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1 **Novel species of the oomycete *Olpidiopsis* potentially threaten European red algal**
2 **cultivation**

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16

17 **Abstract**

18 The rapid growth of marine macroalgal cultivation amplifies the potential impacts of
19 seaweed diseases. Here, we combine microscopy and molecular analysis to describe
20 two novel European species, *Olpidiopsis palmariae* and *O. muelleri* spp. nov., that infect
21 the commercially important red algae *Palmaria* and *Porphyra*, respectively. A Scottish
22 variety of *Olpidiopsis porphyrae*, a devastating pathogen of *Pyropia* previously thought
23 to be restricted to Japanese seaweed farms, is also described as *O. porphyrae* var.
24 *scotiae*. In the light of their destructiveness in Asian farms, together with the global
25 expansion of algal cultivation and pertaining seed trade, *Olpidiopsis* pathogens should
26 be treated as a serious threat to the sustainability of red algal aquaculture. Our findings
27 call for the documentation of seaweed pathogens and the creation of an international
28 biosecurity framework to limit their spread.

29
30 **Keywords:**

31 Aquaculture, Algal Disease, Algal Parasite, Barcoding, Biosecurity, *Olpidiopsis*,
32 Oomycete, Rhodophyte.

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44 **Introduction**

45 In the last 25 years, the production of *Pyropia* (formerly *Porphyra*), the alga extensively
46 used as sushi wrap in Asiatic cuisine, has more than tripled, mostly due to a rapid
47 expansion in China and Korea (FAO 2014, <http://www.fao.org>). Over the same period,
48 the production of seaweeds grown for their jellifying properties (carageenophytes and
49 agarophytes, including eucheumatoids and *Gracilaria*) has increased fifteen-fold. Many
50 more species, including *Palmaria palmata* (traditionally eaten as dulse in the UK) are
51 subjected to cultivation trials.

52 A recent analysis conducted in Korea showed that alongside the intensification of
53 production, disease management is a growing concern for farmers, and now contributes
54 up to half the running cost of a farm (Kim *et al.*, 2014); in the last three years, three new
55 species of pathogens infecting *Pyropia* have been described (Kim *et al.*, 2016; Klochkova
56 *et al.*, 2016b). This echoes a well-known pattern in agriculture and animal aquaculture,
57 whereby diseases are discovered when species first become cultivated in large scale
58 (e.g. for invertebrate aquaculture, Brasier, 2008; Stentiford *et al.*, 2011; Stentiford *et al.*,
59 2017). Therefore, the identification and characterisation of pathogens infecting algal
60 crops is becoming a research priority to underpin the sustainable development of the
61 industry (Cottier-Cook *et al.*, 2016). To ensure the conservation of native algal
62 biodiversity, it is also indispensable to understand the potential disease-mediated
63 interplay between crops and wild stocks (e.g. presence of pathogen reservoirs and wild-
64 crop cross-contamination, Loureiro *et al.*, 2015).

65 Amongst the most devastating pathogens of Asian *Pyropia* farms are the oomycetes
66 *Olpidiopsis porphyrae* and *O. pyropiae*, that are investigated in Japan and Korea (Arasaki,
67 1947 ; Arasaki, 1960; Park *et al.*, 2001; Ding *et al.*, 2005; Sekimoto *et al.*, 2008; Kim *et al.*,
68 2014; Klochkova *et al.*, 2016a; Kwak *et al.*, 2017). Additionally, *Olpidiopsis bostrychia*
69 was found infecting *Bostrychia moritziana*, a small red alga of the West Indian Ocean
70 mangroves (West *et al.*, 2006; Sekimoto *et al.*, 2009). Recently, records of *Olpidiopsis*
71 *feldmanni* and *Olpidiopsis heterosiphoniae* were also published (Fletcher *et al.*, 2015;
72 Klochkova *et al.*, 2017). To date, these marine endoparasites of red algae are the only
73 members of the *Olpidiopsis* genus characterised both molecularly and morphologically;
74 they form a monophyletic clade (commonly recognised as the order Olpidiopsidales),

75 most closely related to the Anisolpidiales and Haliphthorales (Gachon *et al.*, 2017).
76 Additional older records of holocarpic pathogens of red algae have been reviewed
77 elsewhere (Dick, 2001; Beakes *et al.*, 2014), but molecular information is lacking for all
78 of them. Likewise, and despite their destructiveness in Asia, there is no morphology-
79 based nor any molecular report of *Olpidiopsis* pathogens of red algae in Europe.

80 Taking into account the growing number of seaweed cultivation initiatives in European
81 waters, we set out to assess if the genus *Olpidiopsis* could represent a potential threat to
82 this developing aquaculture sector. Here, a modest sampling campaign of wild algal
83 populations and *Palmaria* cultivation facilities led us to the identification in Scotland of
84 two novel *Olpidiopsis* species, and of one species previously only reported in Japan
85 (Sekimoto *et al.*, 2008).

86 **Methods**

87 ***Biological material***

88 Details of all records of marine *Olpidiopsis* and their respective hosts are given in
89 Table S1, alongside with sampling location, GPS coordinates, host culture collection ID,
90 and Genbank sequence accession number (when applicable).

91 ***Palmaria palmata*** blades were collected from cultivation lines seeded in the wild (Isle
92 of Kerrera, Scotland, UK) in April 2015 and cultivated in 10.000 L fibreglass tanks for
93 two months (ambient light and temperature, continuous flow of seawater 7L/min).
94 Fertile *Palmaria* tetrasporophytes developed in those conditions, together with
95 epiphytic *Ectocarpus* filaments. *Olpidiopsis* Isolate 1 was encountered parasitizing
96 *Palmaria* tetraspores retained on epiphytic *Ectocarpus* filaments. Though stable
97 cultures were not established, the pathogen was successfully propagated into healthy
98 tetraspores as follows: healthy fertile tetrasporophytes were rinsed and maintained
99 overnight at 10°C in filtered sterile sea water, and a tetraspore suspension was obtained
100 by gentle centrifugation (2000g for 10min at 10°C). In an inoculation procedure
101 adapted from Strittmatter *et al.* (2013), a droplet of healthy tetraspore suspension was
102 placed in a 50 mm diameter, 20 mm-deep petri dish, covered with a 40- μ m mesh cell
103 strainer containing the infected material. The propagation of the parasite into the
104 tetraspores beneath the cell strainer was monitored by bright field microscopy and this
105 material was used for histological staining and DNA extraction.

106 ***Porphyra sp.*** blades infected with several *Olpidiopsis* parasites were collected from
107 various locations in Scotland (Table S1), namely the Shetland Islands (Isolate 2), Seil
108 Island (Isolate 4) and Oban.

109 ***Polysiphonia sp.*** specimens (infected with *Olpidiopsis* Isolate 3) were collected at low
110 tide (Clachan Bridge, Scotland, UK) and observed using conventional bright field
111 microscopy (Zeiss Observer Z1). Infected algal tips were dissected for subsequent DNA
112 extraction.

113 ***Histological staining***

114 Observations were made on fresh material, or samples fixed in 2% formaldehyde and
115 0,2% glutaraldehyde in phosphate buffered saline). Cell wall structures and nuclei were
116 stained with Calcofluor white and SYBR-Green, respectively (Gachon *et al.*, 2017).

