Elucidating the diversity of microeukaryotes and epi-endophytes in the brown algal holobiome

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Abstract

Background

Brown algae (Phaeophyceae) are essential species in coastal ecosystems where they form kelp forests and seaweed beds that support a wide diversity of marine life. Host-associated microbial communities are an integral part of phaeophyte biology. The bacterial microbial partners of phaeophytes have received far more attention than microbial eukaryotes. The pre-requisite to understand the ecology of phaeophytes and their host-associated microbes is to know their diversity, distribution and community dynamics. To our knowledge, this is the first study to investigate phaeophyte-associated eukaryotes (the eukaryome) using broadly targeting 'pan-eukaryotic' primers and high throughput sequencing (HTS). Using this approach, we aimed to unveil the eukaryome of seven large common phaeophyte species. We also aimed to assess whether these macroalgae harbour novel eukaryotic diversity and to ascribe putative functional roles to the host-associated eukaryome, based on taxonomic affiliation and phylogenetic placement.

Results

Our sequence dataset was dominated by phaeophyte reads, from the host species and potential symbionts. We also detected a broad taxonomic diversity of eukaryotes in the phaeophyte holobiomes, with OTUs taxonomically assigned to ten of the eukaryotic major Kingdoms or supergroups. A total of 265 microeukaryotic and epi-endophytic operational taxonomic units (OTUs) were defined, using 97% similarity cut off during clustering, and were dominated by OTUs assigned to Stramenopiles, Alveolata and Fungi. C Almost one third of the OTUs we detected have not been found in previous molecular environmental surveys, and represented potential novel eukaryotic diversity.

This potential novel diversity was particularly diverse in phylogenetic groups comprising heterotrophic and parasitic organisms, such as labyrinthulids and Oomycetes, Cercozoa, and Amoebozoa.

Conclusions

Our findings provide important baseline data for future studies of seaweed-associated microorganisms, and demonstrate that microeukaryotes and epi-endophytic eukaryotes should be considered as an integral part of phaeophyte holobionts. The potential novel eukaryotic diversity we found and the fact that the vast majority of macroalgae in marine habitats remain unexplored, demonstrates that brown algae and other seaweeds are potentially rich sources for a large and hidden diversity of novel microeukaryotes and epi-endophytes.

Keywords [3-10]

Symbiome, epiphyte, endophyte, protist, microeukaryote, eukaryome, Phaeophyceae, seaweed, holobiont, fungi

Background

Seaweeds are essential to the health of our planet, providing core ecosystem services, and food and shelter to a wide diversity of marine life, from microbes to mammals, [1, 2 and references therein]. Brown algal seaweeds (Phaeophyceae) are Stramenopiles, a

81 radiation of eukaryotes which is phylogenetically distant from red- and green algal
82 seaweeds (Archaeplastida; Chlorophyta and Rhodophyta). The phaeophytes are one of
83 the most diversified groups of benthic algae and comprise approximately 2000 species,
84 encompassing complex multicellular species which have diversified and evolved since
85 the Mesozoic Era ~250 MYA [2, 3]. Large phaeophytes, especially those in the orders
86 Laminariales, Fucales and Tilopteridales are dominant members of intertidal and
87 shallow subtidal ecosystems worldwide, where they function as ecosystem engineers
88 forming complex underwater forests. These coastal ecosystems are some of the most
89 productive habitats on the planet [4], where seaweed beds and kelp forests are
90 indispensable biodiversity hotspots, which also provide habitat and nursery for a wide
91 range of taxa, including many commercially important species [5]. Phaeophytes have
92 been shown to accommodate up to 100.000 individuals of different invertebrate species
93 per square metre [6].

94 Phaeophytes and other seaweeds also harbour a wide diversity of microbial
95 epibionts and endobionts, comprising eukaryotes [7-9], prokaryotes [10-12] and viruses
96 [13-16]. These, together with the host, are collectively referred to as the seaweed
97 holobiont and can be considered as a localized ecosystem living on and in a host [10,
98 17-19]. It is increasingly recognized that host-associated microbial communities
99 (microbiomes) are an integral part of the host biology, exerting diverse and strong
100 influences on their hosts [19, 20]. While the term microbiome usually refers mostly or
101 exclusively to bacteria, it is important to consider the whole microbial symbiome,
102 including microbial eukaryotes (microeukaryotes) and larger epiphytic, epizootic, and
103 endophytic symbionts, to enable a comprehensive understanding of holobiont
104 functioning [19, 21].

105 Symbiotic interactions in its broadest sense refers to all intimate ecological
106 interactions between two species [22]. According to the fitness effect on the members
107 involved in the symbiotic relationship, the associations cover a wide spectrum of
108 interactions along the gradual continuum between positive mutualism and negative
109 parasitism. Some symbiotic interactions are obligate while other are more temporary
110 and fluctuating, and the nature of the interaction can vary depending on the symbiont's
111 interactions with its hosts, other symbionts and environmental conditions [20]. To date,
112 bacterial symbionts of phaeophytes have received most study [e.g., 10, 11, 12, 23], and
113 have been shown to represent complex and highly dynamic relationships that can have
114 everything from fundamental to detrimental effects on their hosts [10, 24-28].

115 Microeukaryotes in the phaeophyte symbiome are far less well known. Only a
116 small number of studies have investigated these associations, mostly using traditional
117 culturing/cell isolation methods or targeted molecular approaches. These studies
118 demonstrate that a broad taxonomic diversity of microeukaryotes are associated with
119 phaeophytes, including surface dwelling heterotrophic diatoms, dinoflagellates and
120 ciliates [8], 'naked amoeba' [29], epiphytic and endophytic diatoms [30-32], and green
121 algal endophytes [33], in addition to parasitic or saprotrophic labyrinthulids [34, 35],
122 oomycetes [7, 36, 37], phytomyxids [38-40] and fungi [9, 41-44]. The nature of these
123 microeukaryote-host relationships is mostly unknown, although some symbionts can
124 have detrimental effects on their macroalgal hosts, for example phytomyxids [38-40],
125 oomycetes [7, 36, 37] and chytridiomycete fungi [41]. Other microeukaryotes are
126 suspected to have a beneficial effect on their hosts, for example fungi that are mutualists
127 [9, 45, 46] and fungal endophytes that might protect seaweeds against pathogenic
128 protists [44].

129 Adding to the complexity of the microeukaryotic biodiversity associated with
130 phaeophytes, their thalli are often overgrown with a wide variety of smaller seaweeds
131 which represents a continuum between epiphytism and endophytism. These epi-
132 endophytes can have negative effects such as imposing physical and physiological
133 stress on their host, or positive ecosystem effects such as increased available habitats
134 and food for both macroscopic and microscopic life [47]. As macroalgal symbiomes
135 comprise multi-cellular algae as epiphytes [48, 49] and potentially pathogenic
136 endophytes [50-53] in addition to microeukaryotes, the almost complete lack of
137 knowledge of these eukaryotes in a holobiont context/perspective is a fundamental
138 knowledge gap which needs to be filled in order to improve our understanding of
139 phaeophyte holobionts.

140 The overall aim of this study was to unveil the eukaryome of seven large
141 phaeophytes which are key components in coastal ecosystems: *Fucus vesiculosus*, *F.*
142 *serratus*, *Himanthalia elongata* and *Ascophyllum nodosum* (order Fucales), *Laminaria*
143 *digitata* and *Saccharina latissima* (order Laminariales) and *Saccorhiza polyschides*
144 (order Tilopteridales). We achieved this aim by using using broadly-targeted pan-
145 eukaryotic primers and High Throughput Sequencing (HTS). This application in host
146 environments has so far been limited due to the technical limitation of co-amplifying
147 host DNA when using 'universal' eukaryotic primers [20]. However, with sufficient
148 sequencing depth now tractable to amplify host-associated eukaryotes much more
149 comprehensively than was previously possible, this will become increasingly
150 established as a standard tool for investigating host-associated eukaryomes [54, 55].
151 We also aimed to assess whether phaeophytes harbour a novel or differentially
152 structured diversity of (micro)eukaryotes, and to ascribe putative functional roles to the
153 microeukaryotes associated with these phaeophytes (e.g., putative parasites).

154

155 **Results**

156 **Composition of the brown algal eukaryome**

157 A total of 256 eukaryotic Operational Taxonomic Units (OTUs) were defined by
158 UPARSE and post-UPARSE trimming. Their read abundance in each library are shown
159 in Supplementary Table S1. Each library represented five phaeophyte samples pooled
160 according to species and geographic location, and is hereafter referred to as samples.
161 The richness recovered from each sample ranged from 26 to 101 OTUs, and the
162 taxonomic profile of these are shown as proportional OTU abundance in Fig. 1,
163 Supplementary Table S1. The sequence dataset was dominated by phaeophyte reads
164 (97.3% of all sequences, Supplementary Table S2). Phaeophytes also displayed the
165 highest OTU richness (18.9% of the total OTUs; Fig. 2, and accountable for 27.3-
166 70.4% of the OTUs per sample; Supplementary Table S2). Nevertheless, there was a
167 broad taxonomic diversity of microeukaryotes in the phaeophyte samples with OTUs
168 taxonomically assigned to ten of the eukaryotic major Kingdoms or supergroups (Fig.
169 1, Fig. 2).

170

171 **Phylogenetic diversity of eukaryotic OTUs**

172 Phylogenetic analyses were conducted for taxonomic groups displaying either high
173 taxonomic diversity and/or taxonomic groups previously documented to be in a
174 symbiotic relationship (*sensu lato*) with phaeophytes. The resulting phylogenies and
175 heat maps of proportional read abundance (log_{10}) of each OTU in the different samples
176 are shown in Figs. 3-7 and Supplementary Figs 1-4, which include OTUs and the closest
177 sequence matches in NCBI GenBank as of 05.05.2020 (re-blasted 11.04.2021).

178 For some taxonomic groups, the heatmaps indicated that the majority of OTUs
179 were only detected associated with one phaeophyte sample, such as Cercozoa (Fig. 4),
180 Fungi (Fig. 5), Ciliophora (Supplementary Fig. 1), Apicomplexa and Perkinsea
181 (Supplementary Fig. 2), Dinoflagellata (Supplementary Fig. 4) and Amoebozoa and
182 Centroheliozoa (Supplementary Fig. 3). However, most of these taxonomic groups also
183 comprised OTUs that were found in multiple samples, across host orders and
184 geographic locations (e.g., the fungal ascomycete OTU 226; Fig. 5, the ciliate OTU 89;
185 Supplementary Fig. 1, and the centroheliozoan OTU 13; Supplementary Fig. 3 were
186 found in samples belonging to Fucales, Tilopteridales and Laminariales from both
187 Norway and the UK). For other taxonomic groups such as Bacillariophyta (Fig. 6) and
188 Labyrinthulomycetes & Oomycetes (Fig. 3), the heatmaps show that a large proportion
189 of the OTUs were detected in multiple samples.
190 Several OTUs clustered with reference sequences that have been found
191 associated with various hosts in previous studies, such as the labyrinthulid and
192 oomycete OTUs clustering with *Aplanochytrium* (Labyrinthlida) and Olpidiopsidales
193 (Fig. 3), and the cercozoan vampyrellid OTUs clustering with a reference sequence
194 previously detected in samples of brown alga (Fig. 4).
195
196 **Novel diversity in the brown algal eukaryome**
197 We found that ~30% of the OTUs had low percentage identity (<95 %) with any known
198 close relatives in reference sequence databases and represent potential novel taxonomic
199 diversity (Supplementary Table 1). The majority of OTUs taxonomically and
200 phylogenetically placed within Fungi, Bacillariophyta and ciliates displayed high
201 percentage identity (>95 %) to known reference sequences (Fig. 5; Fig. 6; and
202 Supplementary Fig. 1, respectively). Conversely, within Labyrinthulomycetes and

203 Oomycetes (Fig. 3), Cercozoa (Fig. 4) and Amoebozoa and Centroheliozoa
204 (Supplementary Fig. 3), about 50% of the OTUs had low similarity to known reference
205 sequences (<95 %) which indicated that these OTUs represented potential novel
206 diversity. Some of this potential novel diversity was represented by specific clades in
207 the phylogenetic trees, such as a well-supported labyrinthulid clade within
208 Thraustochytrida that encompassed four OTUs with very low percentage identity
209 (ranging between 84.1% to 89.6%). Similarly, within Cercozoa, eight of the nine
210 Vampyrellida, Phytomyxea, and Novel Clade 9 OTUs had low similarity to reference
211 sequences (from 85.2% to 93%; Fig 4).
212
213 **Green (Chlorophyta), red (Rhodophyta) and Brown (Phaeophyceae) algae:**
214 In addition to protist taxa, we also detected OTUs from green, red, and (non-host-
215 derived) brown algae (Fig. 7). The majority of the nine green algal OTUs (Fig. 7) were
216 very similar (>99% identity) to characterized and sequenced Ulvales taxa such as
217 *Ulvella* (formerly *Acrochaete*) leptochaete and *Umbraulva japonica*. Only a single
218 green alga (OTU 264) displayed low similarity (90.4%) to reference sequences, and
219 clustered with *Lithotrichon pulchrum*, Ulvales (Fig. 7). Similarly, the five red algal
220 OTUs (Fig. 7) displayed >98% similarity to taxa including *Ceramium* sp. and
221 *Cryptopleura ramosa*.
222 There were in total 50 phaeophyte OTUs. Significant levels of microdiversity
223 in the V4 OTUs were seen in the phaeophyte clade. OTUs corresponding to the host
224 species were inferred on the basis of their phylogenetic proximity to, and high
225 proportions of reads associating with, reference sequences from that host, either from
226 GenBank or sequences generated in this study. Host OTUs are indicated by labeled
227 brackets on Fig. 7. Many of the OTUs derived from the host species were present in

228 samples from multiple host species, albeit generally represented with lower read
229 abundance in the 'non-host' samples.

230 However, other OTUs did not cluster with host-derived sequences, or from the
231 longer 18S sequences generated by cloning amplicons from the host. The 26 OTUs in
232 the "uncharacterized clade" on Fig. 7 were clearly distinct from any characterized or
233 environmental sequences in reference sequence databases. This clade encompassed
234 OTUs that were mainly detected in the *Fucus* sp. samples, but six of them were also
235 detected in lower abundance in other phaeophyte samples (Fig. 7). Additionally, seven
236 OTUs in the *A. nodosum* clade may be host sequences but are obviously different in
237 sequence from the cloned sequences generated from that host, and OTUs 2 and 326,
238 presumably deriving from that host (Fig. 7).

239 Some OTUs grouped strongly with known epiphytic lineages, such as the three
240 OTUs clustering with genera *Pylaiella* and *Halothrix*. These OTUs were found in all,
241 or in the majority, of the different pheaophyte hosts (Fig. 7). In the same clades were
242 the brown algal endophytes isolated and sequenced by Attwood (2005), published here
243 for the first time, and represented on Fig. 7, all in the 'brown endophytes' clade. These
244 endophytes were identified as *Myrionema strangulans*, isolated from *Fucus serratus*,
245 *Mastocarpus stellatus*, *Chorda filum*, *Osmundea pinnatifida*, and *Chondrus crispus*;
246 *Microspongium tenuisimum*, isolated from *Osmundea osmunda* and *C. crispus*;
247 *Streblonema* sp. isolated from *M. stellatus*; *Mytriotrichia clavaeformis* and *Ectocarpus*
248 sp. from *C. crispus*; and *Chorda filum* from a larger *C. filum* individual. Light
249 microscopy images of the isolated endophytes are shown in Fig. 8.

250 Three of our OTU sequences occurred in the same clade as these endophytes:
251 OTU 9 and some 18S clone sequences from *S. polyschides* clustering with *Pylaiella*,
252 and OTUs 70 and 146, clustering most closely with *Myriotrichia* and *Myrionema* (Fig.

