

Note

Algae 2020, 35(1): 79-89
<https://doi.org/10.4490/algae.2020.35.2.25>

Open Access



Occurrence and pathogenicity of *Pythium* (Oomycota) on *Ulva* species (Chlorophyta) at different salinities

María-Luz Herrero^{1,*}, May Bente Brurberg^{1,2}, Darío I. Ojeda³ and Michael Y. Roleda^{4,5}

¹Department of Molecular Plant Biology, Norwegian Institute of Bioeconomy Research (NIBIO), PB 115, NO-1431 Ås, Norway

²Department of Plant Sciences, Norwegian University of Life Sciences (NMBU), P.O. Box 5003 NMBU, 1432 Ås, Norway

³Department of Forest Genetics and Biodiversity, Norwegian Institute of Bioeconomy Research (NIBIO), PB 115, NO-1431 Ås, Norway

⁴Department of Algae Production, Norwegian Institute of Bioeconomy Research (NIBIO), PB 115, NO-1431 Ås, Norway

⁵The Marine Science Institute, College of Science, University of the Philippines, Diliman 1101, Quezon City, Philippines

Pythium species are ubiquitous organisms known to be pathogens to terrestrial plants and marine algae. While several *Pythium* species (hereafter, *Pythium*) are described as pathogens to marine red algae, little is known about the pathogenicity of *Pythium* on marine green algae. A strain of a *Pythium* was isolated from a taxonomically unresolved filamentous *Ulva* collected in an intertidal area of Oslo fjord. Its pathogenicity to a euryhaline *Ulva intestinalis* collected in the same area was subsequently tested under salinities of 0, 15, and 30 parts per thousand (ppt). The *Pythium* isolate readily infected *U. intestinalis* and decimated the filaments at 0 ppt. Mycelium survived on *U. intestinalis* filaments for at least 2 weeks at 15 and 30 ppt, but the infection did not progress. Sporulation was not observed in the infected algal filaments at any salinity. Conversely, *Pythium* sporulated on infected grass pieces at 0, 15, and 30 ppt. High salinity retarded sporulation, but did not prevent it. Our *Pythium* isolate produced filamentous non-inflated sporangia. The sexual stage was never observed and phylogenetic analysis using internal transcribed spacer suggest this isolate belongs to the clade B2. We conclude that the *Pythium* found in the Oslo fjord was a pathogen of *U. intestinalis* under low salinity.

Key Words: macroalgae; oomycete; pathogen

Abbreviations: CMA P₅ARP, cornmeal agar amended with 5 mg L⁻¹ pimaricin, 250 mg L⁻¹ ampicillin, 10 mg L⁻¹ rifampicin, and 100 mg L⁻¹ pentachloronitrobenzene; COI, cytochrome *c* oxidase subunit I; HSD, honestly significant difference; ITS, internal transcribed spacer; MQ, Milli Q; PCA, potato carrot agar; Por, *Porphyra*; Pyr, *Pyropia*; Pyt, *Pythium*; ppt, parts per thousand; *rbcL*, ribulose biphosphate carboxylase large subunit; V8A, eight vegetables juice agar

INTRODUCTION

Pythium Pringsheim is a genus of parasitic and saprophytic oomycetes, which form a distinct phylogenetic lineage of fungus-like eukaryotes. They are filamentous and microscopic and reproduce both sexually and asexually. Oomycetes are placed under the kingdom Chrom-

ista, which includes a diverse groups of photosynthetic organisms such as the large brown seaweeds and microscopic diatoms (Cavalier-Smith 2018).

Pythium species are ubiquitous organisms found in aquatic and terrestrial habitats from the tropics to the



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received October 22, 2019, Accepted February 25, 2020

*Corresponding Author

E-mail: maria.herrero@nibio.no

Tel: +47-92065048, Fax: +47-64946110

Polar regions. *Pythium* species are best known as plant pathogens (Hendrix and Campbell 1973), but they have also been described as pathogens of crabs and fish (Czeczuga 1996), mammals (Gaastra et al. 2010), and algae (Van der Plaats-Niterink 1981). They play an important role as debris decomposers and they have been isolated from dead insects, decaying plant material, water and soil (Van der Plaats-Niterink 1981).

The genus *Pythium* was divided in 11 clades (from A to K) based on the internal transcribed spacer (ITS) region and the D1 to D3 regions of the nuclear large ribosomal subunit (Lévesque and De Cock 2004). One of the clades, clade K, was later segregated from the genus and described as the new genus *Phytopythium* (Bala et al. 2010). Furthermore, Uzuhashi et al. (2010) proposed to segregate the genus *Pythium* into five different genera.

The first *Pythium* species described infecting a marine alga was *Pyt. marinum* Sparrow found on red algae *Ceramium rubrum* (= *Ceramium virgatum* Roth) in Denmark (Sparrow 1934). A *Pythium* sp. causing disease in the cultivated red alga *Porphyra tenera* [= *Pyropia tenera* (Kjellman) N. Kikuchi, M. Miyata, M. S. Hwang & H. G. Choi] (nori) was reported from Japan in 1947 (Arasaki 1947), and this was later described as *Pyt. porphyrae* Takahashi and Sasaki (Takahashi et al. 1977). *Pyt. marinum* has been reported from North America and Europe mostly infecting *Porphyra* (= *Pyropia*) species (Kazama and Fuller 1977, Aleem 1980, Kerwin et al. 1992). *Pyt. porphyrae* is reported as the causal agent of the red rot disease causing substantial economical losses in nori production in Asia (Kim et al. 2014), and it has been also isolated from natural populations of *Pyropia plicata* W. A. Nelson in New Zealand (Diehl et al. 2017) and *Pyr. acanthophora* (E. C. Oliveira & Coll) M. C. Oliveira, D. Milstein & E. C. Oliveira in the Philippines (Dumilag 2019).

There are other *Pythium* species found in marine environments: *Pyt. grandisporangium* Fell and Master (Fell and Master 1975, De Cock 1986, Kurokawa and Tojo 2010), *Pyt. chondricola* De Cock (De Cock 1986, Lee et al. 2015), *Pyt. aquatile* Höhnk, *Pyt. salinum* Höhnk (Van der Plaats-Niterink 1981), and *Pyt. undulatum* H. E. Petersen (Thompson 1982). Several of them have been isolated from algae. For example, *Pyt. grandisporangium* and *Pyt. undulatum* were isolated from the brown alga *Fucus distichus* Linnaeus while *Pyt. chondricola* was isolated from several decaying red algae such as *Chondrus crispus* Stackhouse, *Pyr. yezoensis* (Ueda) M. S. Hwang & H. G. Choi and an unknown species, and from a decaying green alga *Ulva lactuca* Linnaeus (De Cock 1986, Lee et al. 2015).

