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1 **Pathogens of brown algae: culture studies of *Anisolpidium ectocarpii* and *A. rosenvingei***  
2 **reveal that the Anisolpidiales are unflagellated oomycetes.**

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12

13 **Running title:** *Anisolpidium* is a uniciliate oomycete genus

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15

16

17 **Abstract**

18 Using laboratory cultures, we have documented the life cycle of *Anisolpidium ectocarpii*, a  
19 pathogen of *Ectocarpus* and other filamentous brown algae, and presented preliminary  
20 observations on *Anisolpidium rosenvingei*, a pathogen of *Pylaiella littoralis*. Consistent with  
21 earlier reports, the zoospores of both species have a single anterior flagellum, which justified  
22 the placement of *Anisolpidium* amongst the Hyphochytriales (Hyphochytridiomycota). We  
23 have also shown that *A. ectocarpii* can complete its infection cycle in a broad selection of  
24 species from various brown algal orders, whereas *A. rosenvingei* seemingly exhibits a strict  
25 specificity for unilocular sporangia of *P. littoralis*. Unexpectedly, nuclear (18S rRNA) and  
26 mitochondrial (*cox 1*, *cox2*) markers regroup *A. ectocarpii* and *A. rosenvingei*, into a hitherto  
27 unrecognised monophyletic clade within the oomycetes (Oomycota), most closely related to  
28 the Olpidiopsidales. The *Anisolpidium* genus is therefore entirely distinct from the  
29 Hyphochytridiomycota and represents the first confirmed instance of an anteriorly uniciliate  
30 oomycete. Finally, we suggest that a valid morphological criterion to separate true  
31 hyphochytrids from oomycetes is the timing of zoospore cleavage. Given the evidence, we  
32 propose to transfer the Anisolpidiales from the Hyphochytriales to the Oomycetes.

33

34 **Keywords:**35 *cox2*, *cox1*, 18S rRNA, *Ectocarpus*, *Pylaiella*, host range, Hyphochytriales, Anisolpidiales

## 36 **Introduction**

37 Algae are susceptible to disease outbreaks both in natural and cultivated populations, hence  
38 their pathogens are increasingly recognised as key ecosystem drivers (Gachon *et al.*, 2010).  
39 Diseases also cause significant economic damage, the severity of which is worsening with the  
40 development of seaweed aquaculture worldwide (Kim *et al.*, 2014; Loureiro *et al.*, 2015).  
41 Despite renewed interest, the biodiversity and physiology of intracellular eukaryotic  
42 pathogens of seaweeds remain poorly known. These pathogens span several protistan groups  
43 that are themselves little studied (oomycetes, hyphochytrids, chytrids and plasmodiophorids;  
44 see Gachon *et al.*, 2010; Neuhauser *et al.*, 2011). In addition, they tend to be inconspicuous in  
45 the field, their diagnosis requires specialist training and pathogen-host systems are difficult to  
46 bring into stable laboratory culture. Furthermore, many reports of these pathogens in the  
47 literature date back many decades and generally consist of rather limited descriptions of field-  
48 collected material.

49 The genus *Anisolpidium* was originally described by Karling (1943) and tentatively regrouped  
50 with *Canteromyces stigeoclonii*, a pathogen of green freshwater algae within the class  
51 *Anisolpidiaceae* (Sparrow, 1960; Adl *et al.*, 2012). Soil-dwelling *Anisolpidium saprobium*  
52 (Karling) isolated from pollen grains and freshwater *Anisolpidium elongatum* (Karling) have  
53 been reassigned to *Hyphochytrium saprobium* and *Hyphochytrium elongatum* (Dick, 2001).

54 In total, six marine species, all pathogens of filamentous brown algae, are currently  
55 recognised in the genus *Anisolpidium* (reviewed in Marano *et al.*, 2012): *A. ectocarpii* Karling  
56 on *Ectocarpus* and *Hincksia* spp.; *A. minutum* (H. E. Petersen) M. W. Dick on *Chorda filum*  
57 (Linnaeus) C. Agardh; *A. sphacellarum* (Kny) Karling on *Sphacelaria* spp., *Chaetopteris*  
58 *plumosa* (Lyngbye) Kützing, and *Cladostephus spongiosus* (Hudson) C. Agardh; *A. joklianum*  
59 (M.W. Dick) on *Hincksia granulosa*; *A. olpidium* (H. E. Petersen) M. W. Dick on *E.*  
60 *siliculosus*; and *A. rosenvingei* (H. E. Petersen) Karling on *Pylaiella littoralis*. *Anisolpidium* is

61 a widespread, perhaps even cosmopolitan genus reported in Japan, the North American East  
62 coast and throughout European waters (reviewed in Marano *et al.*, 2012). In an  
63 epidemiological study conducted over several years, Küpper & Müller (1999) found a strong  
64 seasonal correlation between the prevalence of the pathogen *A. rosenvingei* in sporangia of  
65 the filamentous brown alga *P. littoralis* and the breakdown of the algal population in late  
66 autumn.

67 Most *Anisolpidium* species have been described using field-collected material and the limited  
68 available information suggests that they share a common infection cycle. Species delimitation  
69 is mostly based on the respective algal host, and ill-defined morphological characters such as  
70 the size of the plasmodia inside their host. The Anisolpidiaceae as defined by Karling (1943)  
71 have tentatively been placed by Dick (2001) within the order Hyphochytriales  
72 (Hyphochytridiomycota or hyphochytrids), an order defined on the basis of a single anteriorly  
73 directed flagellum, as opposed to the chytrids that have a posterior flagellum. Besides the  
74 Anisolpidiaceae, the two other families currently recognised in the Hyphochytriales (Sparrow,  
75 1960) are the freshwater, soil-dwelling or facultative parasites Hyphochytridiomycetaceae and  
76 Rhizidiomycetaceae. Sequencing of the 18S rRNA gene showed that these two families were  
77 monophyletic and they were proposed to be the closest relatives to the oomycetes (Hausner *et*  
78 *al.*, 2000; Beakes *et al.*, 2014). To date, no molecular information is available for any  
79 representative of the Anisolpidiaceae. In the most recent classification of eukaryotes (Adl *et*  
80 *al.*, 2012), the Hyphochytriales are tentatively included among the typically biflagellated  
81 stramenopiles, although this speculative placement has been a matter of debate for many years  
82 (Beakes *et al.*, 2012).