253 7). All of these were strongly associated with *F. vesiculosus* and *S. polyschides*
254 individuals, but they were present in almost all host samples.
255
256 **Analyses of community composition**
257 The clustering of samples was consistent irrespective of whether host OTUs were
258 included in the analyses (Fig 9: A-B). The three *F. vesiculosus* samples clustered with
259 *F. serratus* and *S. polyschides* in both plots (Fig 9: A-B), which indicated that these
260 have a similar OTU composition. The *A. nodosum* sample showed less similarity with
261 *Fucus* and *S. polyschides* when the phaeophyte OTUs were excluded from the analysis
262 (Fig. 9, A-B). *Himanthalia elongata* did not cluster with any other phaeophyte hosts
263 and appeared highly dissimilar from the others both including and excluding
264 phaeophyte OTUs (Fig. 9; A-B). Analyses of similarity (ANOSIM) was highly
265 significant both with and without phaeophyte OTUs included (p-value 7e-04 and 0.03
266 respectively), which supported the dissimilarity observed between the phaeophyte host
267 genera in the NMDS plots (Fig. 9: A-B).
268 Dissimilarity between host genera (SIMPER values) ranged from 31.4%
269 (*Laminaria* spp. vs *Saccharina* spp.) to 99.9% (*Ascophyllum* spp. vs *Himanthalia* spp.)
270 in the dataset including phaeophyte OTUs (Table 1, Supplementary Table S3), where
271 certain host derived OTUs (OTU 1, 2, 3 and 5) were the main contributors to the
272 observed dissimilarity. These results also supported some of the OTUs inferred to
273 derive from the host species based on their phylogenetic placement and read abundance
274 (Table 1, Fig. 7). When phaeophyte OTUs were excluded the host genera were all
275 highly dissimilar, with SIMPER values ranging from 83.3% (*Ascophyllum* spp. vs
276 *Saccorhiza* spp.) to 99.4% (*Ascophyllum* spp. vs *Himanthalia* spp.). The main
277 contributors to the observed dissimilarity between the phaeophyte host genera were

278 green algal, red algal, fungal and oomycete OTUs in addition to an unassigned OTU
279 (Table 2). There were also several OTUs assigned to a wide range of taxonomic groups
280 that had some contribution to the dissimilarity (Supplementary Table S3).

281 IndVal analyses of the dataset including phaeophyte OTUs, indicated that six
282 OTUs were preferentially associated with *Ascophyllum* (Table 3), of which five were
283 taxonomically assigned to Phaeophyceae. Four of these OTUs clustered within the
284 clade labelled "*A. nodosum*" in the phylogenetic tree (Fig. 7, Table 3), while the fifth
285 OTU was placed in the "Uncharacterised" clade, represented with reads in all
286 phaeophyte libraries (Fig. 7). The final OTU (25) was a fungal OTU found in all the
287 libraries except *L. digitata* (UK), but with higher read abundance in *Ascophyllum* than
288 in the other host genera (Fig. 5). OTU 25 was also the only one found to be significant
289 by the IndVal analysis of the dataset excluding phaeophytes (p-value 0.0446; Table 2).
290

291 **Shared OTUs**

292 Although our analyses suggested a large degree of dissimilarity between the different
293 phaeophyte hosts (Table 1, 2), 70 OTUs were found in multiple samples across the host
294 orders Fucales, Laminariales and Tilopteridales (Fig. 10, Table 4). Of these, 25 OTUs
295 were detected in samples belonging to all of the three host orders, of which 15 belonged
296 to Phaeophyceae, including the three brown algal endophyte OTUs (Fig. 7), three OTUs
297 from the "uncharacterized" clade (Fig. 7) and several host OTUs that were found in
298 high abundances in the respective host samples, but also present in lower abundance in
299 several of the other phaeophyte samples. The shared OTUs also included five diatoms
300 which clustered with known epiphytes and endophytes of macroalgae in the
301 phylogenetic tree (Fig. 6, Table 4).

302 Twenty OTUs were found in samples belonging to Fucales and Laminariales
303 (Table 4), including Fungi, Oomycetes and Labyrithulomycetes (Table 4, Fig. 5, 3).
304 The 22 OTUs that overlapped between Fucales and Tilopteridales comprised several
305 diatom and green algal OTUs (Table 4, Fig 6, 7), and two Oomycete OTUs that
306 clustered within Olpidiopsidales (Table 4, Fig. 3). The OTUs that were unique to
307 Fucales, Tilopteridales and Laminariales are shown in Supplementary Table S4.
308

309 **Discussion**

310 **Broad diversity of microeukaryotes associated with brown algae**

311 To our knowledge, this is the first study that has investigated the diversity of eukaryotes
312 associated with brown algae using high throughput sequencing. Our findings show that,
313 although the sequence data was dominated by brown algal reads, we were able to
314 identify a broad diversity of microeukaryotes representing most of the main branches
315 in the eukaryotic tree of life (Fig 1, 2).

316 Some of the brown algae we investigated in this study displayed a higher
317 diversity of microeukaryotes compared to others. Particularly, we detected a higher
318 diversity associated with *Fucus vesiculosus*, *F. serratus* (Fucales) and *Saccorhiza*
319 *polyschides* (Tilopteridales) than *Laminaria digitata* and *Saccharina latissima*
320 (Laminariales). There are several factors/variables which may have affected the
321 observed diversity and community composition. Density and composition of
322 microorganisms associated with brown algae have been shown to vary at several scales:
323 e.g., among host species, between individuals of the same host species, different thallus
324 areas of individual hosts, the kind of surface tissue available for microepiphyte
325 colonization, different habitats and location in the intertidal zone, seasons, different
326 stages of the life cycle, and different longevity of the host [e.g., 19, 25, 32, 56, 57-59].

327 In addition, the interaction between the settlers affects the formation of specific
328 communities [47, 56], including chemical interactions and signalling between the
329 microbial symbionts [44, 60, 61]. Seaweeds themselves also, to various degrees have
330 physical and chemical antifouling defence mechanisms which prevents settlement of
331 microorganisms and epiphytes [31, 62, 63]. For this study, we may also have randomly
332 sampled different microhabitats of the brown algal thalli, resulting in a highly variable
333 diversity of microeukaryotes.

334 The community composition of microeukaryotes associated with *Himanthalia*
335 *elongata* stood out as very dissimilar to the other phaeophytes (Fig. 9 B). This seaweed
336 differs from the other phaeophytes in the type of life cycle and the tissue type sampled
337 for this study. *H. elongata* is biannual, but the elongated thongs which accounts for
338 ~98% of the thallus is reproductive tissue that degrades at the end of the season
339 (reproductive season from late July to ~January), leaving only the vegetative basal disc
340 [64]. The tissue type sampled from *H. elongata* were therefore different and most likely
341 younger than the tissues sampled from the other phaeophytes, which could explain the
342 distinctive and low diversity of microeukaryotes detected in this host. A recent study
343 also reported low diversity of bacteria associated with *H. elongata* [65].

344

345 **Eukaryotic epiphytes and endophytes in the seaweed holobiont:**

346 Seaweed surfaces provide nutrient-rich and important habitats for epiphytic and
347 endophytic organisms, from unicellular microbes such as bacteria and protists to
348 multicellular, usually filamentous seaweeds [e.g., 10, 12, 47, 56, 66]. These
349 associations can be either facultative (i.e. the epi-endophytes can grow on a variety of
350 biotic and/or abiotic substrata) or obligate (dependent on one or more specific hosts).
351 Like many biological categorizations, the boundaries between epiphytes and

352 endophytes in real-life can be blurred; the nature of the association can be affected by
353 both biotic and abiotic factors, and can range from mutualism to parasitism [47, 67,
354 68].
355
356 **Microeukaryotic epi-endophytes:**
357 Diatoms are regularly found in the biofilm on seaweed surfaces, where they live as
358 epiphytes, and often occur in large numbers [32, 69-71]. Although many diatoms
359 associated with phaeophytes are epiphytes, there are also studies which have
360 demonstrated that some diatom species, such as the ones belonging to the genera
361 *Navicula* and *Cocconeis* can live as endophytes, intracellularly in tissues of both brown
362 algae and red algae [30, 72-74]. Mayombo *et al*. [75, 76] recently suggested that some
363 diatom taxa might have adapted to an endophytic life to avoid the antifouling
364 mechanisms of their hosts. Several of the OTUs from our study which were
365 taxonomically and phylogenetically assigned to different species of Naviculales,
366 Cocconeidales and Fragilariales (Fig. 6), were present in multiple samples/hosts
367 independent of geographic location. Our molecular data in combination with previous
368 observational studies [30, 32, 72-76] supports the hypothesis that some diatom species
369 such as *Navicula* sp. and *Cocconeis* sp., might have a more intimate, potentially
370 endophytic, association with seaweeds. This, however, remains to be tested in future
371 studies.
372 Ciliates represent another ubiquitous group of microeukaryotes living as
373 epiphytes in the biofilm on seaweeds [8, 77], and was also the dominating group of
374 alveolates in our study (Fig. 2). Many ciliates are predators/grazers feeding on bacteria
375 and microeukaryotes [8], whereas other ciliates can be involved in symbiotic
376 interactions [78]. Most of the ciliates detected in our study were only found in single

377 samples (Supplementary Fig. 1), which might indicate that these ciliates were "random
378 visitors". A few of the OTUs were, however found in several samples from both
379 Norway and the UK, and were highly similar to reference sequences from different
380 marine environmental samples, which suggest that these ciliates are generalist biofilm
381 formers on different organisms and substrates. However, one of these OTUs (OTU 256,
382 Spirotrichea, Supplementary Fig. 1) was almost identical to a reference sequence found
383 in samples of *Ascophyllum nodosum* used as live bait wrapping [79], which could point
384 towards that some of these ciliates have a closer association with brown algae. As
385 emphasised by del Campo et al. [20], "it is likely that many microeukaryotes routinely
386 detected or observed/cultured from environmental samples may also be host associated,
387 even if transiently".
388
389 **Host-associated phaeophytes, chlorophytes and rhodophytes**
390 In addition to the phaeophyte OTUs clearly deriving from the host species, we detected
391 a diversity of other sequence types from putative brown algal epi-endophytes (Fig. 7).
392 The diversity of 'OTUs' apparently deriving from the host organisms was much higher
393 than expected. This may partly be caused by artefactual sequence variants amplified to
394 a discernible extent by the depth of Illumina sequencing. But the distinctiveness of
395 some of these OTUs from obviously host-derived sequences was so great in some cases
396 (e.g., the strongly *Fucus*-associated 'uncharacterised' clade in Fig. 7) that we propose
397 that these represented hitherto unknown epi-endobionts. In other cases we have
398 conservatively ascribed OTU diversity clustering with host sequences (e.g., the clades
399 bracketed as *A. nodosum* and *H. elongata* in Fig. 7) as being host-derived, but there
400 remains the possibility that they represented different organisms.

401 Some of the phaeophyte OTUs detected in our study, such as the ones clustering
402 with epiphytic *Pylaiella* and *Halothrix* and the other phaeophyte epi-endophytes (Fig.
403 7), clearly also represented epi-/endophytic lineages [e.g., 48, 58, 80]. These OTUs
404 were detected in all the phaeophyte hosts included in this study, indicating a broad
405 distribution and host range.

406 By analysing sequences from cultured phaeophyte symbionts of brown algae
407 and red algae (Attwood 2005) we show that the epi-endophyte genera *Microspongium*,
408 *Myriotrichia*, *Myrionema*, and *Streblonema* form a clade with *Chorda* and *Ectocarpus*
409 (growing as epi-endophytes), and previously sequenced phaeophyte epi-endophytes
410 *Pylaiella*, *Halothrix*, and *Scytosiphon*, which all together form a maximally supported
411 clade with *Saccorhiza*. While this significantly expands the 18S sequence
412 representation of phaeophyte epi-endophytes, it does not account for the vast majority
413 of host-associated phaeophyte sequences in our data that are not very obviously host-
414 derived. Further culturing and is situ experiments are required to explain these
415 suggestive findings, which might represent microscopic life-stages (zoospores,
416 gametophytes, gametes and juvenile sporophytes) of brown algae growing epi-
417 /endophytically on substrata, including on macroscopic sporophytes of other seaweeds
418 [81-84]. This can also explain why the host-derived OTUs were detected in lower
419 abundance in the other brown algal samples (Fig. 7).

420 Another possible explanation is that small filamentous brown algae were
421 growing epi-endophytically in the larger brown algal hosts [48, 85]. Such endophytic
422 infections are described as common diseases of brown algae [e.g., 52, 53, 67, 68, 86].
423 Hosts containing brown algal endophytes can either be asymptomatic, show weak
424 symptom such as dark spots or warts, or present severe symptoms such as
425 morphological changes to the brown algal host tissues [52, 67]. Substrata and brown

426 algal thalli often contain many more brown algal endophytes than expected based on
427 visual inspections prior to culturing [47, 53, 85].
428 The green algal OTUs were all similar to reference sequences of taxa that are
429 known to be endophytes in red (*Chondrus crispus Mastocarpus* stellatus, *Osmundea*
430 spp. and *Dumontia contorta*) and brown algae (*Fucus serratus* and *Chorda filum*) [33,
431 87, 88] .
432 All red algal OTUs were similar to reference sequences of taxa that are common
433 epiphytes on seaweeds and a variety of other substrata. Several of the taxonomic names
434 for the reference sequences included in the phylogeny are out of date (Fig. 7):
435 *Ceramium* spp. is an aggregate of species (i.e. *Ceramium rubrum* in Fig. 7) which are
436 members of the order Ceramiales together with *Calithamnion* spp., *Polysiphonia* sensu
437 lato (*Polysiphonia fucoides* is now *Vertebrata fucoides*) and *Cryptoleura* spp. Red algal
438 endophytes are common in other rhodophytes and chlorophytes (REF!). We did not,
439 however find any endophytic rhodophytes in our study, which might indicate that
440 phaeophytes are not prone to endophytic infections by rhodophytes, but instead are
441 more susceptible to endophyte infestations by chlorophytes and endophytic
442 phaeophytes.
443
444 **Novel diversity and putative parasites**
445 We demonstrate that brown algal holobionts encompass potential novel eukaryotic
446 diversity, as almost one third of the OTUs we detected have not been found in molecular
447 environmental surveys before. This was more pronounced in some phylogenetic groups
448 than in others, in particular the Labyrinthulids and Oomycetes (Fig. 3), Cercozoa (Fig.
449 4), Amoebozoa and Centroheliozoa (Supplementary Fig. 3). Some of the OTUs were
450 taxonomically and phylogenetically placed with known and putative parasites of

451 seaweeds. Our findings and the fact that the vast majority of macroalgae in marine
452 habitats remain unexplored, demonstrates that brown algae and other seaweeds are
453 potentially rich sources for a large and hidden diversity of novel microeukaryotes.

454 Labyrinthulomycetes are heterotrophic Stramenopiles which are abundant and
455 diverse in a wide range of marine and freshwater habitats where they play important
456 roles as saprotrophs/decomposers [89, 90]. Some Labyrinthulomycetes are also known
457 as important symbionts (parasites, mutualists or commensals) of marine organisms,
458 including brown algal seaweeds [91-93]. Although most thraustochytrids are free
459 living, a few species have been associated with disease in marine metazoans, including
460 the quahog parasite QPX which parasitizes clams. Several of the OTUs in our study
461 clustered in three highly supported clades within Labyrinthulida (Fig 3), and had high
462 similarity to *Aplanochytrium* labyrinthulids associated with various hosts such as corals
463 [94], the pseudoparenchymatous brown alga *Elachista* sp. [93], seagrass [95] and sea
464 stars [96].

