RESEARCH ARTICLE

High-Temperature-Tolerant Fungus and Oomycetes in Korea, Including *Saksenaea longicolla* sp. nov.

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ABSTRACT
Global temperatures are steadily increasing, leading to significant changes in microbial diversity and ecology. In the present study, we isolated high-temperature-growing fungi and fungi-like group (Oomycota) strains from freshwater environments of Korea and identified them based on cultural, morphological, and multilocus phylogenetic analyses. As a result, we introduce *Saksenaea* (Fungi) isolates as a new species, *Saksenaea longicolla* sp. nov. and record *Phytophthora chlamydospora* and *P. lagoariana* (Oomycota) new to Korea. In the growth experiments, they exhibited high-temperature tolerance, which can grow at 35–40 °C but become inactive at 4 °C and below. This study confirms the presence of high-temperature-tolerant fungi and oomycetes in Korea and suggests that the Korean climate conditions are changing in favor of these species. This indicates that climate warming is altering microbial distributions in freshwater environments.

1. Introduction

Global warming is altering microbial ecology, diversity [1–3], physiology [4–6], and interaction with abiotic and biotic factors [4,7,8]. For terrestrial Fungi and Oomycota (previously classified under Fungi, but now under Straminipila), climate-changing effects have been established concerning their growth, reproduction, physiology, and distributions [9–13]. However, the impact on the aquatic ecosystem, which is one of the important habitats for fungi and oomycetes, has been poorly studied, although it would result in changes in biotic communities and abiotic environmental factors [14–16]. Fungi and oomycetes are abundant in aquatic ecosystems where they play key roles in food web dynamics and carbon cycle [17–19]. They will give feedback to changes in climate gradient that may have a significant impact on aquatic environmental changes.

Ecological changes as a consequence of climate change on the Korean Peninsula are inevitable. According to the Intergovernmental Panel on Climate Change (IPCC), Korea can expect extended dry periods with warmer temperatures and frequent extreme precipitation events [20–22]. As such, the subtropical zone in Korea is expanding, which will produce significant changes in the ecosystem.

Interestingly, extreme cold events are also becoming more frequent [23], caused by the loss of Arctic sea ice and the change in the Arctic atmosphere [24–26]. This unprecedented phenomenon may give rise to considerable changes in Korea's ecosystems. To date, there were relatively few studies focusing on these ecological changes and even fewer on fungal and oomycete communities despite their crucial ecological roles.

Saksenaea, a filamentous fungal genus characterized by large, flask-shaped sporangia, is affiliated with Mucorales [27]. Since its first description from forest soil in India [27], members of *Saksenaea* have been isolated mainly in soil habitats, but also diverse environments such as on the coast in Costa Rica [28] and Taiwan [29], a banana plantation in Honduras [30], a groundnut field in Israel [31], a pineapple field in Okinawa, Japan [32], tree nurseries in Georgia, USA [33], and forests in Panama [34] and Brazil [35]. *S. dorisiae* was recently described in freshwater [36]. This species has been found only in the temperate continental region (the Republic of Serbia), while other members of *Saksenaea* described so far have been isolated in subtropical and tropical regions. Serbia is one of the countries experiencing a warming trend with climate change [37], but it seems to be premature to mention any effect of climatic change on *S. dorisiae*

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Supplemental data for this article can be accessed here.

because this species has been discovered in well water at a depth of 65 m [36]. It should be noted that *Saksenaea* species are often involved in severe human and animal mucormycosis in tropical and subtropical regions [38–40], certainly made possible because of their high-temperature tolerance, growing at 35–40 °C [35,41].

The genus *Phytophthora* belongs to the order Peronosporales of the Oomycota. *Phytophthora* is currently categorized in ten clades, according to their phylogenetic relationships, morphological and physiological characteristics [42]. Many species of *Phytophthora* are well known, such as the pathogens which cause sudden oak death [43], alder dieback [44], root rot [45], and late blight of potatoes and tomatoes [46]. *Phytophthora* species occur abundantly also in riparian ecosystems, irrespective of disease [47–51]. Especially, two phylogenetic clades, 6 and 9, of *Phytophthora* [42,52,53] include many saprotrophic species that are abundantly distributed in littoral zones of rivers and lakes, or forested streams [48,49,51,54]. They play important roles on food webs in aquatic ecosystems as a decomposer of plant debris or prey for zooplankton by trophic transfer to higher levels [19]. A unique part of these two clades is that they are characterized in terms of containing high-temperature-tolerant species [19,42,48,49,55]. Their optimum temperature ranges mostly from 25 to 30 °C, but they are capable of growing at 35–40 °C. The high-temperature-tolerant species have been discovered mostly in hot climates, or where the littoral zones can reach high temperatures [48,55–57], although there are some wide temperature-tolerant species within clade 6, e.g., *P. chlamydospora* [58], *P. riparia* [59], which have often been recorded even in a cold climate.

Our microbial survey discovered high-temperature-growing fungal and oomycete isolates from freshwater, soil sediment, and decaying plant leaf matter originating from the reservoirs and mountain streams of Korea. These isolates were investigated for cultural and morphological features, and their optimum and minimal/maximal temperatures for growth were determined. Molecular sequencing analyses were carried out to determine their phylogenetic positions using the internal transcribed spacer (ITS) and large subunit (LSU) rDNA, and translation elongation factor 1 (*TEF1α*) regions for *Saksenaea*, and cytochrome c oxidase subunit I (*cox*1) and subunit II (*cox*2) mtDNA regions for *Phytophthora*. As a result, a novel species of *Saksenaea* and two species of *Phytophthora*, previously unrecorded in Korea, are reported in the present study.

2. Materials and methods

2.1. Isolation of fungal and oomycete strains

Fungal and oomycete isolates were collected from freshwater environments in Korea, including water, soil sediment, and decaying plant leaves. The collection details are outlined in Table 1, and climate details for the collection sites are given in Supplementary Table S1. For *Saksenaea* isolates, a dilution plating method was used for soil samples at a 1:10 dilution. The diluted soil suspension was spread onto solid surfaces of two agar media: potato dextrose agar (PDA; Difco, Detroit, MI, USA) and V8 agar (V8A; 200 mL clarified V8 juice, 10 g $CaCO_3$, 15 g agar, 800 mL deionized water). The growth of bacteria and other fungi was suppressed by adding antibiotics to the media of *Saksenaea* isolates 5 ppm benomyl, 25 ppm nystatin, 100 ppm penicillin G, 10 ppm pimaricin, 100 ppm streptomycin, and 100 ppm chloramphenicol. For *Phytophthora* isolates, water samples were distributed to both agar plates using a simple plating technique. The decaying plant leaves were cut into 3–5 mm^2 pieces after washing with distilled water and were placed on both types of solid growth media. Bacterial and fungal growth was suppressed by adding 15 ppm rifampicin and 20 ppm nystatin

Table 1. Collection details and GenBank accession numbers for *Saksenaea* and *Phytophthora* isolates investigated in the present study.