83 Here, we report the establishment of stable laboratory cultures of *A. ectocarpii* and *A.*  
84 *rosenvingei*. Since these organisms are only known from historic drawings, we documented  
85 the life history of *A. ectocarpii* using a combination of bright field and epifluorescence

86 observations. In addition, we evaluated the host specificity of *A. ectocarpii*. We also assessed  
87 the phylogenetic position of the species using three molecular markers, all of which reveal  
88 that *Anisolpidium* falls within the oomycetes. Thus we note that oomycetes and hyphochytrids  
89 cannot be distinguished by the presence of an anteriorly uniflagellate zoospore; instead we  
90 suggest that the timing of zoospore cleavage might be a better criterion to separate the two  
91 classes.

92

93 **Material and methods**94 ***Biological material***

95 Specimens of *Ectocarpus* sp. infected with *Anisolpidium ectocarpii* were collected in South  
96 Chile (Table 1). In the laboratory, *A. ectocarpii* was transfected into a male *Ectocarpus* .  
97 gametophyte (strain CCAP 1310/56). Successive cleaning steps removed all eukaryotic  
98 contaminants. A culture of the original *Ectocarpus* host was established in parallel (strain *Ec*  
99 *QU 67-23*). Finally, the parasite was re-transfected into this original host, leading to the host-  
100 parasite system strain *Anisolpidium / Ectocarpus QU 67-5*. The cultures were maintained at  
101 10 to 12°C under daylight-type fluorescent light at 10  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , with a 12h:12h  
102 photoperiod. Culture medium was sterilized sea water containing 20 mL L<sup>-1</sup> Provasoli  
103 enrichment. Parallel attempts with *A. ectocarpii* found in *Hinckesia sandriana* (Zanardini) P.C.  
104 Silva failed, and only preserved samples for DNA sequencing remained (strain *A. ectocarpii*  
105 *PM 76-6*).

106 *A. rosenvingei* was encountered at two occasions in the tidal flats near Roscoff, France, in *P.*  
107 *littoralis* growing on *Fucus serratus* Linnaeus receptacles. Culture conditions for this  
108 pathogen were as described above but because of the high host-specificity of *A. rosenvingei*  
109 towards unilocular sporangia of *P. littoralis*, the following modifications were necessary.

110 A unialgal clonal culture of healthy *P. littoralis* from Roscoff was established as a routine  
111 source for unilocular sporangia. Repeated co-incubations were then undertaken of this new  
112 host with *A. rosenvingei* plasmodia in detached unilocular sporangia in order to remove all  
113 eukaryotic contaminants, whilst maintaining the pathogen. From this point onward, two  
114 different techniques allowed the survival of clean host-pathogen cultures in the laboratory: 1)  
115 a pulse system, by successive co-incubation with a new host culture in one to two week

116 intervals, and 2) a continuous system by selecting apical parts of fertile *P. littoralis* filaments  
117 with infected unilocular sporangia in one to two week intervals for continuous growth.

118 In contrast to *A. rosenvingei*, the broad host spectrum of *A. ectocarpii* tolerated a convenient  
119 long term culture system. Transfection to a female gametophyte clone of *Macrocystis pyrifera*  
120 (strain CCAP 1323/1) resulted in a stable host-pathogen co-culture, which only needed supply  
121 of fresh host biomass in one to two monthly intervals (strain *A. ectocarpii* QU 467-2).

122 Evaluation of host compatibility followed the techniques described by Müller *et al.* (1999). *M.*  
123 *pyrifera* gametophytes infected with *A. ectocarpii* were co-incubated with a culture of the  
124 potential host in 60 mm plastic Petri dishes. Interactions were deemed susceptible if mature  
125 and discharged *A. ectocarpii* plasmodia with exit tubes were detected in cells of the new host.  
126 The earliest infection symptoms appeared after five days, and were subsequently recorded  
127 with digital photography. For additional documentation, permanent mounts of samples with  
128 acetocarmine staining were prepared and mounted in Karo® syrup. A selection of microscope  
129 slides have been deposited in the Natural History Museum of London (BM), under the  
130 accession numbers BM000701847-BM000701849.

### 131 ***Histological staining***

132 Beta 1-3 and 1-4 glucans were stained with a commercially available Calcofluor white  
133 solution containing Blue Evans as a counterstain. Samples were incubated for 5 to 10 min in a  
134 0.01 mg mL<sup>-1</sup> calcofluor solution, rinsed in sterile seawater and observed with a DAPI filter  
135 (excitation: 365 nm, beam splitter: 395 nm, emission: long pass 420 nm). Alternatively, the  
136 non-specific cellulose stain Congo Red was used, at a final concentration of 0.1 mg mL<sup>-1</sup>,  
137 followed by observation under differential interference contrast microscopy. Aniline blue  
138 staining was performed as in Tsirigoti *et al.* (2014). In order to stain *Anisopodium* nuclei,  
139 samples were first fixed 1 h on ice with Microtubule Stabilizing Buffer (Katsaros & Galatis,

140 1992), followed by several washes in methanol until the algal filaments were discoloured. The  
141 samples were then transferred into sterile seawater containing a  $10^{-4}$  dilution of a commercial  
142 Sybr Green solution (Sigma Aldrich), sometimes also containing aniline blue. Samples were  
143 mounted in SlowFade (Life Technologies) and observed on a Zeiss LSM 510  
144 epifluorescence microscope coupled to an Axiocam HR digital camera, using a FITC filter  
145 (excitation BP 450-490 nm; beam splitter 510 nm; emission LP 515 nm).

#### 146 ***Molecular taxonomy***

147 DNA was extracted using a CTAB and phenol-chloroform method or Qiagen DNeasy minikit  
148 as detailed in Gachon *et al.* (2009). The *cox2* marker was amplified using the *cox2HF* -  
149 *cox2HR* primer pair as described in (Hudspeth *et al.*, 2000). The *cox1* marker was amplified  
150 using *Fm85mod* and *CoxleUp* (Robideau *et al.*, 2011). The 18S rRNA marker was amplified  
151 using the new primers *F139* (5' AGTCTATTTGATAGTACCTTACTAC 3') and *R1233* (5'  
152 CAATCCTTACTATGTCTGG 3') with an annealing temperature of 45°C. After Sanger  
153 sequencing, the resulting chromatograms were proofread manually in Geneious V6.1.8  
154 (Kearse *et al.*, 2012) or BioEdit (Hall, 1999) and virtually translated (*cox1/cox2*).  
155 Stramenopile-wide alignments were used to produce unrooted trees to ascertain the initial  
156 branching position of *A. ectocarpii* and *A. rosenvingei* before final selection of suitable in and  
157 outgroup species (Supplementary Info 1). Phylogenetic analyses were performed in MEGA v.  
158 7 (Kumar *et al.*, 2016). Model tests were performed on the alignments in order to find the best  
159 substitution models for subsequent maximum likelihood (ML) analysis. For the 18S rRNA,  
160 Tamura-3-parameter was used (Tamura, 1992) with a discrete Gamma distribution to model  
161 evolutionary rate differences among sites. The rate variation model allowed for some sites to  
162 be evolutionarily invariable. For *cox1* and *cox2* the La Gascuel model (2008) with discrete  
163 gamma distribution was used the general reversible mitochondrial model (Adachi &  
164 Hasegawa, 1996) with discrete gamma distribution and for *cox2* the La Gascuel model (2008)  
165 with discrete gamma distribution was used. Additionally maximum parsimony analysis was

