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Research Article

The first detection of the invasive macroalga *Codium fragile* subsp. *fragile* (Suringar) Hariot in Western Australia

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Abstract

Codium fragile subsp. *fragile* (Suringar) Hariot is a large, dichotomously branched green alga that has invaded many temperate locations around the globe. Despite its wide distribution and pest status, there are few accounts of *Codium fragile* subsp. *fragile* that provide morphological descriptions, including illustrations, for comparison. In 2008, a seaweed sample suspected to be *Codium fragile* subsp. *fragile* was collected from Albany, Western Australia. As this taxon superficially resembles other local erect and dichotomously branching *Codium* species, a detailed morphological and molecular analysis of the suspect alga was conducted. Investigations confirmed the identity of this alga as the invasive subspecies *Codium fragile* subsp. *fragile*. This represents the first record of this species in Western Australia.

Key words: Albany, algae, bioinvasion, Codiaceae, pest, Ulvophyceae

Introduction

Codium fragile subsp. *fragile* (Suringar) Hariot, 1889 [formerly *C. fragile* subsp. *tomentosoides* (van Goor) Silva, 1955; see Maggs and Kelly 2007] is a large, dichotomously branched green alga that has spread dramatically during the last century (Trowbridge 1998). Regarded as native to the coastal waters of Japan and Korea (Provan et al. 2005; Scheibling and Gagnon 2006; Lyons and Scheibling 2009), this alga has spread to temperate seas virtually worldwide. In Australia, *C. fragile* subsp. *fragile* has invaded most temperate coasts; it was first reported from Victoria at Corner Inlet in 1995 and has spread through Victoria, South Australia and Tasmania (Figure 1). In eastern Australia *C. fragile* subsp. *fragile* infests numerous coastal regions, to such an extent that attempting eradication was considered futile (Campbell 1999). Based on the alga's temperature and salinity tolerances, Trowbridge (1999) predicted that *C. fragile* subsp. *fragile* would spread to wave-protected bays, lagoons, and estuaries in New South Wales, Victoria, Tasmania, South Australia, and Western Australia.

In 2008, a seaweed sample collected by the Western Australian Department of Fisheries (DoF) from the Port of Albany was suspected to be the invasive alga *Codium fragile* subsp. *fragile* based on gross morphology. More detailed microscopical analysis confirmed the tentative identification as *Codium fragile* subsp. *fragile*. This species and subspecies had not been recorded from Western Australia previously, despite several extensive marine pest surveys (CRIMP 1997). *Codium fragile* subsp. *fragile* was identified by Hayes et al. (2005) as one of the ten most damaging potential domestic target species based on overall impact potential (economic and environmental). The species was collected as one of the key target marine pest species identified at the time by the Australian

Figure 1. Map of reported *Codium fragile* subsp. *fragile* distributions in Australia. *Codium fragile* subsp. *fragile* was first reported from Victoria at Corner Inlet in 1995 (A) and spread to Port Phillip Bay (1997) (B), Western Port (1998) (C) and then D'Entrecasteaux Channel in Tasmania (1999) (D). It subsequently spread to Adelaide, South Australia (E) then Albany Port, Western Australia (2008) (F).

National System for the Prevention and Management of Marine Pest Incursions. The tendency of this species to overgrow and smother oyster beds has earned it the nickname 'oyster thief' (Naylor et al. 2001). *Codium fragile* subsp. *fragile* will often make its home on the shells of oysters, scallops, and clams. This can cause problems because an attached adult plant can hinder the movement and feeding of the shellfish (Trowbridge 1998). In cases where the attached plant is relatively large and wave exposure is high, the shellfish can be swept away with the plant.

After the initial detection, delimiting surveys of the area confirmed the alga's presence in a relatively small section of the port. Specimens collected during the later surveys were subjected to DNA sequence analyses to confirm their identity and to genetically characterize the Albany population. This paper documents the initial discovery and provides a morphological and molecular description of *Codium fragile* subsp. *fragile* in Western Australia to facilitate ready identification, and a comparison with *Codium spinescens* Silva and Womersley, 1956, the only local species with spinous utricles and likely to be misidentified as *C. fragile* subsp. *fragile*.

