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4 Discovery of *Flabellia petiolata* (Halimedaceae, Chlorophyta) in the southern British Isles: a  
5 relict population or a new introduction?

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21 ABSTRACT

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23 The known distribution of seaweed species often requires updating after findings of new  
24 records outside the known range. The green algal family Halimedaceae is predominantly  
25 distributed in tropical and subtropical regions. *Flabellia petiolata* was considered endemic to  
26 the Mediterranean, but has also been recorded in the Canary Islands and Cape Verde. Recent  
27 marine biodiversity surveys by the citizen science group Seasearch in England led to the  
28 surprising discovery of plants that morphologically resemble *F. petiolata*. In this work we  
29 aimed to clarify the taxonomic identity of these specimens using morphological and  
30 molecular data. We obtained two *rbcL* and four *tufA* sequences for the English specimens  
31 that were identical to the two and nine sequences, respectively, available in GenBank for  
32 Mediterranean specimens of *F. petiolata*. This result is in agreement with morphological  
33 observations. Therefore, molecular and morphological data confirm that *F. petiolata* is  
34 established in southern England. Whether it is a new introduced species or a relict population  
35 is uncertain and we conclude that it is a cryptogenic species in England. This study provides  
36 an example of the contribution of citizen science to the better understanding of marine  
37 biodiversity.

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40 *Keywords:* Atlantic Europe; Bryopsidales; citizen science; cryptogenic; new record; non-  
41 native species; *rbcL* sequences; relict species; *tufA* sequences

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## 45 **1. Introduction**

46 The recently redefined family Halimedaceae includes 19 genera and about 150 species  
47 predominantly distributed in tropical and subtropical regions, with some species extending to  
48 warm temperate coasts (Cremen et al., 2018; Guiry and Guiry, 2019). *Flabellia* is placed in  
49 the tribe Udoteaceae and includes the single species *F. petiolata* (Turra) Nizamuddin, which  
50 is common and widely distributed in the Mediterranean Sea (Rodríguez-Prieto et al., 2013;  
51 Cremen et al., 2018; Guiry & Guiry, 2019). It has also been recorded in the Canary Islands  
52 and Cape Verde (Gallardo et al., 2016; John et al., 2004), as well as at Cádiz (Spain), on the  
53 mainland European Atlantic coast adjacent to the Mediterranean Sea (Seoane-Camba, 1965).  
54 More doubtful disjunct records include Sudan and Polynesia (Papenfuss, 1968; Tsuda and  
55 Walsh, 2013). Thalli in *F. petiolata* are non-calcified and consist of a stoloniferous basal  
56 system that bears a stipe terminating in a flabelliform blade (Nizamuddin, 1987). Among the  
57 Halimedaceae, *Udotea*, *Rhipiliopsis*, *Rhipilia* and *Rhipidosiphon* resemble *Flabellia* in  
58 morphology, but they are distributed in the Indo-Pacific and the Atlantic Americas and do not  
59 occur in mainland Europe. Thus, *Flabellia* is morphologically distinctive among the green  
60 seaweeds from mainland Europe.

61 Biogeographically, the British Isles are a transition zone between the cold-temperate  
62 NE Atlantic region and the warm temperate NE Atlantic subregion 1 (van den Hoek and  
63 Breeman, 1990), resulting in a particularly rich seaweed diversity. Such diversity is among  
64 the earliest studied globally and the best characterized worldwide, including foundational  
65 studies by Stackhouse (1795, 1797, 1802), Greville (1830), Harvey (1857) and Newton  
66 (1931). These classical works have subsequently been updated in floristic accounts including  
67 morphological and, in some cases, molecular studies (e.g. Maggs and Hommersand, 1993;  
68 Brodie et al., 2007). As a result, recent additions to the flora of this region are often restricted  
69 to the detection of newly introduced species (e.g. Fletcher & Manfredi, 1995; Bunker, 2014)

70 or the discovery of new cryptic or semi-cryptic species that are morphologically very similar  
71 to previously known species (Brodie and Irvine, 1997; Leliaert et al., 2009; Walker et al.,  
72 2009). The most recent floristic account of the green seaweeds in the British Isles included 99  
73 species (Brodie et al., 2007). In the order Bryopsidales, four genera have previously been  
74 recorded: *Bryopsis*, *Codium*, *Derbesia* and *Ostreobium* (Brodie et al., 2007; Cocquyt et al.,  
75 2010). Unexpectedly, during recent subtidal marine biological surveys in England, a species  
76 that morphologically resembles *Flabellia petiolata* was collected at three sites up to 50 km  
77 apart. The objective of this work is to clarify the taxonomic identity of this species using  
78 morphological and molecular evidence.

