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FUNGAL DISEASES

# Two new species, *Phytophthora nagaii* sp. nov. and *P. fragariaefolia* sp. nov., causing serious diseases on rose and strawberry plants, respectively, in Japan

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**Abstract** A new disease of rose was noticed in Chiba Prefecture of Japan in 1968, and the pathogen was initially identified as *Phytophthora megasperma* based on morphological characteristics. Similar *Phytophthora* isolates have since been collected from rose plants in Chiba, Kanagawa, and Shizuoka Prefectures. In 2005, several *Phytophthora* isolates were recovered from crowns of strawberry plants in Hokkaido Prefecture. These were considered to be members of a new species. In this study, we re-examined all these isolates using morphological and physiological studies and a multilocus phylogenetic analysis. The rose and strawberry isolates were mostly similar morphologically and physiologically, with some exceptions. The rose isolates differed significantly from *P. megasperma* sensu stricto and other related *Phytophthora* species. The rose and strawberry isolates had external proliferation of sporangia, characteristic funnel-shaped oogonia, predominantly paragynous antheridia, and fast growth rates of 10.5 mm/24 h at an optimum temperature of 28 °C. In the multilocus phylogenetic tree constructed using sequences from the rDNA ITS regions, rDNA LSU, and the translation elongation factor 1-α, β-tubulin and *cox*I genes, they formed a distinct monophyletic group in clade 7 with strong bootstrap support. The rose and strawberry isolates separated into two distinct groups. The results indicate that the rose and strawberry isolates constitute two separate species, designated here as *Phytophthora nagaii* and *P. fragariaefolia.*

**Keywords** Crown rot · Fast growing · Oomycetes · *Phytophthora* · Phylogeny · Rose · Strawberry

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## Introduction

Rose (*Rosa odorata*) is an important ornamental plant in Japan, where 16,800 ha of garden area are devoted to its growth (Ministry of Agriculture, Forestry and Fisheries, Japan, 2011). In 1968 in Yachiyo City of Chiba Prefecture, a new disease, characterized by wilting, leaf yellowing, and defoliation, was discovered in hydroponically grown rose seedlings (Nagai et al. 1978). Five years later, the same symptoms were observed in cuttings in a commercial greenhouse elsewhere in Chiba Prefecture. Subsequently, the disease was found in Shizuoka and Kanagawa Prefectures. In all these cases, pathogen isolates were collected and identified as *P. megasperma* on the basis of morphological characteristics (Nagai et al. 1978).

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*Phytophthora megasperma* was first described by Drechsler (1931) and characterized by its homothallism, paragynous antheridia, and relatively large oogonia and nonpapillate sporangia. Since then, any *Phytophthora* having these characteristics was initially identified as *P. megasperma* Drechsler. Tompkins et al. (1936) extended this concept by isolating smaller spores from other species. Later, many isolates collected from alfalfa, soybean, and red clover (Erwin 1965; Hildebrand 1959; Pratt 1981) were identified as *P. megasperma*. Hansen et al. (1986) studied all of these isolates and recognized that *P. megasperma* consisted of "six emerging biological species groups". These groups were ALF (pathogenic to alfalfa), SOY (pathogenic to soybean), CLO (pathogenic to clover), DF (pathogenic to Douglas-fir), AC (isolated from rosaceous fruit trees), and BHR (pathogenic to a broad range of hosts). Later, the first three groups were recognized as *P. trifolii*, *P. sojae*, and *P. medicaginis*, respectively, by Hansen and Maxwell (1991). The BHR group was recognized as *P. megasperma* sensu stricto (Cooke et al. 2000), DF as *P. sansomeana*, and AC as *P. rosacearum* (Hansen et al. 2009). In the same study, Hansen et al. (1986) characterized two Japanese rose isolates as a unique group, different from these six groups of the *P. megasperma* complex.

Another important crop, strawberry (*Fragaria* × *ananassa*), is extensively cultivated in greenhouses in Japan. In 2005, crown rot symptom appeared in the young seedlings of strawberry in a field in Hokkaido. The causal agent was temporarily named *Phytophthora* sp. *fragaefolia* based on morphological features and a sequence analysis of its rDNA ITS regions (Shirai et al. 2006).

In oomycetes such as *Phytophthora*, species identification based on morphological characteristics alone is not always reliable, because the morphological characters are variable and overlapping. Recent molecular techniques provide more rapid and accurate methods for species identification and delimitation in *Phytophthora*. In the first comprehensive phylogenetic study of *Phytophthora* by Cooke et al. (2000), 10 clades were proposed, and this was supported by subsequent studies (Blair et al. 2008; Kroon et al. 2004, 2012; Naher et al. 2011).

The first objective of this study was to characterize and compare the rose isolates collected at different times and locations to determine their similarity with one another and with *P. megasperma* sensu stricto. The second objective was to characterize the strawberry isolates collected at different times to determine their similarity with one another. Finally, we used both morphological characteristics and a multilocus phylogenic study to identify the *Phytophthora* species group(s) for the rose and strawberry isolates.

## Materials and methods

### Collection and maintenance of isolates

Twenty-four isolates were examined in this study (Table 1). Twelve isolates were collected from basal stems and root of rose plants in Chiba, Kanagawa, and Shizuoka Prefectures between 1968 and 2004. Twelve isolates were collected from diseased crowns of strawberry plants in Hokkaido in 2005. All isolates were maintained on corn meal agar (CMA; 20 g of corn meal, 1 L of water, steamed for 30 min, extracted by filtering, added to 20 g agar in 1 L of water and autoclaved for 1 h at 121 °C, at 20–25 °C until use).

### Morphology and growth rate

Three rose isolates (NBRC 109131, NBRC 109137 and NBRC 109139) that were collected at various times (2004, 2000, and 1974, respectively) and two isolates (MAFF 244054 and NBRC 109138) that were collected from different locations (Kanagawa and Shizuoka) were chosen for morphological characterization. In addition, four strawberry isolates were selected: HSP502 and NBRC 109709 were from strawberry cultivar Summer rubi in Kinausu, Kuriyama, Hokkaido and HSP506 and NBRC 109712 from cultivar Tochiotome in Minami Gakuden, Kuriyama, Hokkaido. Asexual and sexual structures were produced in grass leaf blade cultures (Waterhouse 1967). Autoclaved grass blades were placed on V8 agar [V8A; 200 mL V-8 juice (Campbell Soup Co., Camden, USA), with 3 g of $CaCO_3$ was centrifuged for 5 min at 21,880×*g*, then 20 g agar was mixed with the supernatant and brought to 1L with distilled water] with one of the isolates. After 2 days incubation at 25 °C, the colonized blades were transferred to autoclaved pond water (1:2 pond water to distilled water) and incubated at 20 and 25 °C. Especially for the strawberry isolates, to induce the formation of sporangia, water was changed every day and incubated at 20 °C. Sexual structures were also observed on V8A and CMA. Growth patterns were observed on V8A, CMA, and potato dextrose agar (PDA) after 6 days incubation at 20 °C in the dark. Mycelial growth rates were measured on three replicate plates of V8A medium. For each replicate, a 6-mm mycelial disk, collected from the margin of a colony on V8A, was placed at one edge of a round plate. The plates were incubated at 3, 5, 10, 15, 20, 25, 28, 30, 33 and 35 °C. Colony size was measured after 6 days.

### DNA extraction

The isolates were allowed to grow for 7 days on V8A at 25 °C. A small piece of a mycelial mat from the advancing margin was collected in 100 µL of 50 % (v/v) PrepMan

**Table 1** Voucher information for rose and strawberry isolates sequenced for molecular phylogenetic analysis in this study