Sequence ID	Culture	Species	Source	Location	GenBank Acc. No. *TEF1α*/ LSU/ ITS/*cox*1/*cox*2
C17	C17	*Saksenaea* sp.	Soil sediment in the reservoir	Jeollabuk-do, Gunsan	MW401666/MW391838/MW393835/-/-
Sak-06	Sak-06	*Saksenaea* sp.	Soil sediment in the reservoir	Jeollabuk-do, Gunsan	MW401667/MW391839/MW393836/-/-
Sak-07	NNIBRFG21789	*Saksenaea* sp.	Soil sediment in the reservoir	Jeollabuk-do, Gunsan	MW401668/MW391840/MW393837/-/-
Sak-19	Sak-19	*Saksenaea* sp.	Soil sediment in the reservoir	Jeollabuk-do, Gunsan	MW401669/MW391841/MW393838/-/-
Sak-21	KACC48577	*Saksenaea* sp.	Soil sediment in the reservoir	Jeollabuk-do, Gunsan	MW401670/MW391842/MW393839/-/-
W694	NNIBRFG9321	*Phytophthora chlamydospora*	Water in the mountain stream	Jeollabuk-do, Imsil	-/-/MW411353/MW411356
W655	NNIBRFG9322	*Phytophthora lagoariana*	Water in reservoir	Chungcheongnam-do, Yeongi	-/-/MW411351/MW411354
W675	KACC48557	*Phytophthora lagoariana*	Decaying leaf in the mountain stream	Jeollabuk-do, Namwon	-/-/MW411352/MW411355

to the media of *Phytophthora* isolates. The inoculated plates were incubated for 3 days at 25 °C in the dark. From the outgrowing mycelia, the new hyphal tips were isolated and transferred onto new agar plates. Representative cultures were deposited at the Nakdonggang National Institute of Biological Resources (NNIBR) or the Korean Agricultural Culture Collection (KACC).

2.2. Morphological analysis

Cultural characteristics were investigated 3 days after inoculation of the isolates onto PDA, V8A, corn meal agar (CMA; Difco), malt extract agar (MEA; Difco), and Czapek solution agar (CZA; Difco). Microscopic structures were observed and photographed using an Olympus BX53F microscope (Olympus, Tokyo, Japan) equipped with a DigiRetina 16 M digital camera (Tucsen, Fuzhou, China), and a Leica M205C microscope (Leica, Wetzlar, Germany) equipped with a Dhyana 400DC camera (Tucsen).

2.3. Determination of temperature range for radial growth

To determine the optimal and minimal/maximal temperatures for growth, three strains (Sak-07, W655, and W694) were inoculated by transferring five replicates for each isolate onto four different media (PDA, V8A, MEA, and CZA) at 4, 10, 15, 20, 25, 30, 35, 40, and 45 °C, with additional temperatures of 37 and 42 °C for the *Saksenaea* strain (Sak-07). The colony radial growth was measured every day for 7 days and expressed as mm/day. Relative growth rates and standard deviation were calculated in Microsoft Excel.

2.4. DNA extraction, amplification, and sequencing

Genomic DNA of the strains was extracted using the MagListo 5 M plant Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions. A MM400 mixer mill (Retsch GmbH, Haan, Germany) with glass beads was used to disrupt their culture tissues at 30 Hz/s for 15 min. PCR amplification of *Saksenaea* strains was performed for ITS region with primers ITS1 and ITS4 [60], LSU rDNA region with NL-1 and NL-4 [61], and *TEF1α* with MEF-11 and MEF-41 [62]. For *Phytophthora* strains, two mitochondrial genes, *cox*1 and *cox*2 mtDNA, were amplified with two oomycete-specific primer sets, OomCox1-levup and OomCox1-levlo [63] for *cox*1, and cox2-F [64] and cox2-RC4 [65] for *cox*2. DNA amplicons were

sequenced by Macrogen Inc. (Seoul, Korea) after their purification using the AccuPrep PCR Purification Kit (Bioneer).

2.5. Phylogenetic analysis

All sequences were edited using the DNAStar software package 5.05 (DNAStar, Inc., Madison, WI, USA). A BLASTn search was carried out to investigate the sequence similarities with their reference sequences in NCBI GenBank. Multilocus phylogenetic analysis was performed using a combined dataset of *TEF1α*, ITS, and LSU rDNA for *Saksenaea* isolates and using a dataset of *cox*1 and *cox*2 mtDNA sequences for *Phytophthora*. In addition, the reference sequences of the type of authentic isolates of *Saksenaea* and *Phytophthora* were included in each dataset. The sequences of the individual marker were aligned using MAFFT 7 [66] and then concatenated in SequenceMatrix v1.7.8 [67]. Phylogenetic trees were constructed in MEGA 7.0 [68]. For *Saksenaea* isolates, minimum evolution (ME) using the Kimura-2 model and maximum likelihood (ML) inferences using the General Time Reversible model were performed. For *Phytophthora* isolates, the Tamura-Nei model was used for both ME and ML analyses. Bootstrapping analysis was performed with 1000 replicates.

