possess characteristic morphological features. However, as many researchers have commented (Bliding, 1968; Kapraun, 1970; Mshigeni and Kajumulo, 1979; Tanner, 1980; Koeman and van den Hoek, 1981; Phillips, 1988; Coat *et al.*, 1998; Blomster *et al.*, 1999; Malta *et al.*, 1999), almost all morphological features may

vary depending on different environmental conditions in respective localities, so that it is not always possible to identify field specimens precisely. It is desirable to analyze the range of variation for each morphological feature in response to varying environmental factors such as light intensity, seawater temperature, salinity and nutrient levels by laboratory-culture experiments.

According to Malta *et al.* (1999), the three morphologically distinct types of free-floating *Ulva* growing in the southwestern Netherlands, which have been identified as *U. lactuca*, *U. rigida* and *U. scandinavica* based on morphology, are referred to a single species, *U. scandinavica*, by their molecular phylogenetic analysis using the ITS2 sequences. On the other hand, at least five different species with free-floating thalli of *Ulva* exist in Japanese waters, which may reflect long, diverse coastlines extending north and south, under the influence of various currents, two warm currents (the Kuroshio and the Tsushima Current including its terminal branches) and two cold currents (the Oyashio and the East Sakhalin Current). Three climatic (tropical, warm temperate and cold temperate) regions are recognized in Japanese waters, whereas the southwestern Netherlands belongs exclusively to the cold temperate region (Michanek, 1979).

Acknowledgments

I would like to thank Dr. M. Miyata and other staff of the Natural History Museum and Institute, Chiba, for their invitation to present this paper at the 14th Natural History Symposium on "Present and Future on Molecular Biology of Useful Seaweeds".

References

- Abbott, I. A. and Hollenberg, G. J. 1976. Marine Algae of California. 827 pp. Stanford University Press, Stanford.
- Arasaki, S. 1984. A new aspect of *Ulva* vegetation along the Japanese coast. *Hydrobiologia* 116/117: 229-32.
- Bae, E. H. and Lee, I. K. 2001. *Umbraulva*, a new genus based on *Ulva japonica* (Holmes) Papenfuss (Ulvaaceae, Chlorophyta). *Algae* 16: 217-31.
- Bliding, C. 1963. A critical survey of European taxa in Ulvales. Part I. *Capsosiphon*, *Percursaria*, *Blidingia*, *Enteromorpha*. *Op. Bot. Soc. bot. Lund.* 8: 1-160.

