#### **Benthic Marine Algae on Japanese Tsunami Marine Debris** – a morphological documentation of the species

### Part 2. The Brown Algae

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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 **Copyright:** 2017, CC BY-NC (attribution, non-commercial use). For photographs, please credit G.I. Hansen or those noted on the slides.

**Printing:** For better pdf printing, please reduce to letter (11" x 8.5") size, landscape orientation.

#### Citations to be used for this series:

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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. (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. <u>https://doi.org/10.1016/j.marpolbul.2017.06.053</u>
- Hansen, G.I. (2013). Some Marine Algae on Tsunami Debris. OSU Scholars Archive, Corvallis, a poster. <u>http://ir.library.oregonstate.edu/concern/defaults/ns064b84v</u>



Seaweeds on the Tohoku coast near Misawa, Japan, including Saccharina japonica and Undaria pinnatifida.

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

#### Special codes provided in each description:

- Definitions of terms and abbreviations (not provided below) are given in Part 1 of this series.
- \* = Species that have been sequenced. We also list: the genes sequenced (p. 4), the debris item numbers (Appendix 1), and the collection numbers (with the descriptions).
- # = 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 <u>www.AlgaeBase.org</u> (accessed July 2017). <u>Distribution Codes</u>: G (Globally widespread) = species that appear to be naturally widespread globally, occurring in different oceans and on multiple continents; 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 this study was 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 debris algae was generously 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 debris item database and provided his BF item numbers to all JTMD researchers. Cynthia Trowbridge gave valuable comments on the overall project. 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 the following taxonomic experts for helpful advice and identification assistance with the brown algae: S.M. Boo, S.G.A. Driasma, S. Loiseaux-de Goër, G.T. Kraft, D.G. Müller, A.F. Peters, W.F. Prud'homme van Reine, and J.R. Sears.

**Contents:** The Brown Algae – A Checklist of the Species on JTMD, The Species Descriptions, References, and Appendices 1-3.

### The Brown Algae

#### A Checklist of the Species

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

#### KEY:

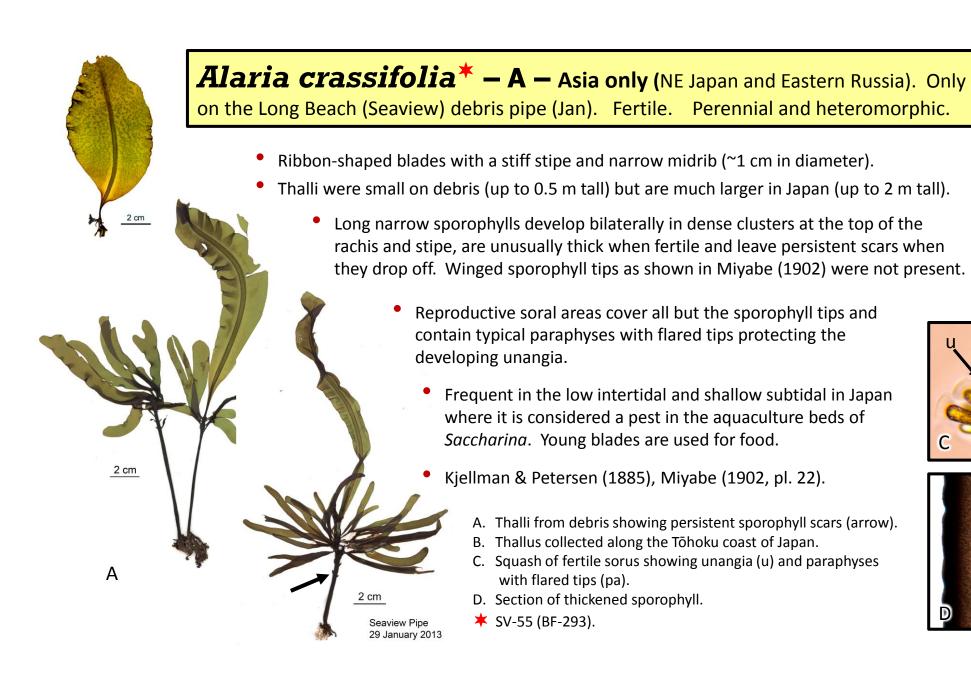
- Pg = page number
  JTMD = Japanese Tsunami Marine Debris
  Global = general global occurrence
  A = Asian only
  A+ = Asian but also introduced by human activities globally
  NP-P = Northwest and Northeast Pacific, some with Alaska and S. Pacific occurrences
  G = globally widespread, including species with rare global occurrences
  NEP = Northeast Pacific occurrence (Washington
- to Mexico)
- y = species occurring in the NEP
- y-s = yes but only California and/or Mexico
- n = species not known in the NEP
- DNA = genes sequenced or expert assistance
- Gene codes: (1) cox1, (2) cox3, (3) ITS rDNA,
   (4) rbcL, (5) psbC
- \* = further study required
- # = monographic expert assistance

#### **The Species Descriptions**

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

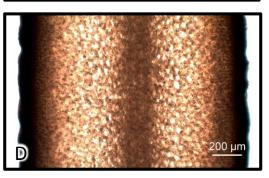


Pg	Brown Algae on JTMD	Global	NEP	DNA
5	Alaria crassifolia Kjellman in Kjellman et Petersen	А	n	(4)
6	Analipus japonicus (Harvey) M.J. Wynne	NP-P	у	(2)
7	Costaria costata (C.Agardh) De A. Saunders	NP-P	ý	(2)
8	Desmarestia japonica H. Kawai et al. in Yang et al.	А	n	(1, 3)
9	Desmarestia viridis (O.F. Müller) J.V. Lamouroux	G	у	
10	The Ectocarpus and Kuckuckia conundrum			
11	Ectocarpus acutus Setchell et N.L. Gardner	G	у	(2)*
12-13	Ectocarpus arctus Kützing	G	n	
14	Ectocarpus crouaniorum Thuret in Le Jolis	G	n	(2, 4)
15	Ectocarpus cf. penicillatus (C. Agardh) Kjellman	G	n	(2)*
16	<i>Ectocarpus siliculosus</i> var. <i>pygmaeus</i> (J. Areschoug) T. Gallardo cpx.	G	у	(2)*
17-18	Feldmannia irregularis (Kützing) Hamel	G	ý	(2)*
19-20	Feldmannia mitchelliae (Harvey) HS. Kim	G	y	(1, 2, 4)
21	Hecatonema cf. streblonematoides (Setchell et N.L.Gardner) Loiseaux	NP-P	ý	#
22	Hincksia granulosa P.C. Silva in Silva, Meñez et Moe	G	y	
23-24	Hincksia ovata (Kjellman) P.C. Silva in Silva, Meñez et Moe	G	y	
25	Hincksia sandriana (Zanardini) P.C. Silva in Silva, Meñez et Moe	G	ý	
26-28	Kuckuckia cf. spinosa (Kützing) Kornmann cpx.	G	n	(2)*
29	Mutimo cylindricus (Okamura) H. Kawai et T. Kitayama	A+	y-s	(2)
30-31	Petalonia fascia (O.F. Müller) Kuntze cpx.	G	y	(1, 2, 4)
32	Petalonia zosterifolia (Reinke) Kuntze	G	n	(1, 2, 3, 4)
33	Petroderma maculiforme (Wollny) Kuckuck	G	у	(4)
34	Protectocarpus speciosus (Børgesen) Kornmann in Kuckuck	G	n	#
35	Pseudolithoderma cf. paradoxum Sears et Wilce	G	n	#
36-37	Punctaria latifolia Greville cpx.	G	у	(1, 2, 4)*
38	Saccharina japonica (Areschoug) Lane, Mayes, Druehl et Saunders	A+	n	(2, 3)
39	Scytosiphon gracilis Kogame	A+	y-s	(1, 2, 4)
40-41	Scytosiphon lomentaria (Lyngbye) Link cpx.	G	y	(2)
42	Sphacelaria rigidula Kützing	G	ý	(4, 5) #
43	Sphacelaria solitaria (Pringsheim) Kylin	G	n	-
44-45	Undaria pinnatifida (Harvey) Suringar	A+	y-s	(2)
46-48	Unknowns		-	-
49-56	References. 57-58 Appendix 1. Tsunami Debris Items. 59 Appendix	2. Ectocarpi	us – Kucku	ckia cox-3
60-61	Appendix 3. The JTMD <i>Ectocarpus</i> and <i>Kuckuckia</i> samples sequenced.	1		





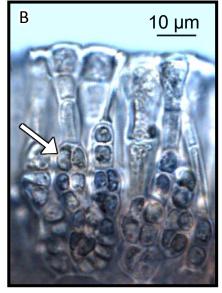




#### **Analipus japonicus**\* – NP-P – Asia (R, J, K) and AK-CA. Abundant on 2 debris items (May, Jun). Fertile. Pseudo-perennial (the crust overwinters) and isomorphic. With epiphytic Pyropia yezoensis.



- Erect flattened to tubular axes reaching 10-20 cm. in height, ~1 mm in diameter, and arising from a perennial thick canaliculate crust.
  - Axes are covered by abundant short unbranched laterals to 1 cm in length and often epiphytised (A).
    - On debris, including the Agate Beach Dock, the species bore intercalary, often biseriate, plurangia (B, arrow).
    - Although the same morphological species occurs on both coasts of the N. Pacific, studies of Nelson (1980) have shown that populations in the NE Pacific bear almost entirely plurangia while those in Asia bear both plurangia and unangia.



Section through fertile branch with intercalary plurangia (arrow).

- Comparative sequencing has indicated that the NE and NW Pacific populations are closely related, but that 6 base pairs in the *cox*3 gene do separate them (Hanyuda *et al.*, 2017). The debris specimen was the Japanese haplotype.
- Abbott & Hollenberg (1976, fig. 146); Nelson (1982a & b), Tokuda et al. (1994), Okamura (1908, pl. XXXV, as Chordaria abietina).
- \* AB-5778 (BF-1). Not sequenced, Nye (BF-59).

**Costaria costata\* – NP-P –** Asia (J, K), AK-CA. Only on the Agate Beach derelict dock (Jun). "5-mid-ribbed kelp". A sequence variant of NEP populations. Sterile. Heteromorphic and usually annual.

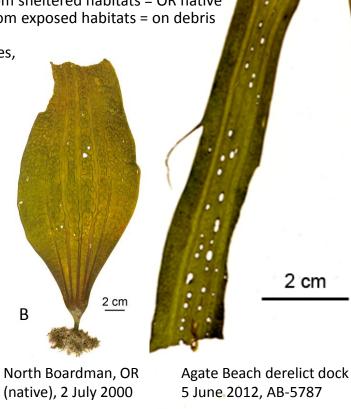


Costaria bed on the Tohoku coast of Japan

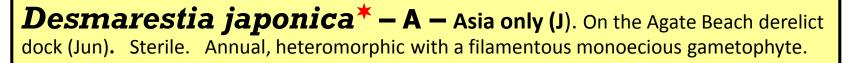
- "Searsucker" kelp reaching 2 m long in Japan (on debris limited limited to 1 m) with a large digitate holdfast (A).
- With 5 midribs, 3 on top and 2 below and bullations typically forming between the ribs.
- The 2 forms of Miyabe & Nagai (1940) were observed: B – f. latifolia, described as from sheltered habitats = OR native C - f. *cuneata*, described as from exposed habitats = on debris

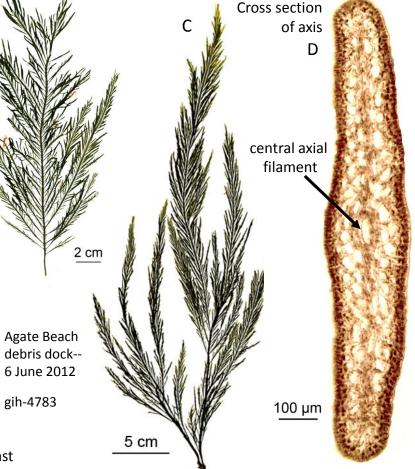
В

- The debris thalli had long stipes, acute bases, and long narrow blades, 2-5 cm wide (C). Oregon thalli had broader,
- more ovate blades with with rounder bases (B).
- Postels & Ruprecht (1840, tab. XXIV), Okamura (1925, pl. CCXXVI), Nagai (1940).
- **\*** AB-5787 + pressing (BF-1) = sequence variant of OR & NEP material. (Sequence variants occur on both coasts, but we are not yet certain how they relate to morphology.)









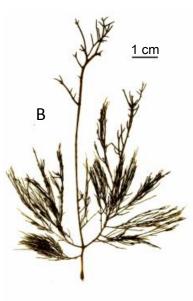
- Erect, highly branched dark brown thalli up to 1 (2) m long in Japan (A).
- Narrow, opposite branches, 2-6 mm in diameter, with a central midrib (B-C).
- Terminal and marginal trichothallic filaments that initiate growth were not apparent in the JTMD material.
- Axes and branches contain a large central axial filament surrounded by corticating filaments that make up the pseudoparenchymatous structure (D).
- Vacuoles contain sulfuric acid.
- Similar morphologically to widespread D. ligulata, but molecular sequences, chromosomes, and subtle culture features indicate that this is a separate species unique to Japan.
- Yang *et al*. (2014), Peters & Müller (1986).
- AB-5A (BF-1); gih-5783 pressings at left.

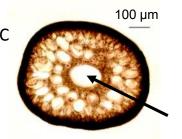
# **Desmarestia viridis** – **G** (Globally widespread) – Asia (R, J, C, K), Arc, AK-WA, CA-MX, SA, A-Arc, ENA, EUR, Medit. Only on the Agate Beach derelict dock (Jun). Sterile. Annual and heteromorphic.



Possibly requires a fully saline sheltered habitat, explaining its rarity in Oregon.

- "Stringy acid weed" a sulfuric acid containing *Desmarestia*.
- On the "100 worst invaders in the Mediterranean" list of Streftaris & Zenetos (2006).
- A highly branched thallus typically reaching 0.5 m in length in the NE Pacific (A, B).
- Trichothallic growth and a large central axial filament characterize the genus (C).
- Terete to slightly flattened axes, 1-2 mm broad, typically with primarily opposite, delicate and highly divided lateral branches.
- Abbott & Hollenberg (1976, fig. 187), Fletcher (1987. figs. 85, 86), Boo *et al*. (2010), Verlaque *et al*. (2015), Peters *et al*. (1997).
- AB-11 (BF-1), but the DNA extraction failed.