The morphology of *Pyt. marinum*, *Pyt. porphyrae*, and *Pyt. chondricola* is very similar and there is controversy about their separation into three species (Park et al. 2000, Lévesque and De Cock 2004). The utilization of molecular methods in the identification of *Pythium* has not completely resolved the issue. Sequences of the ITS region, currently used in oomycetes taxonomy, are the same for *Pyt. porphyrae*, *Pyt. chondricola*, and *Pyt. marinum* isolated from marine habitats (Park et al. 2001, Lévesque and De Cock 2004). On the other hand, when comparing cytochrome *c* oxidase subunit I (COI) sequences, it was possible to discriminate among *Pyt. porphyrae* and *Pyt. chondricola* (Robideau et al. 2011, Lee et al. 2015). Recent studies further showed that cytochrome *c* oxidase subunit 2 sequences can also distinguish between these two species (Lee et al. 2017).

Several *Pythium* species, e.g., *Pyt. marinum*, *Pyt. porphyrae*, and *Pyt. chondricola*, are pathogenic to the gametophytic life stage of *Porphyra* and *Pyropia* species (Kazama and Fuller 1977, Kerwin et al. 1992, Diehl et al. 2017, Lee et al. 2017), but were unable to infect the conchocelis life stage of these species (Kerwin et al. 1992, Diehl et al. 2017). Artificial infections of *U. lactuca* with *Pyt. marinum* and of *U. fasciata* Delile, *U. pertusa* (= *U. australis* Areschoug), *U. prolifera* O. F. Müller with *Pyt. porphyrae* in half-strength seawater have been unsuccessful (Kerwin et al. 1992, Uppalapati and Fujita 2000). Artificial infection of *Ulva* sp. with *Pyt. porphyrae* in 1/4 strength seawater has also been unsuccessful (Diehl et al. 2017).

Other *Pythium* species, e.g., *Pyt. adhaerens* Sparrow, *Pyt. angustatum* Sparrow, *Pyt. diclinum* Tokunaga (syn. *Pyt. gracile* Schenk sensu Middleton), *Pyt. mamillatum* Meurs and *Pyt. tenue* Gobi are pathogenic to freshwater green algae (Van der Plaats-Niterink 1981). However, not much is known about pathogenicity of different *Pythium* species on marine green seaweeds. Previously, *Pyt. chondricola* was found on *U. lactuca* (De Cock 1986), but its pathogenicity was not reported.

Here we present the morphological and molecular characteristics of a *Pythium* strain isolated from an *Ulva* species collected from Oslo fjord. We also investigated the potential of the isolated *Pythium* strain to grow and sporulate and its pathogenicity at different salinities on another *Ulva* species (i.e., *U. intestinalis* Linnaeus) collected from the same area.

MATERIALS AND METHODS

Ulva collection

Filaments of euryhaline *Ulva* species were collected in an intertidal area of Oslo fjord (59°43'18.2" N, 10°43'39.1" E) in August and October 2013. On site, the algae were exposed to extreme salinity fluctuation due to persistent freshwater outflow from a nearby eutrophic stream (Årungselsva), seasonal rainfall, and tidal exchange. Fjord seawater salinity was 29 parts per thousand (ppt).

Isolation of *Pythium*

Ulva filaments collected in August 2013 were examined for the presence of oomycetes. Single threads were carefully separated and immersed in Petri dishes containing sterile Milli-Q (MQ) water. After 4 days, aseptate hyphae with appressoria formation were observed on one of the filaments. Part of the filament was stained with cotton blue for better observation of the hyphal growth (Fig. 1A). The other part of the filament was plated onto semi-selective medium for oomycetes CMA P₅ARP (cornmeal agar amended with 5 mg L⁻¹ pimarinic acid, 250 mg L⁻¹ ampicillin, 10 mg L⁻¹ rifampicin, and 100 mg L⁻¹ pentachloronitrobenzene) (Ferguson and Jeffers 1999). The plate was incubated at room temperature and mycelial growth was transferred onto V8 juice agar (V8A), containing 200 mL V8 juice (Campbell Soup Co., Camden, NJ, USA), 800 mL MQ H₂O, 3 g CaCO₃, and 15 g agar. The isolate was maintained on pieces of V8A submerged in vials with MQ water at 5 and 15°C. The isolate was deposited in the culture collection of NIBIO at Ås, Norway, with accession No. 231095 and in the Westerdijk Fungal Biodiversity Institute collection, Netherland, with accession No. CBS 139622.

Molecular identification of *Ulva* spp. and *Pythium* sp.

Total genomic DNA was extracted from *Pythium* mycelia grown on V8A or from algal filaments using the Qiagen DNeasy Plant Mini Kit (Hilden, Germany), and DNA segments were amplified by PCR using Taq polymerase and designated primers. For *Pythium*, the ITS was amplified and sequenced in both directions using primers ITS5 and ITS4 (White et al. 1990), and part of the COI gene using the oomycete-specific primers OomCoxILevup and Fm85mod (Robideau et al. 2011). A fragment of the ribulose-bisphosphate carboxylase large subunit (*rbcL*)

gene was targeted using primers *rbcLa-F* and *rbcLa-R* for the *Ulva* samples (Kuzmina et al. 2012). The amplicons were sequenced by GATC Biotech, Germany, and the raw data were assembled and analyzed using the Lasergene Sequence Analysis Software (DNASTAR Inc., Madison, WI, USA). The program BLASTN (National Center for Biotechnology Information, Bethesda, MD, USA) was used to search existing DNA databases for sequences showing similarity to the obtained sequences for the three genes. The best hits obtained from the blast searches from the sequences of *Pythium* and *Ulva* were downloaded and used in the phylogenetic analyses. We also included representative sequences from *Pythium* specimens (Robideau et al. 2011, Uzuhashi et al. 2015). In the case of *Ulva*, representative sequences of *Ulva* were selected from a previous analysis of Kang et al. (2014). The sequences for the three gene sets (ITS, COI, and *rbcL*) were aligned and manually inspected in AliView (Larsson 2014). Phylogenetic relationships were inferred on each separate aligned matrix with maximum likelihood as implemented in RAxML ver. 8.2 (Stamatakis 2014) using the GTRCAT model, 1,000 bootstrap and default settings. *Pythium undulatum* and *Umbraulva olivascens* (= *Umbraulva dangardii* M. J. Wynne & G. Furnari) were used as outgroups for the phylogenetic analyses. Obtained trees were edited with FigTree (Rambaut and Drummond 2016). All new sequences generated in this study are deposited in the GenBank database under accession Nos. MK644144, MK644145, MK644146, and MK644147.

