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Chapter 5

SYSTEMATICS AND TAXONOMIC KEYS FOR THE MARINE GREEN ALGAL FAMILY MONOSTROMATACEAE

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ABSTRACT

Marine green algal family Monostromataceae consists of single cell-layered green seaweeds distributed throughout the world, yet identification of it being one of the most challenging. Monostromatic green seaweeds are all edible and are commercially cultivated in Japan for centuries. A thorough understanding of its systematics is indeed a necessity for any empirical investigation. Working dichotomous taxonomic key for this family is presented for the first time for the ready benefit of field phycologists. Recent advances in the systematics of this family with a special emphasize on the Japanese varieties are also presented.

ALGAE, WHAT ARE THEY?

Algae, which can be roughly defined as a photoautotrophic eukaryote (with the exception of embryophytes) is a ubiquitous and most abundant of the primary producers in the ocean - an area covering 71% of earth. While their biomass constitutes only a meager portion (<1%) the algae are accounted for almost 41% of the world's carbon fixation (Bolin *et al.*, 1977). Despite its overwhelming importance in the biosphere our knowledge of the algal biodiversity remains limited due to several factors such as phycologically unexplored geographical areas, describing new species being time-consuming, and a reduction in the numbers of researchers as well as funds to support algal systematics.

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Because of their complex and diverse life cycles, algae have been extensively studied to answer fundamental questions in biology (*e.g.*, *Chlorella*, in discovery of light-independent reactions in photosynthesis [Bassham *et al.*, 1950]), and ecology of terrestrial and aquatic biomes alike. Fossil remains of algae with $CaCO_3$ or silica deposit in their exoskeletons (*e.g.*, diatoms and coccolithophorids) are used routinely for the paleoclimactic reconstructions and thereby to predict climate change. In addition, algae have had a long history in biochemical (*e.g.*, agar-agar from *Gelidium*, carrageenan from *Chondrus*), pharmaceutical (β-carotene from *Dunaliella*, Arachidonic acid from *Parietochloris*) and food (Kombu, Nori and Wakame from seaweeds) industries. In the wake of competing demands during diminishing fossil energy resources, global warming and the world food crisis, algae has attracted much attention lately from the researchers and environmentalists alike as a potential source of renewable energy (the so called "algal biofuel"), as a candidate for carbon capture and sequestration (CCS), and as a future food source.

Classically, algae were classified based on color and distinct morphology (*e.g.*, "red algae lack motile stages"). However, this was found to be insufficient while dealing with the daunting problem of the interrelationships between different groups of eukaryotes. A problem while interpreting phylogeny based on morphology is the difficultly in selecting key character states and assigning phylogenetic values to them, especially in the distantly related groups with substantial morphological differences and of questionable homology. The plasticity of morphological characters resulted in either misclassification or over-classification of recognizable groups that led to a general confusion among phycologists. A significant advancement in the algal systematics took place with the advent of electron microscopy during mid 20th century, when phycologists began to characterize the ultrastructure of algal cells. Since then, the ultrastructural details -especially that of the plastids and the motile cells (flagellar roots and basal bodies) - have been widely taken as a reliable character state in cladistic analysis of various algal taxa because these characteristics were found to be evolutionarily more conserved (Friedl, 1997). By the end of the 20th century, DNA-based molecular systematics had largely superseded ultrastructure–based systematics and it had been shown that the morphological and biochemical diversity of the algae ~~results~~ from their polyphyletic origins within the eukaryotic lineage of the tree of life (Bhattacharya and Medlin, 1995; Stiller and Hall, 1997). The latest among proposed classification schemes for the tree of life, the "six kingdom" model based on molecular, ultrastructural and paleontological evidences (Cavalier-Smith, 2004), groups algal taxa into three kingdoms of the bikonts (ancestrally uniciliate eukaryotes); *viz.*, Protozoa, Chromista and Plantae (Figure 1).

Algae are tremendously diverse; the existence of nine distinct lineages, which are mentioned below, is good evidence for its diversity (Figure 1). Major lineages (divisions) are the Chlorophyta (green algae), Rhodophyta (red algae), Euglenophyta, Chlorarachniophyta, Glaucophyta, Heterokonta, Cryptophyta, Haptophyta, and the dinoflagellates (within the Alveolata). The latter four are grouped together as Chromalveolates or chromophyte algae because they contain various xanthophylls -that make them appear yellow or brown- in addition to the light harvesting pigments chlorophyll *a* and *c*. The algae not only include the world's largest protist giant kelp (*Macrocystis* spp in Heterokonta, that can reach up to 30m in length), but also many unicellular, bacteria- sized coccoid algae (*e.g.*, *Micromonas* spp in Chlorophyta, that have only 1-3 μm cell length).