166 performed on all three datasets. Bootstrap re-sampling was set to 100 replicates. Sequence  
167 data of all *Anisolpidium* strains were deposited in Genbank (see Table 1 for accession  
168 numbers) and the alignments are given in Suppl. Info. 2-4.

169

170

171 **Results**

172 Life history and infection cycle

173 Field-collected material was morphologically identified as *Anisolpidium ectocarpii* Karling  
174 (Figs 1, 2) and *A. rosenvingei* (not shown). Subsequent observations were based on laboratory  
175 cultures of *A. ectocarpii* in its original host *Ectocarpus* sp. (strain *A. ectocarpii* QU 67-5) or  
176 in female gametophytes of *Macrocystis pyrifera* (strain *A. ectocarpii* QU 467-2). Both  
177 cultures contained naturally-occurring commensal bacteria but were devoid of eukaryotic  
178 contaminants.

179 The release of infectious zoospores from *A. ectocarpii* sporangia could be triggered by  
180 transferring the culture from 10°C into fresh medium at room temperature. Within a few  
181 minutes, zoospores started to move inside the sporangium and escape in files through the exit  
182 tubes. The swimming spores were globular bodies of 2.5 µm diameter, and became slightly  
183 pyriform after a few minutes (Figs 3, 4). Nile Red staining indicated that they contain lipid  
184 globules (not shown). They had one anterior flagellum, which pulled the spore forward by  
185 rapid meandering oscillations (Suppl. Information 5). The liberated zoospores remained active  
186 for about 30 minutes at room temperature before encysting at the surface of a new host cell  
187 (Fig. 5). *A. ectocarpii* then penetrated into the algal cell and developed as an intramatrical,  
188 unwallled plasmodium with a conspicuously granulose cytosol and a subspherical shape (Fig.  
189 6). The algal cell components disaggregated rapidly and cell remnants were already visible at  
190 this stage, with a typical chestnut brown colour. The growing plasmodium then differentiated  
191 a thin cell wall, thus becoming spherical, whilst its cytoplasm became more uniformly  
192 granular (Fig. 7). The pathogen progressively filled the host cell, closely moulding its shape  
193 (Fig. 8) and differentiating one or several protruding exit tube(s) (Fig. 9); the final  
194 plasmodium size depended on the cell type and the number of infections per host cell  
195 (detailed below). For example, spore initials in *Ectocarpus* plurilocular sporangia were

196 commonly infected, and led to distinctively smaller pathogen sporangia compared to when  
197 somatic cells were infected (Fig. 10). Throughout its growth, the pathogenic thallus was  
198 monocentric and did not propagate to neighbouring algal cells. Likewise, *A. rosenvingei*  
199 developed intracellularly, with a strict tissue specificity for unilocular sporangia of *P.*  
200 *littoralis* (Figs 11, 12). Spore release typically occurred within 6-10 days following infection  
201 (Fig. 13) and monoflagellation of the spores was observed. Virus-infected *P. littoralis* could  
202 also undergo lysogeny, leading to the production of viral particles in the algal unilocular  
203 sporangia. Though the association between both pathogens was not obligate, *A. rosenvingei*  
204 infection of virus-producing unilocular sporangia was observed both in field material (Fig.  
205 14) and in culture (not shown). Finally, and similar to *A. ectocarpii*, co-infection of the same  
206 host cell by *A. rosenvingei* was also frequent (Fig. 15).

207 Young unwalled *A. ectocarpii* plasmodia did not react to any of the cell wall stains examined,  
208 (Fig. 16). Typical of holocarpic pathogens, the developing thallus fully converted into a  
209 sporangium with exit tube(s) that ruptured the wall of the dead host cell at full maturity. The  
210 differentiating exit tubes were labelled by Congo red (Fig. 17); the entire cell wall of mature  
211 sporangia reacted to this stain (Fig. 18), whilst spores gave a weak and inconsistent labelling.  
212 However, these structures remained unstained when methylene blue was used (not shown).  
213 The thin cell wall progressively built up by the early spherical unwalled thallus was most  
214 easily visualised using the  $\beta 1 \rightarrow 3$  and  $\beta 1 \rightarrow 4$  glucan stain calcofluor white (Fig. 19). Mature  
215 exit tubes were most intensely labelled (Fig. 20), strongly suggesting that the pathogen  
216 sporangium forces its way out of the host cell. Calcofluor also stained the encysted *A.*  
217 *ectocarpii* zoospores, revealing thin infectious germ tubes (ca. 1  $\mu\text{m}$  in length) that penetrate  
218 into the algal cell perpendicularly to its cell wall (Fig. 21). Therefore, we recommend  
219 calcofluor as a rapid and convenient method to locate pathogen structures, in particular the  
220 otherwise inconspicuous spores encysted at the surface of the algal cells. In addition, localised  
221 modifications of the algal cell wall were visible in the immediate vicinity of encysted *A.*

222 *ectocarpii* spores both with calcofluor (Fig. 22, arrowhead) and aniline blue (Fig. 23). *A.*  
223 *ectocarpii* structures were only weakly stained with aniline blue ( $\beta$ 1 $\rightarrow$ 3 glucans), with again  
224 the most intense labelling at the basis of exit tubes (Fig. 24).

225 The dynamics of nuclear division during vegetative growth was followed using Sybr-Green  
226 (Figs 25-40). Consistent with the rapid degeneration of the host cell mentioned above (Fig. 6,  
227 also visible in Fig. 16), the nucleus of the algal host cell was rarely seen past the 2-nucleus  
228 plasmodium stage (Fig. 26). Throughout the plasmodium development, nuclei became smaller  
229 and more compact (see the progression between Figs 25-34). All nuclei within the syncytium  
230 were always at the same stage, and divisions were synchronous (Figs 35-38). The observed  
231 trails of fluorescence during the anaphase suggest closed mitosis, a frequent trait of syncytial  
232 organisms (Fig. 37).

