Host pathogen interaction between European strains of red algae 'Bangia' and oomycete Olpidiopsis porphyrae var. scotiae, and phylogenetic analysis for European 'Bangia' strains

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MASTER OF RESEARCH (AWARDED BY UHI)

Award date:
2022

Awarding institution:
University of the Highlands and Islands

Host pathogen interaction between European strains of red algae '*Bangia*' and oomycete *Olpidiopsis porphyrae* var. *scotiae*, and phylogenetic analysis for European '*Bangia*' strains

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December 2021

A thesis presented for the degree of Master by Research in marine science (Algal Biotechnology Biology and Ecology)

Scottish Association for Marine Science

University of Highlands and Islands

University of the Highlands and Islands
Oilthigh na Gàidhealtachd agus nan Eilean

SAMS
Scottish Association for Marine Science

Declaration

I declare that all the material contained in this thesis is my own work that took place at Scottish Association for Marine Science.

Abstract

Olpidiopsis is one of the main disease causal agents affecting *Neoprophyra* (commonly known as Nori) sea farms. A recent study described a Scottish variety in the Scottish water *Olpidiopsis porphyrae* var. *scotiae.* I studied the resistance of 11 red algae ('*Bangia*') strains against this new described variety by using microscopy inspection and qPCR assay. Results from microscopy inspection showed significant effect for '*Bangia*' strains on incubation period (days from inoculation until observation of the first symptom) and the percentage of infection (the number of infected cells as percentage). qPCR assay showed also significant effect for the '*Bangia*' strain on normalized Pfaffl ratio suggesting different levels of resistance for different '*Bangia*' strains. Moreover, I studied the phylogeny for 20 '*Bangia*' strains collected from France and Scotland by using *rbc*L and *cox*1 molecular markers and I compared the phylogenetic position of our '*Bangia*' strains with available information for the phylogeny of '*Bangia*' strains around the world. My study showed that eight strains are belong to '*Bangia*'2 clade, 11 strains belong to '*Bangia*'3 clade, and one freshwater strain belongs to '*Bangia*' clade. Overall, there was different resistance reaction from different '*Bangia*' strains against the pathogen suggesting different levels of host susceptibility which is not related to the clade.

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Acknowledgements

This project was funded by GlobalSeaweedSTAR program, and I would like to transfer my gratitude to all of the people in this program for their fruitful efforts and help.

At the end of my project, I would like to thank Prof. **Claire Gachon`s research team** for their help and collaboration during my work in Scottish Association for Marine Science. It was a great opportunity to work with this kind of scientific collaborative friendly team.

I would like to thank my supervisor Dr **Janina Brakel** for her continuous help, collaboration, and direct support during the work.

Many thanks for Professor **Claire M.M Gachon** for her efforts, and scientific support in addition to her great support and contribution to secure accommodation for me and my family under such difficult circumstances.

I would like also to thank Dr **Marie-Mathilde Perrineau** for her kind help and valuable practical advice when needed.

I would like to convey me sincere gratitude also to the great people I met at SAMS especially Dr **Anuschka Miller** for her support during early stages**,** Professor **Bhavani Narayanaswamy** for her kind support and help for me and my family to overcome all the difficulties, Dr **Anna Kane** for her kind help.

Finally, I would like to mention the great help and support I received from **Peter Lemont**.

I am sorry to leave SAMS where I could find the most friendly atmosphere during my scientific career, *I missed my first family when I left Syria and I will miss my second family when I leave SAMS.*

Basem Attar December 2021.

1. Introduction

1.1 Algae in nature and seaweed farming

Algae are aquatic organisms that release oxygen by photosynthesis. They are either unicellular, colonial organisms, or constructing filaments and colonies. They exist in marine and freshwater habitats and differ in size that range from 0.2-2 μm in diameter -like picoplankton- to 60 m length like giant kelp. According to their size they are classified into micro- and macroalgae (Guiry, 2012; Barsanti & Gualtieri, 2005).

Three phylogenetic groups of macroalgae are recognised Rhodophyta, Phaeophyta and Chlorophyta. These differ in their pigmentation (Manzelat et al. 2018). Green algae (Chlorophyta) contains an estimated 6,000-8,000 species most of them are freshwater algae. Most species of this group are macroalgae but there are also some unicellular and colonial green algae. Brown algae (Phaeophyta) estimated to contain 1,500-2,000 species most of them as marine species (Chapman 2013), the giant kelp (Macrocystis) is brown alga. Red algae (Rhodophyta) contain between 4,000-5,000 species most of them are marine (Chapman 2013).Seaweed farming is considered an important food production method especially in Asia. During the past century the seaweed farming expanded rapidly Table *1* (Junning et al. 2021), gaining importance in human food production, animal feed, chemicals and biofuel production (Tiwari and Troy 2015).

Table 1. Source: FAO. 2021c. Fishery and Aquaculture Statistics. Global seaweeds production by production source 2019 (FishStatJ).

Table. Global seaweed production, 2019	Total seaweed production (farmed and wild)		Seaweed cultivation	
Country/area	Tonnes (wet weight)	Share of world production (%)	Tonnes (wet weight)	Share in farmed and wild production (%)
World	**35 762 504**	**100.00**	**34 679 134**	**96.97**
Asia	**34 826 750**	**97.38**	**34 513 223**	**99.10**
1. China	*20 296 592*	*56.75*	*20 122 142*	*99.14*
2. Indonesia	*9 962 900*	*27.86*	*9 918 400*	*99.55*
3. Republic of Korea	*1 821 475*	*5.09*	*1 812 765*	*99.52*
4. Philippines	*1 500 326*	*4.20*	*1 499 961*	*99.98*
5. Democratic People's Republic of Korea	*603 000*	*1.69*	*603 000*	*100.00*
7. Japan	*412 300*	*1.15*	*345 500*	*83.80*
8. Malaysia	*188 110*	*0.53*	*188 110*	*100.00*
Rest of Asia (7 countries/territories)	42 047	0.12	23 344	55.52
Americas	**487 241**	**1.36**	**22 856**	**4.69**
6. Chile	*426 605*	*1.19*	*21 679*	*5.08*
Peru	36 348	0.10	-	-
Canada	12 655	0.04	-	-
Mexico	7 336	0.02	10	0.14
United States of America	3 394	0.01	263	7.75
Rest of the Americas (6 countries)	904	0.00	904	100.00
Europe	**287 033**	**0.80**	**11 125**	**3.88**
9. Norway	*163 197*	*0.46*	*117*	*0.07*
France	51 476	0.14	176	0.34
United Kingdom*	---	----	----	----
Ireland	29 542	0.08	42	0.14
Russian Federation	19 544	0.05	10 573	54.10
Iceland	17 533	0.05	-	-
Rest of Europe (5 countries)	5 741	0.02	217	3.78
Africa	**144 909**	**0.41**	**117 791**	**81.29**
10. United Republic of	*106 069*	*0.30*	*106 069*	*100.00*

Tanzania				
Zanzibar	104 620	0.29	104 620	100.00
Tanzania (mainland)	1 449	0.00	1 449	100.00
Morocco	17 591	0.05	273	1.55
South Africa	11 155	0.03	2 155	19.32
Madagascar	9 665	0.03	8 865	91.72
Rest of Africa (2 countries)	430	0.00	430	100.00
Oceania	**16 572**	**0.05**	**14 140**	**85.32**
Solomon Islands	5 600	0.02	5 600	100.00
Papua New Guinea	4 300	0.01	4 300	100.00
Kiribati	3 650	0.01	3 650	100.00
Australia	1 923	0.01	-	-
Rest of Oceania (3 countries)	1 099	0.00	590	53.66

* The FAO database has no record of production for the UK. In 2013 the wild harvest seaweeds in UK estimated between 2000-3000 dry tonnes, (Capuzzo and McKie 2016).

Asia is one of the main centres of seaweed production where the seaweeds are used for human food like *Neoporphyra* (nori), *Neopyropia*, *Undaria* and *Saccharina* (Kim et al. 2017), or for the extraction of chemicals like agar-agar and carrageenan (*Gracilaria* spp. for agar, and *Kappaphycus* and *Eucheuma* spp. for carrageenan) (Francavilla et al. 2013; Lim et al. 2017). The usage of seaweeds as human food began in the third century in China (L.-E. Yang, Lu, and Brodie 2017). The highest consumption of seaweeds is in Asia, but the export of seaweeds products was increased to Europe, Africa and North America after global popularization of Sushi consumption (Chen, J., Xu 2005).

In Europe, seaweeds production still on the first steps comparing with Asia, however, it is expected to expand for its potential positive contribution with European economy and positive impact on environment (Commission 2012; Hasselström et al. 2020).

1.2 Bangiales order

Bangiales is an order of red algae with a global distribution range and includes the most valuable seaweeds crop species such as *Neoporphyra* (Nori) (Brodie, J. A. & Irvine 2003; Mumford, T. F. & Miura 1988).

Most of species under order Bangiales are marine species, only one of 130 currently accepted species is a freshwater species (Sutherland et al. 2011).

In order Bangiales there is single family (Bangiaceae), the family contains two genera that were classified depending on the morphology of gametophytes. The unbranched filaments are classified under genus *Bangia* Lyngb (Müller, Cannone, and Sheath 2005; Nelson, W. A., Farr, T. J. & Broom 2005).

The taxonomy of algae was based on classical taxonomy according to morphological characters such as dimensions and colours, however it is not reliable method as most of algal species and specially Bangiales has the same nature of filaments (Mueller et Al 1998; Xu et al., 2016).

The best widely used technique to detect and discriminate algal species is the molecular technique that allows researchers from classifying and studying the genetic and phylogenetic relation and history of algal species (Mueller et al 1998; Xu et al. 2016).

Initially, only two genera were recognised according to morphological characters depending on the filaments in *Bangia* and bladed taxa in *Porphyra*, however, this classification was not supported by molecular phylogenetic studies (Broom et al. 1999; Müller et al. 2019; Oliveira et al. 1995). In a recent molecular study by using SSU rDNA and *rbc*L genes, fifteen genera were included under order Bangiales seven filamentous and eight foliose genera (Sutherland et al. 2011). The phylogram of these genera are shown in Figure 1. And the latest update for Bangiales genera was as shown in Table 2 (Sutherland et al. 2011; L. E. Yang et al. 2020).

Table 2. The Bangiales genera that revised by (Sutherland et al. 2011; L. E. Yang et al. 2020) by using SSU and rbcL and COI-5P molecular markers filamentous genus written in red, and folios are written in blue.

Genus	**shape**	**Habitat**	**Domestication Status**
Minerva W. A. Nelson	Filamentous	Marine	Wild
Dione W. A. Nelson	Filamentous	Marine	Wild
Miuraea N. Kikuchi, S. Arai	Folios	Marine	Wild
Bangia Lyngb.	Filamentous	Freshwater	Wild
''*Bangia*'' 1.	Filamentous	Marine	Wild
Clymene W. A. Nelson	Folios	Marine	Wild
Porphyra C. Agardh.	Folios	Marine	Wild and aquacultures
Lysithea W. A. Nelson	Folios	Marine	Wild
Pseudobangia K. M. Müller et Sheath.	Filamentous	Marine	Wild
Fuscifolium S. C. Lindstrom	Folios	Marine	Wild
''*Bangia*'' 2.	Filamentous	Marine	Wild
Boreophyllum S. C. Lindstrom	Folios	Marine	Wild
Wildemania De Toni.	Folios	Marine	Wild
''*Bangia*'' 3.	Filamentous	marine	Wild
Pyropia J. Agardh.	Folios	marine	Wild and aquaculture
Calidia L.-E.Yang & J.Brodie	Folios	Marine	Wild
Neoporphyra J.Brodie & L.-E.Yang	Folios	Marine	Wild and aquaculture
Neopyropia J.Brodie & L.-E.Yang	Folios	Marine	Wild and aquaculture
Uedaea J.Brodie & L.-E.Yang	Folios	Marine	Wild