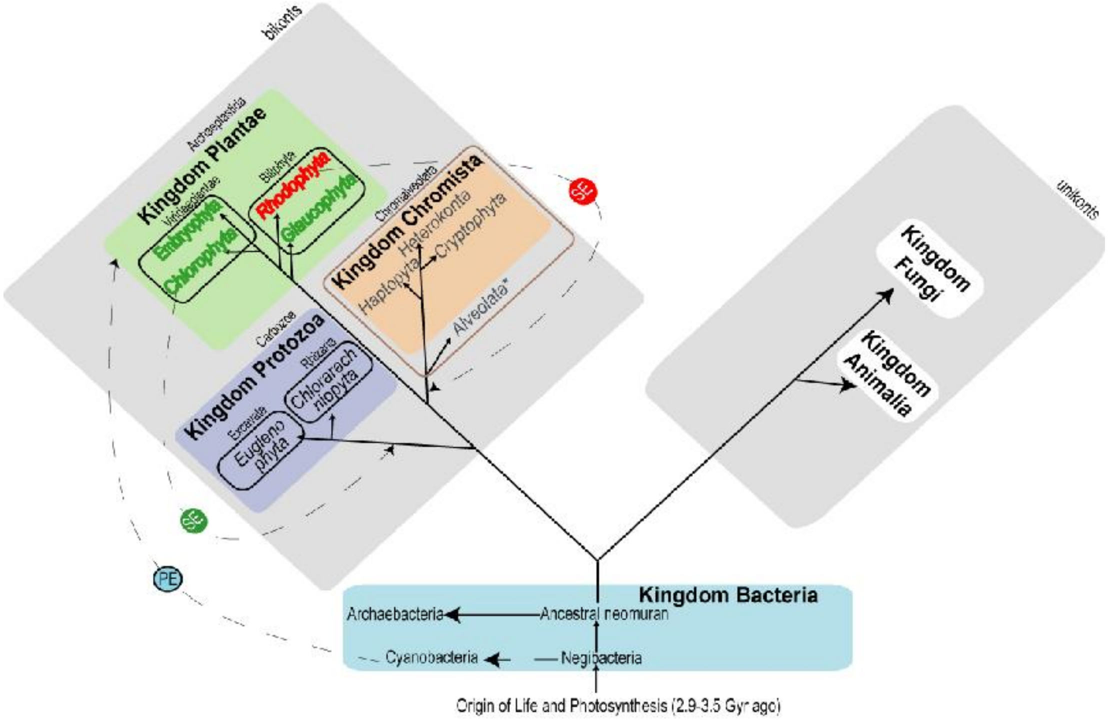

Figure 1. The tree of life based on Cavalier-Smith's "six kingdom" model (redrawn from Cavalier-Smith, 2004 to emphasize kingdoms relevant to algae). Nine algal lineages are indicated in italics. Although a chromalveolate, lineage Alveolata (indicated by an asterisk) belongs to the kingdom Protozoa. PE and SE indicate hypothetical primary and secondary endosymbiotic (endocytobiotic) events respectively, explaining the origin of plastids.

Division Chlorophyta: The Green Algae

Of the nine algal lineages, Chlorophyta is by far the most diverse, with around 16,000 extant species and up to 100,000 species that are estimated to be discovered (Andersen, 1992). Chlorophyta are also found in the greatest diversity of habitats; open oceans, coasts, freshwater, soils, inside shells, on the trees and animals, and even in the lower atmosphere (Bold and Wynne, 1985; Schlichting Jr., 1974). This lineage is estimated to be 1500 million years old (Yoon *et al.*, 2004). The size and structure of the body (thallus) show varying levels of complexity ranging from microscopic motile (e.*g.*, nanoplankton) or nonmotile forms (*e.g.*, lichen phycobionts) to filaments (*e.g.*, *Spirogyra*), bicellular (*e.g.*, *Euastropsis*), giant animal-like colonies (*e.g.*, *Volvox*) and complex levels of tissue differentiation (pseudo-parenchymatous, parenchymatous, or thalloid). Cells can be either uninucleate (*e.g.*, *Monostroma*) or coenocytic (*e.g.*, *Caulerpa* and *Bryopsis*).

Division Chlorophyta (Pascher, 1914) is characterized by the presence of double membrane-bound plastids containing chlorophylls *a* and *b*, accessory pigments β-carotene and xanthophylls (as in bryophytes) and a unique stellate structure linking 9 pairs of microtubules in the basal body of flagella, when present (Mattox and Stewart, 1984; Sluiman, 1985; Bremer *et al.*, 1987; Kenrick and Crane, 1997). Flagella lack tripartite tubular hairs (Moestrup, 1982; Mattox and Stewart, 1984). Main storage product is starch (amylose and amylopectin) and is stored inside plastids as grains (Dodge, 1973). Cell walls, when present, are usually composed of cellulose (Graham and Wilcox, 2000).