3. Results

3.1. Cultural and morphological analyses

Colonies of five *Saksenaea* strains (C17, Sak-06, Sak-07, Sak-19, and Sak-21) formed a radiate pattern on five agar media (PDA, V8A, MEA, CMA and CZA) at 25 °C in the dark and grew colorless with aerial hyphae (Figure 1); aerial hyphae were denser on PDA, V8A, and MEA than on CMA and CZA. Radial growth with a circular layer was observed on the reverse side of the colony on PDA. Hyphae were hyaline, sparsely septate, and smooth. Asexual structures, including sporangiophores, sporangia, and sporangiospores, formed only on CZA. The sporangiophores were erect, unbranched, and formed singly. The sporangia were hyaline, flask-shaped, multispored, and developed terminally. The sporangiospores were mostly bacilliform or narrow middle rod-shaped, but rarely trapezoid. The cultural and morphological characteristics were close to those of *S. dorisiae*, *S. oblongispora*, and *S. trapezispora*. However, the sporangiophores of *S. trapezispora* were significantly longer than those of other species, including the Korean strains. The sporangia of *S. oblongispora* were smaller than those of *S. dorisiae* and the Korean ones. *Saksenaea dorisiae* was distinguished from the present strains by the narrow
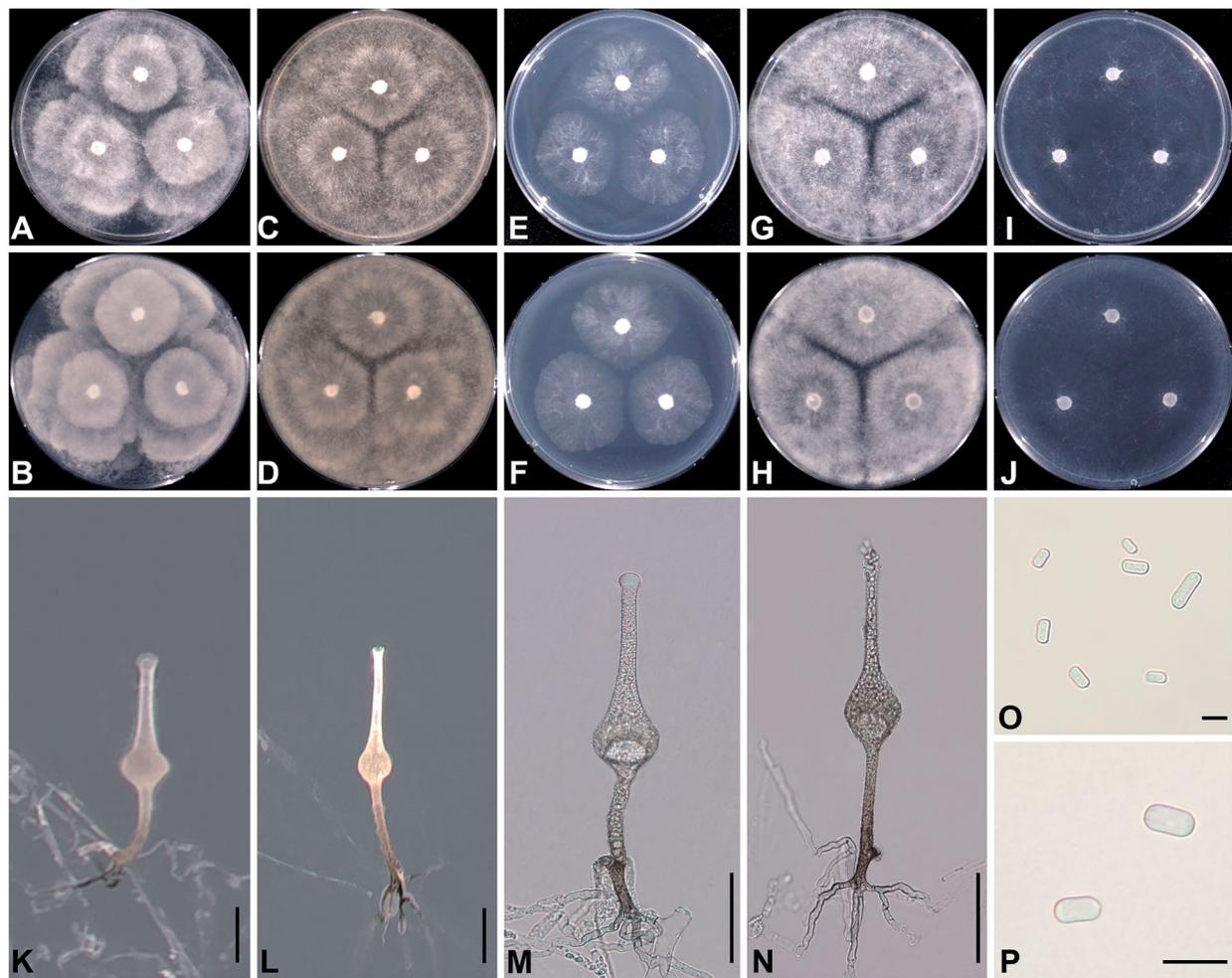
Figure 1. Cultural and morphological characteristics of *Saksenaea longicolla* sp. nov. NNIBRFG21789 (SAK-07) on PDA (A, B), V8A (C, D), CMA (E, F), MEA (G, H), and CZA (I, J) after 72 h at 25 °C (A, C, E, G, I: observed view; B, D, F, H, J: reverse view). Microscopic structures: sporangiophore under a stereoscopic microscope (K, L) and under a light microscope (M, N), sporangiospores (O, P). Scale bar = 50 μm for K–N, 5 μm for O and P.

Table 2. Morphology and hyphal growth temperature of *Saksenaea* strain Sak-07 and their morphologically close species.

Morphology characteristics	*S. trapezispora* (UTHSC DI 15-1)	*S. oblongispora* (CBS 133.90)	*S. dorisiae* (BiMM-F232)	Sak-07 (NNIBRFG21789)
Sporangiophores				
Length (μm)	150–230	80–100	(75–)85–100(–130)	(58–)78–135(–168) (av. 107)
Width (μm)	12–15	6–10	(6–)7–10(–12)	(5.1–)6.6–13.9(–21.7) (av. 10)
Sporangia				
Length (μm)	50–140	70–110	(70–)90–160(–190)	(57–)73–152(–198) (av. 113)
Width of columellae (μm)	–	–	15–35	(23–)32–54(–74) (av. 43)
Necks				
Length (μm)	30–100	60–90	70–100	(35–)53–114(–151) (av. 83)
Width (μm)	–	–	6–10	(6.8–)7.3–12.7(–18.1) (av. 10)
Sporangiospores				
Length (μm)	(4–)5.5–7.5(–8)	5–6.5	(4.5–)5.0–5.5(–6.0)	(5.0–)5.6–6.8(–7.9) (av. 6.2)
Width (μm)	(2.5–)3.5–4	3–4.5	(2.0–)2.5–3.0(–3.5)	(2.5–)2.8–3.5(–3.9) (av. 3.1)
Average (μm)	7 × 3.5	–	5.1 ± 0.4 × 2.8 ± 0.3	6.2 ± 0.6 × 3.1 ± 0.3
Growth temperature on CZA				
Lower limit	≥15 °C	≥15 °C	≥12 °C	≥10 °C
Upper limit	<40 °C	<42 °C	<40 °C	<42 °C
Optimum range	25–35 °C	25 °C	20–35 °C	25–37 °C

widths of sporangiophores, neck, and sporangia, and the somewhat smaller size of sporangiospores. The distinguishing morphological features between the Korean isolates and the closely related species of *Saksenaea* are summarized in Table 2.

All *Phytophthora* isolates grew colorless on PDA, V8A, CMA, MEA, and CZA at 25 °C in the dark, with fewer aerial hyphae. Colonies of W694 formed a radiate pattern on all media. Sporangia were non-papillate, ovoid to obpyriform, formed on

unbranched sporangiophores, and displayed commonly internal proliferation. Chlamydospores formed either terminally or intercalary and were observed abundantly at 25–30 °C. These characteristics were identical to those in the original description of *Phytophthora chlamydospora* [69]. The strains of W655 and W675 were identified as *Phytophthora lagoariana* based on the colony growth pattern and morphological features described by Wallace [70]. The colonies formed a vague rosaceous pattern on PDA, V8A, and MEA, but a radiate pattern on CMA and CZA. The strains grew submerged on CMA and CZA, while they formed a colorless mat on PDA, V8A, and MEA. These strains produced ovoid to obpyriform, non-papillate, and internally proliferating sporangia. Hyphal swelling was often observed, and chlamydospores were rarely produced. The colony patterns of *P. chlamydospora* (W694) and *P. lagoariana* (W655) on PDA,

V8A, and CMA, along with morphological characteristics, are shown in Figure 2.