117 ***DNA extraction sequencing and molecular phylogeny reconstruction***

118 DNA extraction of infected *Palmaria* tetraspore suspensions, infected *Porphyra* sp., and
119 infected *Polysiphonia* algal tips was performed according to Strittmatter *et al.* 2013.
120 Oomycete Cox1, Cox 2 and 18S markers were amplified (according to Gachon *et al.*,
121 2017) and individually subjected to phylogeny reconstruction with a representative
122 subset of published oomycete sequences. NCBI accession numbers of all sequences used
123 for phylogeny reconstruction are given in Table S2. Alignments were generated using
124 the MAFFT algorithm and manually corrected prior to phylogenetic analysis in MEGA v.
125 7 (Kumar *et al.*, 2016). Model tests were performed on each alignment prior to
126 maximum likelihood (ML) analysis to find the best substitution models. For the 18S
127 rRNA, Tamura-3-parameter was used (Tamura, 1992) with a discrete Gamma
128 distribution to model evolutionary rate differences among sites. The model by Le &
129 Gascuel was used with discrete gamma distribution for cox1 and cox2 markers (Le *et*
130 *al.*, 2008). The rate variation model allowed for some sites to be evolutionarily
131 invariable for the cox2 alignment. Maximum parsimony analysis was also performed on
132 all three datasets. Bootstrap re-sampling was set to 500 replicates. *Palmaria* and
133 *Polysiphonia* SSU markers were amplified using primers according to Saunders *et al.*
134 (2013), and default Neighbour Joining trees were generated in Geneious R6 (Fig. S7).

135 **Results and discussion**

136 ***A novel Olpidiopsis pathogen identified in Palmaria palmata cultivation facilities***

137 Isolate 1, hereafter identified as *O. palmariae* sp. nov., was discovered when monitoring
138 fertile *Palmaria palmata* tetrasporophytes grown in tanks (Fig. 1a,b and Fig. S1a). The
139 blades were colonised by brown filamentous epiphytes (*Ectocarpus* sp.) and displayed
140 an unusual punctuated aspect due to the germination of tetraspores before their release
141 (Fig S1b). Numerous free *Palmaria* tetraspores, as well as some young gametophytes
142 (Fig. 1b), were found entangled in the brown filamentous epiphytes. Most tetraspores
143 appeared dead and devoid of any cellular content, suggestive of an infection with an
144 intracellular holocarpic pathogen (arrows on Fig. 1b). Various developmental stages of

145 the parasite thallus were observed (Fig. 1c-h): young unwalled thalli in degrading red
146 algal cell structures (Fig. 1c), as well as older, granulous thalli filling entirely dead
147 *Palmaria* tetraspores (arrow on Fig. 1d). Each granular thallus developed an exit tube in
148 the course of spore differentiation (arrowhead on Fig. 1e). Some dead *Palmaria*
149 tetraspores contained mature pathogen sporangia with individualised encysted spores
150 (Fig. 1f) and multiple infections of the same tetraspore were frequent (Fig. 1g-h). Each
151 mature empty sporangium displayed one single exit tube of varying length (Fig. 1h). We
152 successfully propagated the pathogen by co-incubating infected material with freshly
153 released *Palmaria* tetraspores, enabling us to better observe its development (Fig. 2).
154 Uninfected tetraspores had a granulous content, with often an asymmetric repartition
155 of chloroplasts (Fig. 2a). We did not succeed in observing the penetration of the
156 pathogen into the red algal cell, and the earliest recognisable stage of infection was
157 characterised by a small refringent globule (ca. 2 μm in diameter) surrounded by a
158 spherical structure within the tetraspore cytosol (Fig. 2b). We assume that this perfectly
159 spherical shape is conferred by a prominent vacuole (arrow on Fig. 2c) that occupies
160 most of the thallus biovolume. Small vesicles (arrowheads on Fig. 2c) appeared at this
161 stage. Each thallus grew outwards very rapidly, as shown in a 21 min time course (Figs.
162 2d to 2f). During this period, increasing digestion of the red algal cell structures was
163 evident and absorption vesicles formed at the algal-pathogen interface, suggestive of
164 rapid incorporation of algal material by the parasite. The diameter of the absorption
165 vesicles increased with time, and they fused with the central globule (arrowheads on
166 Fig. 2e and 2f), suggesting that the latter is a storage structure. Once the tetraspore
167 content was fully assimilated, each thallus differentiated a cell wall (arrow on Fig. 2g)
168 and underwent radical ultrastructural changes. The refringent globule and the vacuole
169 both receded in size and became fragmented, leaving space for an increasingly dense
170 cytosol (Fig 2g-h). Just before sporogenesis, only dense cytoplasm was recognisable in
171 the once highly vacuolated thallus (Fig. 2i).

172 SYBR Green staining revealed that walled thalli are multinucleate syncytia (Fig. 3a):
173 Similar to recent observations conducted on the closely related pathogen *Anisolpidium*
174 *ectocarpii* (Gachon *et al.*, 2017), the nuclei of the younger stages were ca. 2.5 μm in
175 diameter with condensed peripheral material (arrowheads), whereas more mature
176 stages had dense compact nuclei ca. 1 μm in diameter (arrow). All nuclei within a

177 thallus were always at the same stage, therefore we assume that nuclear divisions are
178 synchronous, as is often the case in syncytial organisms. Multiple infections of the same
179 tetraspores were prevalent in our co-incubation experiment. In tetraspores containing
180 multiple parasites, SYBR Green and calcofluor white staining did not hint to sexual
181 fusion between antheridia and oogonia (Fig. 3b and c). Each sporangium produced one
182 exit tube. Calcofluor staining revealed that the length of the exit tubes varied widely
183 from 3 to ca. 20 μm (Fig. 3d, the inset shows the same infected spore in a different focal
184 plane). Remnants of encysted spores were observed on the surface of some host cells.
185 Those were 3 μm in diameter and had a thin, calcofluor-positive cell wall (Fig. 3e). Very
186 thin needle-like structures reminiscent of the penetration structure of *A. ectocarpii*
187 (Gachon *et al.*, 2017) were observed, through which spore content was injected into the
188 *Palmaria* host (arrowheads on fig 3e). It is unclear whether these infectious spores
189 encyst at the surface of the algal host directly following their release from the
190 sporangium, or if diplanetism might exist.

191 Comparable to other holocarpic oomycetes, the syncytium segmented to produce
192 spores (Fig. 2g-i). The dehiscence of one pathogen sporangium was observed, as well as
193 subsequent spore differentiation (Fig. 3f-h). Spores were about 3 μm in diameter and
194 assumed a light amoeboid movement inside the sporangium. Their release outside the
195 sporangium took a few minutes. As soon as the spores reached the outside medium,
196 they extended two straight flagella of unequal length within a minute or less (Fig. 3f and
197 g). During this process, the spores first assumed a triangular shape (Fig. 3f) and
198 progressively became spherical (h and i). The mature flagella were 3 and 8-10 μm ,
199 respectively, and bore a vesicle at their apex and tips (Fig. 3f-i, white arrowheads),
200 probably due to rapid reorganisation of their plasma membrane.

201 To the exception of individual cells of developing female gametophytes, no infection was
202 observed on any of the other *Palmaria* life stage available in culture (tetrasporophytes,
203 and embryos). In the absence of a stable supply of *Palmaria* tetraspores, this stage-
204 specificity of the parasite prevented its long-term laboratory cultivation.

205 Morphologically similar parasites were observed again several times in other
206 populations of *Palmaria palmata* grown in tanks, and on September 7th, 2015 on
207 individuals collected in Seil Island (Supplementary Table S1).

