## **Benthic Marine Algae on Japanese Tsunami Marine Debris**

- a morphological documentation of the species

## Part 3. The Green Algae and Cyanobacteria

Gayle I. Hansen (hansengi@outlook.com), Oregon State University, USA

With DNA determinations\* by Takeaki Hanyuda (<u>hanyut@kobe-u.ac.jp</u>) & Hiroshi Kawai (<u>kawai@kobe-u.ac.jp</u>), Kobe University, Japan





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#### **Other publications supported:**

- The Scholars Archive presentations above provide photographic documentation for the species included in the following publications. The poster is a pictorial overview of some of the larger debris algae made for teaching.
- Hansen, G.I., Hanyuda, T. & Kawai, H. (2017, in Review). The invasion threat of benthic marine algae arriving on Japanese tsunami marine debris in Oregon and Washington, USA.
- Hanyuda, T., Hansen, G.I. & Kawai, H. (2017, in Press). Genetic identification of macroalgal species on Japanese tsunami marine debris and genetic comparisons with their wild populations. Marine Pollution Bulletin. https://doi.org/10.1016/j.marpolbul.2017.06.053
- Hansen, G.I. (2013). Some Marine Algae on Tsunami Debris. OSU Scholars Archive, a poster. <u>http://ir.library.oregonstate.edu/concern/defaults/ns064b84v</u>

### **Codes, Definitions & Abbreviations + Acknowledgements & Contents**

#### Special codes provided in each description:

- > Definitions of terms or abbreviations (not provided below) are given in Part 1 of this series.
- Species that have been sequenced (genes used, BF debris item and collection numbers are provided see p. 4, Appendix 1, and the text).
- # = Identification was assisted by a monographic expert(s). Their names are provided on the species page.

**Approximate identifications:** The names for the Japanese Tsunami Marine Debris (JTMD) algae and cyanobacteria shown on the following pages are derived from: morphological accounts on the species, personal observations, and the genetic sequences (when available). Although the sequences and morphology often precisely match known species, situations can occur where either the sequences or the morphology vary slightly from the known observations and absolute identifications are impossible to determine. For these samples, I use the following qualifying terms to indicate approximate identifications: *sensu* X = an identification according to scientist X; *cf.* = refer to (the most probable species identification); *cpx.* = a clade or group of closely related species that includes the unnamed isolate. The term *cpx.* includes both: (1) morphological variants = species with identical sequences that have different morphology, and (2) sequence variants = species that are morphologically correct with the literature whose sequences do not match exactly those for the same species deposited in GenBank. These variant types are noted in the text.

Longevity: Life span data is from the literature and observations in the NEP. If it is estimated, a "~" precedes the most likely type (annual, perennial, ephemeral).

- Distributions: See part 1 for all sources. Global distributions follow www.AlgaeBase.org (accessed July 2017). Distribution Codes: G (Globally widespread) = species that appear to be naturally widespread globally, occurring on multiple continents and in different oceans; A (Asian-only) = species occurring only in Asia, from Russia to the Philippines; A+ (Asian+) = Asian species that have also been exported globally by human activities; NP-P (North Pacific-P) = species limited primarily to both the NE and NW Pacific but with some occurrences in Alaska and the S. Pacific.
- Distribution Abbreviations:. Afr = Africa; AK = Alaska; A-Arc = Antarctic; Arc = Arctic; Aus = Australia; BC = British Columbia; Bra = Brazil; C = China; Car = Caribbean; ENA = Eastern North America; EUR = Europe including the British Isles; EUR-Arc = Europe and the European Arctic; HA = Hawaii; IO = Indian Ocean (including Indonesia); J = Japan; K = Korea; Med = Mediterranean; MX = Mexico; NEP = Northeast Pacific; NZ = New Zealand; OR = Oregon; Phil = Philippines; R = Eastern Russia; SA = South America (both coasts); Viet = Vietnam; WA = Washington. For brevity, we have excluded some island groups and Arctic areas. For more thorough distribution coverage, see the continually updated: www.algaebase.org
- Acknowledgements: Financial support for studies on the algae of tsunami debris were provided by Oregon Sea Grant, the Ministry of the Environment of Japan through the North Pacific Marine Science Organization (PICES), and personal savings. Collection assistance for the algae was provided by John Chapman, Russ Lewis, Nancy Treneman, Jessica Miller, Thomas Murphy and the state and volunteer agencies in Washington and Oregon responsible for debris removal. Jim Carlton kept the BF (biofouling) item database. Judy Mullen (OSU libraries) provided essential and often obscure literature for the study. The US-EPA provided laboratory space for the Oregon part of the project. Additional credits are due to Mike Guiry and AlgaeBase.org for global distributions, reference information, and nomenclature advice and also to these monographic experts for advice and identification assistance with the green algae and cyanobacteria: C. Boedeker, R. Nielsen, J.R. Sears, and S. Shalygin.

**Contents:** The Green Algae and Cyanobacteria – A Checklist of the Species on JTMD, The Species Descriptions, References, and Appendix 1.

### The Green Algae and Cyanobacteria

### A Checklist of the Species

**on JTMD** & their global and NEP distributions

#### KEY:

- Pg = page number
  JTMD = Japanese Tsunami Marine Debris
  Global = general global occurrence
  A = Asian only
  A+ = Asian but also introduced by human activities globally
  NP-P = Northwest and Northeast Pacific, some with Alaska and S. Pacific occurrences
- G = globally widespread, including species with rare global occurrences
- NEP = Northeast Pacific occurrence (Washington to Mexico)
- Y = occurring in the NEP

N = not known in the NEP

- **DNA** = genes sequenced or expert assistance
- Gene codes: (1) ITS rDNA, (2) rbcL, (3) 18S rDNA, (4) 28S rDNA, (5) atp H-I, (6) trnA-N

\* = further study required

**#** = monographic expert assistance

### **The Species Descriptions**

Please use the page number or ^F to call up the individual species.



