

# Phylogeny of the genus *Pythium* and description of new genera

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**Abstract** Phylogeny of the genus *Pythium* is analyzed based on sequences of the large subunit ribosomal DNA D1/D2 region and cytochrome oxidase II gene region of *Pythium* isolates and comprehensive species of related taxa belonging to the Oomycetes. The phylogenetic trees show that the genus *Pythium* is a highly divergent group and divided into five well- or moderately supported monophyletic clades. Each clade is characterized by sporangial morphology such as globose, ovoid, elongated, or filamentous shapes. Based on phylogeny and morphology, the genus *Pythium* (s. str.) is emended, and four new genera, *Ovatisporangium*, *Globisporangium*, *Elongisporangium*, and *Pilasporangium*, are described and segregated from *Pythium* s. lato.

**Keywords** Molecular phylogeny · rDNA · Sporangia · Taxonomy

## Introduction

The genus *Pythium* belongs to the family Pythiaceae, order Pythiales, class Oomycetes, phylum Oomycota, and kingdom Chromista (Kirk et al. 2008). The genus is widely

distributed throughout the world, and appropriately 150 species have been described (Kirk et al. 2008). The members are amphibious and ubiquitous and occupy several ecological niches (van der Plaäts-Niterink 1981). Several species are known as pathogens of various plants, and many of them show much wider host ranges than those of other related genera, such as *Phytophthora*. They generally cause rot of fruit, roots, and stems, and pre- or postemergence damping-off of seeds and seedlings. Other pathogenic species are restricted to one or closely related host species; for example *P. porphyrae* M. Takah. & M. Sasaki causes red rot of marine red algae (Takahashi et al. 1977). A few species can cause disease in restricted environments; for example *P. okanoganense* P. E. Lipps causes snow rot under snow (Lipps 1980). In addition to these plant pathogens, *P. guiyangense* X. Q. Su is a parasite of mosquito larvae (Su 2006) and *P. insidiosum* De Cock, L. Mend., A. A. Padhye, Ajello & Kaufman is a mammalian pathogen (de Cock et al. 1987). On the other hand, many species are known to inhabit various soils, such as cultivated and uncultivated fields including forest, pastures, or arid places as saprophytes; however, *Pythium* species in uncultivated fields have not been the subject of much study. Consequently, the distribution, ecological roles, and physiological features of the species have not been sufficiently elucidated. Recently, many new *Pythium* species have been described based on strains isolated from uncultivated fields or seminatural environments in several countries (Nechwatal and Oßwald 2003; Allain-Boulé et al. 2004; Ko et al. 2004; Nechwatal et al. 2005; Nechwatal and Mendgen 2006; Paul 2006; Belbahri et al. 2008; de Cock et al. 2008; Moralejo et al. 2008; Paul and Bala 2008; Uzuhashi et al. 2009). This suggests that more unidentified species exist in soils of uncultivated fields, and thus a survey of *Pythium* species is warranted. Investigations of

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*Pythium* species in these relatively unexplored habitats are important to understand not only their ecological roles and physiological features but also the taxonomy and phylogeny of the genus.

The genus *Pythium* has the following morphological characteristics (van der Plaäts-Niterink 1981): Hyphae are hyaline and coenocytic without cross septa. Two types of sporangia are present, filamentous and globose. Zoospores develop in a vesicle, which is formed at the tip of a discharge tube derived from a sporangium. Oospores are formed in smooth or ornamented oogonia after fertilization with paragynous or hypogynous antheridia. The oospore fills the whole oogonium (plerotic) or has some space between the walls of the oogonium and oospore (aplerotic). The formation of zoospores within a vesicle is characteristic of *Pythium* and different from morphologically similar genera, such as *Phytophthora* and *Halophytophthora*. On the other hand, the process of zoospore formation is also similar in the genus *Lagenidium*, but this genus shows endobiotic and holocarpic features that have not been reported in any *Pythium* species (Dick 2001a).