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## 81 **2. Materials and methods**

82 The Isle of Wight and surrounding areas in the Solent (southern England) have regularly been  
83 explored for more than 30 years since the Nature Conservancy Council, Marine Conservation  
84 Society and Marine Nature Conservation Reviews surveys of the 1970s to 1990s (Downie  
85 and Davies, 1991).

86 The UK citizen science diving group Seasearch (<http://www.seasearch.org.uk>) has  
87 surveyed many parts of the Dorset and Hampshire coasts (e.g. Isle of Wight, Hampshire,  
88 2007: <http://www.seasearch.org.uk/downloads/HantsIOW2007summary.pdf>) for the last  
89 three decades (Fig. 1). Seasearch is a partnership between the Marine Conservation Society  
90 (MCS), The Wildlife Trusts, statutory nature conservation bodies and others. In 2013 Lin  
91 Baldock took part in a Hampshire Seasearch dive in Alum Bay, Isle of Wight, coordinated by  
92 the Hampshire and Isle of Wight Wildlife Trust. In 2015, Natural England commissioned  
93 Fugro (<https://www.fugro.com>) to survey the subtidal in Alum Bay (Table 1). Survey  
94 techniques were similar, based on diver observations of biotopes including presence of

95 conspicuous marine macroalgae. Lin Baldock took part in Dorset Seasearch surveys of  
96 Weymouth Bay coordinated by the Dorset Wildlife Trust.

97         Specimens resembling *Flabellia petiolata* were observed for the first time in 2013 and  
98 subsequently observed and collected in 2015 (both formalin-preserved and pressed). Records  
99 were obtained from Weymouth Bay in every year between 2016 and 2019 (Fig. 1, Table 1).  
100 Materials collected during the 2018 survey were preserved in silica gel desiccant for DNA  
101 extraction and pressed as herbarium specimens. Voucher specimens collected in 2015 were  
102 deposited at the Natural History Museum, London (BM013844022).

103         DNA was extracted from silica gel-dried material following an adapted  
104 cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). PCR  
105 amplification of the *rbcL* and *tufA* plastid genes was carried out using primers 7F/1391R and  
106 GF4/R, respectively (Verbruggen et al., 2009; Saunders and Kucera, 2010). Reactions were  
107 performed in a total volume of 25  $\mu$ l, consisting of 5  $\mu$ l 5 $\times$  MyTaq<sup>TM</sup> reaction buffer, 0.7  $\mu$ l  
108 10  $\mu$ M of forward and reverse primers, 0.125  $\mu$ l 1U  $\mu$ l<sup>-1</sup> My Taq<sup>TM</sup> DNA Polymerase  
109 (Bioline, London, UK), 17.475  $\mu$ l MilliQ<sup>®</sup> water and 1  $\mu$ l template DNA. The PCR profile  
110 consisted of initial denaturation (93°C for 3 min), 35 cycles of denaturation (94°C for 30 s),  
111 primer annealing (45°C for 30 s), and extension (74°C for 90 s) and final extension (74°C for  
112 5 min). The PCR products were purified and sequenced by the sequencing service of the  
113 University of A Coruña (Spain).

114         Two *rbcL* and four *tufA* new sequences were analysed together with the two and nine  
115 sequences available in GenBank, respectively (Table 1). Sequences were aligned using  
116 Muscle in Geneious 6.1.8 (Kearse et al., 2012). The *rbcL* alignment was 1428 nucleotides  
117 long in total, and new sequences were 1256-1265 bp long. The *tufA* alignment was 871  
118 nucleotides long in total, and new sequences were 796-871 bp long.

119

## 120 **3. Results**

### 121 **3.1 Molecular identification**

122 The two *rbcL* and four *tufA* sequences determined in this study for samples collected in  
123 England were identical and were also identical to the two and nine, respectively, existing  
124 sequences of *Flabellia petiolata* from the Mediterranean Sea (Table 1).