| Isolate no. | NBRC no. | MAFF no. | CBS no. | Other no. | Year of collection | Place of collection in Japan | Host | Cultivar | DNA database accession numbers | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | rDNA ITS | rDNA LSU | *cox*I | β-tubulin | EF1-α |
| CH04PHR11 | | MAFF 244046 | | | 2004 | Chiba | Rose | | AB688355 | AB688498 | AB688225 | AB736247 | AB736246 |
| CH04PHR12[a] | NBRC 109131 | MAFF 244047 | CBS 133248 | | 2004 | Chiba | Rose | | AB688356 | AB688499 | AB688226 | AB736249 | AB736248 |
| CH94PHR1 | NBRC 109132 | MAFF 244048 | | | 1994 | Chiba | Rose | | AB688357 | AB688500 | AB688227 | AB736251 | AB736250 |
| CH95PHR10 | NBRC 109133 | MAFF 244049 | | | 1995 | Chiba | Rose | | AB688358 | AB688501 | AB688228 | AB736253 | AB736252 |
| CH95PHR16 | NBRC 109134 | MAFF 244050 | | | 1995 | Chiba | Rose | | AB688359 | AB688502 | AB688229 | AB736255 | AB736254 |
| CH95PHR17 | NBRC 109135 | MAFF 244051 | | | 1995 | Chiba | Rose | | AB688360 | AB688503 | AB688230 | AB736257 | AB736256 |
| CH00MKR1 | NBRC 109136 | MAFF 244052 | | | 2000 | Chiba | Rose | | AB688361 | AB688504 | AB688231 | AB736259 | AB736258 |
| CH00MKR2 | NBRC 109137 | MAFF 244053 | | | 2000 | Chiba | Rose | | AB688362 | AB688505 | AB688232 | AB736261 | AB736260 |
| CH02PHR1 | | MAFF 244054 | | | 2001 | Kanagawa | Rose | | AB688363 | AB688506 | AB688233 | AB736263 | AB736262 |
| CH97PHR1 | NBRC 109138 | MAFF 244055 | | | 1997 | Shizuoka | Rose | | AB688364 | AB688507 | AB688234 | AB736265 | AB736264 |
| P-A | | MAFF 244056 | | P6466 | 1968 | Chiba | Rose | | AB688365 | AB688508 | AB688235 | AB736267 | AB736266 |
| P–B | NBRC 109139 | MAFF 244057 | | | 1974 | Chiba | Rose | | AB688366 | AB688509 | AB688236 | AB736269 | AB736268 |
| HSP501 | | | | MSm1 | 2005 | Kuriyama, Hokkaido | Strawberry | Summer rubi | AB305060 | AB761576 | AB761570 | AB761588 | AB761582 |
| HSP502 | | | | KSm1 | 2005 | Kuriyama, Hokkaido | Strawberry | Summer rubi | AB305061 | AB761577 | AB761571 | AB761589 | AB761583 |
| HSP503 | | | | KSt1 | 2005 | Kuriyama, Hokkaido | Strawberry | Sachinoka | AB305062 | AB761578 | AB761572 | AB761590 | AB761584 |
| HSP504 | | | | KSt2 | 2005 | Kuriyama, Hokkaido | Strawberry | Sachinoka | AB305063 | AB761579 | AB761573 | AB761591 | AB761585 |
| HSP505 | | | | KSt3 | 2005 | Kuriyama, Hokkaido | Strawberry | Sachinoka | AB305064 | AB761580 | AB761574 | AB761592 | AB761586 |
| HSP506 | | | | Mto1 | 2005 | Kuriyama, Hokkaido | Strawberry | Tochiotome | AB305065 | AB761581 | AB761575 | AB761593 | AB761587 |

**Table 1** continued

| Isolate no. | NBRC no. | MAFF no. | CBS no. | Other no. | Year of collection | Place of collection in Japan | Host | Cultivar | DNA database accession numbers | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | rDNA ITS | rDNA LSU | *cox*I | β-tubulin | EF1-α |
| CH05NSU11[a] | NBRC 109709 | MAFF 244058 | CBS 135747 | | 2005 | Kuriyama, Hokkaido | Strawberry | Summer rubi | AB819580 | AB819581 | AB819582 | AB819579 | AB819578 |
| CH05NSU21 | NBRC 109710 | MAFF 244059 | | | 2005 | Kuriyama, Hokkaido | Strawberry | Summer rubi | AB819585 | AB819586 | AB819587 | AB819584 | AB819583 |
| CH05NSU31 | NBRC 109711 | MAFF 244060 | | | 2005 | Kuriyama, Hokkaido | Strawberry | Summer rubi | AB819590 | AB819591 | AB819592 | AB819589 | AB819588 |
| CH05MT11 | NBRC 109712 | MAFF 244061 | | | 2005 | Kuriyama, Hokkaido | Strawberry | Tochiotome | AB819600 | AB819601 | AB819602 | AB819599 | AB819598 |
| CH05MT31 | NBRC 109713 | MAFF 244062 | | | 2005 | Kuriyama, Hokkaido | Strawberry | Tochiotome | AB819595 | AB819596 | AB819597 | AB819594 | AB819593 |
| CH05MT40 | NBRC 109714 | MAFF 244063 | | | 2005 | Kuriyama, Hokkaido | Strawberry | Tochiotome | AB819605 | AB819606 | AB819607 | AB819604 | AB819603 |

[a] Type isolates

Ultra Reagent (Applied Biosystems, Foster City, USA) and heated to 100 °C for 10 min. After 3 min at room temperature, the sample was centrifuged at 21,880×*g* for 3 min. The supernatant was transferred to another tube containing 100 µL TE buffer (10 mM Tris–HCl, pH 7.5 and 0.1 mM EDTA) for PCR.

## DNA amplification

The rDNA ITS regions, rDNA large subunit (LSU) genes, and the genes for translation elongation factor (EF) 1-α, β-tubulin and cytochrome *c* oxidase subunits (*cox*I) were amplified using the primer sets listed in Table 2. For the rDNA ITS and rDNA LSU PCRs, we used 25 µL reaction mixtures containing 1 µL DNA, 0.2 µM of each primer, 0.4 mg/mL BSA, 0.2 mM dNTP, 0.625 U of TaKaRa *Taq* DNA polymerase (Takara Bio, Otsu, Japan), and PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$). The reactions for the EF1-α, β-tubulin and *cox*I genes were the same except that 0.4 mM dNTP and 2 µM of each primer were used. The PCR reactions were carried out with a 2700 DNA thermal cycler (Applied Biosystems). The reaction sequence consisted of 3 or 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, annealing at the appropriate temperature (Table 2), and extension for 1 min at 72 °C, and a final extension for 10 min at 72 °C. PCR product sizes were confirmed using gel electrophoresis.

## DNA sequencing

The PCR products were purified using the GeneElute PCR clean-up kit (Sigma-Aldrich, St. Louis, MO, USA) and the manufacturer's instructions. Each 10 µL sequencing mix contained 1 µL purified PCR product, 1 µL primer (25-fold dilution of primer used in first PCR), 4 µL Ready Reaction Mix (Applied Biosystems) and 4 µL of sterile distilled $H_2O$. Sequencing was then performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), with the following thermocycler program: 96 °C for 1 min; followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min; and a final incubation at 10 °C. The reaction products were purified by ethanol precipitation then analyzed using an ABI 3100 DNA Sequencer (Applied Biosystems). The sequences were edited using the ChromasPro version 1.33 software (Technelysium Pty Ltd., Tewantin, Australia), and the consensus sequences were used for alignment analysis. The DNA sequence data were deposited in the DDBJ (accession numbers shown in Table 1).

## TA cloning

In some cases, when it was difficult to obtain clear data by direct sequencing of the PCR products, the purified

**Table 2** Primers used for DNA amplification and sequencing in this study

| Locus | Primer pair | Primer sequence (5′-3′) | Annealing conditions | | References |
|---|---|---|---|---|---|
| | | | Temperature (°C) | Time (s) | |
| rDNA ITS + rDNA LSU | UN_UP18S42 | CGTAACAAGGTTTCCGTAGGTGAAC | 68 | 45 | Bakkeren et al. (2000) |
| | UN_Lo28S576B | CTCCTTGGTCCGTGTTTCAAGACG | | | |
| rDNA ITS | ITS2[a] | GCTGCGTTCTTCATCEATGA | 55 | 30 | White et al. (1990) |
| | ITS4[a] | TCCTCCGCTTATTGATATGC | | | |
| rDNA LSU | NL1 | GCATATCAATAAGCGGAGGAAAAG | 55 | 60 | O'Donnell (1993) |
| | NL4 | GGTCCGTGTTTCAAGACGG | | | |
| *cox*I | FM85-mod2 | CYTCHGGRTGWCCRAAAAACCAAA | 52 | 30 | Robideau et al. (2011) |
| | Oom-COI-Lev-up | TCAWCWMGATGGCTTTTTTCAAC | | | |
| β-tubulin | TUBUF2_for | CGGTAACAACTGGGCCAAGG | 60 | 30 | Kroon et al. (2004) |
| | TUBUR1_rev | CCTGGTACTGCTGGTACTCAG | | | |
| Translation elongation factor 1-α | ELONGF1_for | TCACGATCGACATTGCCCTG | 60 | 30 | Kroon et al. (2004) |
| | ELONGR1_rev | ACGGCTCGAGGATGACCATG | | | |
| TA cloning | M13M4 | GTTTTCCCAGTCACGAC | 55 | 30 | |
| | M13Rv | CAGGAAACAGCTATGAC | | | |

[a] Primers used for sequencing only

products were cloned in the pT7Blue T (TA cloning) vector (TaKaRa Bio) according to the manufacturer's instructions. The cloned region was amplified using primer set M13M4 and M13Rv. The thermocycler program for amplification was 94 °C for 5 min; followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; with a final extension at 72 °C for 10 min. The products were sequenced as described earlier.

## Phylogenetic analysis

One representative isolate was selected for various representative species of the genus *Phytophthora*, and sequence data for these isolates were obtained from the NCBI. The accession number for each sequence is listed in Supplemental Table 1. We covered all species that either have been described or are awaiting description and that have sequence data available for the rDNA ITS regions, rDNA LSU, EF1-α, β-tubulin and *cox*I genes. Blair et al. (2008) provided a phylogenetic analysis of *Phytophthora* species that is well accepted by *Phytophthora* researchers; we thus selected all of our international representative isolates from that study. Finally, a combined tree was constructed using the sequences of these 5 regions or genes. The analysis included 65 representative *Phytophthora* species and the 12 rose and 12 strawberry isolates. *Pythium vexans* (P3980) was used as the outgroup.