The genus *Pythium* was originally described by Pringsheim in 1858, and *P. monospermum* Pringsh. was selected as the type species. Since then, the classification has been changed by several researchers based on morphological characteristics. Fischer (1892) divided the genus into three subgenera, *Aphragmium*, *Nematosporangium*, and *Sphaerosporangium*, based on sporangial morphology. The first subgenus was composed of species with noninflated filamentous sporangia without a septum that delimited sporangia and hypha. The second subgenus included species with filamentous sporangia delimited from the vegetative hyphae by septa. The last subgenus was characterized by (sub-)globose sporangia delimited by septa from the vegetative hyphae. Schröter (1897) first emphasized the importance of differences between filamentous and globose shapes and combined two subgenera, *Aphragmium* and *Nematosporangium*, into one genus, *Nematosporangium*, and treated *Sphaerosporangium* as the genus *Pythium*. Two subgenera, *Eupythium* and *Artotrogus* were also placed within the genus *Pythium* by Schröter (1897) based on the structure of the oogonial wall. Subsequently, several *Pythium* species were transferred to *Nematosporangium* (Sideris 1931; Yachevskij and Yachevskij 1931); however, the genus *Nematosporangium* was considered an illegitimate taxon because the type species of *Pythium*, *P. monospermum*, defined by Pringsheim, was included in *Nematosporangium*. Therefore, all members are included in the genus *Pythium* in the current taxonomy (van der Plaäts-Niterink 1981); however, it is clear that *Pythium* is composed of two morphological groups clearly differentiated from each other by

filamentous or globose sporangia, thus the infrageneric classification of *Pythium* should be examined in detail.

Taxonomy of *Pythium* species is generally based on morphological characteristics, such as the shape and size of sporangia and oogonia, the extent of oospore in the oogonium (plerotic or aplerotic), the number of antheridia per oogonium, and the position of the antheridium in relation to the oogonium (Waterhouse 1963; van der Plaäts-Niterink 1981; Dick 1990). However, members of *Pythium* are considered a very difficult group for species delimitation and identification because these characteristics are often very similar among different species and sometimes not formed on an agar medium. Therefore, recently, molecular methods have been used for species identification to supplement the morphological taxonomy by many researchers who have mainly analyzed the ribosomal DNA (rDNA) region. The results revealed that sequences of the rDNA internal transcribed spacer (ITS) region were very different among *Pythium* species (Wang and White 1997; Matsumoto et al. 1999; Lévesque and de Cock 2004; Kageyama et al. 2005). Thus, sequence data of this region had been frequently used to identify and classify *Pythium* species.

Molecular data have also been used for phylogenetic analyses of *Pythium* and related genera based on the rDNA large subunit (LSU) D1/D2 and ITS,  $\beta$ -tubulin, or mitochondrial cytochrome oxidase II (*coxII*) gene (Briard et al. 1995; Matsumoto et al. 1999; Panabieres et al. 1997; Martin 2000; Petersen and Rosendahl 2000; Dick 2001b; Voglmayr 2003; Villa et al. 2006; Belbahri et al. 2008). Many reports have shown that *Pythium* appears to be a nonmonophyletic group that includes several monophyletic groups, and the species are clustered according to sporangial morphology. One monophyletic group is characterized by filamentous inflated or noninflated sporangia and another group by globose sporangia. These results reveal that the genus consists of several groups supported by both morphology and phylogeny. Thus, this suggests the necessity of taxonomic revision of the genus.

The objective of this study was to clarify the taxonomy of the genus *Pythium* by morphological and phylogenetic examinations based on *Pythium* isolates (Table 1). For this purpose, *Pythium* species were isolated from various soil samples collected from cultivated and uncultivated fields in Japan and were analyzed phylogenetically based on the sequences of two different genes, LSU rDNA D1/D2 region and *coxII*. To evaluate the phylogenetic relationship between *Pythium* and other genera and phylogenetic relationships among *Pythium* species, comprehensive sequence data from the GenBank database were used for analyses. Based on the relationships between morphology and phylogeny, taxonomy of the genus *Pythium* is revised, and a new taxonomic revision is proposed.