233 Multiple infections of the same host cell were commonly observed, leading to the successful,  
234 yet not necessarily synchronous, differentiation of one to five sporangia per host cell (see for  
235 example one empty and one dehiscing sporangium in the same host cell on Fig. 33). In  
236 extreme cases however, over fifteen plasmodia were observed in a single host cell (Figs 31-  
237 32). The number of spores produced per *A. ectocarpii* sporangium varied from a handful to  
238 several hundred for the biggest observed sporangia. Finally, it was repeatedly noted that *A.*  
239 *ectocarpii* spores preferentially encyst on the filament cells close to the apical tips of  
240 *Ectocarpus* filaments and *M. pyrifer* gametophytes, although no quantification of this  
241 phenomenon was attempted (Figs 39-40).

242

#### 243 **Host range of *A. ectocarpii* and *A. rosenvingei*.**

244 Co-incubation experiments with representative brown algal cultures spanning 28 species  
245 across ten brown algal orders revealed an unexpectedly broad host spectrum for *A. ectocarpii*  
246 (Table 2). Algal strains were deemed susceptible if they allowed completion of the full life

247 cycle of the pathogen, i.e. penetration of spores, development of plasmodia, and spore release  
248 through exit tubes (Figs 41-59). Susceptibility was the most frequent outcome of the  
249 experiments, across all orders tested. However, we encountered several algal strains that  
250 departed from this pattern, especially among uniseriate filamentous species. There was a  
251 complete absence of any visible interaction for *Botrytella uvaeformis*, *Leptonematella*  
252 *fasciculata*, *Feldmannia irregularis*, *Pylaiella littoralis*, *Cutleria multifida*, *Microzonia*  
253 *velutina*, *Sphacelaria* sp. and *Sphacelaria rigidula*, although another *Sphacelaria* isolate was  
254 successfully infected.

255 In sharp contrast to *A. ectocarpii* both *A. rosenvingei* isolates tested infected exclusively  
256 unilocular sporangia initials of *P. littoralis*. This strict host and cell specificity required  
257 intense observations of unilocular sporangia of *Pylaiella* at the initial stage of the study.  
258 Among field samples from Perharidy, in addition to *A. rosenvingei*, we found evidence for a  
259 DNA virus. Our material agreed in all light-microscopic details with the symptoms known for  
260 the PlitV-1 virus infecting *P. washingtoniensis* C.C. Jao in Alaska, as described in detail by  
261 Maier et al. (1998). The virus genome is integrated into the nuclear genome of the host and  
262 systemically present in each somatic host cell. It becomes virulent exclusively in unilocular  
263 sporangia, and causes their cytosol to appear translucent and unstructured. In field samples  
264 and by infection experiments we could verify that virion formation and plasmodium  
265 development of *A. rosenvingei* could occur simultaneously in the cytoplasm of a unilocular  
266 sporangium initial on a virus-infected *Pylaiella littoralis* specimen. Since this aspect extends  
267 beyond the scope of the present study, we refer to Fig. 14 as a representative field sample. A  
268 series of permanent light microscopic mounts documenting the Perharidy *Pylaiella* virus have  
269 been deposited in the Paris Natural History Museum (D.G. Müller 2015, accession numbers  
270 PC0723465-PC0723473 and PC0723476).

271

272 *Anisolpidium ectocarpii* and *A. rosenvingei* belong to the oomycetes  
273 Partial sequence information for the 18S rRNA and *cox1* genes was successfully obtained for  
274 *A. ectocarpii* CCAP 4001/1, *A. rosenvingei* Ros and *A. rosenvingei* Per; partial *cox2*  
275 sequences were obtained for *A. ectocarpii* CCAP 4001/1, *A. ectocarpii* PM 76-6 and *A.*  
276 *rosenvingei* Ros. Our phylogenetic analyses of all three markers (Figs 60-62) placed *A.*  
277 *ectocarpii* and *A. rosenvingei* within the oomycetes. The assumed placement of *Anisolpidium*  
278 within the hyphochytrids was rejected, as seen from the phylogenetic analysis of 18S rRNA  
279 and *cox2*. All *Anisolpidium* strains formed a monophyletic group with the three markers  
280 investigated. Despite limited bootstrap support, both the 18S rRNA and *cox1* marker placed  
281 the three strains of *Anisolpidium* on a long branch, sister to the Olpidiopsidales. The *cox2*  
282 marker lacked sufficient resolution to decipher the relationship between the *Anisolpidium* and  
283 *Olpidiopsis* genera, but the tree topology retrieved was also consistent with the above  
284 interpretation. Additionally, four environmental 18S sequences most closely related to  
285 *Anisolpidium* were retrieved from Genbank (Table 3). All originate from coastal marine  
286 habitats (pelagic or sediment), with a geographic coverage encompassing California,  
287 Greenland and the Mediterranean Sea. Two of those sequences fell within the same clade as  
288 the three *Anisolpidium* strains whereas the other two formed a sister clade (Suppl. Info. 6).

289

## 290 **Discussion**

291 Our observations of *A. ectocarpii* and *A. rosenvingei* in culture fully matched the meticulous  
292 description of the type material given by Karling (1943). Novel aspects were the localised  
293 thickenings of the algal cell wall at the pathogen penetration site, which are consistent with a  
294 localised defence reaction similar to the one recently described in brown algal cells infected  
295 by the oomycete *Eurychasma dicksonii* (Tsirigoti et al., 2015). As originally reported by  
296 Karling, mitotic divisions were synchronous. However, we found that nuclear counts often

297 deviated from the exponential progression that would be expected if nuclei numbers doubled  
298 at each division. Extensive observations, especially in multiple-infected host cells, suggested  
299 that such non-canonical nuclei numbers were accounted for by the degeneration of some  
300 parasite nuclei, with no evidence of any thallus fusion as recently reported in *Olpidiopsis*  
301 *pyropiae* (Klochkova et al., 2015). Depending on resource availability from the host cell and  
302 the resulting plasmodium size, this phenomenon would provide a possible mechanism to  
303 regulate the number of spores produced per *A. ectocarpii* sporangium.