Cross section of axis revealing large central axial filament (arrow).

Eroded specimen from the Agate Beach debris dock. (BF-1)

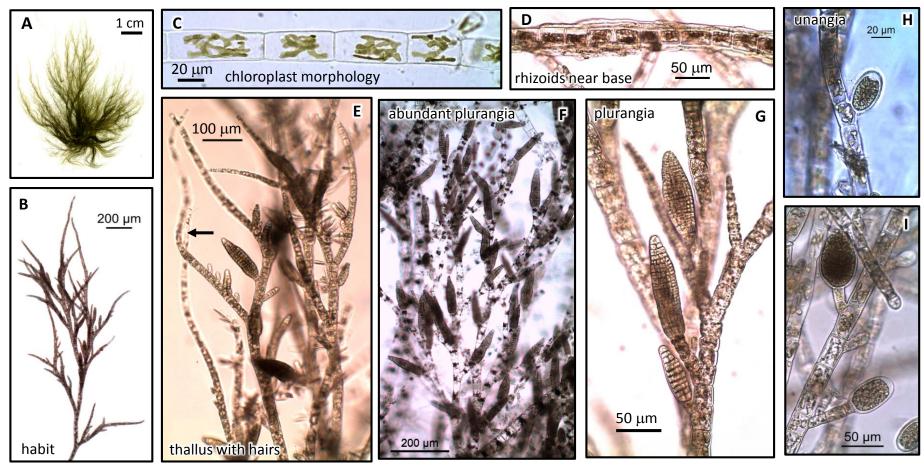
## The *Ectocarpus* and *Kuckuckia* Conundrum

The species in *Ectocarpus* and *Kuckuckia* were not only the most abundant on debris but also the most difficult to identify. Due to their morphological similarity, frequent deteriorated state and problematic genetic characterization, many of the final identifications in this group of species are approximate.
 Some of the problems encountered with the identifications:

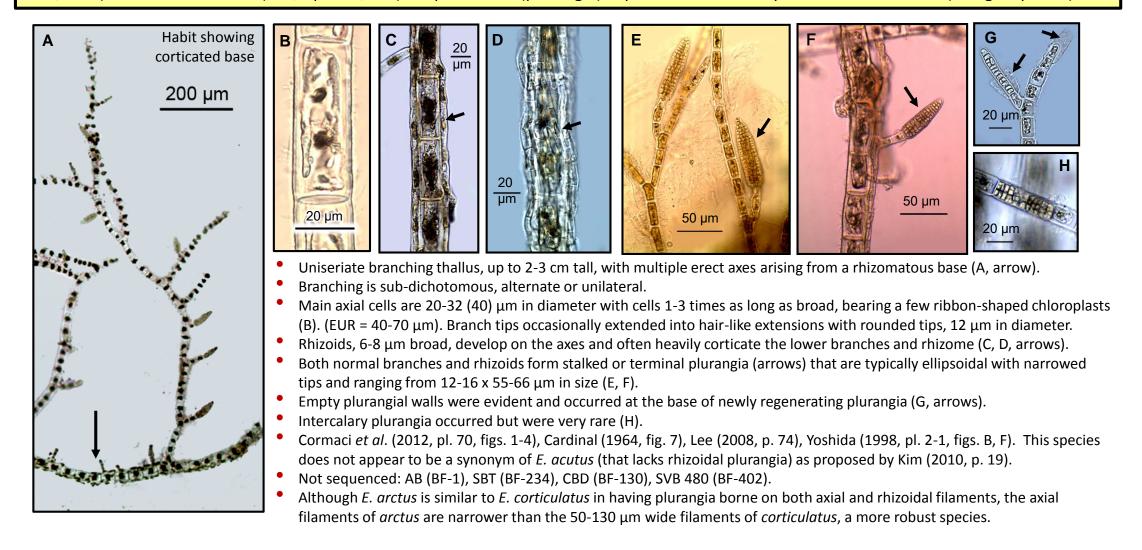
- Only partially disintegrated material without visible chloroplasts (important in generic identification) was present for some species.
- The literature descriptions occasionally conflict. Over the years, different authors have applied slightly different features to identify the species and some have provided incorrect synonymies. These errors are then passed along by others in identification keys, etc. For this study, we provide references for the species identifications and try to use the following traditional features (when available) for our morphological identifications: chloroplast structure; shape, size and location of plurangia and unangia; filament diameter, cell width to length ratio, and shape of the filament apex; rhizoid presence; and the occurrence and abundance of hairs (differentiating phaeophycean hairs and pseudohairs when possible).
- Field collections and separation of species. In field collected material, species are often intermixed, and they must be separated under a microscope for sequencing. For species that intertwine and attach to one another, separation is difficult. Although specific genes can be targeted for some species in these mixed samples, this is not always successful so contamination might account for some of our DNA discrepancies.
- Sequencing data could not be obtained from some samples due to their small size or to extraction difficulties.
- Possible sequence errors in GenBank. The sequences deposited in GenBank occasionally come from misidentified species. For easily confused species, it is important that sequences (preferably multiple gene sequences) are obtained and deposited in GenBank from holotype (or lectotype) material so that truly accurate identifications are possible. Although research is underway in this area, it has happened for very few species in the *Ectocarpus* complex. We are looking forward to having access to the sequences of all of the holotypes (or lectotypes) in this group and also to additional culturing research on the species that will help us determine the stable morphological features useful in identification.
- Determining Ectocarpus and Kuckuckia species names for this project. Hanyuda et al. (2017) have produced a phylogenetic tree of the Ectocarpus complex, including the JTMD samples. JTMD species are in 5 of the clades, but names could be provided for only 2 of these: Ectocarpus crouaniorum and Kuckuckia spinosa. The morphological features of the JTMD samples grouped in these 2 known clades and in the 3 unnamed clades have been used, along with the morphological literature, to provide tentative names for the species. See Appendix 2 and 3 for this Ectocarpus and Kuckuckia tree and for the JTMD specimens used in this study.
- It is our hope that the final naming of the species in this abundant and complex group will be facilitated by our JTMD studies, including both the sequences provided by our molecular study (Hanyuda *et al., 2017*) and by the photographic documentation in this account.

**Ectocarpus acutus** - G (Globally widespread) - Asia (C, K), BC-MX, SA, Afr. On 12 debris items (Jan-May). Reproductive (plurangia, unangia). Spring ephemeral, isomorphic. \* Wal-1 (BF-196); RE-686, 690, 699 (BF-533); Bev-113 (BF-288). Not sequenced: GB (BF-23), MP (BF-36). All are in Hanyuda clade *E*. sp. 2 (see Appendix 2 and 3). Kim & Lee (1992a, figs. 9, 11 A-D as *penicillatus*), Kim (2010, fig. 2), Setchell & Gardner (1922, figs. 36-39).

Thalli are 4-5 cm tall with alternate branching and secund branches near tips, laterals that are often slightly reflexed, pointed apices, and occasional hairs (A, B, E). Chloroplasts are ribbon-shaped (C), and rhizoids envelope the axis near the base (D). Plurangia are cylindroconical with rounded tips (E-G). Unangia are ellipsoidal (H, I). Both are stipitate or sessile and often occur near the base. Diameters: axes (16-40 µm), rhizoids (8 µm), plurangia (16-22 x 70-122 µm), unangia (20-40 x 38-60 µm).

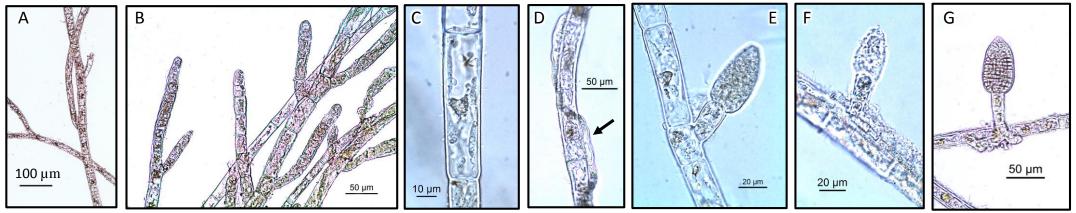


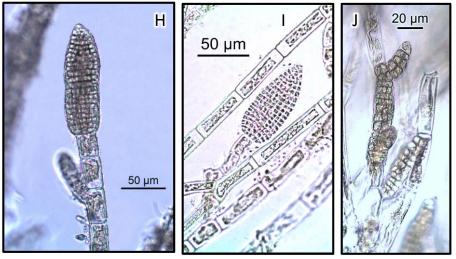
**Ectocarpus arctus 1** (including *E. siliculosus* var. arctus) – **G** (Globally widespread) – Asia (R, J, K), ENA, EUR, Med). On 6 debris items (Feb, Apr-Jun, Oct). Reproductive (plurangia). Ephemeral. Similar species: *E. corticulatus* (a larger species).



### **Ectocarpus arctus 2<sup>\*</sup>** – but cox3 sequencing indicated the *Kuckuckia* clade of Hanyuda. **\***Wal-2 (BF-196) was

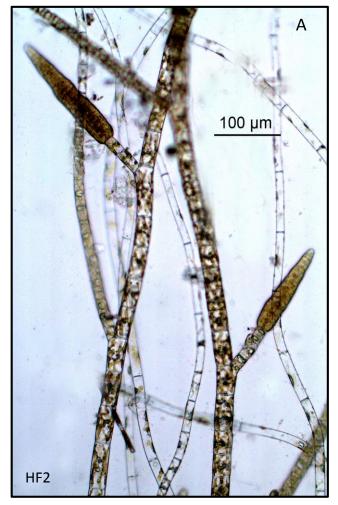
partially disintegrated, but it had the clear diagnostic characteristics of *E. arctus:* narrow filaments and small plurangia born on both normal and enveloping rhizoidal filaments. The sample appeared pure, but it sequenced as a *Kuckuckia spinosa*. There are several possibilities for this: (1) the genus may be highly variable morphologically, (2) the *cox*3 gene may not separate the *Kuckuckia* clade well, (3) sample contamination. Note that the synonymy of *Ectocarpus spinosa* Kuetzing (1843) (=*Kuckuckia*) with *Ectocarpus arctus* Kuetzing (1843) was suggested by Ardissone (1886) but was later rejected by Kuckuck (1958, p. 174).

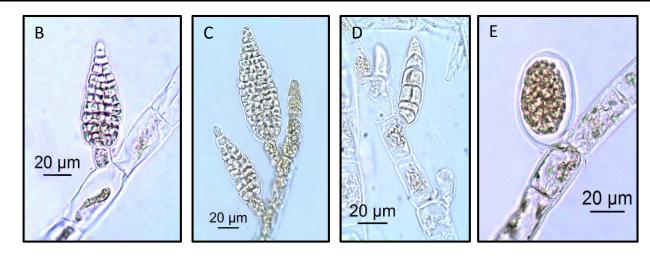




- Although disintegrating and with *arctus* morphology, *cox*3 sequencing of these samples clearly indicated this species was a *Kuckuckia*.
- Uniseriate filamentous thalli had scarce subdichotomous branching below and clustered branches near the rounded tips (A, B).
- Filaments were 14-20 (32) μm broad and straight to barrel-shaped. *E. arctus* in Japan measures 24-32 (40) μm wide and in EUR, 40-70 μm; *E. corticulatus* is 50-100 μm.
- Chloroplasts were narrow, ribbon-shaped, branched, and few in number (C).
- Corticating filaments lightly enveloped some of the axes (D, arrow).
- Plurangia were ovate to cylindrical, 28-40 x 68-92 μm, and formed on both lateral branches (E, H) and on corticating rhizoids (F, G, I). They were rarely intercalary (J).
- No phaeophycean hairs with basal mersitems, known for *Kuckuckia*, were visible.
- **\*** Wal-2 (BF-196) is shown.

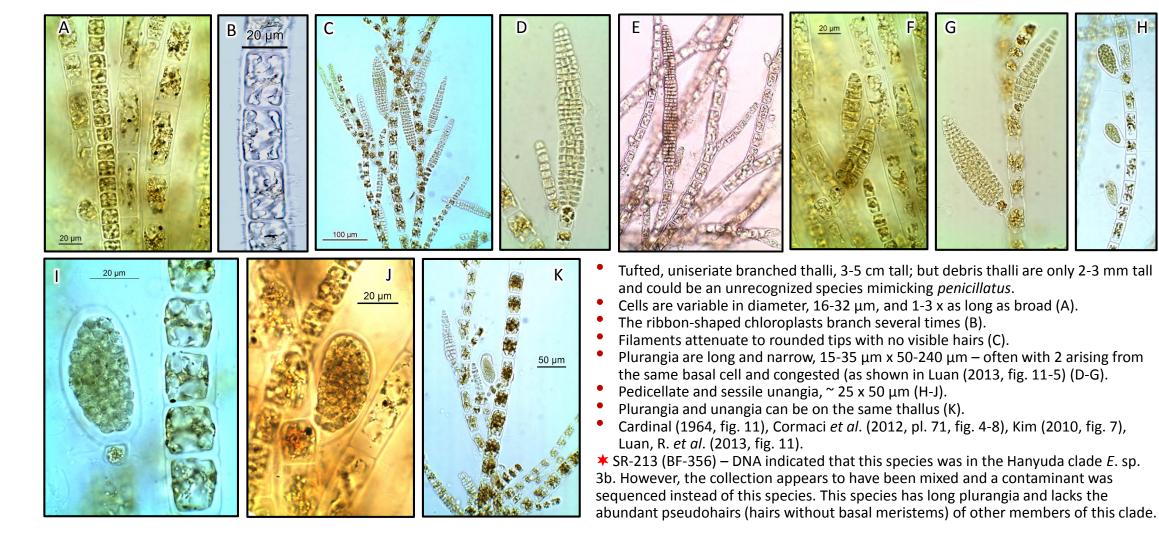
**Ectocarpus crouaniorum**\* – **G** (Globally widespread) – Asia (K), EUR, Med, SA (Ch), but probably overlooked elsewhere. On 5 debris items (Jan-Apr). Ephemeral, isomorphic. Reproductive (plurangia and unangia). \* NC-5 (BF-208). Not sequenced: HF2-650 (BF-526), Fal-932 (BF-652).