125

### 126 **3.2 Morphological description**

127 Thalli consist of a stoloniferous base that bears a terete unbranched stipe terminating in a flat  
128 and flabelliform blade up to 18 mm in length and 15 mm in width (Figs 2A-C, 3A-C). They  
129 are non-calcified, dark green in colour, with a rigid texture. The stoloniferous base consists of  
130 almost unpigmented irregularly branched filaments while the stipe is composed of densely  
131 aggregated filaments (Fig. 2C). The blade is monostromatic, with concentric zonation,  
132 formed by parallel filaments (Fig. 3D) bound together by irregularly branched filaments (Fig.  
133 3E). Filaments are dichotomously branched and inserted at an acute angle (Fig. 3F). Older  
134 blades typically had frayed margins and some were entirely overgrown by epibiota (Fig. 2C).  
135 In some specimens, erect filaments formed a loose aggregation rather than a cohesive blade  
136 (Fig. 3B). Chloroplasts are spindle-shaped (Fig. 3G). Reproductive structures were not  
137 observed.

138

### 139 **3.3 Habitat and distribution in the British Isles**

140 *Flabellia petiolata* was observed in 2013 and every year between 2016 and 2019, and  
141 collected in 2015 and 2018 in the subtidal (3-16 m depth) at three sites, two of them ca. 3 km  
142 apart and the third site ca. 50 km distant from them, in southern England (Fig. 1, Table 1).  
143 These sites are characterized by elevated current speeds (up to 3 knots), high water turbidity  
144 and limestone or chalk bedrock. *F. petiolata* was found attached to the rocks, forming a turf

145 with red algae such as *Aglaothamnion tenuissimum* (Bonnemaison) Feldmann-Mazoyer,  
146 *Chondria dasyphylla* (Woodward) C.Agardh, *Halurus flosculosus* (J. Ellis) Maggs &  
147 Hommersand, *Heterosiphonia plumosa* (J. Ellis) Batters, *Phyllophora crispa* (Hudson)  
148 P.S.Dixon and *Xiphosiphonia ardreana* (Maggs & Hommersand) Savoie & G.W.Saunders.  
149 Sponges were also common, and the turfs were covered by a layer of silt.

150         Some Weymouth Bay specimens supported epiphytic bryozoans (Ctenostomatida,  
151 Crisiidae, *Chartella papyracea*), tunicates (*Aplidium punctum*), didemnids and the clavelinid  
152 *Pycnoclavella aurilucens*. Small foliose and filamentous red algae were also present.

153

#### 154 **4. Discussion**

155 Molecular and morphological data confirmed that the specimens collected in England  
156 correspond unequivocally to *Flabellia petiolata*. *RbcL* and *tufA* sequences from England  
157 were identical to the sequences available in GenBank from the Mediterranean Sea, its type  
158 locality (Turra, 1780). Morphologically, *F. petiolata* from England shares the main characters  
159 reported for the species at various Mediterranean locations (Nizamuddin, 1987; Rodríguez-  
160 Prieto et al., 2013; Cormaci et al., 2014). The major morphological difference detected is that  
161 the maximum size of English specimens was considerably smaller (up to 18 mm in length)  
162 than Mediterranean specimens (up to 9 cm; Nizamuddin, 1987). *F. petiolata* was observed in  
163 England in several years (2013, every year between 2015 and 2019), evidencing well-  
164 established populations. However, reproductive structures were never observed in the English  
165 specimens, suggesting that populations are mainly maintained through vegetative growth and  
166 fragmentation. This is not unusual in *F. petiolata*, where reproduction is rarely observed  
167 (Rodríguez-Prieto et al., 2013). Likewise, other stoloniferous or turf-forming seaweeds often  
168 reproduce mostly by vegetative growth of basal parts and fragmentation (Ceccherelli and  
169 Cinelli, 1999; Díaz-Tapia et al., 2011). Moreover, the habitat is more restricted in England

170 (subtidal, 3-18 m depth, at sites with elevated current speeds, the substrate is limestone or  
171 chalk and covered by silt) than in the Mediterranean (from the surface to 100 m depth;  
172 Rodríguez-Prieto et al., 2013). The species would appear to thrive at low light levels at  
173 considerable depths in clear Mediterranean waters as well as in the shallow turbid waters  
174 prevailing at the English sites.