**Table 1** Species and GenBank accession number of the *Pythium* isolates used in this study

Isolate no.		Isolate origin		Species <sup>a</sup>	GenBank accession No.		
Origin	International	Substrate	Locality		ITS	D1/D2	<i>coxII</i>
<i>Pythium</i>							
UZ352	MAFF 241099	Soil (uncultivated)	Hokkaido, Japan	<i>P. acanthicum</i>	AB468763	AB468698	AB468889
UZ364	MAFF 241100	Soil (uncultivated)	Hokkaido, Japan	<i>P. acanthicum</i>	AB468764	AB468699	
UZ051	MAFF 241101	Soil (cultivated)	Nganano, Japan	<i>P. aphanidermatum</i>	AB468765	AB468700	AB468890
UZ216	MAFF 240154, NBRC 103117	Soil (cultivated)	Gunma, Japan	<i>P. aquatile</i>	AB359909	AB468701	AB468891
UZ264	MAFF 240156, NBRC 103118	Soil (cultivated)	Okinawa, Japan	<i>P. catenulatum</i>	AB468766	AB468702	AB468892
UZ159	MAFF 241102	Soil (cultivated)	Kyoto, Japan	<i>P. dissotocum</i>	AB468767	AB468703	AB468893
UZ357	MAFF 241115	Soil (uncultivated)	Hokkaido, Japan	<i>P. torulosum</i>	AB468780	AB468718	AB468905
OPU1445	MAFF 241116	Orchard grass	Hokkaido, Japan	<i>P. vanterpoolii</i>	AB468783	AB468721	AB468908
OPU1446		Wheat	Hokkaido, Japan	<i>P. volutum</i>	AB468786	AB468724	AB468911
OPU797	MAFF 241119	Soil (uncultivated)	Okinawa, Japan	<i>Pythium</i> sp.40	AB468768	AB468704	
OPU1448	MAFF 241120	Orchard grass	Hokkaido, Japan	<i>Pythium</i> sp.41	AB468818	AB468756	AB468937
OPU1449	MAFF 241121	Orchard grass	Hokkaido, Japan	<i>Pythium</i> sp.42	AB468819	AB468757	AB468938
UZ156	MAFF 241122	Soil (cultivated)	Nganano, Japan	<i>Pythium</i> sp.2	AB468787	AB468725	AB468912
UZ190	MAFF 241125	Soil (cultivated)	Hokkaido, Japan	<i>Pythium</i> sp.3	AB468790	AB468728	AB468915
UZ379	MAFF 241142	Soil (uncultivated)	Fukushima, Japan	<i>Pythium</i> sp.16	AB468807	AB468745	AB468929
UZ419	MAFF 241147	Soil (uncultivated)	Fukuoka, Japan	<i>Pythium</i> sp.23	AB468812	AB468750	AB468933
UZ655	MAFF 241151	Soil (uncultivated)	Miyagi, Japan	<i>Pythium</i> sp.28	AB468816	AB468754	AB468935
ZSF0011		Soil (uncultivated)	Nganano, Japan	<i>Pythium</i> sp.43	AB468820	AB468758	AB468939
ZSF0093		Soil (uncultivated)	Nganano, Japan	<i>Pythium</i> sp.47	AB468824	AB468762	
<i>Ovatisporangium</i>							
UZ215	MAFF 241117	Soil (cultivated)	Gunma, Japan	<i>O. vexans</i> = <i>P. vexans</i>	AB468784	AB468722	AB468909