All sequences were first aligned using the Clustal X multiple sequence alignment software (Thompson et al. 1997). Phylogenetic analysis was performed by maximum parsimony (MP) and neighbor joining (NJ) with the PAUP* version 4.0b10 software (Swofford 2002). The MP analysis was performed for 1000 replications with different random starting points using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Alignment gaps were treated as missing data, and all characters were unordered and had equal weight. Tree bisection and reconstruction was used as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The best tree topology of the MP trees was established using the Kishino–Hasegawa likelihood test (Kishino and Hasegawa 1989) with PAUP*. The trees have been deposited in the TreeBASE database (accession S14497; http://treebase.org).

## Pathogenicity tests

Two rose isolates (NBRC 109131 and NBRC 109138) and two strawberry isolates (HSP501 and HSP503) were each used for pathogenicity test on both rose and strawberry. For the rose inoculations, the four isolates were grown in PD broth at 25 °C in the dark for 4 days. Rose cuttings (4 for each replication) were then placed in plastic packs

containing water (4 cm depth) such that the basal parts of the cuttings were submerged. A mycelial mat from the PD cultures was then added to the water in each pack, and the packs were incubated at 25 °C for 4 days. For the strawberry inoculations, hemp seeds (25 each) were incubated on PDA plates with each isolate for 3 days. Strawberry seedlings (25 for each replication) were placed in trays containing 3 L of water, and the hemp seeds were added to the water. The inoculated seedlings were incubated at 21 °C for 4 days.

The experiments were conducted with three replications. Disease severity was calculated according to the formula of Watanabe et al. (2007; see notes in Table 5).

## Results

### Phylogenetic analysis

The phylogenetic tree constructed by MP, based on the sequences of the rDNA ITS regions, rDNA LSU, EF1-α, β-tubulin, and *cox*I genes, was remarkably similar to those obtained in previous studies (Blair et al. 2008; Cooke et al. 2000; Kroon et al. 2012; Naher et al. 2011). Ten distinct clades were formed (Fig. 1). Our 24 isolates (12 rose and 12 strawberry) formed a separate monophyletic group in clade 7 with high bootstrap support of 100. We found high levels of homology (99.6 %) between the rose and strawberry isolates in their rDNA ITS regions. However, the rose and strawberry isolates positioned separately within this group with bootstrap values of 97 and 99.8, respectively. In the NJ tree (figure not shown), the rose and strawberry isolates formed similar groups with high bootstrap values of 97 and 99.9, respectively. In a sequence variation analysis, all rose isolates showed nearly identical sequences with one another and were identified as the strawberry isolates. Between the two groups, there were some sequence differences: 3 bp in the rDNA ITS regions, 3 bp in the EF-1α gene, 3 bp in the β-tubulin gene, and 6 bp in the *cox*I gene. All of the rDNA LSU sequences were identical. Both rose and strawberry isolates were phylogenetically referred to as new species, designated as *P. nagaii* and *P. fragariaefolia*, respectively.

### Taxonomic description of *Phytophthora nagaii*

*Phytophthora nagaii* M. Z. Rahman, S. Uematsu, T. Takeuchi, K. Shirai & K. Kageyama, sp. nov. (MycoBank MB 804991) (Figs. 1, 2, 3, 4, 5, 9)

Etymology: *nagaii* = Y. Nagai, the first to isolate this oomycete.

Type: Japan, Chiba Prefecture, from leaf and stem blight of rose (*Rosa* sp.), 1968, collector S. Uematsu. Isolate NBRC H-13102-holotypus (freeze-dried specimen). Ex-type strains are NBRC 109131 = CBS 133248. The DDBJ accession numbers of the DNA sequences of the rDNA ITS regions, rDNA LSU, EF1-α, β-tubulin, and *cox*I genes are: AB688356, AB688499, AB736248, AB736249, and AB688226, respectively.

The species produced typically nonpapillate, noncaducous sporangia abundantly in grass blade cultures. These terminal sporangia were mostly single (Fig. 2a, b) and less frequently in simple sympodia (Fig. 2c). The sporangia were usually ellipsoid (Fig. 2a) often with tapering bases and eccentric basal sporangiophore attachment points (Fig. 2b). The lengths and widths of the sporangia ranged from 26.8–79 μm × 23.7–55.4 μm (mean 46.7 ± 13.8 μm × 35.4 ± 7.8 μm), and the length to width ratio was 1:1.35. Zoospores were discharged through large exit pores of 14.7 ± 5.5 μm (range 6.7–24.6 μm) diameter (Fig. 2d–e). The zoospores were limoniform to reniform when motile and became spherical upon encystment. Sporangia proliferated internally, either extended (Fig. 2g) or nested (Fig. 2h), or externally (Fig. 2i). Spherical, intercalary chlamydospores produced in water cultures after 45 days (Fig. 2j). The thin walled (1–2 μm) chlamydospores were around 33 μm (range 30–40 μm) in diameter. Mostly lateral, rarely intercalary and terminal hyphal swellings were produced in both grass blade cultures and on agar media. Hyphal swellings were spherical (Fig. 2k, 1 left), 25 ± 9 μm (range 21–28 μm) in diameter, or subspherical (Fig. 2l right).

The species was homothallic; gametangia were produced abundantly in single grass leaf blade cultures, CMA cultures, and V8A cultures. Oogonia were produced terminally (Fig. 3a), often laterally sessile (Fig. 3b) and intercalary (Fig. 3c). They were mostly spherical or nearly spherical, and occasionally funnel-shaped with tapering bases and short stalks (Fig. 3d). Elongated, curved oogonial stalks were also observed (Fig. 3e). The oogonia were 40 ± 4.7 μm (range 32.2–53.3 μm) in diameter. Oospores were aplerotic, with an average diameter of 37.6 ± 4.3 μm (range 29.5–47.7 μm). The oospore walls were about 3.3 ± 0.5 μm thick. The antheridia were predominantly paragynous (Fig. 3f–k) and sometimes amphigynous (Fig. 3l) along with an occasional finger-like protrusion (Fig. 3k). They were spherical (Fig. 3f–h), or ellipsoid (Fig. 3k), with mean lengths and widths of 19.1 ± 3.5 and 13.5 ± 1.2 μm (ranges 13.5–28.1 and 10.7–15.3 μm). They were occasionally irregular in shape (Fig. 3c). Generally, one (Fig. 3h) and occasionally, two antheridia (Fig. 3i) fused laterally with an oogonium. The colony was radial with aerial mycelia and smooth growth all over the plate (Fig. 4a–c). The growth was fast on V8A medium with a rate of 11.8 mm/24 h at the optimum temperature of 28 °C (Fig 5). On V8A, the isolate grew at all temperatures between and including 5 and 33 °C.


**Fig. 1** Phylogenetic tree showing the relationships of *Phytophthora nagaii* and *P. fragariaefolia* within the genus *Phytophthora*, based on five nuclear sequences (rDNA ITS regions, rDNA LSU, EF1-α, β-tubulin, and *cox*I genes). The tree was inferred by maximum parsimony and rooted with *Pyhtium vexans* (P3980). The support values for maximum parsimony and neighbor joining are shown side by side