175 This finding considerably expands northward the known distribution range of *Flabellia*  
176 *petiolata*, a species with warm-water affinities that was considered endemic to the  
177 Mediterranean Sea (Nizamuddin, 1987). Two main hypotheses would explain this new  
178 finding and the resulting disjunct distribution: 1) this is a recent introduction to England or 2)  
179 this is a relict population that has previously been overlooked.

180 The introduction hypothesis would explain why this distinctive and attractive species  
181 has never been found before in southern England, and is consistent with the lack of genetic  
182 divergence in *rbcL* and *tufA* relative to Mediterranean specimens. Potential introduction  
183 vectors, as for other non-native seaweeds, might be aquaculture activities, shipping or an  
184 aquarium escape (Williams and Smith, 2007; Mineur et al., 2014; Thomsen et al., 2016;  
185 Vranken et al., 2018). However, *Flabellia petiolata* was found in relatively pristine locations  
186 in England. Sites are more than 5 km from harbours and there is no cultivation of Pacific  
187 oyster, *Magellana gigas* (Thunberg, 1793), on the Isle of Wight (Herbert et al., 2012); the  
188 closest aquaculture facilities where Pacific oysters are cultivated are in Poole and Portland  
189 Harbours, 15-25 km respectively from the sampling sites. In the event of an introduction of *F.*  
190 *petiolata* mediated by these vectors, the finding of specimens growing on or close to human-  
191 made facilities would be expected, as most introduced seaweeds occur in these habitats  
192 (Arenas et al., 2006; Díaz-Tapia et al., 2017; Wolf et al., 2018). However, surveys in such  
193 habitats (e.g. Portland and Poole harbours) did not record *F. petiolate* (Fig. 1). Two public  
194 aquaria including tropical tanks are in the area where *F. petiolata* was found (Weymouth and

195 Bournemouth), but again, they are more than 10 km from the sampling locations. Moreover, a  
196 recent risk assessment of aquarium introductions in Europe showed that *F. petiolata* is not  
197 among the species found in European aquaria or provided by suppliers (Vranken et al., 2018).  
198 Also, the potential introduction seems unlikely considering that the species was found in  
199 locations up to 50 km apart. If *F. petiolata* were introduced, it must have an ability for rapid  
200 and long distance spread or multiple introduction events have occurred over a short  
201 timeframe. The only species of the tribe Udoteae that has been recorded as introduced is  
202 *Udotea argentea* in Hawaii (Bailey-Brock and Magalhaes, 2010). Therefore, *F. petiolata*  
203 would be the second record of an introduced species in this tribe if its non-native character in  
204 England is confirmed.

205         The hypothesis of *Flabellia petiolata* representing a relict species in England implies  
206 that its distribution was potentially wider in Atlantic Europe in the past, probably during  
207 warmer periods in the Holocene (11,700 years before present (BP) to now). A plausible  
208 hypothesis is that its distribution was reduced during cold periods in the Holocene and it only  
209 persisted in the NE Atlantic in refugia. Interestingly, previous studies on seaweeds suggested  
210 the existence of a Pleistocene (2.5 My to 11,700 years before present) glacial refugium in the  
211 Western English Channel, consistent with the genetic diversity and structure of the analysed  
212 species (Provan et al., 2005; Hoarau et al., 2007; Maggs et al., 2008). Similarly, this region  
213 could have acted as a refugium during more recent cold periods in the Holocene. The  
214 isolation of English and Mediterranean populations only recently would explain the absence  
215 of genetic differentiation between them in the *rbcL* gene, which mutates slowly (Clegg,  
216 1993), and in the more variable *tufA* gene (Oliveira-Carvalho et al., 2012). Further studies  
217 using more variable molecular markers and wider collection of specimens might provide  
218 further support for this hypothesis. *F. petiolata* is not the only example of warm-water  
219 species with a disjunct distribution on European Atlantic coasts, as the scenario is similar for