3.2. Phylogenetic analysis

Based on a BLASTn search at the NCBI GenBank, the Korean isolates of *Saksenaea* were determined to be closest to *S. trapezispora* (UTHSC DI 15-1), with sequence similarities of 90% (229/254 bp) in ITS rDNA, 98% (687/703 bp) in LSU rDNA, and 99% (493/498 bp) in *TEF1α*. ME and ML analyses of a concatenated alignment of ITS, LSU, and *TEF1α* sequences were performed to infer the phylogenetic relationships between the Korean isolates and previously published *Saksenaea* species. As the ME and ML trees were congruent, only the ME tree is shown in Figure 3B. The multilocus tree revealed that the Korean isolates formed a distinct clade with high bootstrapping values in ME (100%) and ML (99%) analyses. They further grouped with *S. dorisiae, S.*
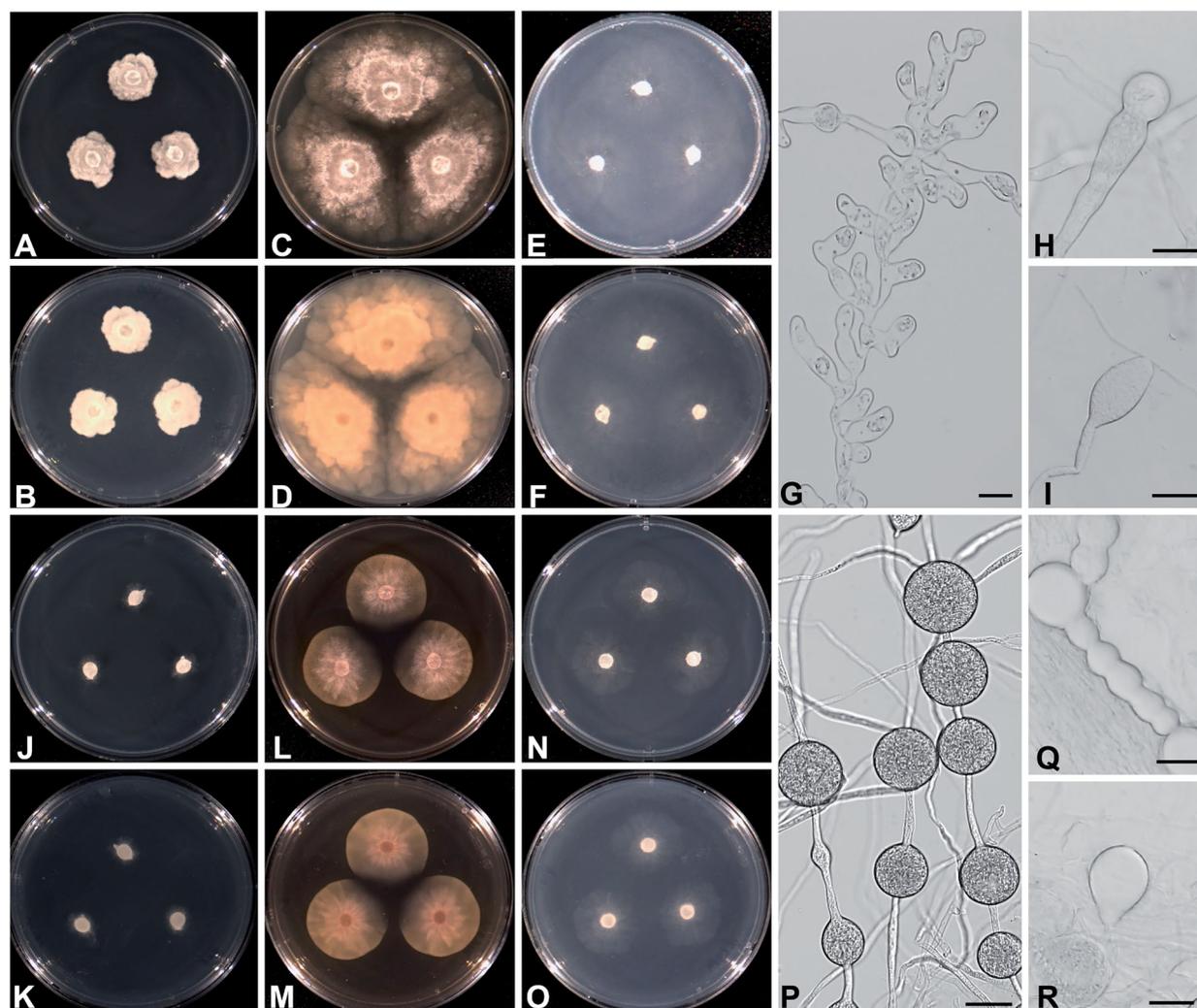
Figure 2. Cultural and morphological characteristics of *Phytophthora lagoariana* NNIBRFG9322 (W655) (A–I), *P. chlamydospora* NNIBRFG9321 (W694) (J–R) on PDA (A, B, J, K), V8A (C, D, L, M), and CMA (E, F, N, O) after 72 h at 25 C° (A, C, E, J, L, N: observed view; B, D, F, K, M, O: reverse view). Microscopic structures observed under a light microscope: hyphal swellings (G, H), and sporangium (I) of *P. lagoariana;* chlamydospores (P, Q), and sporangium (R) of *P. chlamydospora*. Scale bar = 13.4 μm for G and H, 27 μm for I, P-R.
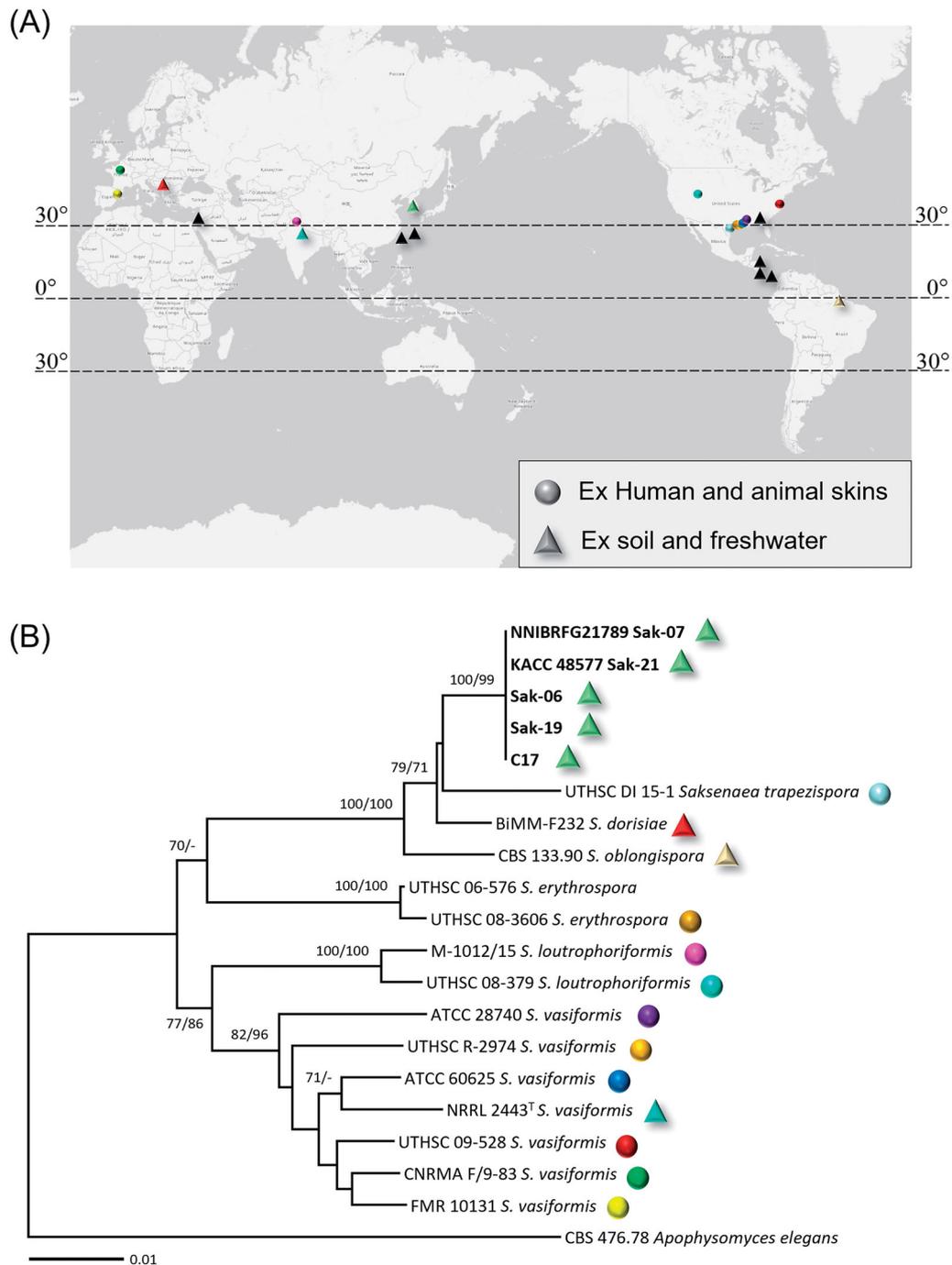
Figure 3. (A) Geographic distribution of *Saksenaea* species. Circle means the records of *Saksenaea* species or isolates from infected humans and animals. Triangle means the isolates from soil and freshwater, and black triangles mean the isolates with no sequence data in GenBank. (B) Multi-gene phylogenetic tree of *Saksenaea* species from the minimum evolution analysis of a concatenated alignment of three loci (*TEF1α*, ITS, LSU rDNA). Bootstrapping values (minimum evolution BP/maximum likelihood BP) higher than 70% were given above or below the branches (1,000 replicate). The strains isolated in Korea are shown in bold. *Apophysomyces elegans* was used as an outgroup. The scale bar equals the number of nucleotide substitutions per site.

