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Rapid Communication

First confirmed occurrence of *Codium fragile* (Suringar) Hariot in the Iberian Peninsula coast of Portugal

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Abstract

Invasive macroalgae represent a major threat to marine ecosystems worldwide. *Codium fragile* ssp. *fragile* is one of the most invasive species in the world. The species has deleterious impacts on marine ecosystems, but can also coexist with other *Codium* congeners, with neutral impact. Several invasive macroalgae have been described to occur along the west coast of the Iberian Peninsula, including *C. fragile*. However, despite its presence being noted in citizen science surveys, the occurrence of *C. fragile* on the coast of Portugal was not confirmed (except in the Azores Islands). The cryptic nature of this invasive macroalga has led to several misidentifications, and strict taxonomic approaches must therefore be used to confirm sightings. Previous intensive field sampling (2017–2018) aimed at recording the presence of different macroalgae did not find *C. fragile* along the Portuguese coast, northwards from Praia do Matadouro. Here, we report the first confirmed record of *C. fragile* on the coast of Portugal, in the Avencas Marine Park. Identification of the alga was confirmed by microscopic examination of the utricles. Early detection of invasive species is essential, and further research should be carried out in the surrounding areas in order to better understand the invasion pathways to enable correct application of management plans.

Key words: biological invasions, macroalgae, Avencas Marine Park, MPA, cryptic species, Chlorophyta, *Codium fragile* subsp. *fragile*

Introduction

In the European Union, over 39% of alien marine species of special concern to at least one EU Member State are macroalgae (EASIN 2021). Therefore, its management (preferably early detection and prevention) should be in the forefront of invasive species policies (Goldsmit et al. 2018). However, management of such species is extremely difficult, especially for cryptic invasive species (Morais and Reichard 2018). The genus *Codium* is one important example of cryptic species complex that has led to historical misidentification due to its high degree of morphological and functional plasticity (Provan et al. 2008). These challenges are especially present in

regions where they coexist with native *Codium* congeners (Trowbridge 2001; Rojo et al. 2014). In European waters the genus includes four native species (*C. adherens* C. Agardh, *C. bursa* (Olivi) C. Agardh, *C. tomentosum* Stackhouse, *C. vermilara* (Olivi) Delle Chiaje), and one invasive species C. *fragile* (Suringar) Hariot, the last including two subspecies C. *fragile* subsp. *fragile* (Suringar) Hariot and subsp. *atlanticum* (A.D. Cotton) P.C. Silva. Hence, the presence of other native congeners has led to errors in species identification that have resulted in misinformation about the number of extant subspecies, and their invasiveness (Provan et al. 2008).

The green algae *Codium fragile* ssp. *fragile* (hereafter *C. fragile*) has a siphonaceous thallus differentiated in medulla and cortex. Microscopically the medulla consists of imbricated multinucleated filaments and the cortex consists of multiple bladder-like structures (utricles), by which species can be differentiated (Chapman 1999; Kusakina et al. 2006). This alga is commonly found on both rocky and sand substrates and tolerates a wide range of environmental conditions (Trowbridge 2001; Drouin et al. 2011). Although *C. fragile* (as well as other members of the genus *Codium*) is considered a valuable food product, it is also among the most invasive species worldwide, with both positive and negative effects on associated communities (see Katsanevakis et al. 2014 for detailed impacts).

Codium fragile originated in Japan and was first recorded in Europe in 1845, in County Donegal, Northern Ireland (Provan et al. 2008). Subsequently, spread throughout Europe (on the coasts of the North Atlantic Ocean and Mediterranean Sea), to the east coast of America and north-east Pacific and Australasian shores (Trowbridge 2001; Provan et al. 2005). In this context, while *C. fragile* was rapidly observed in the north-west Atlantic in the absence of native congeners, the cryptic nature of the species led to an unnoticed spread on the north-east Atlantic coast (interspecific cryptic invasion) and on the north-east Pacific and Australasian shores (intraspecific cryptic invasion) (Trowbridge 2001; Morais and Reichard 2018). In coastal areas of the north-east Atlantic Ocean, *C. fragile* has been reported to occur from Norway to the Canary Islands (Spain), with an important gap in its distribution along the coast of Portugal (Tyberghein et al. 2012; Blanco et al. 2020, 2021). Nevertheless, *C. fragile* has been reported to occur in the Azores Islands, Portugal (Tittley and Neto 2005). Only one study in mainland Portugal reported wild collection of *C. fragile* (Raja et al. 2016), although neither microscopic nor genetic approaches were used for identification. In addition, no records of *C. fragile* in Portugal were reported in an extensive database including 284 records of the species in European waters (Tyberghein et al. 2012). Likewise, Blanco et al. (2021) did not report *C. fragile* occurrences among approximately 1000 sampled *Codium* (*unpublished data*) in the 31 locations surveyed along the Portuguese coast (i.e. n = 57 sites). Therefore, the present study provides the first known microscopically verified record of *C. fragile* in the Iberian Peninsula, on the coast of Portugal.