220 other species such as *Cladophora retroflexa* (Bonnemaison ex P.Crouan & H.Crouan) Hamel,  
221 *C. battersii* C.Hoek, *Codium bursa* (Olivi) C.Agardh and *Halopithys incurva* (Hudson)  
222 Batters (Maggs and Hommersand, 1993; Brodie et al., 2007). Their distribution also ranges  
223 from the Mediterranean to southern England, but they are absent at least along the western  
224 Atlantic Iberian Peninsula (Guiry and Guiry, 2019), where the summer seawater temperature  
225 is lower than in surrounding areas as a consequence of cold water upwelling events (Relvas et  
226 al. 2007). Palaeoceanographic reconstructions indicate that the intensity of this upwelling  
227 system varied during the Holocene (Martins et al., 2007; Pena et al., 2010). Thus, this  
228 temporally variable system could have allowed the northward expansion of *F. petiolata* and  
229 other warm-water seaweed species from the Mediterranean or northern Africa during periods  
230 of weak or absent upwelling, and cause the local extinction of these species in the western  
231 Atlantic Iberian Peninsula during stronger upwelling periods. The most recent weak  
232 upwelling included the Medieval Warm Period (c. 950-1250) and was followed by a strong  
233 upwelling during the Little Ice Age (c. 1300-1850) (Álvarez et al., 2005; Martins et al.,  
234 2007). This strong upwelling persists at present and could be acting as a barrier to the  
235 dispersal of warm-water seaweed species from glacial refugia in the Mediterranean and the  
236 Western English Channel, perpetuating their disjunct distribution.

237 A question remains if *Flabellia petiolata* is a relict species: why has it previously been  
238 overlooked despite the long tradition of seaweed studies in the British Isles and regular  
239 surveys in the study area? A plausible explanation is that it has persisted as inconspicuous  
240 stoloniferous basal parts and loosely aggregated filaments that maintained the populations by  
241 vegetative growth and fragmentation. Such loosely aggregated filaments of *F. petiolata* were  
242 observed in the sampling sites in addition to blades. Interestingly, recent metabarcoding  
243 studies of kleptoplasts of a sacoglossan sea slug in Hawaii revealed the presence of an  
244 invasive siphonous green algae at some sites where it had never been observed during visual

245 surveys because it persists as diminutive thalli (Wade and Sherwood, 2018). Similarly, *F.*  
246 *petiolata* could be present in England as small thalli that had remained undetected. Probably  
247 the flabelliform blades develop only under particular environmental conditions or for short  
248 periods.

249 In conclusion, three well-established populations of the warm-water species *Flabellia*  
250 *petiolata* were found in England and it is unclear whether it represents a new introduced  
251 species or is an overlooked native. Therefore, we consider this species as cryptogenic, as its  
252 origin is unknown or speculative (Carlton, 1996). This new record is an example of the  
253 relevance of the potential contribution of citizen science, i.e. the involvement of non-  
254 professionals in scientific research and environmental monitoring (Chandler et al., 2017), to  
255 improve our understanding of marine diversity. Initiatives developed by associations  
256 involving volunteers, such as Seasearch, contribute to increase the network of biodiversity  
257 observers, increasing the chance of revealing previously undetected diversity or  
258 environmental change (Cigliano and Ballard, 2017).

259

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276

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438 **Table 1.** Collection information and GenBank accession numbers for the material used in this  
 439 study.  
 440

Collection site, date, habitat, survey organizers & collectors or Reference	GenBank Accession Number	
	<i>rbcL</i>	<i>tufA</i>
Alum Bay, Isle of Wight, England; 12.x.2013; subtidal 4 m; Seasearch; LB	-	-
Alum Bay, Isle of Wight, England; 10.ix.2015; subtidal 3 m; Fugro; AB	-	-
White Nothe Reef, Weymouth Bay, England; 06.08.2016; subtidal 12 m, Seasearch; LB	-	-
Durdle Door Reef, Weymouth Bay, England; 05.08.2017; subtidal 18m, Seasearch; LB	-	-
Lulworth Banks, Weymouth Bay, England; 11.08.2018; subtidal 16 m; Seasearch; LB	MN150015-6	MN481501-4
Durdle Door Reef, Weymouth Bay, England; 04.08.2019; subtidal 18 m; Seasearch, LB	-	-
Cala Corbs, Palamós, Cataluña, Spain; Verbruggen et al., 2009	FJ432640	-
Cala Corbs, Palamós, Cataluña, Spain; Cremen et al., 2019	MH591083	MH591083