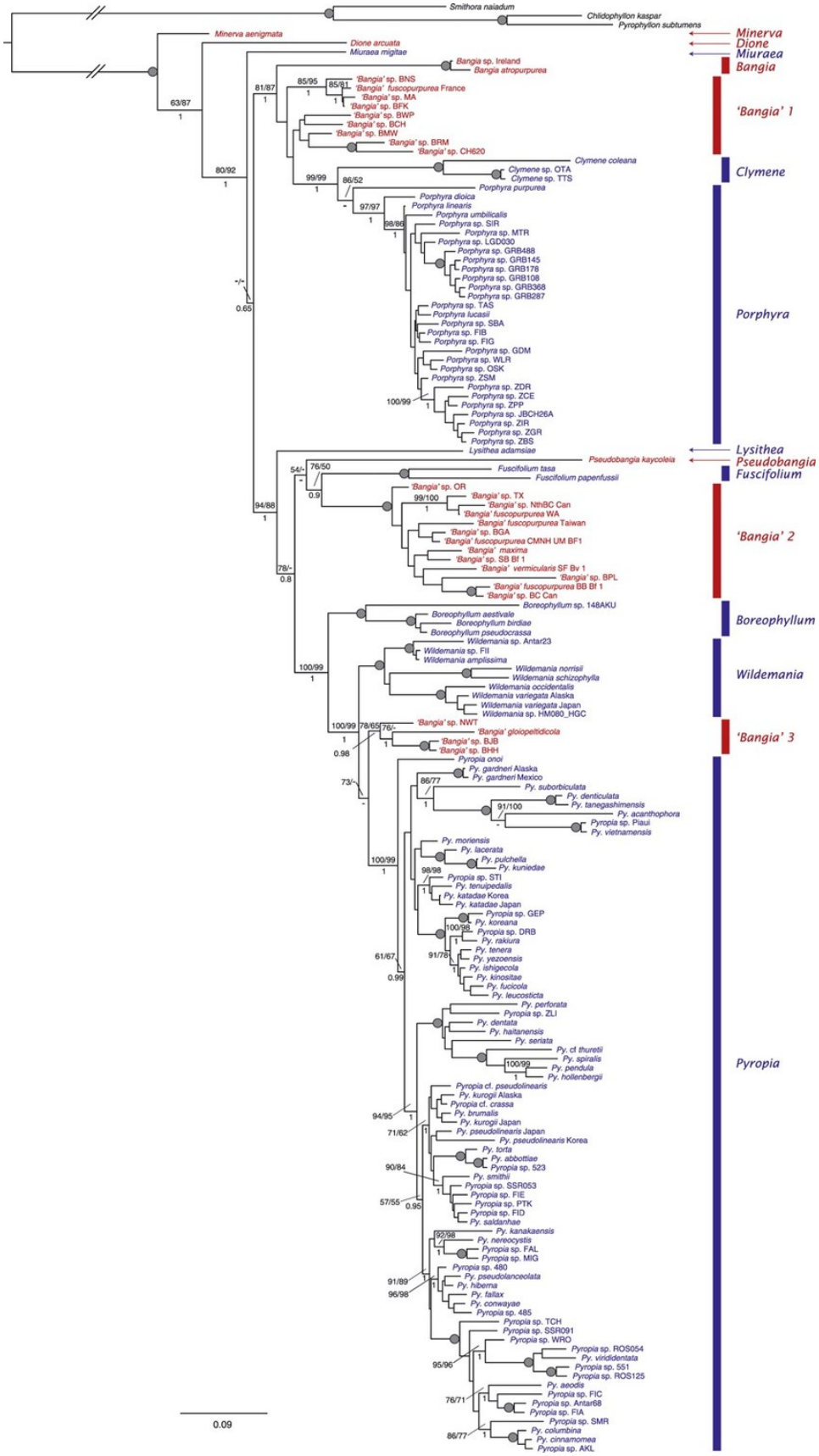

Figure 1. Phylogram of 157 Bangiales taxa calculated from the concatenated nuclear SSU ribosomal RNA (nrSSU) and RUBISCO LSU (rbcL) data set under RAxML. The values shown above the nodes are the bootstrap values for RAxML and GARLI, and below the nodes are the Bayesian PP values. The nodes supported at 100% RAxML/ 100% GARLI / 1 PP are marked as gray circles. Genera are indicated by lines on the right of the tree and monotypic genera by arrows. The filamentous taxa are shown in red, while the foliose taxa in blue (Sutherland et al. 2011).

In the most recent molecular study, the genus *Pyropia* redefined to four new genera *Calidia*, *Neoporphyra*, *Neopyropia*, and *Uedaea* (L. E. Yang et al. 2020) Table 2 .

1.3 Main Challenges of seaweed production

As any production process, the production of seaweed is affected by the occurrence of different pests and diseases as a direct result of production intensification (Kim et al. 2014; Loureiro, Gachon, and Rebours 2015a; Tsiresy et al. 2016). For instance 25-30% of production from *Saccharina japonica* was lost in China due to an appearance of diseases (X. Wang et al. 2013), moreover, 10% of *Porphyra* (Nori) production that was around 1.5 billion US$ in Japan and Korea was lost due to oomycetes pathogens *Olpidiopsis* spp. and *Pythium* spp. (Ding and Ma 2005), and reduction of 15-20% in yield with 20-30% in quality of seaweeds was reported in Korea (Park, Kakinuma, and Amano 2001). In Korea it has been reported that Korean *Pyropia* farms production was reduced by 20% in some regions and the essential cause of loss was the oomycete pathogen *Olpidiopsis pyropiae* (Kim et al. 2014; Klochkova et al. 2012, 2016a). Strategies to control the diseases and decrease the losses are cost intensive very expensive for the farmer like the complete removal of seedlings, or negatively impact the local and global environment like chemical treatments (Klochkova et al. 2012; Loureiro, Gachon, and Rebours 2015b).

Diseases outbreak in marine environments are yet likely to increase as a result of intensive aquaculture of algae as these provide favourable conditions for disease development (Gachon et al. 2010). Algal pathogens are diverse, e.g., there are viruses (Suttle 2007), bacteria (Ashen and Goff 2000; Gachon et al. 2010; G. Wang et al. 2008), amoebae and small heterotrophic flagellates (Sime-Ngando, Lefèvre, and Gleason 2010), oomycetes (Strittmatter, Gachon, and Küpper 2008), Chytridiomycetes, dinoflagellate phytoplanktonic algae and obligatory algal endophytes (Ibelings et al. 2004; Park, Yih, and Coats 2004; Rasconi et al. 2009).

Compared to pathogens of land plants, more attention should be paid to do more studies about algal pathogens and their interaction with algae. Our current study is a trial to shed more light on host pathogen interaction between an oomycete *Olpidiopsis* and different strains of filamentous red algae (*Bangia*) of the order Bangiales. This thesis focuses on basal marine oomycetes that infect red algae of the economically relevant order of Bangiales.

1.4 *Olpidiopsis* an algal pathogen

Olpidiopsis is one of the main disease causal agents affecting *Neoporphyra* sea farms since many decades (Cho YC 1986), and from that date till present it became the most devastating limiting factor for seaweeds in the sea farms (Klochkova et al. 2012). Two known species causing *Olpidiopsis* blight disease in Asian *Neoporphyra* and *Neopyropia* farms are *Olpidiopsis porphyrae* and *O. pyropiae* (Arasaki 1947, 1960; Badis et al. 2018; Klochkova et al. 2016b). *Olpidiopsis* spp. have a wide host range and can affect many algal species for instance, *Olpidiopsis porphyrae* species can affect *Neopyropia yezoensis*, *Neopyropia tenera*, *Neoporphyra seriata*, and *Bangia fuscopurpurea* (Sekimoto et al. 2008). Symptoms on *Neoporphyra* spp. blades appear as non-regular blades shape with scattered green spots that become bigger in size and lighter in colour with the infection progress, in the later infection stage the spots form holes that destroy the shape of the blade (Kim et al. 2014; Migita 1969).

While *Olpidiopsis* spp. have been recognised as a relevant algal pathogen in Asia, it exists now in Scotland in both seaweed farms and in the wild. In Scotland the two *Olpidiopsis* species infecting red algae are *Olpidiopsis palmariae* infecting *Palmaria palmata*, and *O. mulleri* spp. nov., infecting *Porphyra*. Moreover there is new described Scottish variety in Scotland *Olpidiopsis porphyrae* var. *scotiae*, that was reported previously just in Japan (Badis et al. 2018).

This Scottish variety of *Olpidiopsis porphyrae* was virulent on the Korean red algal cultivar *Neopyropia yezoensis* (previously *Pyropia*), on the other hand a Korean *Olpidiopsis porphyrae* isolate was able to infect wild '*Bangia'* sp. collected from Scotland. Suggesting the potential negative effect from exported crops that might be infected with *Olpidiopsis* and will probably affect the native European algae in the future (Badis, Klochkova, Strittmatter, et al. 2019). The isolated *Olpidiopsis* from wild Scottish *Bangia* was obtained from gametophyte life stage (Badis et al. 2018). In nature, *Bangia* can be found in two different life stages: gametophyte (haploid, male or female), or sporophyte (diploid) as shown in Figure 2 (Notoya and Iijima 2003).

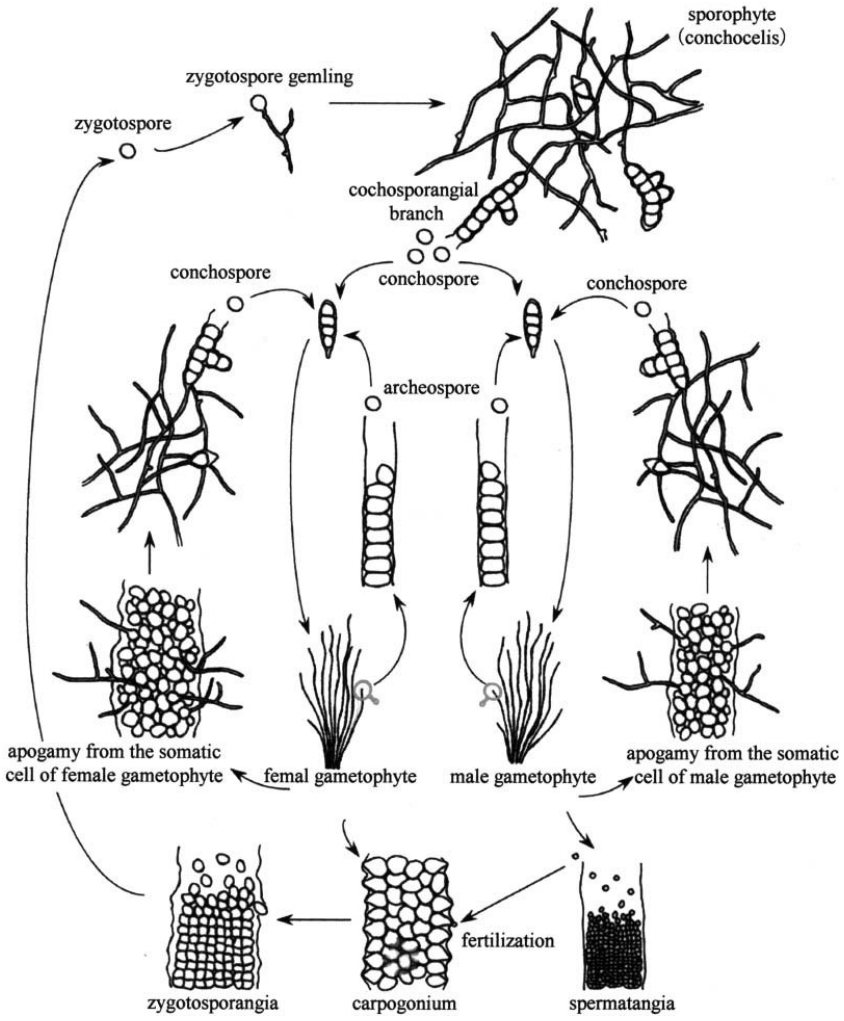

Figure 2. Summarized life history of Bangia atropurpurea (Notoya and Iijima 2003).

1.5 Research question

Given the concern of potential negative impacts from *Olpidiopsis* spp. on wild algae and algal production in Europe (Badis et al. 2019), a better understanding of the interaction between *Olpidiopsis* spp. and European red algae is needed, to identify the wild algae strains with high resistance which will provide the opportunity to foster research and breeding for more resistant strains for algae cultivation. In addition to identify a reliable protocol to assess *Olpidiopsis* infection to control the movement of red algae pathogens and try to find any available source of resistance from the wild that could be used in the future to limit and control the widespread and negative impact of *Olpidiopsis* on both seaweed farms and in the wild.

Recent efforts by Y. Badis and D. Müller resulted in collection and isolation of 20 wild '*Bangia*' strains from Scottish water (west coast) and Atlantic coast of France (Bretagne) (see Tab 1). '*Bangia*' strains were frequently found to host *Olpidiopsis* spp. in both regions. Y. Badis established techniques to maintain *Olpidiopsis* spp. in '*Bangia'* strains in the laboratory, giving rise to a model system to explore red algae – oomycete interaction (unpublished work).

As explained about the efforts to collect *Bangia* strains and *Olpidiopsis* isolates and I mentioned to potential negative effect of *Olpidiopsis* on red algal populations in Europe and Scotland, however, our knowledge about phylogenetic relationship of these '*Bangia'* strains is still limited. Moreover, there are limited studies investigating the interaction between red algae and oomycetes pathogens (Badis, Klochkova, Brakel, et al. 2019). Therefore, the objectives of our study are:

1- Determine the resistance of 11 red algae ('*Bangia'*) strains collected from France and Scotland to the newly described oomycete isolate *Olpidiopsis porphyrae* var. *scotiae*.