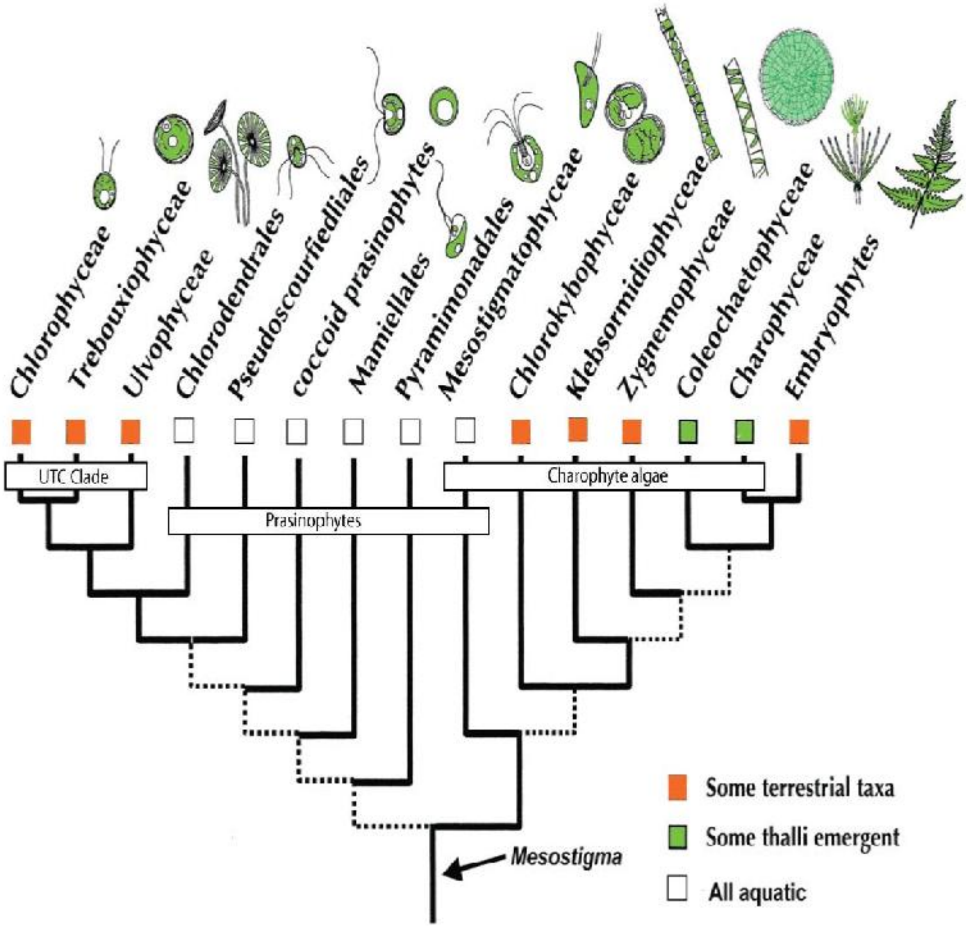

Figure 2. Internal lineages of the green algae inferred from DNA sequence data (redrawn from Lewis and McCourt, 2004).

Green algae have evolved into three major clades; charophyte algae, prasinophytes and "UTC clade" (Figure 2). Some taxonomists elevate the charophyte clade to a rank of division, *i.e.*, Charophyta (Cavelier-Smith, 2004), while the others consider this clade as a class within Chlorophyta. Charophytes, although relatively poor in species diversity, are believed to be the ancestral group of the Embryophytes-a clade consisting of over 100,000 species (Pickett-Heaps, 1969). Charophytes show great diversity in cellular organization, from flagellate unicells and filaments (branched or unbranched) to fairly complex forms, sometimes referred to as parenchymatous. Prasinophytes, also known as "ancestral green flagellate" (AGF), are widely regarded to be the most primitive green algae (Mattox and Stewart, 1984). Oldest fossils of green algae ever discovered are phycoma stage of prasinophytes, dated 1.2 bya (Tappan, 1980). Some of the best-known bloom-forming marine planktonic algae are prasinophytes (O'Kelly *et al.*, 2003). Prasinophytes are currently considered to be representatives of early diverging clades of uncertain taxonomic affinity (Fawley *et al.*, 2000). The name of the third clade, "UTC", stands for the three groups it contains; *viz.*, Ulvophyte, Trebouxiophyte and Chlorophyte groups. Vast majority of the described species of green algae are part of this triad. Monophyly of UTC clade has been strongly supported by various molecular studies based on nrDNA 18S (nucleoribosomal DNA gene for ribosomal small subunit) and has indicated that the Ulvophyte group is sister to the other two groups (Mishler *et al.*, 1994; Friedl, 1995; Krienitz *et al.*, 2001). These three groups are generally considered to be three distinct classes of green algae, *viz.*, Ulvophyceae, Trebouxiophyceae and Chlorophyceae (Mattox and Stewart, 1984).