Methods

The Albany marine area, which includes King George Sound, Princess Royal Harbour, and Oyster Harbour, is located on the south coast of Western Australia approximately 418 km southeast of the state capital, Perth (Figure 1). Albany was one the first sites of European settlement in Western Australia, being colonised in 1826. The port predominantly handles bulk products; the main export trade being grain (43% in 2011) and woodchips (45% in 2011) (Albany Port Authority 2013). The port currently has four operational berths (combined total length of 825 m), a tug boat marina, and seven anchorage positions.

The Albany area underwent comprehensive marine-pest surveys in the late 1990's by the now defunct Centre for Research on Introduced Marine Pests (CRIMP); however *C. fragile* subsp. *fragile* was not recorded at that time (CRIMP 1997). In a recent overview, 25 introduced marine species were documented from the Albany marine area (Huisman et al. 2008).

The initial Town Jetty sample was identified by JMH based on morphology. Measurements of internal structures (utricles, medullary filaments, reproductive structures) were made on portions of the plant 2 cm from the apex, as dictated by Trowbridge (1999). In total, fourteen (1 at Town Jetty, 13 in tug harbour) (Supplementary material Table S1) specimens of *C. fragile* subsp. *fragile* were collected. Anatomical observations on these samples were made by FNH and JMH, and DNA analyses by RRMD. All specimens have been pressed onto herbarium sheets and lodged with the Western Australian Herbarium (PERTH), with a small collection retained at Western Australian Department of Fisheries for reference purposes.

Molecular analysis

Eight Australian *Codium* samples, including five from Western Australia, were newly sequenced for the molecular analysis, targeting the region *rps*3-*rpl*16 (UCP6) that was previously used in subspecies delineation in *Codium fragile* (Brodie et al. 2007) (Supplementary material Table S1). Methods for DNA extraction, PCR and sequencing follow Dixon et al. (2012) with the exception of