208 ***A novel Olpidiopsis parasite infecting both wild Porphyra and Polysiphonia***

209 Isolate 2, hereafter identified as *O. muelleri* sp. nov., stemmed from a female
210 gametophyte of *Porphyra* sp. harvested in the Shetland Islands due to its discoloured
211 margin (Fig. S1c,d). The infection was spatially restricted to the fertile margin where
212 red female gametes could still be found (Fig. 4a, arrowheads). The pathogen thalli were
213 granular and completely filled their host algal cell, which displayed an almost spherical
214 shape and altered brownish to greenish pigmentation (arrows). Each of the sporangia
215 developed a single and highly vacuolated exit tube (inset on Fig. 4b). Calcofluor white
216 staining of the same infected margin revealed a high density of infection (Fig. 4b, main
217 picture), and highlighted numerous *Olpidiopsis* empty sporangia with exit tubes of
218 varying length (ca. 20-80 μm). We did not observe any evidence of multiple infections of
219 the same algal cell. Numerous additional observations of *Olpidiopsis* parasitizing
220 *Porphyra* sp. were recorded from various blades collected in Oban and its surrounding
221 (Fig. S2) but they were not submitted to DNA barcoding.

222 Isolate 3, hereafter identified as *O. muelleri* var. *polysiphoniae*, was observed on
223 *Polysiphonia stricta*. The host alga displayed slightly curved growing tips (Fig. 4c), of
224 which several were infected (Fig. 4d). The parasite first developed as an apparently
225 unwallled granulous syncytium with granulous, greyish content which sometimes
226 completely filled the infected algal tip (Fig. 4d); spanning several algal cells, the cell wall
227 of which was still discernible (arrowheads on Fig. 4e). When infecting three-
228 dimensionally branched algal tips, the parasite thallus was lobed (arrow on e). Multiple
229 and perfectly spherical vacuoles could often be seen (Fig. 4f). The vacuolated thalli
230 displayed a clearly-defined wall (arrow). Additional smaller unwallled thalli could be
231 found in the same infected tip (arrowheads), suggesting multiple infections. Mature
232 parasite sporangia with individualized spores of ca. 5 μm in diameter were also
233 observed (Fig. 4g). Remains of excysted spore cell walls reminiscent of the honeycomb
234 structure typical of *Eurychasma* could be observed within empty sporangia (Fig. 4h),
235 and the latter typically displayed one or two short exit tubes (arrows in Fig. 4h). This
236 honeycomb structure is suggestive of sequential spore encystment/excystment, and
237 therefore of diplanetism. Spore release was not observed, and in the absence of
238 potential alternative hosts, the limited material prevented us from documenting further
239 the life cycle of this parasite.

240 ***A new variety of Asian Olpidiopsis parasite is present in Scotland***

241 Isolate 4, hereafter molecularly identified as *O. porphyrae* var. *scotiae*, was observed on
242 wild *Porphyra* blades collected from Easdale, Argyll, Scotland. Infected blades were
243 crinkled (Fig. S1e), resulting from localised necrotic lesions (arrows on Fig. S1f).
244 Diseased tissue revealed patches of infected host cells (Fig. 5a and higher magnification
245 on Fig. 5b). *Porphyra* cells containing pre-mature parasite thalli appeared light pink due
246 to degradation of algal pigments (dark arrows). The parasite thallus ultimately filled the
247 host cell, where remaining greenish algal material was compacted at the periphery
248 (dark arrowheads). The centre of infected patches usually displayed collapsed dead
249 cells of *Porphyra* (double arrowheads). Multiple infections of the same host cell were
250 frequently observed (Fig. 5c). In such multiple infections, calcofluor staining of the
251 parasite cell wall did not reveal any thallus fusion (inset in Fig. 5c), although further
252 ultrastructural work is needed to ascertain this result. Neighbouring dead collapsed
253 *Porphyra* cells (double arrowheads in Fig. 5c) were also calcofluor-positive, thus
254 revealing empty parasite sporangia. All empty sporangia observed displayed single exit
255 tubes of varying length (ca. 5-30 μm). SYBR-Green staining revealed fully grown
256 syncytial sporangia containing numerous nuclei (Fig. 5d). The number of nuclei (and
257 thus of infectious propagules) seemingly depended on the overall size of differentiating
258 sporangia, although no precise quantification was attempted. Diseased material was
259 incubated with fresh spores released by the same blade, leading to the observation of
260 successful spore infections (Fig. 5e).

261 **Molecular phylogeny unveils two novel *Olpidiopsis* species and unknown varieties**
262 **of Asian *Olpidiopsis* strains**

263 18S sequences were obtained for all four isolates described above. We were also
264 successful in obtaining a Cox2 sequence for Isolates 2 and 4 and a Cox1 sequence for
265 Isolate 4. All sequences generated in this study were submitted to Genbank (Table S1)
266 and used for Maximum Likelihood and Maximum Parsimony tree reconstruction (Fig. 6
267 and Fig. S3). All markers consistently grouped our four isolates with the known marine
268 *Olpidiopsis* parasitizing red algae, within a single clade (red arrow on Fig. 6 and Fig. S3),
269 most closely related to *Haliphthoros*, *Halocrusticida* and *Halodaphnea* (Haliphthorales).
270 Though the bootstrap values are mediocre, our 18S data further suggest that marine
271 *Olpidiopsis* species are split in three distinct clades, that we hereafter refer to as the

272 “*bostrychiae*”, “*pyropiae*” and “*porphyrae*” lineages. The parasite of *P. palmata* (Isolate 1)
273 was most closely related to *O. porphyrae*, with 98.8% identity on exons of the 18S
274 sequence. Its unique host, zoospore differentiation and 18S sequence set it aside from *O.*
275 *porphyrae* and we therefore propose to name it *Olpidiopsis palmariae* sp. nov. The 18S
276 sequence obtained for Isolate 2 was closest to *O. bostrychiae* (97% identity,
277 AB363063.1) while the amino acid Cox2 sequence formed a long branch clustered with
278 *O. porphyrae* (85% identity versus 72% identity with *O. bostrychiae*, Fig. S5). Therefore,
279 we conclude that isolate 2 is a novel species that we name *Olpidiopsis muelleri* sp. nov.
280 While no Cox2 sequence was obtained for the *Polysiphonia* parasite Isolate 3, its 18S
281 sequence was 100% identical to *O. muelleri* (Isolate 2). In contrast to all members of the
282 “*bostrychiae*” and “*porphyrae*” lineages, Isolate 3 forms honeycomb structures strongly
283 suggestive of diplanetism. This specific phenology argues against Isolate 3 being
284 conspecific with any of the *Olpidiopsis* already described, especially *O. muelleri*.
285 However, we were unable to observe zoospore behaviour on *O. muelleri* (Isolate 2).
286 Therefore, our conservative interpretation is to consider Isolates 2 and 3 as conspecific
287 until more molecular or morphological evidence is obtained. In order to take into
288 account its different host and phenology compared to Isolate 2, we refer to Isolate 3 as
289 *O. muelleri* var. *polysiphoniae*. Finally, the 18S and Cox1 sequences of Isolate 4 were
290 100% identical to *O. porphyrae*. Its virtually translated Cox2 sequence was 99.4%
291 identical to *O. porphyrae* (one substitution over 180 amino acid residues). Introns were
292 also detected in the 18S sequence, some of which were conserved with *O. porphyrae*
293 and/or with *O. porphyrae* var. *koreanae* (Fig. S6). Taking into account its original intron-
294 exon structure, a feature already used to erect the Korean variety *O. porphyrae* var.
295 *koreanae* (Kwak *et al.*, 2017), we refer to this isolate as *O. porphyrae* var. *scotiae*.