- Bliding, C. 1968. A critical survey of European taxa in Ulvales. II. *Ulva*, *Ulvaria*, *Monostroma*, *Kornmannia*. Bot. Notiser. 121: 535-629.
- Blomster, J., Maggs, C. A. and Stanhope, M. J. 1998. Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. J. Phycol. 34: 319-40.
- Blomster, J., Maggs, C. A. and Stanhope, M. J. 1999. Extensive intraspecific morphological variation in *Enteromorpha muscoides* (Chlorophyta) revealed by molecular analysis. J. Phycol. 35: 575-86.
- Blomster, J. 2000. Molecular and morphological approaches to the evolutionary history of the *Enteromorpha-Ulva* species complex. W. & A. de Notbeck Foundation Sci. Rep. 20: 1-24.
- Bory, J. B. G. M. 1823. *Percursaria*, *Pilayella*. Dict. Class. Hist. Nat. 4: 393.
- Clemente y Rubio, S. de R. 1807. Ensayo sobre las variedades de la vid comun qua vegetan an Andalucia. 324 pp. Madrid.
- Coat, G., Dion, P., Noailles, M. C., De Reviers, B., Fontaine, J. M., Bergaer-Perrot, Y. and Loiseaux-De Goür, S. 1998. *Ulva armoricana* (Ulvales, Chlorophyta) from the coasts of Brittany (France). II. Nuclear rDNA ITS sequences analysis. Eur. J. Phycol. 33: 81-6.
- Dion, P., De Reviers, B and Coat, G. 1998. *Ulva armoricana* sp. nov. (Ulvales, Chlorophyta) from the coasts of Brittany (France). I. Morphological identification. Eur. J. Phycol. 33: 73-80.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution 39: 783-91.
- Fletcher, R. T. 1996. The occurrence of "green tide". In Schramm, W. and Nienhuis, P. H. (Eds), Marine Benthic Vegetation - Recent Changes and the Effects of Eutrophication. pp. 7-43. Springer Verlag, Berlin.
- Gayral, P. 1959. Premières observations et réflexions sur des Ulvacées en culture. Botaniste 43: 85-100.
- Guiry, M. D and Nic Dhonncha, E. 2002. AlgaeBase. World-wide Web electronic publication. [http://www.algaebase.com/default.html]
- Hiraoka, M. and Enomoto, S. 1998. The induction of reproductive cell formation of *Ulva pertusa* Kjellman (Ulvales, Ulvophyceae). Phycol. Res. 46: 199-203.
- Hori, T. 1994. An Illustrated Atlas the Life History of Algae. Vol. 1 Green Algae. 367 + 51 pp. Uchida Rokakuho, Tokyo.
- Kapraun, D. F. 1970. Field and cultural studies of *Ulva* and *Enteromorpha* in the vicinity of Port Aransas, Texas. Contrib. Mar. Sci. 15: 202-85.
- Koeman, R. P. T. and van den Hoek, C. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. Br. Phycol. J. 16: 9-53.
- Kudo, T. and Notoya, M. 1999. The free-floating *Ulva* species growing in the calm seas. Case of the Sea Park of Yokohama City. In Notoya, M. (Ed.), Use of *Ulva* and Restoration of the Environment. pp. 55-70. Seizando, Tokyo. (in Japanese)
- Link, H. K. 1820. Epistola de algis aquaticis in genera disponendis. In Nees von Esenbeck, C. G. (Ed.), Horae physicae berolinenses. pp. 1-8, pl. 1. Bonn.
- Linnaeus, C. 1753. Species plantarum, Tomus II. 561-1200 pp. Laurentius Salvius, Holmiae, Stockholm.
- Malta, E. J., Draisma, S. G. A. and Kamermans, P. 1999. Free-floating *Ulva* in the southwest Netherlands: species or morphotypes? A morphological, molecular and ecological comparison. Eur. J. Phycol. 34: 443-54.
- Michanek, G. 1979. Phytogeographic provinces and seaweed distribution. Bot. Mar. 22: 375-91.
- Migita, S. and Fujita, Y. 1987. The life history of *Ulva fasciata* Delile (Chlorophyceae, Ulvales) in culture. Jan. J. Phycol. 35: 226-30.
- Mshigeni, K. E. and Kajumulo, A. A. 1979. Effects of the environment on polymorphism in *Ulva fasciata* Delile (Chlorophyta, Ulvaceae). Bot. Mar. 22: 145-8.
- Müller, O. F. 1778. Icones plantarum. Flora danicae. Vol. 5, fasc. 13, pp. 8., pls. 721-80. Copenhagen.
- Ohno, M. 1999. *Ulva* and extensive biomass. In Notoya, M. (Ed.), Use of *Ulva* and Restoration of the Environment. Seizando, pp. 1-15. Tokyo. (in Japanese)
- Okamura, K. 1921. Illustrations of the Marine Algae of Japan. Vol. IV, No. 4, pp. 79-83, pl. 169-70. Keigyosha, Tokyo.
- Phillips, J. A. 1988. Field, anatomical and developmental studies on southern Australian species of *Ulva* (Ulvaceae, Chlorophyta). Aust. Syst. Bot. 1: 411-56.
- Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. In Watanabe, A. and Hattori, A. (Eds), Cultures and Collections of Algae. Proceedings of U. S.- Japan Conference, The Japanese Society of Plant Physiologists, pp. 63-75. Tokyo.
- Shimada, S., Horiguchi, T. and Masuda, M. 1999. Phylogenetic affinities of genera *Acanthopeltis* and *Yatabella* (Gelidiales, Rhodophyta) inferred from molecular analyses. Phycologia 38: 528-40.
- Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), Version 4.

- Sinauer Associates, Inc., Sunderland, Massachusetts.
- Tan, I. H., Blomster, J., Hansen, G., Leskinen, E., Maggs, C. A., Mann, D. G., Sluiman, H. J and Stanhope, M. J. 1999. Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, *Ulva* and *Enteromorpha*. *Mol. Biol. Evol.* 16: 1011-8.
- Tanner, C. E. 1980. *Chloropelta* gen. nov., an ulvaceous green alga with different type of development. *J. Phycol.* 16: 128- 37.
- van den Hoek, C., Mann, D. G. and Jahns, H. M. 1995. *Algae. An Introduction to Phycology*. 623 pp. Cambridge University Press, New York.
- Wulfen, F. X. 1803. *Cryptogama aquatica*. *Archiv für die Botanik* 3: 1-64, pl. 1.
- Yoshida, T. 1998. *Marine Algae of Japan*. 1222 pp. Uchida Rokakuho Publishing, Tokyo. (in Japanese)
- Zuker, M. 1989. On finding all suboptimal foldings of an RNA molecule. *Science* 244: 48-52.
- (Manuscript received 15 December 2003; accepted 20 March 2003.)