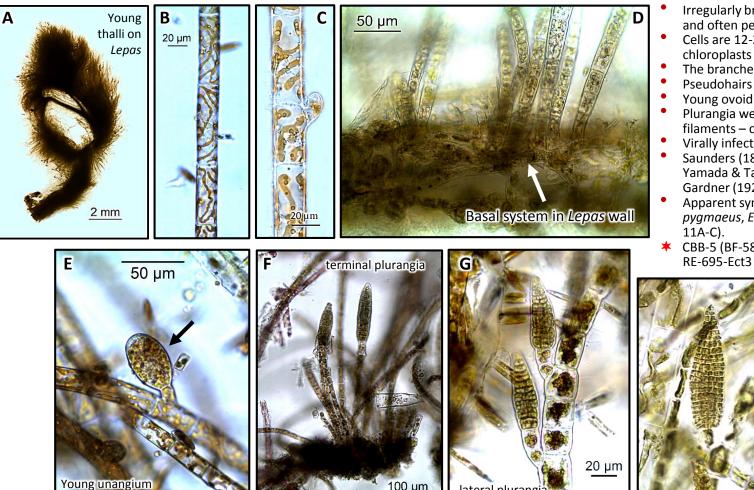
- Unilateral, alternate or irregularly branched filaments often with wide-angle branching that can reach 1-5 cm. in height (A). Rhizoids (not seen) occur near the thallus base.
- Axes were 30-40 µm in diameter near the base, 18-24 µm above and continue to narrow extending into long hyaline hairs, 11 µm in diameter.
- Cells were typically 1.5-2 times as long as broad and contained vertical ribbon-shaped plastids.
- Plurangia were terminal on long laterals (A), diagnostic for the species, and on shorter pedicels (B,C) or occasionally sessile in our material; 30-50 x 80-150 μm. Plurangia also occasionally had narrowed conical apices, a feature only shown in Cardinal (1964, fig. 8) and Cormaci *et al.* (2012, pl. 67, fig. 4).
- Unangia (E) were sessile and 35 x 50 μm in size (small for the species).
- Cardinal (1964, fig. 8 as *confervoides* var. *crouanii*); Cormaci *et al.* (2012, pl.67, figs. 1-4); Hamel (1939, fig. 2f), Kuckuck (1964, p. 12/228) and Peters *et al.* (2010, figs. 5-10).
- Similar species drawings of *E. parvus*: Saunders (1898, pl. XXII, fig. 1., *E. siliculosus parvus* var. nov.), Norris (2010, fig. 89).

# **Ectocarpus cf. penicillatus**<sup>\*</sup> – G (Globally widespread) – Asia (J, C, K), SA, ENA, EUR, Med, A-Arc. On 6 debris items as hemispherical epiphytic tufts on *Scytosiphon* (Mar, May-Jul, Oct). Fertile. Isomorphic, ~ephemeral. Characterized by slender plurangia up to 240 µm long and cylindrical or tapering to a point. Very problematic with mixed descriptions in the literature.



### Ectocarpus siliculosus var. pygmaeus cpx.\* - G (Globally widespread) - Asia (J, K, C, with

synonyms listed below), AK-MX, SA (Br), ENA, EUR, Med. On 22 debris items (Jan-Jul). On Lepas anatifera, the pelagic goose neck barnacle, and on other algae. Reproductive (plurangia and unangia). Ephemeral and Isomorphic.

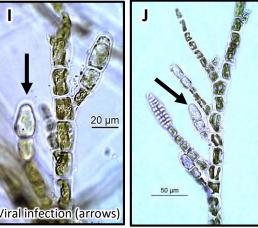


100 µm

lateral plurangia

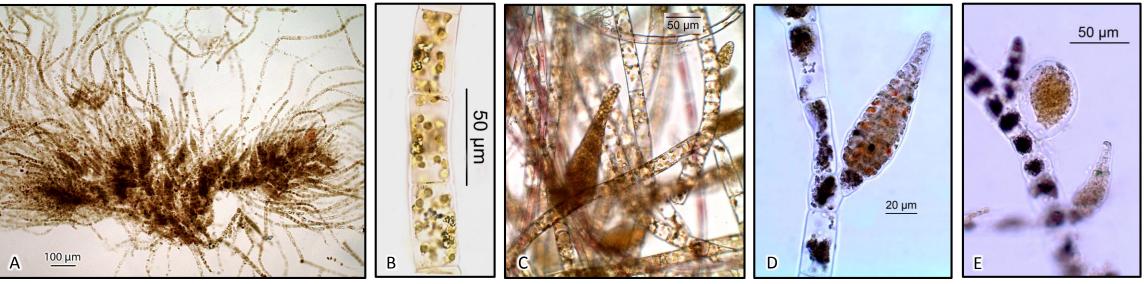
- Irregularly branched filaments, ~0.5-1.5 (3.0) cm tall on debris, epiphytic on and often penetrating the walls of *Lepas* and also larger algae (A).
- Cells are 12-25 µm in diameter, 1:1-1:3, and have variable band-shaped chloroplasts (B. C).
- The branched basal filaments penetrate into the host wall (D).
- Pseudohairs (without noticeable meristems) were present in larger thalli.
- Young ovoid unangia (38x44 µm) were present (E).
- Plurangia were terminal on younger filaments but often lateral in on mature filaments – cylindro-conical and 20-30 x 50-100 µm (F. G. H).
- Virally infected plurangia were found in one collection (I, J, arrows).
- Saunders (1898, pl. XV, figs. 5-9), Cormaci et al. (2012, pl. 72, figs. 1-3), Yamada & Tanaka (1944, fig.3, as E. yezoensis), Ohta (1973, fig.6), Setchell & Gardner (1922, pl. 48, fig 32, as *commensalis*), Cardinal (1964, fig. 10).
- Apparent synonyms: E. commensalis (non parvus), E. confervoides var. pygmaeus, E. dimorphus, E. yezoensis, Kuckuckia kylinii of Kim (2010, fig.
- CBB-5 (BF-58), LB-108 (BF-285), SR-223 and 234 (BF-356), LBF-405 (BF-462), RE-695-Ect3 and 698 (BF-533), Hanyuda clade E. sp. 3a.

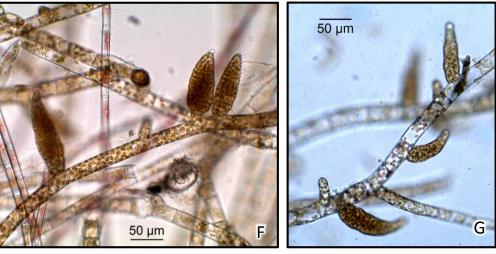




## **Feldmannia irregularis** 1<sup>\*</sup> – G (Globally widespread) – Asia (R, J, C, K, Phil), Aus, NZ, SA, AK-CA, ENA, Car, Afr,

EUR, Med, Arc. In turf on 3 derelict boats (May, Mar). Fertile (plurangia, unangia). Ephemeral and isomorphic.

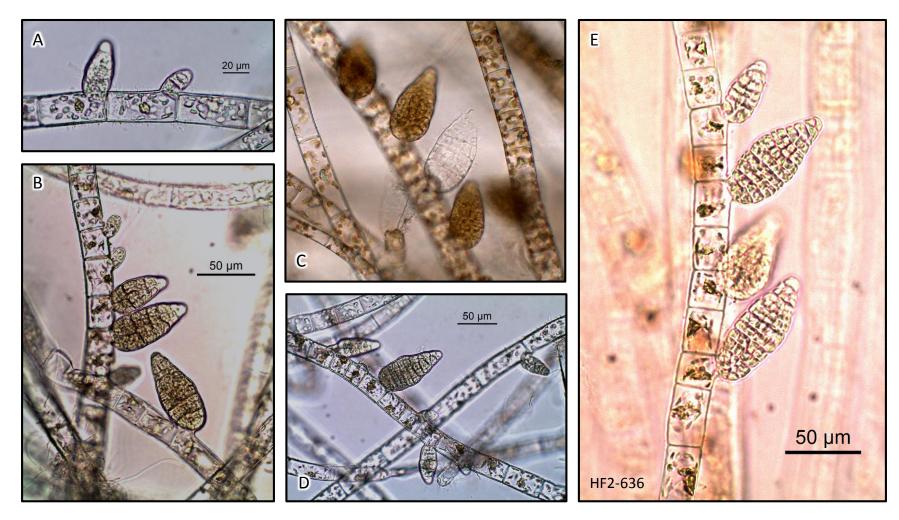




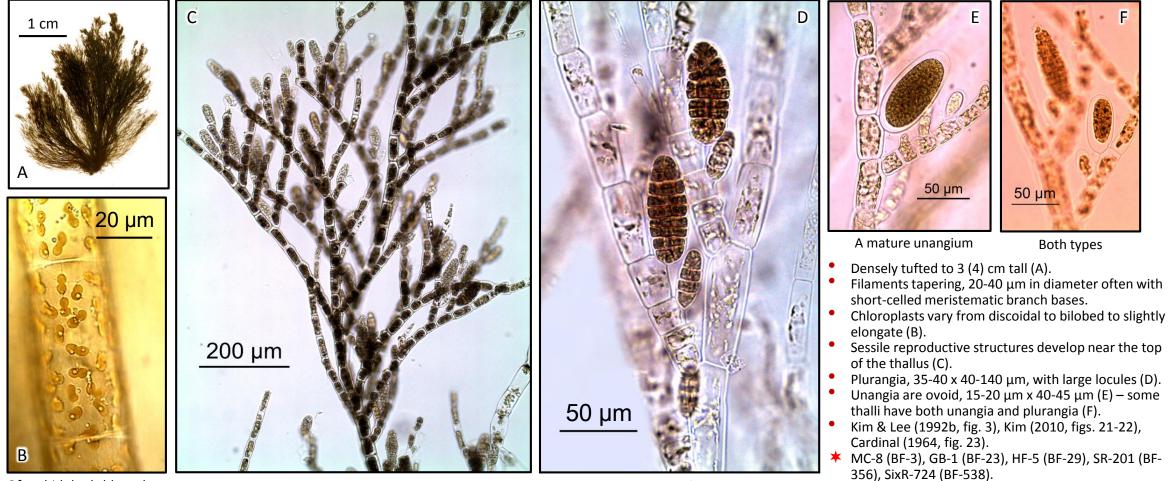
- Tufted filamentous brown alga occurring in the turf of derelict boats (A).
- Thalli 2-5 mm tall with filaments up to 24 µm wide bearing discoidal chloroplasts (B).
- Typically branched 1-3 times near the base with long hair-like filaments extending upward.
- Elongate-conical plurangia, curved or straight, form near the thallus base and occasionally in the upper branches, typically below the growth zones (C, D, F, G).
- Plurangia: sessile or rarely pedicellate, 20-40 µm x 90-150 µm (D).
- Unangia: sessile, globose and up to 50  $\mu$ m in diameter (E).
- Kim & Lee (1994, fig. 1A-B, fig. 2A-C), Cardinal (1964, fig. 29), Sauvageau (1933, fig. 24-26), Stegenga & Mol (1983).
- Similar species: Acinetospora filamentosa (Noda) Yaegashi et al. (2015, fig. 2), a species originally described with ellipsoidal (not globose) unangia (Noda, 1970, fig. 3, 1980, fig. 33). Our species is morphologically similar to Yaegashi's images, but Hanyuda's sequencing indicates that our species is true F. irregularis.
- SVB-404 (BF-402). Not sequenced: HF2 (BF-526), NC (BF-208).

### **Feldmannia irregularis 2**, the *arabicus* form – HF2-636 (Mar)

Branching uniseriate filaments with discoidal chloroplasts – intermixed with normal *Feldmannia irregularis*. Axes are 20-24 μm in diameter; sessile plurangia reach 24 x 68 μm with slightly narrowed tips (A-E). Identical to drawings of Kuckuck (1963, fig. 6) for this species and illustrated by Kim & Lee (1994, fig. 4D-E).



# **Feldmannia mitchelliae**\* – G (Globally widespread) – Asia (J,C,K, Phil), IO, Aus, NZ, SA, CA-MX, ENA, Car, Afr, EUR, Med. Abundant on 24 debris items (all debris months). Fertile – some thalli with both plurangia and unangia. Ephemeral and isomorphic.



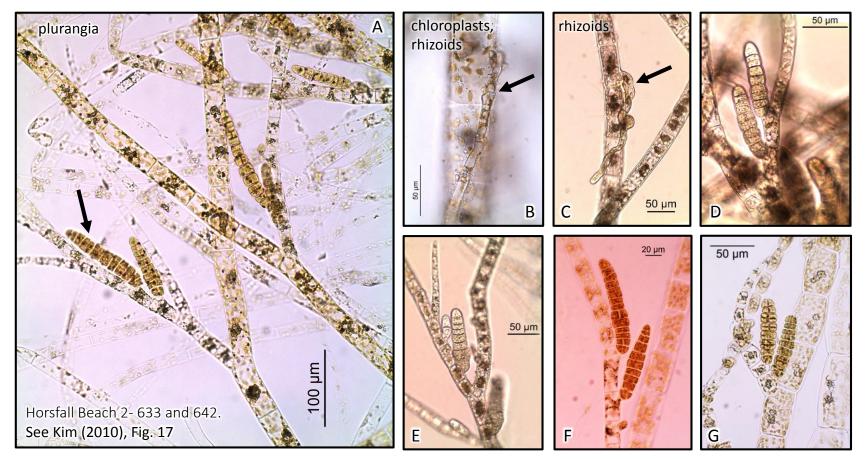
Often bi-lobed chloroplasts

Upper branches with some unilateral branching

Arrangement of plurangia

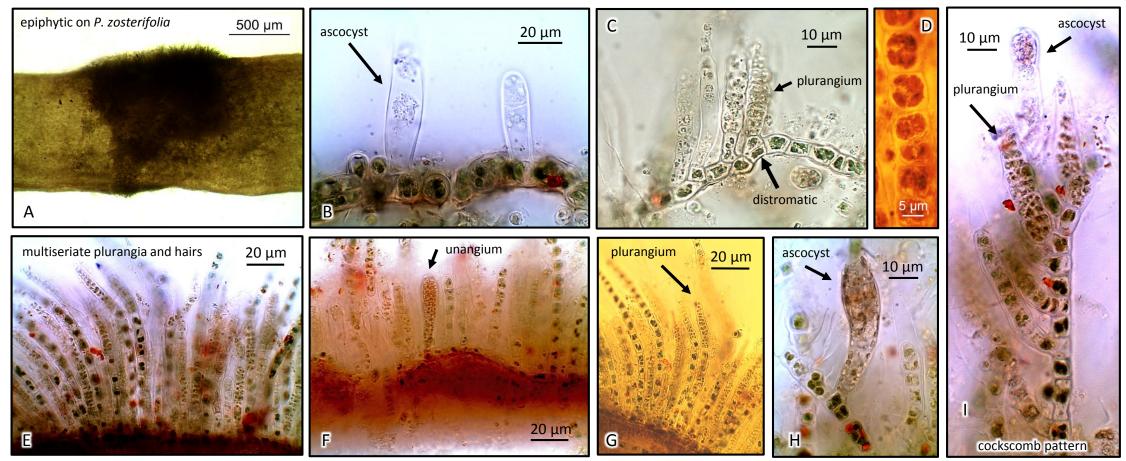
# **Feldmannia mitchelliae 2** – the *indica* form<sup>\*</sup> – True *F. indi*ca is also globally widespread: known in Asia (J, C, K, Phil), IO, Aus, NZ, SA, CA-MX, ENA, Car, Afr. This form was on 2 of the 24 debris items. Reproductive (plurangia) and ephemeral.