Of the five classes of green algae, only Ulvophyceae is predominantly marine and it contains almost all known marine green macroalgae (the so-called green seaweeds). Ulvophyceae (Mattox and Stewart, 1984) is one of the algal classes that are exclusively classified based on ultrastructural characteristics. Members of this class are characterized by the presence of one or two pairs of flagella without mastegonemes in the motile cells, basal bodies having four microtubular rootlets arranged in stellar pattern, cell division by furrowing with centric, persistent and closed mitotic spindle fibers, and diplobiontic life cycle in free-living forms (O'Kelly and Floyd, 1984; Bold and Wynne, 1985). Various forms of thallus are present in Ulvophyceans; *e.g.*, filamentous (branched or unbranched), membranous (distromatic or monostromatic) or tubular. Cells can be either uninucleate or coenocytic. Some taxa have heavily calcified thalli (*e.g.*, *Halimeda*) and some have abundant deposits of orange pigment that make them appear brown or orange (*e.g.*, *Trentepohlia*). So far, molecular data (nrDNA 18S and *rbcL*- the gene encoding large subunit of RuBisCO-ribulose-1,5-bisphosphate carboxylase/ oxygenase) has not been able to provide solid support for the recognition of a monophyly of the class Ulvophyceae *sensu* Mattox and Stewart (Zechman *et al*., 1990; Watanabe *et al*., 2001; López-Bautista and Chapman, 2003; Lewis and McCourt, 2004). Within this class, there are five groups (orders) recognized by many contemporary classification schemes, *viz.*, Ulotrichales, Ulvales, Bryopsidales (Caulerpales) and Dasycladales, Cladophorales (including Siphonocladales), and Trentepohliales (Guiry and Guiry, 2008; Index Nominum Algarum, 2009), as illustrated in Figure 3.

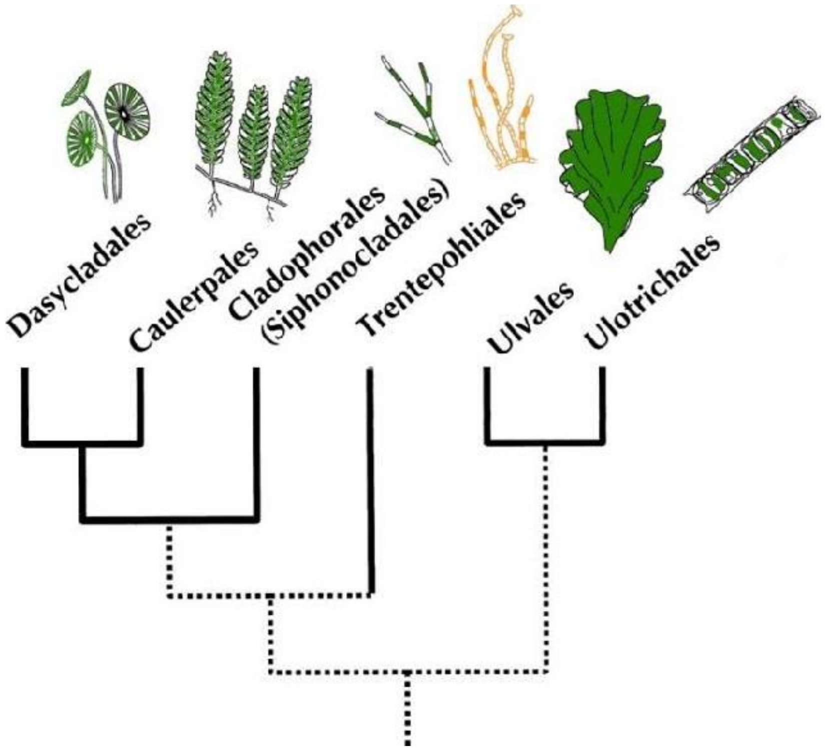

Figure 3. Internal lineages of Ulvophyceae based on DNA sequences (adapted from Lewis and McCourt, 2004).

Orders Ulotrichales (Borzi, 1895) and Ulvales (Blackman and Tansley, 1902) contain many green seaweed genera commonly seen across the world. Traditionally, Ulotrichales included only unbranched filaments and Ulvales included branched filaments or blades, but as ultrastructural and molecular data became available, several Chlorophycean taxa were added to either of these orders. Morphological forms in Ulotrichales include branched filaments (as in *Urospora*), unbranched filaments (as in *Ulothrix*) and membranous monostromatic blades (*Monostroma*). Most of the Ulotrichalean species have heteromorphic diplohaplontic alternation of generation with typical microscopic sporophytes known as *Codiolum*. Based on the presence of *Codiolum* stage, Kornmann (1973) erected a new class within Chlorophyta, the Codiolophyceae. However, the International Code of Botanical Nomenclature (ICBN) rejected this class because no Latin diagnosis was provided. Mattox and Stewart (1984) did not recognize this class as well because order Ulvales was not included. Genus named *Codiolum*, characterized based on aforementioned *Codiolum* stage, was later found to be sporophytic stage in the life cycle of *Urospora*, a later synonym. Taxonomic situation of Ulotrichales is problematic, especially because circumscription of this order, and its grouping under class Ulvophyceae was based on ultrastructural studies conducted in just one species, *Ulothrix zonata* (O'Kelly and Floyd, 1984). Ultrastructural characteristics of the type species of *Ulothrix*, *U. tenuissima* Kützing is unknown and because ICBN established that order name should be connected to the name of type genus and its type species; taxonomic validity of this order is still uncertain. On the other hand, members of the Ulvales have isomorphic alternation of generations. Morphologic switch between tubular (as in *Enteromorpha*) and membranous distromatic blade (as in *Ulva*) forms of Ulvales have been suggested to have evolved multiple times (Tan *et al*., 1999). Molecular work indicated that the genera *Ulva* and *Enteromorpha*, two of the familiar Ulvacean seaweeds worldwide, are monophyletic (Hayden and Waaland, 2002; Hayden *et al*., 2003). Several systematic studies argue in favor of merging Ulvales into Ulotrichales (*e.g.,* Papenfuss, 1960). Molecular phylogenetic studies in general show that these two orders are monophyletic, although bootstrap support for Ulotrichales is weak or absent (Watanabe *et al*., 2001; Friedl and O'Kelly, 2002; Hayden and Waaland, 2002; O'Kelly *et al*., 2004a, 2004b, 2004c; Lindstrom *et al*., 2006).