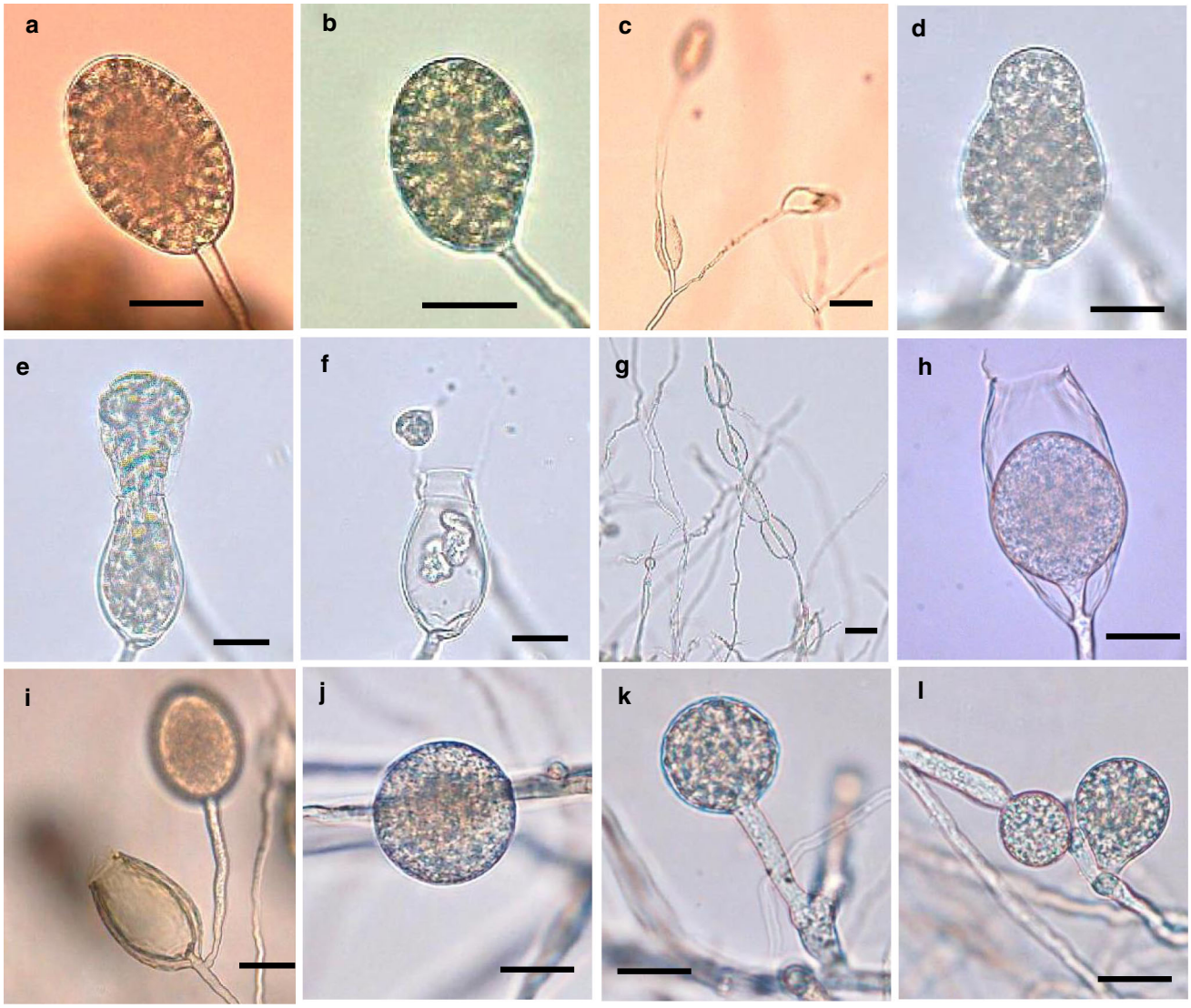


**Fig. 2** Morphology of asexual structures in *Phytophthora nagaii*. **a** Ellipsoid nonpapillate terminal sporangium. **b** Ellipsoid nonpapillate sporangium with eccentric basal attachment to the sporangiophore. **c** Simple sympodial sporangiophore. **d** Sporangium just before zoospore discharge and subsequent **e** zoospore discharge in a thin membranous vesicle from the same sporangium. **f** Empty sporangium with ruptured membrane. **g** Internally extended proliferation at low magnification. **h** Internally nested proliferation. **i** External proliferation. **j** Intercalary spherical chlamydospore. **k** Terminal hyphal swelling. **l** Spherical intercalary (*left*) and subspherical lateral (*right*) hyphal swellings. *Scale bars* 20 µm

## Comparison of morphological and physiological characters with morphologically similar species

Rose isolates were previously morphologically identified as *P. megasperma* (Nagai et al. 1978), but they differ morphologically from *P. megasperma* (Table 3). Because the new taxon is a fast growing species (growth rate 11.8 mm/24 h) and possessing external proliferation, while *P. megasperma* grows slower (6.7 ± 1 mm/24 h) and lacks external proliferation. *P. nagaii* can be differentiated from other morphologically similar species previously identified as *P. megasperma* sensu lato, like *P. rosacearum*, *P. gregata*, *P. sojae*, *P. sansomeana*, and *P. constricta*, respectively (Table 3). *P. rosacearum* can easily be differentiated from *P. nagaii* by the absence of chlamydospores, a higher optimum growth temperature (30 °C), a slower growth rate (6 mm/24 h), and its characteristic rosette petaloid colony pattern. *P. gregata* (clade 6) can be readily distinguished by the absence of external proliferation, smaller oogonia (36.8 ± 4.1 µm in diameter), partially self-fertile sexuality, and slower growth rate (6.8 ± 1 mm/24 h). *P. sojae* (Kaufmann and Gerdemann 1958) can be readily differentiated from the new species by the absence of chlamydospores and their slow growth rate <5 mm/24 h (Erwin and Ribeiro

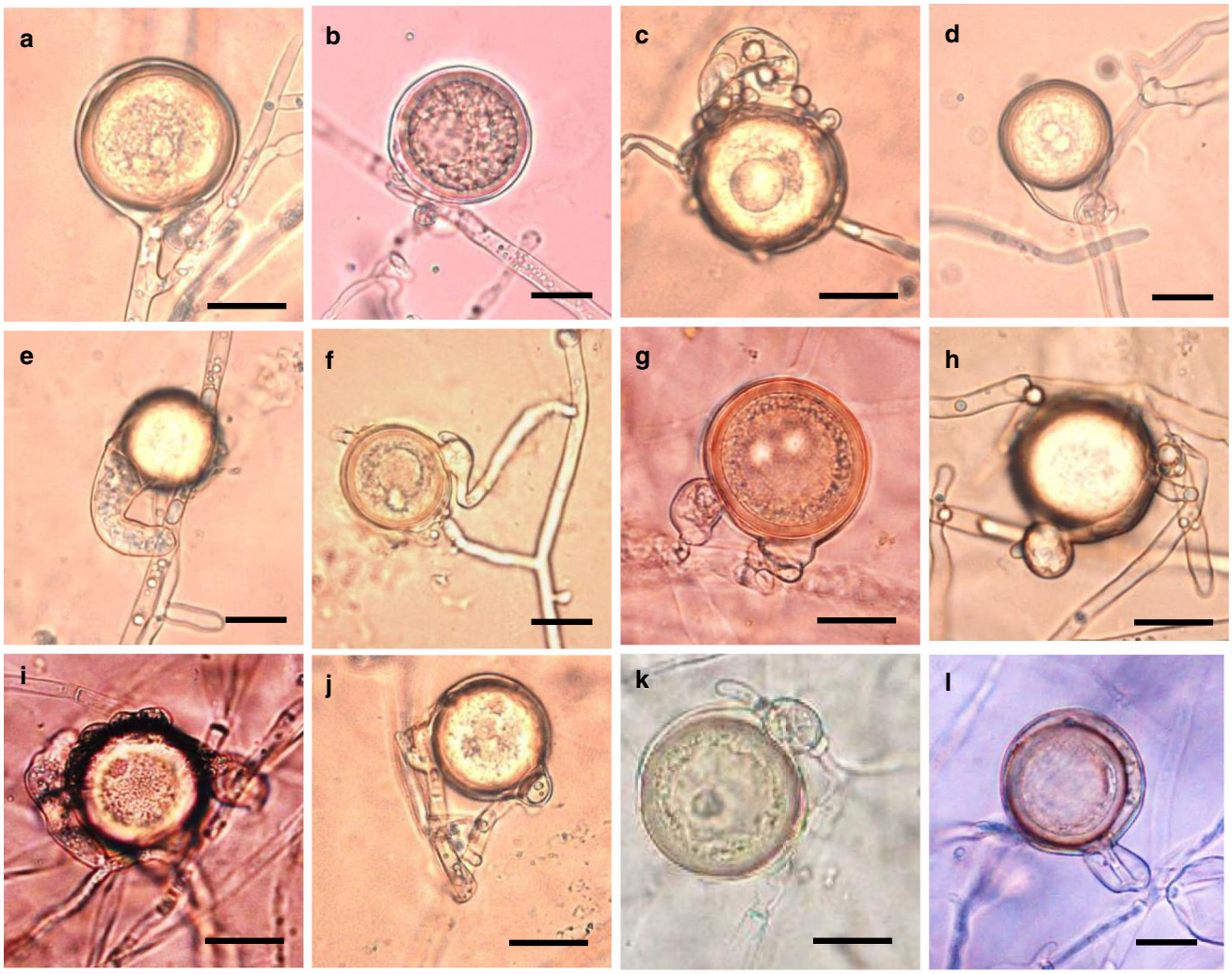


**Fig. 3** Morphology of sexual structures in *Phytophthora nagaii* **a** terminal spherical oogonium with aplerotic oospore. **b** Lateral spherical oogonium with aplerotic oospore. **c** Intercalary spherical oogonium. **d** Funnel-shaped oogonium with curved base. **e** Elongated curved oogonial stalk. **f** Paragynous diclinous antheridium with lateral attachment. **g** Paragynous antheridium with apical attachment. **h** Broadly laterally attached antheridium. **i** Two antheridia coiled around one oogonium. **j** Two laterally attached antheridia with one oogonium. **k** Antheridium with finger-like protrusion. **l** Amphigynous antheridium. *Scale bars* 20 µm

1996). *P. sansomeana* (clade 8) can be differentiated from *P. nagaii* by the absence of chlamydospores and slower growth rate (7–10 mm/24 h). *P. constricta* (clade 9) differs from *P. nagaii* by its characteristic constriction in the sporangiophore, absence of external proliferation, slower growth rate (6 ± 0.2 mm/24 h), and lower optimum growth temperature (22.5 °C).