Pg	Green Algae on JTMD	Global	NEP	DNA
5	Acrosiphonia arcta (Dillwyn) Gain	G	Y	(1)
6	Blastophysa rhizopus Reinke	G	Ν	#
7	Blidingia minima var. minima (Nägeli ex Kützing) Kylin	G	Y	(1, 2)
8	Blidingia subsalsa (Kjellman) Kornmann et Sahling ex Scagel et al.	G	Y	
9	Bolbocoleon piliferum Pringsheim	G	Y	
10	Bryopsis plumosa (Hudson) C.Agardh cpx.	G	Y	(2)*
11	Bryopsis stolonifera W.J. Lee, S.M. Boo et I.K. Lee	А	Ν	
12	Cladophora albida (Nees) Kutzing	G	Y	(1, 3, 4) #
13-14	Cladophora vagabunda (Linnaeus) Hoek (including C. parriaudii C. Hoek)	G	Y	(1, 3, 4) #
15	Codium fragile subsp. fragile (Suringar) Hariot	A+	Y	(2)
16	Entocladia polysiphoniae Setchell et N.L.Gardner	NP-P	Y	
17	Epicladia flustrae Reinke cpx. (including E. phillipsii R. Nielsen)	G	Ν	#
18	Halochlorococcum moorei (N.L. Gardner) Kornmann et Sahling	G	Y	
19	Ulothrix implexa (Kützing) Kützing (including U. subflaccida Wille)	G	Y	
20	The Ulva complex and its problematic taxonomy			
21-22	Ulva australis Areschoug	A+	Y	(1, 2, 5, 6)
23-24	Ulva compressa Linnaeus	G	Y	(1, 2)
25	Ulva intestinalis Linnaeus cpx.	G	Y	(1, 2)*
26	Ulva lactuca Linnaeus	G	Y	(1, 2)
27	Ulva linza Linnaeus	G	Y	(1)*
28-29	Ulva prolifera O.F. Müller	G	Y	(1, 2)*
30-31	Ulva simplex (K.L. Vinogradova) H.S. Hayden et al. sensu Ogawa	G	Ν	(1, 2)*
32	Cyanobacteria on JTMD	Global	NEP	DNA
33	Calothrix scopulorum C.Agardh ex Bornet & Flahault cpx	G	Ν	#
33	Chroococcus submarinus (Hansgirg) Kovácik	G	Ν	#
34	Lyngbya confervoides C. Agardh ex Gomont	G	Ν	#
34	Scytonematopsis crustacea (Thuret ex Bornet et Flahault) Koválik et Komárek cpx.	G	Y	#
35-41	References for the Green Algae and Cyanobacteria 42-43 Appendix 1. Tsunar	ni Debris Items		

**Acrosiphonia arcta<sup>\*</sup> – G (Widespread) –** Asia (R,J,C), Arc, AK-CA, SA (Chile, Argentina), A-Arc, ENA, EUR. On 3 debris items (Jan, May, Jul). Sterile on debris. Annual and heteromorphic with a *Codiolum*-like sporophyte.





- Partially disintegrated, this coarse branching uniseriate green alga reached only 3-5 cm in height on debris (B); in Europe, 10-15 cm.
- The filaments were somewhat stiff and branching was subdichotomous near the base (C) and alternate or unilateral near the tips (D).
- The filaments ranged from 48-92 µm in diameter and had broadly rounded tips (A).
- An abundant growth of rhizoids occurred at the base, but there was no evidence of the hook-like cells characteristic of some species of *Acrosiphonia*.
- Apparent rhizoid initiation is detected in D (arrow).
- Polyhedral pyrenoids, characteristic of Acrosiphonia, were visible even in partially disintegrated material (F, arrows).
- Scagel (1977, pl. 37, figs. A-D), Vinogradova (1979, figs. 52-53), Klochkova *et al.* (2009, pl. 12, figs. 1-6), Brodie *et al.* (2007, fig. 15), Kornmann & Sahling (1977, pls. 7-8), Sussmann *et al.* (1999).
- Sequences of ITS1 and ITS2 indicted that this species was a part of the Acrosiphonia arcta complex.
- \* Nye-6 (BF-59/61). Not sequenced: SV-15-2 (BF-293), FC-751 (BF-652).



**Blastophysa rhizopus<sup>#</sup> – G (Widespread) –** Asia (R, J, K), Aus, SA (Brazil), ENA, Car, Afr, EUR, Med. In the turf on 5 debris items (Mar-May), but not sequenced. Reproductive. Thought to be pseudo-perennial with apomictic reproduction.

The large coenocytic globose to irregular lobed cells,  $30-90 \ \mu m$  in diameter, were scattered singly or in small clusters in the turf among the holdfast cells of *Polysiphonia* (A). The alga appeared to be epiphytic and not endophytic.

 The coenocytic cells contained multiple globular chloroplasts (arrow) that nearly all contained a single central pyrenoid surrounded by starch grains (B).



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- Empty cells (C, arrow) indicated the earlier release of reproductive propagules. Quadriflagellate zoospores and coenocytes are known in this species (Burrows, 1991; lima & Tatewaki, 1987).
- Both filaments and tubes (hairs) extended from the larger coenocytic cells (D, G, arrows).
- The filaments were cellular, contorted, occasionally branched and from 14-20 µm wide (E, F).
- The flexible tubes (often referred to as hairs) were acellular, 1-2 μm in diameter and seemed to arise from both the large multinucleate cells and the irregularly contorted filaments (G, H, I).
- Reinke (1889), pl. 23; Brodie *et al*. (2007, fig. 88), Cormaci *et al*. (2014, pl. 88, figs. 3-5), Abbott & Huisman (2004, fig. 34), Kraft (2007, fig. 53), Kim *et al*. (2014, fig. 5 D-F, lima & Tatewaki (1987, figs. 1-24).
- Not sequenced: Ump (BF-545), SixR (BF-538), RE (BF-533), HF2 (BF-526), SVB (BF-402).
- **#** J.R. Sears provided information of the life history.