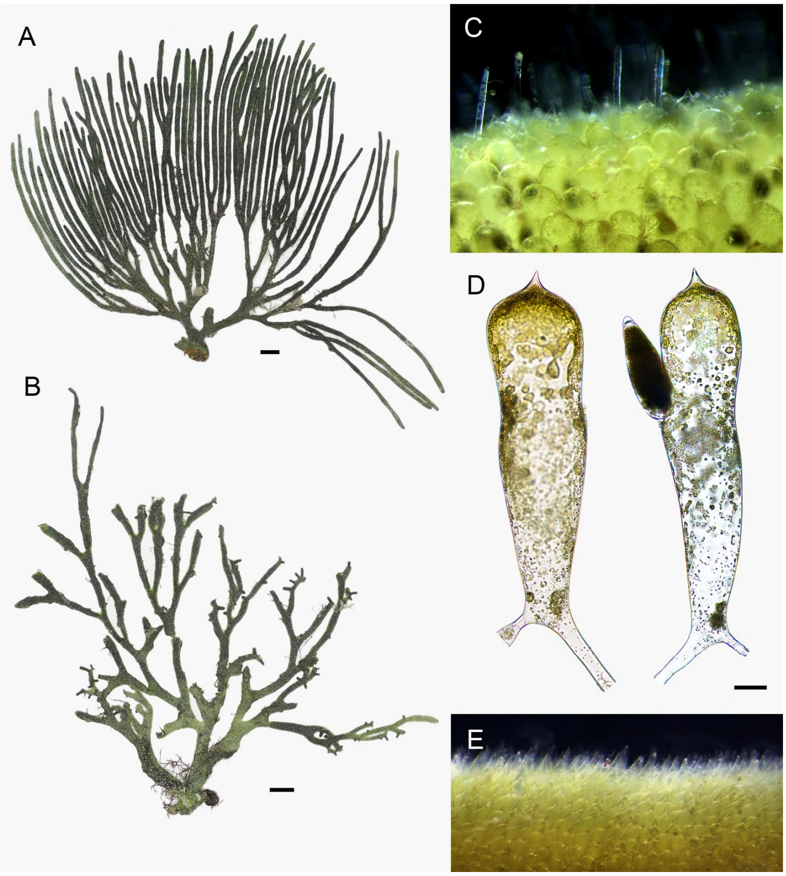

Figure 2. A-D. *Codium fragile* subsp. *fragile.* A, B. Pressed specimens showing habit variation (scale = 1cm). C. Surface view of thallus with hairs and utricular spines visible. D. Utricles showing slight medial 'waist'; that on the right has a lateral gametangium (scale = 100 µm). E. *Codium spinescens*, surface view showing utricular spines for comparison. Photographs by J.M. Huisman.

a secondary DNA purification step. Primers were derived from Provan et al. (2008) (primers for *rps*3-*rpl*16 (5′–3′) For: CCMGAHCCCATHCGDGTTTC, Rev: GGBMGHTTWAATGGHGCHGAWAT; annealing temperature 56°C).

The *rps*3-*rpl*16 sequences were between 386 and 457 base pairs long. *Codium* sequences downloaded from GenBank from Provan et al. (2008) and Verbruggen et al. (2007) were aligned with the new sequences and cut at each terminus, resulting in an alignment 370 nucleotides long (Supplementary data).

Results

Despite its global distribution and receiving much scrutiny due to its pest status, there are surprisingly few accounts of *Codium fragile* subsp. *fragile* that provide morphological descriptions, including illustrations, for comparison (e.g. Silva 1957; González and Santelices 2004). The utricles of the Western Australian specimens agree well with the diagnostic description given by Silva (1957), with only minor variations: utricles were slightly shorter, to 1050 µm; slightly narrower to 400 µm in maximum diameter; spines slightly shorter to 68 µm; and gametangia slightly narrower, 72–92 µm in diameter. The description given by González and Santelices (2004) is also similar, with slight differences: utricles slightly shorter to 1060 µm; slightly narrower to 392 µm in maximum diameter; spines longer, 78–138 µm in length; and gametangia slightly more broad, 123–197 µm in diameter. Below we give a detailed description to facilitate identification and comparison.

Morphology

The Western Australian samples had thalli that were medium to dark green, firm, erect, and repeatedly and regularly dichotomously branched, to 30 cm high. Branches were highly tomentose, generally terete, 5 mm in diameter basally, attenuating to 2–4 mm in diameter near branch apices; mostly with a relatively large and firm basal holdfast (Figure 2 – A, B). Utricles easily disassociated, quite variable in size and shape between thalli, and from the same specimen, with

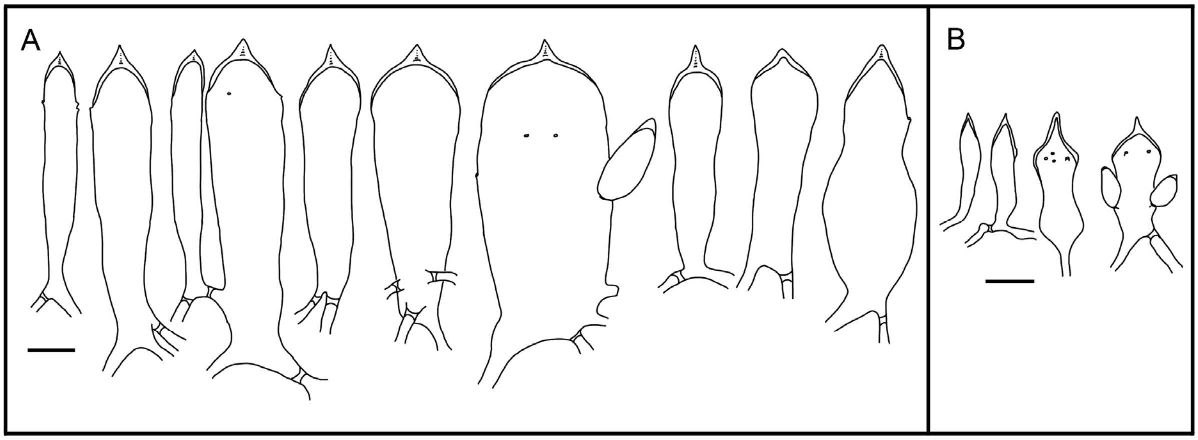

Figure 3. A. *Codium fragile* subsp. *fragile*, a selection of utricles showing variation in shape (scale = 200 µm). B. *Codium spinescens*, utricles drawn at the same scale as those in Fig. A for comparison, considerably smaller than those of *C. fragile* subsp. *fragile* (scale = 200 µm). Drawings by F.N. Hart.