Torre Faro, Messina, Italy; Miladi et al., 2018	-	MF172077
Banyuls-sur-Mer, France; Händeler et al., 2010	-	GU592533-8
Cap D'Antibes, France; Sauvage et al., 2016	-	KU361871

441

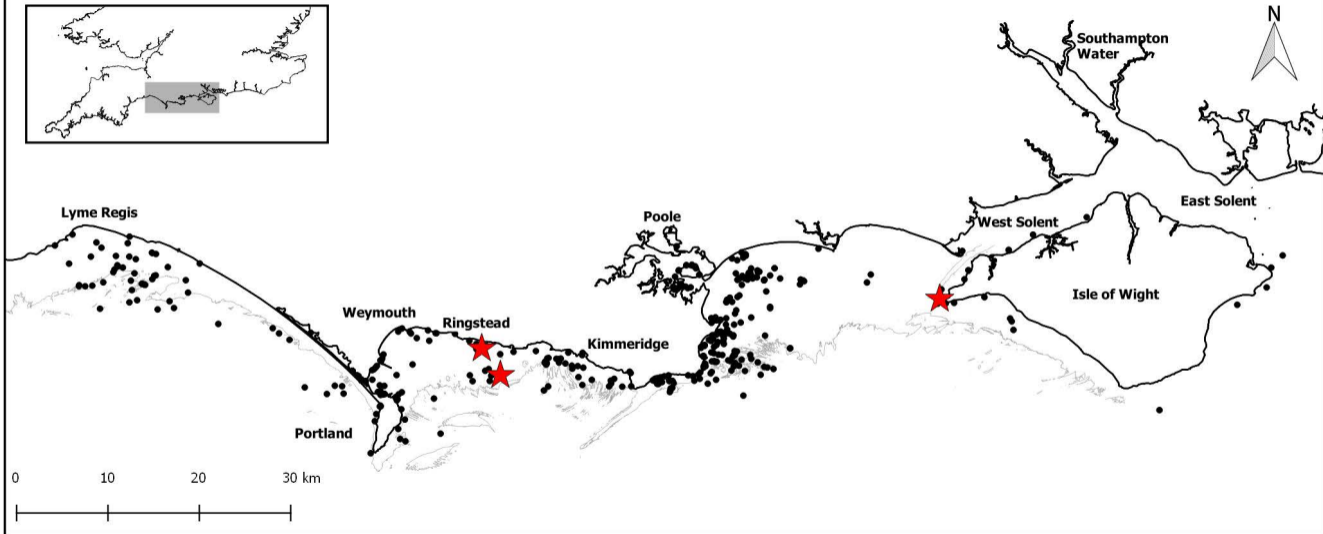
442 Collectors: LB (Lin Baldock), AB (Alison Bessell)

443 **Figure legends**

444 Fig. 1. Distribution of *Flabellia petiolata* in England (stars) and Seasearch survey dives  
445 undertaken in Dorset and Hampshire waters since 2000 by LB indicated by black dots. Grey  
446 line represents the – 30 m contour (below chart datum).

447 Fig. 2. *Flabellia petiolata*. (A) Blades on a silty limestone reef (Weymouth Bay). (B) Blades  
448 among loosely aggregated filaments on a silty chalk reef (Alum Bay). (C) Blades, one  
449 completely covered by a diverse epibiontic assemblage (Weymouth Bay). Scale bars: B, 20  
450 mm; C, 5 mm.

451 Fig. 3. *Flabellia petiolata*. (A) Herbarium specimens (Weymouth Bay). (B) Specimen with a  
452 blade and loose aggregated filaments (Alum Bay). (C) Blade consisting of parallel filaments  
453 and with concentric zonation. (D) Detail of the filaments forming the blade. (E) Warty  
454 appendages which bind filaments to form the coherent blade. (F) Dichotomous branching of a  
455 filament at an acute angle. (G) Spindle-shaped chloroplasts. Scale bars: A, B, 20 mm; C, 5  
456 mm; D, 500  $\mu\text{m}$ ; E-G, 50  $\mu\text{m}$ . Figures 5, 9 and 10 were provided by N. Owen.



Lyme Regis

Weymouth

Ringstead

Kimmeridge

Poole

Portland

West Solent

Isle of Wight

East Solent

Southampton  
Water

N

0 10 20 30 km