296

297 **Conclusion**

298 Here we describe two pathogens, *Olpidiopsis palmariae* and *O. muelleri* spp. nov. and
299 report two novel varieties *O. muelleri* var. *polysiphoniae* and *O. porphyrae* var. *scotiae*
300 from Scotland. In the light of our modest sampling efforts, this work illustrates the
301 widespread occurrence of undocumented *Olpidiopsis* species infecting both wild and
302 cultivated algae in Europe. The destructiveness of *O. pyropiae* and *O. porphyrae* in Asia
303 demonstrates the risk posed by these oomycete pathogens for the red seaweed industry
304 (Ding and Ma, 2005; Kim *et al.*, 2014; Kwak *et al.*, 2017). Accordingly, disease

305 management has become an integral part of farm design and operation in Asia; most
306 recently, insurance schemes have been set-up to protect farmers against worsening
307 crop losses (Cottier-Cook *et al.*, 2016). Our repeated observations of *O. palmariae* in
308 cultivation facilities in Scotland, combined with multiple accounts by growers of
309 hitherto unexplained seeding failures, highlights the real possibility of yield-limiting
310 epidemic outbreaks in the nascent Western aquaculture industry, and echoes repeated
311 reports of *Petersenia* diseases in Canadian *Chondrus* production facilities (Craigie *et al.*,
312 1996 and refs therein). This first European report of an *Olpidiopsis* species which is
313 already known to be highly destructive in Asia, opens the question of the potential
314 economic impact that non-native pathogens could have on Asian crops, especially if they
315 were introduced as a result of unregulated seed movements. Taken together, our
316 findings call for a much more systematic documentation of seaweed pathogens and the
317 creation of an international biosecurity framework to monitor and limit their spread.

318 **Taxonomy**

319

320 ***Olpidiopsis palmariae* Y. Badis & C.M.M. Gachon sp. nov.**

321 Vegetative thalli endobiotic, spherical, 2.3–4 µm in diameter when young, 10–50 µm
322 before cell-wall thickening and zoospore cleavage; completely filling host cell at
323 maturity, single discharge tube of variable length (3.5 to 30 µm) protruding from the
324 algal tetraspore; Multiple infections frequent (typically 2-5 thalli in one host cell in
325 holotype material), resulting in angular sporangia after cell wall thickening; zoospores
326 maturing outside of zoosporangium, triangular to spherical at maturity, 2.5–3.5 µm in
327 diameter, laterally biflagellate; flagella perpendicular and of unequal length (3 and 8-10
328 µm, respectively), differentiating upon discharge of the spores from the sporangium and
329 bearing at least temporarily submicrometric vesicles at their apical extremity; resting
330 spores unknown; obligate endoparasite in *Palmaria palmata* (Rhodophyceae). Only
331 observed infecting tetraspores or very young (2-8 cells) gametophytes.

332 HOLOTYPE: Registration number BM001222129 (Population of infected *Palmaria*
333 tetraspores in formaldehyde/glutaraldehyde TEM buffer), National History Museum,
334 London (NHM). Type specimens are specific of *Palmaria palmata* tetraspores.

335 ILLUSTRATIONS WITH ANALYSIS: Fig. 2

336 PARATYPES: MuseumID (resin-embedded EM specimen), National History Museum,
337 London (NHM).

338 TYPE LOCALITY: Kerrera Island, Oban, Scotland, United Kingdom.

339 TYPE CULTURE: None

340 ETYMOLOGY: Named after its algal host.

341 GenBank accession number: KY403502 (18S)

342

343 ***Olpidiopsis muelleri* Y. Badis & C.M.M. Gachon sp. nov.**

344 Vegetative thalli endobiotic, spherical, 10–50 µm in diameter and zoospore cleavage;
345 completely filling host cell at maturity; single vacuolated discharge tube (variable
346 length, 20 to 80 µm) protruding from the host algal cortex at maturity; zoospores not
347 observed; obligate endoparasite in *Porphyra* sp. gametophyte.

348 HOLOTYPE: Registration number BM001222128 (Fragment of infected *Porphyra* blade
349 (Fig. S1.c-d) in formaldehyde/glutaraldehyde TEM buffer), National History Museum,
350 London (NHM).

351 ILLUSTRATIONS WITH ANALYSIS: Fig 4a-b.

352 ISOTYPES: Additional fragment of the same infected *Porphyra* blade (Fig. S1.c-d) in
353 formaldehyde/glutaraldehyde TEM buffer), National History Museum, London (NHM).

354 TYPE LOCALITY: Lunna, Shetland Islands, Scotland, United Kingdom. (60°24'; -1°07')

355 TYPE CULTURE: None.

356 ETYMOLOGY: Named after Professor Dieter. G. Mueller, in recognition of his pioneering
357 contribution to the study and laboratory cultivation of marine oomycetes.

358 Genbank accession numbers: KY403503 (18S) and KY403508 (Cox2)

359

360

361 ***Olpidiopsis muelleri* var. *polysiphoniae* Y. Badis & C.M.M. Gachon var. nov.**

362 Vegetative thalli endobiotic, elongated, 10–50 µm, restricted to algal tips, completely
363 filling several adjacent cells, causing slight hypertrophy of algal tips, possibly lobed in
364 bifurcated algal tips; thalli naked in early stages, walled and vacuolated in later stages;
365 1-2 short discharge tubes (5 µm) protruding from the host algal cortex at maturity;
366 Zoospores sub-spherical, 3 to 5 µm in diameter, diplanetic, maturing and encysting in
367 sporangium. Remains of excysted spore cell walls visible in empty sporangium, forming
368 an irregular honeycomb structure. Obligate endoparasite in apices of *Polysiphonia* sp.

369 ICONOTYPE: Fig. 4c-h
370 TYPE LOCALITY: Atlantic Bridge, Seil Island, Scotland, United Kingdom. (56°17'; -5°58')
371 TYPE CULTURE: None.
372 ETYMOLOGY: Named after *Olpidiopsis muelleri*, on the basis of its identical 18S
373 sequence, and the specific host of this isolate.
374 Genbank accession number: KY403501 (18S).

375

376 ***Olpidiopsis porphyrae* var. *scotiae* Y. Badis, G.H. Kim, T.A. Klochkova & C.M.M.**
377 ***Gachon* var. nov.**

378 Vegetative thalli endobiotic, spherical to ellipsoidal, 2.5–6 µm in diameter when young,
379 12–30 µm in size when mature before spore cleavage; with 1 discharge tube protruding
380 from the host algal cortex at maturity. Zoospores spherical to reniform, 2.2–3.5 µm in
381 size, biflagellate, motile, maturing in sporangium; two flagella of unequal length,
382 inserted sub-apically, positioned at 45° angle. Dormant cysts spherical to ovoid, non-
383 motile, without flagella, maturing in sporangium. Armored resting spores absent; sexual
384 reproduction absent. Obligate endoparasite in *Porphyra* and *Pyropia* spp. 18S rRNA
385 gene sequence contains 5 group I introns.