465 Certain Oomycetes are common parasites of brown and red algae [37, 97-99],
466 and *Olpidiopsis* species have been shown to have cosmopolitan occurrence and broad
467 host ranges [98, 100]. In this study, we found OTUs clustering within Olpidiopsidales.
468 Some of these were highly similar to reference sequences of *Anisolpidium rosenvingei*
469 and *A. ectocarpii* (Fig 3), which are parasites of filamentous brown algae [36]. Others
470 displayed high similarity to *Pontisma lagenidioides* and "*Ectrogella*" (lnfLicSC1,
471 SC2-a, SC3 and SC4) which parasitizes the diatom *Licmophora* sp. and the red algae
472 *Ceramium virgatum* [101, 102]. Further, we detected two novel OTUs with no close
473 relatives among available reference sequences (OTU 214; 90.9 % identity to JN635125
474 and OTU 290; 92.4 % identity to KT273921, Fig. 3), of which the closest known
475 relative is an Oomycete parasitizing diatoms [101, 103].

476 Cercozoan diversity was dominated by vampyrellid amoebae, with four OTUs
477 forming a distinct clade with GU385680, which was previously detected on
478 *Ascophyllum nodosum* used as live bait wrapping [79]. Vampyrellids exhibit a wide
479 diversity of feeding strategies and often feed omnivorously; it is striking that this clade
480 comprises only lineages associated with brown algae, suggesting a specific association.
481 Phytomyxids, biotrophic parasites of angiosperms, oomycetes, and stramenopile algae
482 [39], were represented by two OTUs in a clade of marine sediment-derived sequences,
483 so there is no direct evidence for a specific interaction with macroalgae. OTU 242
484 groups with endomyxan Novel Clade 9 [104], members of which are found in a wide
485 range of environment types and have been hypothesized to be parasites of algae.
486 Fungi associated with brown algae can have everything from detrimental to
487 beneficial effects on their hosts [105, and references therein]. Two of the Ascomycota
488 OTUs clustered with taxa known to be parasites of brown algae such as *Sarocladium*
489 and *Acremonium* [106] (Fig. 5). Several of the basidiomycete OTUs clustered with
490 reference sequences of *Cryptococcus* and *Cystofilobasidium* that have previously been
491 found associated to various marine invertebrates, algae and seaweeds (Fig. 5) [107-
492 110]. In addition, within Chytridiomycota there were several OTUs that clustered with
493 *Rhizophydium* sp., which comprise parasites with broad host ranges known to infect
494 both macroalgae and protists [111, 112]. One OTU (OTU 76; Fig. 5) also displayed
495 high similarity to *Chytridium polysiphoniae*, a parasite of brown algae [113, 114]. The
496 final chytrid OTU clustered with *Mesochytrium penetrans*, a parasite of the unicellular
497 green algae *Chlorococcum minutum* [115], indicating that this could represent a parasite
498 of one of the green algae found in our study, or that relatives of *M. penetrans* have a
499 broader host range than currently known.
500

501 **Conclusions**

502 Brown algal holobionts are major marine habitat formers that provide food and shelter
503 for diverse communities of both macro- and microorganisms. Our findings demonstrate
504 that microeukaryotes should be considered as an integral part of brown algal holobionts,
505 and provides important baseline data for future studies of seaweed-associated
506 microorganisms. The prerequisite to understand the ecology of brown algae and their
507 host-associated microbes, is to know their diversity, distribution and community
508 dynamics. Consequently, in order to obtain an understanding of the interdependence of
509 all the different players in brown algal holobionts it is critical to include the so far
510 largely neglected eukaryotic and microeukaryotic partners in future holobiont
511 investigation. It is however also important to remember that microeukaryotes interact
512 not only with the host but also with the prokaryotes and viruses that share the same host
513 environment, and future holobiont surveys should ideally include all of these
514 components.

515

516 **Methods**

517 **Sample collection**

518 Five individuals each of *Ascophyllum nodosum*, *Fucus vesiculosus*, *Laminaria digitata*
519 and *Saccharina latissima* were sampled from the Oslofjord (59°40′26.9″N,
520 10°35′13.199″E), while five individuals each of *F. vesiculosus*, *F. serratus*
521 *Himanthalia elongata*, *Saccorhiza polyschides* and *L. digitata* were collected at
522 Newton's Cove (Dorset, UK: 50°36′18″N, 2°26′58″W) in October 2015. Samples were
523 collected by free diving and shore-based collection, kept cool in separate containers of
524 seawater while transported to molecular laboratories where they were immediately
525 subjected to subsampling of tissues for molecular analyses.

526 All samples were handled under laminar flow hoods to limit their exposure to
527 airborne contaminants. The brown algae were rinsed in sterile artificial seawater (ASW)
528 to remove loosely attached organisms (but not true epibionts) and debris from the
529 surface. This was done in three consecutive steps by vortexing the samples in sterile 50
530 mL tubes with ASW for 5-10 seconds. The rinsed individuals were placed in sterile
531 petri dishes, and one subsample of the algal thallus (approximately 1 cm^2 squares) per
532 individual was excised and placed in separate 2 mL tubes. All algae looked healthy; we
533 did not specifically target potentially infected tissues.
534 The samples were freeze-dried under sterile conditions by perforating the
535 sample tube lids and placing the sample tubes inside sterile culture flasks with 0.2 um
536 filter caps (Thermo Scientific™ Nunclon™). After freeze-drying, samples were
537 weighted, placed in new 2 mL sample tubes, and stored at -80 °C until DNA extraction.
538
539 **Endophyte sampling**
540 As part of a previous student project (Attwood 2005), brown algal endophytes were
541 selected from the B. Rinkel collection at The Natural History Museum, London. The
542 samples were isolated from hosts as shown in Table 5. Partial 18S-ITS1 rDNA
543 sequences were amplified using the phaeophyte-specific primer DICI [116] and the
544 general primer TW7 [117]. The 18S region of this amplicon did not overlap with the
545 V4 region sequenced by the metabarcoding approach, so longer 18S amplicons were
546 generated from the brown macroalgal samples used for this study as described below,
547 to enable as much direct comparison of our newly generated sequences with those from
548 the endophytes. Full details of the DNA extraction, PCR, and sequencing methods used
549 for the endophytes are given in Attwood 2005.
550

551 **Molecular methods**

552 The freeze-dried samples were mechanically disrupted by adding two sterile tungsten
553 carbide beads to each sample tube and tissue-lyzed at 20Hz for 2 minutes, or longer,
554 until the tissues were completely pulverised. Thereafter, a modified lysis buffer [118]
555 was added to the tubes proportional to sample weight. This lysis buffer contains
556 antioxidant compounds such as PVP (Polyvinylpyrrolidone) and BSA (Bovine Serum
557 Albumin) that bind polyphenols, and has high salt concentration which decrease the
558 levels of coextracted polysaccharides. The samples were homogenised and divided in
559 two or more tubes so that the sample used for DNA isolation did not exceed 20 mg dry
560 weight tissue. DNA was extracted following the protocol by Snirc *et al*. [118].

561 PCR amplification of the V4 region of the 18S rDNA gene was performed with
562 'pan eukaryotic' primers; the forward primer used was a slightly modified version of
563 the TAReuk454FWD1 primer (5'-CCAGCASCYGCGGTAAT**W**CC-3') and reverse
564 primer TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') [119]. PCR reactions (25
565 µl) contained 1x KAPA HiFi HotStart ReadyMix (Kapa Biosystems), 0.4 µM of each
566 primer, 0.5 mg/mL Bovine serum albumin (BSA; Promega) and 1 µl genomic DNA.
567 The PCR program had an initial denaturation step at 98°C for 2 min, 15 cycles of 30 s
568 at 98°C, 30 s at 53°C and 45 s at 72°C, then 20 similar cycles except that the annealing
569 temperature was 48°C, and a final elongation step at 72°C for 10 min. DNA was titrated
570 into several dilutions to take into account potential inhibiting substances from the
571 brown algae. Each brown algal individual sample was amplified separately. PCR
572 products were purified and eluted (20 µl) with ChargeSwitch PCR Clean-Up kit
573 (ThermoFisher Scientific), and quantified with the dsDNA BR Assay Kit and Qubit 2.0
574 Fluorometer (ThermoFisher Scientific), before they were pooled equimolarly,
575 according to species and geographic location; e.g., the purified PCR products of five

576 *Fucus vesiculosus* individuals from Norway were pooled into one sample before
577 sequencing library preparation.

578 Sequencing libraries were prepared using the TruSeq Nano DNA Library
579 Preparation Kit at The Natural History Museum, London UK, following the
580 manufacturer's protocol. High throughput sequencing was conducted on a MiSeq v3
581 flow cell using 2x300 bp paired-end reads. Base calling was performed by Illumina
582 Real Time Analysis v1.18.54 and was demultiplexed and converted to FastQ files with
583 Illumina Bcl2fastq v1.8.4.

584 The complete sequencing dataset is available at the European Nucleotide
585 Archive under the study accession number XXXXXXXX (http://www.ebi.ac.uk/ena/
586 data/view/ XXXXXXXX).

587 To obtain full length 18S sequences of the brown algal host species, three DNA
588 isolates from each brown algal species were subjected to PCR amplification using the
589 general eukaryotic 18S primers NSF83 (5'-GAAACTGCGAATGGCTCATT-3' [120])
590 and 1528R (5'-TCCTTCTGCAGGTTCACCTAC-3' [121]) utilising illustra™
591 PuReTaq Ready-To-Go™ PCR beads (GE Healthcare). PCR reactions contained 1
592 Illustra PuReTaq Ready-To-Go-bead, 1µl of DNA template, 0.5 µM of each primer,
593 filled to 25µl with water. PCR settings were: initial denaturation at 94°C for 2 min; 35
594 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s, elongation at 68°C
595 for 1.5 min; final elongation step at 68°C for 15 min. The PCR products were cloned
596 using the TOPO TA Cloning Kit for Sequencing (Invitrogen), according to the
597 manufacturer's protocol. The clones were grown overnight in Luberia-Bertoni (LB)
598 media amended with 50 µg/mL ampicillin. From each brown algal species, 20-30
599 bacterial colonies/cloned fragments, were subjected to PCR reactions with the vector
600 primers T7 and M13R and using ca 0.5 µl of the bacterial suspension as template. PCR

601 settings were: initial denaturation at 94°C for 10 min; 30 cycles of denaturation at 94°C
602 for 30 s, annealing at 53°C for 1 min, elongation at 72°C for 2 min; final elongation
603 step at 72°C for 10 min. The PCR products were cleaned using illustra™ ExoProStar
604 1-Step (GE Healthcare) and then Sanger sequenced (GATC Eurofins Genomics,
605 Germany).
606
607 **Bioinformatics**
608 The sequence data was processed using a workflow for MiSeq amplicon data
609 (https://github.com/ramalok/amplicon_processing). In short, prior to downstream
610 analyses error correction was done with BayesHammer [122], before merging the
611 paired-end reads with PEAR [123]. Thereafter, quality filtering, length check and fasta
612 conversion (from fastq to fasta) was done with USEARCH [124] before reads were put
613 into the correct 5' – 3' direction using profile hidden Markov models (hmmer-3.0rc2).
614 Dereplication, sorting by abundance and discarding of singletons was done using
615 USEARCH 64bit version. Clustering of reads into Operational Taxonomic Units
616 (OTUs) was done using the UPARSE algorithm as implemented in USEARCH,
617 applying a 97% sequence similarity threshold. *De-novo* and reference-based chimera
618 checking was performed and chimeric sequences discarded. OTUs were taxonomically
619 assigned using BLASTn against the PR2 v. 4.12.0 [125], GenBank [126] and SILVA
620 SSU [127] databases. We used a relaxed BLAST search strategy (BLASTn parameters:
621 -evalue 0.0001 -perc_identity 75) to retrieve distant sequences, and only retrieved the
622 best hit per query sequence. The PR2 taxonomy was used for taxonomy assignment for
623 the majority of the following analyses.
624
625 **"Post-UPARSE trimming" & Diversity analyses**

626 Species (OTU) composition analyses and further trimming of the dataset was done
627 using R v. 3.6.3 (R Core Team 2013). As we expected the sequence dataset was heavily
628 dominated by brown algal host reads, and we did therefore not normalise the dataset
629 because this would have led to removal of true diversity since most of the other OTUs
630 had very low read numbers compared to the brown algal OTUs. Further, all OTUs
631 assigned to Metazoa were removed from the dataset in addition to OTUs assigned to
632 land plants. The 57 OTUs (16.3% of the total number of OTUs) that matched those of
633 Metazoa are shown in Supplementary Figure S5, Supplementary Table S5.The trimmed
634 dataset, containing only non-metazoan eukaryotes, was used for further analyses.
635 The taxonomic composition of OTUs was visualized using the ggplot2 package
636 v. 3.3.2. [128]. To investigate how the composition of (micro)eukaryotes associated
637 with the different brown algal hosts, Bray-Curtis pairwise distances were calculated
638 using vegan v. 2.5-6 [129] for all brown algal samples (including/excluding brown algal
639 OTUs) and visualised using non-metric multidimensional scaling (NMDS) cluster
640 analyses (ggplot2 package v. 3.3.2 [128]). Analysis of similarities [ANOSIM; 130] was
641 calculated using vegan v. 2.5-6 [129] to test if there was a statistical significant
642 differences between clusters of libraries corresponding to different brown algal host
643 genera. The similarity percentage [SIMPER; 130] was calculated using the
644 simper.pretty.R script [131] to identify OTUs that contributed the most to the
645 dissimilarity observed. Indicator species analyses (IndVal) were carried out using the
646 indicspecies package v. 1.7.9. [132], to identify OTUs most preferentially associated
647 with specific brown algal host genera. Venn diagrams intersects were computed using
648 the function overLapper and plotted using the function vennPlot in the R-package
649 systemPipeR v. 1.24.3 [133].
650

651 **Phylogenetic analysis**

652 All OTUs were places into a global eukaryotic alignment of near full-length 18S
653 reference sequence using MAFFT v7.300b with the 'einsi' option for global homology
654 and long gaps for the reference sequences [134]. The shorter OTUs from the V4 region
655 (300-450 bp) were added to the reference alignment using the –addfragment option in
656 MAFFT and ambiguously aligned sites were removed with TrimAl [135] with –gt 0.3
657 and –st 0.001. Phylogenetic trees were built with RAxML v8.0.26 under the
658 GTRGAMMA model and the autoMRE bootsopping criteria [136]. Based on a
659 combination of the phylogenetic affinities of the OTUs and the taxonomic assignment
660 from PR2, individual phylogenies of the most abundant/diverse groups of
661 microeukaryotes – and those with known associations with brown algae - were
662 constructed: i.e Fungi, Oomycetes & labyrinthulids, Cercozoa, diatoms, red, brown,
663 and green algae, ciliates, other alveolates, Amoebozoa, and centrohelid heliozoans.

664 Individual sequence datasets were constructed including OTUs from each group
665 whose taxonomic annotation and phylogenetic position were concordant, and their
666 closest Blastn matches from GenBank. Sequence alignments were carried out in
667 MAFFT as described above. Phylogenetic trees were built with MrBayes v.3.2.6 [137].
668 Two separate MC3 runs with randomly generated starting trees were carried out for 4
669 million generations each with 1 cold and 3 heated chains. The evolutionary model
670 applied a GTR substitution matrix, with a 4-category autocorrelated gamma correction.
671 All parameters were estimated from the data. The trees were sampled every 1000
672 generations and the first 1 million generations discarded as burn-in. All phylogenetic
673 analyses were carried out on the Cipres server [138]. Heatmaps were made for all
674 phylogenetic trees to display the proportional read abundance (log_{10}) for each OTU in
675 the different samples.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The dataset generated are available in XX repository, under the accession XXXX-XXXX (link to dataset).