The "indica" form is characterized by discoidal chloroplasts (B) and sessile, long, narrow plurangia (A, D-G). Our form developed rare rhizoids (B, C, arrows). This isolate was found to be a morphological variant of *F. mitchelliae* since DNA sequences could not separate the two. See Kim (2010, fig.17) for *F. indica* in Korea. **\*** HF2-633 (BF-526), RE-691 (BF-533); not sequenced HF2-642 (BF-526) (all Mar).

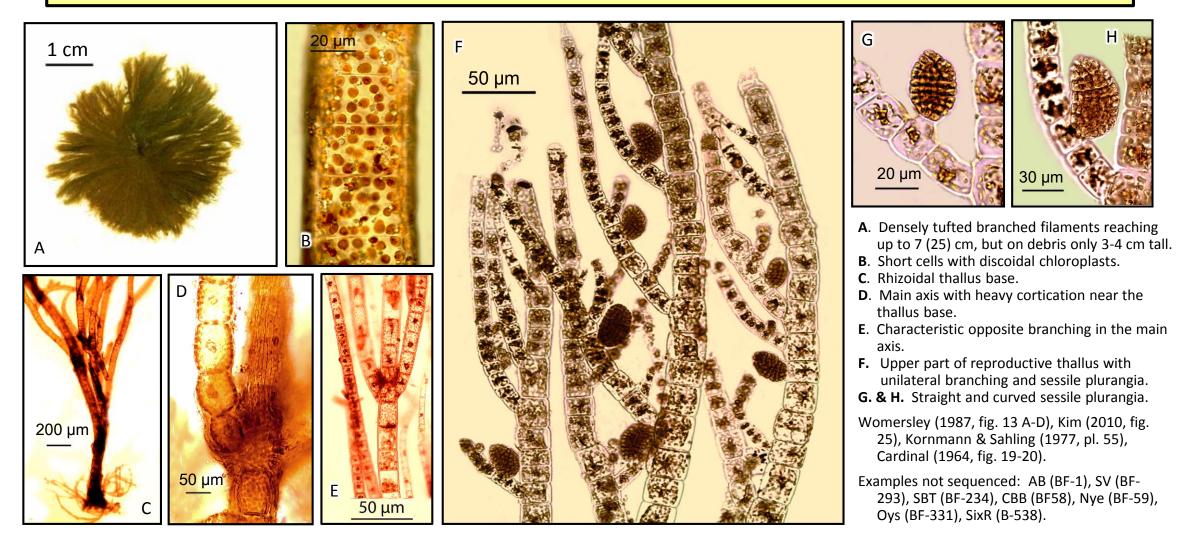


# **Hecatonema cf. streblonematoides<sup>#</sup> – NP-P –** Asia (J), AK-MX, SA (Ch). Only on the Seaview boat (May), SVB (BF-402). Not sequenced. Reproductive (multiseriate plurangia and sessile unangia). Ephemeral (year around in CA) and heteromorphic.

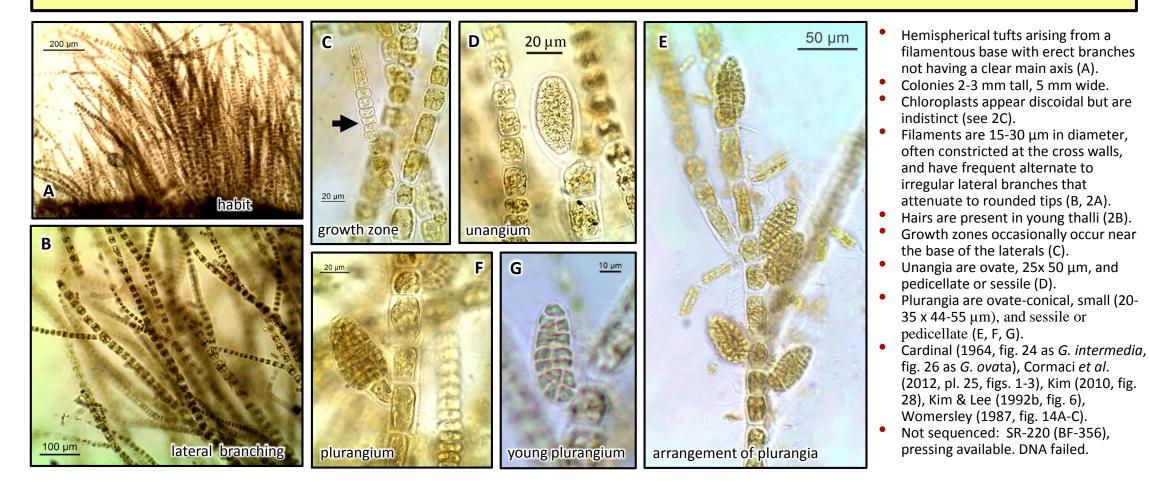
Forming 1-10 mm cushions on *Petalonia zosterifolia* (A). Partially distromatic basal layer (arrow) producing upright filaments, uni-multiseriate plurangia, unangia and ascocysts (B, C, E-H). Older uprights occasionally produce a cockscomb unilateral arrangements of plurangia and ascocysts (I). With discoidal chloroplasts (D).



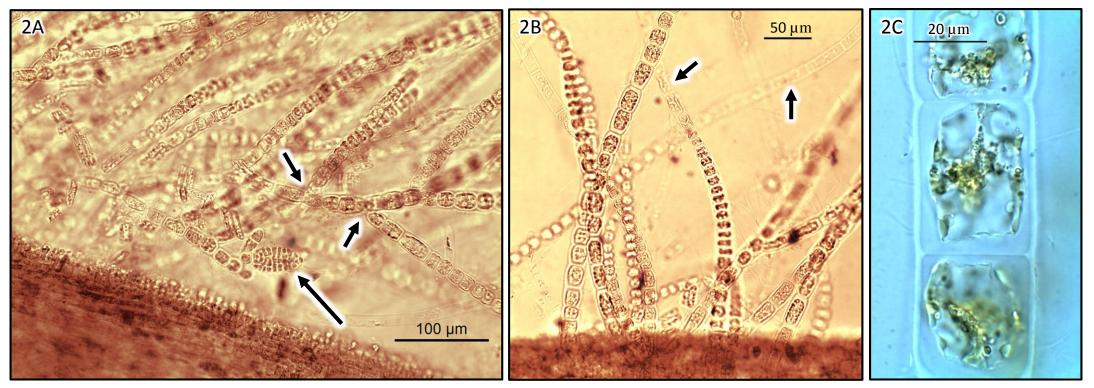
# = Loiseaux-de Goër (4/21/2017 pers. com) confirmed the morphological identity of this species from pictures but also stated that sequencing and comparison with the type would be critical for absolute identification. See Loiseaux (1970), fig. 2. **Hincksia granulosa** – **G** (Globally widespread) – Asia (J, C, K), Aus, SA, AK-MX, ENA, Afr, EUR, Med. On 7 debris items (Jan-Jun). Reproductive (only plurangia were observed). Ephemeral (Mar-Dec in EUR – Cardinal, 1964).



**Hincksia ovata 1 – G (Widespread) –** Asia (R, J, K, C), Aus, SA (Arg, Ch), AK-WA, ENA, EUR, Med, IO. Epiphytic on *Scytosiphon* on 1 debris item (Apr). Cardinal indicates Mar-Sep in EUR. Fertile (plurangia and unanagia). ~Ephemeral. Kim & Boo (2010) say thalli are isomorphic. Guiry (2017) indicates uniphasic direct development occurs. This sample has the spiral-alternate branching form of Kim & Lee (1992b, fig. 6c) and the *Giffordia intermedia*-form of Cardinal (1964, fig. 25), both without opposite plurangia that are often considered diagnostic for the species.



Hincksia ovata 2 – additional pictures. All specimens from SR-220 (Apr) sample.

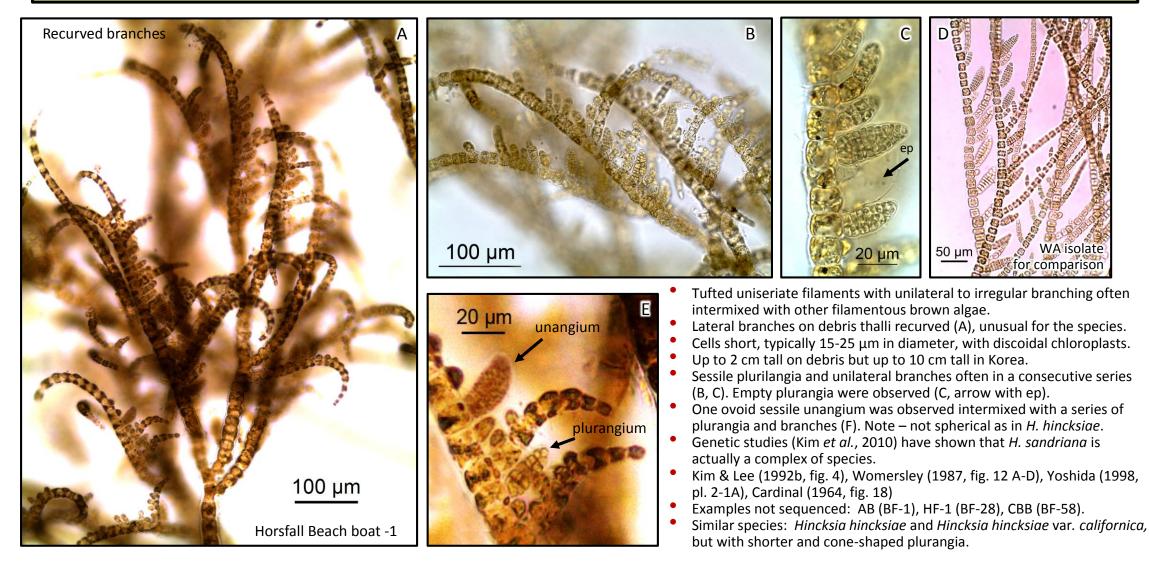


Young tuft with plurangium (long arrow) and alternate branching (short arrows)

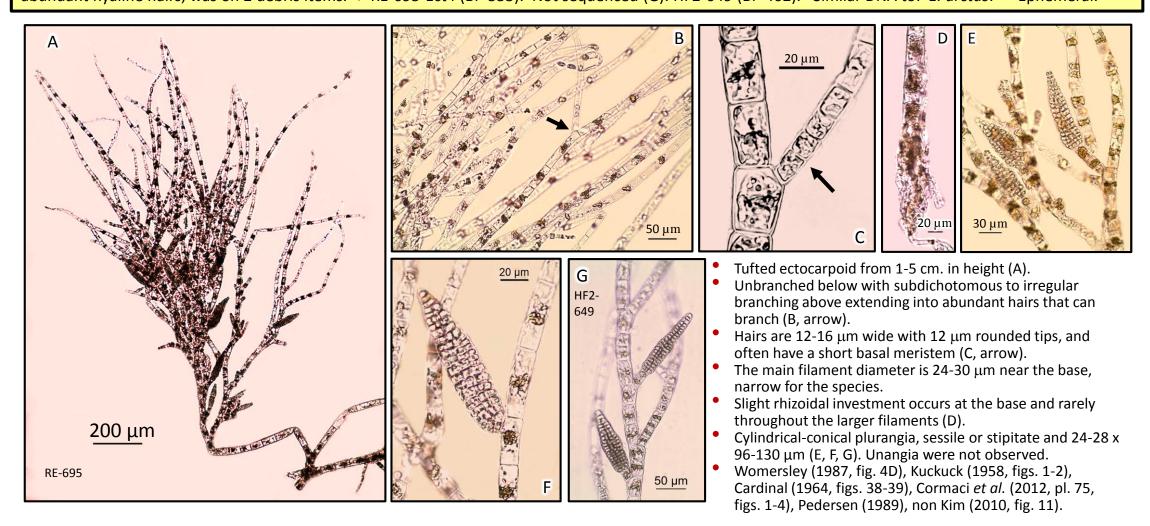
Young filaments with some bead-like cells and the production of hyaline hairs (arrows)

Plasmolyzed filament with indistinct discoidal chloroplasts

**Hincksia sandriana** – **G** (Globally widespread) – Asia (J, C, K), Aus, BC-Mx, Aus, IO, SA, ENA, Afr, EUR, Med. On 3 debris items (Feb, May, Jun). Mar-Dec in EUR (Cardinal, 1964). Reproductive with both plurangia and unangia. Ephemeral.