Monostromataceae

All single cell-layered green algae with blade-like thallus were traditionally grouped under the eponymous genus *Monostroma* (Thuret, 1854). Kunieda (1934) erected family Monostromaceae (later synonym for Monostromataceae Kunieda ex Suneson, 1947) to include this genus. Various and often contradictory taxonomic revisions have resulted in over-classification as well as lack of a clear-cut systematic placement of this Ulvophycean group.

For example, there are two lectotypifications in *Monostroma*; first recognizing *M. oxyspermum* (Papenfuss, 1960), and second recognizing *M. bullosum* (Kornmann, 1964). There were at least three ordinal placements of this family as well, *viz.*, Codiolales (Kornmann, 1964; van den Hoek *et al*., 1995), Ulotrichales (Gayral, 1964; Chapman and Chapman, 1973; Floyd and O'Kelly, 1990; Gabrielson *et al*., 2000; Graham and Wilcox, 2000) and Ulvales (Bliding, 1968; Vinogradova, 1974; Bold and Wynne, 1985).

Systematic history of Monostromataceae has been reviewed in great detail by Tatewaki (1969) and O'Kelly *et al. (*1984). In summary, the following are the major revisions. Gayral (1964) grouped *M. angicava*, *M. grevillei* and *M. bullosum* under *Ulvopsis* due to shared ontogeny (Disc-Sac-Blade; DSB) and swarmer release (simultaneously through an irregular rent), and reserved genus *Monostroma* only for asexual species with typical ontogeny (presence of a filament stage) and zoid release mechanism (*en-masse* without pore). Inversely, Kornmann (1964) and Bliding (1968) proposed to remove asexual members from *Monostroma*. Vinogradova (1969) erected two monotypic genera *Protomonostroma* and *Gayralia,* to accommodate the asexual members *M. undulatum* and *M. oxyspermum,* respectively. *Monostroma fuscum* and *M. obscurum* have been reclassified with resurrected genus *Ulvaria* in order Ulvaceae because of the isomorphic life cycle pattern (Gayral, 1964). *Monostroma leptodermum* and *M. zostericola* have been grouped under the new genus *Kornmannia* due to shared life cycle and ontogenetic patterns and placed under Ulvales due to typical flagellate release mechanism in which swarmers are released one by one through a gametangial exit pore (Bliding, 1968).

Monostroma groenlandicus has been included in the genus *Capsosiphon* due to similarity in habit (cylindrical gametophyte), thallus ontogeny (filament-tube) and swarmer release mechanism (*en-masse*, enclosed within hyaline sheath; Vinogradova, 1969).

Taxonomic confusion has largely been caused by a lack of synapomorphic character due to polyphyly in this group of algae. Many studies have concluded that Ulvophycean algae show considerable phenotypic plasticity and therefore diagnostic character such as "monostromatic blade" is not taxonomically reliable.

For example, green algae belonging to *Prasiola* have macroscopic monostromatic thalli that closely resemble *Kornmannia*, however they belong to an entirely different class (Trebouxiophyceae). A green-tide forming single cell-layered algae that superficially resembles *Monostroma* isolated from west coast of Finland turned out to be a morphotype of tubular *Ulva* (Blomster *et al*., 2002). Abiotic factors such as nutrient supply (Valiela *et al.*, 1997) and salinity (Reed and Russsel, 1978) are believed to be inducing morphological changes in green algae.

Phenotypical polymorphism induced by biotic factors is known in green algae for a long time. Bonneau (1977) discovered that adding extracts containing marine bacteria isolated from thallus of *Ulva* to axenic cultures of the same algae resulted in a change of its morphology from blade to tube. Recently, specific bacterial strains isolated from *M. oxyspermum* had been demonstrated to induce morphogenetic changes in the axenic cultures of this alga, as well as *Ulva pertrusa* and *Ulva intestinalis*. Through phylogenetic analyses, these strains had been identified as belonging to *Cytophaga-Flavobacterium-Bacteroides* (CFB) complex (Matsuo *et al*., 2003).

Further investigations on this bacterium lead to biochemical characterization of a morphogenetic inducer (Thallusin), and confirmation of its importance for the natural growth of these algae (Matsuo *et al*., 2005).

A diagnostic dichotomous key to aid in the identification of various monostromatic green algal species is presented in Table 1. As one can observe, field identification of these algae is extremely challenging and is limited only to a few species; the rest demands life cycle and other culture experiments that are often time-consuming.