Competing interest

The authors declare that they have no competing interests

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Authors' contributions

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701

702

703 **Figure titles and figure legends**

704 **Figure 1. Diversity of eukaryotes in brown algal holobionts.**

705 Proportional abundance of OTUs taxonomically assigned to eukaryotic major
706 Kingdoms or 'supergroup' in the brown algal samples of *Fucus serratus*, *F.*
707 *vesiculosus*, *Ascophyllum nodosum*, *Himanthalia elongata*, *Saccorhiza polyschides*,
708 *Laminaria digitata* and *Saccharina latissima*. The sampling location for each brown
709 alga is shown in parenthesis; NO = Norway and UK = The United Kingdom, together
710 with the total number of OTUs per library. The asterisk (NO*) represent *F. vesiculosus*
711 sampled in Norway, May 2013. All other samples were collected in October 2015.

712

713 **Figure 2. Abundance of eukaryotic taxonomic groups.**

714 Percentage representation of OTUs assigned to the different taxonomic groups at (A)
715 Kingdom or 'supergroup' level and at lower taxonomic level (B) in all of the brown
716 algal samples combined. To see the percentage of reads and percentage of OTUs for all
717 the taxonomic groups per brown algal sample, see Supplementary table S2.

718

719 **Figure 3. Oomycetes and Labyrinthulids phylogeny with heatmap representing**
720 **proportional read abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of
721 Oomycetes and Labyrinthulomycetes with bootstrap values from maximum likelihood
722 analysis added. Black circles represent maximal support (posterior probability = 1, and
723 bootstrap support = 100%). OTUs from this study are shown in bold. The percentage
724 identity to the most similar reference sequence is shown in parenthesis after each OTU.
725 Host or type of environment the reference sequences were retrieved from in previous

726 studies are listed in parenthesis after each GenBank accession number. The scale bar
727 represents 0.2 substitutions per site. Abbreviations used for descriptions of
728 environment: env.S = environmental sample, marine sediment; env.W = environmental
729 sample, marine water; env.FW = environmental sample, fresh water; env.So =
730 environmental sample, soil. The heatmap illustrates the $\log_{10}$ read abundance for each
731 OTU in the brown algal samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum*
732 *nodosum*, *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and
733 *Saccharina latissima*. The sampling location for each brown alga is shown in
734 parenthesis; NO = Norway and UK = The United Kingdom. All samples were collected
735 in October 2015, except *F. vesiculosus* (NO*) which was sampled in Norway, May
736 2013.
737
738 **Figure 4. Cercozoa: phylogeny with heatmap representing proportional read**
739 **abundance ($\log_{10}$) of OTUs per sample**. Bayesian phylogeny of Cercozoa with
740 bootstrap values from maximum likelihood analysis added. Black circles represent
741 maximal support (posterior probability = 1, and bootstrap support = 100%). OTUs from
742 this study are shown in bold. The percentage identity to the most similar reference
743 sequence is shown in parenthesis after each OTU. Host or type of environment the
744 reference sequences were retrieved from in previous studies are listed in parenthesis
745 after each GenBank accession number. The scale bar represents 0.1 substitutions per
746 site. Abbreviations used for descriptions of environment: env.S = environmental
747 sample, marine sediment; env.W = environmental sample, marine water; env.FW =
748 environmental sample, fresh water; env.BW = environmental sample, brackish water;
749 env.S/W = environmental sample, marine sediment or water; env.So = environmental
750 sample, soil. The heatmap illustrates the $\log_{10}$ read abundance for each OTU in the

751 brown algal samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*,
752 *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina*
753 *latissima*. The sampling location for each brown alga is shown in parenthesis; NO =
754 Norway and UK = The United Kingdom. All samples were collected in October 2015,
755 except *F. vesiculosus* (NO*) which was sampled in Norway, May 2013.
756
757 **Figure 5. Fungi: phylogeny with heatmap representing proportional read**
758 **abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of Fungi with bootstrap
759 values from maximum likelihood analysis added. Black circles represent maximal
760 support (posterior probability = 1, and bootstrap support = 100%). OTUs from this
761 study are shown in bold. The percentage identity to the most similar reference sequence
762 is shown in parenthesis after each OTU. Host or type of environment the reference
763 sequences were retrieved from in previous studies are listed in parenthesis after each
764 GenBank accession number. The scale bar represents 0.07 substitutions per site.
765 Abbreviations used for descriptions of environment: env.S = environmental sample,
766 marine sediment; env.W = environmental sample, marine water; env.FW =
767 environmental sample, fresh water; env.So = environmental sample, soil; env.A =
768 environmental sample, air. The heatmap illustrates the log_{10} read abundance for each
769 OTU in the brown algal samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum*
770 *nodosum*, *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and
771 *Saccharina latissima*. The sampling location for each brown alga is shown in
772 parenthesis; NO = Norway and UK = The United Kingdom. All samples were collected
773 in October 2015, except *F. vesiculosus* (NO*) which was sampled in Norway, May
774 2013.
775

776 **Figure 6. Bacillariophyceae: phylogeny with heatmap representing proportional**
777 **read abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of diatoms with
778 bootstrap values from maximum likelihood analysis added. Black circles represent
779 maximal support (posterior probability = 1, and bootstrap support = 100%). OTUs from
780 this study are shown in bold. The percentage identity to the most similar reference
781 sequence is shown in parenthesis after each OTU. Host or type of environment the
782 reference sequences were retrieved from in previous studies are listed in parenthesis
783 after each GenBank accession number. The scale bar represents 0.06 substitutions per
784 site. Abbreviations used for descriptions of environment: env.S = environmental
785 sample, marine sediment; env.W = environmental sample, marine water; env.Si =
786 environmental sample, sea ice; GC = gut content. The heatmap illustrates the log_{10} read
787 abundance for each OTU in the brown algal samples of *Fucus serratus*, *F. vesiculosus*,
788 *Ascophyllum nodosum*, *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria*
789 *digitata* and *Saccharina latissima*. The sampling location for each brown alga is shown
790 in parenthesis; NO = Norway and UK = The United Kingdom. All samples were
791 collected in October 2015, except *F. vesiculosus* (NO*) which was sampled in Norway,
792 May 2013.
793
794 **Figure 7. Brown-Red-Green: phylogeny with heatmap representing proportional**
795 **read abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of Phaeophyceae,
796 Rhodophyta and Chlorophyta with bootstrap values from maximum likelihood analysis
797 added. OTUs from this study are shown in bold. The percentage identity to the most
798 similar reference sequence is shown in parenthesis after each OTU. Host or type of
799 environment the reference sequences were retrieved from in previous studies are listed
800 in parenthesis after each GenBank accession number. The longer 18S sequences

801 generated by cloning and sanger sequencing in this study are shown in bold and
802 includes which brown algal host species they were amplified from. The brown algal
803 endophytes isolated and sequenced by Attwood (2005) are all in the 'brown
804 endophytes' clade and are shown in capital letters. The scale bar represents 0.7
805 substitutions per site. The heatmap illustrates the $\log_{10}$ read abundance for each OTU
806 in the brown algal samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*,
807 *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina*
808 *latissima*. The sampling location for each brown alga is shown in parenthesis; NO =
809 Norway and UK = The United Kingdom. All samples were collected in October 2015,
810 except *F. vesiculosus* (NO*) which was sampled in Norway, May 2013.
811
812 **Figure 8. Brown algal endophytes**
813 Light microscopy images of phaeophyte endophytes isolated from *Fucus serratus*,
814 *Mastocarpus stellatus*, *Chorda filum*, *Osmundea pinnatifida*, and *Chondrus crispus*. (I)
815 *Streblonema* sp.: A – terminal sporangium, showing apical spore release; B – branching
816 thallus from spherical cells; C – growth pattern of new filament; D – discoid parietal
817 plastid. (II) *Myrionema strangulans*: A – Phaeophyceae hair; B – plurilocular
818 sporangia; C- two different cell shapes; D – unilocular lateral sporangium. (III)
819 *Microspongium tenuisimum*: A - plurilocular sporangia releasing zoospores; B -
820 Phaeophyceae hair; C – bifurcating branch; D – ovate cell, showing plate-like plastid
821 with prominent pyrenoids; E – waisted cross cell wall. (IV) *Mytriotrichia clavaeformis*:
822 A - Phaeophyceae hair; B – clubbed terminal cell; C - unilocular clustering sporangia;
823 D – empty clubbed terminal sporangium. (V) *Ectocarpus* sp.: A – ribbon-shaped
824 chloroplast; B – pyrenoid; C – pseudohairs; D – pseudohairs shoing growth from

825 filament; E – branching growth form. (VI) *Chorda filum*: A – densely pigmented cells;
826 B – prostrate clumps of branched filaments.
827
828 **Figure 9. Community composition**. Non metric multidimensional scaling (NMDS)
829 analysis of the composition of eukaryotic OTUs in the brown algal samples based on
830 Bray-Curtis pairwise distances. (A) including brown algal/Phaeophyceae OTUs and
831 (B) excluding brown algal/Phaeophyceae OTUs.
832
833 **Figure 10. Shared and unique OTUs in the brown algal orders**.
834 Venn diagrams displaying the number of shared and unique OTUs between the brown
835 algal host orders Fucales, Laminariales and Tilopteridales. To see the taxonomic
836 assignment for the shared OTUs see Table 4, and for the unique OTUs see
837 Supplementary Table S4.
838
839
840 **Supplementary figures:**
841
842 **Supplementary figure 1. Ciliates: phylogeny with heatmap representing**
843 **proportional read abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of
844 Ciliates with bootstrap values from maximum likelihood analysis added. Black circles
845 represent maximal support (posterior probability = 1, and bootstrap support = 100%).
846 OTUs from this study are shown in bold. The percentage identity to the most similar
847 reference sequence is shown in parenthesis after each OTU. Host or type of
848 environment the reference sequences were retrieved from in previous studies are listed
849 in parenthesis after each GenBank accession number. The scale bar represents 0.2

850 substitutions per site. Abbreviations used for descriptions of environment: env.S =
851 environmental sample, marine sediment; env.W = environmental sample, marine water;
852 env.FW = environmental sample, fresh water; env.WW = environmental sample, waste
853 water; env.BW = environmental sample, brackish water; env.S/W = environmental
854 sample, marine sediment or water; env.MBF = environmental sample, marine biofilm;
855 env.FBF = environmental sample, freshwater biofilm; env.So = environmental sample,
856 soil. The heatmap illustrates the $\log_{10}$ read abundance for each OTU in the brown algal
857 samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*, *Himanthalia*
858 *elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina latissima*. The
859 sampling location for each brown alga is shown in parenthesis; NO = Norway and UK
860 = The United Kingdom. All samples were collected in October 2015, except *F.*
861 *vesiculosus* (NO*) which was sampled in Norway, May 2013.
862
863 **Supplementary figure 2. Apicomplexa: phylogeny with heatmap representing**
864 **proportional read abundance ($\log_{10}$) of OTUs per sample**. Bayesian phylogeny of
865 Apicomplexa and perkinsids with bootstrap values from maximum likelihood analysis
866 added. Black circles represent maximal support (posterior probability = 1, and bootstrap
867 support = 100%). OTUs from this study are shown in bold. The percentage identity to
868 the most similar reference sequence is shown in parenthesis after each OTU. Host or
869 type of environment the reference sequences were retrieved from in previous studies
870 are listed in parenthesis after each GenBank accession number. The scale bar represents
871 0.3 substitutions per site. Abbreviations used for descriptions of environment: env.S =
872 environmental sample, marine sediment; env.W = environmental sample, marine water;
873 env.FW = environmental sample, fresh water; env.I = environmental sample, ice; GC
874 = gut content. The heatmap illustrates the $\log_{10}$ read abundance for each OTU in the

875 brown algal samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*,
876 *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina*
877 *latissima*. The sampling location for each brown alga is shown in parenthesis; NO =
878 Norway and UK = The United Kingdom. All samples were collected in October 2015,
879 except *F. vesiculosus* (NO*) which was sampled in Norway, May 2013.
880
881 **Supplementary figure 3. Amoebozoa and Centroheliozoa phylogeny with heatmap**
882 **representing proportional read abundance (log_{10}) of OTUs per sample**. Bayesian
883 phylogeny of Amoebozoa and Centroheliozoa with bootstrap values from maximum
884 likelihood analysis added. Black circles represent maximal support (posterior
885 probability = 1, and bootstrap support = 100%). OTUs from this study are shown in
886 bold. The percentage identity to the most similar reference sequence is shown in
887 parenthesis after each OTU. Host or type of environment the reference sequences were
888 retrieved from in previous studies are listed in parenthesis after each GenBank
889 accession number. The scale bar represents 0.2 and 0.08 substitutions per site for
890 Amoebozoa and Centroheliozoa respectively. Abbreviations used for descriptions of
891 environment: env.FWS = environmental sample, freshwater sediment; env.W =
892 environmental sample, marine water; env.So = environmental sample, soil. The
893 heatmap illustrates the log_{10} read abundance for each OTU in the brown algal samples
894 of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*, *Himanthalia elongata*,
895 *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina latissima*. The sampling
896 location for each brown alga is shown in parenthesis; NO = Norway and UK = The
897 United Kingdom. All samples were collected in October 2015, except *F. vesiculosus*
898 (NO*) which was sampled in Norway, May 2013.
899

900 **Supplementary figure 4. Dinoflagellates: phylogeny with heatmap representing**
901 **proportional read abundance (log_{10}) of OTUs per sample**. Bayesian phylogeny of
902 Dinoflagellates with bootstrap values from maximum likelihood analysis added. Black
903 circles represent maximal support (posterior probability = 1, and bootstrap support =
904 100%). OTUs from this study are shown in bold. The percentage identity to the most
905 similar reference sequence is shown in parenthesis after each OTU. Host or type of
906 environment the reference sequences were retrieved from in previous studies are listed
907 in parenthesis after each GenBank accession number. The scale bar represents 0.08
908 substitutions per site. Abbreviations used for descriptions of environment: env.S =
909 environmental sample, marine sediment; env.W = environmental sample, marine water;
910 env.BW = environmental sample, brackish water. The heatmap illustrates the log_{10} read
911 abundance for each OTU in the brown algal samples of *Fucus serratus*, *F. vesiculosus*,
912 *Ascophyllum nodosum*, *Himanthalia elongata*, *Saccorhiza polyschides*, *Laminaria*
913 *digitata* and *Saccharina latissima*. The sampling location for each brown alga is shown
914 in parenthesis; NO = Norway and UK = The United Kingdom. All samples were
915 collected in October 2015, except *F. vesiculosus* (NO*) which was sampled in Norway,
916 May 2013.
917
918 **Supplementary figure 5. Diversity of metazoan OTUs detected in brown algal**
919 **holobionts.**
920 Proportional abundance of OTUs taxonomically assigned to Metazoa in the brown algal
921 samples of *Fucus serratus*, *F. vesiculosus*, *Ascophyllum nodosum*, *Himanthalia*
922 *elongata*, *Saccorhiza polyschides*, *Laminaria digitata* and *Saccharina latissima*. The
923 sampling location for each brown alga is shown in parenthesis; NO = Norway and UK

924 = The United Kingdom. The asterisk (NO*) represent *F. vesiculosus* sampled in

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Table 1. Summary of pairwise SIMPER analyses between brown algal host samples grouped at genus level. Showing the OTUs that contributed to more than 10% of the observed dissimilarity between host genera according to SIMPER, when Phaeophyceae OTUs were included in the analyses.