2- Develop a novel qPCR assay, in addition to microscopic assays using inverted microscope and fluorescent microscope to quantify oomycete *Olpidiopsis porphyrae* infection in '*Bangia'* strains.
3- Determine the phylogenetic position of 20 '*Bangia*' strains available at SAMS and compare the position of these strains with global *Bangia* strains around the world.

2 Materials and Methods

2.1 Biological material

20 red algae *Bangia* strains were obtained from colleagues at SAMS and Culture Collection of Algae and Protozoa (CCAP). The name of strains, collection sites and isolators are shown in the Table 3. All algal strains were clonal isolates, and they were in the gametophytic life stage when I applied my experiments. The inoculation experiments were realised with algal archespores and young unicellular filaments. Moreover, one *Olpidiopsis* isolate (*Olpidiopsis porphyrae* var. *scotiae*)(Badis et al. 2018) maintained on MFB5 *Bangia* strain was obtained from the same laboratory, as Olpidiopsis is an obligate biotrophic pathogen and it needs to be maintained on the host culture. Algal material was grown inside 650 mL suspension culture flasks (Greiner bio-one) containing half strength modified Provasoli's medium in filtered seawater (West and McBride 1999). Flasks were placed inside 10° C culture room, under 12 hours light regime, 1054 Lux. To keep algal filaments vital the medium was refreshed every 15 days.

Table 3. Information about Bangia strains obtained from microbiology lab at SAMS

Original strain name	Sampling location	Sampling date	Isolator	Phylogenetic study	Pathogenicity and qPCR experiment
BOP 1-A	Oban Pier, Scotland, UK	2018	Dieter Müller	+	-
BOP 1-B	Oban Pier, Scotland, UK	2018	Dieter Müller	+	-
BOP 2-F	Oban Pier, Scotland, UK	2018	Dieter Müller	+	-
BOP 3-D	Oban, Scotland, UK	2018	Dieter Müller	+	-
BMD 3-2	Oban, Scotland, UK	2018	Dieter Müller	+	-
BMD 3-19	Oban, Scotland, UK	2018	Dieter Müller	+	-
BG 2-10	Ganavan, Scotland, UK	2018	Dieter Müller	+	+
BG 2018 YB-D1	Ganavan, Scotland, UK	2018	Yacine Badis	+	+
BSBR 2018 YB-C1	Dunstaffnage, Scotland, UK	2018	Yacine Badis	+	+
Bangia Easdale A	Easdale, Scotland, UK	2018	Yacine Badis	+	+
Bangia Easdale B	Easdale, Scotland, UK	2018	Yacine Badis	+	+
FR1		2018	Yacine Badis	+	-
BAA 2018-D	An Amied, Brittany, France	2018	Yacine Badis	+	+
MFB4	Quiberon,Brittany, France	2018	Yacine Badis	+	+
MFB5	Quiberon, Brittany, France	2018	Yacine Badis	+	+
Bper	Peraridhy, Brittany, France	2019	Yacine Badis	+	-
Btrev	Trevigon, Brittany, France	2019	Yacine Badis	+	+
BAA 2019 A	An Amied, Brittany, France	2019	Yacine Badis	+	+
CCAP 1361/3	Gourock, Scotland, UK	2012	Campbell	+	+
CCAP 1361/2	S. Bass Island, Lake Erie, Ohio, USA	1973	Ott	+	-

Olpidiopsis isolate was maintained by transferring infected filaments to small petri dish containing healthy MFB5 *Bangia* filaments every ten days in the same medium mentioned above and incubated on the same culture room.

2.2 Microscopy test for 'Bangia' strain susceptibility to *Olpidiopsis porphyrae* var. *scotiae*.

The objective of the experiment was to assess levels of resistance of 11 strains of filamentous Bangiales ('*Bangia*' sensu lato) against one *Olpidiopsis* isolate by using an inoculation technique described by (Strittmatter et al. 2016) with some modification.

The experiment was realised in 14.5 cm (Greiner bio-one) plastic petri dishes with a 40 µM falcon cell strainer containing infective material (*Olpidiopsis porphyrae* var. *scotiae*) in MFB5 host strain) being placed centrally and glass coverslips (24x50mm dimensions) with attached *Bangia* spores surrounding these. Each petri dish provided a space for 5 glass coverslips. 11 *Bangia* strains were selected based on the availability of sporulating material (Table 3). Out of 21 Bangia strains I could induce sporulation for 11 strains by incubating them inside shaking incubator (New Brunswick™ Innova® 44/44R - Stackable Incubator Shaker) with the following conditions: 12:12 hours light-dark circle with 15˚C at light, 10˚C at dark with continuous shaking at 40 rpm, 5707 Lux.

The experimental design was an incomplete block design, each Petri dish contained five *Bangia* strains on five coverslips that are attached to the bottom of Petri dish by using melted paraffin. The five *Bangia* strains included two reference strains that were present in each Petri dish. I chose EASDALE B and MFB5 as reference strains, as these released the highest numbers of spores during primary trials. The experiment was run with five replications. Each replication consisted out of three Petri dishes (3 x 3 strains + 2 reference strains = 11 strains), thus in total 15 petri dishes were prepared. In addition, as a control to the five inoculated

replicates - one set of 3 Petri dishes containing all '*Bangia'* strains was not inoculated and serve as an inoculation control.

Coverslips were attached to the bottom of Petri dishes by using 100 µl of melted paraffin on one side of the coverslip, 30 seconds later the paraffin was solidified, and coverslips were fixed very well to the petri dish. Moreover, the 40 µm falcon cell strainer was attached to the bottom of each Petri dish by using 200 µl of melted paraffin, the cell strainers were placed exactly in the middle of each Petri dish in equal distance to all coverslips Figure 3.

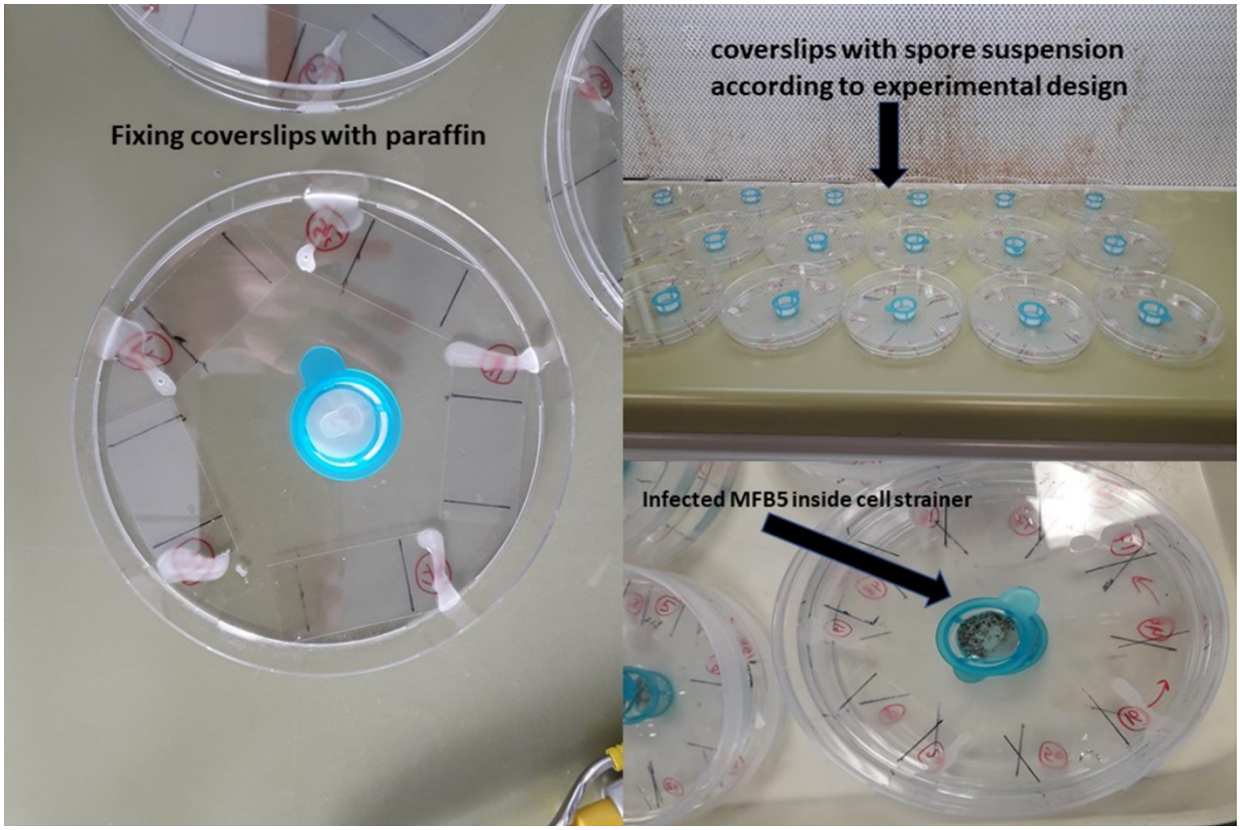

Figure 3. Microscopy experimental set up, to left attaching the coverslips and cell strainer to the bottom of Petri dish, the upper right distribution of algal spore suspension according to experimental design for each Petri dish and the lower right is inoculation with infected MFB5 strain that was placed inside the cell strainer.

Spore suspension was prepared from each *Bangia* strain by harvesting the maximum amount of biomass from the 650 mL culture flasks using 70 µm falcon cell strainer and placing this algal material inside 2 ml Eppendorf tube, vortex the tube for one minute and obtain the liquid from the tube by using another 70 µm cell strainer. The liquid was transferred to another 2 mL Eppendorf tube and centrifuged for one minute on maximum speed so that the spores could be obtained from the bottom of the tube. 600 µl from the bottom of the tube was transferred very carefully to the coverslip inside petri dish according to experimental design. The spore suspension was left 48 hours incubating on the coverslips giving spores time to settle and attach, a 40 ml of half strength modified Provasoli's enriched medium was added to each Petri dish, and approximately equal amount of ten days inoculated (MFB5 strain)

'*Bangia*' filaments was placed in the middle of the 40 µm cell strainer as a source of *Olpidiopsis* spores for infection Figure 3.

For control treatment the cell strainers contained healthy MFB5 '*Bangia'* filaments. The Petri dishes were agitated gently four times during daytime to insure equal distribution of zoospores in the Petri dishes.

Starting from third day after inoculation petri dishes were checked under the inverted microscope (Zeiss ID03 Inverted Phase Contrast Microscope) by using 10 X magnification in a daily basis to record incubation period and latent period. I defined incubation period as number of days from inoculation until the observation of the first visible signs of *Olpidiopsis* infection. These looked like some cells (one or more) of the *Bangia* filament were discoloured and the algal cell seemed empty from inside or contained different cell content comparing with the healthy cells in the filament. I defined latent period as number of days from inoculation until the observation of first mature *Olpidiopsis* sporangia.

Sometimes exit tubes were visible, but partly these were difficult to identify in the Petri dish. Images of signs of infection that were classified as incubation and latent periods are shown in Figure 4.

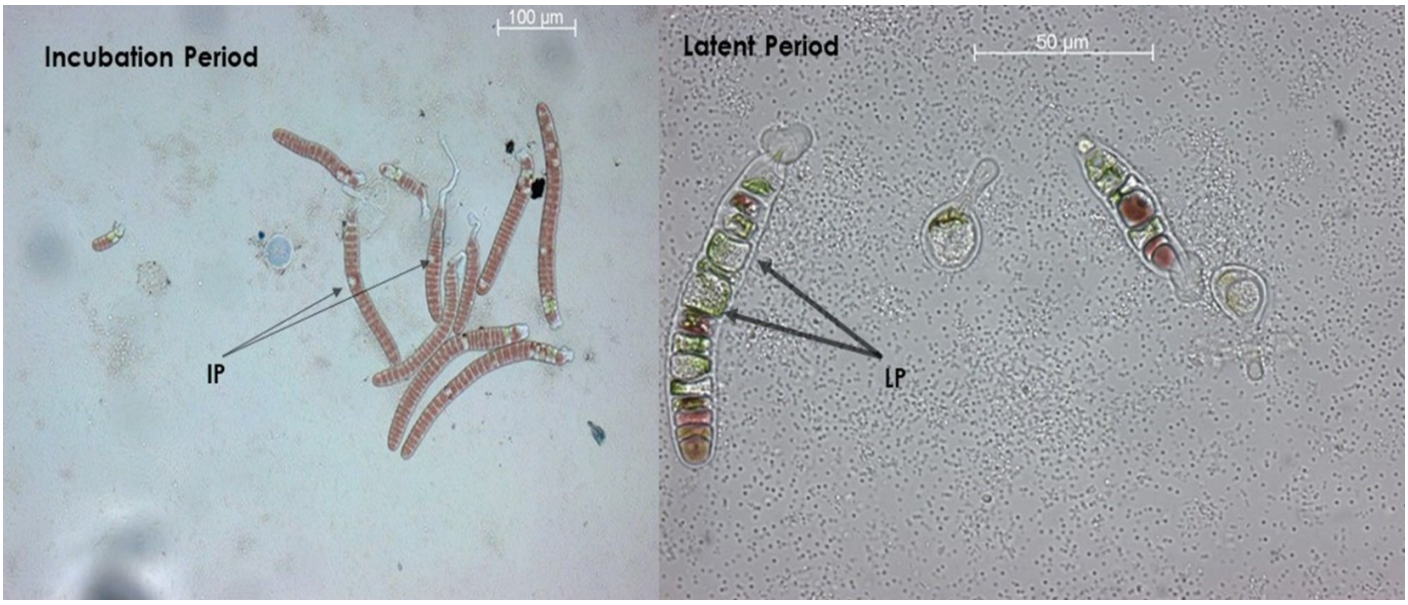

Figure 4.Incubation Period (IP) the number of days from inoculation until first symptom is clear on Bangia filament, and Latent period (LP) the number of days between inoculation and the formation of mature Olpidiopsis sporangia and exit tubes. on Bangia infected filaments after inoculation with Olpidiopsis.