## Taxonomic description of *Phytophthora fragariaefolia*

*Phytophthora fragariaefolia* M. Z. Rahman, S. Uematsu, T. Takeuchi, K. Shirai & K. Kageyama, sp. nov. (MycoBank MB 804990) (Figs. 1, 4, 6, 7, 8, 9)

Etymology: *fragariaefolia* = generic name of the host plant, strawberry (*Fragaria* × *ananassa*).

Type: Japan, Hokkaido Prefecture, from crown of strawberry (*Fragaria* × *ananassa*), 2005, collector T. Takeuchi. Isolate NBRC H-13133-holotypus (freeze-dried specimen). Ex-type NBRC 109709 = CBS 135747. The DDBJ accession numbers of the sequences of the rDNA ITS regions, rDNA LSU, EF1-α, β-tubulin, and *cox*I genes are AB819580, AB819581, AB819578, AB819579, and AB819582, respectively.

The species produced typically nonpapillate, noncaducous, terminal sporangia abundantly in water cultures both singly (Fig. 6a, b) and in simple sympodia (Fig. 6c). The sporangia were usually ellipsoid terminal (Fig. 6a), sometimes with lateral attachment (Fig. 6b), or distorted (Fig. 6d–f). The lengths and widths of the sporangia were 27.8–58.9 × 14.4–42.9 µm (average

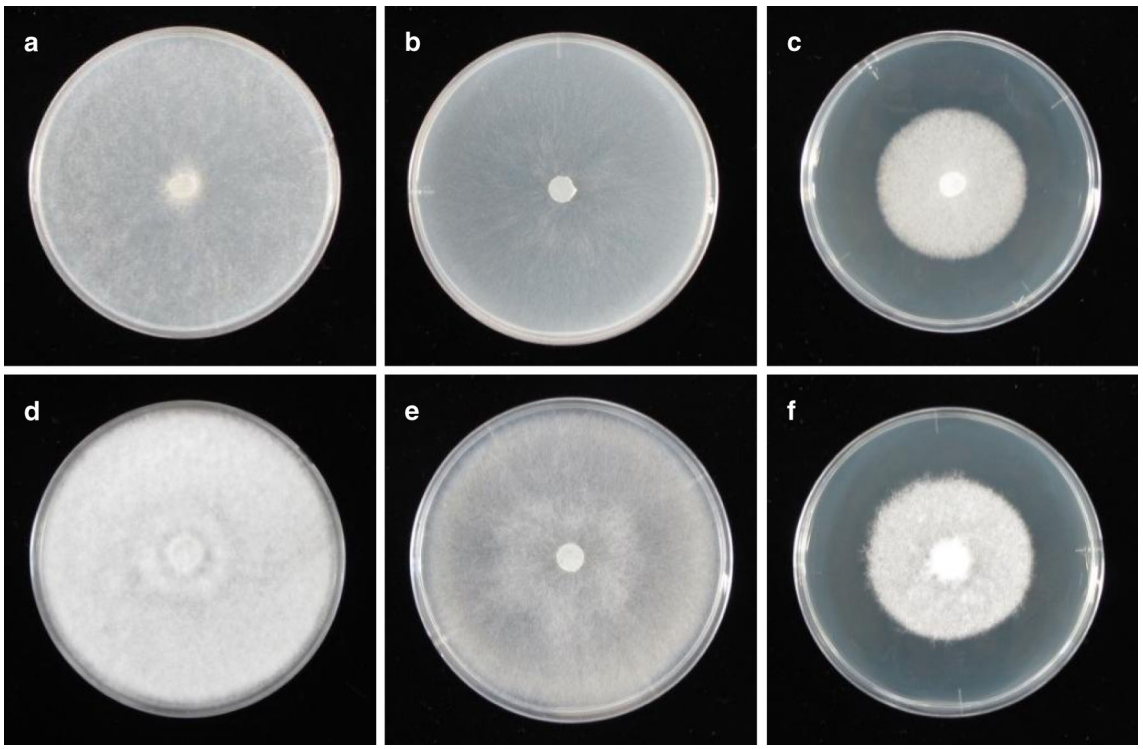


**Fig. 4** Cultures of type isolates of the new species after 7 days growth at 28 °C. *Phytophthora nagaii* (CBS 133248): **a** Aerial mycelia on V8A. **b** Aerial mycelia on CMA. **c** Fluffy aerial mycelia on PDA. *P. fragariaefolia* (CBS 135747): **d** Aerial mycelia onV8A. **e** Aerial mycelia on CMA. **f** Fluffy aerial mycelia on PDA

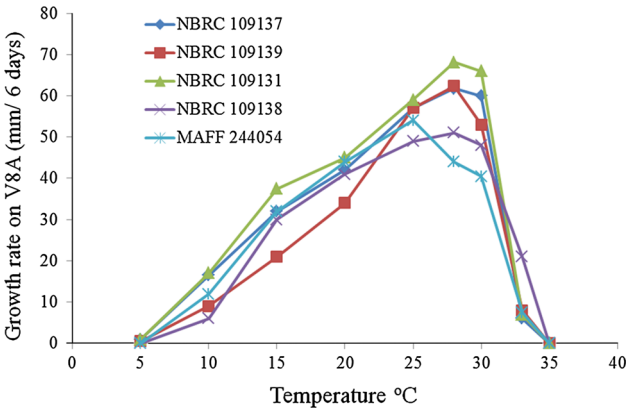


**Fig. 5** Growth rates of *Phytophthora nagaii* at different temperatures on V8A

38.5 ± 10.3 × 27.2 ± 7.62 µm), and the length to width ratio was 1:1.4. The sporangia proliferated internally, either extended (Fig. 6g) or nested (Fig. 6h), or externally (Fig. 6i). Zoospores were discharged through large exit pores of 7–19 µm (average 12.6 ± 3.6 µm) diameter (Fig. 6f–h). The zoospores were limoniform to reniform when motile and became spherical upon encystment. Spherical, intercalary chlamydospore produced in water cultures after 45 days (Fig. 6j). The thin walled (1–2 µm) chlamydospores were around 32.2 ± 5 µm (range 30–42 µm) in diameter. Hyphal swellings were produced abundantly in grass leaf blade and agar cultures. The swellings were spherical and produced terminally (Fig. 6k) or laterally (Fig. 6l).

The species was homothallic; gametangia were produced abundantly in single grass blade cultures, CMA cultures, and V8A cultures. Oogonia were terminal (Fig. 7a), often lateral and sessile (Fig. 7b) or intercalary (Fig. 7c). They were mostly spherical, sometimes funnel-shaped with tapering bases and short stalks (Fig. 7d), or often distorted with beak-like structures (Fig. 7e–g). The oogonia were 26.3–44.1 µm (average 35.4 ± 6 µm) in diameter. Oospores were aplerotic, with an average diameter of 29 ± 4.7 µm (22.4–34.5 µm). The oospore walls were about 2.9 ± 0.8 µm thick. The antheridia were predominantly paragynous (Fig. 7h–j) and sometimes amphigynous (Fig. 7k, l), both terminal (Fig. 7e), often with finger like protrusion (Fig. 7h, l). Their mean lengths and widths were 12.8–27.4 and 11.6–18.7 µm (average 18 ± 3.6 × 15.2 ± 2.2 µm). Sometimes one (Fig. 7i) or two antheridia (Fig. 7j) coiled around one oogonium. The colony was radial with aerial mycelia and smooth growth over the entire plate (Fig. 4d–f). The growth was fast on V8A medium with a rate of 11.3 mm/24 h at the optimum temperature of 25 °C. On V8A, the isolate grew at 5–33 °C (Fig. 8).

