Phytopythium and Pythium Species (Oomycota) Isolated from Freshwater Environments of Korea

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ABSTRACT
Oomycetes are widely distributed in various environments, including desert and polar regions. Depending upon different habits and hosts, they have evolved with both saprophytic and pathogenic nutritional modes. freshwater ecosystem is one of the most important habitats for members of oomycetes. Most studies on oomycete diversity, however, have been biased mostly towards terrestrial phytopathogenic species, rather than aquatic species, although their roles as saprophytes and parasites are essential for freshwater ecosystems. In this study, we isolated oomycete strains from soil sediment, algae, and decaying plant debris in freshwater streams of Korea. The strains were identified based on cultural and morphological characteristics, as well as molecular phylogenetic analyses of ITS rDNA, cox1, and cox2 mtDNA sequences. As a result, we discovered eight oomycete species previously unknown in Korea, namely Phytopythium chamaehyphon, Phytopythium litorale, Phytopythium vexans, Pythium diclinum, Pythium heterothallicum, Pythium inflatum, Pythium intermediate, and Pythium oopapillum. Diversity and ecology of freshwater oomycetes in Korea are poorly understood. This study could contribute to understanding their distribution and ecological function in freshwater ecosystem.

1. Introduction
Oomycota, a monophyletic group of filamentous microbes under the kingdom Straminipila, is ubiquitous in the oceans [1,2], freshwater [3,4], and terrestrial environments [5,6] throughout the world. Aquatic oomycetes are often referred to as “water molds” and play significant roles on aquatic ecosystems, where most oomycetes are saprotrophic, responsible for decomposing and recycling of organic materials. Additionally, their endophytism in aquatic plant and algae [7,8] is worthy of notice. Nevertheless, the traits of aquatic oomycetes regard to saprophytism and endophytism are largely unknown, in comparison with the parasitic species that infect fish, fish eggs [9,10], and algae [11,12].

In Korea, aquatic oomycetes relating to fish and algae diseases have been recently recorded, e.g., Achlya [3], Pythium [12,13], and Saprolegnia [14]. However, diversity of saprotrophic or endophytic oomycetes in Korea remains still poorly understood.

Two genera of oomycetes, Pythium and Phytopythium, have a variety of nutritional modes and ecological niches with varying environmental tolerances. They are well distributed as soil-borne plant pathogens or saprophytes [5,6] but also distributed widely in freshwater systems [15,16]. Many species of Pythium are potentially pathogens causing economically important diseases, such as damping-off, root rots of plants [17–19], and red rot of algae [12]. The genus Pythium with coenocytic, hyaline mycelia is characterized by unique mode of differentiation and discharge of zoospores, which distinguishes this genus from Phytophthora; sporangial protoplast of Pythium normally enters through a tube to a vesicle, and then zoospores are differentiated and discharged from the rupture of the vesicle [20,21]. Species of Pythium are currently categorized in 10 clades, according to their phylogenetic relationships, morphological characters, and host preferences [22].

Members of Phytopythium were originally classified in clade K of Pythium, according to molecular phylogenetic subdivision of Pythium sensu lato by Lévesque and De Cock [22]. They are similar to Pythium species in the mechanism of the zoospore differentiation and release, but produce globose to ovoid, papillate sporangia, often with internal proliferations, like Phytophthora [23]. As a result, a new genus Phytopythium intermediate between Phytophthora and Pythium was introduced.
to separate it from other groups of *Pythium* [23,24]. *Phytopythium* species were further subdivided into three clades [25,26].

To understand the diversity, distribution, and ecological functions of freshwater oomycetes, we isolated numerous strains from freshwater streams of Korea. Eight oomycete species were identified from the soil sediment, algae, and decaying plant debris in freshwater environments; *Phytopythium chamaehyphon*, *Phy. litorale*, *Phy. vexans*, *Pythium diclinum*, *P. heterothallicum*, *P. inflatum*, *P. intermedium*, and *P. oospillum*. These species were previously unrecorded in Korea. In the present study, their molecular phylogenetic and morphological characteristics were investigated.