Growth of *Pythium* and determination of cardinal temperatures

The *Pythium* isolate was subsequently grown on V8A and on potato carrot agar (PCA) (Van der Plaats-Niterink 1981) for morphological observations and growth studies. The maximum, minimum, and optimum temperatures for growth as well as the radial growth per day at different temperatures were determined on PCA as described by De Cock and Lévesque (2004) with small modifications. Agar plates were inoculated in the center and placed at room temperature. After 24 h, the plates were incubated at 1, 5, 10, 15, 20, 25, 30, 35, 37, and 40°C. Two PCA plates were used for each temperature. Radial growth was measured at two perpendicular diameters every 24 h for 3 days. After 3 days, the plates without mycelial growth were placed at room temperature for 1 week to check if growth resumed.

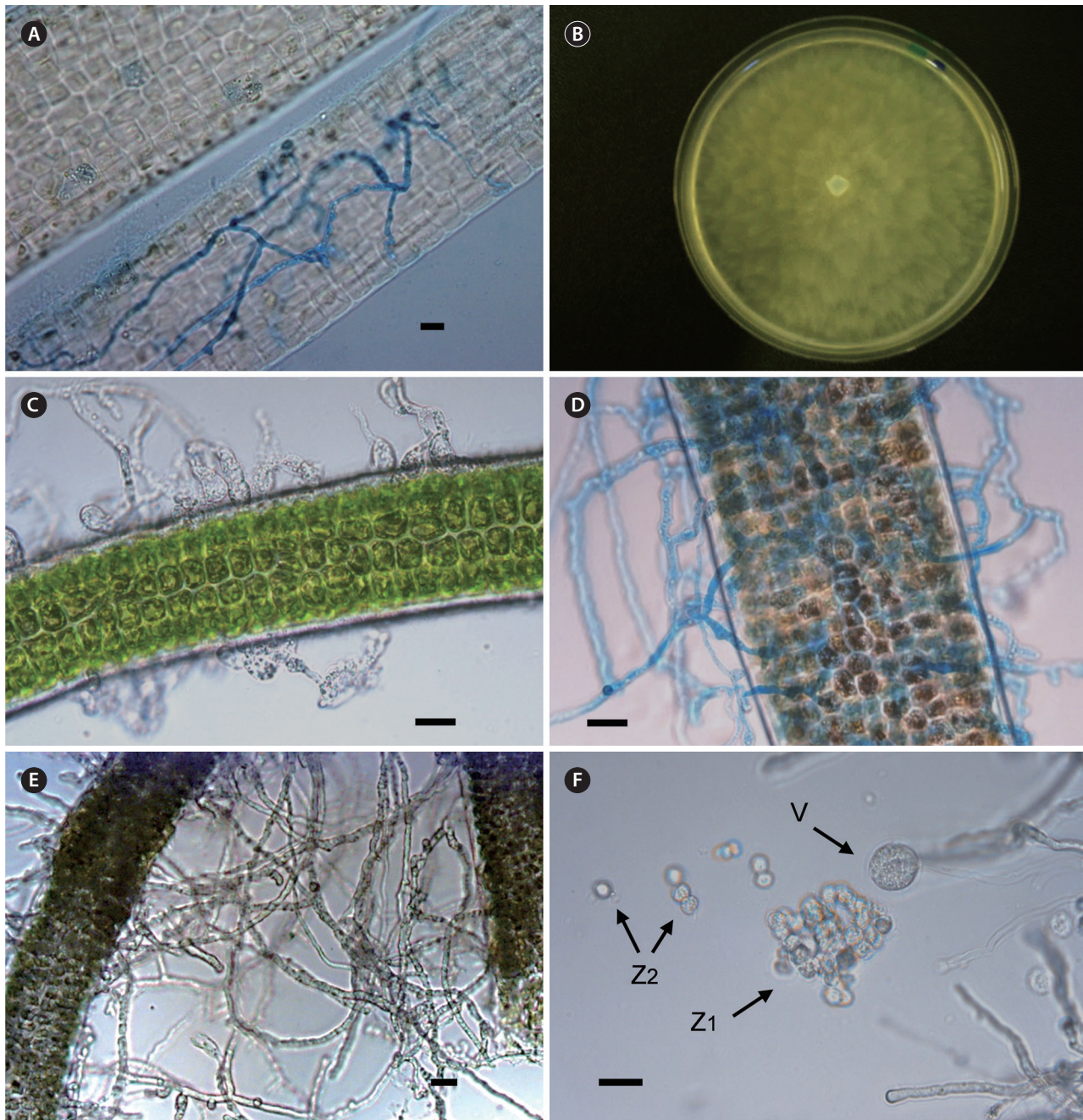


Fig. 1. *Pythium* sp. isolate NIBIO 231095. (A) Hyphae infecting a filament of *Ulva* collected in an intertidal area of Oslo fjord. *Pythium* hyphae are stained with cotton blue. (B) Colony pattern on potato carrot agar after 5 days at 20°C in the dark. (C-E) Pathogenicity test. (C) Appressoria formation on the surface of *Ulva intestinalis* after inoculation. The inoculation was made by putting *Ulva* filaments in contact with a growing *Pythium* colony. (D) Mycelial growth on the surface of *U. intestinalis* 5 days after inoculation and incubation at room temperature (22-23°C) in fresh water. *Pythium* hyphae are stained with cotton blue. (E) Mycelial growth from *U. intestinalis* 2 weeks after inoculation at room temperature (22-23°C) in fresh water. (F) Sporulation of *Pythium* sp. in grass water culture 2 h after immersion of grass pieces in fresh water at room temperature. V, vesicle; Z1, clump of realized zoospores; Z2, realized detached zoospore. Scale bars represent: A, 10 µm; C-F, 20 µm.