UZ309	MAFF 241118	Soil (uncultivated)	Kyoto, Japan	<i>O. vexans</i> = <i>P. vexans</i>	AB468785	AB468723	AB468910
UZ230	MAFF 241127	Soil (uncultivated)	Nagano, Japan	<i>Ovatisporangium</i> sp.1	AB468792	AB468730	AB468917
UZ248	MAFF 241128	Soil (uncultivated)	Okinawa, Japan	<i>Ovatisporangium</i> sp.2	AB468793	AB468731	AB468918
UZ287	MAFF 241138	Soil (cultivated)	Okinawa, Japan	<i>Ovatisporangium</i> sp.3	AB468803	AB468741	AB468925
UZ392	MAFF 241144	Soil (uncultivated)	Fukuoka, Japan	<i>Ovatisporangium</i> sp.4	AB468809	AB468747	
UZ612	MAFF 241149	Soil (uncultivated)	Nagano, Japan	<i>Ovatisporangium</i> sp.5	AB468814	AB468752	
<i>Globisporangium</i>							
UZ067	MAFF 241103	Soil (cultivated)	Nagano, Japan	<i>G. irregulare</i> = <i>P. irregulare</i>	AB468769	AB468705	
UZ370	MAFF 241104	Soil (cultivated)	Hokkaido, Japan	<i>G. irregulare</i> = <i>P. irregulare</i>	AB468770	AB468706	AB468894
OPU1450	MAFF 241105	Wheat	Hokkaido, Japan	<i>G. iwayamae</i> = <i>P. iwayamae</i>	AB299388	AB468707	AB468895
UZ233	MAFF 240155, NBRC 103881	Soil (uncultivated)	Nagano, Japan	<i>G. macrosporum</i> = <i>P. macrosporum</i>	AB359910	AB468708	AB468896
UZ041	MAFF 241106	Soil (cultivated)	Nagano, Japan	<i>G. nunn</i> = <i>P. nunn</i>	AB468771	AB468709	AB468897
OPU1443	MAFF 241107	Orchard grass	Hokkaido, Japan	<i>G. okanoganense</i> = <i>P. okanoganense</i>	AB468817	AB468755	AB468936
OPU1438	MAFF 241108	Wheat	Hokkaido, Japan	<i>G. paddicum</i> = <i>P. paddicum</i>	AB468772	AB468710	AB468898
OPU466	CBS 157.64	Soil	Adelaide, Australia	<i>G. paroecandrum</i> = <i>P. paroecandrum</i>	AY598644	AY598644	
UZ354	MAFF 241109	Soil (uncultivated)	Hokkaido, Japan	<i>G. rostratifingens</i> = <i>P. rostratifingens</i>	AB468773	AB468711	AB468899
OPU1440	MAFF 241110	Wheat	Hokkaido, Japan	<i>G. rostratifingens</i> = <i>P. rostratifingens</i>	AB468774	AB468712	AB468900
OPU1441	MAFF 241111	Wheat	Hokkaido, Japan	<i>G. rostratum</i> = <i>P. rostratum</i>	AB468775	AB468713	AB468901
UZ150	MAFF 240027	Soil (cultivated)	Gunma, Japan	<i>G. spinosum</i> = <i>P. spinosum</i>	AB468776	AB468714	AB468902
UZ405	MAFF 241112	Soil (cultivated)	Fukuoka, Japan	<i>G. spinosum</i> = <i>P. spinosum</i>	AB468777	AB468715	