After 15 days of inoculation, the experiment was stopped by fixing biological material with RNAlater. The medium over all coverslips was removed gently by using 50 ml electric pipet, 30 ml of RNA later was added carefully to each Petri dish by using electric pipet and the petri dishes were incubated for five minutes. After five minutes RNAlater. was removed, and the coverslips were washed once by sterilized filtered seawater. Seawater was removed and the coverslips were incubated in 70% ethanol for five minutes to remove the pigments from algal filaments. Ethanol was subsequently removed, and coverslips were washed once again with sterilized filtered seawater. The coverslips were cut off from paraffin attachment on the Petri dishes using diamond pen and were stained by 1% Calcofluor White staining (Fluka analitical) in seawater by placing one drop of Slow Fade® (Life technologies) and one drop of calcofluor white dye on microscope slide and inverting the coverslips carefully over microscope slide.

The stained algal material on coverslips was inspected with fluorescence microscopy to quantify the infection. Infected and not infected cells were observed and counted under the fluorescence microscope (zeiss axioskop 2 plus microscope) with the DAPI block filter (350 nm) the UV light was obtained from Cool-Led PE-300 fluorescent light unit. To count

infected and uninfected cells after 48 hours of staining (Figure 5). I selected randomly three fields under microscope by using 20 x lens, 35% UV light with DAPI filter, and counted 50 cells from each field, determining the average of infected cells as percentage of the overall counted cells. Images were recorder by using the camera (Zeizz, Axio Cam HRC) with Axiovision Rel 4.7.1 software.

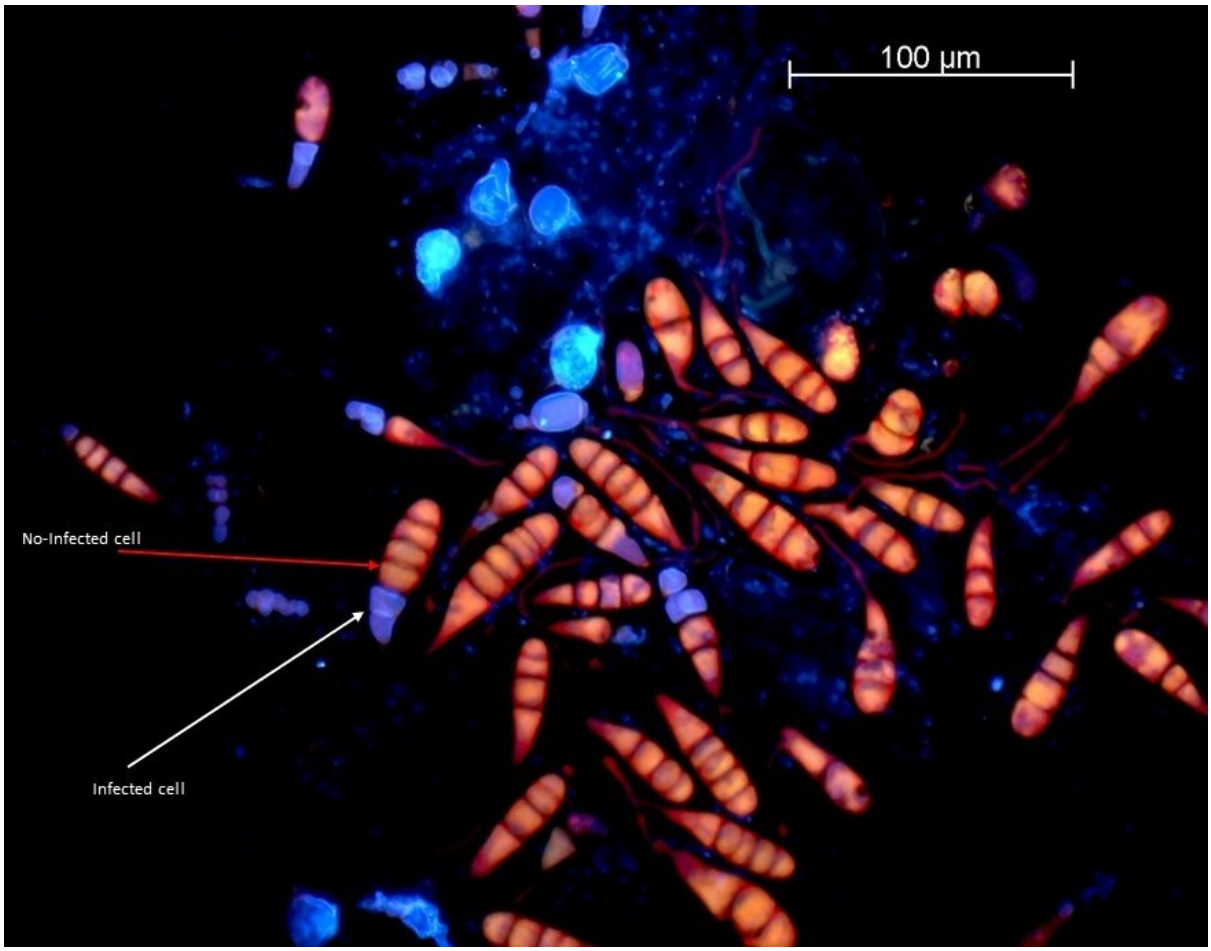

Figure 5.Bangia cells under florescent microscope after staining with calcofluor white, infected cells are blue, uninfected cells are red. The percentage was calculated as the average for three microscopic fields each field was calculated as number of infected cells out of total number as percentage.

2.3 qPCR experiment and DNA extraction

To test the susceptibility of *Bangia* strains for *Olpidiopsis porphyrae* var. *scotiae* isolate using qPCR technique a similar experiment was performed as described above. It had the same experimental design but differed in the age of *Bangia* algal strains at the time of inoculation. In this experiment, inoculation by the *Olpidiopsis* isolate was realised 45 days after the preparation of *Bangia* spores on the coverslips. This timing was to ensure a growth of enough biomass for DNA extraction, so after 45 days the *Bangia* spores were grown to form 3-4 cm long unicellular filaments.

The experiment was run and inoculated as described above and after 15 days of inoculation the trial was fixed by using RNAlater. as described and DNA was extracted from all of coverslips after fixing the experiment as follow: Under sterile conditions each one of the coverslips was detached from Petri dishes by using a diamond pen, each coverslip was transferred to clean sterile 9cm diameter plastic Petri dish and was carefully smashed to small pieces. *Bangia* biomass and glass pieces of each sample were transferred to 2mL safe lock Eppendorf tube containing sterilized small metal beads (4.5mm). Samples were then freeze dried for 48 hours by using freeze dryer (Christ Alpha 1-2 LD plus), with the Eppendorf tubes covered with parafilm containing small holes to allow water to escape during freeze drying process. Similarly, the biomass from falcon cell strainers was harvested and treated in the same way.

For the DNA extraction, freeze dried samples were ground with a Qiagen tissue lyser (30 round per second/2 minutes). DNA was extracted by using a CTAB DNA extraction with the following specifications:

700 µl of preheated CTAB (60˚C) buffer was added to each Eppendorf tube in addition 2.5µl of proteinase K solution (Promega,20mg/ml). The tubes were incubated for 30 minutes in

60˚C and were shaken regularly. A 700 µl of Phenol:CHCl3:Isoamyl alcohol (25:24:1, pH=8) was added to each tube and the tubes were shaken gently by inverting 30-40 times and were then centrifuged for five minutes at the maximum speed (1600 RCF), the supernatant was transferred to 2ml tube and 700 µl of CHCl3:Isoamyl alcohol (24:1) was added to each tube, the tubes were shaken gently by inverting and centrifuged again for five minutes at maximum speed.

The obtained supernatant was transferred into 1.5ml tube and one volume of ice-cold isopropanol (around 600 µl) was added to each tube. The tubes contents were mixed gently by inverting and were incubated for one hour in dark under room temperature.

The tubes were centrifuged after that for 30 minutes and the supernatant was removed carefully to avoid disturbing the white pellet.

The pellet was washed by adding 250 µl ice-cold 70% molecular grade ethanol and turning the tubes to wash the pellet from all sides. Finally, the tubes were centrifuged for 5 minutes at maximum speed and the supernatant was removed carefully, the pellets were allowed to be air dried and 100 µl molecular grade water was added to each tube. The tubes were incubated overnight in the fridge at 4˚C, and the DNA concentration was measured by nanophotometer device (IMPLEN P300 Nanophotometer). Subsequently, the concentration of the DNA was adjusted to a concentration of 1ng/µl by dilution with molecular grade ultra-pure water.

2.4 Testing *Olpidiopsis* and *Bangia* qPCR primers (Olp F3/R3, Bang F9/R9) to quantify *Olpidiopsis*

Two sets of primers were designed each one set to amplify *Olpidiopsis* DNA and other set to amplify *Bangia* DNA. *Bangia* DNA was used as reference as *Olpidiopsis* is always grown on *Bangia*. Primer3plus software was used to design the primers from SSU sequence data that was issued by the team for *Bangia* strains and SSU sequences for *Olpidiopsis porphyrae* var.

scotiae that is available on GenBank (accession number KY403506) (Badis, Klochkova, Strittmatter, et al. 2019). For the primer design, primer conditions in Primer3 plus were set to primer length 20-25 nts, TM 58-60˚C, and amplification length 150-200bp.

Selected primers were tested against available sequences on NCBI nucleotide database by the primer blast algorithm to discard primers that matched nontargeted organisms.

Three sets of primer pairs were designed and tested to amplify *Olpidiopsis* isolates, in addition nine sets of primer pairs were tested to amplify *Bangia* strains. Based on efficiency and R^2 values one pair of primers was selected for *Olpidiopsis* (Olp F3/R3) and one pair was selected for *Baniga* (Ban F9/R9) as shown in the Table 4.

Table 4. qPCR primers that were designed to amplify Olpidiopsis isolate and Bangia strains.

Primer	**Sequence (5'->3')**	**Length (bp)**	**TM**	**CG%**	**self complementarity***	**Self 3' complementarity***	**product size**	**non targeted organisms****
Olp F3	AGGCGAGACTACCTGGAACA	20	60.25	55	3	1		No match
Olp R3	ACGTCCCTTAAGCCTCGTTT	20	59.32	50	6	1	155 bp	No match
Ban F9	GTTGGGGGCATTCGTATTTCA	21	58.9	47.62	4	1		Dominantly matching red algae (diverse species of Bangiales), no hit with *Olpidiopsis*
Ban R9	CCAGTCGGCATCGTTTATGG	20	59.1	55	3	0	196bp	Dominantly matching red algae (diverse species of Bangiales), no hit with *Olpidiopsis*

* Unitless score, 0 indicates no reasonable local alignment between two oligos, for more details see https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plusHelp.cgi.

** primers were tested against NCBI nr database (https://www.ncbi.nlm.nih.gov/tools/primer-blast).

To validate developed qPCR primers these were tested against a dilution series of DNA extracted from MFB5 *Bangia* strain that was inoculated with *Olpidiopsis porphyrae* var. *scotiae* isolate.

The DNA was diluted in six dilutions steps the first dilution was undiluted DNA from the extraction with series of dilutions as follow 0.33, 0.11, 0.03, 0.01 and 0.004.

A concentration of 1ng/µl DNA obtained from healthy MFB5 *Bangia* strain was included as control to test whether pathogen`s qPCR primers are amplifying the host`s DNA.

For each pair of primer, the qPCR plate contained triplicates from each dilution in addition to triplicates from MFB5 *Bangia* strain and negative control (molecular grade water).

qPCR mix final volume was 10 µl contained 2.5 µl of DNA template and was prepared by using SYBR Green qPCR kit (Fast Start Essential DNA Green Master Roche) as shown in Table 5 table. The thermocycle for qPCR reaction is shown in Table 6.