Growth of *Pythium* at different salinities

CMA plates were prepared by using sterile MQ water or saline solutions at 5, 10, 15, 20, 25, 30, and 35 ppt. The saline solutions were prepared by dissolving Sea Salt (Sigma, St. Louis, MO, USA) in sterile MQ water. The plates were inoculated at the border and incubated at 20°C in the dark. The radial growth along a dish diameter was measured every 24 h for 4 days. Four Petri dishes were used per each salinity.

Sporulation of *Pythium* in water culture under different salinities

Sporulation at different salinities was studied in grass water cultures. The isolated *Pythium* was grown on PCA plates for 2 days. Thereafter, 1-2 cm pieces of autoclaved lawn grass were placed on top of the growing *Pythium* colonies. After 24 h, the grass pieces infected with *Pythium* were placed in 9 mm Petri dishes containing ca. 20 mL of sterile MQ water or saline solutions at 15 and 30 ppt. The saline solutions were prepared by dissolving Sea Salt (Sigma) in sterile MQ water. The plates with the infected grass pieces immersed in different saline concentrations were incubated at room temperature (22-23°C). The presence or absence of vesicles and zoospores was checked after 2 and 5 h. Observations were made with an invert microscope that allows a quick check of the dishes without disturbance of the samples or the need of slides preparations. The dishes were periodically observed for formation of sexual stages for 2 weeks. The experiment was performed twice.

Pathogenicity assays

The pathogenicity of the isolated *Pythium* was tested on a filamentous *Ulva* species collected in October 2013. The *Ulva* filaments were carefully examined to make sure that they were not infected with oomycetes. Two pathogenicity tests were performed. In the first test, *Ulva* filaments were placed on the growing margin of a *Pythium* colony on PCA. The day after, the infected filaments were transferred to MQ water and two different saline concentrations (15 and 30 ppt). Non-inoculated filaments were placed in MQ water as controls. The filaments were periodically observed for 2 weeks, using an inverted microscope, in order to detect mycelium development and sporulation. To obtain photos, slide preparations were made for use in an upright microscope. After 2 weeks the filaments were transferred onto CMA P₅ARP to check the

survival of the *Pythium* sp. at the different saline concentrations. In the second test, the algal filaments were infected with zoospores. Small Petri dishes (5 cm diameter) were perforated with small orifices in the angle formed by the bottom and the wall of the dishes. These dishes were placed inside larger dishes (9 cm diameter) filled with fresh water. Grass pieces inoculated with *Pythium*, as described above, were placed inside the smaller dish and algal filaments in the larger dishes. There was no direct contact between the algal filaments and the grass pieces. The *Pythium* infecting the grass pieces produced zoospores that could move from the small dishes to the larger ones through the orifices. The filaments were observed periodically for 2 weeks as in the former assay.

Data handling and statistical analysis

All data except radial growth under different salinities were analyzed qualitatively. Radial growth data were tested for homogeneity of variance (Levene Statistics) and normality (Kolmogorov-Smirnov test). Thereafter, the statistical significance of differences in radial growth as affected by salinity treatment was tested using analysis of variance (ANOVA, $p = 0.05$). This was followed by Tukey honestly significant difference (HSD) test ($p < 0.05$) to find means that are significantly different from each other. Statistical analyses were done using the SPSS 11.5 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Ulva identification

The *Pythium*-infected *Ulva* filaments collected in August 2013 (accession No. MK644145) had a 99.7% sequence identity to the *rbcL* gene of *Ulva linza* Linnaeus (accession No. KP233764.1). The *Ulva* filaments collected in October 2013 (accession No. MK644146) and used in the pathogenicity test had 100% sequence identity to the *rbcL* gene of several voucher specimens of *U. intestinalis*. The *Ulva* sample where *Pythium* was isolated from belongs to the LPP (*linza-procera-prolifera*) complex (Shimada et al. 2008). *Ulva linza* and *U. prolifera* are currently accepted taxonomically while *U. procera* (K. Ahlner) H. S. Hayden, Blomster, Maggs, P. C. Silva, Stanshope & Waaland is currently regarded as a synonym of *U. prolifera* (Guiry and Guiry 2019). This group is taxonomically not well resolved and thus the *Ulva* sample was not conclusively identified. On the other hand, the *Ulva* used for