oblongispora, and *S. trapezispora*, with the maximum supporting values in both analyses.

In BLASTn searching results for *Phytophthora* isolates, the *cox*2 (538 characters) and *cox*1 (640 characters) sequences of the Korean isolate W694 were identical to the authentic isolate CPHST BL156 (MH136867.1) of *Phytophthora chlamydospora* in *cox*1 sequence and P3176 (JF771548.1) in *cox*2. The isolates W655 and 675 matched the isolate P8223

(HQ261441.1 in *cox*1, HM534974.1 in *cox*2) of *Phytophthora lagoariana* with sequence similarities of 100% (634/634 bp) in *cox*1 and 99.8% (535/536 bp) in *cox*2. To infer the phylogenetic relationship between the authentic isolates of ten previously established clades of *Phytophthora* and the Korean isolates, ME and ML trees were constructed based on *cox*1 and *cox*2 mtDNA sequences. The phylogenetic tree based on the *cox*1 dataset is shown in
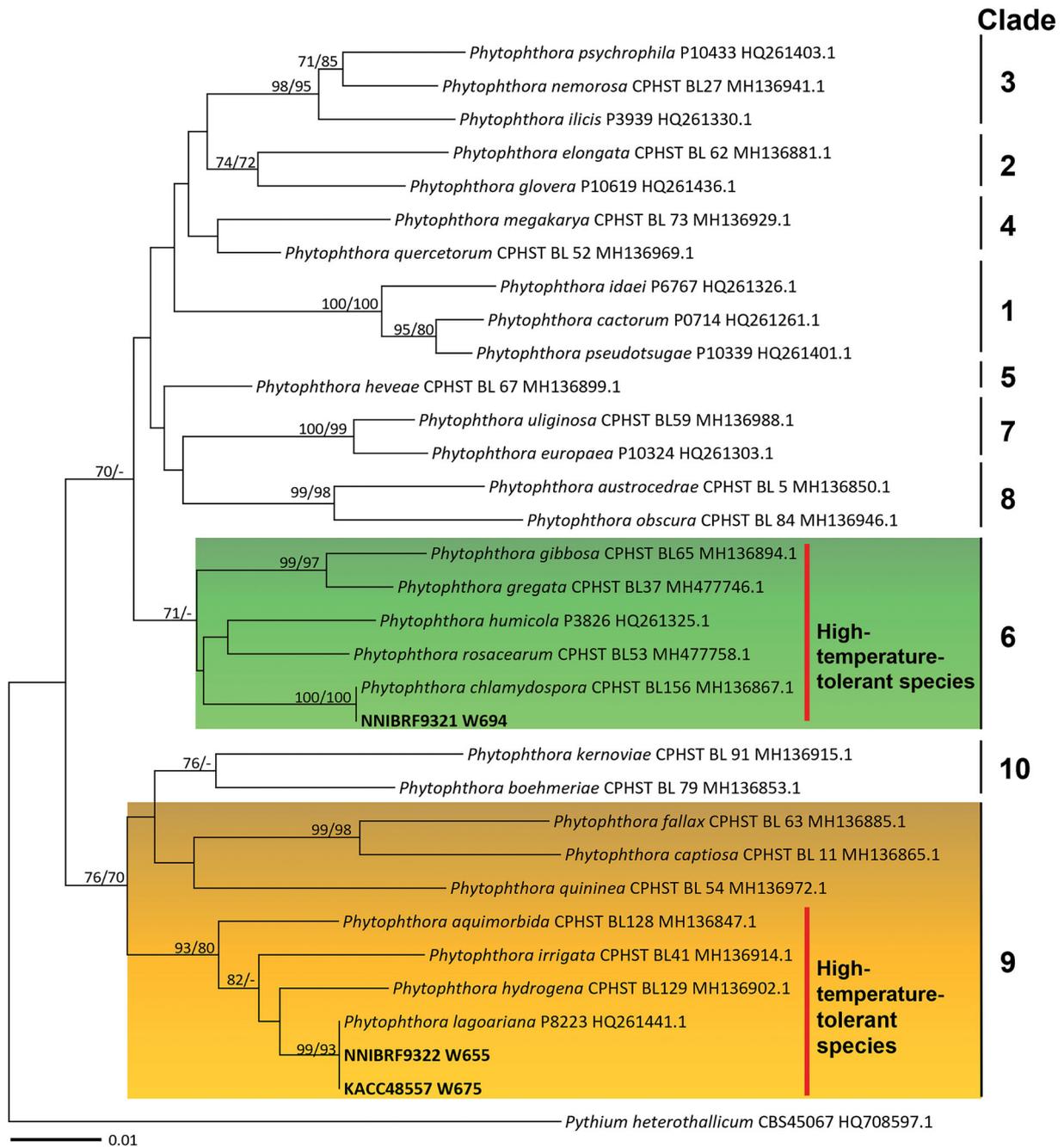
Figure 4. Phylogenetic tree of *Phytophthora* species from the minimum evolution analysis based on cytochrome oxidase subunit I mtDNA sequences. Bootstrapping values (minimum evolution BP/maximum likelihood BP) higher than 70% were given above or below the branches (1,000 replicate). The strains isolated in Korea are shown in bold. *Pythium heterothallicum* was used as an outgroup. The scale bar equals the number of nucleotide substitutions per site.