After validation of primers each DNA sample (1ng/ µl) from the inoculation experiment was run in triplicate once with *Bangia* primers and another time with *Olpidiopsis* primers. Both primers for each DNA sample were run on the same plate. qPCR assay was run by using Roche qPCR light cycler machine (LightCycler 96), and the obtained data was exported from the machine to the desktop software (LightCycler 96) for analysis. The data was analysed by software by Rel quantification analysis, the efficiency and maximum Cq values were1.97, 31.49 and 2.03, 27.00 for *Bangia* and *Olpidiopsis* respectively. In the software Bangia strains were considered as reference to calculate the ratio between the target DNA (*Olpidiopsis*) and reference DNA (*Bangia*) and express the final result as a ratio of these two DNA. The formula for ratio was according to (Pfaffl 2001) with slight modification to suite the target of our experiment and it was calculated follow:

$$Normalized\ Pfaffl\ ratio = \frac{\mathrm{EOlpi}^{\Delta \mathrm{Cq\ olp}}}{(\mathrm{EBang}^{\Delta \mathrm{Cq\ Bang}}) + (\mathrm{EOlpi}^{\Delta \mathrm{Cq\ olp}})}$$

where EOlpi is the *Olpidiopsis* primers efficiency (2.03), and EBang is the *Bangia* primers efficiency (1.93).

Table 5. qPCR mix for final volume of 10 µl.

Reagent	**Amount**	**Final concentrastion**
Water, PCR Grade	1.7 µl	
Forward qPCR primer (10 µM)	0.4 µl	2.5 µM
Reverse qPCR primer (10 µM)	0.4 µl	2.5 µM
Master Mix, (FastStart Essential DNA Green Master). (Roche)	5 µl	
Total Volume	7.5 µl	

Table 6.Thermocycle for qPCR reactions.

	Temp. °C	Ramp (°C/s)	Duration (s)	Acquisition Mode
Pre-incubation	95	4.4	600(2)	None
3 Steps Amplification	No of Cycles 45			
	95	4.4	10(1x3)	None
	60	2.2	10(1x3)	None
	72	4.4	10(1x3x6)	Single
Melting	95	4.4	10	None
	65	2.2	60	None
	97	0.1	1	5 Readings/°C

2.5 Phylogenetic analysis of Bangia strains

The objective of this part of the study was to determine the phylogenetic positions of 20 '*Bangia'* strains tested in this study collected from France and Scotland and compare them with available data for '*Bangia'* around the world.

DNA was extracted from the strains as described above with slight modification. We used here liquid nitrogen to manually grind biomass with mortar and pestle. DNA was extracted from 11 strains the strains are BAA 2018-D, BG 2018 YB-D1, BG2-10, BMD3-19, BMD3-4, BOP1-A, BOP1-B, BOP2-F, BOP3-D, BSBR 2018 YB, Btrev and EASDALE A. rbcL gene (Ribolose-1,5-biphospate carboxylase-oxygenase large chain) was amplified for the strains BG2-10, BMD3-19, BMD3-4, and BOP1-B by PCR by using one rbcL primer pair rbcL F57 (forward: GTAATTCCATATGCTAAAATGGG) / rbcL rev-new (reverse: ACATTTGCTGTTGGAGTYTC) (Saunders and Moore 2013). The final PCR volume was 15 µl and the PCR mix was prepared and PCR thermocycle was adjusted as shown in Table 7 and Table 8 respectively.

Additionally, the SSU gene was amplified for 9 Bangia strains (BAA 2019A, BAA 2018D, BG 2018 YB-D1, BG2-10, BSBR 2018 YB, Btrev, CCAP 1361/2, EASDALE A, and EASDALE B) by using SSU primer pair G01 (forward 5′-CACCTGGTTGATCCTGCCAG–3′)/ G07 (reverse 5′-AGCTTGATCCTTCTGCAGGTTCACCTAC–3′) (Saunders and Moore 2013), the final PCR volume was 15 µl, the PCR master mix and thermocycle are shown in Table 9 and Table 10 respectively.

Moreover, the *cox*1(cytochrome oxidase 1) gene was amplified for 10 *Bangia* strains (BOP1-B, BOP3-D, BMD3-19, BG2-10, BOP1-A, BOP2-F, BSBR 2018 YB, EASDALE A, Btrev and BAA 2018-D) by using *cox*1 primer pair *cox*1 GAZ F1 (forward 5′-

TCAACAATCATAAAGATATTGG–3′) / *cox*1GAZ R1 (reverse 5′-ACTTCTGGATGTCCAAAAAAYCA-3′)(Saunders 2005), the final PCR volume was 15 µl and the PCR master mix The PCR thermocycle and master mix are shown in Table 11 and Table 12 respectively. PCR products for all primers were checked by gel electrophoresis (50 minutes,120 volts) on 1.5% agarose gel in 1X TBE buffer (sample volumes in the gel 5 µl PCR product mixed with 1µ loading dye (Thermo Scientific 6x DNA loading dye that was mixed with Promega Diamond Nucleic Acid dye 1µ/830µl), and one well contained 2 µl of 100 bp Gene ruler (Thermo Scientific Gene Ruler 100bp), electrophoresis products were pictured on ultraviolet radiation.

Table 7.PCR mix for 15 µl total volume for the strains that amplified by rbcL primers.

Reagent	**Amount**	**Final concentration**
Qiagen Taq master mix	7.5 µL	
Primer forward (5 µM)	1.2 µL	0.4 µM
Primer reverse (5 µM)	1.2 µL	0.4 µM
H_2O	3.6 µL	
DNA (either 1:10 or 1:100 dil)	1.5 µL	
Final volume	**15 µL**	

Table 8. rbcL primers PCR thermocycle

	PCR parameters		
DNA markers	*rbc*L		
PCR steps	Temp °C	Time	Cycles
Pre-denaturation	94	2 min	
Denaturation	94	1 min	30
Annealing	47	1 min	
Elongation	72	2 min	
Final Elongation	72	10 min	

Table 9.PCR mix for 15 µl total volume for the strains that amplified by SSU primers.

Reagent	**Amount**	**Final concentration**
Qiagen Taq master mix	7.5 µL	
Primer forward (5 µM)	1.2 µL	0.4 µM
Primer reverse (5 µM)	1.2 µL	0.4 µM
H_2O	3.6 µL	
LongAmp Taq polymerase	0.05 µl	
DNA (either 1:10 or 1:100 dil)	1.5 µL	
Final volume	**15 µL**	

Table 10. SSU primers PCR thermocycle

	PCR parameters		
DNA markers	SSU		
PCR steps	Temp °C	Time	Cycles
Pre-denaturation	94	2 min	
Denaturation	94	1 min	35
Annealing	55	1 min	
Elongation	72	2 min	
Final Elongation	72	10 min	

The PCR products were cleaned by ExoSAP-IT (Applied biosystems by Thermo Fisher scientific) clean up kit and were sent for Sanger sequencing to LGC genomics.

The sequences were received and the chromatograms were trimmed by using Geneious software (R11) and aligned with sequencing data available at SAMS for the remaining 16 strains available at SAMS and all of these sequences were aligned with the 157 taxa sequences described by (Sutherland et al. 2011) by using Geneious software (global alignment with free end gaps 65% similarity). Finally, the phylogenetic analysis was realized with the software MegaX (v10.2.5) using Maximum likelihood tree method with 1000 bootstraps replication. The optimal substitution type model was determined resulting in General Time Reversable model, and Gamma Distributed with Invariant Site (G+I) (Kumar et al. 2018).

Table 11.PCR mix for 15 µl total volume for the strains that amplified by cox1 primers.

Reagent	**Amount**	**Final concentration**
PCR Taq master mix (Qiagen) (1.5 mM MgCl2)	7.5 µL	
$MgCl_2$ solution (25 mM)	0.3 µL	2.0 mM
Primer forward (5 µM)	1.2 µL	0.4 µM
Primer reverse (5 µM)	1.2 µL	0.4 µM
H_2O	3.6 µL	
LongAmp Taq polymerase	0.05 µL	
DNA (either 1:10 or 1:100 dil)	1.5 µL	
Final volume	**15 µL**	

Table 12. cox*1 primers PCR thermocycle*

	PCR parameters		
DNA markers	*Cox1*		
PCR steps	Temp °C	Time	Cycles
Pre-denaturation	94	2 min	
Denaturation	94	30 sec	35
Annealing	50	30 sec	
Elongation	72	1 min	
Post-elongation	72	10 min	

2.6 Statistical analysis and data visualisation

Statistical analysis for the data was performed by using R software (RStudio, version 1.4.1717). To analyse the effect of *Bangia* strains on incubation period, latent period, percentage of infection and normalized Pfaffl ratio I used linear mixed-effect model (package `lme4`, lmer command) (Bates et al. 2015), the models were tested against the null-hypothesis model to find the significant level for the effect of *Bangia* strain identity, Model was validated visually by inspecting distribution of residuals against fitted values and response variable, as overall data distribution of residuals.

The Tukey post-hoc test was applied when there was significant effect for *Bangia* strain identity to pairwise compare *Bangia* strains to find significant differences in response variable (package 'emmeans') (Lenth 2021).

3 Results

3.1 Results of microscopy test for '*Bangia*' strain susceptibility to *Olpidiopsis porphyrae* var. *scotiae*.

All *Bangia* strains were infected by the *Olpidiopsis porphyrae* var. *scotiae* isolate and the symptoms were clearly visible under the inverted microscope.

One *Bangia* strain (EASDALE A) was excluded from microscopy results and from statistical analysis as it produced very few spores (one spore for each coverslip) that were infected in the beginning but lost during staining process.

Incubation period, which is the number of days from inoculation until the observation of the first *Olpidiopsis* infection symptom ranged between five and 12 days and the linear mixed-effect model showed that *Bangia* strain identity had a significant effect on the incubation

(p=0.01, df=49.9, t-ratio=3.751) Figure 6.

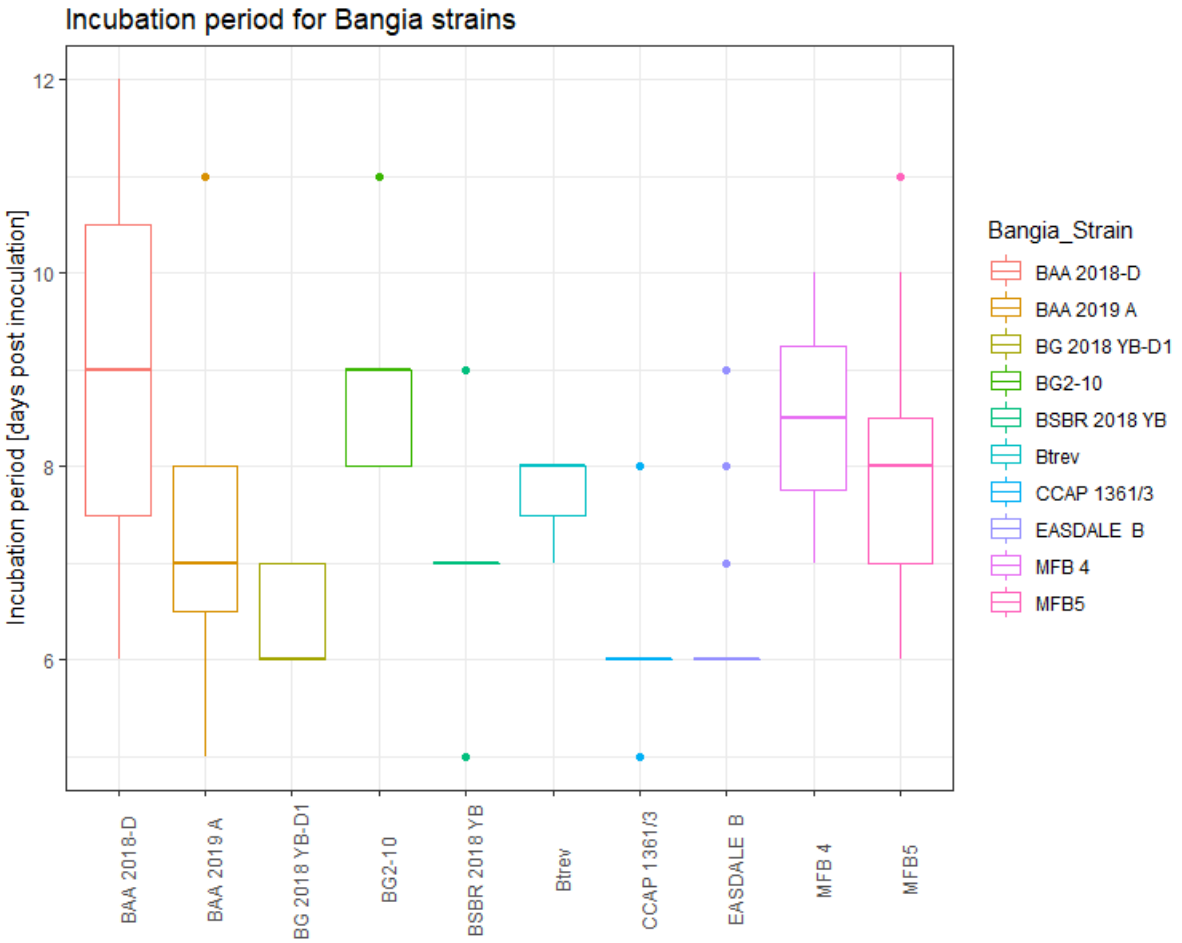

Figure 6. Incubation period for ten Bangia strains. inoculated with Olpidiopsis porphyrae var. scotiae as daily inspection under inverted microscope. Data was collected as daily basis three days after inoculation to monitor the number of days between inoculation and observation of first symptom.