2. Materials and methods

2.1. Isolation

Oomycete isolates were collected from diverse freshwater environments of Korea, including soil sediment, algae, and decaying plant debris such as leaf, seed, and stem (Table 1). To isolate oomycete strains from soil sediment, a dilution plate method was used (1:10 dilution rate). The diluted soil suspension was distributed throughout a potato dextrose agar (PDA; 39 g potato dextrose agar powder in 1 L of deionized water) and V8 agar (V8A; 50 mL clarified V8 juice, 2 g calcium carbonate, 15 g agar powder, and 950 mL deionized water) plate. To restrict bacterial and fungal growth, rifampicin (15 ppm) and nystatin (20 ppm) were added to the media. Algae, decaying plant leaves, stems, and seed peels were washed with distilled water and cut into of 3 – 5 mm² under sterile condition. These prepared samples were placed onto both types of agar plates. The inoculated plates were incubated for 1–3 days at 25 °C in the dark. Hyphal tips were observed microscopically, isolated from the outgrowing mycelia, and transferred onto new agar plates. The isolates were incubated for 3–5 days at 25 °C before genomic DNA extraction.

2.2. Cultural and morphological analysis

Cultural characteristics were investigated 2–3 days after inoculating the isolates on PDA, V8A, and corn meal agar (CMA; 17 g corn meal in 1 L of deionized water) at 25 °C in the dark. To induce the formation of sporangia or oospores sterile distilled water was added to the surface of colony after the incubating for 7–14 days, and the isolates were placed at 5 °C for 24 h. Microscopic structures of prepared isolates were observed under an Olympus BX53F microscope (Olympus, Tokyo, Japan) and photographed using a DigiRetina 16 M digital camera (Tucsen, Fuzhou, China).

2.3. DNA extraction, amplification, and sequencing

Genomic DNA of the tissues cultured on medium was isolated using the MagListo 5 M plant Genomic DNA Extraction Kit (Bioneer, Daejon, Korea) based on a technology of magnetic bead, according to the manufacturer’s instruction. Polymer chain reaction (PCR) amplifications were performed for the internal transcribed spacer (ITS) rDNA region using primers ITS1 and ITS4 [27] and cytochrome c oxidase subunit I (cox1) region using primers OomCox1-levup and OomCox1-levlo, respectively [28]. In addition, cytochrome c oxidase subunit II (cox2) was amplified using primer set cox2-F [29] and cox2-RC4 [30]. Amplicons were purified using an AccuPrep PCR Purification Kit (Bioneer) and were sequenced by Macrogen Inc. (Seoul, Korea). All sequences have been registered with the National Center for Biotechnology Information (NCBI) GenBank (Table 1).

2.4. Phylogenetic analysis

The sequences were edited using the DNAStar software package version 5.05 (DNAStar, Inc., Madison, WI) and blasted to search their reference sequences with homology against the public sequence database of the NCBI. The sequence data including the resulting reference sequences and the previously published sequences of the type or authentic isolates of *Phytopythium* and *Pythium* species were aligned using MAFFT 7 [31], with the Q-INS-i algorithm [32]. Minimum evolution (ME) and maximum likelihood (ML) analyses were used to construct phylogenetic trees with MEGA 6.0 [33], using the Tamura-Nei model with bootstrapping analysis of 1000 replicates.