304 Anteriorly monoflagellated zoospores, hitherto believed to be a hallmark of hyphochytrids,  
305 were clearly detected in both *A. ectocarpii* and *A. rosenvingei*. Unexpectedly, all three  
306 nuclear and mitochondrial markers used here point to *A. ectocarpii* and *A. rosenvingei*  
307 defining a novel monophyletic clade within the oomycetes. We therefore propose that the  
308 order Anisolpidiales, represented by the marine members of the genus *Anisolpidium*, should  
309 be transferred into the oomycetes (subphylum Oomycota) and given equal status to the  
310 Olpidiopsidales and Haliphthorales (as defined by Beakes *et al.*, 2014). The former order,  
311 erected by Dick (2001), regroups obligate, initially plasmodial, holocarpic endoparasites of  
312 plants, algae and oomycetes, of which only marine species have been characterised  
313 molecularly. The latter order contains three marine genera of molluscan and crustacean  
314 parasites, that produce polycentric hyphal structures and can be cultivated saprophytically on  
315 agar. The status of the freshwater *Canteriomyces* and soil-dwelling *Anisolpidium saprobium*  
316 Karling remains unresolved,

317 It is noteworthy that all available molecular data derived from both our cultures and  
318 environmental samples fully agree with the concept of Anisolpidiales as erected by M.W.  
319 Dick (2001), which solely groups marine, obligate endoparasites of marine algae.

320 Despite being unexpected, the closeness of the relationship between *Anisolpidium* and  
321 *Olpidiopsis* agrees with the observation of Johnson (1957), who already highlighted the

322 morphological similarities between their sporangia. Another similarity between both genera is  
323 that zoospores are cleaved within the sporangia before the exit tubes are opened, in contrast to  
324 terrestrial hyphochytrids, many of which release a naked, partially differentiated, protoplasm  
325 into a restraining vesicle or directly into the environment (e.g. *Hyphochytrium catenoides*  
326 (Karling, 1939) and *Rhizidiomyces apophysatus* (Karling, 1943). Additionally, the central  
327 vacuole witnessed in *Anisolpidium* species (Karling, 1943), might be similar to that found  
328 recently in *Olpidiopsis bostrychiae* S. Sekimoto, T. A. Klochkova, J. A. West, G.W. Beakes  
329 & D. Honda (Sekimoto *et al.*, 2009) and *Olpidiopsis feldmannii* (Fletcher *et al.*, 2015), though  
330 it is absent in *Olpidiopsis porphyrae* (Sekimoto *et al.*, 2008) and *Olpidiopsis pyropiae*  
331 (Klochkova *et al.*, 2015). Finally, the genus *Olpidiopsis* was originally defined by the  
332 occurrence of sexual union between two contiguous thalli originating from different zoosporic  
333 infections (see details in Dick, 2001), which echoes the intracellular sexual conjugation of  
334 young uninucleate parasitic thalli described in *A. ectocarpii* (Johnson, 1957; Karling, 1977).  
335 Unfortunately, we were unable to observe such conjugation events in our cultures, and our  
336 observations of *A. ectocarpii* nuclear divisions (Fig. 25-40) did not suggest any non-mitotic  
337 division pattern. Therefore, the sexual potential of our *Anisolpidium* cultures remains  
338 unresolved; likewise, sexuality in marine *Olpidiopsis* species remains to be described.  
339 However, a distinct possibility is that as data become available, intracellular sexual  
340 conjugation in host cells might appear as a defining feature (synapomorphy) of the  
341 Olpidiopsidales and Anisolpidiales.

342 To the best of our knowledge this is the first report of an anteriorly uniflagellate oomycete,  
343 yet flagellae were lost several times amongst Oomycota in obligate pathogenic clades such as  
344 *Hyaloperonospora*, *Bremia*, and most *Peronospora* spp, as well as *Geolegnia* and some  
345 *Haptoglossa* and *Myzocytiopsis*. Further variation is reported, for example the presence of two  
346 smooth flagellae in *Haptoglossa dickii* (Beakes & Glockling, 1998), of two distinct  
347 biflagellate patterns in *Olpidiopsis* (Whittick, 1972; West *et al.*, 2006; Sekimoto *et al.*, 2008)

348 and of differently flagellated zoospores at different life stages (e.g. *Saprolegnia* primary  
349 spores vs secondary spores), suggesting that there is ample scope for variation in flagellation  
350 amongst oomycetes. Among the heterokonts (Heterokonta), loss or strong reduction of the  
351 posterior smooth flagellum is well documented in *Pelagomonas*, *Dictyota* (Kawai, 1992) and  
352 in diatoms. In the latter group, the male gamete of all species investigated thus far has a single  
353 tinselled flagellum. Hence, the loss of one flagellum in *Anisolpidium* represents the third  
354 independent such event reported in the heterokonts. However, electron microscopy  
355 investigation on *Anisolpidium* spores would be necessary in order to investigate a loss or  
356 potential vestiges of the second flagellum.

357 As can be seen in Table 2, *A. ectocarpii* has a broad host spectrum, including several brown  
358 algal orders with heteromorphic life histories, where both the micro- and macroscopic  
359 generations are successfully attacked. Similarly, Müller *et al.* (1999) found that *Chytridium*  
360 *polysiphoniae*, only described as infecting sporangia of *Pylaiella littoralis* in the field,  
361 actually could infect 29 brown algal species across different brown algal orders. *Eurychasma*  
362 *dicksonii* (Oomycota) has a similarly broad host range in marine Phaeophyceae (Müller *et al.*,  
363 1999), as well as the Plasmodiophorid *Maullinia ectocarpii* I. Maier, E. R. Parodi, R.  
364 Westermeier et D. G. Müller (Maier *et al.*, 2000). Over the years, our studies have revealed  
365 that *M. ectocarpii*, *E. dicksonii* and *A. ectocarpii* all infect *Macrocystis* gametophytes in  
366 laboratory cultures, begging to question as to what their impact might be on natural kelp beds.  
367 The contrasting host spectra exhibited by our *A. rosenvingei* and *A. ectocarpii* isolates provide  
368 further evidence against the widely-held view that all intracellular biotrophic pathogens are  
369 necessarily highly host specific. Furthermore, the broad host range observed for *A. ectocarpii*  
370 as well as other marine oomycetes and plasmodiophorids correlates with a unique ability to  
371 perform cross-kingdom host shifts (extensively discussed in Beakes *et al.*, 2012; Neuhauser *et*  
372 *al.*, 2014).