Latent period ranged between 8 days 15 days. The linear mixed-effect model showed no significant effect of *Bangia* strain identity on the latent period (p=0.3, df=9, chisq= 10.588) (Figure 7).

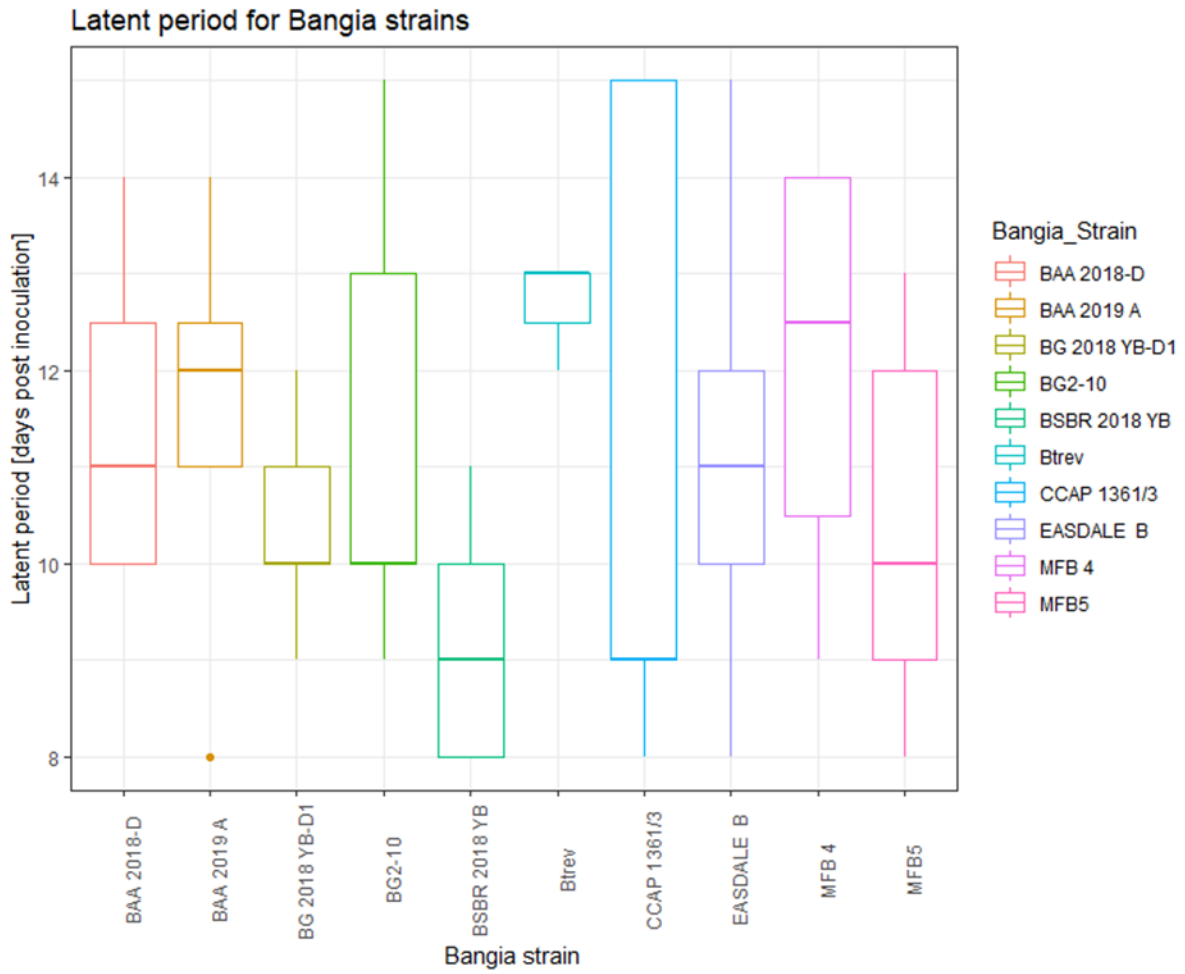

Figure 7. Latent period for ten Bangia strains. inoculated with Olpidiopsis porphyrae var. scotiae as daily inspection under inverted microscope. Data was collected as daily basis three days after inoculation to monitor the number of days between inoculation and the formation of sporangia, exit tubes and zoospores.

The linear mixed-effect model showed that the effect of *Bangia* strain identity on the percentage of infection detected through calcofluor white staining after 16 days post inoculation was significant (p=0.001, df=9, chisq=27.003). The percentage of infection ranged between 2-100% infected samples on some coverslips Figure 8. The mean percentage of infection ranged between 7-50%, it was 7% for CCAP1361/3 strains and 50% for MFB5 strain. The pairwise comparison (Tukey post hoc test) between Bangia strains showed significant differences between MFB5 and EASDAL-B strains (P=0.004, df=34.3, t-ratio= -4.3265) and between CCAP1361/3 and MFB5 strains (P=0.02, df=39.6, t-ratio= -3.655).

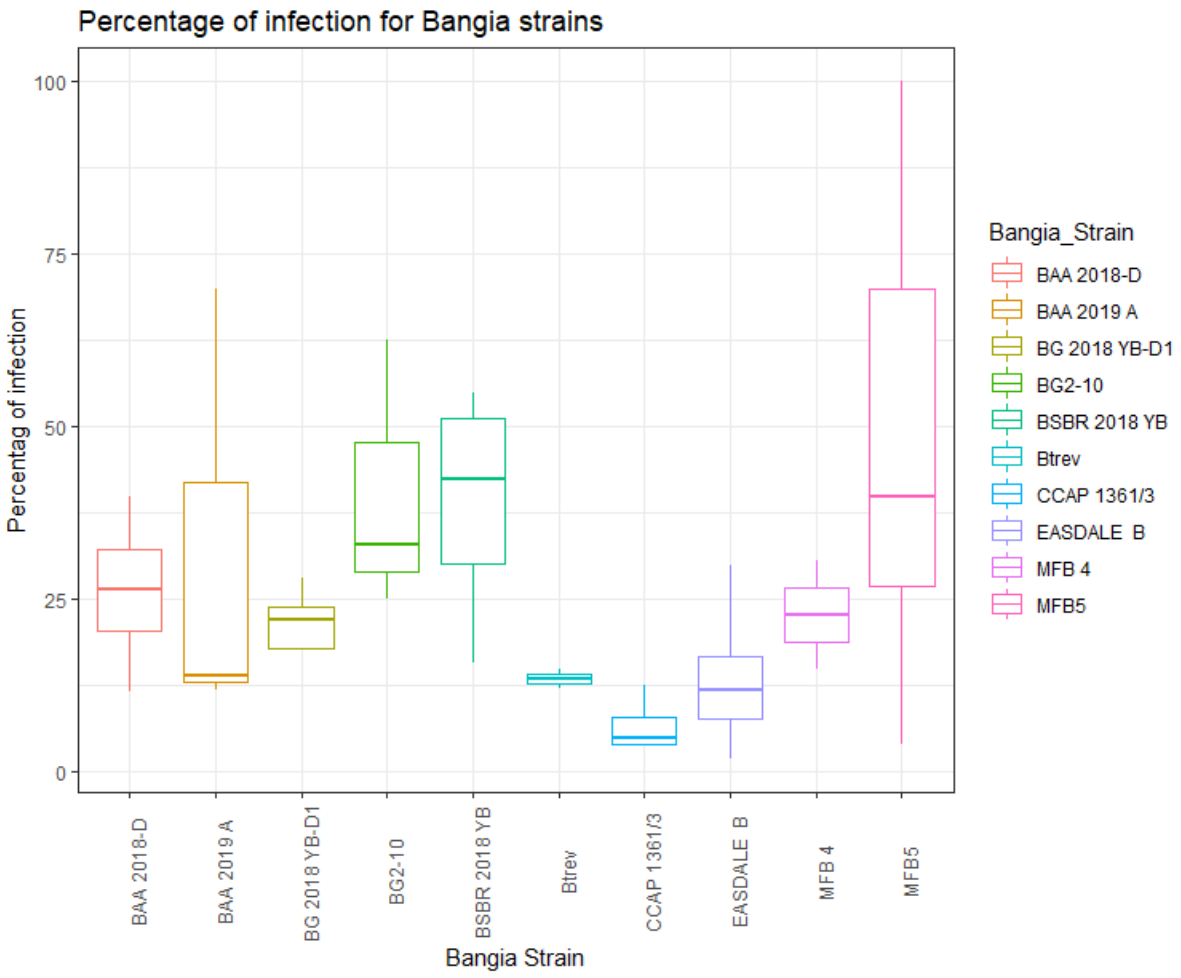

Figure 8. Percentage of infection for ten Bangia strains detected through Calcofluor white staining after 15 days post inoculation the percentage was calculated as the number of infected cells that coloured blue out of the total number of counted cells.

3.2 Validation of qPCR assay

The primer pair *Bangia*_F9/*Bangia*_R9 (Table 4 above in materials) amplified all *Bangia* strains. The efficiency and R^2 for this pair were 1.93 and 0.99 respectively. Efficiency and amplification were validated between the Cq values 21.24 to 31.49 Figure 9. The melting curves were similar between all tested *Bangia* strains Figure 10.