緑藻アオサ・アオノリ類の形態と分子情報

畠田 智¹⁾・平岡雅規²⁾・能登谷正浩³⁾

¹⁾北海道大学創成科学共同研究機構
〒060-0810 札幌市北区北10条西 8 丁目
E-mail : sshimada@sci.hokudai.ac.jp

²⁾高知大学海洋生物教育研究センター
〒781-1164 高知県土佐市宇佐町井尻194

³⁾東京大学海洋科学部
〒108-8477 東京都港区南4-5-7

緑藻アオサ類は、これまで食品として、また海岸でもっとも目立つ海藻類の1つとして日本人に親しまれてきた。市場規模はおおよそ200-300トン(10-20億円)で、最近では食品だけでなく水質浄化のためのバイオフィルターや、血液凝固抑制剤、オキシリピン生成阻害剤などの医薬品として有効利用されている。一方で、浮遊アオサなどは環境問題として取り上げられている。分類学的には、アオサ類は体の作りがあまりにも単純で分類形質が少なく、しかもその形質状態が生育環境で大きく変化し、混乱が生じている。アオサ種の分類形質として用いられてきた外形、分枝の有無、藻体の厚み、細胞サイズ、基部の細胞形、藻体縁辺部分の鋸歯の有無、ピレノイドの数は環境によって変化してしまうことが報告され、分類学や生態学だけでなく応用面でも重要なアオサ類に長い間、「名前が付けら

れない」といった分類学的混乱が生じている。

最近では、アオサ類の系統比較や種分類は核コードのITS領域や葉緑体コードrbcL遺伝子の塩基配列比較で行われる。データも蓄積されてきており、種の分子同定もできるようになってきている。アオサ類の種に関する問題を解決するためにははじめられたITS領域の分子系統解析で、意外な事実が判明した。アオサ属とアオノリ属は海藻類の中でも有名な属で、2層の藻体からなるアオサ属と中空の藻体であるアオノリ属は形態的にも区別しやすかった。しかし、ITS領域の分子系統学的解析で2属は互いに混ざり合い、両属とも単系統にはならなかった。演者らの日本産の株を含めたITS領域及びrbcL遺伝子による系統解析でも同様な結果だった。実は、形態的なデータもこの結果を支持している。2層の藻体からなるはずのアオサ属に所属する*Ulva curvata*は基部が中空になっていたり、中空の藻体であるはずのアオノリ属に所属するウスバアオノリは基部及び縁辺を除いて2層になっていたりするなど、両属の中間形を持った種が存在する。また、*Ulva linearis*は世代によって2層になったり中空になったりする。アオサ属のタイプ種である*Ulva lactuca*でさえ、無菌培養下では中空になる。アオノリ属という有名な属であるが、将来この名前は消えてしまうことになるだろう。

緑藻、主にアオサ類が浅海沿岸域で大量繁殖することを「赤潮」に対比させ「グリーンタイド」と呼んでいる。近年、世界各地の沿岸域でこの現象が認められ、集積したアオサ類の腐敗による悪臭と沿岸の生態系や環境に悪影響を及ぼしていることが報告されている。演者らは、ITS領域の塩基配列を比較することでグリーンタイドを引き起こす種類が判らないか試みた。鹿児島湾、大村湾、博多湾、浦ノ内湾、広島湾、三河湾、金沢湾、根室湾からグリーンタイドを引き起こしている浮遊アオサを採集し、ITS領域の塩基配列を決定し系統解析を行った。その結果、グリーンタイドを引き起こしている浮遊アオサは5つのクレードに分かれ、それぞれ固着性の*U. lactuca*, *U. pertusa*, *U. armoricana*, *U. fasciata*および*Ulva sp.*と単系統となった。つまり、日本におけるグリーンタイドは5種類のアオサから構成されていたのだ。日本全国に生育しているアナアオサは、やはり全国的なグリーンタイド種であった。*Ulva armoricana*型のアオサ種が昔から日本に自生していたのかどうかかわからないが、少なくともこれまで報告はなく、外国から船に乗って運ばれてきたのかもしれない。*U. lactuca*は日本におけるその存在が疑問視されてきたが、これまでの報告通り北方種で北海道の浮遊アオサになっていた。これら5種類の浮遊アオサを、20℃, 16h: 8h(明: 暗), PESの条件で振とう培養すると、表面から見た細胞の特徴において5種類のアオサ類に細胞レベルでの違いが現れた。

ただし、このような違いも、環境によって変異するため野外藻体の同定には使えない。野外藻体で同定を可能にするには、様々な環境要因（温度、塩分濃度、光強度、栄養塩濃度など）に関して幾つか条件を変えた培養実験を行い、分類形質の形質状態がどのように変化するかを理解しなくてはならない。残念ながら、現在のところグリーンタイドを引き起こすアオサ種の特定は野外藻体の形態観察だけでは難しいようだ。