**Table 3** Morphological characters, dimensions and temperature-growth relations of *Phytophthora nagaii*, and other morphologically similar species in clades 6, 7, 8 and 9

| Character | *Phytophthora nagaii*[a] | *P. megasperma*[b] | *P. rosacearum*[c] | *P. gregata*[b] | *P. sojae*[d] | *P. sansomeana*[b] | *P. constricta*[e] |
|---|---|---|---|---|---|---|---|
| Clade position | Clade 7 | Clade 6 | Clade 6 | Clade 6 | Clade 7 | Clade 8 | Clade 9 |
| Hyphal swellings | Spherical irregular, clustered | Globose or angular catenulate or clustered | | Globose, elongated angular, partly catenulate | Spherical or ellipsoid, terminal or intercalary | | Ellipsoid and formed occasionally |
| Mean diameter (μm) | 25 ± 9 | 25.4 ± 1.8 | | 14.8 ± 3.8 | | | |
| Chlamydospore | Present | | Absent | | Absent | Absent | |
| Sporangiophore | Unbranched or simple sympodial | | Loosely sympodial | | Simple or sparingly branched | | Sporangiophore becomes constricted toward the base of sporangium |
| Sporangia | Terminal, ellipsoid, ovoid eccentric basal point | Ovoid, obpyriform | Ovoid, obpyriform | Ovoid, limoniform, obpyriform | Terminal, typically ovoid or ellipsoid | Ovoid, obpyriform | Ovoid, broadly ovoid to turbinate |
| Size (μm) | 26.8–79 × 23.7–55.4 | 37–84 × 35–56 | 56 × 37.3 | 25.7–102.3 × 14.8–50.7 | 23.3–88.8 × 16.6–51.8 | 56 × 36.8 | |
| | 46.7 ± 13.8 × 35.4 ± 7.8 | 59.3 ± 8.8–42.8 ± 4.5 | | 51.0 ± 13.8 × 30.5 ± 5.9 | 58 × 38.3 | | 59.8 ± 8.7 × 48.8 ± 7.4 |
| L/B ratio | 1.35 | 1.39 ± 0.2 | 1.5 | 1. 67 ± 0.32 | 1.51 | 1.5–1.6 | 1.2 ± 0.09 |
| Papilla | Nonpapillate | Nonpapillate | Nonpapillate | Nonpapillate | Nonpapillate | Nonpapillate | Nonpapillate, semipapillate |
| Proliferation | Internal, extended nested or external | Internal nested and extended never external | Typically internal, occasionally lateral from beneath the sporangium | Internal extended and nested, never external, sporangium partly branching inside empty sporangium | Germinate directly by germ tube or indirectly by zoospores | Typically internal, occasionally lateral from beneath the sporangium | Internal, usually nested rarely extended |
| Exit pore, width (μm) | 14.7 ± 5.5 | 12.4 ± 1.2 | | 10.7 ± 2.7 | | | |
| Range (μm) | 6.7–24.6 | 11.8–12.4 | | 8.4–14.1 | | | |
| Oogonia | Smooth | Smooth | Smooth | Smooth | Smooth, typically globose, | Smooth | Tapering base, elongated |
| Shape | Spherical, funnel-shaped base, distorted | | | | Not funnel-shaped at base | | And slightly eccentric |
| Range (μm) | 32.2–53.3 | 27–52 | 29–34 | 23.9–50.9 | 28.3–45.8 | 37–45 | 46–50.4 |
| Mean diameter (μm) | 40 ± 4.7 | 41.8 ± 2.4 | 31.5 | 36.8 ± 4.1 | 36.9 | 41 | 49.0 ± 4.4 |
| Sexual system | Homothallic | Homothallic | Homothallic | Homothallic or partially self-fertile | Homothallic | Homothallic | Homothallic |

**Table 3** continued

| Character | *Phytophthora nagaii*[a] | *P. megasperma*[b] | *P. rosacearum*[c] | *P. gregata*[b] | *P. sojae*[d] | *P. sansomeana*[b] | *P. constricta*[e] |
|---|---|---|---|---|---|---|---|
| Oospore | Aplerotic | Usually aplerotic | | Usually aplerotic | Nearly filled the oogonium | | Slightly aplerotic, nearly plerotic |
| Range (μm) | 29.5–47.7 | 23–42 | | 21.4–45.3 | 19.2–38.3 | | 38.9–41.8 |
| Mean diameter (μm) | 37.6 ± 4.3 | 33.8 ± 2.4 | | 31.6 ± 4.0 | 31.4 | | 40.4 ± 4.3 |
| Wall thickness | 3.3 ± 0.5 | 3.31 ± 0.4 | | 2.65 ± 0.81 | 2.4 | | 2.9 ± 1 |
| Antheridum | Mostly paragynous, some amphigynous | Paragynous and amphigynous | Predominantly paragynous | Predominantly paragynous | Paragynous | Predominantly paragynous | Exclusively paragynous |
| L × B mean (μm) | 19.1 ± 3.5 × 13.5 ± 1.2 | 13 ± 1.5 × 10.4 ± 1.3 | | 17.1 ± 3 × 11.0 ± 1.8 | | | 16.9 ± 2.4 × 13.3 ± 2.1 |
| Range (μm) | 13.5–28.1 × 10.7–15.3 | 10.7–15.8 × 8–13 | | 10.6–24.9 × 7.6–17.8 | | | |
| Optimum growth (°C) | 28 | 22.5–25 | 30 | 25 | 20 | 25–27 | 22.5 |
| Maximum growth (°C) | 33 | 32.5 | 36 | 32.5 to < 35 | No growth at 5 and 35 °C | 35 | 32.5 |
| Minimum growth (°C) | 5 | | | | | | |
| Growth rate (mm/d) | 11.8 | 6.7 ± 0.1 | 6 | 6.8 ± 0.1 | <5[f] | 7–10 | 6.0 ± 0.22 |

[a] Measured in this study

[b] Type specimen information from Jung et al. (2011)

[c] Type specimen information from Hansen et al. (2009)

[d] Species description from Kaufmann and Gerdemann (1958)

[e] Type specimen description from Rea et al. (2011)

[f] Growth rates from Erwin and Ribeiro (1996)

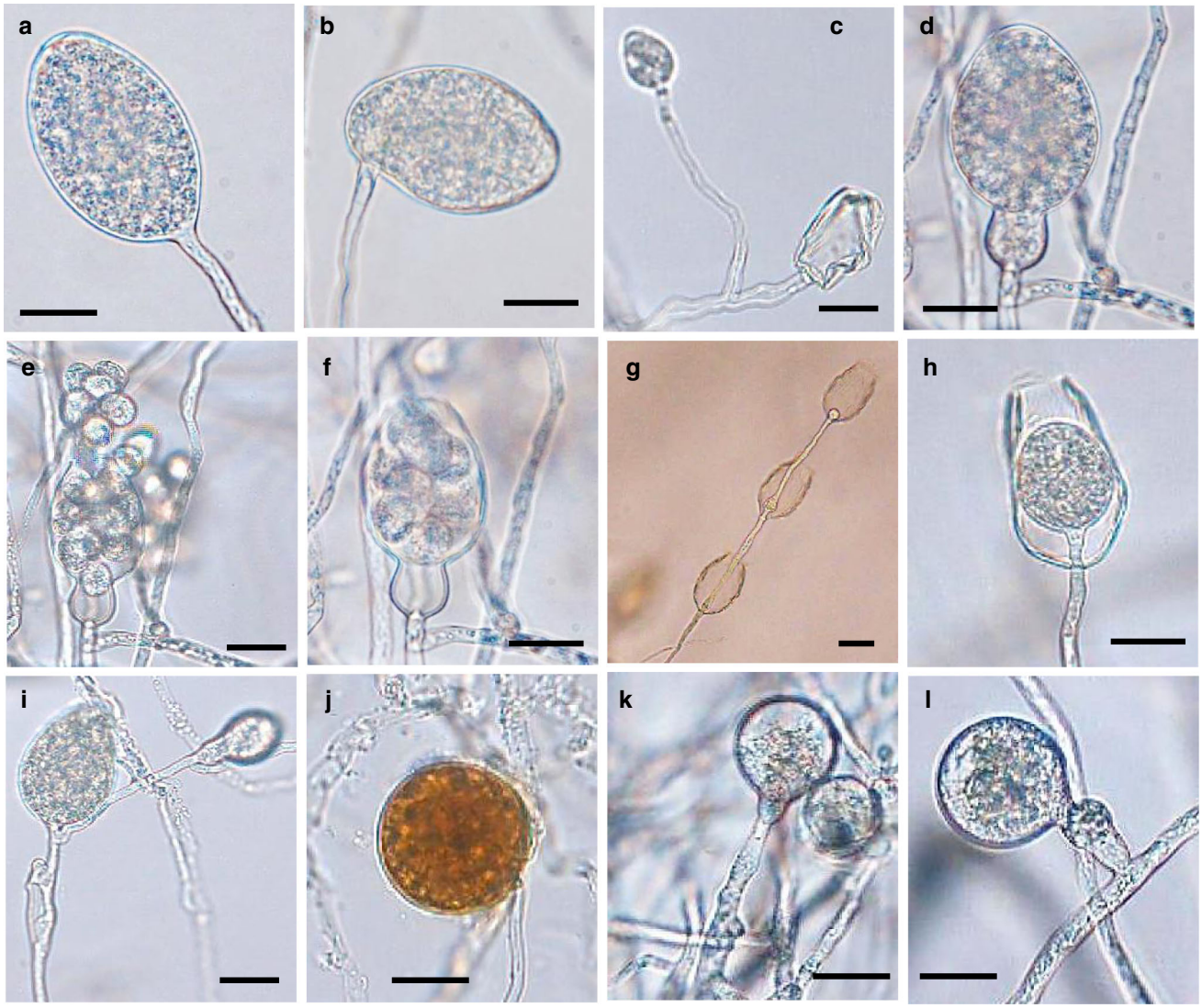


**Fig. 6** Morphology of asexual structures in *Phytophthora fragariaefolia*. **a** Ellipsoid nonpapillate terminal sporangium. **b** Ellipsoid nonpapillate sporangium with eccentric basal attachment to the sporangiophore. **c** Simple sympodial sporangiophore. **d** Distorted nonpapillate sporangium and subsequent **e** zoospore discharge from the same sporangium; **f** the distortion remained even after discharge. **g** Internal extended proliferation at low magnification. **h** Internally nested proliferation. **i** External proliferation. **j** Spherical chlamydospore. **k** Terminal spherical hyphal swelling in water. **l** Lateral hyphal swelling in water. *Scale bars* 20 μm