Table 1. Diagnostic dichotomous key for the monostromatic green algae[1,2]

- **1.** Fronds macroscopic, tubular, wall of tube one cell thick in cross section : **2**
- **1.** Fronds macroscopic, bladelike, monostromatic in cross section : **4**
  - **2.** No rhizoidal filaments at the base of tube; Tube arising from parenchymatous cushion; Eulittoral to supralittoral. ***Blidingia*** (in part)
  - **2.** Rhizoidal filaments at the base of tube; Tube arising initially from uniseriate filament; Sublittoral, Eulittoral or supralittoral : **3**
- **3.** Fronds are hollow except at base and ends of branches; pale to bright green. ***Ulva*** (in part)
- **3.** Fronds are often filled with gelatinous substance and rarely hollow; Fronds very slender at base and broadened towards apex, up to 1mm in diameter and 5-10 cm in height; Yellowish or brownish green; Estuarine supralittoral. ***Capsosiphon groenlandicus***
  - **4.** Blade typically less than 5 cm in height, cells typically less than 8 µm in diameter : **5**
  - **4.** Blade typically larger than 5 cm in height, cells typically larger than 8 µm in diameter : **7**
- **5.** Blades typically less than 2 cm in height, narrowing to short stipiate region at base; Epilithic in supralittoral zone; Zoids released from fronds are nonphototactic, biflagellate . ***Prasiola*** (in part)
- **5.** Blades typically 3-5 cm with ragged outline; Often epiphytic on seagrasses, *Fucus* or *Halosaccion*; Zoids released from fronds are nonphototactic, quadriflagellate; Marine eulittoral/sublittoral; Spring ephemeral; DSB ontogeny : **6**
  - **6.** With tubular stipe, Cells are 6-8 µm in diameter. ***Kornmannia leptoderma***
  - **6.** Without tubular stipe, Cells are 2.5-5 µm in diameter. ***Kornmannia zostericola***
- **7.** Limnetic; Plants usually monoecious; Gametes are biflagellate, phototactic; Heteromorphic alternation; Shell-boring *Codiolum*-sporophyte; DSB Ontogeny. ***Monostroma bullosum***
- **7.** Marine or Estuarine : **8**
  - **8.** Zoids released from the fronds are nonphototactic, quadriflagellate; Zoids exhibit characteristic clumping behavior; Marine eulittoral/sublittoral; Spring ephemeral; Dimorphic asexual life cycle with *Codiolum*-sporophyte (sexual life cycle not reported); *En masse* zoid release by wall dissolution; Zoidangia disintegrates upon zoid release; FB ontogeny. ***Protomonostroma undulatum***
  - **8.** Zoids released from the fronds are phototactic, biflagellate : **9**
- **9.** Life cycle is isomorphic alternation (asexual life cycle not reported); Dark or bright green; Turns to olive green or black upon drying; Singular zoid release through pore; Zoidangia remain on blade after zoid release; FSB ontogeny. ***Ulvaria obscura***
- **9.** Life cycle is either heteromorphic alternation or monomorphic asexual: **10**
  - **10.** Prostrate disc stage in the ontogeny (DSB); *En masse* zoid release by irregular rent; Zoidangia remain in blade after zoid release : **11**
  - **10.** Erect filament stage in the ontogeny (FB or FSB); *En masse* zoid release by wall dissolution; Zoidangia disintegrates upon zoid release : **12**
- **11.** Life cycle is monomorphic asexual (sexual life cycle not reported); Marine; Reported only from temperate to polar regions. ***Monostroma arcticum****

  **M. antarticum* may be a related species but life cycle information is not known
- **11.** Life cycle is dimorphic alternation (asexual life cycle not reported); Marine eulittoral/ sublittoral; Shell-boring *Codiolum*-sporophyte: ***Monostroma grevillei**** or ***M. angicava****

  *Discerning between these two species is difficult or impossible, may be synonymous.
  - **12.** FB Ontogeny; Spring ephemeral; Marine or estuarine eulittoral; Life cycle is sexual or asexual; If sexual, dimorphic alternation; Non shell-boring *Codiolum*-sporophyte. ***Monostroma kuroshiensis***
  - **12.** FSB ontogeny : **13**
- **13.** Present year round; Typically estuarine eulittoral; Life cycle is asexual (sexual lifecycle not reported); Thallus often sac shaped. ***Monostroma oxyspermum***
- **13.** Spring ephemeral; Life cycle is dimorphic alternation (asexual life cycle not reported); Non shell-boring *Codiolum*-sporophyte; Marine or estuarine eulittoral. ***Monostroma nitidum***

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[1] Information based on following references: Gayral, 1964; Bliding, 1968; Tatewaki, 1969; Gabrielson *et al.*, 2000; Guiry and Guiry, 2008.

[2] Expansion of abbreviations: DSB: Disk-Sac-Blade; FSB:Filament-Sac-Blade; FB:Filament-Blade.