3. Results and discussion

In 2016 and 2018, a total of 15 oomycete strains were isolated from various freshwater environments in Chungcheongbuk-do, Jeollabuk-do, and Jeollanam-do in Korea. All oomycete species were found in a pond except for *P. inflatum* that was found in mountain streams, and their substrates varied in decaying leaves, algae surface, and soil sediments in freshwater (Table 1). The isolates were identified based on their cultural, morphological, and molecular genetic characteristics. Phylogenetic relationships between the Korean oomycete isolates and previously published authentic
| Species         | Strain no. | Sequence ID | Source                  | Location                  | Water environment | Date        | GenBank no.                  |
|-----------------|------------|-------------|-------------------------|---------------------------|-------------------|-------------|-----------------------------|
| Phytopythium    | NNIBRFG9358 | W672        | Decaying leaf           | Namwon, Jeollabuk-do      | Mountain stream   | Sept 2018 | MK796010/MK802171/MK802184 |
| chamaehyphon    |            |             |                         | (35°27’80"N 127°35’30"E) |                   |             |                             |
| Phy. litorale   | NNIBRFG9359 | W595        | Algae                   | Wanju, Jeollabuk-do       | Mountain stream   | May 2018   | MK796011/MK802172/MK802185  |
| Phy. vexans     | W630       | W630        | Decaying leaf           | Muju, Chungcheongbuk-do   | Mountain stream   | May 2018   | MK796012/MK802173/MK802186  |
|                 |            |             |                         | (35°57’31"N 127°14’08"E) |                   |             |                             |
|                 |            |             |                         | Muju, Chungcheongbuk-do   | Mountain stream   | May 2018   | MK796012/MK802173/MK802186  |
|                 |            |             |                         | (35°57’36"N 127°41’43"E) |                   |             |                             |
| Pythium diclinum| NNIBRFG9363 | W652        | Decaying leaf           | Yeongdong, Jeollabuk-do   | Mountain stream   | May 2018   | MK796016/MK802177/MK802190  |
|                 |            |             |                         | (36°03’16"N 127°49’37"E) |                   |             |                             |
| Phy. heterothalicum | NNIBRFG9364 | W637        | Soil sediment           | Muju, Chungcheongbuk-do   | Mountain stream   | Jun 2018   | MK796018/MK802179/MK802192  |
|                 |            |             |                         | (35°57’36"N 127°41’43"E) |                   |             |                             |
| Phy. intermedium| NNIBRFG9365 | W704        | Decaying leaf           | Imsil, Jeollabuk-do       | Mountain stream   | Sept 2018  | MK796019/MK802180/MK802193  |
|                 |            |             |                         | (35°39’25"N 127°20’57"E) |                   |             |                             |
| Phy. oopapillum  | NNIBRFG9366 | W631        | Algae                   | Imsil, Jeollabuk-do       | Mountain stream   | May 2018   | MK802182/MK802197            |
|                 |            |             |                         | (35°57’36"N 127°41’43"E) |                   |             |                             |
|                 |            |             |                         | Muju, Jeollabuk-do        | Mountain stream   | May 2018   | MK802183/MK802198            |

ITS: internal transcribed spacer rDNA; cox1: cytochrome oxidase subunit I mtDNA; cox2: cytochrome oxidase subunit II mtDNA.
isolates were inferred using ML and ME analyses of the ITS rDNA, cox2, and cox1 mtDNA sequences. As topologies constructed by the two analyses were congruent, only the ME tree was shown for each locus (ITS in Figure 1, cox2 in Figure 2, and cox1 in Figure 3). Eight oomycete species isolated in the present study were placed in two major groups, representing two different genera, Phytopythium and Pythium; six isolates, W595, W630, W672, W707, W708, and W714, grouped in Phytopythium, whereas nine isolates, W254, W257, W631, W633, W637, W652, W654, W704, and W710, grouped in Pythium (Figures 1-3).

Phytopythium is a distinctly monophyletic genus, which has been segregated from the genus Pythium by phylogenetic analysis of multilocus sequences [24]. Additional phylogenetic studies by Baten et al. [25] and Jesus et al. [26] have identified three subclades within Phytopythium. Morphologically, Clades 1 and 2 differ from Clade 3 by having papillate sporangia as well as internal sporangial proliferation [25,26]. Each of three Phytopythium species isolated in Korea was assigned to the three clades; W672 was matched with Phy. chamaehyphon (CBS 25930), W595 and W630 with Phy. litorale (CBS118360), W707, W708, and 714 with Phy. vexans (CBS11980). The groupings of Phytopythium isolates from Korea with their reference isolates were supported by high bootstrap values (100/100% in ITS, 100/99–100% in cox2, and 100/99–100% in cox1 trees). Phy. vexans (W707, W708, and W714) produced non-papillate sporangia without

Figure 1. Minimum evolution tree of Phytopythium and Pythium species based on internal transcribed spacer rDNA sequences. Achlya aquatica and A. sparrowii are used as outgroup. The isolates collected in Korea are shown in bold. Bootstrapping values (minimum evolution BP/maximum likelihood BP) higher than 70% are given above or below the branches (1000 replicate). The scale bar equals the number of nucleotide substitutions per site.
proliferation, thus supporting its phylogenetic placement in Clade 3 [25]; *Phy. litorale* (W595 and W630) and *Phy. chamaehyphon* (W672), which have papillate sporangia, belong to Clade 1 and 2, respectively. In addition, *Phy. litorale* (W595 and W630) revealed a chrysanthemal colony pattern, which differs from other species that grew in a radiate pattern.