		OTUs contributing >10 % to the dissimilarity between brown algal hosts (Including Phaeophyceae OTUs)					
	Phylum	Stramenopiles					
	Class	Phaeophyceae					
	PR2 taxonomy	*Silvetia siliquosa*	*Fucus gardneri*	*Fucus gardneri*	*Silvetia siliquosa*	*Saccorhiza polyschides*	*Agarum clathratum*
	Inferred host taxonomy	*Ascophyllum nodosum*	*Fucus spp.*	*Fucus vesiculosus*	*Himanthalia elongata*	*Saccorhiza polyschides*	*Laminariales (L. digitata + S. latissima)*
SIMPER comparison	Dissimilarity (%)	OTU 2	OTU 49	OTU 41	OTU 5	OTU 3	OTU 1
Fucus – Ascophyllum	74.4	41.3	8.4	4.8			
Fucus – Himanthalia	99.8	20.0	5.1	9.6	36.6		
Fucus – Saccorhiza	97.9	21.1	5.4	10.1		35.8	
Fucus – Laminaria	96.7	21.0	5.4	10.1			38.3
Fucus – Saccharina	97.3	15.2	3.9	7.4			54.3
Ascophyllum – Himanthalia	99.9	54.9	11.7		19.7		
Ascophyllum – Saccorhiza	99.8	56.6	12.1			18.7	
Ascophyllum – Laminaria	99.9	56.4	12.1				20.8
Ascophyllum – Saccharina	99.9	47.3	10.1				33.3
Himanthalia – Saccorhiza	99.7				45.0	41.6	
Himanthalia – Laminaria	99.7				44.7		45.8
Himanthalia – Saccharina	99.8				31.3		61.6
Saccorhiza – Laminaria	98.3					44.2	49.1
Saccorhiza – Saccharina	98.5					30.4	64.6
Laminaria – Saccharina	31.4						

Table 2. Summary of pairwise SIMPER analyses between brown algal host samples grouped at genus level. Showing the OTUs that contributed to more than 10% of the observed dissimilarity between host genera according to SIMPER, when Phaeophyceae OTUs were excluded from the analyses.

		OTUs contributing >10 % to the dissimilarity between brown algal hosts (Excluding Phaeophyceae OTUs)						
	Phylum	Chlorophyta	Rhodophyta	Fungi			Stramenopiles	Unassigned
	Class	Ulvophyceae	Florideo-phycaea	Basidiomycota		Ascomycota	Oomycota	NA
	PR2 tax	Ulvophyceae sp.	*Ceramium rubrum*	Agarico-mycetes sp.	Exobasidio-mycetes sp.	*Torulaspora franciscae*	*Pythium chamaihyphon*	NA
SIMPER comparison	Dissi-milarity (%)	OTU 6	OTU 21	OTU 57	OTU 25	OTU 50	OTU 42	OTU 61
Fucus – Ascophyllum	95.9	33.2	15.7		11.9	4.2		
Fucus – Himanthalia	98.5	44.9						4.8
Fucus – Saccorhiza	94.0	32.6	5.1				5.8	
Fucus – Laminaria	98.5	43.0	3.2					
Fucus – Saccharina	98.7	41.3		12.2				
Ascophyllum – Himanthalia	99.4		41.3		31.1	10.5		4.4
Ascophyllum – Saccorhiza	83.3		13.8		15.9	5.4	7.7	
Ascophyllum – Laminaria	92.9		35.9		28.6	9.8		
Ascophyllum – Saccharina	98.6		36.4	12.8	27.0	9.2		
Himanthalia – Saccorhiza	98.7		13.7				14.2	4.2
Himanthalia – Laminaria	96.5		11.1		3.0			28.6
Himanthalia – Saccharina	98.1			58.2	1.2			17.5
Saccorhiza – Laminaria	91.9		10.4				13.3	
Saccorhiza – Saccharina	98.6		12.2	12.2			12.5	
Laminaria – Saccharina	97.7		7.4	47.2				

Table 3. Indicator species analysis (IndVal) showing OTUs most preferentially associated with the brown algal hosts.

OTU#	Associated with	p-value	Taxonomic assignment
OTU 149	*Ascophyllum*	0.0185 *	Stramenopiles; Ochrophyta; Phaeophyceae; *Silvetia*; *Silvetia siliquosa*
OTU 381	*Ascophyllum*	0.0175 *	Stramenopiles; Ochrophyta; Phaeophyceae; *Silvetia*; *Silvetia siliquosa*
OTU 244	*Ascophyllum*	0.0005 ***	Stramenopiles; Ochrophyta; Phaeophyceae; *Silvetia*; *Silvetia siliquosa*
OTU 2	*Ascophyllum*	0.0020 **	Stramenopiles; Ochrophyta; Phaeophyceae; *Silvetia*; *Silvetia siliquosa*
OTU 49	*Ascophyllum*	0.0127 *	Stramenopiles; Ochrophyta; Phaeophyceae; *Fucus*; *Fucus gardneri*
OTU 25	*Ascophyllum*	0.0446 *	Fungi; Basidiomycota; Ustilaginomycotina; Exobasidiomycetes; Exobasidiomycetes_X sp.
OTU 1	*Laminaria* + *Saccharina*	0.0033 **	Stramenopiles; Ochrophyta; Phaeophyceae; *Agarum*; *Agarum clathratum*

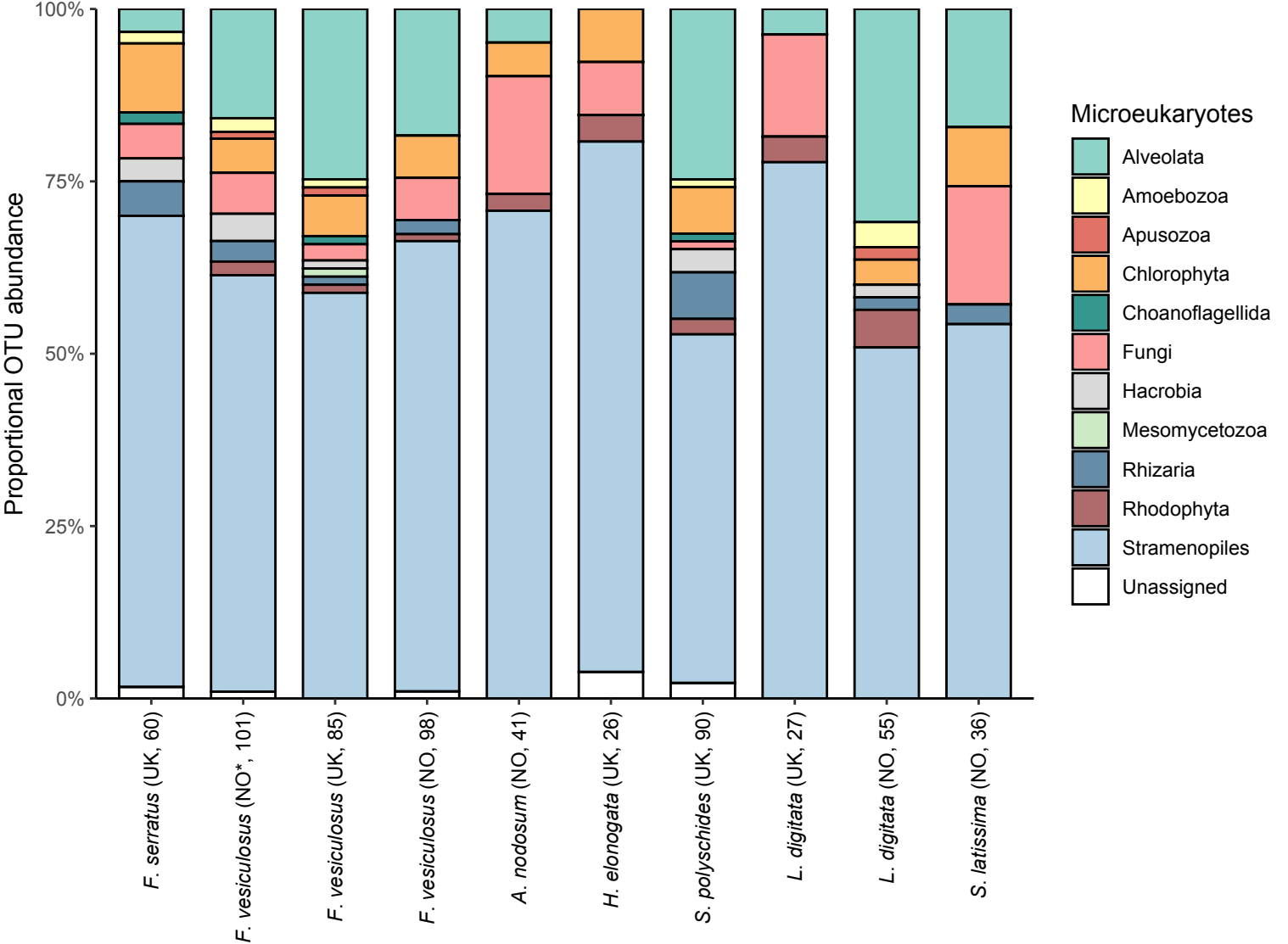
The Associated with column show which library/host species the OTUs were preferentially associated with. Abbreviations used; Ascophyllum = Ascophyllum nodosum, Laminaria = Laminaria digitata, Saccharina = Saccharina latissima. The asterisk associated with IndVal p-values indicat significance at ≤ 0.001 (***), ≤ 0.01 (**), ≤ 0.05 (*).

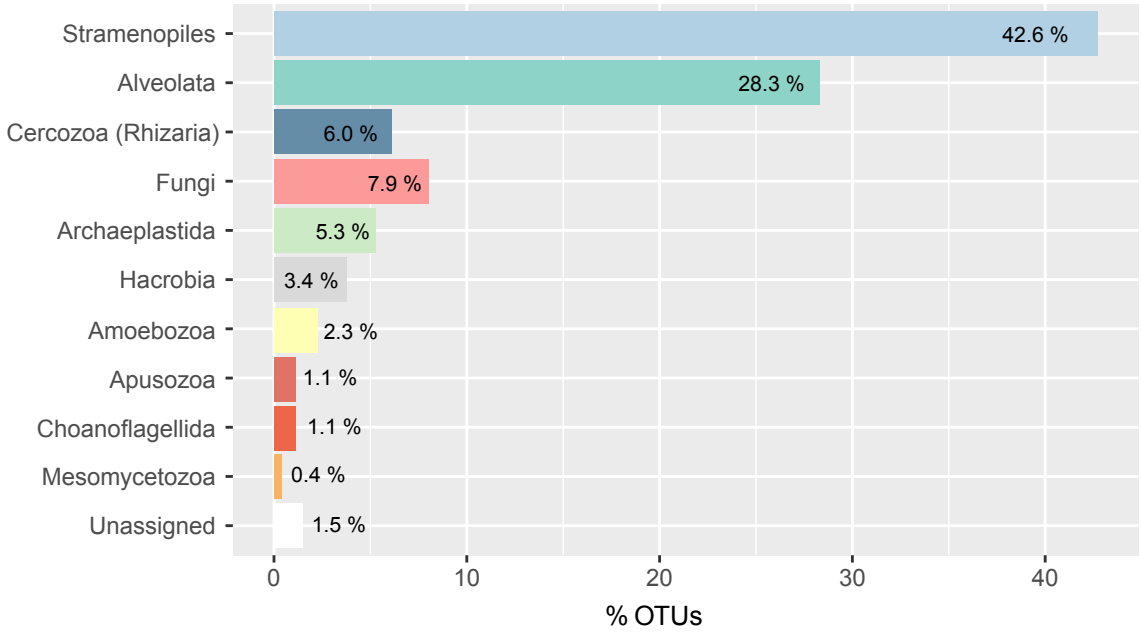
Table 4. Taxonomy of the shared OTUs between the three brown algal orders: Fucales, Laminariales and Tilopteridales, as shown in Venn plot (Fig. 10).

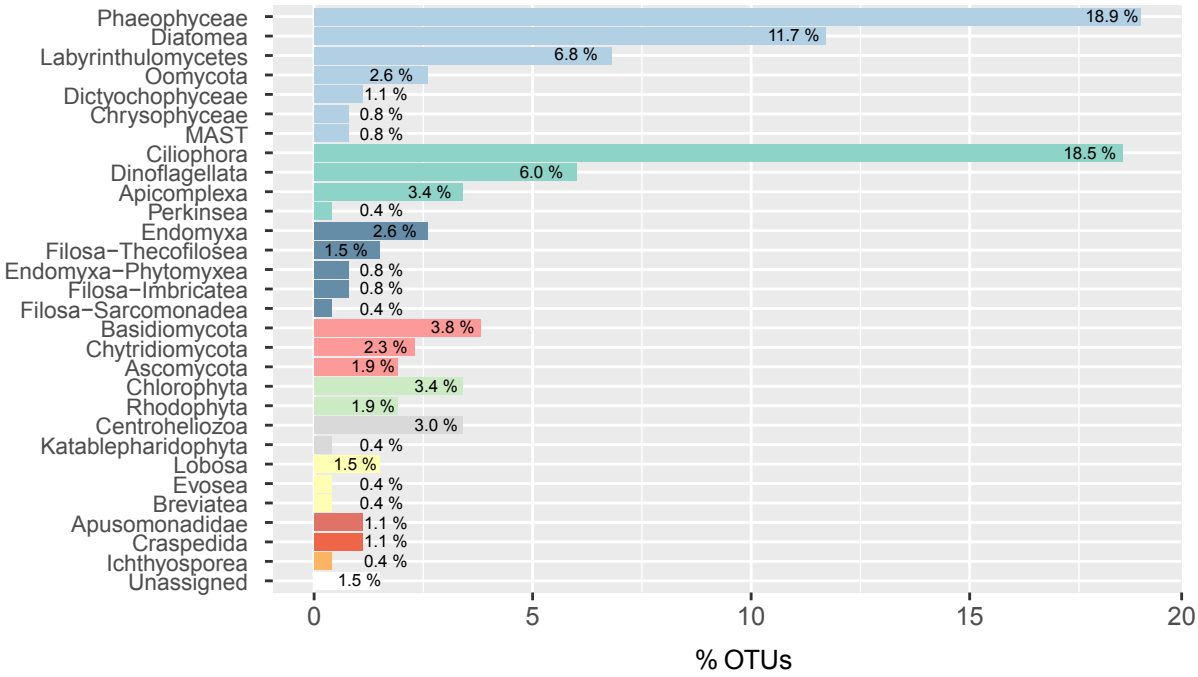
Overlap	**OTU#**	**Taxonomic assignment**		
Fucales + Laminariales + Tilopteridales (25 OTUs)	OTU 256	Alveolata	Ciliophora	Spirotrichea
	OTU 10, OTU 6	Archaeplastida	Chlorophyta	Ulvophyceae
	OTU 21	Archaeplastida	Rhodophyta	Florideophyceae
	OTU 25	Opisthokonta	Fungi	Basidiomycota
	OTU 94, OTU 233, OTU 234, OTU 68, OTU 112	Stramenopiles	Ochrophyta	Bacillariophyta
	OTU 1, OTU 236, OTU 28, OTU 292, OTU 41, OTU 49, OTU 146, OTU 70, OTU 4, OTU 7, OTU 9, OTU 3, OTU 2, OTU 348, OTU 5	Stramenopiles	Ochrophyta	Phaeophyceae
Fucales + Tilopteridales (22 OTUs)	OTU 111, OTU 148, OTU 137, OTU 188, OTU 151	Alveolata	Ciliophora	Oligohymenophorea, Phyllopharyngea, Spirotrichea
	OTU 298	Amoebozoa	Lobosa	*Vermistella* sp.
	OTU 180, OTU 87	Archaeplastida	Chlorophyta	Ulvophyceae
	OTU 110	Rhizaria	Cercozoa	Endomyxa
	OTU 265, OTU 197, OTU 220, OTU 72, OTU 63, OTU 237, OTU 17	Stramenopiles	Ochrophyta	Bacillariophyta
	OTU 300	Stramenopiles	Ochrophyta	Chrysophyceae
	OTU 105	Stramenopiles	Ochrophyta	Phaeophyceae
	OTU 38, OTU 42	Stramenopiles	Pseudofungi	Oomycota
	OTU 166, OTU 173	Unassigned	NA	NA
Fucales + Laminariales (20 OTUs)	OTU 89, OTU 97	Alveolata	Ciliophora	Oligohymenophorea, Phyllopharyngea
	OTU 107, OTU 191	Alveolata	Dinoflagellata	Dinophyceae
	OTU 69	Archaeplastida	Chlorophyta	Ulvophyceae
	OTU 226, OTU 118, OTU 257	Opisthokonta	Fungi	Ascomycota, Basidiomycota
	OTU 93, OTU 172	Stramenopiles	Ochrophyta	Bacillariophyta
	OTU 23, OTU 26, OTU 389, OTU 149, OTU 244, OTU 326, OTU 381	Stramenopiles	Ochrophyta	Phaeophyceae
	OTU 238	Stramenopiles	Opalozoa	MAST-3
	OTU 295	Stramenopiles	Pseudofungi	Oomycota
	OTU 249	Stramenopiles	Sagenista	Labyrinthulomycetes
Laminariales + Tilopteridales (3 OTUs)	OTU 193, OTU 74	Alveolata	Dinoflagellata	Dinophyceae
	OTU 168	Stramenopiles	Sagenista	Labyrinthulomycetes