## Comparison of morphological and physiological characters within and between the rose and strawberry isolates

In general, the species *P. nagaii* and *P. fragariaefolia*, from rose and strawberry, respectively, showed very similar morphological features, temperature reactions, and growth patterns, irrespective of collection location or year and host cultivar. There were a few exceptions (Table 4). Both groups were fast growing (ca. 10.5 mm/24 h on V8A) with high optimal temperatures for growth. Most isolates had optimum temperatures of 28 °C, although the type isolate (CBS 135747) for *P. fragariaefolia* grew maximally at 25 °C. Both the species grew minimally on PDA. No isolates formed patterns on V8A, CMA, or PDA, but fluffy aerial mycelia were found on PDA. The aerial growth of mycelia in *P. fragariaefolia* was higher than those of *P. nagaii* on all media.

*P. nagaii* produced sporangia readily at 20 and 25 °C in grass leaf blade cultures, but *P. fragariaefolia* did not. *P. fragariaefolia* required a temperature of 20 °C and daily water changes. Both species produced ellipsoid sporangia and ovoid sporangia. *P. fragariaefolia* produced some distorted sporangia, which were absent in *P. nagaii*. In *P. nagaii*, the zoospore discharge is a thin-walled, vesicle-like structure, which ruptures from the pressure of discharging zoospores. This was absent in *P. fragariaefolia.* The rose isolates NBRC 109137 and NBRC 109138 produced internally nested sporangia more frequently than the other rose and strawberry isolates tested.

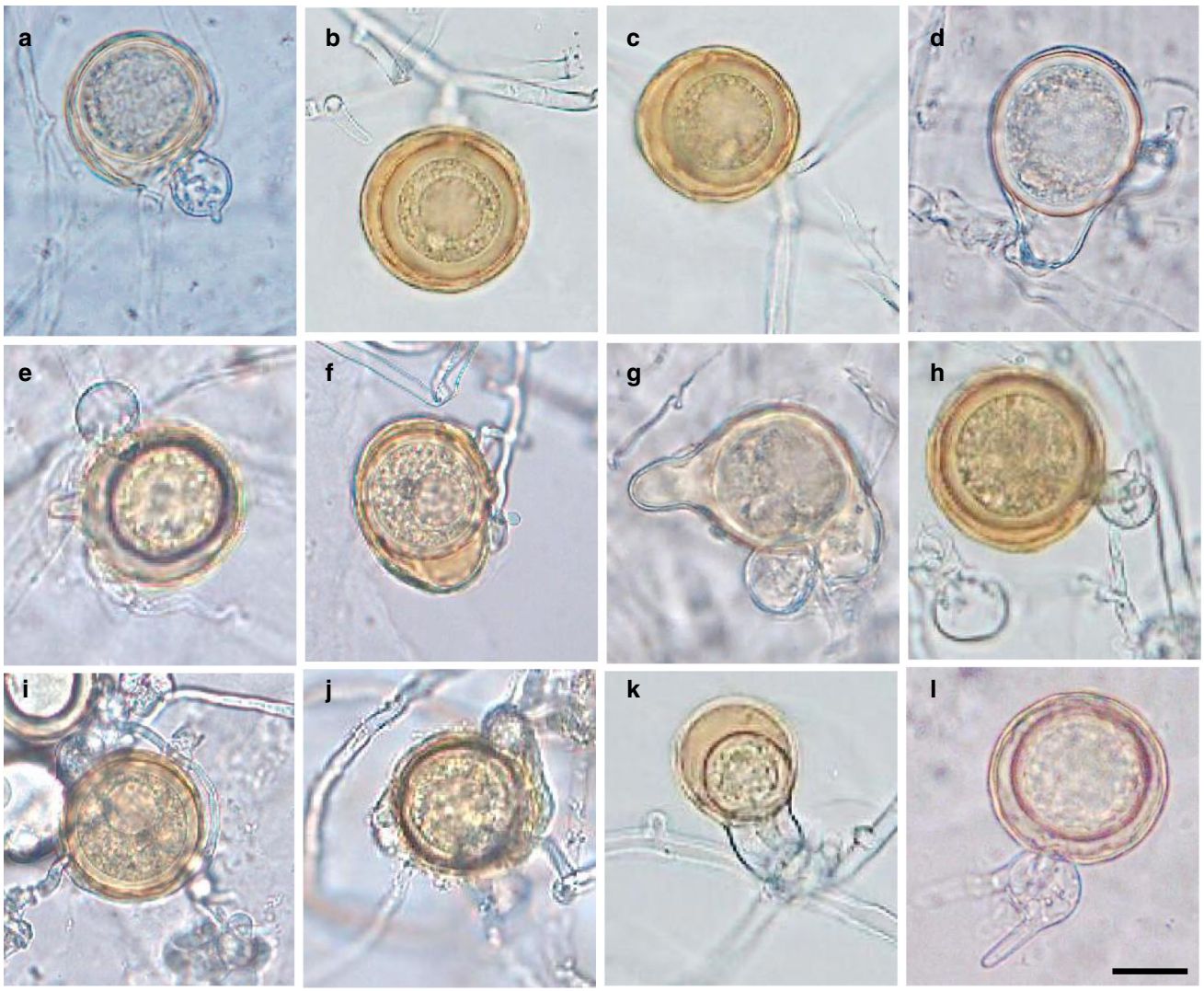


**Fig. 7** Morphology of sexual structures in *Phytophthora fragariaefolia*. **a** Terminal oogonium. **b** Lateral spherical oogonium with aplerotic oospore. **c** Intercalary spherical oogonium. **d** Funnel-shaped oogonium. **e–g** Distorted oogonium. **h** Paragynous antheridium with finger-like protrusion. **i**, **j** Paragynous antheridia coiled around oogonia. **k** Amphigynous antheridium. **l** Antheridium with finger-like protrusion. *Scale bars* 20 μm

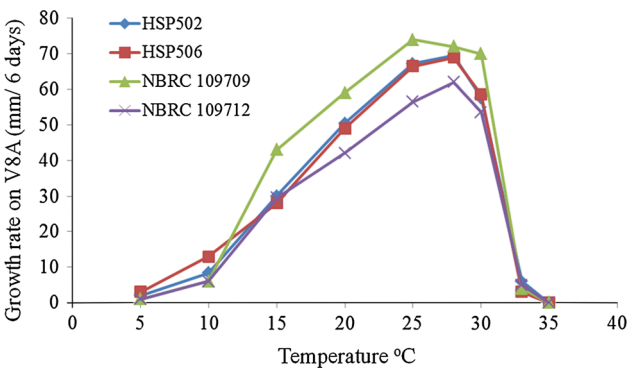


**Fig. 8** Growth rates of *Phytophthora fragariaefolia* at different temperatures on V8A

On the other hand, the rose isolates NBRC 109131 and NBRC 109139 produced spherical oogonia with elongated curved oogonial stalks, and such stalks were absent in the other rose and strawberry isolates. *P. fragariaefolia* produced distorted oogonia along with the spherical and funnel-shaped oogonia, whereas *P. nagaii* did not produce distorted oogonia. At the same time, *P. fragariaefolia* is distinct in the same points at which *P. nagii* is different from its related species.

## Pathogenicity test

The two species, *P. nagaii* and *P. fragariaefolia* were virulent on their respective hosts, rose and strawberry, and


**Fig. 9** Rose cuttings and strawberry seedlings with disease symptoms caused by *Phytophthora nagaii* isolates. **a**, **e** NBRC 109131. **b**, **f** NBRC 109138, *P. fragariaefolia* isolates. **c**, **g** HSP501. **d**, **h** HSP503

caused symptoms that resembled those originally reported (Fig. 9). The rose isolates caused more severe symptoms on rose cuttings than on strawberry plants (Table 5). Similarly, the strawberry isolates caused more severe symptoms on strawberry plants than on rose cuttings. *P. fragariaefolia* seemed to be more host-specialized than *P. nagaii*. The disease severity index was higher (65–74) in strawberry seedlings inoculated with strawberry isolates than in rose cuttings inoculated with rose isolates (52–55). On the other hand, the disease severity index was lower (8–13) in rose cuttings inoculated with strawberry isolates than in strawberry seedlings inoculated with rose isolates (32–35). The pathogens were reisolated from diseased parts of the infected rose cuttings and strawberry seedlings, thus satisfying Koch's postulates. Control cuttings and seedlings remained healthy.