386 COLLECTION: Oban, Scotland; May 2016; by Kim G.H.

387 ICONOTYPE: Fig. 5

388 TYPE LOCALITY: Seil Island, Scotland, United Kingdom. (56°17'; -5°39')

389 ETYMOLOGY: Named after *Olpidiopsis porphyrae*, and the type locality of this isolate.

390 GenBank accession number: KY403504 (18S), KY403506 (cox1), KY403505 (cox2).

391

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403 **Author Contributions**

404 CMMG, GHK, YB and TAK designed the experiments; CMMG and GHK supervised the
405 research; YB, CMMG, GHK and JCS conducted the fieldwork; YB, TAK, MS, AG and PM
406 conducted the laboratory work YB and CMMG wrote the manuscript with contributions
407 from all co- authors. All authors gave final approval for publication.

408

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507

508

509 **Figure Legends**

510 **Fig. 1** *Olpidiopsis palmariae* – Main description

- 511 **a.** Cultivated blade of *Palmaria palmata* with epiphytic tufts of *Ectocarpus* sp. (thick
512 brown arrows); the rare whitish necrotic lesions (white arrow) do not seem related
513 with the *Olpidiopsis* disease. **b.** Numerous tetraspores associated to *Ectocarpus* tufts, as
514 well as some young gametophytes. Most tetraspores appear dead, following an infection
515 with an intracellular holocarpic pathogen (arrows). Inset: mature sporangium within a
516 young gametophyte that is still pigmented. **c-h.** Development stages of the pathogen. **c.**
517 Two young unwalled thalli (arrows) inside a degrading tetraspore. **d.** Thick-walled
518 granulous thallus (arrow), surrounded by two mature empty sporangia (arrowheads).
519 **e.** Sporangium (arrow) containing differentiating spores; Note the long thin exit tube
520 (arrowhead). **f.** Sporangium with individualised encysted spores; note the absence of
521 honeycomb-like structure. **g.** Multiple infections of the same tetraspore are frequent. **h.**

522 Two empty thick-wall sporangia with exit tubes of different length (arrowheads). Bars :
523 b. 20 μm ; c-h. 10 μm .

524 **Fig. 2** *Olpidiopsis palmariae* – Pathogen nutrition inside the tetraspore

525 **a.** Healthy tetraspore of *Palmaria palmata*. **b.** Earliest stages of infection, showing small
526 individual globules (ca. 2 μm in diameter) surrounded by a vacuole **c.** Spherical thalli
527 (arrow) are delimited by a vacuole and contain a refringent central globule and small
528 absorption vesicles (arrowheads). **d-f** Time course over 20 minutes, showing rapid
529 outward growth of each thallus (arrow); note the formation, growth of absorption
530 vesicles at the thallus periphery, followed by their fusion with the central globule
531 (arrowheads). **g-i.** Structural changes in differentiating sporangia: **g-h.** Pathogen cell
532 wall differentiation in fully assimilated tetraspores; note the angular cell walls
533 separating several pathogen thalli; the vacuole and the refringent globule then start
534 receding, progressively leading to the granulous aspect shown in h. **i.** Initiation of
535 cytoplasm segmentation during sporogenesis; the upper sporangium already
536 discharged zoospores. **Legend:** Thick arrow: individual thallus; Thin arrow: Pathogen
537 cell wall; V-shaped arrowhead: vacuole. Arrowhead: absorption vesicle and/or
538 refringent globule. All bars: 10 μm .

539 **Fig. 3** *Olpidiopsis palmariae* – Sporogenesis and infection structures

540 **a.** Sybr-Green staining reveals multinucleate thalli at different stages. A nucleolus is
541 visible on younger stages nuclei (arrowheads), whereas more mature stages have dense
542 compact nuclei (arrows). **b.** Sybr-Green (left) and calcofluor white staining (right) do
543 not hint to thallus fusion **c.** Similar conclusions as in a-b, on a different object. **d.**
544 Calcofluor staining of multiple sporangia showing variability in the length of the exit
545 tubes. Inset: same infected spore in a different focal plane. **e.** Calcofluor staining
546 showing the remains of encysted pathogen spores bearing thin injection needles
547 (arrowheads). All bars. **f-g.** Rapid differentiation of flagella in two freshly released
548 spores. The pictures were taken ca. 1 min apart from each other. **h-i.** Spherical spores
549 with fully mature, straight flagella of unequal length, seen from different angles. In all
550 pictures, the arrowhead points to the bulging membranar structure at the tip of each
551 flagellum. Bars: a-e. 10 μm ; f-i. 2 μm .

552

553 **Fig. 4** *Olpidiopsis muelleri* on *Porphyra* sp. and *Polysiphonia* sp.

554 **a.** Close-up of fertile *Porphyra* sp. blade margin showing cells infected by
555 intracellular parasites (dark arrows) and healthy female cells (arrowheads) **b.**
556 Highly infected region stained with Calcofluor white. Note the varying length of
557 exit tubes (up to 80 μm). Inset: Single vacuolated exit tube (arrow) produced by
558 a by mature sporangium (arrow). **c.** Healthy filament tips of *Polysiphonia stricta*
559 **d.** Unwalled intramatrical thallus in a filament tip of *P. stricta*; Note the tip
560 swelling when compared to c. **e.** Lobed shape of the parasite thallus (arrow)
561 invading several algal cells. Note the visible remnants of the host cell walls
562 (arrowheads). **f.** Spherical vacuoles can often be seen at late stages of infection.
563 Note the clearly-defined wall (arrow), whereas the smaller thalli to the top and
564 bottom right are unwalled (arrowheads). **g.** Encysted spores in mature
565 sporangium. **h.** Empty sporangium with two rather short exit tubes (arrows).
566 Note the remains of excysted spore cell walls forming a coarse honeycomb
567 structure. Bars: a, e-f, and inset in b. 20 μm ; b. 100 μm ; c-g. 50 μm ; h. 10 μm .

568 **Fig. 5** *Olpidiopsis porphyrae* var. *scotiae* on Scottish *Porphyra* sp.

569 **a-b.** Necrotic patch of infected *Porphyra* cells. Note the contrasting pigmentation of
570 infected cells, ranging from pinkish in earlier stages (arrows) to greenish in later stages
571 (arrowheads). Dead collapsed host cells are usually observed in the centre of each patch
572 (double arrowheads). **c.** Multiple infections of *Porphyra* cells were frequently observed.
573 Inset: Calcofluor staining of parasite cell wall did not reveal any thallus fusion; collapsed
574 dead *Porphyra* cells (double arrowheads) are calcofluor positive, revealing empty
575 pathogen sporangia. **d.** Syncytial mature sporangia revealed by SYBR-Green staining. **e.**
576 *Olpidiopsis* thallus (arrow) growing in *Porphyra* spore.