373 Altogether, our host range data also highlight the limitations of current species descriptions  
374 within the genus *Anisolpidium*, which are almost exclusively based on morphological  
375 characters that overlap between species, and observed host specificity in the field. It appears  
376 necessary to reassess diversity and species delimitation with modern tools. For example, the  
377 definition of *A. sphacellarum* as a pathogen of *Sphacelaria* is questioned by the fact that our  
378 *A. ectocarpii* culture can infect at least one *Sphacelaria* isolate. However, the infection  
379 structures that we observed were notably smaller than those reported in *Sphacelaria* spp. in  
380 the field (Strittmatter *et al.*, 2013). It is also noteworthy that the observed preferential  
381 infection of filament tips and apical cells of certain brown algal species by *A. ectocarpii*, for  
382 example apical cells of several *Halopteris*, resonates with some other pathogens, such as  
383 *Olpidiopsis porphyrae*, that recognise different lectins (Klochkova *et al.*, 2012).

384 The biogeography and epidemiology of *Anisolpidium* remain very imperfectly known. This is  
385 expected, as infections of filamentous seaweeds are inconspicuous to the naked eye. It is  
386 therefore unsurprising that this study is a first record of the genus for South America. We  
387 should stress however, that this finding is the outcome of a relatively short field campaign  
388 held in summer, similar to another one recently conducted in Greece that also led to the  
389 finding of *A. ectocarpii* and *A. sphacellarum* (Strittmatter *et al.*, 2013). Taken together with  
390 the available environmental sequences from California, Greenland and the Spanish  
391 Mediterranean Coast, our repeated findings via space- and time-limited sampling efforts  
392 reinforce the notion that *Anisolpidium* pathogens are geographically widespread in marine  
393 environments, perhaps even cosmopolitan. We hope that the unprecedented availability of  
394 cultures and molecular data will foster further research on oomycetes pathogens of marine  
395 algae.

396

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538 **Table 1: Collection details of pathogen and algal strains reported in this study.**539 *An* = *Anisolpidium*, *Ec* = *Ectocarpus*, *Pyl* = *Pylaiella*. Field samples are highlighted in bold italics;

540 derived laboratory cultures are in normal font.

541

Sample name	Description	Genbank Accession number
<b><i>An ectocarpii</i> QU 67</b>	<i>Ectocarpus</i> sp. infected with <i>A. ectocarpii</i> . Sample scraped from the bottom of a fishing boat in Quetalmahue (Chiloé, Chile) on Jan 31 <sup>st</sup> , 2007	
<i>Ec</i> QU 67-23	Clonal, healthy <i>Ectocarpus</i> sp. isolate established from the infected material above. Available from the authors upon request.	
<i>An</i> QU 67-5 (CCAP4001/1)	Monoeukaryotic, non-axenic <i>A. ectocarpii</i> isolate transfected into the clonal strain <i>Ec</i> QU 67-23	KU764786 (18S) KX086261 ( <i>cox1</i> ) KP420743.1 ( <i>cox2</i> )
<i>An</i> QU 467-2	Same monoeukaryotic, non-axenic <i>A. ectocarpii</i> isolate as above, artificially introduced into a clonal female <i>M. pyrifera</i> gametophyte (CCAP 1323/1) for easier maintenance	
<b><i>An PM</i></b>	<i>Hinckesia sandriana</i> infected by <i>A. ectocarpii</i> . Sample obtained by scuba diving in a mariculture installation near Puerto Montt, Chile, at c. 13 m depth on Feb 2 <sup>nd</sup> , 2007	
<i>An PM</i> 76-6	Non-axenic <i>Anisolpidium ectocarpii</i> isolate transfected into the clonal strain <i>Ec</i> QU 67-23. This culture was lost during the course of the study.	KP420744.1 ( <i>cox2</i> )
<b><i>An rosenvingei</i> Ros 2014</b>	Sept 8, 2014, tidal flat outside Marine Station Roscoff. Infected <i>Pylaiella littoralis</i> epiphytic on receptacles of <i>Fucus serratus</i> found in the intertidal.	KU764783 (18S) KU764784 ( <i>cox1</i> ) KU764785 ( <i>cox2</i> )
<b><i>An rosenvingei</i> Per 2015-4</b>	Nov. 2015. Infected <i>Pylaiella littoralis</i> found on Perharidy beach, Roscoff, France.	KU752534 (18S) KU764782 ( <i>cox1</i> )

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545 **Table 2: Broad host specificity of *Anisopidium ectocarpii* as determined by co-incubation experiments with candidate brown algal hosts.**

546 dpi: days post infection

<b>Species</b>	<b>Taxonomic position</b>	<b>Origin of host culture, location, date</b>	<b>Dpi</b>	<b>Response and cell type affected</b>
<i>Ectocarpus</i> sp.	Ectocarpales Ectocarpaceae	New Zealand, 1988	5	susceptible; gametophyte filament
<i>Ectocarpus fasciculatus</i> Kützing	Ectocarpales Ectocarpaceae	Roscoff 1993	5	gametophyte filament
<i>Myriotrichia clavaeformis</i> (Harvey)	Ectocarpales Chordariaceae	Argentina 1995	8	somatic and hair meristem, hair cells, plurilocular sporangia
<i>Botrytella uvaeformis</i> (Lyngbye) Kornmann & Sahling	Ectocarpales Chordariaceae	Helgoland 1960	21	no infection
<i>Leptonematella fasciculata</i> (Reinke) P.C.Silva	Ectocarpales Chordariaceae	Roscoff 1970	8	no infection
<i>Elachista stellaris</i> Areschoug	Ectocarpales Chordariaceae	Canary Islands 1991	6	filament cells
<i>Myriogloea chilensis</i> (Montagne) A.H.Llaña	Ectocarpales Chordariaceae	Chile 1985	5	female gametophyte
<i>Feldmannia irregularis</i>	Ectocarpales	Canary Islands 1994	7	no infection

(Kützing) Hamel	Acinetosporaceae			
<i>Pylaiella littoralis</i> (Linnaeus) Kjellman	Ectocarpales Acinetosporaceae	Drake Passage 1986	13	no infection
<i>Macrocystis pyrifera</i> (Linnaeus) C.Agardh	Laminariales	Chile 1999, CCAP 1323/1	9	male and female gametophytes and juvenile sporophyte
<i>Asterocladon lobatum</i> D.G.Müller, E.R.Parodi & A.F.Peters	Asterocladales	Brazil 1996	7	filament and apical cells
<i>Asterocladon rhodochortonoides</i> (Børgesen) S.Uwai, C.Nagasato, T.Motomura & K.Kogame	Asterocladales	South Africa 1993	8	filament cells
<i>Scytothamnus fasciculatus</i> (J.D.Hooker & Harvey) A.D.Cotton	Scytothamnales	New Zealand 1981	8	microthallus and juvenile macrothallus cells
<i>Tilopteris mertensii</i> (Turner) Kützing	Tilopteridales	Helgoland 1984	5	filament and hair cells
<i>Cutleria multifida</i> (Turner) Greville	Tilopteridales	Canary Islands 1994	13	no infection
<i>Microzonia velutina</i> (Harvey) J.Agardh	Syringodermatales	New Zealand 1981	9	no infection