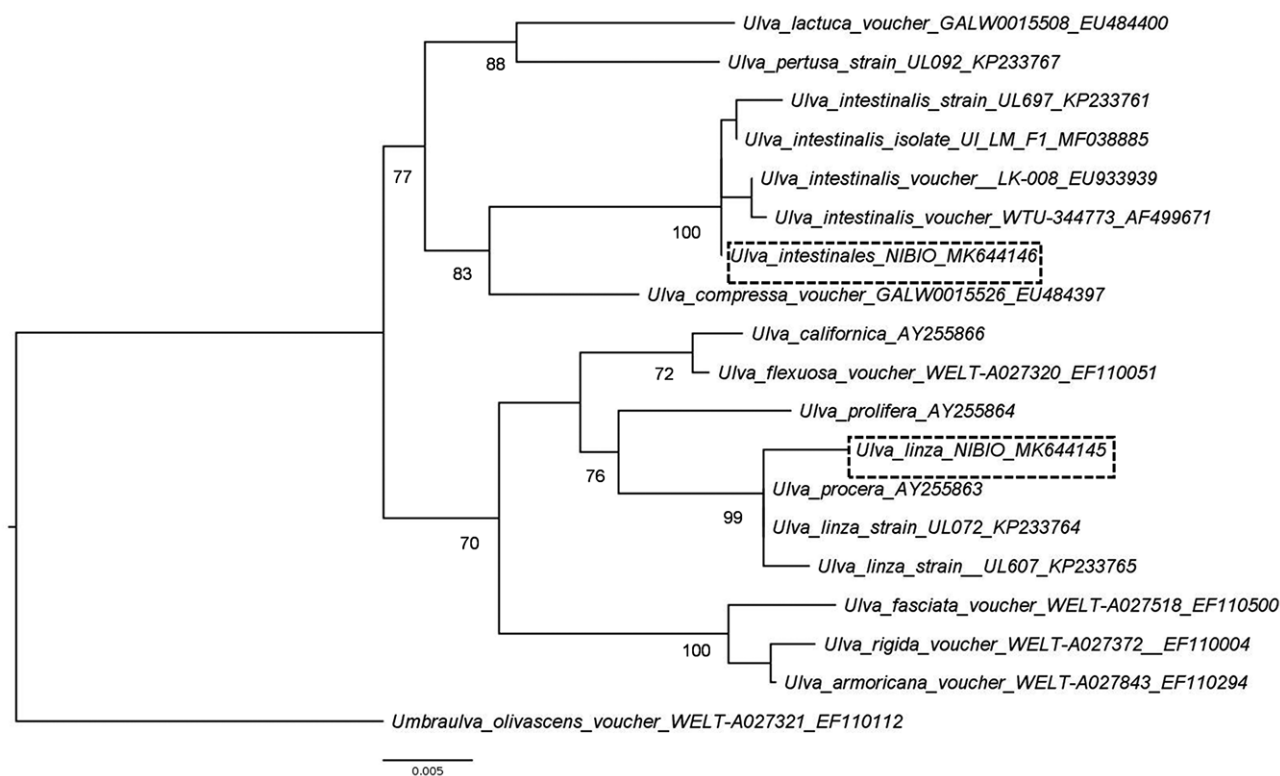


Fig. 2. Maximum likelihood tree of two *Ulva* samples collected in an intertidal area of Oslo fjord inferred from *rbcL* sequences. Numbers at nodes indicates bootstrap support values. GenBank accession numbers are given after each sample in the tree. Scale bar represents substitution rates.

pathogenicity tests clustered with *U. intestinalis* samples with a 100% bootstrap values (Fig. 2). Morphologically, *U. intestinalis* and *U. compressa* Linnaeus have distinguishing characteristics that separate them from other filamentous *Ulva* species. Moreover, sequences of *rbcL* can clearly distinguish these two species (Hayden et al. 2003).

Pythium identification

The ITS sequence of the isolated *Pythium* (accession No. MK644147) has 98.9% sequence identity to isolates deposited in GenBank as *Pyt. dissotocum* (accession Nos. KM061705.1 and KM061706.1); however, these two isolates lack COI sequence in GenBank. The COI sequence of the isolated *Pythium* (accession No. MK644144) has 97.3% identity to *Pyt. brachiatum* isolate UZ00746 (KJ995594.1). Our phylogenetic analyses based on ITS and COI sequences suggest that the Norwegian isolate belongs to the clade B2 of Lévesque and De Cock (2004) (Fig. 3).

Growth of *Pythium* and determination of cardinal temperatures

Pythium sp. colonies showed a chrysanthemum-rose pattern on V8A and PCA (Fig. 1B). The radial growth per day at different temperatures is shown in Fig. 4. At 35°C, limited growth was observed that ceased after day 1; however, growth resumed when culture was transferred to room temperature. At lower temperature, growth ceased at 1°C, but growth resumed when the culture was transferred to room temperature. The main hypha was up to 7 µm in diameter on PCA. The *Pythium* sp. produced filamentous sporangia that was not easily distinguishable from hyphae. The sexual stage was not observed neither in PCA, V8A, nor in grass in water cultures.

Pythium growth at different salinities

Radial growth at different salinities is shown in Fig. 5. Analysis of variance showed that salinity significantly af-