Traditionally, taxonomy of the genus *Pythium* is based on morphology of asexual and sexual structures [21,34,35]. However, the high variability of morphological features and overlapping of different species render it difficult to differentiate *Pythium* species. Advent of DNA sequence-based approaches has allowed the subgroupings of this genus [22], although the insufficient variation in ITS sequences in some morphologically different species and intraspecific heterogeneity within a species have been reported [22,36,37]. As a result, newly generated molecular data of better phylogenetic markers or multigene phylogenies should be combined with the morphological characteristics useful for identification and taxonomy of *Pythium* species [20,22,38,39]. The nine Korean isolates of *Pythium* were assigned to the five species. W254 and W257 were grouped with *P. inflatum* (CBS16868), W631 and W633 with *P. oopapillum* (CBS124053, Lev1619), W637 and W710 with *P. heterothallicum* (CBS45067), W652 and W654 with *P. diclinum* (CBS66479), and W704 with *P. intermedium* (CBS26638, KA2207). Groupings of *Pythium* isolates with their reference isolates were supported by high bootstrap values of

![Minimum evolution tree of Phytopythium and Pythium species based on cytochrome oxidase subunit II mtDNA sequences. Saprolegnia parasitica and Achlya ambisexualis are used as outgroup. The isolates collected in Korea are shown in bold. Bootstrapping values (minimum evolution BP/maximum likelihood BP) higher than 70% are given above or below the branches (1000 replicate). The scale bar equals the number of nucleotide substitutions per site.](image-url)
98–100/90–100% in ITS, 95–100/92–100% in cox2 and 80–100/98–99% in cox1 trees. Among 10 clades previously known within the genus Pythium, three species of Pythium, P. inflatum (W254 and W257), P. oopapillum (W631 and W633), and P. diclinum (W652 and W654), were placed in Clade B, all of which produce filamentous sporangia [22]. Clade B was subdivided into Subclade B1, characterized by species with inflated sporangia, and B2 characterized by species with non-inflated sporangia [22]; P. inflatum and P. oopapillum were affiliated with clade B1 and P. diclinum with B2. Clade B contains a number of waterborne Pythium species isolated from the freshwater habitats [22], including P. inflatum and P. diclinum. P. heterothallicum (W637 and W710), belonging to Clade I, was originally recorded as a soil-borne saprophyte [21], and in the present study it was also found also from a soil sediment sample. P. intermedium (W704) was isolated from a decaying leaf of an herbaceous plant and placed in Clade F which contains plant pathogens with globose sporangia [22]. Most species in Clade F grow very fast
and this fast growth pattern was proved in the present study. Clade A with mainly aquatic species [40,41] was not found in the present study.

In previous studies on the diversity of oomycetes inhabiting the freshwater environment, plants were found to be predominantly utilized by oomycetes [15,40,42,43]. In the present study, decaying plant leaves, seeds, and stems were major substrates of *Phytopythium* and *Pythium* species. Interestingly, *Phy. litorale* and *P. oopapillum* were isolated from the surface of the undetermined algae in mountain streams. Compared with algae-parasitic oomycetes, the traits of algae-saprotrophic oomycetes are largely unknown. It is still uncertain how these species affect aquatic plants and algae and whether they can infect them. However, since diverse oomycetes are found in the aquatic biotic substrates, this means that they play an important role in the circulation and maintenance of the aquatic ecosystem inhabited.

### 3.1. Taxonomy

#### 3.1.1. *Phytopythium chamaehyphon*

**3.1.1.1. Reference.** Abad, de Cock, Bala, Robideau, Lodhi and Lévesque, Persoonia 34: 36 (2014) [MB#563329] (Figure 4).