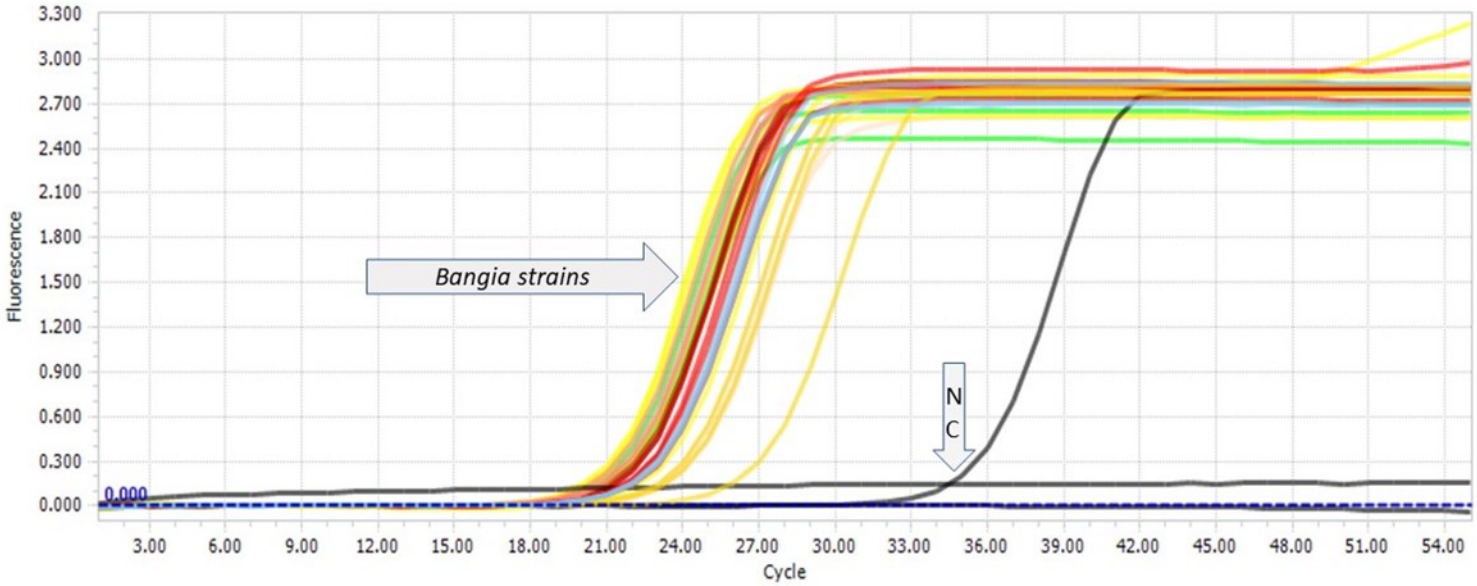

Figure 9. Amplification curves for 11 Bangia strains

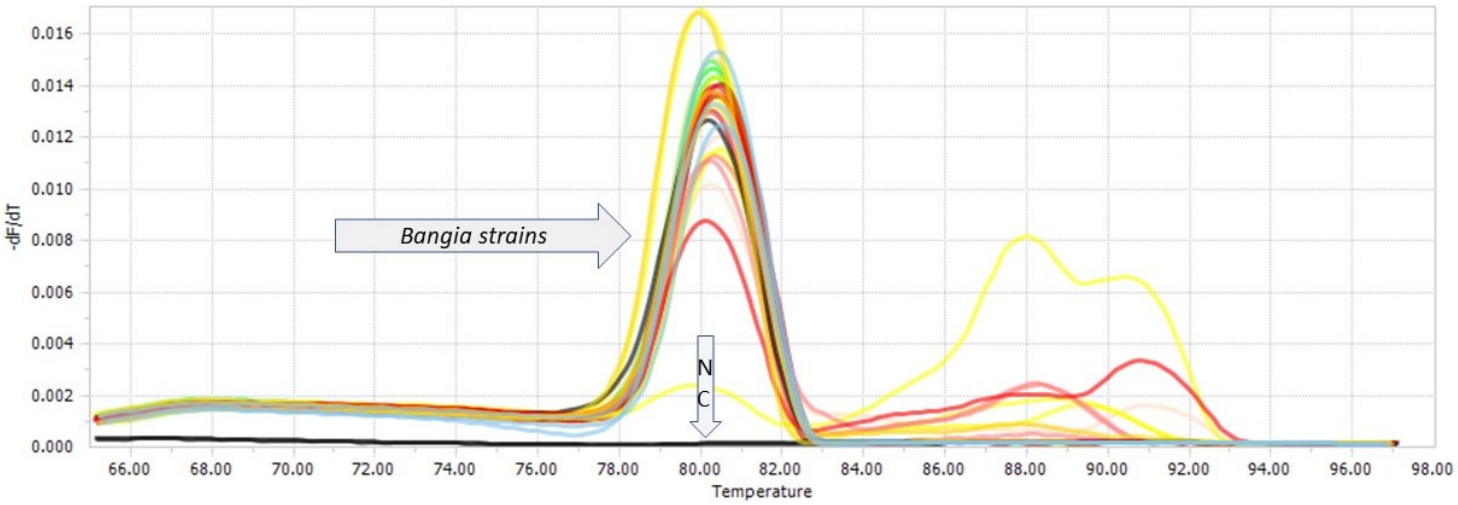

Figure 10. Melting peaks for 11 Bangia strains.

Another pair of primers (*Olpidiopsis*_F3/ *Olpidiopsis*_R3) (Table 4) amplified the *Olpidiopsis* isolate the efficiency and R^2 for this pair were 2.03 and 1 respectively. Efficiency and amplification were validated between the Cq values 18.68 to 27.00 (Figure 11).

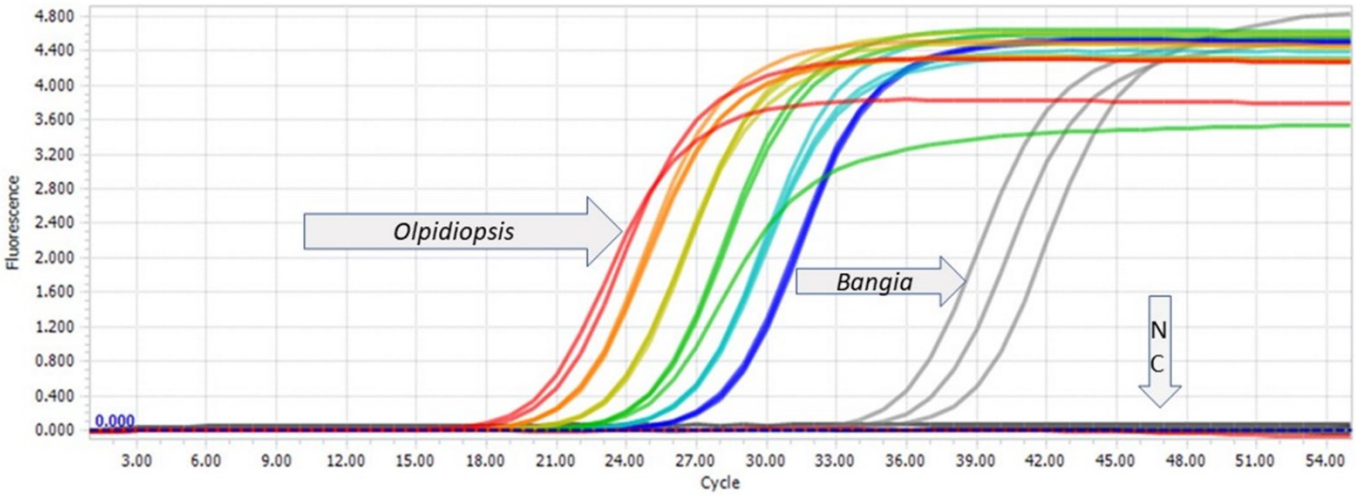

Figure 11. Amplification curves (triplicate) for Olpidiopsis isolate obtained from a dilution range of DNA from the extracted DNA without dilution (in red), dilution of 1/3 (in orange), dilution of 1/9 (in yellow), dilution of 1/27 (in green), dilution of 1/81 (in light blue), dilution of 1/243 (in dark blue). The grey curves represent the amplification of the Bangia DNA used as a control, to confirm that Olpidiopsis primers do not amplify Bangia DNA in the chosen Cq values (between 16.68 and 27.00). The black curves are the negative control (NC) to confirm that Olpidiopsis primers do not form primer dimers (only water with qPCR mix).

3.3 qPCR experiment results

The mean of normalized Pfaffl ratio ranged between 0.04-0.6 for different *Bangia* strains Table 14, Figure 12 , and the linear mixed-effect model analysis showed significant effect for *Bangia* strains on the normalized ratio ($P<0.01$, df=10, chisq=42.588). The pairwise comparison (Tukey post hoc test) showed the significant differences between the strains as shown in Table 13.

Table 13.the differences between Bangia strains according to normalized Pfaffl ratio.

Bangia strain	**P value**	**df**	**t-ratio**
(BAA2018-D) (MFB4)	0.01	33.8	-4.044
(BAA2018-D) (MFB5)	0.007	33.5	-4.199
(BG2018YB-D1) (MFB4)	0.03	35.4	-3.549
(BG2018YB-D1) (MFB5)	0.02	33.2	-3.796
(BSBR2018 YB) (MFB4)	0.009	34.4	-4.092
(BSBR2018 YB) (MFB5)	0.006	36.2	-4.203
(Btrev) (MFB4)	0.04	32.8	-3.497

Table 14. Mean Cq values for Bangia and Olpidiopsis and mean value for normalized Pffafl ratio

BangiaStrain	Mean Cq *Olpidiopsis*	sd* Cq *Olpidiopsis*	Mean Cq *Bangia*	sd* Cq *Bangia*	Mean Normalized Pffafl_Ratio	sd* Pffafl ratio
BAA2018-D	24.36	1.63	21.92	2.72	0.14	0.11
BAA2019A	25.01	1.01	22.91	3.92	0.27	0.35
BG2-10	23.22	2.79	23.40	2.93	0.44	0.34
BG2018YB-D1	25.25	0.38	23.19	2.61	0.09	0.01
BSBR2018YB	24.56	1.82	20.86	1.94	0.11	0.06
Btrev	24.65	1.20	24.07	3.22	0.22	0.17
CCAP1361/3	25.48	1.60	20.88	3.47	0.13	0.16
EASDALEA	26.35	0.86	25.29	0.76	0.40	0.16
EASDALEB	24.13	2.07	24.56	3.72	0.39	0.24
MFB4	22.32	3.45	23.28	2.55	0.68	0.34
MFB5	22.05	3.27	23.36	3.38	0.60	0.29

*sd: standard deviation.

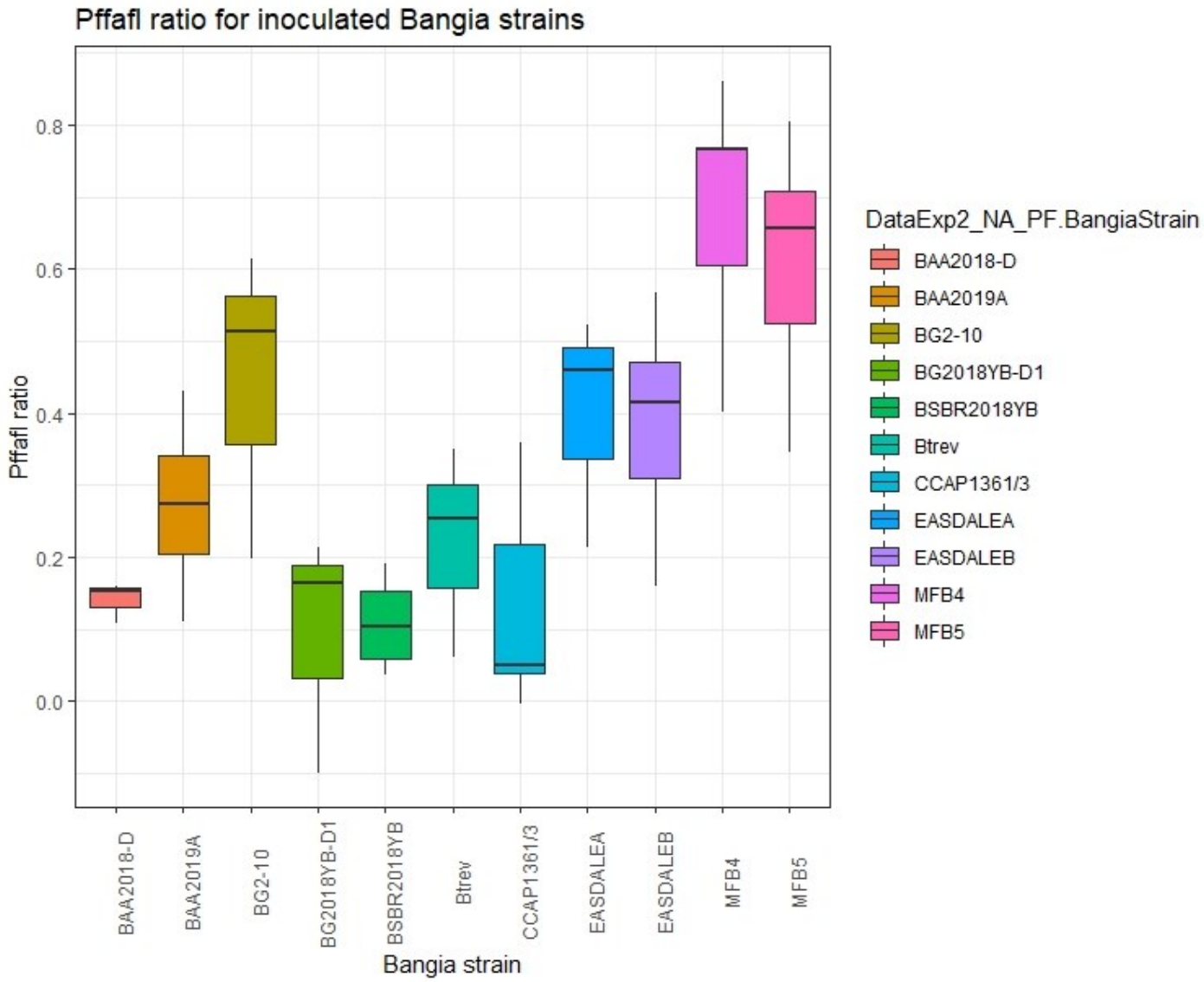

Figure 12. Normalized Pffafl ratio, detected through qPCR assay, for 11 Bangia strains inoculated with Olpidiopsis porphyrae var. scotiae (15 days post inoculation).

3.4 Phylogenetic study results

The obtained sequences from *cox*1 primers had a poor quality so they were not included in the genetic analysis, in addition most of sequences obtained from SSU had also a poor quality and not included in genetic analysis. The good SSU sequences were used to design the qPCR primers as explained above. The obtained sanger sequences are shown in supplementary file S1.

The genetic analysis of *rbc*L sequences for 20 '*Bangia*' strain that were combined with the *rbc*L sequences for Bangiales order obtained from (Sutherland et al. 2011) could discriminate the 20 '*Bangia*' strains into three clades.

Eight Bangia strains were found under '*Bangia*2' clade, and 11 strains were found under '*Bangia*3' clade whereas CCAP1361-2 which belong to freshwater '*Bangia*' was found under *Bangia* clade. Table 15, Figure 13.