**Table 1** continued

Isolate no.		Isolate origin		Species <sup>a</sup>	GenBank accession No.		
Origin	International	Substrate	Locality		ITS	D1/D2	coxII
UZ174	MAFF 241113	Soil (cultivated)	Kagoshima, Japan	<i>G. splendens</i> = <i>P. splendens</i>	AB468778	AB468716	AB468903
UZ307	MAFF 241114	Soil (uncultivated)	Kyoto, Japan	<i>G. sylvaticum</i> = <i>P. sylvaticum</i> <i>G. ultimum</i> = <i>P. ultimum</i>	AB468779	AB468717	AB468904
OPU465	CBS 219.65	<i>Chenopodium album</i>	USA	Var. <i>sporangiferum</i>	AY598656	AY598656	
UZ056	MAFF 240024	Soil (cultivated)	Nagano, Japan	Var. <i>ultimum</i>	AB468781	AB468719	AB468906
Py-2	MAFF 240295	Lettuce	Hyogo, Japan	<i>G. uncinulatum</i> = <i>P. uncinulatum</i>	AB468782	AB468720	AB468907
UZ164	MAFF 241123	Soil (cultivated)	Fukushima, Japan	<i>Globisporangium</i> sp.1	AB468788	AB468726	AB468913
UZ182	MAFF 241124	Soil (cultivated)	Kumamoto, Japan	<i>Globisporangium</i> sp.2	AB468789	AB468727	AB468914
UZ213	MAFF 241126	Soil (uncultivated)	Gunma, Japan	<i>Globisporangium</i> sp.3	AB468791	AB468729	AB468916
UZ318	MAFF 241141	Soil (uncultivated)	Hokkaido, Japan	<i>Globisporangium</i> sp.3	AB468806	AB468744	AB468928
UZ400	MAFF 241145	Soil (uncultivated)	Fukuoka, Japan	<i>Globisporangium</i> sp.3	AB468810	AB468748	AB468931
UZ249	MAFF 241129	Soil (uncultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.4	AB468794	AB468732	AB468919
UZ252	MAFF 241130	Soil (uncultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.5	AB468795	AB468733	
UZ253	MAFF 241131	Soil (uncultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.6	AB468796	AB468734	AB468920
UZ260	MAFF 241132	Soil (uncultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.7	AB468797	AB468735	AB468921
UZ263	MAFF 241133	Soil (cultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.8	AB468798	AB468736	
UZ284	MAFF 241136	Soil (cultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.8	AB468801	AB468739	AB468924
UZ275	MAFF 241134	Soil (uncultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.9	AB468799	AB468737	AB468922
UZ277	MAFF 241135	Soil (cultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.10	AB468800	AB468738	AB468923
UZ285	MAFF 241137	Soil (cultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.11	AB468802	AB468740	
UZ290	MAFF 241139	Soil (cultivated)	Okinawa, Japan	<i>Globisporangium</i> sp.12	AB468804	AB468742	AB468926
UZ304	MAFF 241140	Soil (uncultivated)	Ibaraki, Japan	<i>Globisporangium</i> sp.13	AB468805	AB468743	AB468927
UZ382	MAFF 241143	Soil (uncultivated)	Nagano, Japan	<i>Globisporangium</i> sp.14	AB468808	AB468746	AB468930
UZ416	MAFF 241146	Soil (uncultivated)	Fukuoka, Japan	<i>Globisporangium</i> sp.15	AB468811	AB468749	AB468932
UZ594	MAFF 241148	Soil (uncultivated)	Aichi, Japan	<i>Globisporangium</i> sp.16	AB468813	AB468751	
UZ636	MAFF 241150	Soil (uncultivated)	Miyagi, Japan	<i>Globisporangium</i> sp.17	AB468815	AB468753	AB468934
ZSF0030		Soil (uncultivated)	Nagano, Japan	<i>Globisporangium</i> sp.18	AB468821	AB468759	AB468940
ZSF0069		Soil (uncultivated)	Nagano, Japan	<i>Globisporangium</i> sp.19	AB468823	AB468761	AB468942
				<i>Elongisporangium</i>			
ZSF0056	NBRC 103814	Soil (uncultivated)	Nagano, Japan	<i>Elongisporangium</i> sp.1	AB468822	AB468760	AB468941
				<i>Pilasporangium</i>			
UZ300	MAFF 241059	Soil (uncultivated)	Wakayama, Japan	<i>Pi. apinafurcum</i> = <i>Py. apinafurcum</i>	AB458660	AB458651	AB458820
UZ301	MAFF 241060	Soil (uncultivated)	Wakayama, Japan	<i>Pi. apinafurcum</i> = <i>Py. apinafurcum</i>	AB458657	AB458652	AB458818

<sup>a</sup> Numbers following unidentified isolates indicate morphological groups

## Materials and methods

### Isolation and morphological observation

*Pythium* species were isolated from 79 soil samples collected in 18 prefectures of Japan. Among these samples, 40 were from cultivated fields and 39 from uncultivated fields, such as forests, marshes, naturally grown weeds, and

roadsides. The isolations from soil samples and morphological observations were performed by methods described previously (Uzhashi et al. 2008, 2009).

### DNA extraction, amplification, and sequencing

The 69 isolates of *Pythium* were chosen for molecular phylogenetic analyses based on their morphological

characteristics. Some of them were deposited in the Microbiological Genebank, National Institute of Agrobiological Sciences (MAFF), Japan, and the Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Japan, as shown in Table 1. DNA extractions from these isolates and amplification of the LSU D1/D2 region of the rDNA were prepared by a method described previously (Uzuhashi et al. 2008, 2009). The *coxII* gene was amplified with the primer pair of FM66 (5' TAGGATTTCAAGATCCTGC 3') and FM58 (5' CCACAAATTTCACTACATTGA 3') described by Martin (2000). Reaction mixtures contained 2.5 µl of 10× Ex Taq buffer (20 mM Tris–HCl, pH 8.0; 100 mM KCl), 2.0 µl of 2.5 mM deoxyribonucleotide triphosphate (dNTP) mixture, 2.5 µl of 2 µM each primer, 0.125 µl of Taq polymerase (Takara Bio, Shiga, Japan), 1.0 µl of template DNA, and 14.5 µl sterile distilled water. Amplification was carried out in a polymerase chain reaction (PCR) System 9700 (Applied Biosystems, Tokyo, Japan) according to the following amplification program: an initial denaturation at 95°C for 5 min, followed by 40 cycles including denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min, and the final extension step at 72°C for 7 min. PCR products were purified with Wizard SV Gel and PCR Clean-Up System (Promega, Tokyo, Japan) following the manufacturer's instructions and then used for sequence analysis. All sequence reactions were performed as previously described (Uzuhashi et al. 2008). All sequence data has been deposited in the GenBank (Table 1).