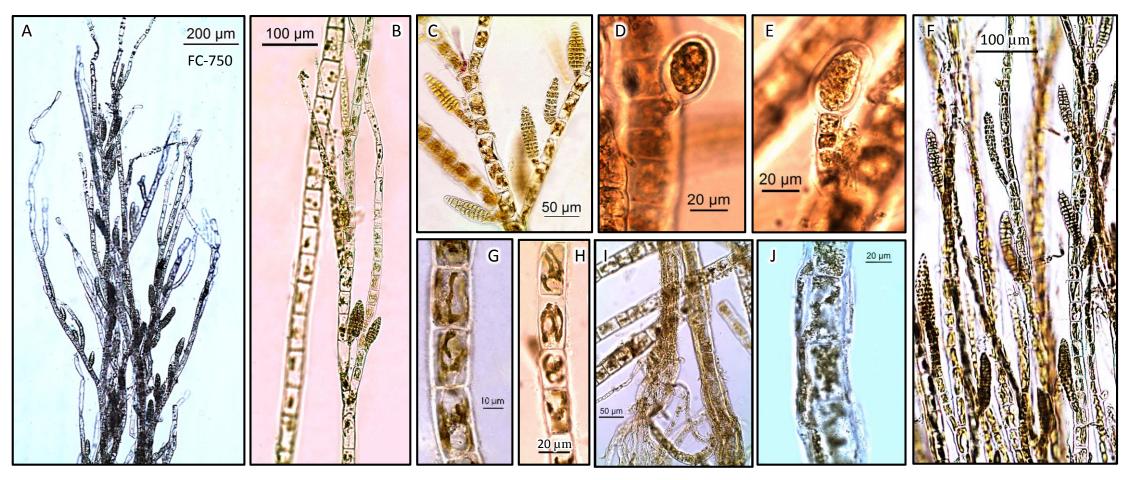


# **Kuckuckia cf. spinosa cpx.** 1<sup>\*</sup> – G (Globally widespread) – Asia (C, K), Aus, SA (Arg, Br), ENA, EUR, Med. In turf or on *Lepas anatifera*. Fertile (plurangia). Isomorphic. On 9 debris items (Jan-May) but variable morphology. Sequenced from 3 items. This form, with abundant hyaline hairs, was on 2 debris items. \* RE-695-Ect4 (BF-533). Not sequenced (G): HF2-649 (BF-462). Similar DNA to: *E. arctus*. ~ Ephemeral.



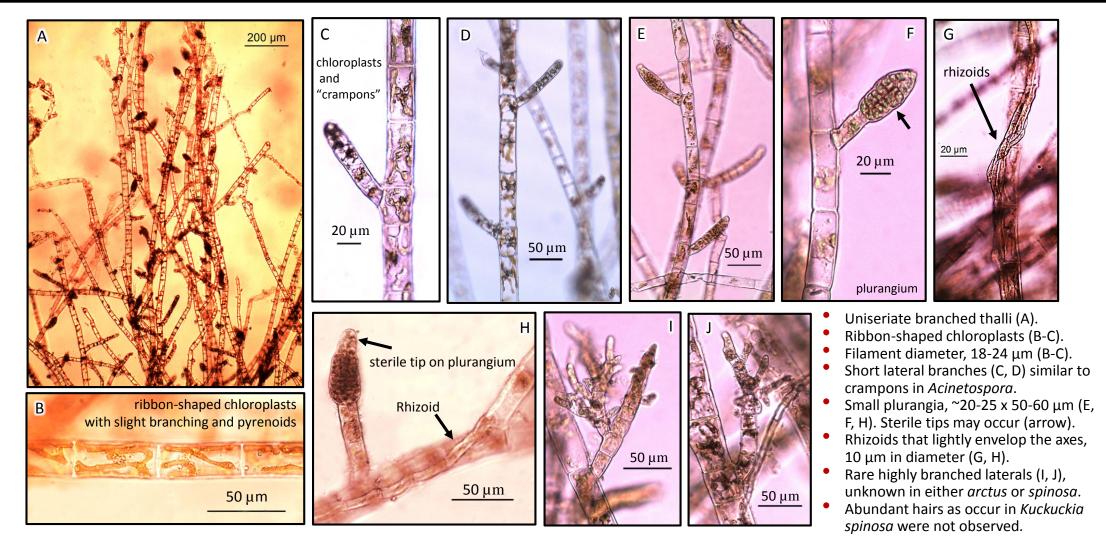
# **Kuckuckia cf. spinosa cpx.** 2\* – A sequence variant occurring in Hanyuda Clade E-sp. 3b. Sequenced from 2 debris items (4 samples) (Jan, Jul). Fertile (plurangia and unangia). This form has the typical characteristics of *Kuckuckia*, but with less extensive hairs than in Form 1 and a growth zone that is rarely present. \*FC-750, 752 and 753 (BF-652), LBF-135 (BF 462), often on Lepas anatifera.

Branched filaments 24-30  $\mu$ m wide with cells having a 1:1 to 1:2 dimension (A, B); hairs were abundant and 12-15  $\mu$ m wide at filament tips but with a limited or absent basal meristem; a rhizoidal base and holdfast were present (I, J), sessile and pedicellate regenerative plurangia 20x80 (160 um)  $\mu$ m (C, F); sessile and pedicellate unangia = 40x26  $\mu$ m (D, E). Cells contained a few longitudinal to spiral ribbon-shaped chloroplasts (G. H), similar to true *Kuckuckia*.

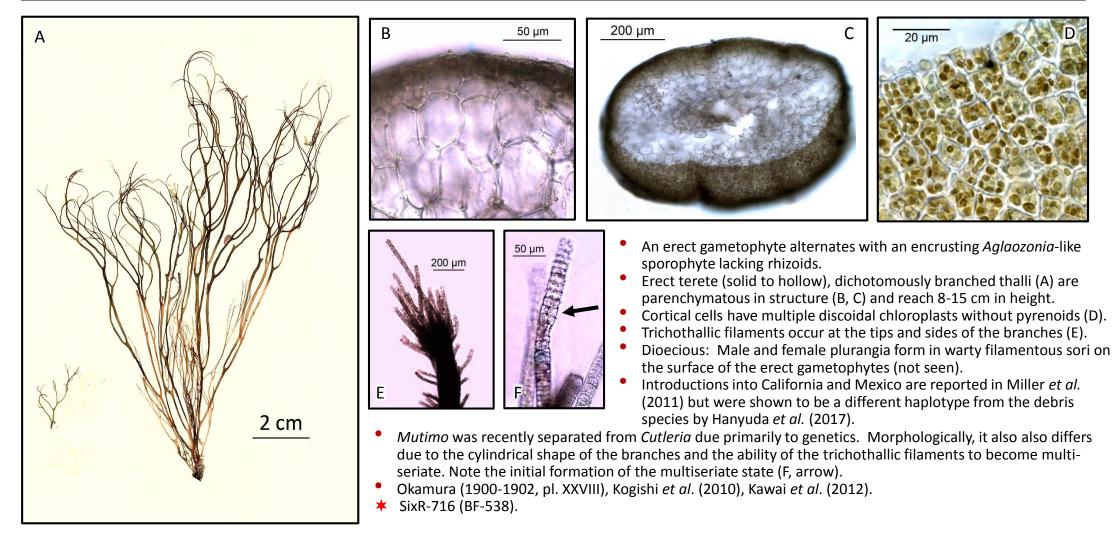


# **Kuckuckia cf. spinosa cpx.** 3<sup>\*</sup> – Sequences indicate *Kuckuckia*. \* SixR-719 (BF-538) (Apr). Not sequenced: SixR-725.

These samples have narrow filaments (like *arctus*), crampon-like laterals and small plurangia. Both also have unusual short highly branched laterals that may be the result of a virus infection (#D. Mueller). Drawings of *E. spinosa* by Kützing (1855, pl. 49) show hairs, small plurangia and also crampon-like laterals (but with more pointed tips).

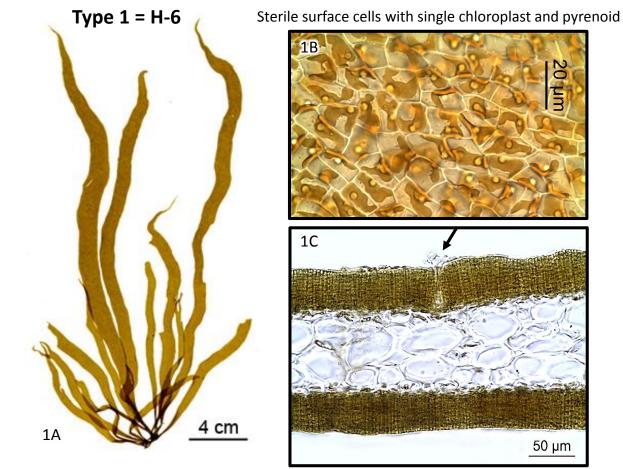


**Mutimo cylindricus** + - A+ - Asia (J, K, Phil) + CA-MX. On 1 debris item (Apr): Sixes River derelict boat. CA (1973) and MX (1990) introductions are a different haplotype than the debris species. Sterile. Annual and heteromorphic.



## **Petalonia fascia cpx.**\* – G (Globally widespread) – Asia (R, J, C, K), Aus-NZ, SA (Ch), AK-MX, ENA, Afr, EUR,

Med. On 24 debris items (Jan-May). Fertile (plurangia). Annual & heteromorphic (with a *Stragularia* crustose sporophyte). Variable both genetically and morphologically. **\***4 of 8 *cox*3 haplotypes known in Japan were found on debris (none of these occur in the NEP) – 3 types are illustrated.



Sterile thalli from Ponsler debris boat, MP-12 (BF-36, Hanyuda H-6) Cross-section of fertile blade. Note surface of plurangia and clustered paraphyses (arrow)

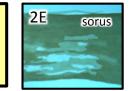


Fertile. Note banding. Seal Rock boat – SR-215 (BF-356, Hanyuda H-6)

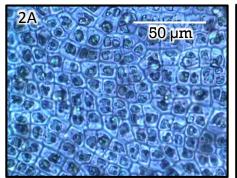
- Type 1, described here, is dominant on debris.
- Lanceolate blades with cuneate bases arising from a small discoidal holdfast (1A, 1D).
- Most thalli have acute or pointed apices (1A).
- Blades are up to 40 cm tall, 1-3 cm wide and 100– 250 μm thick.
- Surface cells are angular and contain a single chloroplast with a single pyrenoid (1B).
- Subcortical cells are also angular (see 1E, p. 31).
- Fertile gametophytes have banded sori (1D) containing densely packed uniseriate plurangia that may be up to 15 cells long (1C).
- Sori may contain clustered paraphyses. (1C, arrow).
- Fletcher (1987, figs. 61A, 62), Kogame (1997), Okamura (1900-1902, pl. X).
- 17 samples sequenced examples: MP-12 (BF-36, H-6), SR-215 (BF 356, H-6), SR-205 (BF-356, H-8), HF2-630 (BF-526, H-5), RE-662 (BF-533, H-2), SixR-727 (BF-538, H-2). Haplotypes (H-xx) are from Hanyuda *et al.* (2017).
- Japanese (including debris) and NEP haplotypes differ by numerous base pairs in the *cox*3 gene, and seem to be separate species (Hanyuda *et al.*, 2017).
- Morphological variation appears to coincide with some of the haplotypes – see Types 2 and 3 (p. 31).



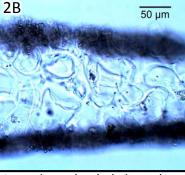
**Petalonia fascia cpx.** — Types 2\* and 3\* — These morphological types are typically larger and have less acute tips than in Type 1 thalli (pg. 30, 1A and 1D). Differences also occur in the sori and subcortical layers. Further study is required.



**Type 2. \***RE-662 (BF-533, Hanyuda H-2): Blades without acute apices (not shown); blade anatomy is below:



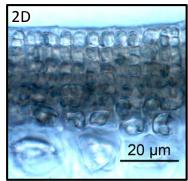
Square to angular surface cells.



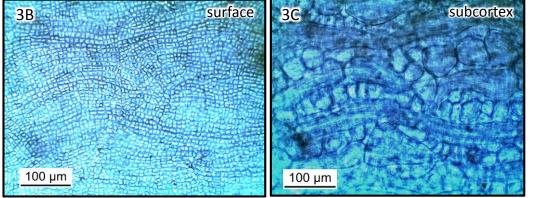
Irregular to hyphal-shaped medullary cells.

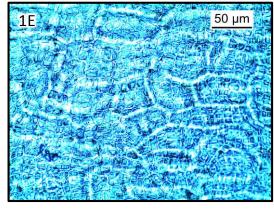
<u>20 µm</u> 2С 0 да работ

Unusually large tip cells on some surface hair clusters.



Plurangia – spore release pattern is in 2E above.

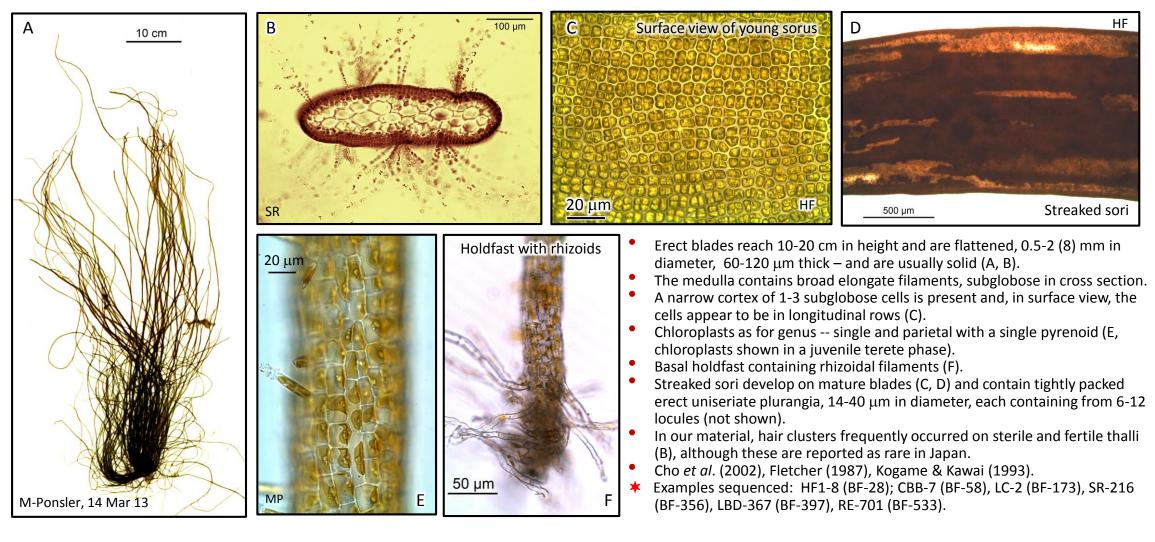




**Type 3**. ★SR-205 (BF-356, Hanyuda H-8) and the large specimen at left (3A). Mature thalli are larger than other *Petalonia* and have less pointed and often slightly curved apices (3A). Surface view (3B) and optical section of subcortex (3C) revealing the undulating pattern of the cell filaments and the cell size variation.