intermixing of slender juvenile and mature utricles (often largely swollen) throughout parts of the thallus: elongated, irregularly cylindrical, clavate, to slightly obpyriform (Figure 2D, 3A), occasionally with a mid-utricular constriction 340–445 µm below utricle apices; utricles from mid-thallus (at dichotomy) 680–900 (–1135) µm long, 105–350 (–465) µm in diameter at utricle/spine margin, attenuating slightly to (70–) 105–290 µm in diameter basally; utricles from basal portions of thallus slightly shorter and narrower, 600–800 (–1085) µm long, 95–320 µm in diameter at utricle/spine margin, attenuating to 85–195 µm in diameter basally. Utricle apices (broadly) rounded, becoming apiculate, with chambered spines up to 78 µm long, less prominent (becoming blunt) on older utricles. Utricle hairs and/or hair scars sporadic to common, 0–3 (–4) per utricle, borne (145–) 185–215 (–260) µm below utricle apices. Medullary filaments generally 29–48 µm in diameter, (1–) 2–3 (–4) subtending each utricle, with the filament plug most commonly adjacent to utricle bases, infrequently may be up to 105 µm distant. Gametangia ovoid to fusiform, 280–320 µm long, 95–135 µm in maximum diameter, borne singly or in pairs on a short pedicel, 450–515 µm below utricle apices.

Codium fragile subsp. *fragile* superficially resembles numerous other Western Australian erect and dichotomously branching *Codium* species, including *C. australicum* Silva, 1956, *C. galeatum* J.Agardh, 1887, *C. harveyi* Silva, 1956, *C. muelleri* Kützing, 1856, *C. silvae* Womersley, 1984, and *C. spinescens* (see Womersley, 1984), all of which have only slight variations in thallus size and branch morphology. Of these species, however, only *C. spinescens* has utricular spines remotely like those of *C. fragile*. *C. spinescens* can be distinguished from *C. fragile* by its branches of a smaller diameter [to 3 mm diam. near base, attenuating to 1–2 mm diam. near tips (*C. spinescens*); to 5 mm diam. near base attenuating to 2–4 mm diam. near tips (*C. fragile*)], and utricles that are smaller, stout and strongly acuminate [compare Figures 2C and 3A (*C. fragile* subsp. *fragile*) with Figures 2E and 3B (*C. spinescens*), these depicted at the same scale]. Thus, in Western Australia at least, *C. fragile* subsp. *fragile* is readily identified following an anatomical examination.

Molecular analyses

As many temperate coasts that have been invaded by *Codium fragile* subsp. *fragile* have native subspecies that are very difficult to distinguish morphologically, Brodie et al. (2007, p 196–197) assigned subspecies to molecular haplotypes of *rps*3-*rpl*16 to aid in identification. The Western Australian *C. fragile* subsp. *fragile* collections were an identical match to the *rps*3-*rpl*16 haplotype for *Codium fragile* subsp. *fragile* assigned by Brodie et al. (2007) (GenBank: EU045560, see alignment in supplementary data). Newly sequenced Victorian and NSW collections also matched *C. fragile* subsp. *fragile* and one Victorian *Codium fragile* collection matched the haplotype *Codium fragile* subsp. *novae-zelandiae* (J. Agardh) Silva, 1956.