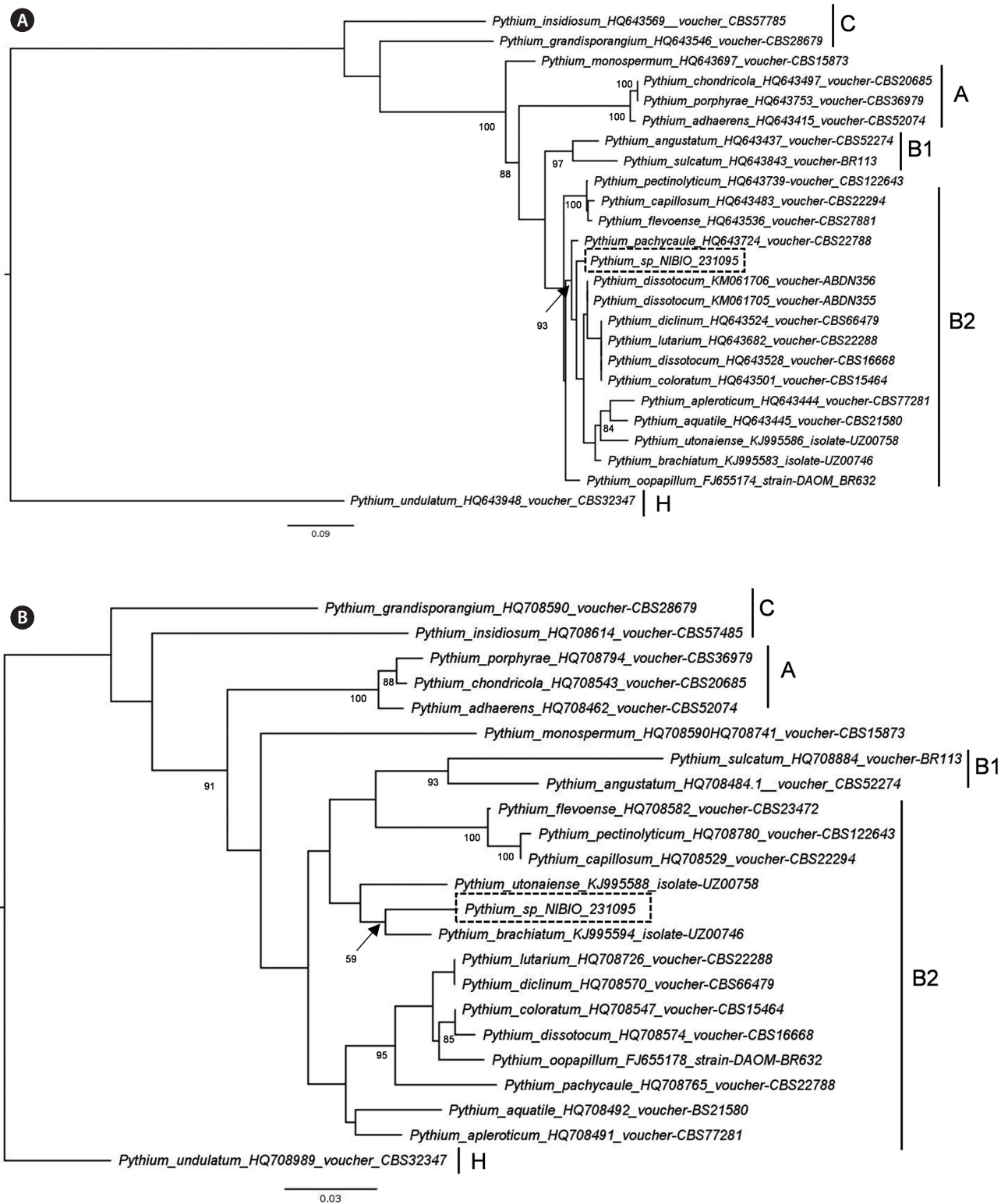


Fig. 3. Maximum likelihood tree of *Pythium* sp. isolate NIBIO 231095 inferred from internal transcribed spacer sequences (A) and cytochrome c oxidase subunit I sequences (B). The sampling includes closely related species belonging to *Pythium* clade B2 (Lévesque and De Cock 2004, Uzuhashi et al. 2015) and species from other clades with filamentous non-inflated sporangia or found in marine environments. Numbers at nodes indicates bootstrap support values above 80, except the location of the NIBIO isolate in B. GenBank accession numbers are given after each species in the tree. Scale bar represents substitution rates.

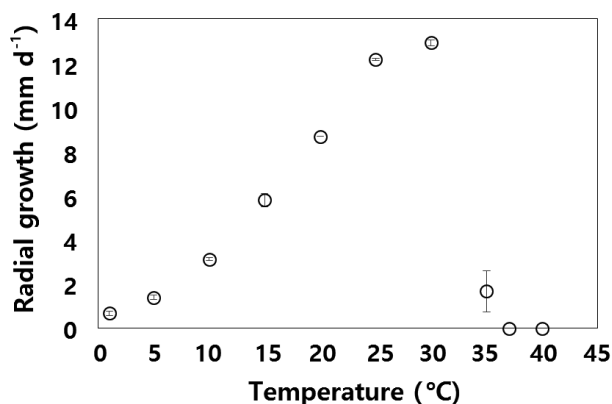


Fig. 4. Radial growth (mm d^{-1}) of *Pythium* sp. isolate NIBIO 231095 on potato carrot agar (0 ppt salinity) at different temperatures. Values are means (\pm standard deviation, $n = 2$), data was not subjected to statistical analysis.

fecting radial growth of *Pythium* sp. ($p < 0.001$). Posthoc Tukey's HSD showed growth was highest at salinity 10 = 15, $10 > 20$, $15 = 20 > 25 = 5 > 35 > \text{zero}$. The sexual stage was not observed at any salinity.

Sporulation at different salinities

The *Pythium* was observed to sporulate in fresh and saline waters, but sporulation was faster in freshwater than in saline water. Already after 2 hours of immersion in fresh water, sporulation was observed in the infected pieces of grass in both performed trials. After 2 hours in salinity 15 ppt, the *Pythium* sporulated in one of the trials and produced only vesicles in the other. In salinity 30 ppt, no sporulation was observed after 2 h; however, in one trial, vesicle formation was observed after 2 h. After 5 h, zoospore release was observed in all treatments.

Pathogenicity test

When *U. intestinalis* filaments were placed in close proximity of the mycelium of a *Pythium* colony, they were readily infected. The hyphae that came in contact with the seaweed formed a large amount of appressoria on the surface of the filaments (Fig. 1C). When *Pythium* in *Ulva* filaments was cultured in fresh water, it grew very well. After 5 days, the *Pythium* had fully colonized the algal filaments (Fig. 1D), and after 2 weeks, the filaments became brownish and completely surrounded by *Pythium* hyphae (Fig. 1E). Conversely, when *Pythium* and *Ulva* filaments were incubated in salinity 15 and 30 ppt, no *Pythium* growth was observed, and after 2 weeks

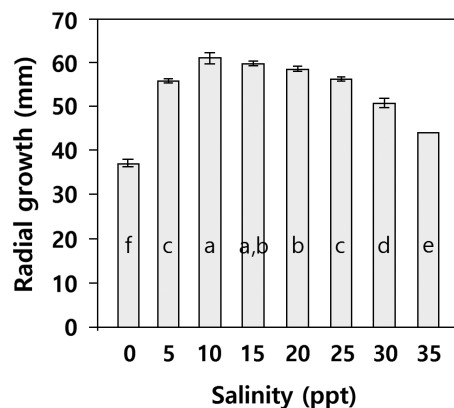


Fig. 5. Effect of salinity on total radial growth of *Pythium* sp. isolate NIBIO 231095 after 4 days on cornmeal agar at 20°C. Values are means (\pm standard deviation, $n = 4$), ANOVA showed significant difference between salinities ($p < 0.001$). Letters show result of Tukey's HSD ($p = 0.05$); different letters refer to significant differences between means.

these algal filaments remained green. The non-infected *Ulva* filaments (controls) placed in fresh water also remained green. After 2 weeks, the infected filaments were plated on CMA P₅ARP and *Pythium* was re-isolated from freshwater and the two saline concentrations. The *Pythium* re-isolated from diseased brown filaments was identified by ITS sequencing, confirming that it was the same *Pythium* sp. that was used for inoculation. Sporulation was never observed in *Ulva* filaments during the 2-week assay period, neither in fresh water nor in any of the saline concentrations.

In the second test, zoospores produced by *Pythium* also readily infected the algal filaments. Chemotaxis was not observed; the zoospores seemed to randomly swim and invade the seaweeds. Appressoria formation and mycelial growth on *Ulva intestinalis* were observed 1 week after inoculation. After 2 weeks, the algal filaments became brownish and there was abundant mycelial growth around the filaments.

DISCUSSION

Ulva intestinalis has typically been found in fresh and saline waters (Edwards et al. 1988). The different ecotypes are adapted to grow in different salinities and populations inhabiting brackish habitats have a wider range of salinity tolerance (Reed and Russell 1979). The survival of *U. intestinalis* at 0 ppt seems to be very variable. Edwards et al. (1988) reported that *U. intestinalis* could survive 6 days at 0 ppt, while Ritchie and Larkum (1985) reported a longer survival period of 3-5 weeks. Conversely, no sur-

vival at salinity < 1 ppt was observed in another study (Martins et al. 1999). Survival in zero or low salinities is promoted and compensated by the presence of high nutrient levels (Kamer and Fong 2001, McAvoy and Klug 2005). However, the *Ulva* filaments thriving in low salinity environments are prone to *Pythium* infection that is of freshwater origin.