Figure 1. Map of the sampling location (the Avencas Marine Park, Portugal) where the specimen of *Codium fragile* was found.

Materials and methods

On July 22, 2020, one specimen of *C. fragile* was identified from the Avencas Marine Park, Portugal (38.686091; −9.357883; Figure 1), where it was caught by hand during field sampling. The Avencas Marine Park was first designated as a Zone of Biophysical Interest in 1998 and later reclassified as a Marine Protected Area (MPA) in 2016 (POOC 2016), in order to increase compliance and enhance conservation outcomes by strengthening the participation of local stakeholders in planning processes. The Avencas Marine Park covers an area of 6 hectares in a highly urbanized coastal zone; it is formed by a calcareous rocky platform characterized by high marine biodiversity, including 32 species of macroalgae (Coelho et al. 2018). Among those algal species, the most abundant are the Rhodophyta *Ellisolandia elongata* (J. Ellis & Solander) K.R. Hind & G.W. Saunders, *Caulacanthus* sp. and *Ceramium* sp., the Chlorophyta *Cladophora* sp., and the Ochrophyta *Sphacelaria rigidula* Kützing and *Stypocaulon scoparium* (Linnaeus) Kützing.

Codium specimen was identified in the laboratory as *C. fragile* by morphological identification of the utricles under a dissecting microscope, following Rojo et al. (2014) methodology (Figure 2B). Both macroscopic and microscopic morphometic data were measured. Macroscopic size of the specimen was measured with a digital caliper to the nearest 0.001 mm. Microscopic size of utricules was measured with an ocular micrometer (Nikon, CFN filar micrometer eyepiece 10xA) to the nearest 1 µm. Wet and dry weights were measured on an analytical balance (Sartorius, ED224S Sartorius AG, Göettingen, Germany) to the nearest 0.1 mg.

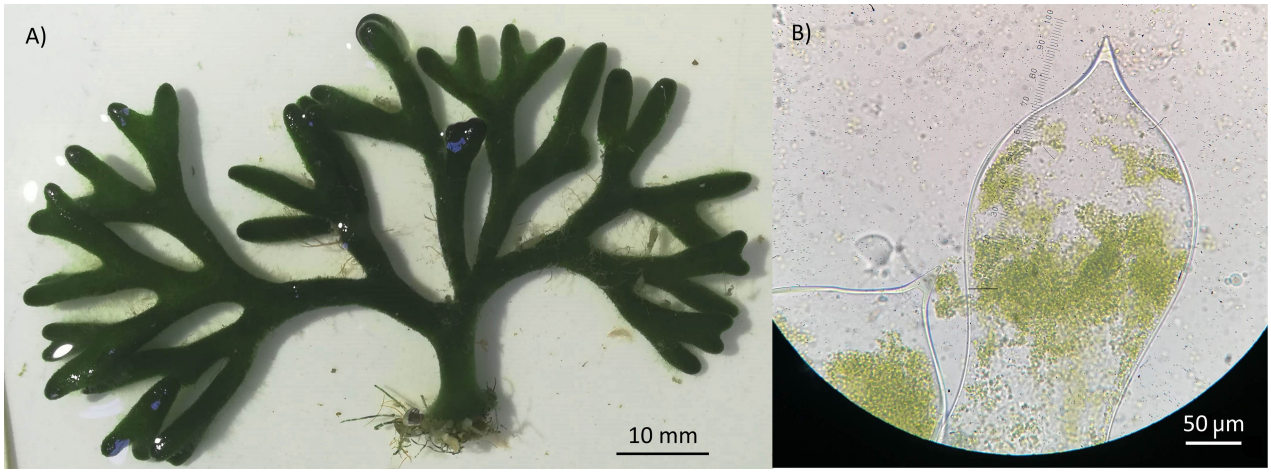

Figure 2. (A) Photograph of the whole specimen of *Codium fragile*, and (B) photomicrograph of utricles used for species-level identification.

Table 1. Macroscopic and microscopic morphometric data of the *Codium fragile* ssp. *fragile* individual collected in the Avencas Marine Park.

Total height (mm)	78.56
Width (mm)	34.77
Diameter of the frond (mm)	64.15
Wet weight (g)	2.8953
Dry weight (g)	0.4568
Mean (± SD) mucron length (µm)	23.86 ± 2.87
Mean (± SD) maximum utricle diameter (µm)	170.37 ± 55.36
Mean (± SD) length of utricle (µm)	503.63 ± 54.01
Mean (± SD) position of hair (or scar) (µm)	287.83 ± 42.91
Number of dichotomies on longest axis	7

Results and discussion

The current record of *C. fragile* in central Portugal (at Avencas Marine Park) represents the first strict taxonomic identification of this invasive species on the coast of mainland Portugal. The record was confirmed as a first occurrence by a search in Scopus and Pubmed with the keywords "*Codium fragile*" AND "Portugal" (date of search May 11th 2021). Nevertheless, the European Alien Species Information Network (EASIN) has reported occurrences of *C. fragile* in mainland Portugal, with records extracted from external databases (GBIF and iNaturalist). The network, as an initiative of the Joint Research Centre of the European Commission, collects data from researchers and also through a citizen science program that reports sightings of alien species, providing easy access to data and information on such species occurring in Europe. Despite the importance of public awareness and social participation, species should be properly identified, especially when dealing with cryptic species. Morphological identification of cryptic species is difficult macroscopically and, therefore, approaches such as microscopic examination and molecular techniques should be conducted by experts for correct identification of such species.