**Type 1. \*** MP-12 (BF-36, H-6, pg. 30). Optical section of the subcortex of Type 1 thalli showing angular cells.

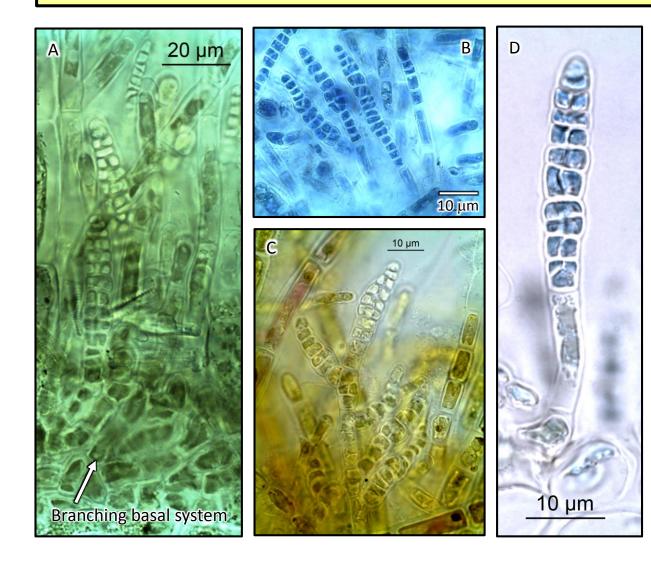
**Petalonia** zosterifolia<sup>\*</sup> – G (Widespread) – Asia (R, J, C, K), Arc, ENA, EUR, Med. On 12 debris items (Jan-May). Reproductive. Annual and heteromorphic with direct development from a microscopic filamentous *Compsonema saxicolum* sporophyte.



#### **Petroderma maculiforme<sup>\*</sup> – G (Globally widespread)** – Asia (R, J), Arc, A-Arc, SA (Argentina, AK (Hansen), WA, MX, ENA, EUR. On the Quail Street JTMD plastic carboy (Mar). Disintegrating with eroded unangia and plurangia. Perennial and isomorphic. Congested thick I cm patches on plastic; soft to scrape off (A-C). Unangia tips were 10-12x30 µm, but rarely 10x50 µm. Single-celled basal layer with no visable rhizoids (E). Eroded cells often contained dark-colored storage material Erect filaments are 8-10 µm broad with rare branching. (F-H). Wilce, et al. (1970, figs. 1 & 2), Waern (1949, pl. 1). Single parietal plate-like chloroplasts were not visible. Eroded terminal unangia & divided plurangia were visible (D). \* Quail Street carboy – QS-620 (BF-656). Divided plurangia 20 µm 10 µm G F ← unangium 100 µm н 10 µm 50 µm 20 µm

### **Protectocarpus speciosus – G (Globally widespread) –** Asia (J, K), IO, ENA, SA, EUR, Med, Canaries.

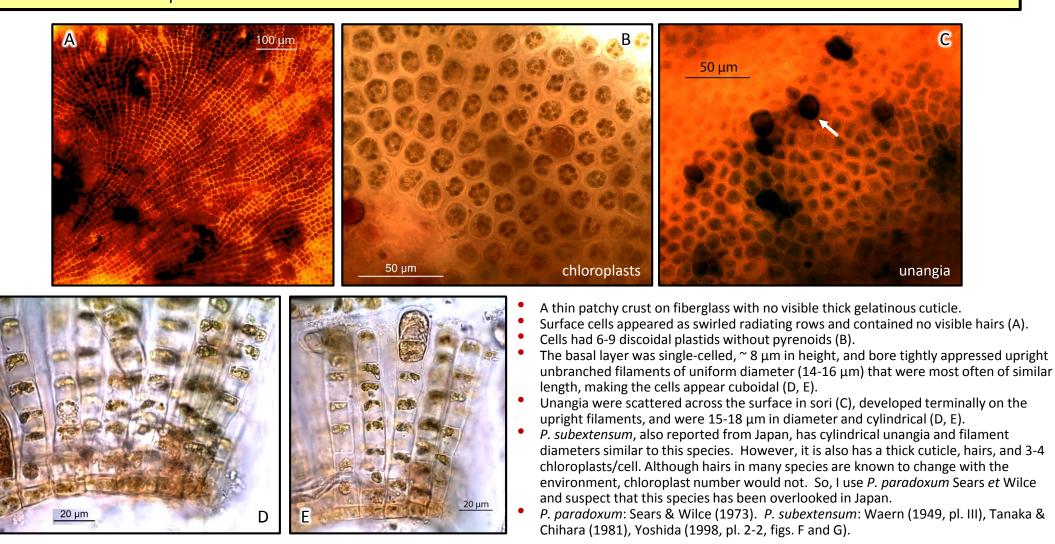
Epiphytic in the turf on *Lepas anatifera* and also on some algae. On 5 debris items (Feb-Jun). Fertile. ~Ephemeral and isomorphic.

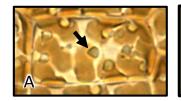


- A branched filamentous and monostromatic basal layer that forms upright, occasionally branched uniseriate filaments 5-10 μm in diameter and only 1-3 mm in height (A).
- Plurangia are uni to multiseriate on upright filaments -- when branching occurs the sporangia are continuous between the uprights and branches (B-F). A cockscomb pattern was occasionally observed (not shown). Unangia were not observed.
- Chloroplasts were not well-preserved in the debris material.
- Kuckuck (1958, p. 66/126, pl. 4), Tanaka (1986), Fletcher (2987, figs. 15-16), Kornmann & Sahling (1977, pl. 158).
- Not sequenced: AB (B-1), HF (BF-28), MP (BF-36), LC (BF-173), SVB (BF-402).

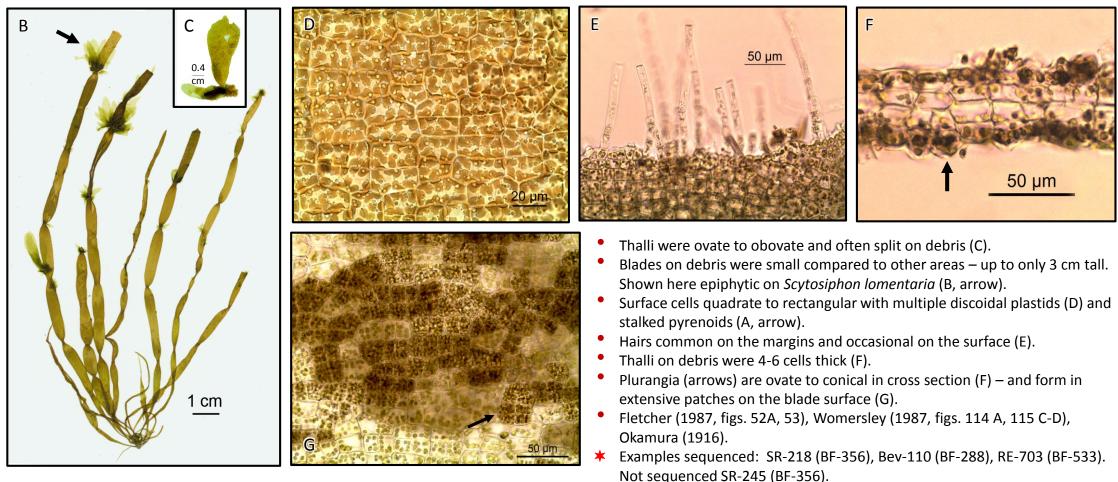


#### **Pseudolithoderma cf. paradoxum – G** (rare global occurrences) – Asia (J? and debris), ENA (Massachusetts). On 1 debris item (Apr) – the fiberglass from the Sixes River Boat – SixR-730 (BF-538). Reproductive (unangia). Perennial and isomorphic.



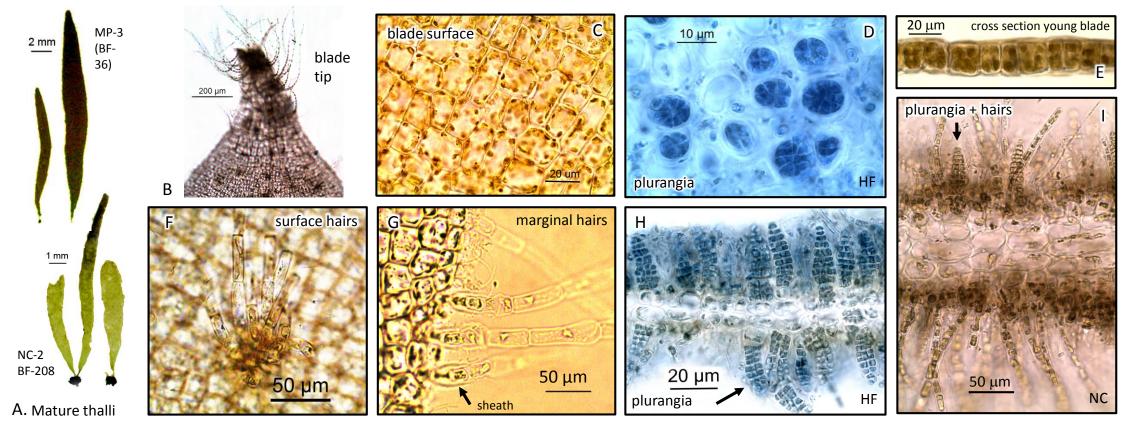


**Punctaria latifolia cpx.**  $\mathbf{l}^* - \mathbf{G}$  (Globally widespread) — Asia (R, J, C, K), Aus, NZ, SA, AK, ENA, Afr, EUR, Med. A common epiphyte on debris on 14 JTMD items (Feb-Jun, Oct). Fertile with only plurangia. Annual. Heteromorphic with direct-development from a microthallus.

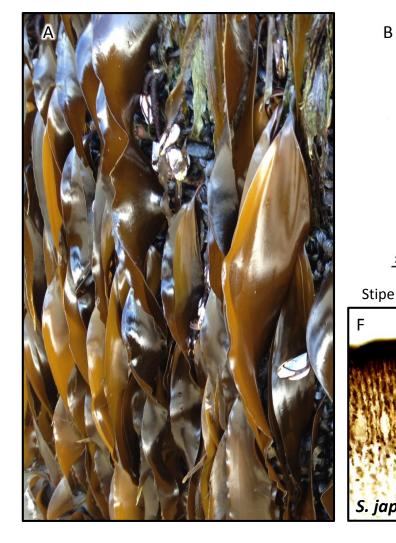


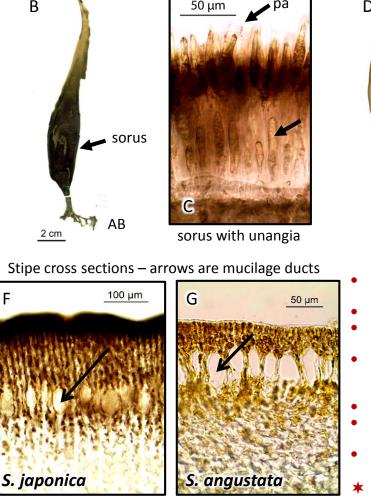
**Punctaria latifolia cpx. 2 – the flaccida form** Asian only (R, J, K). On 4 of the 14 JTMD *latifolia* items. Merged with *P. latifolia* for this study. Fertile with elongate plurangia extending outward 3-4 x the size of the outer cortical cells, similar to illustrations of true *P. flaccida* by Noda (1979, pl. 3, fig. 4 #2-3) and Nagai (1940, pl. II, figs. 12-13; pl. III, fig. 7). Annual – direct development from a microthallus.

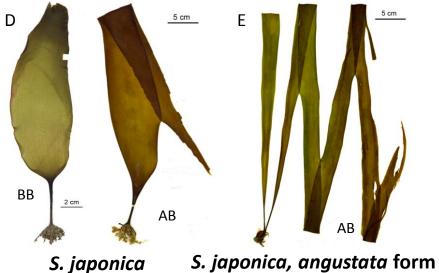
Thalli were lanceolate to oblanceolate often with a 1.5:10 ratio of width to length and reaching 5 cm (A). Blades are 1-4 (6) cells thick (E, I) with cortical cells slightly smaller than medullary cells. Sheathed hairs form in clusters on surface and solo along the margins (B, F, G). Our material was only plurangial and bore tall cylindroconical plurangia (D, H, I). DNA sequences could not differentiate this small form from *P. latifolia*. This similarity also occurs between *P. tenuissima* (not known in Japan) and *P. latifolia* (Parente *et al.*, 2010). Both smaller forms have protrudent plurangia. **\*** Sequenced: MP-3 (BF-36), NC-2 (BF-208). Not sequenced: SJC (BF-39) and HF1 (BF-28).



#### **Saccharina japonica**\*/angustata\* – A+ – Asia (R, J, C, K) + France (*S. japonica*). "Kombu", edible kelp. On 3 debris items (Mar, Jun), including the Agate Beach dock. Fertile. Biennial and heteromorphic. \* 2 nearly identical haplotypes.