## Blidingia subsalsa – G (Widespread) – Asia (R, J), Arc, Aus, SA, AK-CA, ENA, Car, Afr, EUR, Med. On 1 JTMD

item (Oct): CBD (BF-130), not sequenced. Sterile but known to reproduce through asexual quadriflagellate zoospores. Prange (1978) observed small discs with tiny uprights in winter in BC. Erect thalli are year around in San Francisco Bay, CA. ~Ephemeral.



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- Debris thalli were irregularly forked (C) and mature blades could be seen initiating narrow tubular branches from the margins (D).
- Debris thalli were 1-2 cm tall, but in other areas, the blades are known to reach 10-20 (30) cm in height and 2-3 mm in width.
- Cells were small, 4-6 µm in diameter and polygonal with rounded corners. Each contained a single stellate or lobed chloroplast with a central pyrenoid (E).
- Cells were arranged irregularly in broad areas but were in longitudinal rows in the narrow branches. No reproduction was observed.
- Abundant in Spring-Summer, but occurs year around in sheltered areas.
- Kjellman (1883, pl. 31, fig. 1), Setchell & Gardner (1920, pl. 16, fig. 1 as E. micrococca f. subsalsa), Nagai (1940, pl. 1, fig. 9), Bliding (1963, fig.14), Scagel (1966, pl. 22, figs. A-D), Kornmann & Sahling (1978); Thom (1984), DeCew (1997).

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**Bolbocoleon piliferum** – **G** (Widespread) – Asia (R, J, K), IO, SA (Brazil), AK, BC, CA, ENA, Car, EUR, Med. Known year around. Fertility not noticed on debris. Isomorphic life history or asexual with quadriflagelate zoospores. ~Ephemeral.



- A trace of this species was found in debris only on the HF2 debris boat (Mar). No DNA was taken.
- Morphological characteristics of the genus and species were obvious enough for identification.
- Prostrate uniseriate filaments that are irregularly branched (not seen).
- Axial cells 12-16 um wide and 1-4 x the diameter in length.
- Chloroplasts were not clear but are known to be Irregularly lobed and perforated with pyrenoids (A).
- Characteristic *Bolbocoleon*-like hair cells were found on the fragment developing on the apparent axis with expanded onion-shaped basal cells and long hair like extensions.
- Reproduction is reported to occur via 32 bi- or quadriflagellate swarmers developing from mature vegetative cells.
- Brodie, et al. (2007, fig. 22), Kogame & Yoshida (1988). Nielsen (1979), O'Kelly et al. (2004).

#### **Bryopsis plumosa cpx. \* – G** (Widespread) – Asia (R, J, C, K, Viet, Phil), IO, Aus, SA, AK-MX, ENA, Car, Afr, EUR, Med. Found only on the Mosquito Creek dock (Jan), \*MC-41 (BF-8). Similar species = *B. pennata* var. *minor* (*B. pennatula*) and *B. nana*. Annual, heteromorphic.



- Disintegrating thalli on debris were 1-3 cm tall and a form that was similar in appearance to *B. pennatula sensu* Lee and *B. minor* Wynne. However, sequencing indicated that this was *B. plumosa*, a species that can reach 10 cm or more in height. We use cpx. to accommodate this morphological variation.
- The erect, terete and coenocytic thalli were naked toward the base, but had short lateral pinnules, 1–1.5 mm long, occurring near the tips and in irregular groupings along the axis (A). Occasionally scars of old pinnules could be seen along the axis.
- The pinnules were bilaterally arranged, somewhat irregular and never precisely opposite in location (B). Occasionally they appeared double ranked (C).
- The pinnule bases were equally rounded.
- The diameter of the axis was 450-600  $\mu$ m; the pinnules were 100-250  $\mu$ m wide.
- Chloroplasts, when visible, were 6-8 μm in diameter.
- Gametangia were not observed, but reproduction in *B. plumosa* is reported to be sexual by the production of biflagellate anisogamous gametes and asexual through both the production of stephanokont zoospores, aplanospores, and fragmentation.
- Erect gametophytic thalli alternate with a small filamentous sporophyte.
- The similar *B. pennata* var. *minor* is described as a more delicate alga reported by Norris (2010, p. 81) to be up to 3 cm tall with axes only 210-250 μm wide and pinnules 20-30 μm wide and 1 mm long. It is genetically recognized by Lam & Zechman (2006).
- The similar *B. nana*, described by Wynne (2005, fig. 1) can be 410 µm wide with pinnules up to 260 µm wide and 1 mm long, but it is radially branched.
- Scagel (1966, pl. 6, figs D-G, pl. 7, figs. A-E), Burrows (1991, pl. 8), Lee (2008, p. 64-65), Lee *et al.* (1991, fig.1), Womersley (1984, fig. 96C).

**Bryopsis stolonifera** – A (Asian only, Korea) – Found on 1 debris item – the Clatsop Beach dock (Oct), CBD (BF-130). Not sequenced. Reproduction was not evident, but unusual highly branched out-growths (g) on the filaments may act as propagules. Annual.











- Clatsop Beach Dock (A) with a water-line turf of mostly green algae.
   On the dock, the *Bryopsis* thalli were 0.5-1.0 cm tall and composed of cylindrical coenocytic filaments (45-55 μm in diameter) rarely branched below but producing very irregular to radially formed pinnules (30-35 μm broad) near the apices (B-F). Pinnules narrowed at their attachment to the axes.
- Erect shoots developed from a broad stolon up to 100 μm in diameter.
- Mature pinnules were occasionally cut off by a wall from the axis and cross walls were also rarely seen in other areas of the upright filaments (F, I, arrows with c).
- Numerous ovoid chloroplasts, up to 7-8 μm in diameter, filled the coenocytic tubes and were most congested near the tips.
- Highly branched outgrowths occasionally occurred on the filaments possibly vegetative structures for propagation (H, I, arrow with g).
- Our thalli were narrower than those described by Lee *et al*. (1991, fig.
- 3), but they matched perfectly the drawings in this account.