<i>Desmarestia viridis</i> (O.F.Müller) J.V.Lamouroux	Desmarestiales	Helgoland 1978	6	juvenile sporophyte
<i>Arthrocladia villosa</i> (Hudson) Duby	Desmarestiales	Villefranche 1980	10	gametophyte
<i>Carpomitra costata</i> (Stackhouse) Batters	Sporochnales	Villefranche 1981	23	juvenile sporophyte
<i>Perithalia caudata</i> (Labillardière) Womersley	Sporochnales	Tasmania 1984	9	female gametophyte
<i>Sphacelaria</i> sp.	Sphacelariales	Greece, 2009	15	apical and hair cells
<i>Sphacelaria</i> sp.	Sphacelariales	Morocco 1964	19	No infection
<i>Sphacelaria rigidula</i> Kützting	Sphacelariales	Netherlands 1968	20	No infection
<i>Halopteris gracilescens</i> (J.Agardh) Womersley	Sphacelariales	Australia 1981	21	young apical cells
<i>Halopteris</i> sp.	Sphacelariales	Australia 1988	11	apical cells, hair cells
<i>Halopteris congesta</i> (Reinke) Sauvageau	Sphacelariales	New Zealand 1981	27	apical cells
<i>Halopteris paniculata</i> (Suhr) Prud'homme van Reine	Sphacelariales	New Zealand 1981	27	apical cells

<i>Discosporangium mesarthrocarpum</i> (Meneghini) Hauck	Discosporangiales	Greece 2004	17	death of filament cells, no repair
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550 **Table 3**

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552 18S rRNA environmental sequences most closely related to *Anisolpidium*.

Accession	Author	Geographic origin	Identity (%)		
			<i>A. ectocarpii</i> <i>QU</i>	<i>A. ectocarpii</i> <i>Ros</i>	<i>A. ectocarpii</i> <i>Per</i>
EF100276.1	Stoeck <i>et al.</i> , unpub.	oxygen-depleted intertidal marine sediment, upper 2 cm sediment surface, Greenland	97.6	99.0	96.4
EF100297.1	Stoeck <i>et al.</i> ,unpub.	oxygen-depleted intertidal marine sediment, upper 2 cm sediment surface, Greenland	97.6	99.1	96.5
AY381206.1	(Massana <i>et al.</i> , 2004)	coastal surface water, Northwestern Mediterranean, Blanes Bay	91.7	91.6	89.5
JQ781890.1	(Lin <i>et al.</i> , 2012)	Pelagic sample, Monterey Bay, 5 m depth	90.6	90.1	90.5

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## Figure Legends

### **Figs 1-15. Infection cycle of *Anisolpidium ectocarpii* CCAP 4001/1 (1-10) and *A. rosenvingei* (11-15).**

Figs 1-2. Acetocarmine-stained field-collected *Ectocarpus* sp. with developing *A. ectocarpii* intramatrix plasmidia (1, arrowheads) and mature empty sporangia with typical exit tubes (2, arrowheads). Fig. 3. Anteriorly monoflagellated spores (arrowheads). See Supplementary Information 1 for a time-lapse movie illustrating their swimming behaviour. Fig. 4. Detail of a spore (phase contrast). Fig. 5. The arrows point to two *A. ectocarpii* spores encysted at the surface of the same *Ectocarpus* cell. Fig. 6. Two young unwalled plasmodia, with a conspicuously granulous cytoplasm. Fig. 7. Spherical walled thallus, with homogeneous cytoplasm. Fig. 8. Late intramatrix plasmodium filling entirely the host cell, preceding zoosporangium differentiation. Fig. 9. Differentiating exit tube (arrowhead). Fig. 10. Infection of *Ectocarpus* spore initials in plurilocular sporangia. Figs 11-12. Healthy (11) vs. *A. rosenvingei*-infected (12) unilocular sporangia of *Pylaiella littoralis*. Fig. 13. Spore release, 6 days after infection. Fig. 14. Acetocarmine-stained field specimen with virus symptoms (double arrowhead) and *A. rosenvingei* exit tube (arrowheads). Fig. 15. As for *A. ectocarpii*, multiple infections of the same host cell by *A. rosenvingei* are frequent (arrowheads point to exit tubes of sporangia formed in the same host cell).

Scale bars: 1-2, 11-15: 50  $\mu\text{m}$ ; 3, 5-10: 10  $\mu\text{m}$ ; 4: 5  $\mu\text{m}$ .

### **Fig 16-24. Cell wall differentiation of *A. ectocarpii* CCAP 4001/1 and algal defence.**

Figs 16-18. Staining pattern obtained with Congo red. 16. Unlabelled, unwalled young granulose plasmodium. Fig. 17. Differentiating exit tube forcing its way outside the host cell. Fig. 18. Mature empty sporangia in dead algal cells. Note also that encysted spores (arrowheads) are weakly

labelled. Figs 19-22. Calcofluor white. Fig. 19: walled plasmodium (inset: corresponding bright field image). Fig. 20. Empty mature sporangia with a distinctive annular thickening at the basis of the exit tubes. Fig. 21. Encysted spores at the surface of an *Ectocarpus* cell. Note the very thin penetration apparatus (arrowhead). Fig. 22. Cell wall thickening underneath an encysted spore, tentatively attributed to a defence reaction of the alga. Figs 23-24. Aniline blue. Fig. 23: Cell wall thickening (arrowheads) underneath encysted spores, similar to Figs 22. 24. Weak labelling of the sporangium cell wall, again with an annular thickening at the basis of the exit tube. Scale bars: 16-22, 24:10  $\mu\text{m}$ . 23: 20  $\mu\text{m}$ .