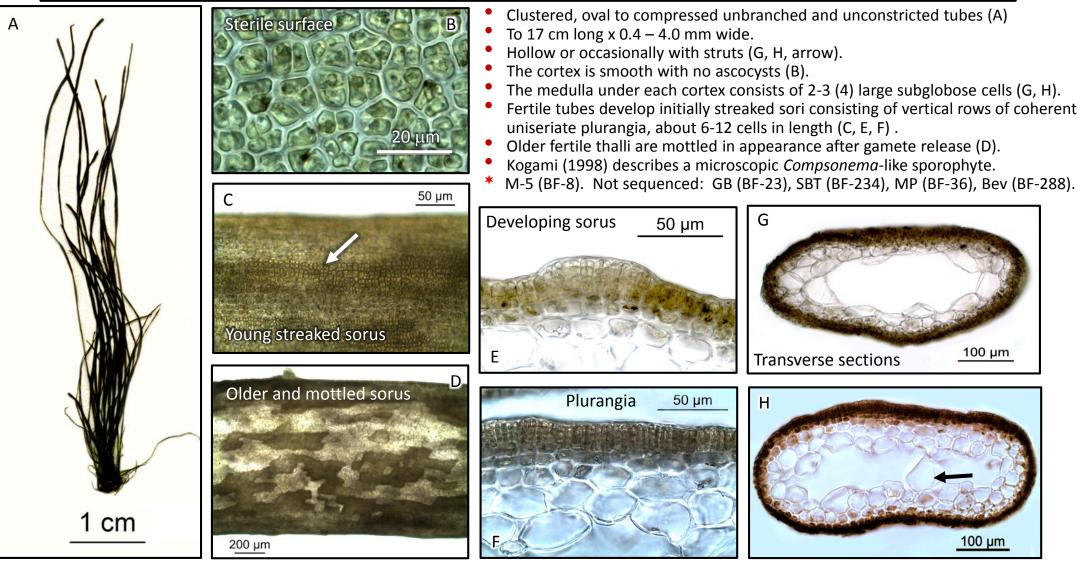




- Both are 1-2(4) m long flat to bullate simple blades with short round stipes (A, B, D, E).
- Reproductive sori form on the lower part of the blade (B, arrow).
- A section of the sorus (C) reveals plurangia (arrow) developing intermixed with typical protective paraphyses (arrow, pa).
- Morphologically, the 2 species differ in:
  - Blade and fascia width (D, E).
  - Mucilage duct location in the stipe (F, G, cross sections).
- Molecularly, the 2 are considered genetic variants of the same species.
- Introduced in 1976 into Thau Lagoon, France, the species has not spread or become invasive.
- Miyabe (1902, pls. I and IX), Okamura, (1925, pl. CCXXI), Okamura & Uyeda (1925), Nagai (1940), Yotsukura *et al*. (2006, 2010); Verlaque *et al*. (2015).
- \* AB-6, 7, 8, 9 (all BF-1), BB-1 (BF-2). Not sequenced: Fish-1 (BF-40).

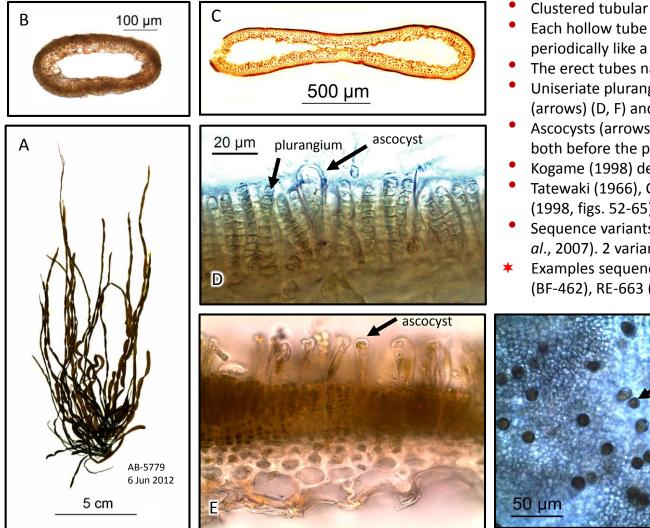
## **Scytosiphon gracilis\* – A+ –** Asia (J, K) + MX, Chile. On 10 debris items (Jan-May).

Similar to Oregon's Scytosiphon dotyi in lacking ascocysts. Fertile (plurangia). Annual and heteromorphic.



## **Scytosiphon Iomentaria cpx.** 1<sup>\*</sup> – G (Globally widespread) – Asia (R, J, C, K, Viet), IO, Aus,

NZ, SA, AK-MX, ENA, EUR, Med, Afr. On 12 debris items (Jan-Jun). Reproductive (plurangia). Annual and heteromorphic.



- Clustered tubular unbranched thalli to 30 or more cm tall (A).
- Each hollow tube is up to 5 mm in diameter and is often crimped periodically like a chain of sausages (A, B, C).
- The erect tubes narrow toward their tips and may be slightly flattened.
- Uniseriate plurangia coat the surface of fertile thalli dotted by ascocysts (arrows) (D, F) and occasional hair clusters (G).
- Ascocysts (arrows), generally submersed in the plurangia, are erumpent both before the plurangia fully develop and after they are released (E).
- Kogame (1998) describes a Microsporangium-like sporophyte.
- Tatewaki (1966), Clayton (1978), Fletcher (1987, figs. 67b, 69); Kogame (1998, figs. 52-65).
- Sequence variants are known on both Pacific coasts and in Europe (Cho *et al.*, 2007). 2 variants on JTMD are illustrated (see the following slide).

hair cluster

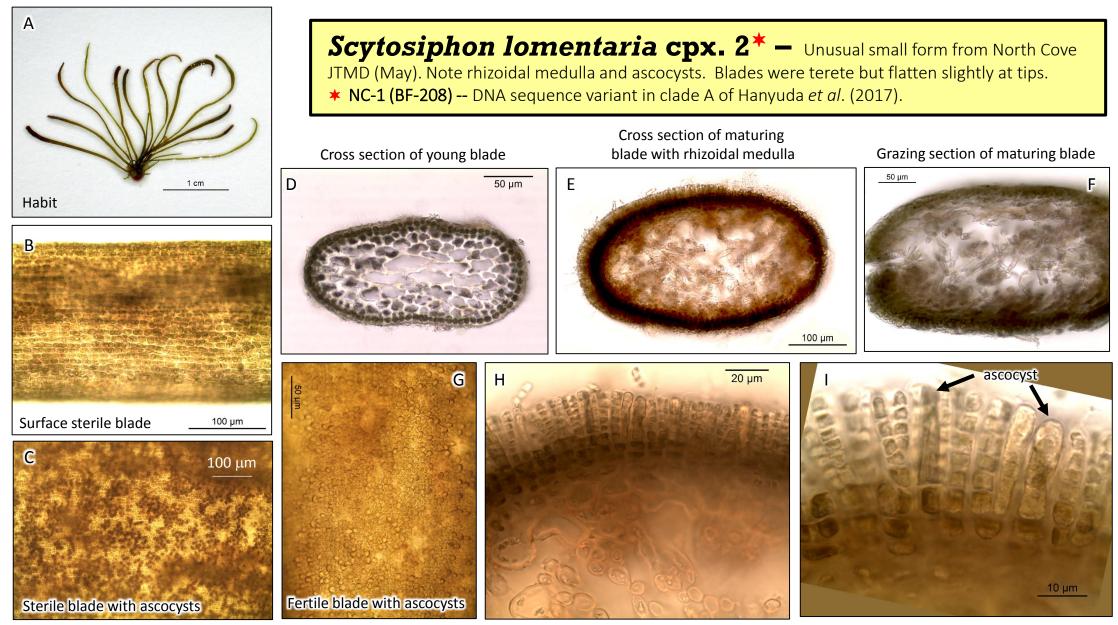
plurangia

Examples sequenced: SR-212 (BF-356), SVB-382 (BF-401), LBF-409 (BF-462), RE-663 (BF-533), (Clade B of Hanyuda *et al*, 2017).

ascocyst

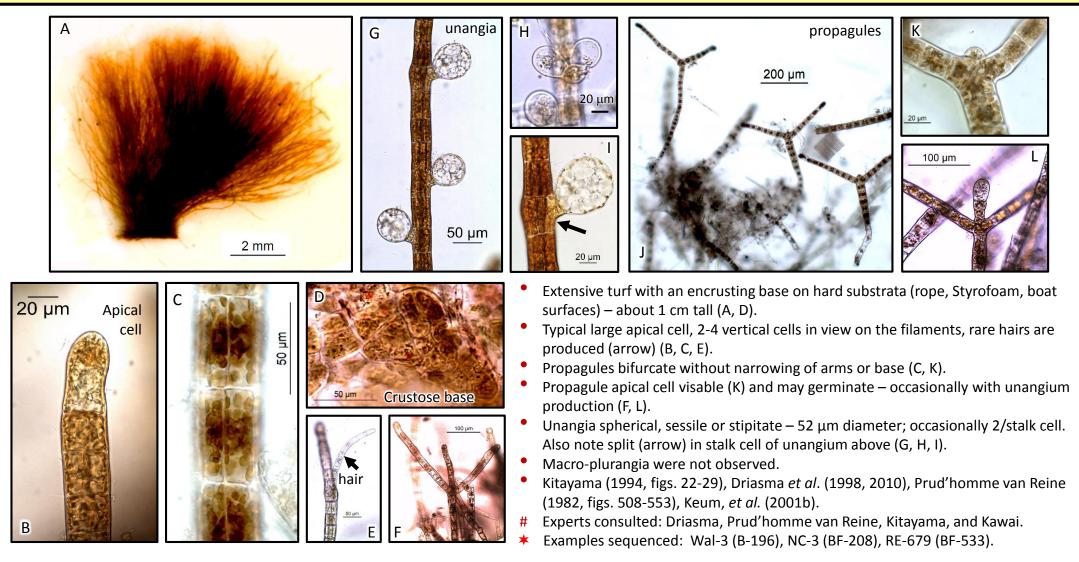
G

50 µm



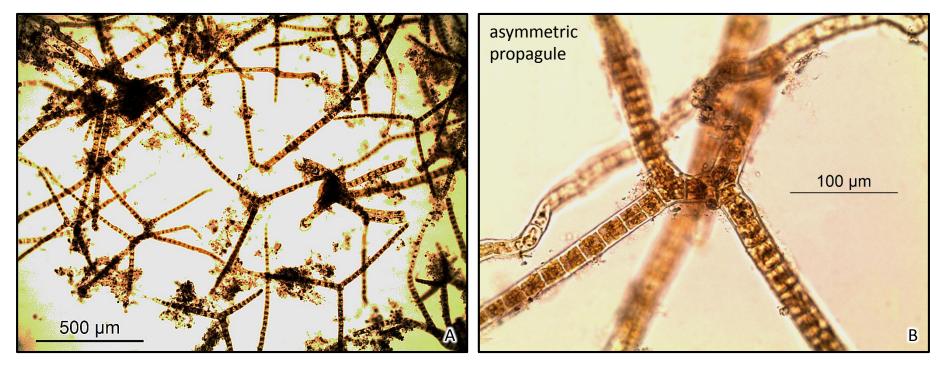
Sections of fertile blades with ascocysts and unbranched and branched uniseriate plurangia

# **Sphacelaria rigidula**<sup>\*#</sup> – **G** (Globally widespread) – Asia (R, J, C, K, Viet, Phil), IO, Aus-NZ, SA, AK-MX, ENA, Car, Afr., EUR, Med. On 8 debris items (Mar-May). Reproductive (propagules and unangia). Perennial and isomorphic.



**Sphacelaria solitaria – G (Widespread) –** Asia (J, K, C, Viet), NZ, EUR. Only on the Mosquito Creek dock (Jan), MC (BF-8). Abundant propagules. Perennial and isomorphic.

- Irregularly branched filaments up to 1.8 cm in height and 35-60 μm in diameter.
- Each tier of the filaments bear 2-5 longitudinal walls in surface view.
- Plants were found in the turf on the dock and were epilithic or epiphytic.
- The asymmetric bi-furcate propagules that characterize this species were present.
- Kitayama (1994, figs. 40-45), Keum et al. (2001a, figs. 1-17), Kylin (1947, non fig. 24D).



# **Undaria pinnatifida**\* – A+ – Asia (R, C, J, K), Aus, NZ, SA (Argentina), CA-MX, EUR, Med. Only on the Agate Beach Dock (Jun). Abundant and fertile on debris. Annual and heteromorphic with a microscopic gametophyte.

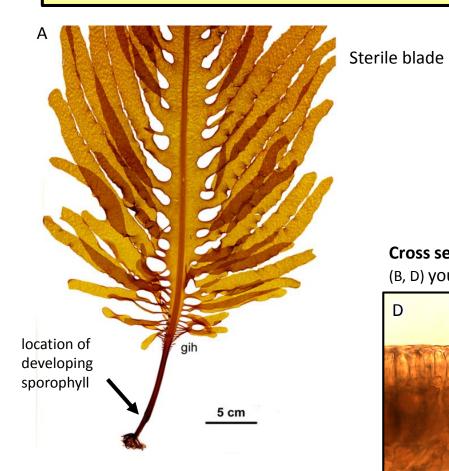


- "Wakame", a food staple in Japan. Wild harvested and cultivated in Asia.
- Sporophytic blades on debris were highly lobed with a central midrib and reached 1+ m tall (A).
- Sporophylls form as a ruffled ring at the base of the stipe but are only fertile when the ruffle expands and becomes mature (B, C). See part 2.
- Thalli can regenerate and reproduce up to 3x/year as documented for NZ.
- Highly invasive listings: "100 world's worse invasive alien species", "100 worst invaders in the Mediterranean", and 3rd among the "most invasive algal species in Europe".
- First recorded in CA in 2000 (the CA-MX haplotype differs from the haplotype on JTMD).
- Okamura (1915, 1926, pl. CCXXVI), Saito (1972), Uwai *et al*. (2006).
- **Sequenced:** AB-5790a (BF-1).





#### **Undaria 2 –** Reproductive development



Fertile sporophyll develops at the base of the stipe and is actively reproductive only after the ruffles mature.