Figure 4. As the topologies constructed by both analyses were congruent, only the ME tree is presented. Three Korean isolates were placed in two different clades of *Phytophthora*, Clade 6 and Clade 9, with high supporting values. The strain W694 fell within *Phytophthora* Clade 6, which tolerates high temperatures, and further matched *P. chlamydospora* but was separated from non-chlamydospore-forming species [48,49]. The isolates of *P. lagoariana*, containing the Korean strains W655 and W675, formed a group with high-temperature-tolerant species of Clade 9. The optimum temperatures for these species range between 25 °C and 30 °C, but they can also grow at 35–40 °C [42,55].

3.3. Temperature range for radial growth

The strain Sak-07 of *Saksenaea* exhibited an optimum growth temperature ranging from 25 °C to 37 °C, with the lowest temperature at 10 °C
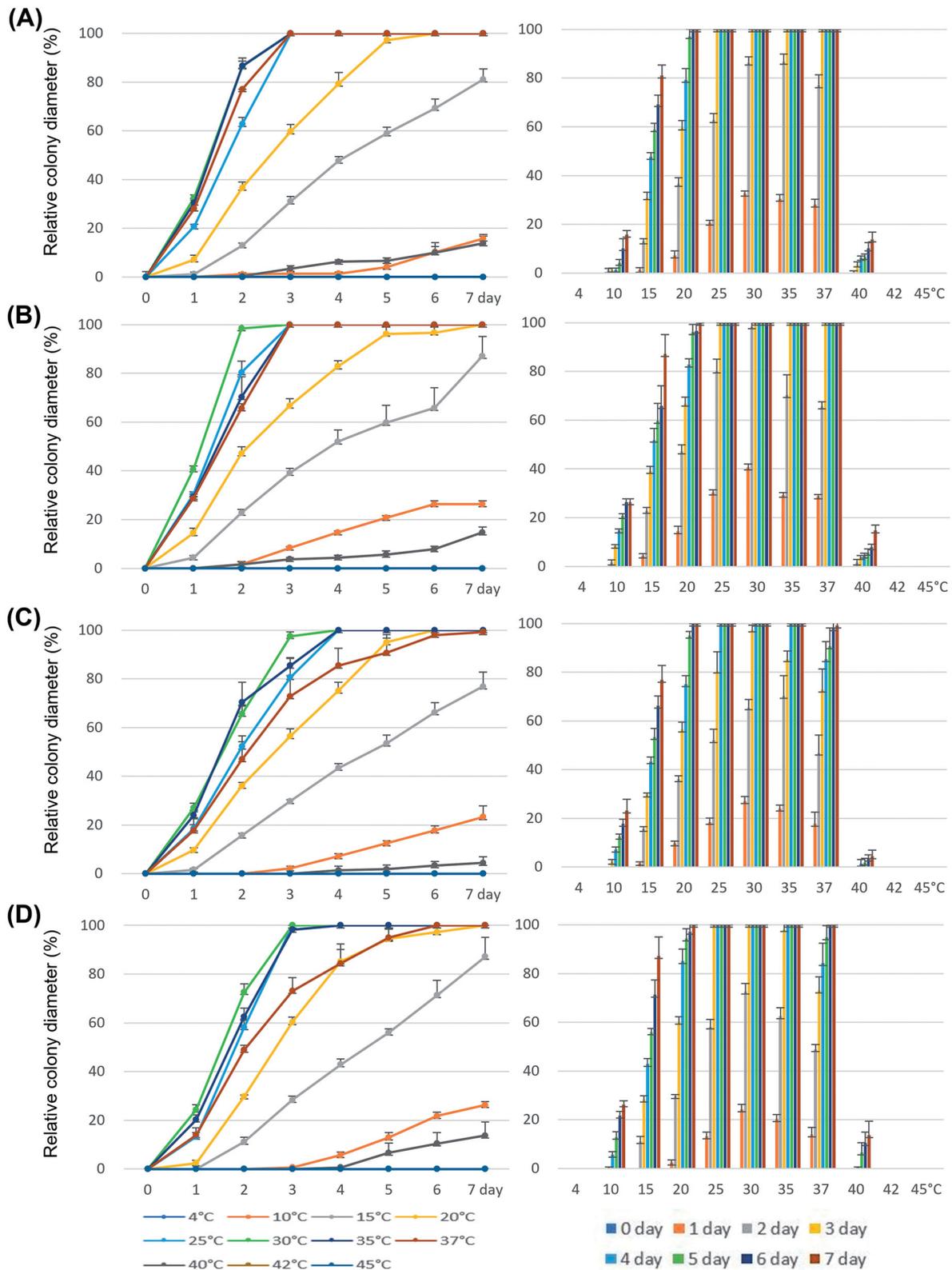
Figure 5. Relative growth rates of *Saksenaea longicolla* sp. nov. NNIBRFG21789 (SAK-07) for seven days at 4, 10, 15, 20, 25, 30, 35, 37, 40, 42, and 45 °C on four media, V8A (A), PDA (B), MEA (C), and CZA (D).

(reaching 20–30 mm in diameter after a week) and the highest at 40 °C (Figure 5). No growth was observed at 4 °C and 42 °C. Mean colony radial growth at 25 °C was observed to be 13.3 mm/day on PDA, V8A, and CZA, but 10 mm/day on MEA. On V8A and PDA, the colony grew as fast at 30–37 °C

as at 25 °C. On MEA and CZA, the growth rate was lower at 37 °C than at 25–35 °C. The growth at 15 °C was moderately fast (reaching 40–50 mm in 4 days).