MONOSTROMA KUROSHIENSIS-NITIDUM COMPLEX

Monostroma kuroshiensis (Yendo) Bast *et al*. and *M. nitidum* share a lot of taxonomic features in common including life cycle, basic thallus ontogeny pattern and gamete release mechanism. Both have heteromorphic diplohaplontic life cycle with leafy gametophytes and *Codiolum*-sporophytes. Gametophyte releases positively phototactic, biflagellate gametes by thallus disintegration. Sexual fusion is anisogamic and subsequently, zygotes become negatively phototactic. Successive divisions and enlargement of the zygote produces a microscopic *Codiolum*-sporophyte. *Codiolum*-sporophyte releases quadriflagellate zoospores that germinate to form either of the gametophytes. Systematically, these two species were distinguished based only upon the frond thickness; *i.e.*, "thicker" species being *M. nitidum* (Wittrock, 1866). There have been several reports on the thallus ontogeny of *M. kuroshiensis*, which is of FB (Filament-Blade) pattern. While this ontogenetic pattern can be considered as a typical characteristic of this complex, there has been one report (Arasaki, 1949) that mentions FSB (Filament-Blade-Sac) ontogeny for *M. nitidum*. Although the differences between them warrant further investigation, we tentatively call these two very similar algae as *Monostroma kuroshiensis-nitidum* complex, or *K-N*.

Commonly found in the eulittoral zones of marine and estuarine environments, algae belonging to this complex are crucial players contributing to the ecology of the coastal ecosystem. Along with other abundant primary producers, *K-N* contributes to the growth and survival of the whole niche of coastal biota. Many small benthic invertebrates, collectively known as meiofauna, utilize the assemblage of *K-N* as a shelter as well. Therefore, a good understanding of the ecophysiology as well as biogeography of this complex has become increasingly important in our overall comprehension of the coastal ecosystem.

Algae belonging to this complex have been used for human consumption in East Asia- especially in Japan- since time immemorial. At present, the *K-N* complex is the most important commercial green algae in Japan, constituting about 90% of the total green algal cultivation (Nisizawa *et al*., 1987). In and around Ise Bay, Mie Prefecture, Central Japan, is the epicenter of *K-N* cultivation, with a vast majority of the edible monostromatic seaweed, known in Japanese as "*hitoegusa*" coming from this region (Figure 4). Seeding method used in the cultivation can either be natural (*e.g.*, Ise Bay) or artificial (*e.g.*, Shimanto Estuary, Kochi Prefecture). In the natural seeding method, plants derived from the naturally deposited zoospores on the culture nets are harvested. On the other hand, in the artificial seeding method a large quantity of zygotes –as obtained from *in vitro* fertilization of isolated gametes by the end of growth period- are grown throughout the summer. The resulting *Codiolum*-sporophytes are then treated with high intensity of light to induce zoospore release. Culture nets are immersed in the concentrated zoospore solution under dark conditions to facilitate successful attachment of released zoospores on the nets. These "seeded" nets are subsequently installed in the attached fabrication of wooden sticks in the coastal waters and the height of nets are adjusted such as to provide adequate immerse-in and drying-out effects with each tidal range. Upon reaching the highest size, thalli are harvested and processed. Harvested thallus is boiled down in soy sauce to make a jam-like product, *tsukudani-nori* while dried sheets (*hoshi-nori*) are used as sushi wraps. Other popular products are roasted (*yaki-nori*) and seasoned (*ajitsuke-nori*) *Monostroma*.

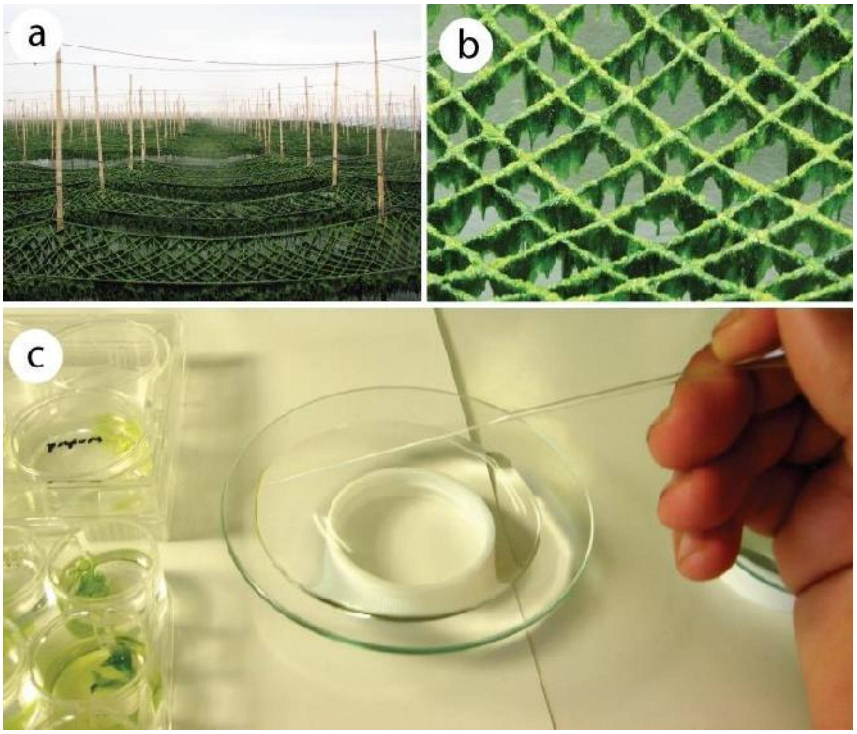

Figure 4. *Monostroma kuroshiensis* from Matsusaka, Mie Prefecture, Japan. a. Fronds growing luxuriously on the intertidal cultivation nets. b. A close-up view on the cultivation net. c. *In vitro* phototactic isolation of gametes using a capillary tube by the first author.