577 **Fig. 6** Molecular phylogeny of marine members of the *Olpidiopsis* genus

578 Maximum Likelihood inference of the phylogeny of all known *Olpidiopsis* 18S sequences
579 using 500 Bootstrap replicates. The tree features four additional environmental 18S
580 sequences (EF100276, EF100297, AY426928, AY789783) identified by blastn searches
581 against Genbank. The red arrow points to the single clade grouping all *Olpidiopsis* and
582 *Anisolpidium* sequences. The Bootstrap values are reflected by the diameter and colour
583 of each node. Scale: number of nucleotide substitutions per site

584

585 **Supporting Information**

586 **Fig. S1** Additional captions of infected host algae

587 **Fig. S2** Additional *Olpidiopsis* sp. observed on a *Porphyra* sp. blade in Oban

588 **Fig. S3** 18S Phylogenetic tree (Maximum Parsimony)

589 **Fig. S4** Cox1 Phylogenetic trees

590 **Fig. S5** Cox2 Phylogenetic Trees

591 **Fig. S6** Intron Content in *Olpidiopsis porphyrae* varieties

592 **Fig. S7** Molecular characterization of some red algal hosts identified in this study

593 **Table S1** Summary table of all known *Olpidiopsis* sequences, OTUs described in this study, as
594 well as selected bibliographical records

595 **Table S2** List of all Genbank accessions used for 18S, Cox1, and Cox2 phylogeny reconstruction

596

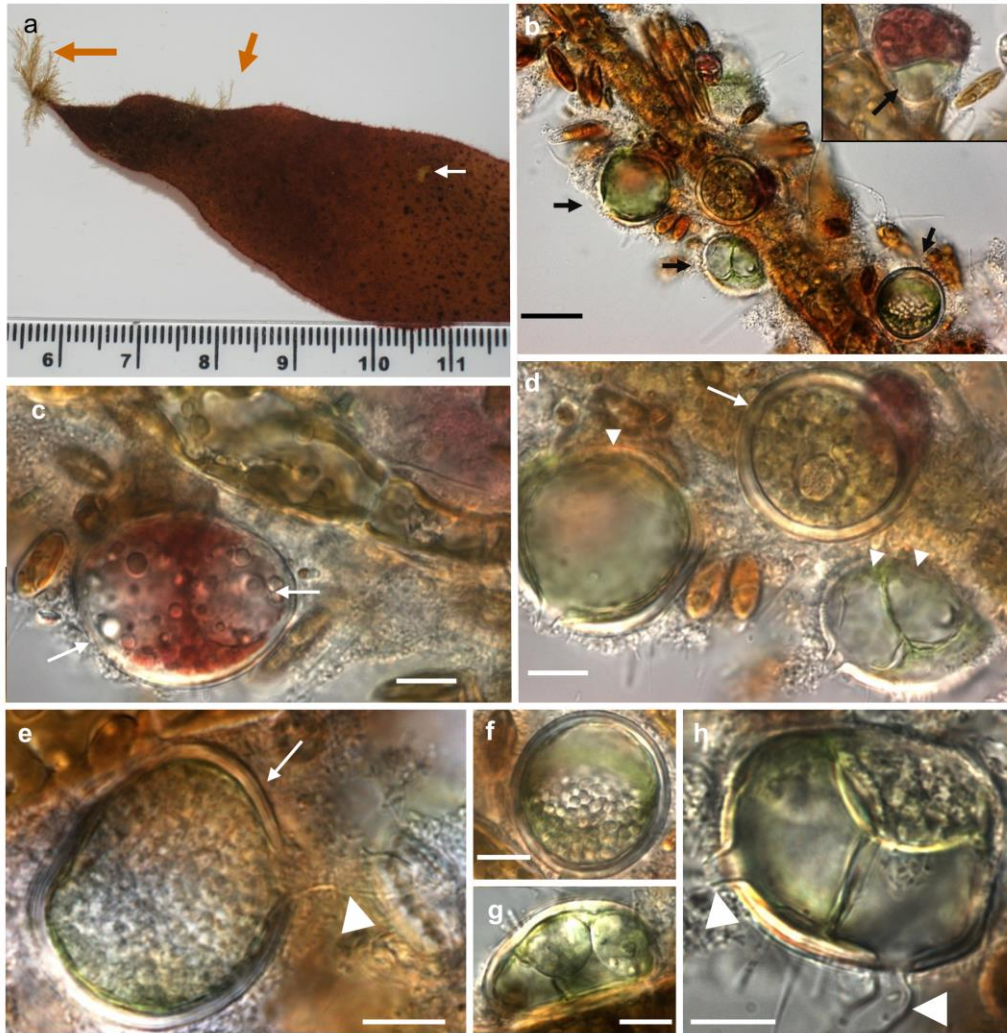


Fig.1 *Olpidiopsis palmariae* - Main description:

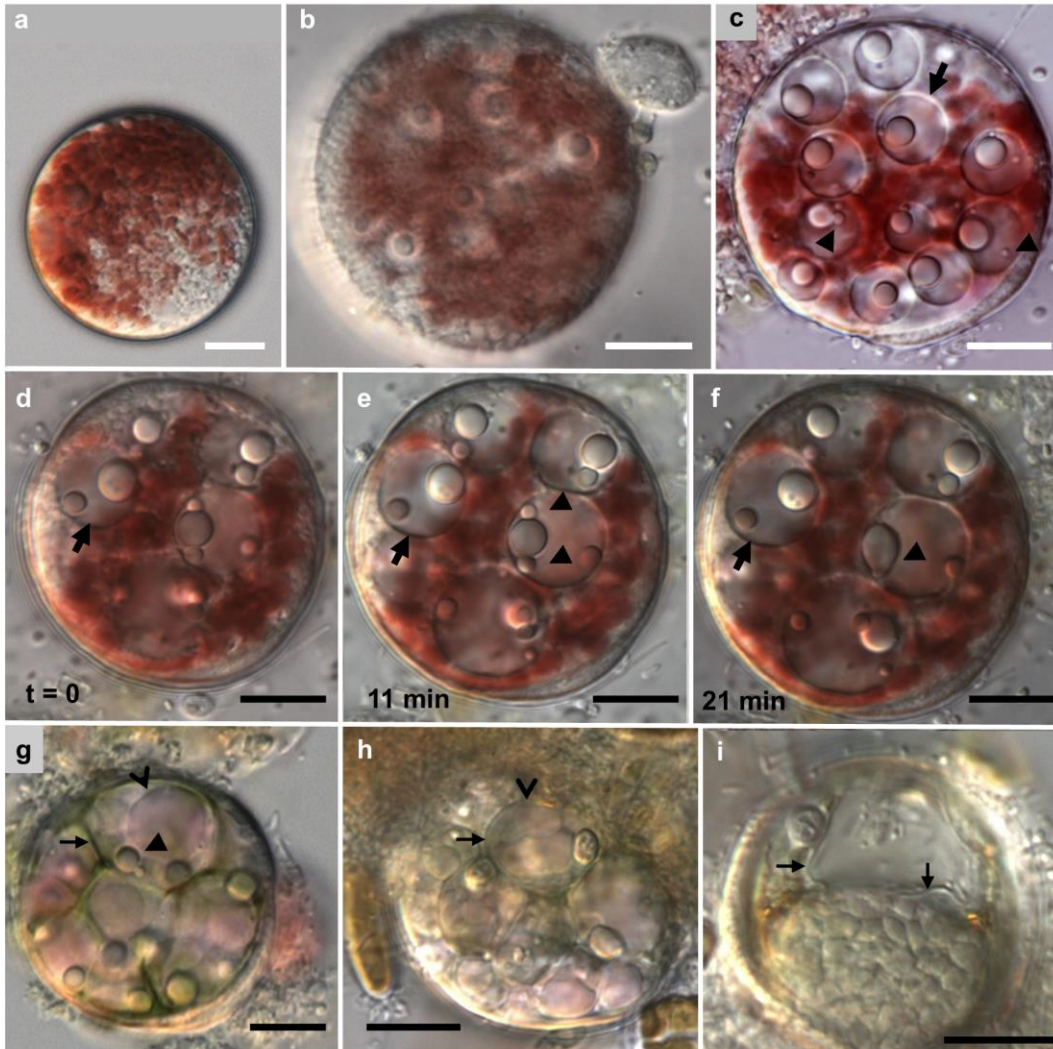


Fig.2 *Olpidiopsis palmariae* - Pathogen nutrition inside the tetraspore

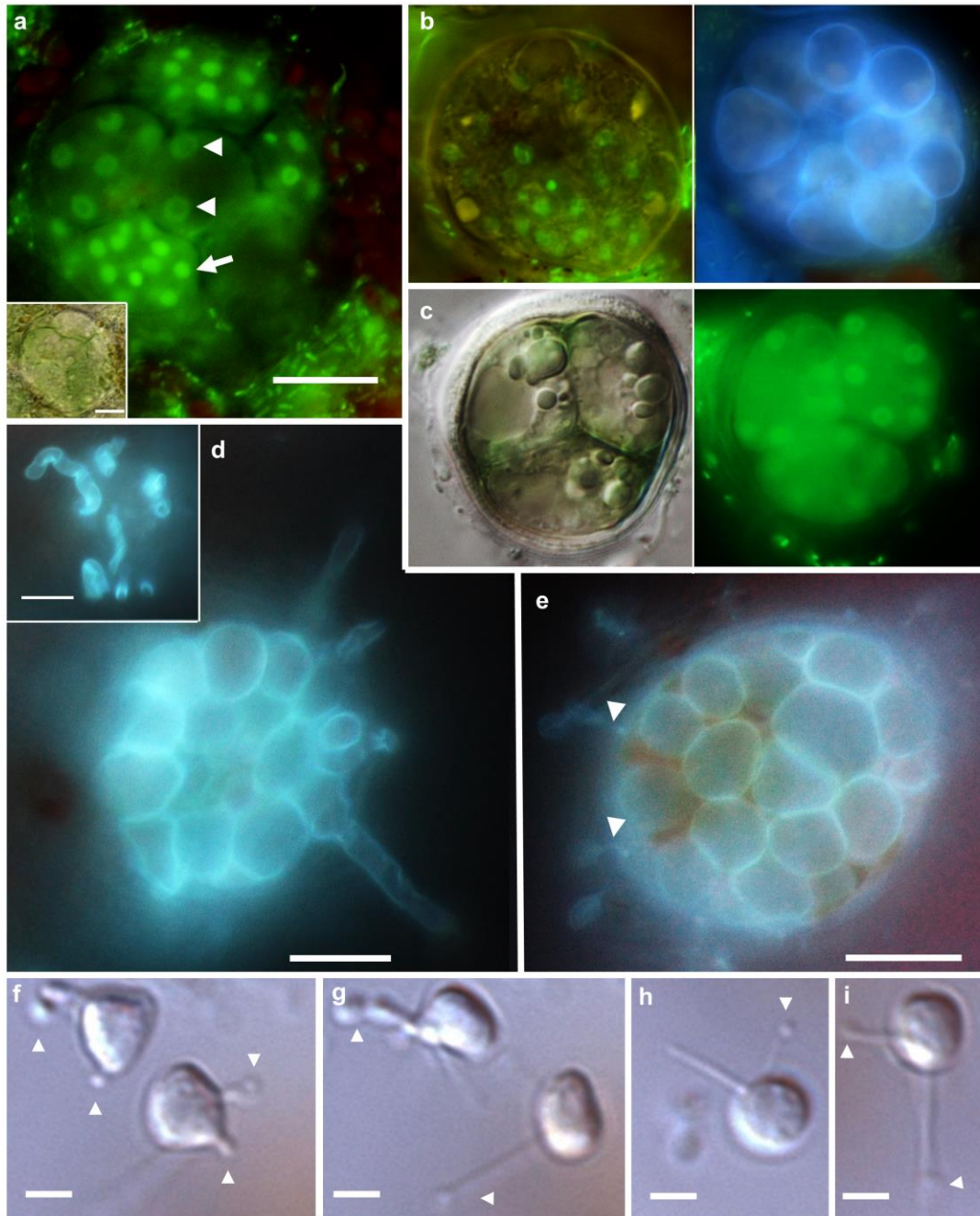


Fig.3. *Olpidiopsis palmariae* - sporogenesis and infection structures

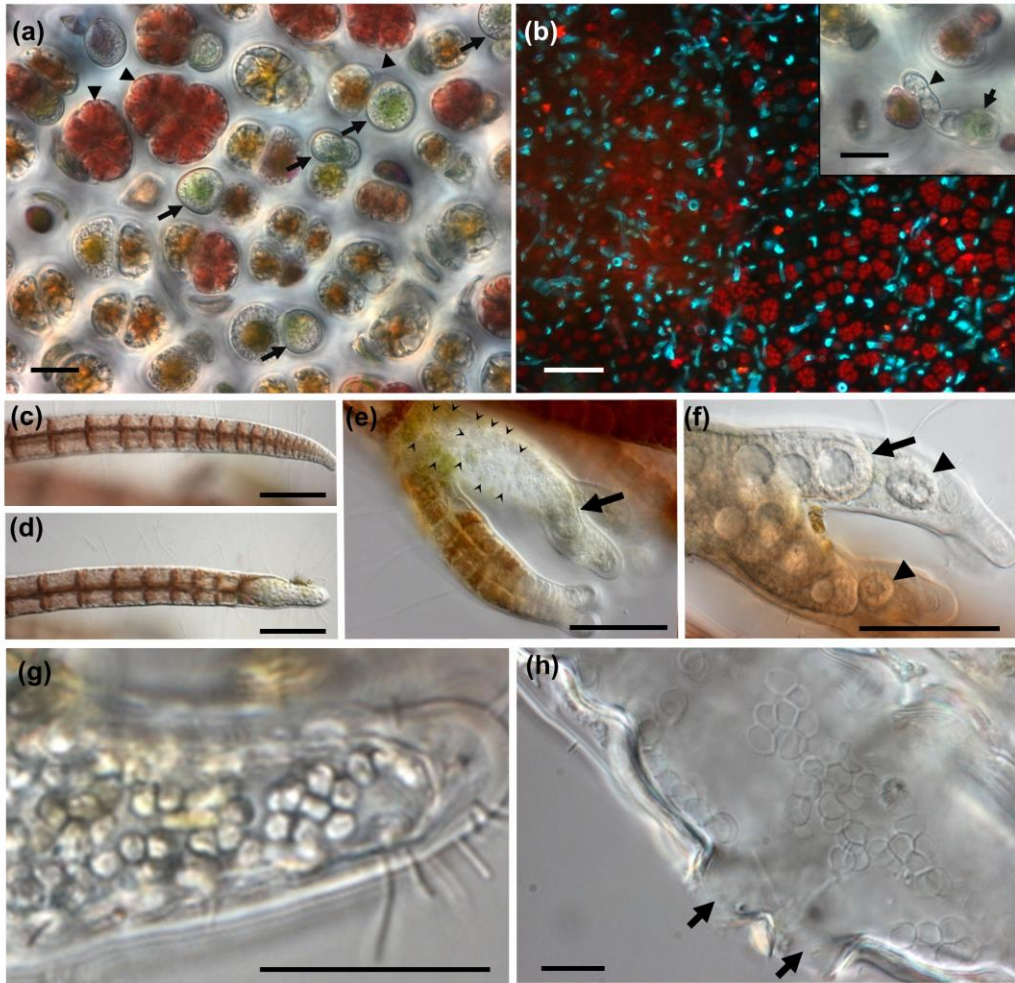


Fig. 4 *Olpidiopsis muelleri* sp. nov. on *Porphyra* sp. and *Polysiphonia* sp.