For *P. lagoariana* strain W655, the optimum temperature ranged from 25 °C to 35 °C (Figure 6A). At
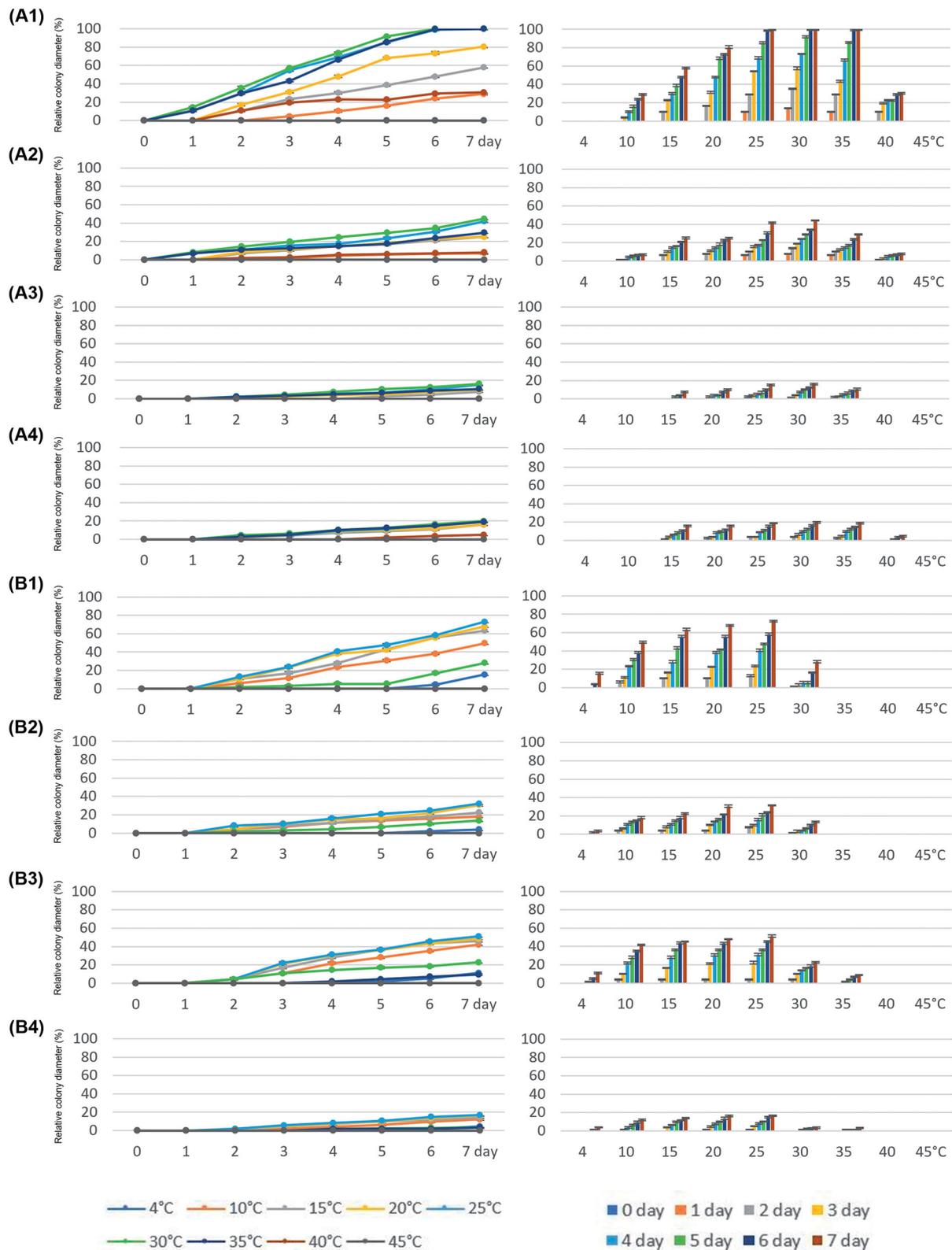
Figure 6. Relative growth rates of *Phytophthora lagoariana* W655 (A1–A4) and *P. chlamydospora* W694 (B1–B4) for seven days at 4, 10, 15, 20, 25, 30, 35, 40, and 45 °C on four media, V8A (A1, B1), PDA (A2, B2), MEA (A3, B3), and CZA (A4, B4).

25 °C, the strain grew fastest on V8A, with an average radial growth of 6.7 mm/day, but slowly on PDA (2.4 mm/day), MEA (0.9 mm/day), and CZA (1.1 mm/day). At 40 °C, the growth rate slowed down to 1.5 mm/day (V8A), 0.4 mm/day (PDA), and 0.2 mm/day (CZA), and no growth was observed on MEA. At 10 °C, it showed limited growth on V8A and PDA and no growth on MEA and CZA.

Compared to the W655, *P. chlamydospora* stain W694 grew substantially slower on all media (Figure 6B). The maximum growth temperature was 35 °C on MEA and CZA, and 30 °C on V8A and PDA. The optimum temperature for W694 ranged from

20 °C to 25 °C on all culture media. It was capable of growing at 4 °C (average 0.5 mm/day) as well as 10 °C (average 1.8 mm/day) on all culture media, although the growth rates were significantly lower. The growth rate of W694 was the highest at 25 °C on V8A. The colony radial growth on V8A was observed to be 4 mm/day at 25 °C. At 10 °C (2.6 mm/day) and 30 °C (1.4 mm/day); the colonies grew faster on V8A and MEA than on other media. At 30 °C on CZA, the strain showed limited growth (0.25 mm/day).

3.4. Taxonomy

Saksenaea longicolla D.J. Lee, B. Nam, & Y.J. Choi, sp. nov.
MycoBank: MB 838397

Etymology. From Latin "longus + collum", characterizing the long neck of sporangiophores.

Description. *Colonies* reaching 65–80 mm after 4 days of incubation on CZA at 37 °C, whitish, with wispy aerial mycelium and abundant sporangiophores. *Colonies* on V8A, PDA, and MEA at 37 °C showing floccose and whitish, with no sporulation. *Hyphae* sparsely septate, hyaline, branched, thin-walled, and measuring 10–12 μm wide. *Sporangiophores* erect, generally arising singly, brown, unbranched, and measuring (58)78–135(168) (average 107) μm long and (5.1)6.6–13.9(21.7) (average 10) μm wide, with distinct spinulose stipe and a profuse dichotomously branched rhizoidal complex. *Sporangia* terminal, multispored, hyaline, flask-shaped, asperulate, and measuring (57)73–152(198) (average 113) μm long and (23)32–54(74) (average 43) μm wide. *Neck* measuring (35)53–114(151) (average 83) μm long, (6.8)7.3–12.7(8.1) (average 10) μm wide, with the apex closed with a mucilaginous plug but dissolved when mature. *Sporangiospores* are mostly bacilliform but rarely trapezoid, somewhat narrower at the middle and measuring (5.0)5.6–6.8(7.9) (average 6.2) μm long, (2.5)2.8–3.5(3.9) (average 3.1) μm wide. *Zygospores* were not observed.

Holotypus. Korea; Jeollabuk-do; Gunsan-si; Miryong-dong (35°57'01"N 126°40'47"E), ex soil sediment in a freshwater reservoir, Sept. 18, 2018, D.-J. Lee and Y.-J. Choi, NNIBRFG21789 (=Sak-07).

Notes. Like other members of *Saksenaea*, *S. longicolla* is high-temperature tolerant. In terms of being unable to grow above 42 °C, *S. longicolla* is close to its phylogenetically related species, *S. trapezispora*, *S.*

oblongispora, and *S. dorisiae* [35,71]. However, the optimum (25–37 °C) and the maximum (40 °C) growth temperatures of *S. longicolla* are higher than those of *S. trapezispora* and *S. oblongispora*. In addition, *S. longicolla* grows slowly at 10 °C, while the latter two species are inactive [35,72]. Morphologically, *Saksenaea longicolla* is similar to *S. trapezispora* [72] and *S. oblongispora* [35], but distinguishable by the size of the sporangiophores, sporangia, and sporangiospores. Both BLASTn-based comparison and phylogenetic analysis of multilocus sequences supported this morphological identification. *Saksenaea* stains have been isolated mainly from the soil of diverse environments such as forest, beach, and grain and fruit fields, as well as animal skin infections. Similarly, *S. longicolla* was isolated from soil sediments of a freshwater reservoir. All species of *Saksenaea* described so far have a tropical and subtropical distribution in America [28,30,33,73,74], Australia [75,76] and southern Asia [29,32,38,77,78]. A warm-temperate site from which *S. dorisiae* has been recorded, the Republic of Serbia is experiencing warming trends [37], similar to a Mediterranean climate. Additionally, as mentioned above, *S. dorisiae* has been discovered in the water at a depth well [36], which is less affected by the air temperature. To date, there has been no record of *Saksenaea* species from cooler regions. Interestingly, the sporangiospores of *S. dorisiae* have been reported to be lively at low temperatures in water [36], which could provide a hint that *S. longicolla* originating from freshwater could survive the cold season of Korea. However, further research is needed to answer this question.