**3.1.1.2. Description.** Colonies were colorless on PDA, V8A, and CMA at 25°C, with low aerial mycelia on PDA and V8A and with submerged growth on CMA, forming a radiate pattern. After 72 h the colony diameter was found to be 35–40 mm on PDA, >70 mm on V8A, and 55–60 mm on CMA. Main hyphae extended up to 5 μm wide. Sporangia were subglobose or oblong, 18–28 μm in diameter; vesicles were 15–30 μm in diameter, containing zoospores; discharge tubes were also observed. Encysted zoospores were 9–10 μm in diameter. Oogonia were terminal or intercalary with an average of 26 μm in diameter. Oospores were aplerotic with an average of 24 μm in diameter; the wall was found to reach up to 2 μm thickness.

**3.1.1.3. Isolate examined.** Korea, Jeollabuk-do; Namweon-si; Inwol-myeon; Inwol-ri (35°27′80″N 127°35′30″E) from a decaying leaf on September 05 2018 by the authors (NNIBRFG9358, W672).

**Note:** The isolate W672 was identified as *Phy. chamaehyphon*. The sequences are nearly identical to the ex-type strain CBS25930 isolated from the papaya in USA [24]. *Phy. chamaehyphon* is close to *Phy. vexans* and *Phy. cucurbitacearum*, but distinguished from *Phy. vexans* by having larger oogonia and from *Phy. cucurbitacearum* by having non-papillate sporangia [21]. This species was...
previously isolated from irrigation water tanks in a greenhouse, with a pathogenicity on greenhouse crops [41].

3.1.2. Phytopythium litorale

3.1.2.1. Reference. Abad, de Cock, Bala, Robideau, Lodhi and Lévesque, Persoonia 34: 37 (2014) [MB#563355] (Figure 4).

3.1.2.2. Description. Colonies formed a broad, colorless chrysanthemal pattern on PDA, V8A, and CMA at 25 °C, with submerged growth on CMA. After 72 h, colony diameter was recorded as 35–40 mm on PDA, 50–55 mm on V8A, and 45–50 mm on CMA. Main hyphae extended up to 5–6 µm wide. Sporangia were abundant, usually globose, sometimes pyriform, terminal, subterminal, or intercalary and measured 16–32 µm in diameter, with a very conspicuous apical papilla. Hyphal swellings were globose with an average of 28 µm in diameter. Sexual structures were not observed.

3.1.2.3. Isolate examined. Korea, Jeollabuk-do; Wanju-gun; Gosan-myeon; Gosanhuyangnim-ro (35°57′31″N 127°14′08″E) from an algae on May 17 2018 (NNIBRFG9359, W595) and Korea, Jeollabuk-do; Jeoksang-myeon; Sanseong-ro (35°57′36″N 127°41′43″E) from a decaying leaf on May 17 2018 (W630) by the authors.

Note: Isolates W630 and W595 are morphologically similar and phylogenetically close to ex-type strain CBS118360 "Phy. litorale", isolated from a lake in Germany [16]. Oogonia or oospores of Phy. litorale, previously known as a heterothallic or sexually sterile strain, were not found from single or dual cultures in a previous study [16], and similarly were not observed in the present isolate also. Phy. litorale was first isolated in the littoral soils of Constance lake in Germany [16], while the present isolate was found from the surface of an undetermined algae in a mountain stream. This study revealed a new habitat for Phy. litorale. The pathogenicity of Phy. litorale to algae or plants was not known.

3.1.3 Phytopythium vexans

3.1.3.1. Reference. Abad, de Cock, Bala, Robideau, Lodhi and Lévesque, Persoonia 34: 37 (2014) [MB#563322] (Figure 4).

3.1.3.2. Description. Colonies were colorless, with rapid growth and cottony aerial mycelia on V8A, slower growth on PDA, and submerged growth, forming a radiate pattern on CMA. After 72 h at 25 °C the colony diameter recorded was 35–40 mm on PDA, >70 mm on V8A, and 55–60 mm on CMA. Main hyphae extended up to 5 µm wide. Sporangia were globose, subglobose, ovoid, or pyriform and occasionally proliferating, intercalary, or terminal with an average of 20 × 18 µm in size. Oogonia were mostly terminal on short side branches, sometimes lateral, or intercalary, globose with an average of 19 µm in diameter.