**Fig. 25-40. Nuclear dynamics of *A. ectocarpii* CCAP 4001/1.**

Arrowheads and arrows point to *A. ectocarpii* and host structures, respectively. All pictures illustrate *Ectocarpus* sp. except Figs 39-40, which represent *M. pyrifera*. Fig. 25. Young uninucleate thallus. Fig. 26. Interphasic nuclei of a binucleate *Anisolpidium* thallus; the arrow points to the nucleus of the infected host cell. Fig. 27. Eight-nucleus stage, corresponding to the granulose stage illustrated in Fig. 16. Note the perinuclear mitochondria visible as minute fluorescent dots. Fig. 28. At a later stage, the syncytium contains small compact nuclei, whilst the younger thallus at the bottom is at a similar stage as in Fig. 27. Fig. 29. Late walled *Anisolpidium* thallus, with multiple small compact nuclei; the arrow points to the nucleus of a healthy algal cell. Fig. 30. Spore encysted at the algal host cell surface (arrowhead). Note also a late-stage walled thallus with compact nuclei on the right. Figs 31-32. Multiple infections of a single host cell that contained sixteen *A. ectocarpii* plasmodia. In Fig. 31 (bright field), numerous empty infectious spores (arrowheads) are encysted at the surface of the host. In Fig. 32, each *A. ectocarpii* plasmodium contains a small number (max. 8) of small compact nuclei, illustrating the regulation of the pathogen development by available host resources. Figs 33-34. Mobile zoospores in a dehiscent sporangium just before release, viewed under bright field (33) and epifluorescence

(34). Figs 35-38. Synchronous mitoses of *A. ectocarpii* nuclei. Fig. 35. Metaphase. Fig. 36. Anaphase. Fig. 37. Late anaphase, with trails of fluorescence suggestive of a closed mitosis. Fig. 38. Telophase. Figs 39-40. Preferential infection of distal end of filaments in *M. pyrifera* gametophytes. Both pictures depict the same field of view under bright field and epifluorescence. Developing (arrowheads on 39 & 40) plasmodia and mature dehiscent sporangia (additional arrowheads on 40) are disproportionately located at the tips of the host filaments. Scale bars: Figs 25-38: 10  $\mu\text{m}$ ; Figs 39-40: 20  $\mu\text{m}$ .

**Fig. 41-59. Host range of *A. ectocarpii* CCAP 4001/1**

Arrowheads point to *A. ectocarpii* structures. Figs 41-43. *Macrocystis pyrifera*: Infected young sporophyte at 12 dpi (41), oogonia on a female gametophyte (42), and mature empty sporangium on a male gametophyte (43). Figs 44-45. *Asterocladon lobatum*: developing plasmodium (44) and mature empty sporangium (45). Figs 46-48. *Myriotrichia clavaeformis*: multiple (46) and single (47) infection in hair cells; infected plurilocular sporangium (48). Figs 49-50. *Myriogloea chilensis* female gametophyte: developing plasmodium (49) and mature sporangium (50). Figs 51-52. *Elachista stellaris*: developing plasmodia. Fig. 53. *Ectocarpus fasciculatus*: mature sporangium at 6dpi. Fig. 54. *Desmarestia viridis* juvenile sporophyte: developing plasmodia and mature sporangium (inset) at 8 dpi. Fig. 55-56. *Tilopteris mertensii*: developing plasmodia in somatic filament (55) and hair cell (56, stained with acetocarmine) at 8 dpi. Fig. 57-58. *Halopteris gracilescens*: infected apical cells containing motile zoospores just before release (57) and empty sporangium (58). Fig. 59. *Halopteris* sp.: apical cell with three mature sporangia. All scale bars: 20  $\mu\text{m}$ .

**Fig. 60.** Maximum-likelihood (ML) tree of 18S rRNA gene sequences of oomycetes including *Anisolpidium ectocarpii* and *Anisolpidium rosenvingei*, the three hyphochytrids *Hyphochytrium*

*catenoides* BR217, ATCC18719 and *Rhizidiomyces apophysatus* and the two marine flagellates *Cafeteria* sp. and *Cafeteria roenbergensis*, which were used to root the tree. Bootstrap values are given for ML and Maximum parsimony (MP) phylogenetic analyses. Bootstrap values in % represent 100 replicates; bootstrap values below 50 are omitted. Branch lengths represent substitutions per site. A/X: bootstrap lower than 50 in ML and no bootstrap support for this branch in MP, B: bootstrap lower than 50 in MP.

**Fig. 61.** Maximum-likelihood (ML) tree of *cox1* protein sequences of oomycetes including *Anisulpidium ectocarpii* and *Anisulpidium rosenvingei*, the brown alga *Ectocarpus* sp., the thraustochytrid *Thraustochytrium aureum* and the marine flagellate *Cafeteria roenbergensis*. *Thraustochytrium aureum* and *Cafeteria roenbergensis* were used to root the tree. Bootstrap values are given for ML and Maximum parsimony (MP) phylogenetic analyses. Bootstrap values in % represent 100 replicates; bootstrap values below 50 are omitted. Branch lengths represent substitutions per site. A: bootstrap lower than 50 in ML, B: bootstrap lower than 50 in MP X: no bootstrap support for this branch in MP.

**Fig. 62.** Maximum-likelihood (ML) tree of *cox2* protein sequences of oomycetes including *Anisulpidium ectocarpii* and *Anisulpidium rosenvingei*, and the hyphochytrid *Hyphochytrium catenoides* which was used to root the tree. Bootstrap values are given for ML and Maximum parsimony (MP) phylogenetic analyses. Bootstrap values in % represent 100 replicates; bootstrap values below 50 are omitted. Branch lengths represent substitutions per site. A: bootstrap lower than 50 in ML, X: no bootstrap support for this branch in MP.

## Legends for Supplementary information

**Supplementary Information 1:** Genbank accession number of all organisms used in the phylogenetic analyses of the markers 18S rRNA, cox1 and cox2.

**Supplementary Information 2:** Multiple sequence alignment of the marker 18S rRNA.

**Supplementary Information 3:** Multiple sequence alignment of the marker cox1.

**Supplementary Information 4:** Multiple sequence alignment of the marker cox2.

**Supplementary Information 5:** Time lapse video of freshly released *A. ectocarpii* zoospores, illustrating their swimming behaviour, in particular their anterior flagellation. Pictures were taken over a 2 min period and the video is accelerated 10 times. The scale bar is indicated on the first image.

**Supplementary Information 6:** Maximum-likelihood (ML) tree of 18S rRNA sequences of environmental sequences (Table 3) and the basal oomycetes *Olpidiopsis pyropiae*, *Olpidiopsis porphyrae*, *Olpidiopsis feldmannii*, *Olpidiopsis* sp., *Anisolpidium ectocarpii*, *Anisolpidium rosenvingei*, *Haliphthoros milfordensis* and *Haliphthoros* sp., The latter two were used to root the tree. Bootstrap values are given for ML and Maximum parsimony (MP) phylogenetic analyses. Bootstrap values in % represent 100 replicates; bootstrap values below 50 are omitted. Branch lengths represent substitutions per site. B: bootstrap lower than 50 in ML, X: no bootstrap support for this branch in ML