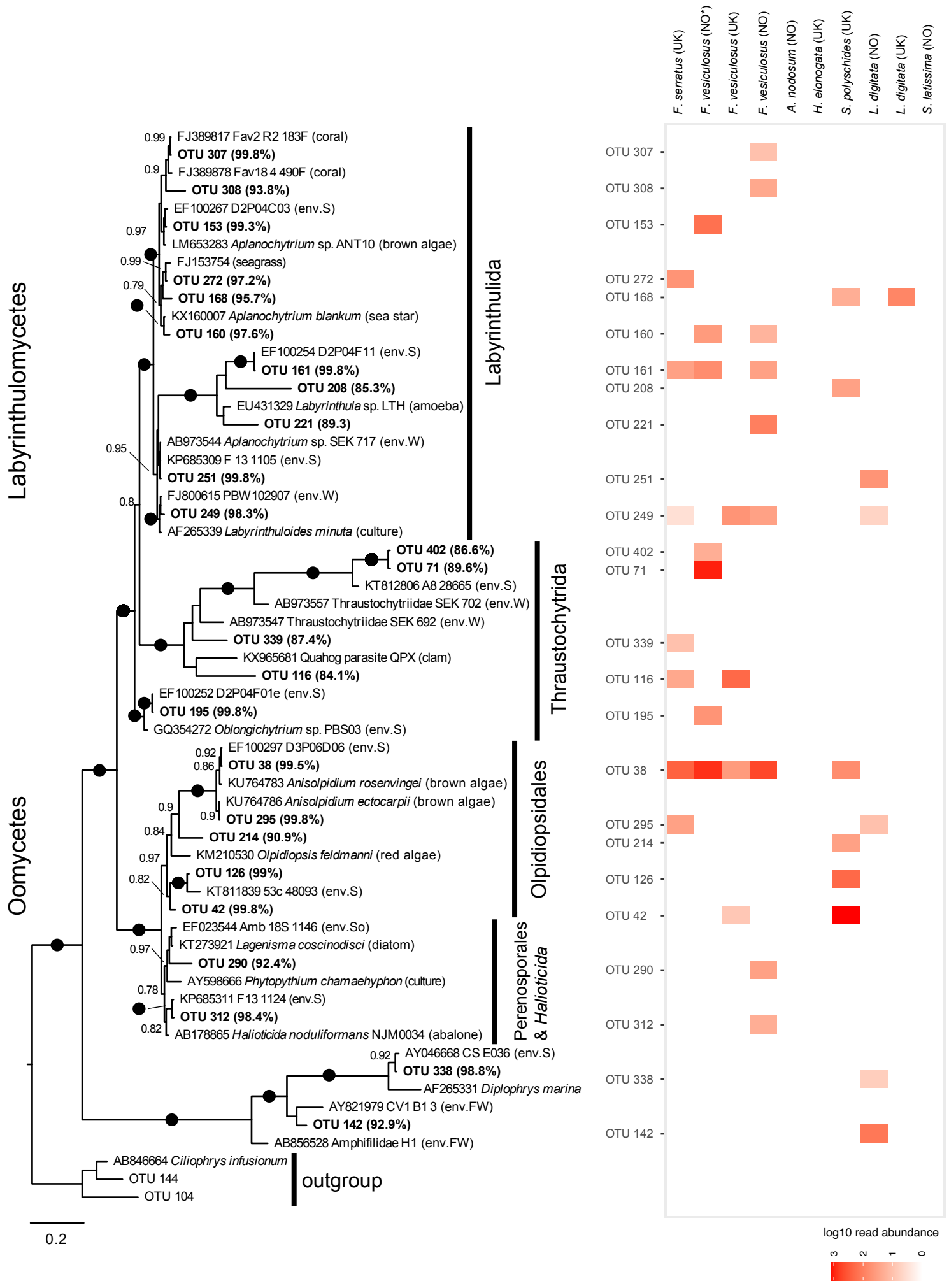
Table 5. Hosts with brown algal endophytes selected for isolation from the B. Rinkel collection at the Natural History Museum, London.

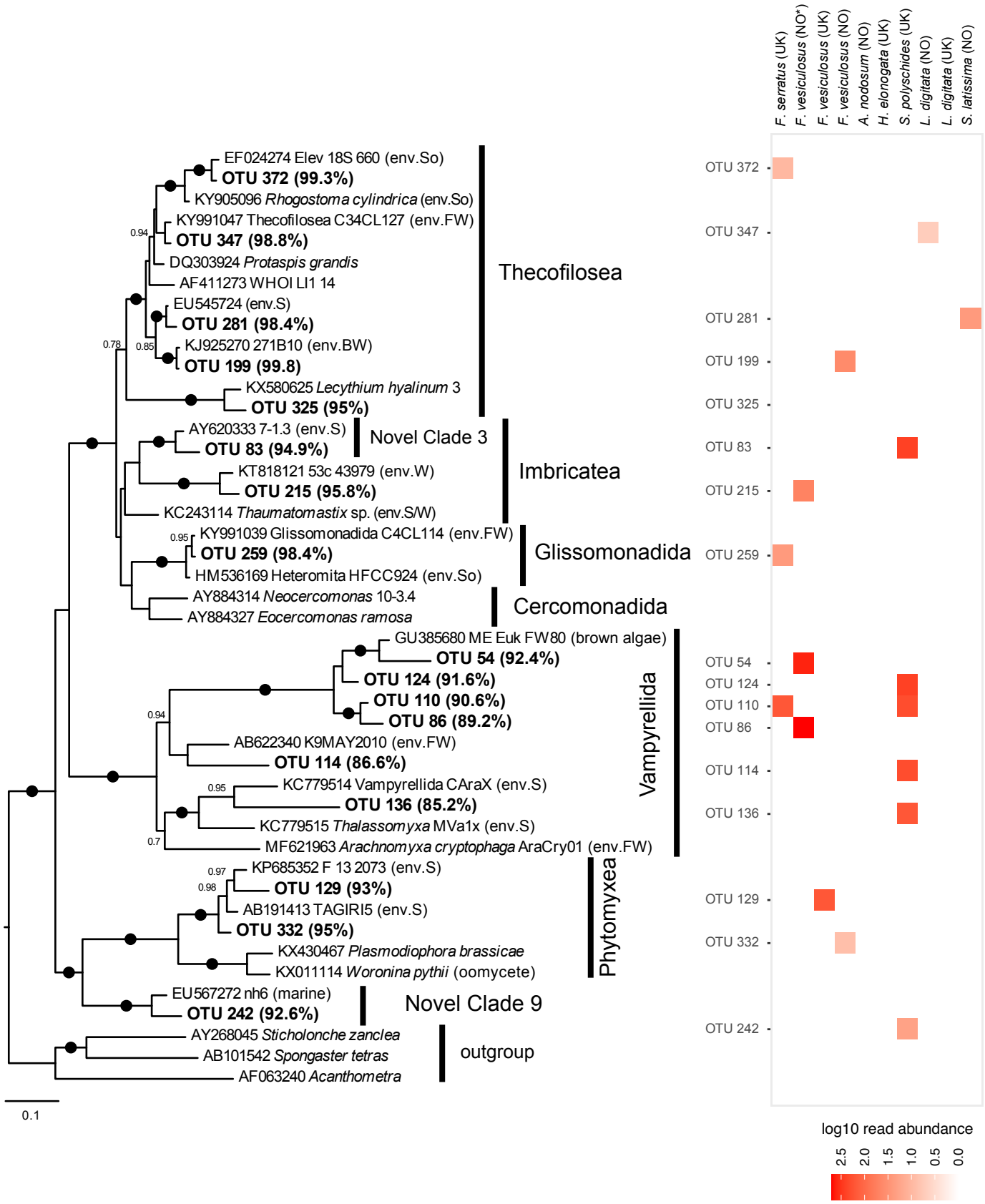
Sample ID	**Host**	**Site name, county**	**Date**	**Collector**
BRE1sn1	*Chondrus crispus*	Sheerness, Kent	22.10.03	J. Brodie
BRE9ls2	*Chondrus crispus*	Lilstock, Somerset	27.10.03	B. Rinkel
BRE38sm11	*Mastocarpus stellatus*	Sidmouth, Devon	28.10.01	B. Rinkel
BRE74ls1	*Fucus serratus*	Lilstock, Somerset	27.10.03	B. Rinkel
BRE98sm12	*Mastocarpus stellatus*	Sidmouth, Devon	28.10.01	B. Rinkel
BRE117sm22	*Osmundea osmunda*	Sidmouth, Devon	28.10.01	B. Rinkel
BRE118sm22	*Osmundea osmunda*	Sidmouth, Devon	28.10.01	B. Rinkel
BRE128hi1	*Osmundea pinnatifida*	Hayling Island, Hampshire	05.02.04	B. Rinkel
BRE152cb2	*Chondrus crispus*	Castle Beach, Pembrokeshire	09.03.04	B. Rinkel
BRE157br1	*Mastocarpus stellatus*	Black Rock, Pembrokeshire	10.03.04	B. Rinkel
E026pz	*Chondrus crispus*	Polzeath, Cornwall	19.06.04	M. Holmes
E030Nb	*Mastocarpus stellatus*	Norway	23.06.04	J. Brodie
E326wr	*Fucus serratus*	West Runton, Norfolk	28.09.04	B. Rinkel
E360sm2	*Chorda filum*	Sidmouth, Devon	28.10.03	B. Rinkel
E361ac	*Chorda filum*	A'Chleit, Bute	10.07.04	B. Rinkel
E365sg	*Mastocarpus stellatus*	Seagreens, Aberdeenshire	16.07.04	B. Rinkel
E368	*Chondrus crispus*	Bouley Bay, Jersey	13.03.05	B. Rinkel
E370	*Chondrus crispus*	Bouley Bay, Jersey	13.03.05	B. Rinkel
E377	*Chondrus crispus*	Greve de Lacq, Jersey	14.03.05	B. Rinkel
LTE19/1	*Osmundea pinnatifida*	Black Rock, Pembrokeshire	10.03.04	B. Rinkel

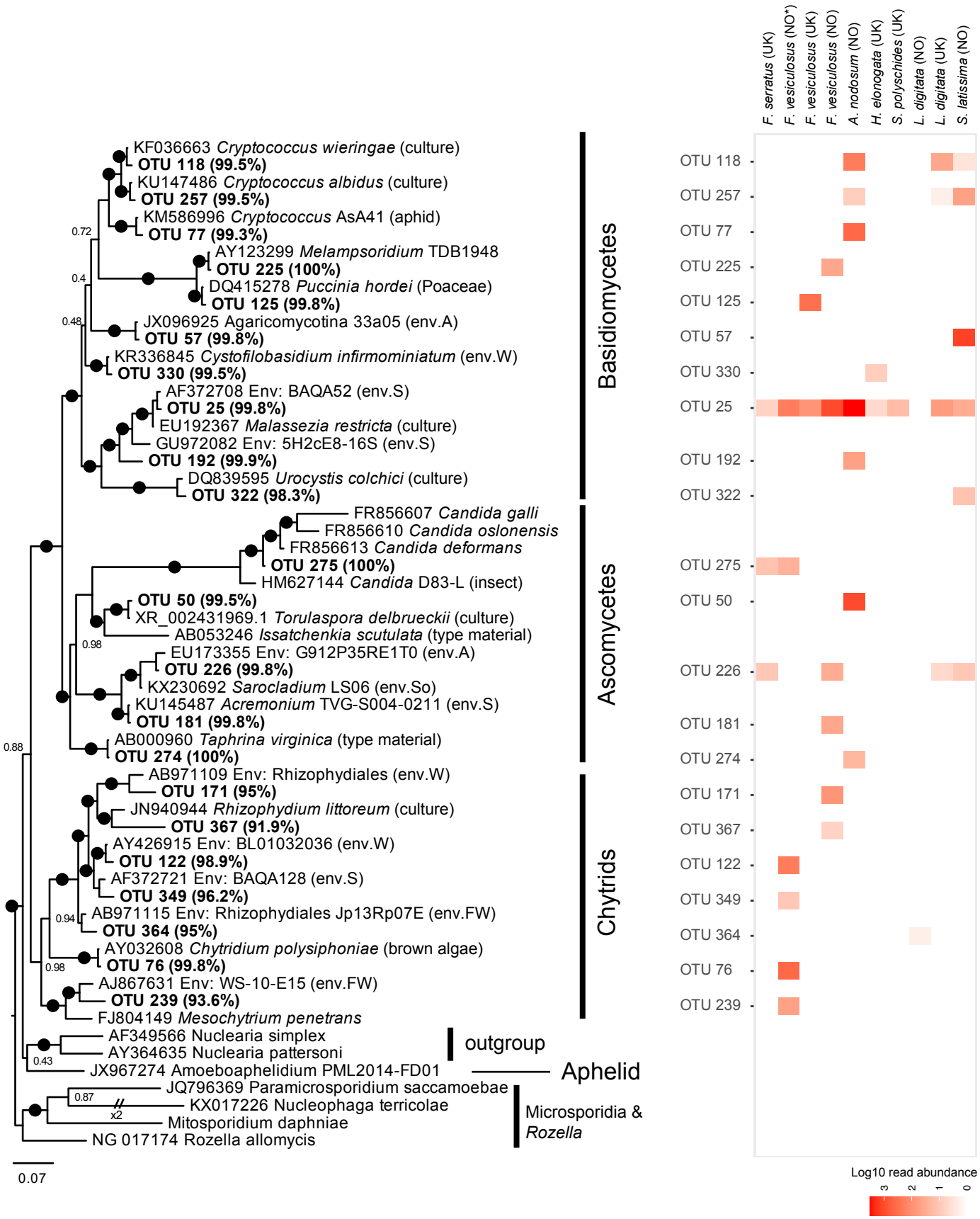

Proportional OTU abundance
100%
75%
50%
25%
0%
F. serratus (UK, 60)
F. vesiculosus (NO*, 101)
F. vesiculosus (UK, 85)
F. vesiculosus (NO, 98)
A. nodosum (NO, 41)
H. elonogata (UK, 26)
S. polyschides (UK, 90)
L. digitata (UK, 27)
L. digitata (NO, 55)
S. latissima (NO, 36)
Microeukaryotes
Alveolata
Amoebozoa
Apusozoa
Chlorophyta
Choanoflagellida
Fungi
Hacrobia
Mesomycetozoa
Rhizaria
Rhodophyta
Stramenopiles
Unassigned