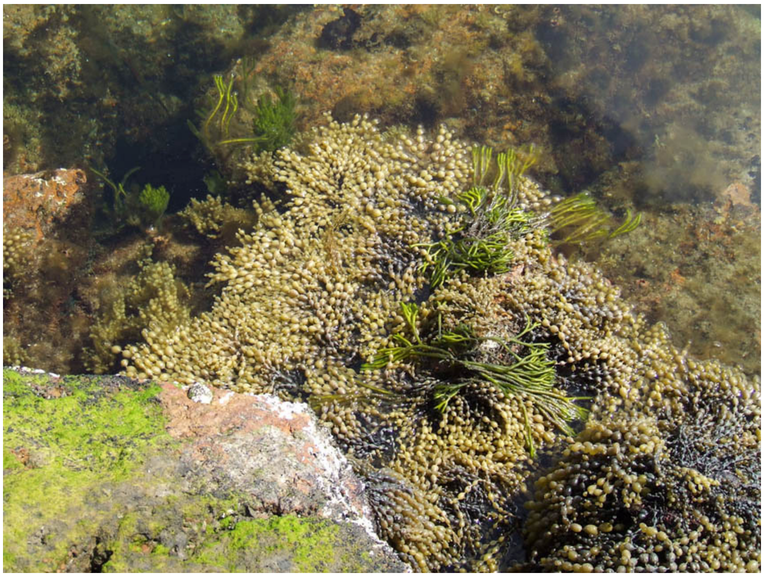

Figure 4. *Codium fragile* subsp. *fragile* as of February 2014 photographed from rock wall in tug pen (Photograph courtesy Rachael Goetze). The common brown alga also present is the native *Hormosira banksii* (Turner) Decaisne, 1842.

Discussion

Our morphological and genetic analyses have confirmed the presence of the invasive alga *C. fragile* subsp. *fragile* in Albany, Western Australia. Based on its invasive nature in other regions, this may represent a serious ecological and economic concern. Since *C. fragile* subsp. *fragile* appeared in the western North Atlantic in 1957 (Bouck and Morgan 1957) it has become a problem species along the east coast of America, growing at densities of up to 170 thalli m^2 and damaging the natural kelp (*Laminaria* spp.) forests, which provide fish nurseries (Trowbridge 1995). In eastern Australia this species infests numerous coastal regions. The invasive success of *C. fragile* subsp. *fragile* can be attributed to various characteristics of the alga's life history and physiological ecology (Begin and Scheibling 2003). The species exhibits various modes of reproduction; it can reproduce sexually, parthenogenetically, and vegetatively (Watanabe et al. 2009). Water currents can and will carry this species over long distances, introducing it to new locations. *Codium fragile* subsp. *fragile* is also tolerant of a range of salinity and water temperature levels. It also thrives in sheltered habitats, such as ports and marinas, which further facilitate human-mediated dispersal (Begin and Scheibling 2003). Regeneration and rapid growth of *Codium* from basal parts or holdfasts confers a competitive advantage over recruits of kelp and other native seaweeds (Scheibling and Gagnon 2006).

The frequency of incursions by invasive species such as *C. fragile* continues to rise, but the lack of robust and reliable genetic databases and the paucity of taxonomic expertise required to rapidly identify species can limit the capacity to respond effectively. Since the initial discovery of this species, surveys by the Department of Fisheries have identified that the number of plants appears to be increasing (Figure 4). As yet the effects of this *Codium* incursion in Albany Port are unknown. The Department of Fisheries (the lead agency for aquatic biosecurity in Western Australia) realises that working in partnership with industry and other stakeholders is essential to protecting the marine waters of Western Australia from non-indigenous species (see Piola and McDonald 2012 for example). As such the Department is working with the Albany Port Authority in determining the current distribution and what effects this species may be having, and how best to manage any secondary spread.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Voucher species identification numbers and collection details of samples examined during this study.
This material is available as part of online article from:
http://www.reabic.net/journals/bir/2015/Supplements/BIR_2015_McDonald_etal_Table_S1.xls

Appendix 1. Sequence alignments for *Codium* species examined in this paper.
This material is available as part of online article from:
http://www.reabic.net/journals/bir/2015/Supplements/BIR_2015_McDonald_etal_Appendix_1.fasta