## Discussion

Here we describe two new *Phytophthora* species isolated from rose and strawberry plants in Japan. These species are named *P. nagaii* and *P. fragariaefolia*, respectively. Both the rose and strawberry isolates are homothallic in nature. They form nonpapillate, noncaducous sporangia with internal proliferation and have paragynous antheridia. Thus they belong to the morphological group V described by Waterhouse (1963). Both species are characterized by the formation of hyphal swellings and chlamydospores, simple sympodial sporangiophores, characteristic ellipsoid sporangia with eccentric basal attachment points to the sporangiophores, internal (extended or nested) and external proliferation, funnel-shaped oogonia, and relatively fast growth. Morphological comparisons between isolates of this species and the over 120 reported taxa and other putative new species indicate that *P. nagaii* and *P. fragariaefolia* are distinct new species.

The rose and strawberry isolates showed mostly similar morphological characteristics with few exceptions. The rose isolates showed a characteristic mode of zoospore discharge, and the strawberry isolates produced distorted sporangia and oogonia. The sequencing results were consistent with the morphological comparisons. All isolates from the same host had identical sequences, with some differences in rDNA ITS regions, EF1-α, β-tubulin and *cox*I genes, respectively.

In the multilocus phylogenetic tree, the two new taxa appeared in clade 7 (Blair et al. 2008; Cooke et al. 2000), which contains Waterhouse (1963) groups V and VI. Two Waterhouse group V members, *P. pistaciae* and *P. sojae* are morphologically similar to the new species. But *P. pistaciae* (Mirabolfathy et al. 2001) and *P. sojae*

**Table 4** Overview of morphological data for isolates of the new *Phytophthora* species

| Trait | *P. nagaii* | *P. fragariaefolia* |
|---|---|---|
| Cardinal growth (°C) | | |
| Minimum | 5 | 5 |
| Maximum | 33 | 33 |
| Optimum | 28 | 25–28 |
| Growth rate (mm/day) | | |
| Range | 9.2–10.4 | 9.8–12 |
| Isolate means | 10.4 ± 1 | 10.8 ± 1 |
| Sporangia | | |
| Length (μm) × width (μm) | | |
| Range | 30.7–82.6 × 17.4–52 | 25–68.3 × 14.7–50 |
| Mean | 56 ± 13 × 39.3 ± 8.3 | 43.6 ± 9.9 × 30.9 ± 7 |
| Length (μm):width (μm) | | |
| Range | 1.2–1.5 | 1.4 |
| Mean | 1.4 ± 1 | 1.4 |
| Discharge pore width (μm) | | |
| Range | 6.7–22.6 | 5–20.9 |
| Mean | 15.3 ± 3.5 | 13.8 ± 3 |
| Mating system | homothallic | homothallic |
| Antheridia | | |
| Type | mostly paragynous, some amphigynous | mostly paragynous, some amphigynous |
| Length (μm) × width (μm) | | |
| Range | 12.5–28.1 × 9–16.7 | 12.8–27.4 × 11.6–18.8 |
| Mean | 18 ± 3–13.1 ± 1.5 | 18 ± 3.7–15.3 ± 2.2 |
| Oogonia | | |
| Diameter (μm) | | |
| Range | 21.9–52.5 | 20.1–55.6 |
| Mean | 39.7 ± 4.6 | 41.8 ± 7.8 |
| Oospore | aplerotic | aplerotic |
| Diameter Range (μm) | 20.8–43.6 | 18–50.3 |
| Mean (μm) | 35.8 ± 4.2 | 35.4 ± 7.2 |
| Wall thickness (μm) | | |
| Range | 1 – 6 | 1.4–6.2 |
| Mean | 3.5 ± 0.7 | 3.9 ± 1 |
| Hyphal swellings | | |
| Length (μm) × width (μm) | | |
| Range | 20.3–28.6 | 22–29.3 |
| Mean | 24.9 ± 1.4–27.8 ± 0.5 | 25.5 ± 1.6–27.9 ± 0.8 |
| Chamydospore | | |
| Diameter (μm) | | |
| Range | 26.8–40.3 | 29.5–44.4 |
| Mean | 33.2 ± 5 | 35 ± 1 |

*P. nagaii*, isolates: NBRC 109131, NBRC 109137, MAFF 244054, NBRC 109138, NBRC 109139; *P. fragariaefolia*: HSP502, HSP506, NBRC 109709, NBRC 109712

**Table 5** Disease severity on rose cuttings and strawberry plants 4 days (at 21 °C) after inoculation with rose or strawberry isolate

| Source host of isolate | Accession nos. | Disease severity[a] | |
|---|---|---|---|
| | | Rose[b] | Strawberry[c] |
| Rose | NBRC 109131 | 55 ± 13 | 35 ± 2 |
| | NBRC 109138 | 52 ± 8 | 32 ± 5 |
| Strawberry | HSP501 | 13 ± 13 | 74 ± 7 |
| | HSP503 | 8 ± 5 | 65 ± 6 |

[a] Disease severity = Σ (disease severity scale × no. of plants at each severity)/(maximum disease severity × total no. of plants) × 100 (Watanabe et al. 2007)

[b] Stem and leaf blight severity based on disease severity scale of 0 = no symptom, 1 = slightly browning, 2 = browning, 3 = slightly defoliated, 4 = severely defoliated, 5 = dead

[c] Leaf blight severity based on a disease severity scale of 0 = no symptom, 1 = young leaf slightly browning, 2 = young leaf browning, 3 = leaf slightly browning, 4 = leaf browning, 5 = blighted

(Kaufmann and Gerdemann 1958) can be readily differentiated from the new species by the absence of chlamydospores and their slow growth rates 3 mm/24 h and <5 mm/24 h, respectively (Erwin and Ribeiro 1996). Another clade 7 member is *P. fragariae*, which causes red core and red stele fruit rot in strawberry. *P. fragariae* can be differentiated from *P. fragariaefolia* by its amphigynous antheridium, low optimum temperature (20 °C) and slow growth rate (1.3–2 mm/24 h) (Kröber 1985).

The rose isolate was originally identified as *P. megasperma* (Nagai et al. 1978). But the rDNA ITS sequence from these rose isolates have only 82–85 % homology with the rDNA ITS regions of *P. megasperma* sensu stricto, indicating that they are not closely related. Hansen et al. (1986) suggested that *P. megasperma* isolated from rose plants imported from Japan was different from other groups of the *P. megasperma* complex, based on the protein pattern, colony type, and growth rate. Our morphological and phylogenetic analyses also indicate that these isolates are members of a new species. Furthermore, rose isolates collected at different times and from different locations were morphologically and phylogenetically identical.

Because rose and strawberry are in the family Rosaceae, the pathogens should fall into the group AC (*P. rosacearum*) according to Hansen et al. (1986). But our new species differ morphologically and phylogenetically from *P. rosacearum*. The results suggest that the size of the oogonium should not be used to identify an isolate as *P. megasperma*. Phylogenetic analyses of nuclear and mitochondrial genes, along with morphological characterization, will provide a useful approach for differentiating the *P. megasperma* at the species level.

Clear differences were observed between rose and strawberry isolates in the pathogenicity tests. Rose cuttings inoculated with rose isolates and strawberry seedlings inoculated with strawberry isolates produced significantly more severe symptoms than those observed when rose cuttings were inoculated with strawberry isolates and strawberry seedlings were inoculated with rose isolates. The symptoms produced in both cases were similar to the originally described symptoms. *Phytophthora* rot of strawberry, induced by *P. nicotianae* var. *parasitica*, was first reported in Shizuoka Prefecture of Japan in 1978 (Suzui et al. 1980). Later, wilting of strawberry caused by *P. cactorum* was reported in Tochigi Prefecture (Ishikawa et al. 1990). Both diseases produce symptoms (wilting of the whole plant) that are similar to those produced by *P. fragariaefolia*. Interestingly, a number of new species recently segregated from *P. megasperma* sensu lato, including *P. medicaginis*, *P. rosacearum*, *P. sansomeana*, *P. sojae*, and *P. trifolii*, have host specificity. The new species, *P. nagaii* and *P. fragariaefolia* are distinctively aggressive on their original host, rose and strawberry.

On the other hand, *P. megasperma* sensu stricto has a wide host range.

Some interesting physiological features are associated with the two newly described species. First, all isolates have minimum growth temperatures of $\geqq$5 °C; in fact, most isolates hardly grow at 5 °C. All the isolates grow well at 25–28 °C, which enables them to acclimatize in moderate temperature zones. Second, these isolates all grow rapidly, with maximum growth rates between 9.2 and 12 mm/24 h. These fast growth rates make them easy to isolate from diseased plants and grow in the laboratory. Third, both species are host-specialized. Both rose and strawberry groups can cause quite severe disease in their respective hosts. On the other hand, as Hansen and Hamm (1983) cautioned, host specializations should be interpreted carefully.

**Acknowledgments** We thank Drs. Yuji Nagai, E. M. Hansen, and Tsuneo Watanabe for kind suggestions.

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