Phytophthora chlamydospora
Brasier, C. and E. Hansen, North American Fungi 10 (2): 3 (2015) [MB#809175].

Description. *Colonies* growing colorlessly and slowly on PDA, V8A, and CMA at 25 °C, with few aerial mycelia, showing a radiate pattern on CMA and V8A, submerged growth on CMA, petaloid on V8A. *Colony* diameter after 72 h reaching 20–25 mm on PDA, 30–35 mm on V8A, and 25–30 mm on CMA. *Sporangiophores* hyalin, unbranched. *Sporangia* sometimes sympodial, obpyriform or ovoid, often elongated, non-papillate, and measuring 45–65 μm long, 30–40 μm wide; internal proliferation. *Chlamydospores* intercalary or terminal, occasionally in sessile form and measuring 16–35 μm in diameter.

Isolate examined. Korea; Jeollabuk-do; Imsil-gun; Seongsu-myeon; Seongsu-ri (35°38'04"N,

127°24'52"E), ex freshwater, 5 Sept 2018, B. Nam and Y.-J. Choi (NNIBRFG9321=W694).

Notes. The present study combining morphological and phylogenetic data revealed that the isolate W694 is identical to *Phytophthora chlamydospora* [69]. The name *P. chlamydospora* was previously known informally as "*P.* taxon Pgchlamydo", but Hansen *et al.* [69] have redesigned this species based on a distinguishing feature of chlamydospore forming at a higher temperature. The Korean isolate also formed chlamydospores abundantly at 25–30 °C but not at 20 °C and below. In the present study, growth experiments verified that the Korean *P. chlamydospora* can grow at 35 °C but not at 40 °C, overlapping with a previous report that this isolate is usually able to survive at 36–37 °C [69]. Members of *Phytophthora* Clade 6, including *P. chlamydospora*, are high-temperature tolerant and related to freshwater environments [79,80], which is in line with the Korean one obtained from the freshwater of a mountain stream. *Phytophthora chlamydospora* is often found in irrigation water, rivers, streams, and riparian areas of forest in Australia [81], North America [51], Europe [82], Africa [50], and Asia [83]. Notably, this species is known as a pathogen and a saprophyte; in North America [84] and Turkey [85], it has been reported as a cause of root and crown rot of almonds. Remarkably, these potentially pathogenic species have inhabited the Korean peninsula.

Phytophthora lagoariana

Wallace, Sydney F. Diversity of Phytophthora Species in Costa Rica's Tropical Forest. University of Maryland (2015).

Description. *Colonies* growing colorlessly on PDA, V8A, and CMA at 25 °C. On V8A forming a colorless mat with limited surface mycelia and a vague rosaceous pattern. On PDA growing slowly, with a rosaceous pattern. On CMA forming a radiate pattern with some aerial mycelium. *Colony* diameter after 72 h reaching >70 mm on V8A, 20–25 mm on PDA, and 55–60 mm on CMA. *Sporangiophores* hyaline extended. *Sporangia* obpyriform or ovoid, often elongated, non-papillate, 40–56 μm long, 25–40 μm wide; internal proliferation. *Hyphal swelling* is often observed. *Chlamydospores* are rarely produced and measuring 21–33 μm in diameter.

Isolates examined. Korea; Chungcheongnam-do; Yeongi-gun; Seo-myeon; Gobok-ri (36°36'29"N, 127°14'47"E), ex freshwater, 1 Jun 2018, B. Nam and Y.-J. Choi (NNIBRFG9322 = W655). Korea; Jeollabuk-do; Namweon-si; Inwol-myeon; Inwol-ri

(35°27'08"N, 127°35'30"E), ex a decaying leaf in freshwater, 5 Sept 2018, B. Nam and Y.-J. Choi (KACC48557 = W675).

Notes. The isolates W655 and W675 were morphologically and phylogenetically identified as *P. lagoariana* [70]. These isolates fell within *Phytophthora* Clade 9, of which many species are high-temperature tolerant but also well adapted to aquatic environments, e.g., *P. hydrogena* [55], *P. irrigata* [86], and *P. aquimorbida* [56]. Similarly, the Korean isolates exhibited a high temperature tolerance, with an optimum growth temperature of 10–35 °C and were isolated from a freshwater reservoir and mountain stream. This species has been previously isolated from aquatic environments in the Cuyabeno Reserve in Ecuador and Carara National Park in Costa Rica [70]. This is the first record of *P. lagoariana* in a nontropical region.

4. Discussion

Here, we report that high-temperature-tolerant fungus (*Saksenaea*) and oomycetes (*Phytophthora*) are present in the temperate but cold winter areas of Korea. *Saksenaea* species and *Phytophthora lagoariana* has previously been reported only in the tropics and subtropics. It is still unclear how they are able to overcome the cold winter of Korea. In the present growth experiment, *S. longicolla* did not grow at 4 °C and below, and *P. lagoariana* stopped growing even at 10 °C. However, the mean winter temperature of the sampling locations (Gunsan, Yeongi, Namwon, and Imsil) of the present study is below 4 °C, with the lowest temperature ranging from −10 to −20 °C and frequent frost events.

Their presence could be related to climate change as the warming in the Korean peninsula continues to accelerate. According to the Korea Meteorological Administration, Korea's average temperature increased by 1.8 °C over the last century. Since 1910, Korea's summer has increased by a month, from 80–110 to 110–140 days. In the sampling locations, the temperature has increased by around 1–2 °C for the past 50 years, and extreme heat events have become more frequent. This is an indication that the subtropical zone, restricted to the southern islands and coast of Korea, is expanding to the north.

Another crucial question is whether *S. longicolla* and two *Phytophthora* species are indigenous to Korea or exotic species. Given that they still prefer to grow at a much higher optimal temperature (25–35 °C) than the yearly mean temperatures (12.0–13.5 °C) of Korea, it seems likely that they may have recently immigrated from other (sub-

)tropical or temperate regions, and then climate warming of Korea has made it easier for them to settle there. In addition, their ability to grow over a broad spectrum of temperatures (10–40 °C for *S. longicolla* and *P. lagoariana* and 4–35 °C for *P. chlamydospora*) may have facilitated more rapid adaptation.

It seems certain that the Korean peninsula's climate conditions are changing in favor of high-temperature-tolerant fungus and oomycetes, which could encourage their presence and dominance in the ecosystems. The freshwater environment is an essential habitat for fungus and oomycetes, which are influential as saprophytes and parasites in aquatic food web structure and dynamics. Their diversity and distribution patterns are impacted by environmental settings with climatic factors, e.g., temperature, precipitation [17,87] that can be variable in global climate change. Understanding their diversity and distribution patterns under climate change is essential, and further research focusing on their ecological and functional traits is required.

Author contributions

Conceived and designed the experiments: B.N., D.J.L. and Y.J.C.; Performed the experiments: B.N. and D.J.L.; Analyzed and interpreted the data: B.N., D.J.L. and Y.J.C.; Wrote the article: B.N., D.J.L. and Y.J.C. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Sequence data analyzed during the present study are available in GenBank.

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