**Cladophora albida**\*# – **G** (Widespread) – Asia (R, J, C, K, Viet, Phil), IO, Aus, NZ, BC-MX, ENA, Car, SA, Afr, EUR, Med. On 7 debris items (Jan, Mar-Apr, Jul, Oct). Fertile. Ephemeral in the NE Pacific and on debris.

200 µm







- Abundant when present on debris. On HF-2, the species formed a turf covering most of the exposed upper deck and sides of the derelict boat.
- The uniseriate branched thalli were 4-14 cm tall (A).
- Thalli were subdichotomous to unilaterally branched with occasional polychotomous divisions of 3-4 branches (B-G).
- Axial filaments were up to 40 μm wide; tip cells ranged from 20-22 (28) μm.
- Cell width to length ratio was about 1:9.
- Tips were typically rounded, but some were slightly acute (F).
- *C. albida* and *C. sericea* are in the same genetic clade (Boedeker *et al.*, 2016).
- Womersley (1984, figs. B-D), Brodie *et al*. (2007, Fig. 58), Hoek (1976, figs. 247, 265), Hoek & Chihara (2000, figs. 55-57), Cormaci *et al*. (2014, pl. 72, fig. 1-3).
- BB-4 (BF-2), HF2-625, 626, 627, 632, 641, 645 (BF-462), SixR-711, 712 (BF-538). Not sequenced: MC (BF-8), CBD (BF-130), LB2 (BF-228), Fal-755 (BF-652).
- **#** C. Boedeker sequenced and confirmed this species. Boedeker *et al.* (2016).

## Cladophora vagabunda<sup>\*#</sup> – G (widespread) – Asia (J, C, K), IO, Aus, NZ, SA, OR, MX, ENA, Car,

Afr, EUR, Med. On 4 debris items (Mar-Apr, Jul). Fertile. Isomorphic and ephemeral (year around in Europe).



- Thalli on debris were typically floating and intertwined in standing seawater in the hold of some of the debris boats.
- The uniseriate branched thalli consisted of long-celled pseudo-dichotomously branched basal filaments with tightly branched lateral fascicles at the cell nodes.
- Branching within the fascicles was unilateral with 2-5 branches arising from a single cell, and the branches were typically falcate or incurved. This growth form is characteristic of several species in the Glomeratae section of *Cladophora*.
- Since the filaments were broken on collection, length was difficult to determine, but some filaments were several cm or longer. Records show that this species can reach 50 cm long in some habitats (Burrows, 1991).
- The main axial cells were 48-56 (88) μm in diameter with striated walls. The apical cells were of variable diameter: (20) 32-48 (64) μm and often appeared somewhat swollen or club-shaped. The cell length to width ratio varied from 3 to 7.
- Reproduction (not shown) is through biflagellate gametes or quadriflagellate zoospores. Asexual akinetes are also reported to occur.
- van den Hoek & Chihara (2000, figs. 76-79), Schneider & Searles, figs. 63-65), Boederker et al. (2016).
- **\*** HF2-631 (BF-626), Fal-751 (BF-652). Not sequenced: RE (BF-533), SixR (BF-538). # C. Boedeker sequenced and confirmed this species.



### Cladophora vagabunda 2 – C. parriaudii Hoek\* –

This form is known only from France. On 1 debris item (Mar): \* RE-702 (BF-533), a new record for the Pacific. Sterile on debris. It normally reproduces asexually via quadriflagellate zoospores and akinetes.

- The debris material was slightly deteriorated and sterile. The species is reported to reproduce asexually via quadriflagellate zoospores and akinete-like filaments that drop off the thallus (van den Hoek, 1963).
- Debris thalli were small, less than 1 cm in height (A). However, only limited material was available for measuring. Intricate floating spongy masses, 2-7 cm in diameter, were originally described for this species but were not seen in our samples.
- The uniseriate thalli had branching that could be irregular, ternate (B) or unilateral (A) near the thallus tips.
- The branch tips were slightly falcate (C, D), but not expanded in diameter as in some *C. vagabunda*.
- Apical cells were rounded and  $\sim 28 \,\mu m$  in diameter on debris (B). In European material, apical cells are reported to be 12-30  $\mu m$  and axes up to 90  $\mu m$  wide.
- Terminal cells were often long, up to 228 µm (9:1, length to width ratio).
- Van den Hoek (1963, pp. 196-199, figs. 636-646), Boedeker *et al*. (2016, fig. 6).
- Since the species in the *vagabunda* s.l. clade (section Glomeratae) are still under investigation and the taxonomy remains problematic (see Boedeker *et al.,* 2016), we retain *C. parriaudii* as a genetic variant in *C. vagabunda* for this study.

## **Codium fragile subsp. fragile\* – A+ (A widespread Asian invader) –** Asia (R, J, C, K), Aus, NZ, WA, CA,

ENA, Afr, EUR, Med. On 5 debris items (Jan-Apr). Asexual reproduction at base; no gametangia observed. Diplontic and perennial. Transported globally with oysters for aquaculture. Nicknamed "oyster thief" since its settlement, buoyancy and weight causes shellfish to be ripped off the substratum.