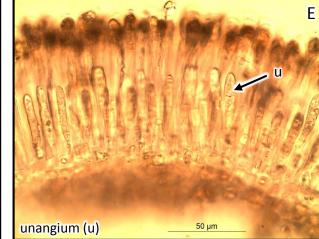
#### Cross sections of:

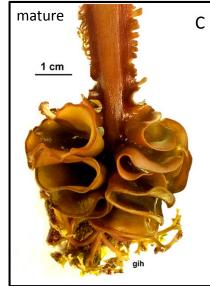
(B, D) young sporophyll



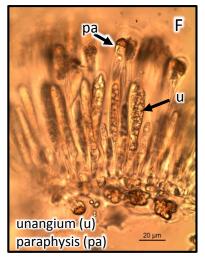


(C, E) maturing sporophyll

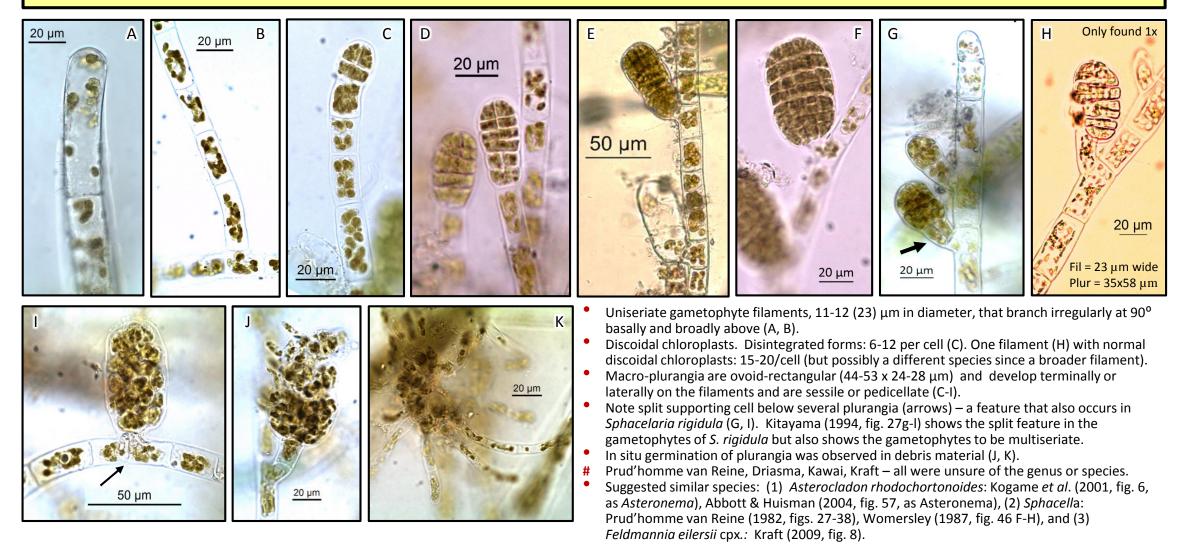




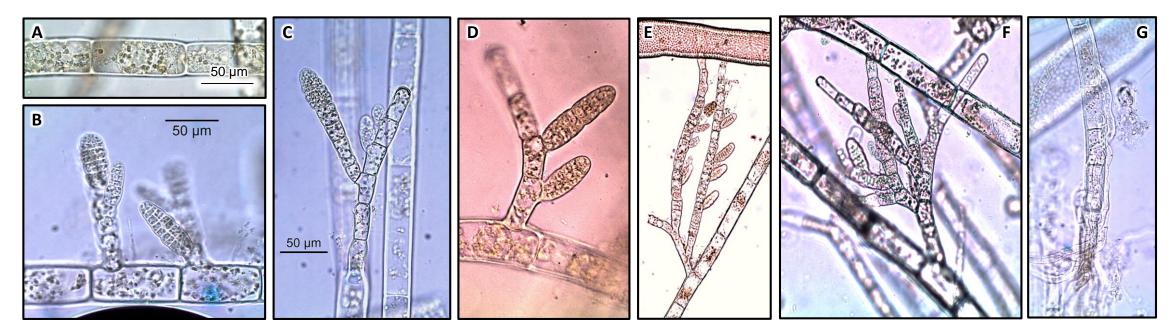
(C, F) mature sporophyll



**Unknown** 1<sup>#</sup> – Mixed with Sphacelaria rigidula in HF2-634 (BF-526), DNA failed. Chloroplasts appear plasmolyzed and abnormal (except H). Possibly an unusual gametophyte of Sphacelaria rigidula with only uniseriate filaments. Other suggestions by experts: Sphacella n. sp, Asterocladon sp., Feldmannia sp.

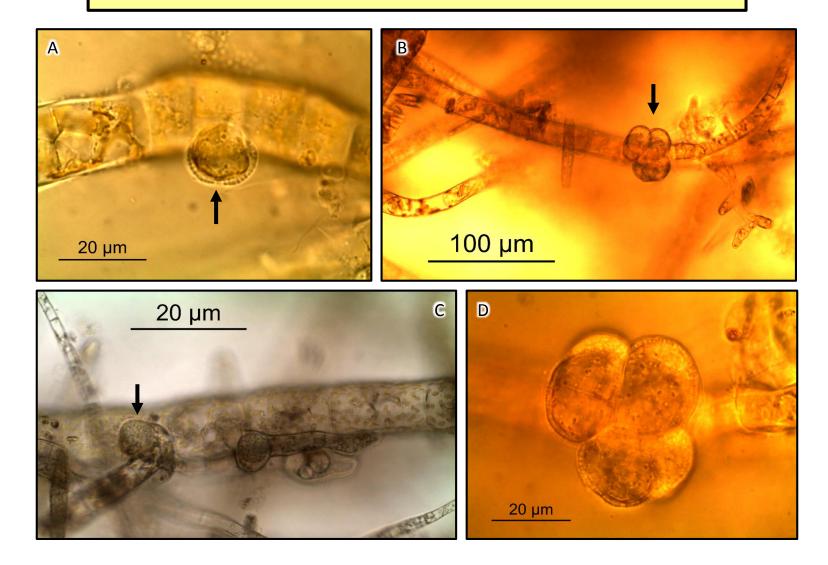


**Unknown 2** — possibly *Feldmannia paradoxa* var. *caespitula* cpx. (including *F. simplex* and *F. socialis*) — **G** (Widespread) — Asia (J, K, C, Pak), AK-MX, ENA, SA, EUR, Med. Fragments only found on one JTMD site: CBD (BF-130). DNA not obtained.



- Small irregularly branching uniseriate filaments with main axes usually straight sided fragments occurred intermixed with other algae in the Clatsop Beach Dock collection. Preserved so no DNA.
- The pieces were too fragmented to be certain they were a single species, and therefore this sample was excluded from the main list.
- Chloroplasts appeared discoidal, although the material was partially disintegrated (A, B).
- Branches were 15-30 µm in diameter, varying from ~30 µm in the main axes to 10-15 µm in the often branched laterals (A-C).
- Small, sessile and stipitate, cylindrical plurangia with rounded apices were born only on the lateral branches and not on the main axes (B-F).
- Rhizoidal filaments occurred at the thallus base (G).
- Cormaci *et al.* (2012, pl. 21, figs. 4-6), Cardinal (1964, fig. 30. as *F. simplex*), Kim & Lee (1994, table 1 similar to *Feldmannia paradoxa* var. *caespitula*).

#### **Unknown 3 –** A wart-like infection of *Feldmannia mitchelliae*.



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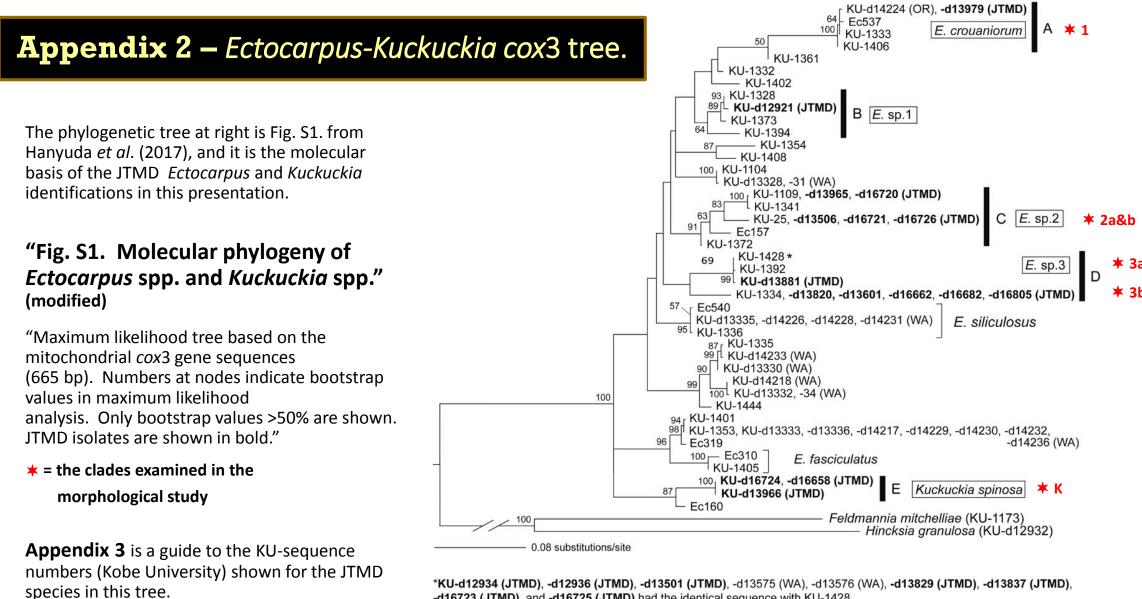
## **Appendix 1 – Japanese Debris Items**

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.

BF #	State	Site Name	Abbrev.	Collection		ltom
			ADDIEV.	Date	Year	Item
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

BF #	State	Site Name	Abbrev.	Collection		Item
ЫГ #			ADDIEV.	Date	Date Year	
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



<sup>-</sup>d16723 (JTMD), and -d16725 (JTMD) had the identical sequence with KU-1428.

## **Appendix 3.** The JTMD *Ectocarpus* and *Kuckuckia* samples sequenced.

The following tables are excerpts from Table S1 of Hanyuda *et al.* (2017). The sequence references are from: Peters *et al.* (2010-*cox*3, 2015-*cox*1), Kogishi *et al.* (2010-*cox*3). Accession numbers are listed in S4 of the 2017 paper.

Genetic identification	Clade in Appendix 2, GIH approximate identification	Apparent morphological variation	Kobe University sequence and silica gel numbers	BF Number	Location, item	Gene Sequenced		
REFERENCE SPECIMENS								
E. crouaniorum	(A) E. crouaniorum		KU-1333		Portdeha, Aran Islands, Ireland	сох3		
<i>E</i> . sp. 1	(B) E. sp-1, near crouaniorum		KU-1373		Tierra del Fuego, Argentina	сох3		
<i>E</i> . sp. 2	(C) <i>E</i> . sp-2a, ~ <i>acutus</i>		KU-1109		Akkeshi, Hokkaido, Japan	cox3		
<i>E</i> . sp. 2	(C) <i>E</i> . sp-2b, ~ <i>acutus</i>		KU-25		Awaji I., Hyogo Pref., Japan	cox3		
<i>E.</i> sp. 3	(D) E. sp-3a, ~pygmaeus		KU-1428		Roscoff, France	cox3		
<i>E</i> . sp. 3	(D) E. sp-3b, sp-with-hairs	K. spinosa	KU-1334		Helgoland, Germany	cox3		
Kukuckia spinosa	(E) Kuckuckia spinosa	E. arctus	Ec-160		Arica, Chile	cox3		

## **Appendix 3 (continued)**

Genetic identification	Clade in Appendix 2, GIH approximate identification	GIH Sample numbers	Kobe Univ. sequence and silica gel numbers	BF Number	Location, item	Gene Sequenced	
JAPANESE TSUNA	JAPANESE TSUNAMI MARINE DEBRIS SAMPLES						
Ectocarpus crouaniorum	E. crouaniorum	NC-5	KU-d13979	BF-208	Cape Arago, OR, boat	rbcL, cox3	
Ectocarpus sp.	E. sp-1, near crouaniorum	SV-15-1	KU-d12921	BF-293	Long Beach, WA, pipe	cox3	
Ectocarpus sp.	E. sp-2a, ~ <i>acutus</i>	Wal-1	KU-d13965	BF-196	Waldport, OR, boat	cox3	
Ectocarpus sp.	E. sp-2a, ~acutus	RE-686	KU-d16720	BF-533	Roads End, OR, boat	cox3	
Ectocarpus sp.	<i>E.</i> sp-2b, ~ <i>acutus</i>	Bev-113	KU-d13506	BF-288	Beverly Beach, OR, pallet	cox3	
Ectocarpus sp.	<i>E.</i> sp-2b, ~ <i>acutus</i>	RE-690	KU-d16721	BF-533	Roads End, OR, boat	cox3	
Ectocarpus sp.	<i>E.</i> sp. 2b, <i>~acutus</i>	RE-699	KU-d16726	BF-533	Roads End, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	CB-5	KU-d12934	BF-58	Clatsop Beach, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	LB-108+	KU-d13501, 75, 76	BF-285	Long Beach, WA, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	SR-223	KU-d13829	BF-356	Seal Rock, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	SR-234	KU-d13837	BF-356	Seal Rock, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	RE-695-Ect3	KU-d16723	BF-533	Roads End, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	RE-698	KU-d16725	BF-533	Roads End, OR, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	SVB-383	KU-d13861	BF-402	Seaview, WA, boat	cox3	
Ectocarpus sp.	E. sp-3a, ~pygmaeus	LBF-405	KU-d13881	BF-462	Long Beach, WA, float	cox3	
Ectocarpus sp.	E. sp-3b, ~penicillatus?	SR-213	KU-d13820	BF-356	Seal Rock, OR, boat	cox3	
Ectocarpus sp.	E. sp-3b, sp-with-hairs	LBF-135	KU-d13601	BF-462	Long Beach, WA	cox3	
Ectocarpus sp.	E. sp-3b, sp-with-hairs	Fal-750	KU-d16662	BF-652	Falcon Cove Beach, OR, boat	cox3	
Ectocarpus sp.	E. sp-3b, sp-with-hairs	Fal-752	KU-d16682	BF-652	Falcon Cove Beach, OR, boat	cox3	
Ectocarpus sp.	E. sp-3b, sp-with-hairs	Fal-753	KU-d16805	BF-652	Falcon Cove Beach, OR, boat	cox3	
Ectocarpus sp.	Kuckuckia	RE-695-Ect4	KU-d16724	BF-533	Roads End, OR, boat	cox3	
Kuckuckia spinosa	Kuckuckia, with highly branched laterals	SixR-719	KU-d16658	BF-538	Sixes River mouth, OR, boat	cox3	
Kuckuckia spinosa	E. arctus	Wal-2	KU-d13966	BF-196	Waldport, OR, boat	<i>cox</i> 1, <i>cox</i> 3	