The ITS sequence of *Pythium* in this study is very similar to the sequences of different *Pythium* species isolated from freshwater samples in the Falkland Islands (Davis 2016). The two isolates with sequences similar to the *Pythium* isolate described here, are both deposited as *Pyt. dissotocum* in GenBank. However, in the work of Davis (2016) these isolates are not morphologically described. The *Pythium* from Oslo fjord belongs to clade B2 of the *Pythium* phylogenetic tree of Lévesque and De Cock (2004). The common feature of different species in clade B2 is the production of filamentous sporangia. This type of sporangium corresponds to the *Pythium sensu stricto* in the classification of Uzuhashi et al. (2010). No other *Pythium* species isolated from marine algae seemed to belong to clade B. In the study of Lévesque and De Cock (2004) there is a *Pyt. marinum* placed in clade B2, but the authors pointed out that the isolate do not have a maritime origin and is probably misidentified. Due to the absence of sexual stage, it is not possible to make a complete morphological description of the *Pythium* isolated from *Ulva* filaments in this study.

The external mycelium developed profusely only when the filaments were placed in fresh water. Sporulation was never observed from mycelium growing from algae at any salinity tested. Zoospores were very easily produced from infected grass pieces. The increase in salinity slightly retarded the *Pythium* sporulation on grass but was able to sporulate at a salinity of 30 ppt. The tolerance of *Pythium* species to salinity is very variable. *Pyt. undulatum* isolated in estuaries from *Fucus distichus* could sporulate well between 0 and 15 ppt, but sporulation rarely occurred at 27 ppt (Thompson 1982), while *Pyt. marinum* infecting *Por. perforata* had difficulties to sporulate at salinities lower than 24 ppt (Kazama and Fuller 1977). In axenic culture, the *Pythium* of Oslo fjord could grow in all salinities tested with maximum radial growth at 10-15 ppt. *Pyt. porphyrae* isolated from *Pyropia* species could also grow at salinities between 0 and 30 ppt with an optimum at 15 ppt (Klochova et al. 2017). *Pyt. grandisporangium* isolates from a saline march in Osaka Bay could grow between 0 and 90 ppt with an optimum between 10 and 30 ppt (Kurokawa and Tojo 2010).

The *Pythium* species found in Oslo fjord is a patho-

gen of filamentous *Ulva* species. The infection does not progress at high salinity, but the *Pythium* can survive in infected algae for at least 2 weeks at 30 ppt. *Ulva* species exposed to fluctuating salinities, especially towards the lower range, are very likely more prone to infections caused by this *Pythium* species. Sporulation on infected algae was never observed, but it could sporulate on grass pieces in fresh water and up to 30 ppt. It is probable that terrestrial plants are hosts of this *Pythium* and floating terrestrial plant debris from freshwater systems could act as vectors infecting intertidal algae exposed to extreme salinity fluctuations.

ACKNOWLEDGEMENTS

This study was supported by the Norwegian Biodiversity Information Centre (Artsdatabanken), project number 70184222.

REFERENCES

- Aleem, A. A. 1980. *Pythium marinum* Sparrow (Phycomycetes) infesting *Porphyra leucosticta* Thuret in the Mediterranean Sea. Bot. Mar. 23:405-407.
- Arasaki, S. 1947. Studies on the rot of *Porphyra tenera* by *Pythium*. Nippon Suisan Gakkaishi 13:74-90 (in Japanese).
- Bala, K., Robideau, G. P., Lévesque, C. A., De Cock, A. W. A. M., Abad, Z. G., Lodhi, A. M., Shahzad, S., Ghaffar, A. & Coffey, M. D. 2010. *Phytopythium* Abad, de Cock, Bala Robideau, Lodhi & Lévesque, gen. nov. and *Phytopythium* Sindhum Lodhi, Shahzad and Levesque, sp. nov. Persoonia 24:136-137.
- Cavalier-Smith, T. 2018. Kingdom Chromista and its eight phyla: a new synthesis emphasising periplastid protein targeting, cytoskeletal and periplastid evolution, and ancient divergences. Protoplasma 255:297-357.
- Czeczuga, B. 1996. Species of *Pythium* isolated from eggs of fresh-water fish. Acta Mycol. 31:151-161.
- Davis, K. S. 2016. Biodiversity of aquatic oomycetes in the Falkland Islands. Ph.D. dissertation, University of Aberdeen, Aberdeen, 266 pp.
- De Cock, A. W. A. M. 1986. Marine Pythiaceae from decaying seaweeds in the Netherlands. Mycotaxon 25:101-110.
- De Cock, A. W. A. M. & Lévesque, C. A. 2004. New species of *Pythium* and *Phytophthora*. Stud. Mycol. 50:481-487.
- Diehl, N., Kim, G. H. & Zuccarello, G. C. 2017. A pathogen of New Zealand *Pyropia plicata* (Bangiales, Rhodophyta), *Pythium porphyrae* (Oomycota). Algae 32:29-39.