- Erect thalli reached 15 cm long on debris (A, F), were terete, spongy in texture, and repeatedly dichotomously branched.
- The branches were ~1 cm in diameter and bore a central filamentous medulla and an outer cortical layer of utricles with mucronate tips (B, arrows, I).
- The utricles often produced hairs (H and J, arrows).
- On debris, the erect thalli arose from a thick, nearly black, turf of intertwined branched filaments (F, C, E, arrows).
- The filaments, ~62 μm in diameter, were coenocytic and contained numerous lenticular chloroplasts, each with 1 (2) small central pyrenoid(s), previously undescribed for the species (G).
- Gametangia were not present, but asexual reproduction was evident by fragmentation and the production of new thalli from the basal turf. The basal filaments formed knots (D) that generated the new young erect thalli (E).
- On the "100 worst invasive species in the Mediterranean" list of Streftaris & Zenetos (2006).
- MC-17 (BF-3). Not sequenced: HF2 (BF526), RE (BF-533), SixR (BF-538), Ump (BF-545).







### Entocladia polysiphoniae – NP-P – Asia

(J), Aus, MX. Epiphytic on *Polysiphonia morrowii* and other algae. Found on 6 different debris items (Mar-Jun, Nov). Sterile. ~Ephemeral.

- This species is likely to be an *Ulvella* species but needs to be cultured and sequenced to determine its correct affiliation (Nielsen *et al.*, 2013).
- The tiny 4-9 μm wide branching filaments are characterized by not coalescing with the neighboring filaments in the endo/epiphytic colony (A).
- The cells in our collections were typically 3-4 μm wide.
- Hairs formed terminally on lateral branch filaments in some colonies (B).
- No reproductive cells were observed, but zoosporangia are reported.
- Ulvella species are known to have isomorphic sexual and asexual life histories.
- Norris (2010, fig. 9B), Noda (1987), Kraft (2007, fig. 11).
- Not sequenced: AB (BF-1), Oys (BF-331), NC (BF-196), Ilw2 (BF-223), LB2 (BF-227, SRT (BF-277).

Similar species: Ulvella viridis – this species has the following features: a more consolidated central filament clustering, 1-2 pyrenoids/cell, filament diameter of 4.4-5.5  $\mu$ m, central cells that are 10.5-16  $\mu$ m, and hairs that are intercalary.

Setchell, W.A. & Gardner, N.L. (1924). Original illustration (C) and description of of *E. polysiphoniae* (p. 718, and pl. 13: fig. 18 with added label).

"Filaments distinct, very crooked, irregularly and much branched, branches often at right angles, arising from the middle of the cell, not coalescing in the center of the thallus to form a disk; cells very variable in shape and size, 4-9  $\mu$ diam., 3-6 times as long as the diameter; chromatophores parietal, pyrenoid single; zoosporangia (?) intercalary; formed from vegetative cells at irregular intervals in the filaments, numerous, up to 24  $\mu$  diam.; thallus up to 1 mm. diam." **Epicladia flustrae cpx.<sup>#</sup>** – G (Widespread) – Asia (R), Aus, BC, ENA, Car, Afr, EUR, Med. On 6 debris items in Oregon and Washington (Jan-May). Fertile. ~Ephemeral. We include *E. phillipsii*, found only in Europe, in *E. flustrae* for this account.







## Halochlorococcum moorei – G (Rare occurrences) – BC

to OR, EUR, probably overlooked elsewhere. Fertile (zoospores) on debris. Ephemeral. On 2 debris item: CBD (BF-130) and SixR (BF-538), October and April. Not sequenced.



- Endophytic in the outer walls of *Blidingia* spp.
- On debris, the single globose cells are (8) 15-32 μm in diameter including a 2 μm thick wall (A-C). Fig. C appears to be young cells.
- The cells contain a single radiating stellate chloroplast with a large central pyrenoid (D).
- A number of cells were fertile, filled with numerous pyriform zoospores, 4-6 µm in diameter (E) discharged through a pore (not seen). The life history is known to be asexual and populations of the species have been found throughout the year in Europe (Burrows, 1991).
- Kornmann & Sahling (1977, figs. 25; 1983, figs. 24-26), Burrows (1991, fig. 9), Taylor (1957, p. 42), Gardner (1917, pp. 382-384), O'Kelly *et al*. (2004, sequence data).

**Ulothrix implexa** (including subflaccida) – G (Widespread) – Asia (R, J, C, K, Viet), Aus, NZ, SA (Ch), AK-CA, ENA, Car, Afr, EUR, Med. On 3 debris items (Feb, Apr) but not sequenced: HF-1 (BF-28), TBT (BF-160), SR (BF-356). Fertile. Heteromorphic with a *Codiolum*-like sporophyte, but both sexual and asexual reproduction is known. Ephemeral.



- Clustered and solitary unbranched uniseriate filaments, 8- 11 (16) μm in diameter (A).
- Chloroplasts are band shaped encircling ~ 2/3 of the cell and containing a single pyrenoid, difficult to see in debris material (B).
- Filaments attached by a single basal cell that is 1:3 in width to length and narrows basally (C, arrow).
- Reproductive gametophytic filaments are broader, reaching 16 μm in diameter and became bead-like (D). Each cell contains up to 16 (32) gametes or asexual zoospores. Propagation also occurs through fragmentation. Burrows (1991) indicates year around in Britain although most abundant in spring and early summer.
- Brodie et al. (2007, figs. 18 & 20), Womersley (1984, fig. 41 (A-C), Lokhorst (1978, figs. 15-17).