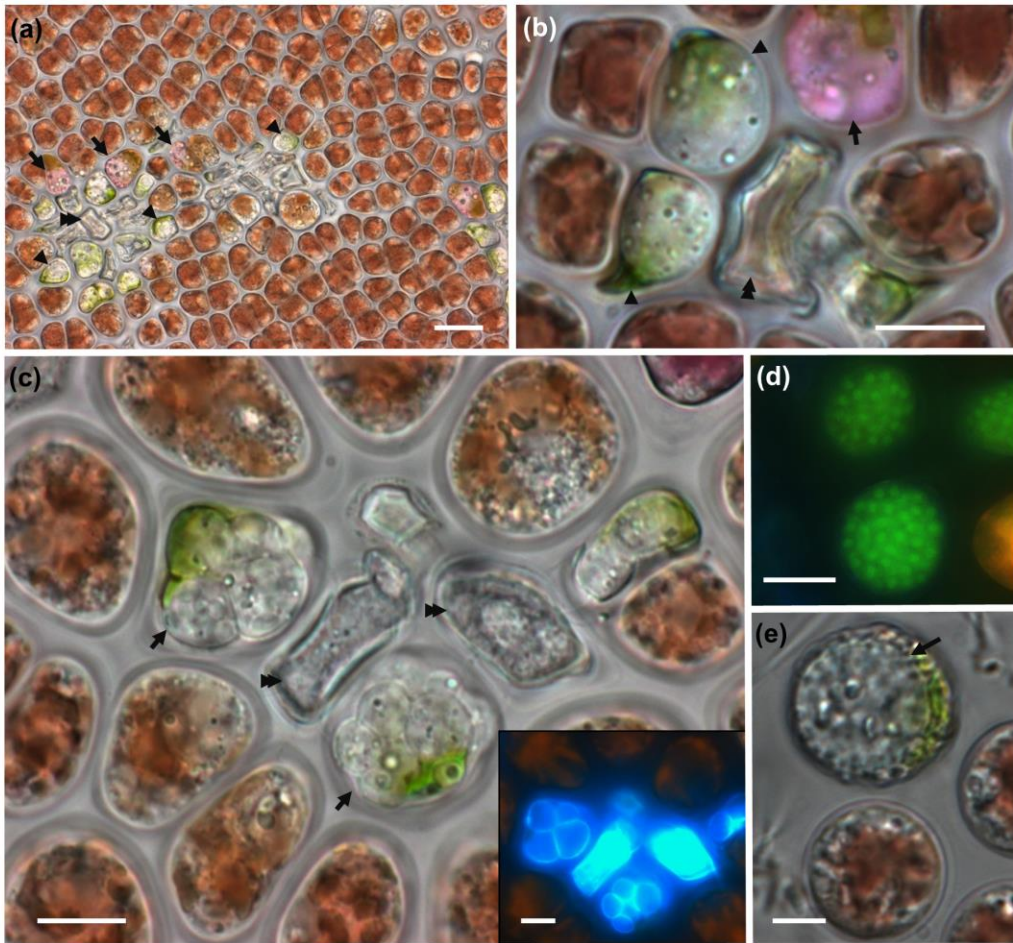


Fig. 5 *Olpidiopsis porphyrae* var. *scotiae* infecting *Porphyra* sp.

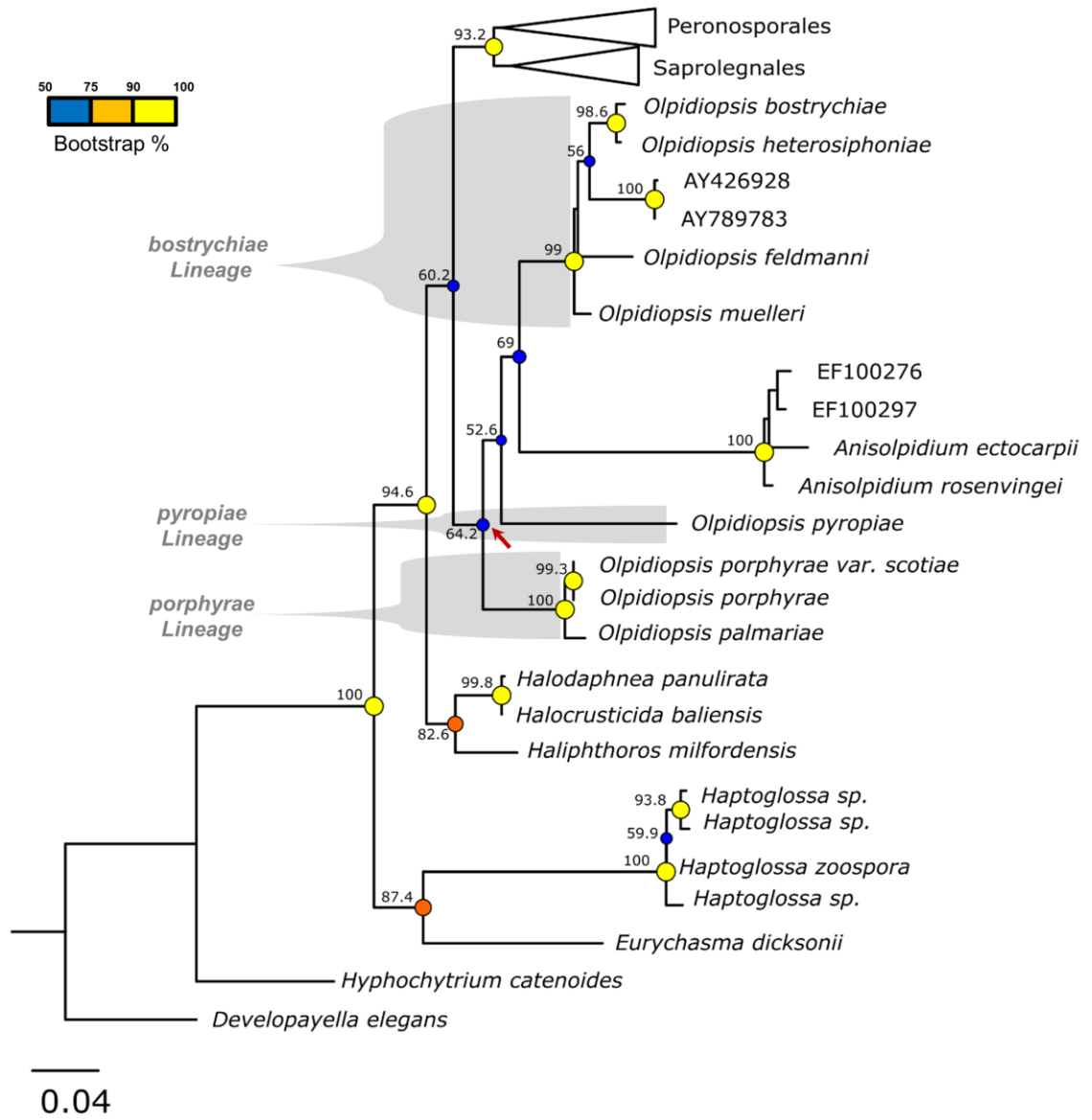


Fig. 6