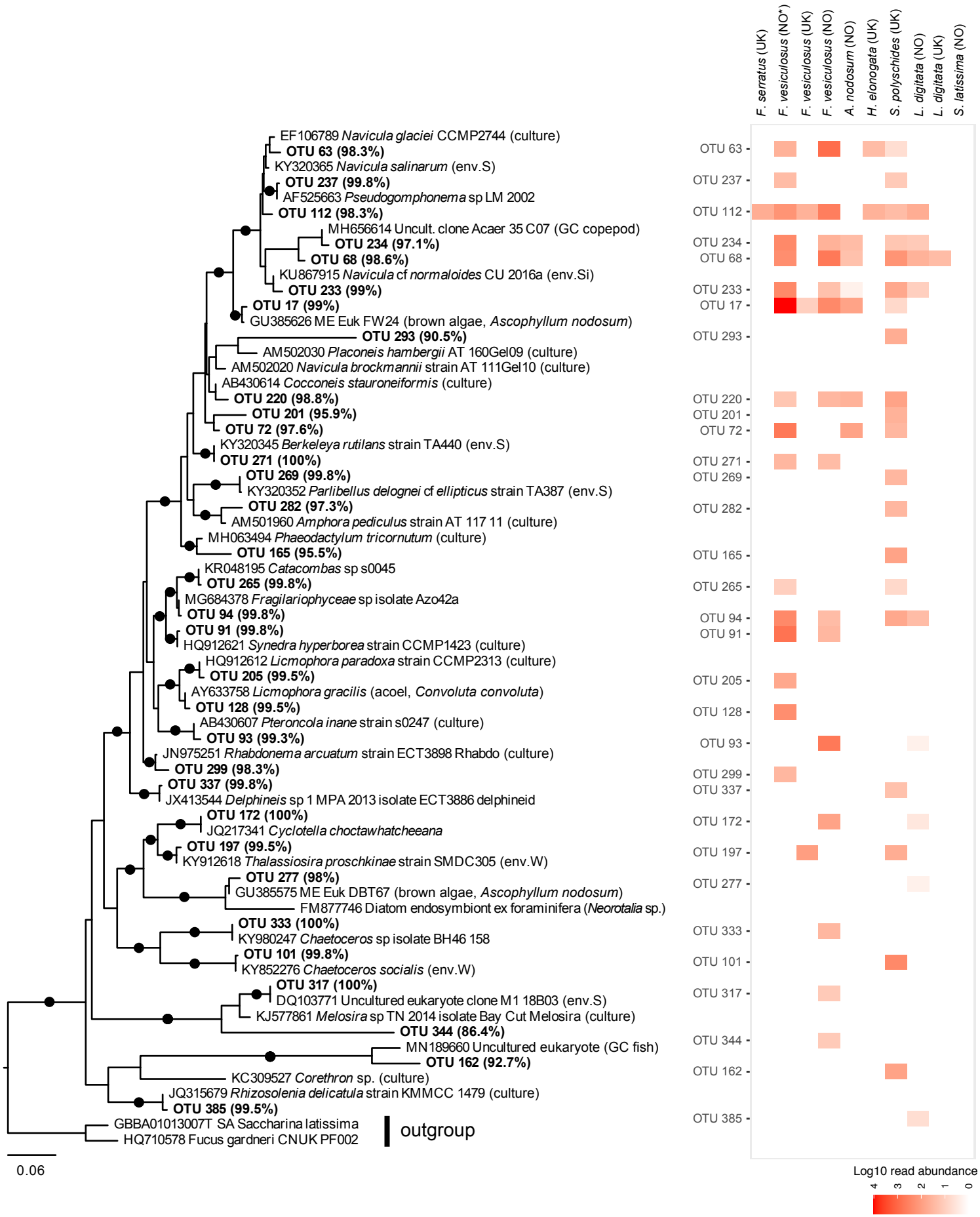

Stramenopiles
Alveolata
Cercozoa (Rhizaria)
Fungi
Archaeplastida
Hacrobia
Amoebozoa
Apusozoa
Choanoflagellida
Mesomycetozoa
Unassigned
42.6 %
28.3 %
6.0 %
7.9 %
5.3 %
3.4 %
2.3 %
1.1 %
1.1 %
0.4 %
1.5 %
0
10
20
30
40
% OTUs

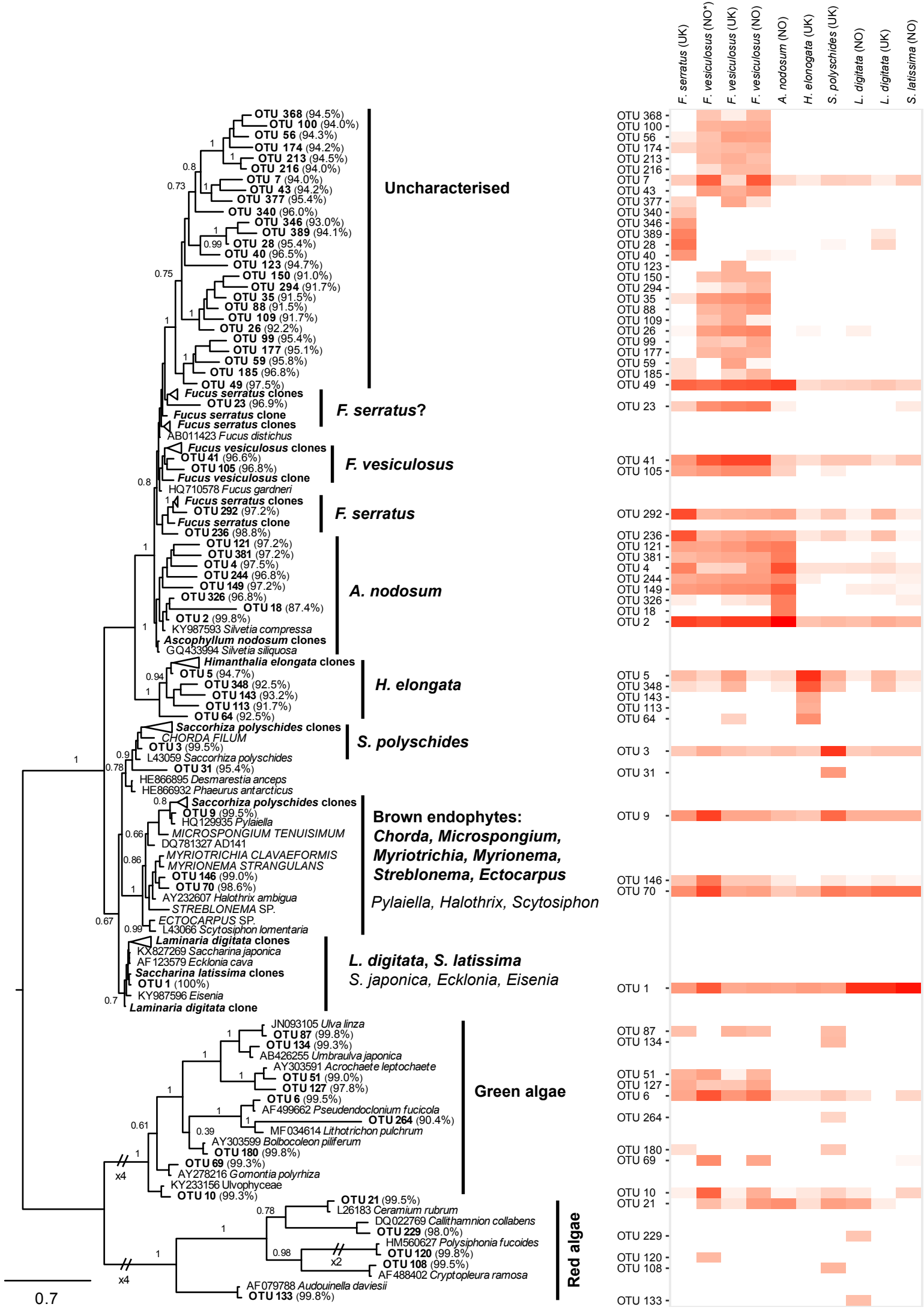

Phaeophyceae
Diatomea
Labyrinthulomycetes
Oomycota
Dictyochophyceae
Chrysophyceae
MAST
Ciliophora
Dinoflagellata
Apicomplexa
Perkinsea
Endomyxa
Filosa−Thecofilosea
Endomyxa−Phytomyxea
Filosa−Imbricatea
Filosa−Sarcomonadea
Basidiomycota
Chytridiomycota
Ascomycota
Chlorophyta
Rhodophyta
Centroheliozoa
Katablepharidophyta
Lobosa
Evosea
Breviatea
Apusomonadidae
Craspedida
Ichthyosporea
Unassigned
18.9 %
11.7 %
6.8 %
2.6 %
1.1 %
0.8 %
0.8 %
18.5 %
6.0 %
3.4 %
0.4 %
2.6 %
1.5 %
0.8 %
0.8 %
0.4 %
3.8 %
2.3 %
1.9 %
3.4 %
1.9 %
3.0 %
0.4 %
1.5 %
0.4 %
0.4 %
1.1 %
1.1 %
0.4 %
1.5 %
0
5
10
15
20
% OTUs

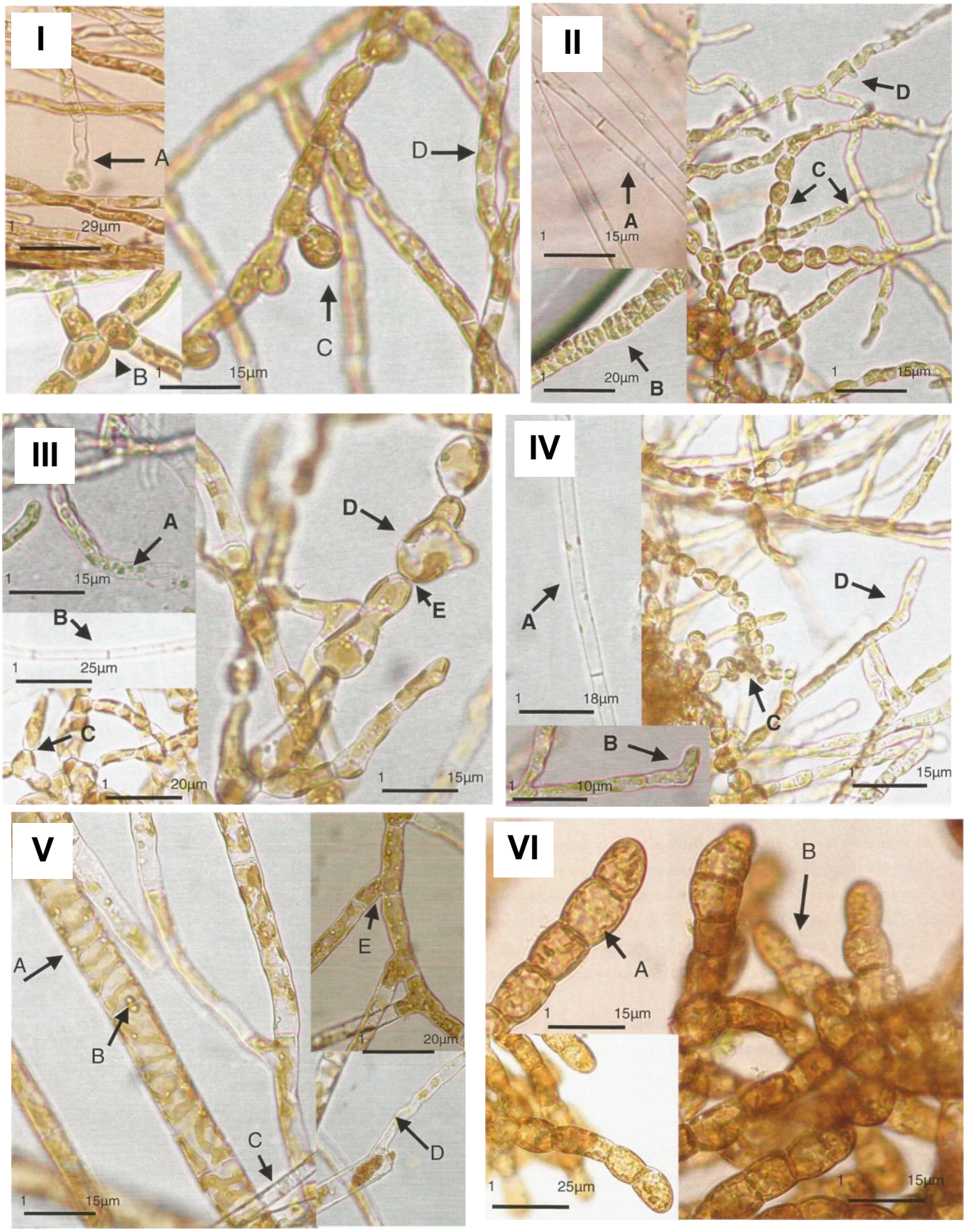

Labyrinthulomycetes
Oomycetes
Labyrinthulida
Thraustochytrida
Olpidiopsidales
Perenosporales & Halioticida
outgroup
FJ389817 Fav2 R2 183F (coral)
OTU 307 (99.8%)
FJ389878 Fav18 4 490F (coral)
OTU 308 (93.8%)
EF100267 D2P04C03 (env.S)
OTU 153 (99.3%)
LM653283 Aplanochytrium sp. ANT10 (brown algae)
FJ153754 (seagrass)
OTU 272 (97.2%)
OTU 168 (95.7%)
KX160007 Aplanochytrium blankum (sea star)
OTU 160 (97.6%)
EF100254 D2P04F11 (env.S)
OTU 161 (99.8%)
OTU 208 (85.3%)
EU431329 Labyrinthula sp. LTH (amoeba)
OTU 221 (89.3)
AB973544 Aplanochytrium sp. SEK 717 (env.W)
KP685309 F 13 1105 (env.S)
OTU 251 (99.8%)
FJ800615 PBW102907 (env.W)
OTU 249 (98.3%)
AF265339 Labyrinthuloides minuta (culture)
OTU 402 (86.6%)
OTU 71 (89.6%)
KT812806 A8 28665 (env.S)
AB973557 Thraustochytriidae SEK 702 (env.W)
AB973547 Thraustochytriidae SEK 692 (env.W)
OTU 339 (87.4%)
KX965681 Quahog parasite QPX (clam)
OTU 116 (84.1%)
EF100252 D2P04F01e (env.S)
OTU 195 (99.8%)
GQ354272 Oblongichytrium sp. PBS03 (env.S)
EF100297 D3P06D06 (env.S)
OTU 38 (99.5%)
KU764783 Anisolpidium rosenvingei (brown algae)
KU764786 Anisolpidium ectocarpii (brown algae)
OTU 295 (99.8%)
OTU 214 (90.9%)
KM210530 Olpidiopsis feldmanni (red algae)
OTU 126 (99%)
KT811839 53c 48093 (env.S)
OTU 42 (99.8%)
EF023544 Amb 18S 1146 (env.So)
KT273921 Lagenisma coscinodisci (diatom)
OTU 290 (92.4%)
AY598666 Phytopythium chamaehyphon (culture)
KP685311 F13 1124 (env.S)
OTU 312 (98.4%)
AB178865 Halioticida noduliformans NJM0034 (abalone)
AY046668 CS E036 (env.S)
OTU 338 (98.8%)
AF265331 Diplophrys marina
AY821979 CV1 B1 3 (env.FW)
OTU 142 (92.9%)
AB856528 Amphifilidae H1 (env.FW)
AB846664 Ciliophrys infusionum
OTU 144
OTU 104
0.2
F. serratus (UK)
F. vesiculosus (NO*)
F. vesiculosus (UK)
F. vesiculosus (NO)
A. nodosum (NO)
H. elonogata (UK)
S. polyschides (UK)
L. digitata (NO)
L. digitata (UK)
S. latissima (NO)
log10 read abundance
3
2
1
0