Intertwined Ulva compressa and prolifera

### The Ulva complex and its problematic taxonomy

- Species in the genus *Ulva* are common in fouling habitats, such as on tsunami debris, and most species are widely distributed.
- They have been under investigation by numerous scientists and, although molecular sequencing has helped to unravel many of the species, problems still exist.
- On debris, many of the *Ulva* species were difficult to determine. The samples often arrived partially disintegrated and chloroplasts could not clearly be discerned. Moreover, similar species would often closely intertwine, and, if the species were not cleanly separated for genetic analysis, the sequences would be contaminated.
- For our study, we sequenced ITS and *rbcL* in many of our samples and tried to apply the most recent morphological features used in species identification. We were able to recognize the following species: *Ulva australis, U. compressa, U. intestinalis* cpx., *U. lactuca, U. linza, U. prolifera,* and *U. simplex*.
- Due to the morphological variability in some of the *Ulva* species, we have included extra pictures so that the available features can be further assessed.
- With additional study and the use of different genes, it is likely that some of the variant forms will be separated as new or different species (see Hanyuda *et al.*, 2017).

**Ulva australis**  $\mathbf{1}^* - \mathbf{A} + \mathbf{-}$  Asia (R, J, C, K, Phil), IO, Aus, NZ, SA, BC-CA, ENA, Afr, Eur, Med. On 4 debris items (Jan-Jun). Noticed in CA in 2004 and now known from BC-CA. JTMD populations are a different haplotype than the NEP populations. Fertile on debris. Pseudoperennial and isomorphic.



- Distromatic blades, 5-15 cm tall on debris (A, B) with smooth margins and occasional holes in the blades.
- In surface view, the cells were irregularly arranged, polygonal with rounded corners, and contained 1-2 pyrenoids (C).
- Thalli often showed evidence of 2 years of growth (B) with thin outer blades about 32 µm in diameter (D, E) and a basal portion reaching 140 µm or more in thickness due to the production of rhizoids (F).
- Easily confused with *U. lactuca*, a similar species but sequences clearly differentiate the two.
- DNA from *U. australis* (as *U. pertusa*) was used to determine the source in Japan of the Seal Rock debris boat (Miller, 2016).
- Hanyuda et al. (2016, as U. pertusa), Verlaque et al. (2015, p. 280).
- \* BB-2 (BF-2), SR-206 (BF-356); not sequenced: Oys (BF-331), MC (BF-8).



### **Ulva australis** 2<sup>\*</sup>- a *fenestrate* form. Found only on the Seal Rock debris boat. \* SR-6355 (BF-356).





Only pressed material was available for study.

- The bright green blades of this sample were broadly ovate to obovate and deeply split. Each was characterized by many irregular and often ragged perforations (A).
- The blades reached 16 cm in height and 10 cm wide and had slightly ruffled margins.
- They were distromatic and averaged 85  $\mu$ m thick, but reached 120  $\mu$ m near the blade base (B). At the blade margins, the cells were ~35  $\mu$ m tall.
- In surface view, the cells were irregularly arranged, polygonal with rounded corners and 12-15 µm in diameter (C).
- The chloroplasts and pyrenoids were indistinct in our material.

**Ulva compressa** 1<sup>\*</sup>- G (Widespread) - Asia (R, J, C, K), IO, Aus, NZ, SA, Arc, AK-MX, ENA, Car, Afr, EUR, Med. The dominant Ulvoid on debris: on 22 debris items. Reproductive on debris. Isomorphic. Year around. **\*** Sequenced: MC-31 (BF-3); NC-4 (BF-208); LB-109 (BF-285); SR-202, 207, 211, 228 (BF-356); SVB-375, 376, 377, 384, 385, 392 (BF-402); LBF 398 (BF-462); HF2-646, 647, 648 (BF-526); RE-665 (BF-533); + not sequenced specimens.



## **Ulva compressa 2 –** additional pictures.



G. Broad bladed basally branched thalli

H. Clear cap-shaped chloroplasts shown with aniline blue staining

- I. Reproductive filament with 5-6 μm wide unreleased zooids
- J. Young thalli arising from a holdfast

**Ulva intestinalis cpx.** \* – G (Globally widespread) – Asia (R, J, C, K, Viet, Phil), IO, Aus, NZ, SA, AK-MX, ENA, Car, Afr, EUR, Med. On the Seal Rock derelict boat (Apr). \* SR-207 & 248 (BF-356). Reproductive. Isomorphic. ~ Ephemeral. A sequence variant. Similar morphologically to *U. intestinalis*, but ITS and tufA sequences indicate it is genetically close to *U. compressa* sensu Ogawa *et al.* (2013). Further study is required.



**Ulva lactuca**\* – **G** (Widespread) – Asia (R, J, C, K, Phil), IO, Aus, NZ, SA, CenA, AK-MX, ENA, Car, Afr, EUR, Med. On 2 debris items – the Agate Beach Dock (Jun), \* AB-10 (BF-1), the *lactuca* form, and SR (Apr), (BF-356), the *fenestrata* form. Reproductive (orange , yellow-green 9) on debris (Bliding, 1968). Isomorphic. Pseudo-perennial. Asexual reproduction by fragmentation.



• Brodie et al. (2007, fig. 4), Kornmann & Sahling (1977, fig. 28), Kraft (2007, fig. 18), Scagel (1966, pl 31, figs. I-K).

**Ulva linza\* – G (Widespread) –** Asia (R, J, C, K), IO, Aus, NZ, SA, AK-MX, ENA, Car, Afr, EUR. On 17 debris items (Jan-Jun). **\***LC-1 (BF-173), Man-126 (BF461). Fertile on debris. Spring-summer annual with asexual reproduction by both 2 and 4 flagellated zooids.







- <sup>2</sup> Unbranched ribbon-shaped to obovate thalli, often with undulating margins above and straight margins below that narrow to a discoidal holdfast (A, B). In some thalli, the margins are planar.
- The blades are distromatic and loosely adnate with the layers nearly always separated at the margins forming a characteristic loop (C).
- Our specimens were up to 20 cm tall and 40 µm thick.
- Surface cells were polygonal (clearly angular), 9-14 μm wide and isodiametric or 12-17 μm long. Cells were often arranged in short, slightly curved, longitudinal rows (D).
- Cells contained a single parietal chloroplast and 1-2 pyrenoids often obscured by starch grains (E).
- Abundant rhizoidal cells developed at the base (F).
- In fertile material, zooids, 5-7  $\mu$ m in diameter, could be seen developing and releasing from cells near the apices and margins of the thallus (G).
- Kim *et al.* (1991, figs. 3-5), Koeman & Hoek (1984, figs. 2-51), Cormaci *et al.* (2014, pl. 15, figs. 1-4), Norris (2010, fig. 23).