In the present report, the collected *C. fragile* was macroscopically measured and morphologically identified under the microscope by the typically

pointed mucron of the utricles (Table 1, Figure 2) (Trowbridge 1996; Armitage and Sjøtun 2016). In this context, while acknowledging the efforts of social science, none of the records reported by the databases included photomicrographs of the utricles, which may have led to species misidentification (Provan et al. 2008; Rojo et al. 2014). Some of the reported occurrences of *C. fragile* in mainland Portugal from the aforementioned databases corresponded to locations identified as being free of *C. fragile* in a recent census involving intensive field sampling, specifically aimed at monitoring invasive macroalgae (Blanco et al. 2021). In the census, up to 100 *Codium* individuals (depending on abundance along 50 m transects) were sampled in each of these locations and identified by utricle morphology, and none of the specimens were reported to be *C. fragile* (Blanco et al. 2021). Hence, since there is no reported evidence of the invasive species among the extensive *Codium* mats known to occur in the area (Blanco et al. 2020, 2021), the visual sightings reported in the citizen science programme are probably misidentifications.

Early detection is key for invasive management and, therefore, the record of *C. fragile* on the Portuguese coast is particularly important. Species distribution models (SDMs) of *C. fragile* at both regional (Blanco et al. 2021) and European (Tyberghein et al. 2012) scales revealed unsuitable marine physicochemical conditions for the species from ria *sensu lato* (Cotton 1956) of Vigo southwards. The wider distribution of *C. fragile* in Europe is related to sea-surface temperature range with an optimum at 17.5 °C (data extracted from SDM response curves using ImageJ) (Tyberghein et al. 2012; however, see Armitage and Sjøtun 2017 for more specific data on temperature requirements). In addition, other factors such as air temperature and dispersal kernels largely explain the current distribution of the species in the northwest Iberian Peninsula with very little influence of anthropogenic factors, which suggests an important role of secondary spread in *C. fragile* distribution in the area (Blanco et al. 2021). Identification of the pathways of spread of *C. fragile* is beyond the scope of the present research; a comprehensive survey of the Avencas Marine Park and other nearby locations is needed to determine whether the specimen belongs to a well-established but unnoticed population, or has spread from nearby (northern or southern) extant populations. However, it is unlikely that the specimen spread from northern populations as no individuals have been reported between Praia do Matadouro (~ 50 km northwards) and Cape Silleiro (~ 400 km northwards) (Blanco et al. 2020, 2021). In addition, sea current direction and geographical features such as the Tagus flow and the Nazaré Canyon may play an important role in describing population dynamics, as are known barriers in the distribution of other species such as *Zostera noltei* Hornemann in the area (Diekmann et al. 2005).

The presence of *C. fragile* in the Avencas Marine Park may respond to different invasion hypotheses. On one hand, following the "biotic resistance

hypothesis" (Elton 1958), the opening of new niches for the establishment of alien species may have responded to the decline in intertidal biodiversity as a result of poor implementation of conservation measures within the MPA (mainly related to high anthropogenic pressure via tourism and illegal fishing) (Coelho et al. 2018). On the other hand, according to the "invasional meltdown hypothesis" (Jeschke et al. 2012), the presence of other alien species (including macroalgae, invertebrates and fish) known to occur in the area (Almada et al. 2017) may increase the likelihood of survival and/or ecological impacts between one another (Simberloff and Von Holle 1999). Biodiversity effects associated to MPAs have been previously reported to have little impact in preventing the spread of invasive macroalgae, including *C. fragile*, in the study area (Blanco et al. 2020). Therefore, we recommend focusing on alien species interactions, especially since other invasive macroalgae have already been detected, namely *Asparagopsis armata* Harvey and *Colpomenia peregrina* Sauvageau (*pers. obs.* and Almada et al. 2017). Understanding such multi-species interactions may help evidencing the potential risk of invasion within an area when increasing the number of alien species (Braga et al. 2018). In addition, when considering cryptic invasive species (such as *C. fragile*) this may be extremely important as cryptic species may take on new roles in the community structure, thus potentially altering ecosystem functioning through biotic interactions (Morais and Reichard 2018). Altogether, the current record represents a breakthrough in the establishment of *C. fragile* in Portugal improving invasive species knowledge and, by including it into SDMs will help ultimately directing efficient management actions.

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Author's contribution

AB: research conceptualization; sample design and methodology; investigation and data collection; writing – original draft. CO: writing – review and editing. MB: writing – review and editing.

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