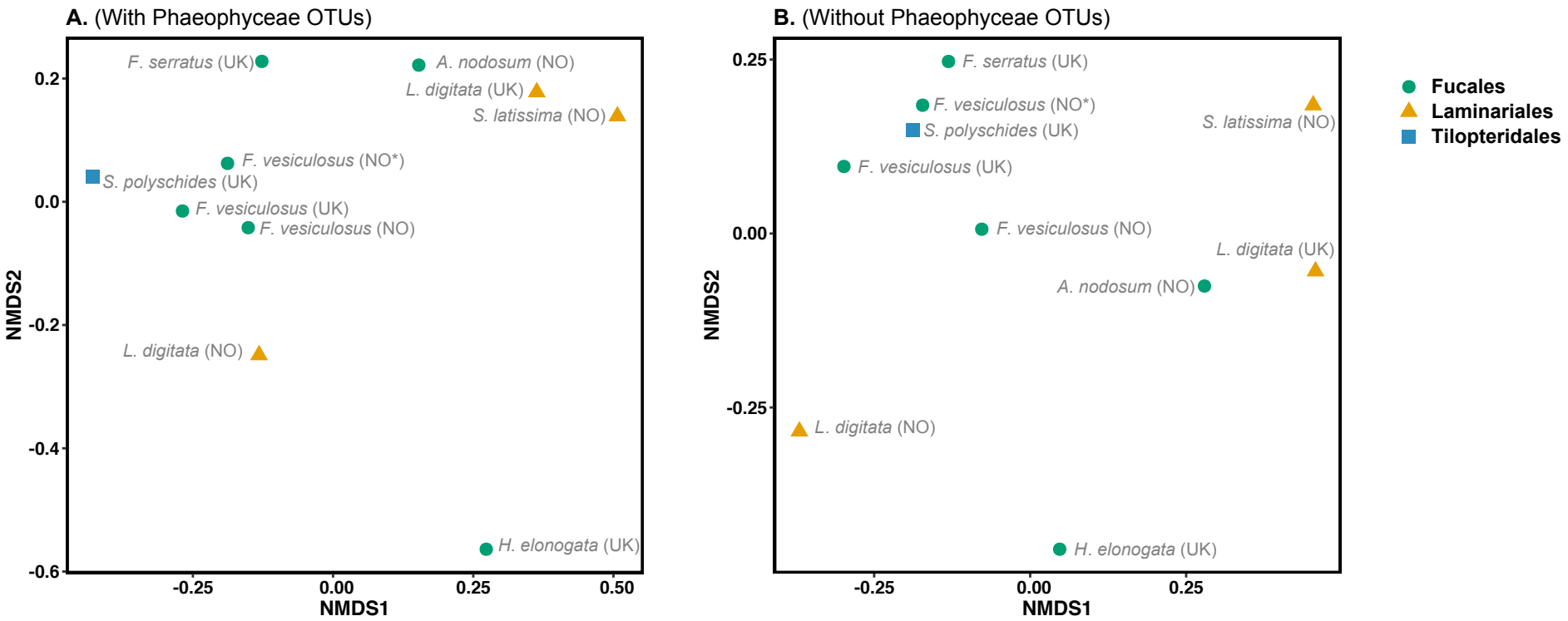

F. serratus (UK)
F. vesiculosus (NO*)
F. vesiculosus (UK)
F. vesiculosus (NO)
A. nodosum (NO)
H. elongata (UK)
S. polyschides (UK)
L. digitata (NO)
L. digitata (UK)
S. latissima (NO)
EF024274 Elev 18S 660 (env.So)
OTU 372 (99.3%)
KY905096 Rhogostoma cylindrica (env.So)
KY991047 Thecofilosea C34CL127 (env.FW)
OTU 347 (98.8%)
DQ303924 Protaspis grandis
AF411273 WHOI LI1 14
EU545724 (env.S)
OTU 281 (98.4%)
KJ925270 271B10 (env.BW)
OTU 199 (99.8)
KX580625 Lecythium hyalinum 3
OTU 325 (95%)
Thecofilosea
AY620333 7-1.3 (env.S)
OTU 83 (94.9%)
Novel Clade 3
KT818121 53c 43979 (env.W)
OTU 215 (95.8%)
KC243114 Thaumatomastix sp. (env.S/W)
Imbricatea
KY991039 Glissomonadida C4CL114 (env.FW)
OTU 259 (98.4%)
HM536169 Heteromita HFCC924 (env.So)
Glissomonadida
AY884314 Neocercomonas 10-3.4
AY884327 Eocercomonas ramosa
Cercomonadida
GU385680 ME Euk FW80 (brown algae)
OTU 54 (92.4%)
OTU 124 (91.6%)
OTU 110 (90.6%)
OTU 86 (89.2%)
AB622340 K9MAY2010 (env.FW)
OTU 114 (86.6%)
KC779514 Vampyrellida CAraX (env.S)
OTU 136 (85.2%)
KC779515 Thalassomyxa MVa1x (env.S)
MF621963 Arachnomyxa cryptophaga AraCry01 (env.FW)
Vampyrellida
KP685352 F 13 2073 (env.S)
OTU 129 (93%)
AB191413 TAGIRI5 (env.S)
OTU 332 (95%)
KX430467 Plasmodiophora brassicae
KX011114 Woronina pythii (oomycete)
Phytomyxea
EU567272 nh6 (marine)
OTU 242 (92.6%)
Novel Clade 9
AY268045 Sticholonche zanclea
AB101542 Spongaster tetras
AF063240 Acanthometra
outgroup
0.94
0.78
0.85
0.95
0.94
0.95
0.7
0.97
0.98
0.1
OTU 372
OTU 347
OTU 281
OTU 199
OTU 325
OTU 83
OTU 215
OTU 259
OTU 54
OTU 124
OTU 110
OTU 86
OTU 114
OTU 136
OTU 129
OTU 332
OTU 242
log10 read abundance
2.5
2.0
1.5
1.0
0.5
0.0

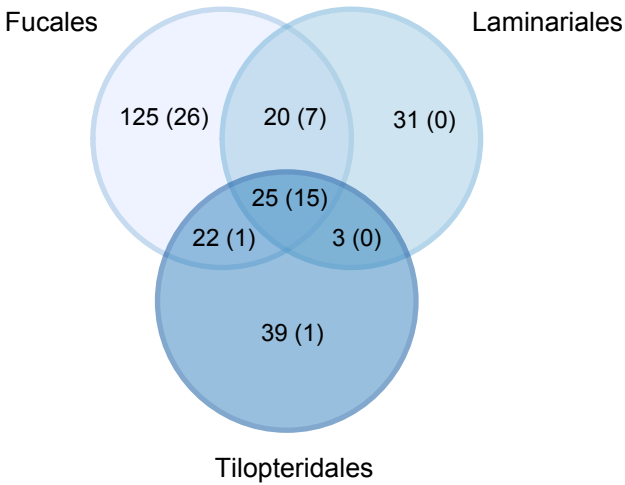

KF036663 Cryptococcus wieringae (culture)
OTU 118 (99.5%)
KU147486 Cryptococcus albidus (culture)
OTU 257 (99.5%)
KM586996 Cryptococcus AsA41 (aphid)
OTU 77 (99.3%)
AY123299 Melampsoridium TDB1948
OTU 225 (100%)
DQ415278 Puccinia hordei (Poaceae)
OTU 125 (99.8%)
JX096925 Agaricomycotina 33a05 (env.A)
OTU 57 (99.8%)
KR336845 Cystofilobasidium infirmominiatum (env.W)
OTU 330 (99.5%)
AF372708 Env: BAQA52 (env.S)
OTU 25 (99.8%)
EU192367 Malassezia restricta (culture)
GU972082 Env: 5H2cE8-16S (env.S)
OTU 192 (99.9%)
DQ839595 Urocystis colchici (culture)
OTU 322 (98.3%)
FR856607 Candida galli
FR856610 Candida oslonensis
FR856613 Candida deformans
OTU 275 (100%)
HM627144 Candida D83-L (insect)
OTU 50 (99.5%)
XR_002431969.1 Torulaspora delbrueckii (culture)
AB053246 Issatchenkia scutulata (type material)
EU173355 Env: G912P35RE1T0 (env.A)
OTU 226 (99.8%)
KX230692 Sarocladium LS06 (env.So)
KU145487 Acremonium TVG-S004-0211 (env.S)
OTU 181 (99.8%)
AB000960 Taphrina virginica (type material)
OTU 274 (100%)
AB971109 Env: Rhizophydiales (env.W)
OTU 171 (95%)
JN940944 Rhizophydium littoreum (culture)
OTU 367 (91.9%)
AY426915 Env: BL01032036 (env.W)
OTU 122 (98.9%)
AF372721 Env: BAQA128 (env.S)
OTU 349 (96.2%)
AB971115 Env: Rhizophydiales Jp13Rp07E (env.FW)
OTU 364 (95%)
AY032608 Chytridium polysiphoniae (brown algae)
OTU 76 (99.8%)
AJ867631 Env: WS-10-E15 (env.FW)
OTU 239 (93.6%)
FJ804149 Mesochytrium penetrans
AF349566 Nuclearia simplex
AY364635 Nuclearia pattersoni
JX967274 Amoeboaphelidium PML2014-FD01
JQ796369 Paramicrosporidium saccamoebae
KX017226 Nucleophaga terricolae
Mitosporidium daphniae
NG 017174 Rozella allomycis
0.72
0.4
0.48
0.98
0.88
0.94
0.98
0.43
0.87
x2
0.07
Basidiomycetes
Ascomycetes
Chytrids
outgroup
Aphelid
Microsporidia & Rozella
F. serratus (UK)
F. vesiculosus (NO*)
F. vesiculosus (UK)
F. vesiculosus (NO)
A. nodosum (NO)
H. elongata (UK)
S. polyschides (UK)
L. digitata (NO)
L. digitata (UK)
S. latissima (NO)
OTU 118
OTU 257
OTU 77
OTU 225
OTU 125
OTU 57
OTU 330
OTU 25
OTU 192
OTU 322
OTU 275
OTU 50
OTU 226
OTU 181
OTU 274
OTU 171
OTU 367
OTU 122
OTU 349
OTU 364
OTU 76
OTU 239
Log10 read abundance
3
2
1
0


F. serratus (UK)
F. vesiculosus (NO*)
F. vesiculosus (UK)
F. vesiculosus (NO)
A. nodosum (NO)
H. elonogata (UK)
S. polyschides (UK)
L. digitata (NO)
L. digitata (UK)
S. latissima (NO)
EF106789 Navicula glaciei CCMP2744 (culture)
OTU 63 (98.3%)
KY320365 Navicula salinarum (env.S)
OTU 237 (99.8%)
AF525663 Pseudogomphonema sp LM 2002
OTU 112 (98.3%)
MH656614 Uncult. clone Acaer 35 C07 (GC copepod)
OTU 234 (97.1%)
OTU 68 (98.6%)
KU867915 Navicula cf normaloides CU 2016a (env.Si)
OTU 233 (99%)
OTU 17 (99%)
GU385626 ME Euk FW24 (brown algae, Ascophyllum nodosum)
OTU 293 (90.5%)
AM502030 Placoneis hambergii AT 160Gel09 (culture)
AM502020 Navicula brockmannii strain AT 111Gel10 (culture)
AB430614 Cocconeis stauroneiformis (culture)
OTU 220 (98.8%)
OTU 201 (95.9%)
OTU 72 (97.6%)
KY320345 Berkeleya rutilans strain TA440 (env.S)
OTU 271 (100%)
OTU 269 (99.8%)
KY320352 Parlibellus delognei cf ellipticus strain TA387 (env.S)
OTU 282 (97.3%)
AM501960 Amphora pediculus strain AT 117 11 (culture)
MH063494 Phaeodactylum tricornutum (culture)
OTU 165 (95.5%)
KR048195 Catacombas sp s0045
OTU 265 (99.8%)
MG684378 Fragilariophyceae sp isolate Azo42a
OTU 94 (99.8%)
OTU 91 (99.8%)
HQ912621 Synedra hyperborea strain CCMP1423 (culture)
HQ912612 Licmophora paradoxa strain CCMP2313 (culture)
OTU 205 (99.5%)
AY633758 Licmophora gracilis (acoel, Convoluta convoluta)
OTU 128 (99.5%)
AB430607 Pteroncola inane strain s0247 (culture)
OTU 93 (99.3%)
JN975251 Rhabdonema arcuatum strain ECT3898 Rhabdo (culture)
OTU 299 (98.3%)
OTU 337 (99.8%)
JX413544 Delphineis sp 1 MPA 2013 isolate ECT3886 delphineid
OTU 172 (100%)
JQ217341 Cyclotella choctawhatcheeana
OTU 197 (99.5%)
KY912618 Thalassiosira proschkinae strain SMDC305 (env.W)
OTU 277 (98%)
GU385575 ME Euk DBT67 (brown algae, Ascophyllum nodosum)
FM877746 Diatom endosymbiont ex foraminifera (Neorotalia sp.)
OTU 333 (100%)
KY980247 Chaetoceros sp isolate BH46 158
OTU 101 (99.8%)
KY852276 Chaetoceros socialis (env.W)
OTU 317 (100%)
DQ103771 Uncultured eukaryote clone M1 18B03 (env.S)
KJ577861 Melosira sp TN 2014 isolate Bay Cut Melosira (culture)
OTU 344 (86.4%)
MN189660 Uncultured eukaryote (GC fish)
OTU 162 (92.7%)
KC309527 Corethron sp. (culture)
JQ315679 Rhizosolenia delicatula strain KMMCC 1479 (culture)
OTU 385 (99.5%)
GBBA01013007T SA Saccharina latissima
HQ710578 Fucus gardneri CNUK PF002
outgroup
0.06
OTU 63
OTU 237
OTU 112
OTU 234
OTU 68
OTU 233
OTU 17
OTU 293
OTU 220
OTU 201
OTU 72
OTU 271
OTU 269
OTU 282
OTU 165
OTU 265
OTU 94
OTU 91
OTU 205
OTU 128
OTU 93
OTU 299
OTU 337
OTU 172
OTU 197
OTU 277
OTU 333
OTU 101
OTU 317
OTU 344
OTU 162
OTU 385
Log10 read abundance
4
3
2
1
0


F. serratus (UK)
F. vesiculosus (NO*)
F. vesiculosus (UK)
F. vesiculosus (NO)
A. nodosum (NO)
H. elonogata (UK)
S. polyschides (UK)
L. digitata (NO)
L. digitata (UK)
S. latissima (NO)
Uncharacterised
F. serratus?
F. vesiculosus
F. serratus
A. nodosum
H. elongata
S. polyschides
Brown endophytes:
Chorda, Microspongium, Myriotrichia, Myrionema, Streblonema, Ectocarpus
Pylaiella, Halothrix, Scytosiphon
L. digitata, S. latissima
S. japonica, Ecklonia, Eisenia
Green algae
Red algae
Log10 read abundance
0.7


I
A
B
C
D
29µm
15µm
II
A
B
C
D
15µm
20µm
15µm
III
A
B
C
D
E
15µm
25µm
20µm
15µm
IV
A
B
C
D
18µm
10µm
15µm
V
A
B
C
D
E
15µm
20µm
VI
A
B
15µm
25µm
15µm


A. (With Phaeophyceae OTUs)
F. serratus (UK)
A. nodosum (NO)
L. digitata (UK)
S. latissima (NO)
F. vesiculosus (NO*)
S. polyschides (UK)
F. vesiculosus (UK)
F. vesiculosus (NO)
L. digitata (NO)
H. elonogata (UK)
NMDS2
NMDS1
0.2
0.0
-0.2
-0.4
-0.6
-0.25
0.00
0.25
0.50
B. (Without Phaeophyceae OTUs)
F. serratus (UK)
F. vesiculosus (NO*)
S. polyschides (UK)
S. latissima (NO)
F. vesiculosus (UK)
F. vesiculosus (NO)
L. digitata (UK)
A. nodosum (NO)
L. digitata (NO)
H. elonogata (UK)
NMDS2
NMDS1
0.25
0.00
-0.25
-0.25
0.00
0.25
Fucales
Laminariales
Tilopteridales


Fucales
Laminariales
125 (26)
20 (7)
31 (0)
25 (15)
22 (1)
3 (0)
39 (1)
Tilopteridales