Table 15.The 20 Bangia strains distribution between 'Bangia' clades 'according to rbcL sequences data.

'*Bangia*' strain	Clade (as defined by (Sutherland et al. 2011))
BMD3-2	'*Bangia*2'
CCAP1361-3	'*Bangia*2'
BOP-2F	'*Bangia*2'
BOP-1A	'*Bangia*2'
BMD3-19	'*Bangia*2'
BOP3-D	'*Bangia*2'
BOP-1B	'*Bangia*2'
EASDALE B	'*Bangia*2'
MFB4	'*Bangia*3'
MFB5	'*Bangia*3'

BG2 -10	'*Bangia*3'
BG2018 YB	'*Bangia*3'
BSBR2018	'*Bangia*3'
Easdale A	'*Bangia*3'
Btrev	'*Bangia*3'
BAA 2018D	'*Bangia*3'
BAA 2019A	'*Bangia*3'
BPER	'*Bangia*3'
FRI	'*Bangia*3'
CCAP1361-2 Fresh water	*Bangia*

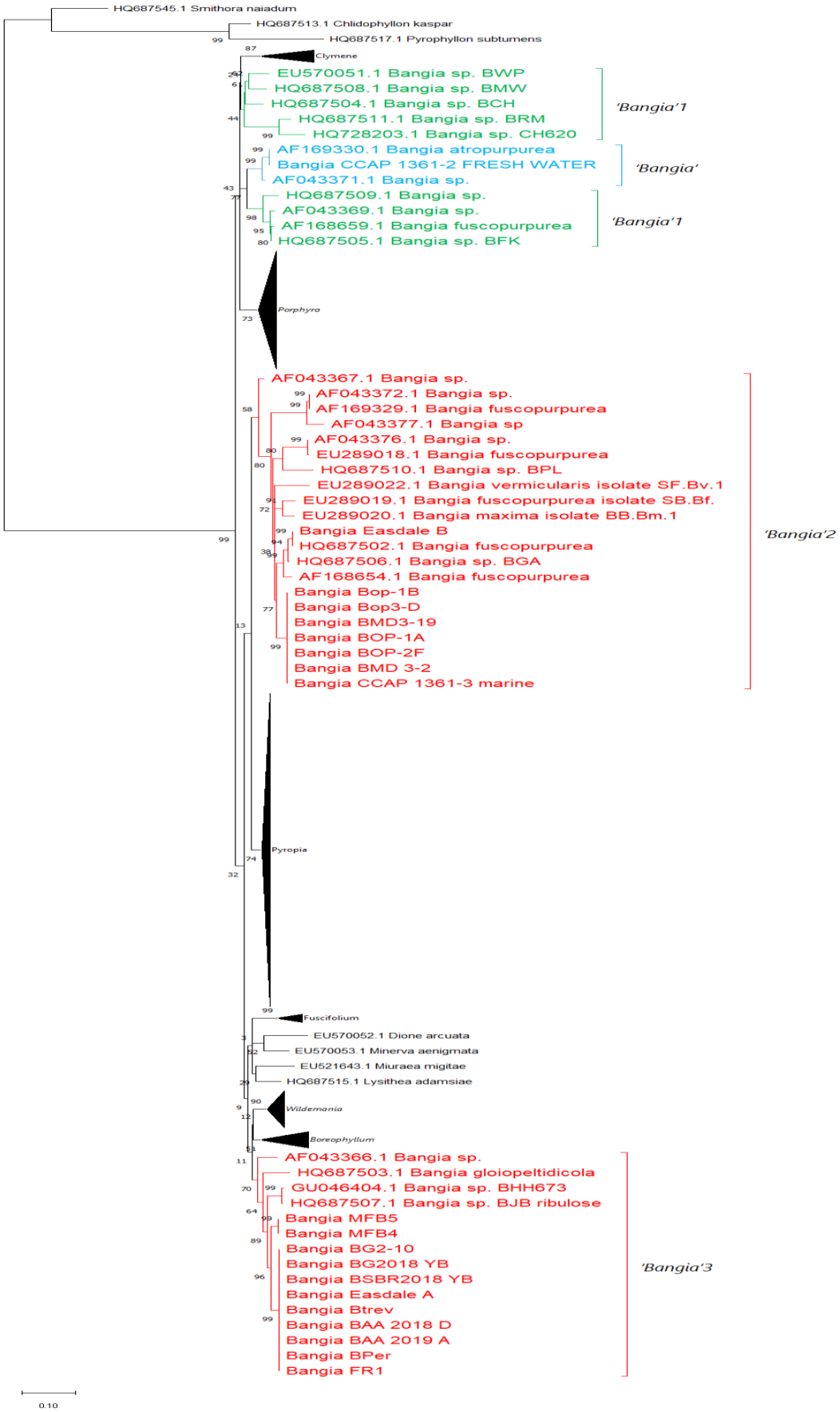

Figure 13. Maximum likelihood rbcL DNA tree (G+I) for 20 'Bangia' strains and comparison with general phylogeny obtained from (Sutherland et al. 2011) by using Mega X software bootstrap method, the 20 'Bangia' strains were distributed into three clades. In total179 sequences were used to generate this phylogenetic tree. Bootstrap values (1000 replicates) are shown at each node (%).

4 Discussion

This study aimed to determine levels of susceptibility of different '*Bangia*' strains against one isolate of *Olpidiopsis porphyrae* var. *scotiae* that is a variant from the species *Olpidiopsis porphyrae* which affects Nori production in Asia. We found significant differences between some '*Bangia'* strains for their susceptibility to this *Olpidiopsis* isolate. The linear mixed effect model showed significant effect for '*Bangia*' strains on both incubation period, percentage of infection and relative DNA amount of the pathogen detected by qPCR.

The results between the two experiments with different methodologies to determine pathogen load (microscopy and qPCR) showed differences in the reaction for some specific algal strains. One limitation in both experiments was that as I was not able to make a standardised source of inoculum to inoculate host materials with equal amounts of *Olpidiopsis* spores, due to the special biological characters for both host and pathogen. The pathogen is a biotrophic obligatory endoparasite, it was not feasible to collect zoospores from mature *Olpidiopsis* sporangia and count standard number of zoospores to be used in inoculation, and it was also difficult to harvest equal amount of '*Bangia'* spores to be inoculated with *Olpidiopsis*. The time was limiting factor for us to try many approaches that allows us from obtaining spores from both organisms. Additionally, the age of `*Bangia*` strains was not the same between the two experiments. There may be a relation between the age of inoculated `*Bangia*` strain and its reaction against the pathogen. We inoculated spores in microscopy experiment after 48 hours of spore suspension preparation, while I inoculated 45 days old filaments in qPCR

experiment. Moreover, the effect of methodology is another factor as it was visual method in microscopy experiment, and I may was not able to detect the minor infection when it was not very clear comparing with qPCR method that is able to detect very small rare amount of pathogen`s DNA. And finally in microscopy experiment there is a potential effect of the staining procedure as I calculated the percentage of infection after fixing the experiment with RNAlater. During this procedure I washed the coverslips and incubated with ethanol and that may have led to losing some infected spores that became out of calculation. As example I excluded EASDALE A strain from microscopy results because it had few number of spores in the beginning of the experiment, I was able to detect incubation and latent period for this amount of spores on the coverslips under the inverted microscope, however, I lost this few spores after staining procedure and was not able to see any spore under fluorescent microscope. On the other hand, both experiments revealed consistently that all '*Bangia'* strains were infected and I could discriminate levels of susceptibility between algae strains. as example MFB5 strain was highly susceptible in both experiments.

In a previous study (Gachon et al. 2009) used qPCR and microscopic observation to investigate the effect of oomycete pathogen *Eurychasma dicksonii* on brown algae strains and found different levels of susceptibility for brown algae against this oomycete, their microscopy and qPCR results were compatible and they had the same results from two methodologies when comparing microscopy experiment with qPCR experiment. The differences between our findings is due to biological differences between pathogens and hosts

in both research as I worked on *Olpidiopsis* pathogen affecting red algae *Bangia* but they worked on *Eurychasma* affecting brown algae.

It is good to find in the future whether the amount of inoculum influences this kind of experiments. Regarding experimental design, I used incomplete block design and I was not able to expose all of our host materials to the same amount of inoculum in the same block. It was not feasible for one Petri dish to contain all *Bangia* strains at the same distance from the source on inoculum (cell strainer in our experiment), so the strains in each one replication were not exposed to the same amount of inoculum materials. I recommend finding a suitable container that will be able to contain all of strains together as one block in one replication and then to repeat the experiment with different design (complete block design) to compare the results.

This study combining fluorescent microscopy inspection and qPCR assay demonstrates that *Olpidiopsis porphyrae* var. *scotiae* which was newly described by (Badis et al. 2018) has a very broad host range and thus, presents a potential limiting factor for red algal cultivation in Scotland. In our study a Scottish oomycete isolate was used to inoculate red algal strains of Scottish and French origin. Algal strains from different locations were infected with the pathogen. So that the Scottish pathogen isolate had a negative effect on French host MFB4 and MFB5 strains, and the last strain was the highly infected in the results of microscopy experiment whereas MFB4 was the highly infected in qPCR experiment. As mentioned in (Badis, Klochkova, Brakel, et al. 2019), these results further support the concern that

introduced pathogen can cause severe damage to natural populations and potential aquaculture activities. Thus, more attention should be paid for the movement of algal materials between the countries inside and outside Europe. Our qPCR assay with newly designed described primers may be a useful tool to be used in quarantine system to monitor the movement of infected algal materials around the world. |However, I validated my primers according to my *Bangia* strains and *Olpidiopsis* isolate, and it will be important to try these primers with more host strains and pathogen isolates and non-targeted DNA in order to expand the usage of our primers and qPCR assay to control and monitor the movement of infected algal materials around the world.

There were previous studies about the interaction between oomycete pathogens and red algae, for instance (Sekimoto et al. 2008) found that *Olpidiopsis porphyrae* infects several stages of red algae that belong to Bangiales order from different algal stages as example *Porphyra yezoensis* blade (gametophyte), *Porphyra yezoensis* conchocelis (sporophyte), and *Bangia fuscopurpurea* conchocelis (sporophyte), different to this study, the study of (Sekimoto et al. 2008) just confirmed that these members of Bangiales order are hosts to *O. porphyrae* pathogen, but did not quantify the levels susceptibility of the host against pathogen. Whereas I detected the infection on different *Bangia* strains and quantified the *Olpidiopsis* pathogen in the infected strains. To the best of my knowledge this study is the first study that quantified *Olpidiopsis* pathogen in red algae (*Bangia*).

The phylogenetic study using *rbc*L sequences gave evidence that the 20 '*Bangia*' strains belong to three different clades '*Bangia' 2* ,'*Bangia' 3* and the original described genus *Bangia* that includes freshwater species when comparing with *rbc*L sequences reported by (Sutherland et al. 2011) Figure 13. And there is no relation between '*Bangia*' clade and the level of susceptibility to *Olpidiopsis* oomycete in both microscopy and qPCR experiment in our phylogenetic tree. For example, MFB4 strain was from `*Bangia*`3 clade and it was highly susceptible while Btrev strain from the same clade was less susceptible. The *rbc*L phylogenetic tree in this study differed from the tree presented in (Sutherland et al. 2011),

for instance the tree in this study positioned *Bangia* within the clade of '*Bangia*'1.

The differences probably stem from additional molecular information that (Sutherland et al. 2011) relied on, as they generated the phylogenetic tree based on both SSU and rbcL sequences where I used just *rbc*L in my study due to limited time factor. To improve the phylogenetic resolution, I suggest more effort to be done in this direction to implement additional molecular markers to study more in depth the phylogeny for '*Bangia*' strains. In our study I tried to implement SSU and *cox*1 sequences to get more markers for phylogenetic analysis but the data for *cox*1 and SSU sequences (reported in supplementary material) could not be generated for all `*Bangia* `strains due to time limitations. Therefore, these were not included in the phylogenetic analysis.

This study was an effort to shed the light on the interaction between red algae strains and *Olpidiopsis* isolate by inoculating 11 '*Bangia*' strains with on *Olpidiopsis* isolate by using incomplete block design, the study showed different levels of susceptibility for the 11 '*Bangia*' strains. We aimed to develop a new qPCR to quantify *Olpidiopsis* in *Bangia* and I could validate the suitable qPCR primers that suited my purpose and was able to quantify the amount of pathogen`s DNA in the host. The phylogenetic study was to find the position of

available *Bangia* strains at SAMS comparing with available information for *Bangia* strains around the world and I positioned the 20 strains in three clades.

To further validate this initial result, more efforts should be done to improve the experimental set-up, e.g. by containing all of algal strains together in one block as complete block design which will improve the experiment.

Also, as a next step in the future it will be helpful to include more pathogen isolates from the same species in the experiment that will give us information not just about the susceptibility of the host but the virulence of the pathogen.

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