3.1.3.3. Isolate examined. Korea, Jeollabuk-do; Imsil-gun; Seongsu-myeon; Seongsu-ri (35°38′05″N 127°25′16″E) from a decaying seed on September 5 2018 (NNIBRFG9360, W707; KACC48560, W708) and Korea, Jeollabuk-do; Imsil-gun; Seongsu-myeon; Sambong-ri (35°39′25″N 127°20′57″E) from a decaying leaf on September 5 2018 (NIBRFGC00 0502053, W714) by the authors.

Note: Three strains W707, W708, and W714, isolated from a decaying seed and leaf in a mountain stream, were identified as Phy. vexans (CBS11980) [24]. Phy. vexans was previously isolated from the plant hosts, such as avocado [44], grapevine [45], apple [46], and also from their rhizospheres. Some previous studies have demonstrated their possible pathogenicity [21]. Interestingly, Phy. vexans has been recorded also from freshwater [42], which is in line with the present result. Morphologically, this species is distinguishable by oogonia smaller than other Phytopythium species.

3.1.4. Pythium diclinum

3.1.4.1. Reference. Tokun., Transactions of the Sapporo Natural History Society 14(1): 12 (1935) [MB#263154] (Figure 5).

3.1.4.2. Description. Colonies were colorless and were found growing rapidly on PDA, V8A, and CMA at 25 °C, with little aerial mycelia on PDA and V8A and with submerged growth, forming a radiate pattern on CMA. After 72 h the colony diameter recorded was 60–65 mm on PDA, >70 mm on V8A, and 60–65 mm on CMA. Main hyphae were seen to extend up to 7 µm wide. Sporangia were filamentous or non-inflated and branched or unbranched. Vesicles varied from small containing 2 zoospores to large containing many zoospores. Encysted zoospores were 6–7 µm in diameter. Oogonia were spherical or ovoid, terminal or subterminal, and occasionally intercalary with an average of 20 µm in diameter. Oospores were aplerotic with 17–19 µm in diameter, and its wall thickness was up to 3 µm.

3.1.4.3. Isolate examined. Korea, Chungcheongbuk-do; Yeongdong -gun; Yonghwa-myeon; Jodong-ri (36°03′16″N 127°49′37″E) from a decaying leaf on May 24 2018 (NNIBRFG9363, W652; KACC48556, W654) by the authors.
Note: *P. diclinum* is characterized by filamentous, non-inflated sporangia, diclinous antheridia, and aplerotic oospore with a thick wall [21]. This species was originally found from rice plants [21], with a pathogenicity regarding damping-off disease of wheat [47], and in addition its pathogenicity to tomato seedling was proved using damping-off test [40]. Interestingly, *P. diclinum* was detected through a freshwater monitoring in Saudi Arabia. The present isolate was found from a decaying leaf in a mountain stream.

### 3.1.5. Pythium heterothallicum

#### 3.1.5.1. Reference.
W.A. Campb and F.F. Hendrix, Mycologia 68: 803 (1968) [MB#338125] (Figure 5).

#### 3.1.5.2. Description.
Colonies grew rapidly at 25°C, as a thin, colorless mat with limited surface mycelium and a radiating pattern on V8A, with a vague rosette pattern on PDA, and with a radiate pattern and some aerial mycelium on CMA. After 72 h the colony diameter was recorded as >70 mm on V8A, 20–25 mm on PDA, and 35–40 mm on CMA. Main hyphae extended up to 7 μm wide. Sporangia were globose, terminal or intercalary and grew up to 28 μm in diameter with a wall thickness of 1–2 μm.