Several independent pharmacological studies suggest that the algae belonging to this complex are potential pharmaceutical agents. Rhamnan sulfate, a water-soluble sulfated polysaccharide isolated from the fronds of *K-N*, has shown to possess antiviral activity against pathogenic viruses such as Human Immunodeficiency Virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1) and cytomegalovirus (HCMV) *in vitro* (Lee *et al*., 1999). Rhamnan sulfate and other sulfated polysaccharides isolated from this complex also have been demonstrated to possess antithrombin activity (Lee *et al*., 1998; Zhang *et al*., 2008). Of several betains isolated from *K-N*, β-homobetain has been shown to lower plasma cholesterol level in rats (Abe and Kaneda, 1973). Hot water extract of the *K-N* had been demonstrated to increase longevity of the animals implanted with leukemia cells (Yamamoto *et al*., 1982). There has also been a successful attempt on the isolation of protoplasts from the vegetative thalli of this complex, for a potential use as seed stock (Chen, 2002).

This ephemeral species had been reported only during the colder part of the year (Kida, 1990). Typically, growth season starts in late autumn and ends in mid spring. The *K-N* complex is observed only along the Central and SW Japanese coasts influenced by the warm water Kuroshio Current. Geographical history of Japan might have had an influence on the distribution pattern of *K-N* complex. After the recession of the last ice age around 10,000 years ago, tremendous rise in sea level resulted in the isolation of the four main islands (*i.e.*, Hokkaido, Honshu, Shikoku, and Kyushu) from the Korean peninsula (Ohta and Yonekura, 1987). Seawater from the Pacific infiltrated to the Sea of Japan and Seto Inland Sea, both of which had been freshwater lakes during the ice age. Re-colonization of the marine algae from Southern Pacific might have taken place during this period along the warmer waters of the Southern and Western Japan, accounted by Kuroshio and Tsushima currents, respectively.

By a novel, polyphasic strategy that involved classical culture-based methods such as mating tests and microscopic investigations of life cycle and ontogeny, as well as modern genome-based techniques such as DNA barcoding, mRNA secondary structure analysis and multi-local phylogeny reconstruction, taxonomic reappraisal of this genus has recently been proposed (Bast, 2010). Natural and cultivated sexually reproducing environmental samples, as well as sexually reproducing and serendipitously discovered asexually reproducing ecotypes of monostromatic green alga from Tosa Bay, Japan were discovered to be conspecific and was identified as *Monostroma kuroshiensis* (Yendo) Bast *et al* (Bast et al, 2009a). Patterns of seasonal fluctuations in its thallus lengths were habitat specific and recur annually. Both appearance and decay of thalli were earlier at high saline habitats, suggesting that salinity positively influences either maturation of sporophytes or senescence of gametophytes (Bast *et al*, 2009b). Gametogenesis in *M. kuroshiensis* occurs in discontinuous patches along the frondal apex and the gametes release synchronously in a posterior faced linear fashion by the dehiscence of gametangial sheath, leading to the thallic disintegration (Bast and Okuda, 2010). The overall primary sex ratio of this alga was about 1:1 which is likely to reflect Fisherian selection (Bast *et al*, 2009c). Results from life cycle, thallus ontogeny, gametangial ontogeny and phylogenetic analyses suggest that this alga is evolutionarily more close to *M. oxyspermum* than other green algae. As there are conspecific members having different life cycles, type of life cycle is not a valid diagnostic character for the species circumscription in Monostromataceae. Life cycle may be an adaptive strategy to increase fitness of this algae during environmental stress. Biogeographic isolates of *Monostroma* sp. distributed along Kuroshio Coast of Japan were panmictic and had similar sequences for the conserved nucleoribosomal DNA (nrDNA) 18S gene, however, a substantial variance was observed in the highly variable first internal transcribed spacer (ITS1) sequences that are suggestive of an emerging sympatric speciation (Bast, 2010).

Whereas differences in morphology, color and habitat of various monostromatic algae remains so feeble, culture-based investigation on its life cycle and thallus ontogeny remains the only method to conclusively identify the members of monostromataceae. Hopefully, recently developed ITS based DNA barcoding (Bast, 2010) will bring about the cryptic monostromatic marine biodiversity. As we expand our search to more and more phycologically unexplored sea shores, the unambiguous green algal biodiversity will keep on unfolding, for which it is expected that the taxonomic key of monostromatic green algae presented herein will be of some help.

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