#### **Ulva prolifera** 1<sup>\*</sup> – G (Widespread) – Asia (R, J, C, K, Viet, Phil), IO, Aus, NZ, SA, AK-MX, Cen-A, ENA, Car, Afr, EUR, Med. On 9 debris items (Jan-June). Year around in Japan, ~ephemeral. In the Qingdao bloom of the 2008 Olympics in China. Reproductive. Isomorphic.



- Gregarious tubular to compressed and often adnate thalli arising from a merged holdfast, a basal pad (A, B, K).
- Reaching 15 cm on debris; thalli in Europe can be 50 cm tall.
- The blades are highly variable in morphology: tubular to ribbon-shaped and thread-like to 2 cm broad. Cell layers were 15-30  $\mu$ m thick (L).
- Often branched or proliferous throughout (B, C) but also unbranched. Our ribbon-shaped thalli (A) resembled narrow U. linza, except at the branched base (K).
- Cells were arranged in distinct longitudinal rows in narrow parts of the thallus but varied in broader areas often precisely aligned in the center of the blades but irregular near the margins (D, M-P).
- Cells were polygonal (often cuboidal), occasionally with rounded corners, and 7-12 x 15-24 μm or longer near the base -- with 1 (2-3) pyrenoids, 3-5 μm in diameter (E).
- New branchlets had a single large tip cell (H, I) as described by Koeman & Hoek (1982). The tips later expanded to have multiple terminal cells (J).
- A rhizoidal base was evident (F), but we did not see the spiral base illustrated in Kornmann & Sahling (1977, pl. 32, fig. B).
- Reproduction was observed in 1 small tubular thallus (G, RE-666). Unreleased zooids were globose and 4-6  $\mu$ m in diameter.
- Ogawa *et al.* (2013), Koeman & Hoek (1982, figs. 34-53), Womersley (1984, figs. 48D, 49H), Brodie *et al.* (2007, fig. 42), Bliding (1963, Types I-III, figs. 19-22), Kim *et al.* (2010, fig. 22), Cormaci *et al.* (2014, pl. 18, figs. 4-7).
- ★ LBF-131 and 132 (BF 462); not sequenced: AB (BF-1), MC (BF-8), HF1 (BF-28), Fish (BF-40), TH (BF-134), Oys (BF-331), SR (BF-462).

## **Ulva prolifera 2 –** Additional pictures

H–J. Young blade tips (H-I with enlarged tip cell). K. Compound holdfast showing merged basal pad (from A on previous page). L. Optical section of blade. M-N. Blade cell arrangements (M-near the blade center, N-near the margin). O-P. Narrow tube cell arrangement with faint pyrenoids



**Ulva simplex** sensu Ogawa 1<sup>\*</sup> – G (Spotty distribution) – Asia (J), R-Arc, EUR, Afr (Morocco). Only on 1 JTMD item – the Waldport debris boat (May). \* Wal-5 (BF-196). Fertile. Summer annual in Japan (Ogawa, 2013). Isomorphic.



### **Ulva simplex sensu Ogawa 2 – Additional pictures.** Branching, branch tips, and reproduction.



In our material, we did not see the twisted base reported in: (1) the type material of *U. simplex* from Murmansk, (2) Typus I of Bliding (1963, fig. 19, as *E. prolifera*), Vinogradova (1979, pl. XVI, figs. 1-5), (3) Cormaci *et al.* (2014, pl. 21, figs. 1-4), and (4) Koeman & Hoek (1982, figs. 2-33). Our material had a straight proliferous base (J) and matched most closely the specimens described in Ogawa *et al.* (2013, figs. 12-16).

# **Cyanobacteria on JTMD**

The taxonomy of the blue-green bacteria is still in transition. Although molecular biology has changed many of the names, numerous species still require study. The identifications that I have provided for the JTMD species follow Komárek & Anagnostidis (1998, 2005) and Komárek (2013). These names will undoubtedly change with time, but the pictures will help to provide a reference for the new names. The wild collections in our study were mixed and difficult to isolate, and culturing facilities were not available. So sequencing was not carried out. **#** S. Shalygin of John Carroll University (Ohio) and the Kola Science Center (Kirovsk, Russia) kindly examined my pictures to confirm that my provisional species and complexes were correct.

### Chroococcus submarinus and Lyngbya confervoides – G – Both globally

widespread and common in turf on JTMD. Reproduction by the release of single cells or hormogonia (short filaments). Ephemeral.



**Chroococcus submarinus** (A). Irregular clusters of spherical cells 15-25 µm cells in diameter in a common slime. Halophilic. (marine/freshwater). Only found in the turf of the HF2 debris boat under *Polysiphonia scopulorum var. villum.* Komárek & Anagnostidis (1998. fig. 400).



**Lyngbya confervoides** (B-C). Clustered trichomes 10-16-25 µm wide in a clear sheath, possibly consisting of more than 1 species. Very short cells, apical cells rounded. Marine. Found in turf on 7 debris items, typically boats and docks where a mature community of seaweeds had developed. Release of hormogonia was evidenced through the occurrence of empty sheaths (arrow). Setchell & Gardner (1919, p. 77-78), Komárek & Anagnostidis (2005, fig. 945).

## Calothrix scopulorum cpx. and Scytonematopsis crustacea cpx. – G – Globally

widespread species common on debris. The trichomes of both possess narrow sheaths and basal heterocysts, and, in one species, heterocysts are also scattered throughout the trichome. Ephemeral, found year around. Asexual reproduction by hormogonia, short filaments created by cell death that slip out of the sheath.