- Dumilag, R. V. 2019. Detection of *Pythium porphyrae* infecting Philippine *Pyropia acanthophora* based on morphology and nuclear rRNA internal transcribed spacer sequences. *J. Gen. Plant Pathol.* 85:72-78.
- Edwards, D. M., Reed, R. H. & Stewart, W. D. P. 1988. Osmoacclimation in *Enteromorpha intestinalis*: long-term effects of osmotic stress on organic solute accumulation. *Mar. Biol.* 98:467-476.
- Fell, J. W. & Master, I. M. 1975. Phycomycetes (*Phytophthora* spp. nov. and *Pythium* sp. nov.) associated with degrading mangrove (*Rhizophora mangle*) leaves. *Can. J. Bot.* 53:2908-2922.
- Ferguson, A. J. & Jeffers, S. N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. *Plant Dis.* 83:1129-1136.
- Gaastera, W., Lipman, L. J., De Cock, A. W., Exel, T. K., Pegge, R. B., Scheurwater, J., Vilela, R. & Mendoza, L. 2010. *Pythium insidiosum*: an overview. *Vet. Microbiol.* 146:1-16.
- Guiry, M. D. & Guiry, G. M. 2019. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: <http://www.algaebase.org>. Accessed May 15, 2019.
- Hayden, H. S., Blomster, J., Maggs, C. A., Silva, P. C., Stanhope, M. J. & Waaland, J. R. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* 38:277-294.
- Hendrix, F. F. Jr. & Campbell, W. A. 1973. *Pythiums* as plant pathogens. *Annu. Rev. Phytopathol.* 11:77-98.
- Kamer, K. & Fong, P. 2001. Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*. *Mar. Ecol. Prog. Ser.* 218:87-93.
- Kang, E. J., Kim, J. -H., Kim, K., Choi, H. -G. & Kim, K. Y. 2014. Re-evaluation of green tide-forming species in the Yellow Sea. *Algae* 29:267-277.
- Kazama, F. Y. & Fuller, M. S. 1977. Colonization of *Porphyra perforata* thallus discs by *Pythium marinum*, a marine facultative parasite. *Mycologia* 69:246-254.
- Kerwin, J. L., Johnson, L. M., Whisler, H. C. & Tuininga, A. R. 1992. Infection and morphogenesis of *Pythium marinum* in species of *Porphyra* and other red algae. *Can. J. Bot.* 70:1017-1024.
- Kim, G. H., Moon, K. -H., Kim, J. -Y., Shim, J. & Klochkova, T. A. 2014. A revaluation of algal diseases in Korean *Pyropia* (*Porphyra*) sea farms and their economic impact. *Algae* 29:249-265.
- Klochkova, T. A., Jung, S. & Kim, G. H. 2017. Host range and salinity tolerance of *Pythium porphyrae* may indicate its terrestrial origin. *J. Appl. Phycol.* 29:371-379.
- Kurokawa, K. & Tojo, M. 2010. First record of *Pythium gran-disporangium* in Japan. *Mycoscience* 51:321-324.
- Kuzmina, M. L., Johnson, K. L., Barron, H. R. & Hebert, P. D. N. 2012. Identification of the vascular plants of Churchill, Manitoba, using a DNA barcode library. *BMC Ecol.* 12:25.
- Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30:3276-3278.
- Lee, S. J., Hwang, M. S., Park, M. A., Baek, J. M., Ha, D.-S., Lee, J. E. & Lee, S. -R. 2015. Molecular identification of the algal pathogen *Pythium chondricola* (Oomycetes) from *Pyropia yezoensis* (Rhodophyta) using ITS and *cox1* markers. *Algae* 30:217-222.
- Lee, S. J., Jee, B. Y., Son, M. -H. & Lee, S. -R. 2017. Infection and *cox2* sequence of *Pythium chondricola* (Oomycetes) causing red rot disease in *Pyropia yezoensis* (Rhodophyta) in Korea. *Algae* 32:155-160.
- Lévesque, C. A. & De Cock, A. W. A. M. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycol. Res.* 108:1363-1383.
- Martins, I., Oliveira, J. M., Flindt, M. R. & Marques, J. C. 1999. The effect of salinity on the growth rate of the macroalgae *Enteromorpha intestinalis* (Chlorophyta) in the Mondego estuary (west Portugal). *Acta Oecol.* 20:259-265.
- McAvoy, K. M. & Klug, J. L. 2005. Positive and negative effects of riverine input on the estuarine green alga *Ulva intestinalis* (syn. *Enteromorpha intestinalis*) (Linnaeus). *Hydrobiologia* 545:1-9.
- Park, C. S., Kakinuma, M. & Amano, H. 2001. Detection of the red rot disease fungi *Pythium* spp. by polymerase chain reaction. *Fish. Sci.* 67:197-199.
- Park, C. S., Sakaguchi, K., Kakinuma, M. & Amano, H. 2000. Comparison of the morphological and physiological features of the red rot disease fungus *Pythium* sp. isolated from *Porphyra yezoensis* from Korea and Japan. *Fish. Sci.* 66:261-269.
- Rambaut, A. & Drummond, A. 2016. Figtree version 1.4.3. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Available from: <http://tree.bio.ed.ac.uk/software/figtree>. Accessed May 15, 2019.
- Reed, R. H. & Russell, G. 1979. Adaptation to salinity stress in populations of *Enteromorpha intestinalis* (L.) Link. *Estuar. Coast. Mar. Sci.* 8:251-258.
- Ritchie, R. J. & Larkum, A. W. D. 1985. Potassium transport in *Enteromorpha intestinalis* (L.) Link: II. Effects of medium composition and metabolic inhibitors. *J. Exp. Bot.* 36:394-412.
- Robideau, G. P., De Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., Désaulniers, N.,

- Eggertson, Q. A., Gachon, C. M. M., Hu, C. -H., Küpper, F. C., Rintoul, T. L., Sarhan, E., Verstappen, E. C. P., Zhang, Y., Bonants, P. J. M., Ristaino, J. B. & Lévesque, C. A. 2011. DNA barcoding of oomycetes with cytochrome *c* oxidase subunit I and internal transcribed spacer. *Mol. Ecol. Resour.* 11:1002-1011.
- Shimada, S., Yokoyama, N., Arai, S. & Hiraoka, M. 2008. Phylogeography of the genus *Ulva* (Ulvophyceae, Chlorophyta), with special reference to the Japanese freshwater and brackish taxa. *J. Appl. Phycol.* 20:979-989.
- Sparrow, F. K. Jr. 1934. Observations on marine Phycomycetes collected in Denmark. *Dansk Bot. Ark.* 8:1-24.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312-1313.
- Takahashi, M., Ichitani, T. & Sasaki, M. 1977. *Pythium porphyrae* Takahashi et Sasaki, sp. nov. causing red rot of marine red algae *Porphyra* spp. *Trans. Mycol. Soc. Jpn.* 18:279-285.
- Thompson, T. A. 1982. Some aspects on the taxonomy, ecology and histology of *Pythium* Pringsheim species associated with *Fucus distichus* in estuaries and marine habitats of British Columbia. Ph.D. dissertation, University of British Columbia, Vancouver, BC, 114 pp.
- Uppalapati, S. R. & Fujita, Y. 2000. Carbohydrate regulation of attachment, encystment, and appressorium formation by *Pythium porphyrae* (Oomycota) zoospores on *Porphyra yezoensis* (Rhodophyta). *J. Phycol.* 36:359-366.
- Uzuhashi, S., Kakishima, M. & Tojo, M. 2010. Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* 51:337-365.
- Uzuhashi, S., Okada, G. & Ohkuma, M. 2015. Four new *Pythium* species from aquatic environments in Japan. *Antonie Van Leeuwenhoek* 107:375-391.
- Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. *Stud. Mycol.* 21:1-242.
- White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Eds.) *PCR Protocols: A Guide to Methods and Amplifications*. Academic Press, New York, pp. 315-322.