#### 3.1.5.3. Isolate examined.
Korea, Jeollabuk-do; Muju-gun; Jeoksang-myeon; Bukchang-ri (35°57′36″N 127°41′43″E) from a soil sediment on May 24 2018.
3.1.6. Pythium inflatum

3.1.6.1. Reference. V.D. Matthews, Studies on the genus Pythium: 45 (1931) [MB#267884] (Figure 5).

3.1.6.2. Description. Colonies were colorless on PDA, V8A, and CMA at 25°C, with little aerial hyphae, forming a radiate pattern; on CMA with submerged growth. After 72 h the colony diameter recorded was 55–60 mm on PDA, >70 mm on V8A, and 55–60 mm on CMA. Main hyphae extended up to 4 μm wide. Sporangia were filamentous, inflated, often swelling, forming irregular or globose outgrowths and often grew out into vegetative hyphae or produced a vesicle and zoospores. Oogonia were globose, smooth and located terminal and intercalary in diameter of 21–25 μm in diameter. One to two antheridia were found per oogonium and were dichinous. Oospores were almost plerotic and 20–25 μm in diameter with a wall thickness of up to 3 μm.

3.1.6.3. Isolate examined. Korea, Jeollanam-do; Gwangsan-gu; Donggok-dong (35°04′59″N 126°46′35″E) from a decaying stem on June 29 2016 (CNFG_2028, W254; W257) by the second author.

Note: P. inflatum is characterized by dichinous antheridia [21]. This species is well-known as a major causal agent of maize stalk rot in China [50] but are also often found from the freshwater environment [51,52]. The present isolate was found from a decaying stem of an herbaceous plant in a pond environment.

3.1.7. Pythium intermedium

3.1.7.1. Reference. de Bary, Botanische Zeitung 39: 554 (1881) [MB#170773] (Figure 5).

3.1.7.2. Description. Colonies grew colorlessly on PDA, V8A, and CMA at 25°C, with aerial hyphae on PDA and V8A and with submerged growth, forming a vague radiate pattern on CMA. After 72 h at 25°C the colony diameter was recorded as >70 mm on PDA, >70 mm on V8A, and 50–55 mm on CMA. Main hyphae extended up to 7 μm wide. Sporangia were globose and grew up to 27 μm in diameter. Encysted zoospores were rarely observed. Hyphal swellings were abundant, terminal or intercalary, and catenulate hyphal swellings were found often.

3.1.7.3. Isolate examined. Korea Jeollabuk-do; Imsil-gun; Seongsu-myeon; Seongsu-ri (35°38′05″N 127°25′16″E) from a decaying leaf on September 5 2018 (NNIBRF9365, W704) by the authors.

Note: Abundant swelling and catenulate hyphae enable the discrimination of P. intermedium from two morphologically similar species, P. sylvaticum [21] and P. atrantheridium [53]. Records of sporangia formation of P. intermedium are rare [34,35]. P. intermedium inhabits diverse plant hosts and their rhizosphere [21], but in the present study it was detected from a decaying leaf in a mountain stream.

3.1.8. Pythium oopapillum

3.1.8.1. Reference. Bala, de Cock & Lévesque, Persoonia 25: 23 (2010) [MB#512818] (Figure 5).

3.1.8.2. Description. Colonies grew rapidly on PDA, V8A, and CMA at 25°C, forming a colorless, vague chrysanthemum pattern. After 48 h the colony diameter was recorded as 45–50 mm on PDA, 65–70 mm on V8A, and 65–70 mm on CMA. Main hyphae extended up to 4.5 μm in diameter. Sporangia were filamentous and inflated, consisting of lobate elements which give rise to vesicles containing 5–24 zoospores. Hyphal body was spherical and located terminal or intercalary. Zoospores were developed within vesicles, and the encysted zoospores were mostly 7–9 μm in diameter. Oogonia were globose and intercalary or subterminal with an average of 21 μm in diameter.

3.1.8.3. Isolate examined. Korea, Jeollabuk-do; Muju-gun; Jeoksang-myeon; Bukchang-ri (35°57′36″N 127°41′43″E) from an algae on September 5 2018 (NNIBRF9366, W631; KACC48552, W633) by the authors.
Note: *P. oopapillum* is close to *P. pachycaule* and *P. coloratum*, but distinguishable by the thick-walled and papillate oospores. This species was first isolated from the soils of cucumber, wheat, alfalfa, and corn fields in Canada [54], but also observed in the rivers of Ukraine and Poland [55], which is in line with the present isolate that was isolated from an undetermined alga in freshwater.

**Disclosure statement**

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