#### Calothrix scopulorum cpx. (A-C) –

- Dark olive-green to black cylindrical trichomes with colorless to brown sheaths and 1-2 basal heterocysts.
- Trichomes are 8-15 μm wide, constricted at the cross-walls near the base and smooth above tapering slightly to a rounded tip.
- Epilithic and epiphytic.
- Our species is typically brown or dark olive, around 15 μm near the base and often with incised basal cells. Common in early fouling stages on plastic, often embedded in crusts of *Tsunamia transpacifica*. Similar to *Calothrix confervicola* as illustrated in AlgaeBase.org.
- On 7 debris items.
- Komárek, J. (2013, fig. 237), Setchell & Gardner (1919, p. 96).

### Scytonematopsis crustacea cpx. (D-F) –

- Trichomes of various colors with sheaths that are hyaline to light brown.
- The species has both basal and intercalary heterocysts.
- Trichomes are 7-25 μm in diameter and attenuate gradually toward the tips. False-branching is rare but may occur.
- Epilithic and epiphytic, and marine.
- Our species had narrow trichomes, 7-12 μm diameter and both basal and intercalary heterocysts. No false branching was observed. This species was found in turf, in the primary fouling areas of boats and also epiphytic on macroalgae (*Sphacelaria*).
- On 8 debris items.
- Komárek, J. (2013, fig. 165), Humm & Wicks (1980, fig. 29).

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## **Appendix 1 – Japanese Debris Items**

Japanese Tsunami Marine Debris (JTMD) items collected for the algal project, including their BF-numbers, state, site name, collection number abbreviations, collection date and year, and item type. All collections were made between Mosquito Creek, WA, and Sixes River, OR. Key: Abbrev.= collecting number abbreviation, BF # = biofouling number of Carlton *et al.* (2017, Table S1), OR = Oregon, WA = Washington.

DF #	State	Site Name	Abbroy	Collection		ltom	
DF #			Abbrev.	Date	Year	ntem	
BF-1	OR	Agate Beach	AB	5-Jun	2012	dock	
BF-2	WA	Ilwaco, Benson Beach	BB	15-Jun	2012	boat	
BF-8	WA	Mosquito Creek	MC	5-Jan	2013	dock	
BF-23	OR	Gleneden Beach, Salishan	GB	6-Feb	2013	boat	
BF-28	OR	Horsfall Beach	HF1	21-Feb	2013	boat	
BF-36	OR	Florence, Muriel Ponsler Park	MP	14-Mar	2013	boat	
BF-39	OR	Cannon Beach, S Jockey Cap	SJC	22-Mar	2013	boat	
BF-40	WA	Long Beach (fish boat)	Fish	22-Mar	2013	boat	
BF-50	OR	Coos Bay North Spit	CBS	22-Apr	2013	boat	
BF-58	OR	Clatsop Beach	CBB	30-May	2013	boat	
BF-59/61	OR	Nye Beach	Nye	30-May	2013	post & beam	
BF-108	OR	Cape Arago, Lighthouse Beach	CA	11-Jul	2013	post & beam	
BF-130	OR	Clatsop Beach	CBD	9-Oct	2013	dock, pontoon	
BF-134	WA	Twin Harbors State Park	TH	17-Jan	2014	boat	
BF-135	OR	Yachats	Yac	18-Feb	2014	boat	
BF-160	OR	Tillamook Bay spit	TBT	26-Apr	2014	tree	
BF-171	OR	Tillamook Bay spit	TB	25-Apr	2014	post & beam	
BF-173	OR	South Beach, Lost Creek	LC	27-Apr	2014	buoy	
BF-188	OR	Cape Lookout Beach	CL	3-May	2014	boat	

## Appendix 1 (continued) – Japanese Debris Items

DE #	State	Site Name	Abbrev.	Collection		ltom
<b>DГ #</b>				Date	Year	llem
BF-196	OR	Waldport	Wal	12-May	2014	boat
BF-208	OR	Cape Arago, North Cove	NC	19-May	2014	boat
BF-223/224	WA	Long Beach, Ilwaco	llw2	29-May	2014	boats 2
BF-227/228	WA	Long Beach	LB2	5-Jun	2014	boats 2
BF-234	OR	South Beach	SBT	9-Feb	2013	tank
BF-235	WA	Long Beach	LBT	1-Mar	2013	tire
BF-277	OR	Seal Rock	SRT	30-Nov	2014	tote
BF-285	WA	Long Beach	LB	4-Jan	2015	boat fragment
BF-288	OR	Beverly Beach	Bev	20-Jan	2015	tote, pallet
BF-293	WA	Long Beach, Seaview	SV	28-Jan	2013	pipe
BF-331	WA	Oysterville	Oys	14-Mar	2014	boat
BF-356	OR	Seal Rock, in ocean	SR	10-Apr	2015	boat
BF-397	WA	Long Beach	LBD	1-May	2015	dock, pontoon
BF-402	WA	Long Beach, Seaview	SVB	12-May	2015	boat
BF-461	OR	Manzanita	Man	2-Mar	2015	tote, basket
BF-462	WA	Long Beach	LBF	4-Jan	2015	float
BF-500	WA	Long Beach	LBT	16-Feb	2016	tote
BF-526	OR	Horsfall Beach 2	HF2	22-Mar	2016	boat
BF-533	OR	Roads End	RE	28-Mar	2016	boat
BF-538	OR	Sixes River mouth	SixR	16-Apr	2016	boat
BF-545	OR	Umqua River mouth	Ump	26-Mar	2016	boat
BF-652	OR	Falcon Cove beach	Fal	26-Jul	2016	boat
BF-656	OR	Quail Street	QS	26-Mar	2016	carboy