### Molecular phylogenetic analyses

All isolates used for molecular phylogenetic analyses are listed in Table 1. In addition to the species isolated in this study, phylogenetically diverse *Pythium* species were chosen based on a previous study (Lévesque and de Cock 2004). Other organisms belonging to the Oomycetes were chosen mainly based on the results of a report by Riethmüller et al. (2002) and analyzed (Table 2). *Saproomyces elongatus* (Cornu) Thaxt. (Rhipidiales in the Rhipidiomycetidae) was chosen as the outgroup on the basis of the results of previous studies (Riethmüller et al. 1999; Petersen and Rosendahl 2000). All sequence data were aligned initially using ClustalX (Thompson et al. 1997) and then visually checked and refined with MacClade 4 (Maddison and Maddison 2000). The complete alignments were deposited in TreeBASE as SN4688 (D1/D2) and SN4689 (*coxII*). All neighbor-joining (NJ) analyses (Saitou and Nei 1987) were performed using PAUP\* 4.0b10 (Swofford 2002). The aligned data were firstly analyzed with Modeltest version 3.7 (Posada and Crandall 1998) using the Akaike information criterion

(AIC; Akaike 1974) to find the most appropriated model of DNA substitution, which was then used to calculate the NJ tree. Support for internal tree nodes was obtained using bootstrap analysis of 100 replicates. All maximum likelihood (ML) analyses were performed using RAxML software version 2.2.3 (Stamatakis 2006). One hundred random sequence additions, as well as 100 bootstrap replicates, were computed with RAxML version 2.2.3 using the GTRMIX algorithm. All phylogenetic trees were visualized by TREEVIEW version 1.6.6 (Page 1996).

## Results and discussion

### Identification of isolates

The 554 isolates of *Pythium* were obtained from 79 soil samples. Among them, 314 isolates were obtained from cultivated fields, and the other 240 isolates were obtained from uncultivated fields. Based on the morphological characteristics observed, the 356 isolates were identified into 18 species. The other 198 isolates could not be identified either because an asexual or sexual stage was not observed or they had unique morphological characteristics differing from those of reported species despite forming several organs of both stages. Further taxonomic examination of these isolates, such as whether or not these isolates should be considered as new species, are necessary in the future. Most isolates from cultivated fields were identified, whereas more than half of the isolates obtained from uncultivated fields were not identified. Although the number of identified isolates was greater in cultivated fields than in uncultivated fields, the number of species identified was almost the same between the two types of fields. These results suggest that more diverse *Pythium* species occupied uncultivated fields than cultivated fields, and many *Pythium* isolates from uncultivated field could not be identified to species.

### Alignment

In phylogenetic analyses based on the D1/D2 region of LSU rDNA, 208 sequences were analyzed, which included 99 sequences from *Pythium* isolates (Table 2). The length of all sequences varied from 545 to 651 bp, and the total length after alignment was 692 bp. The log likelihood of the best ML tree recovered by RAxML was  $-15897.572501$ . The base frequencies were A 0.221360, C 0.177384, G 0.311829, and T 0.289427. In the *coxII* gene, 127 sequences were analyzed, which included 75 sequences from *Pythium* isolates (Table 2). The length of all sequences varied from 554 to 581 bp, and the total length after alignment was 581 bp. The log likelihood

**Table 2** GenBank accession numbers and references of taxa used in this study

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	CoxII	
Oomycetes				
Albuginales				
Albuginaceae				
Genus <i>Albugo</i>				
<i>A. candida</i>	AR 156	AY035539		Riethmüller et al. (2002)
<i>A. evolvuli</i>	AR 377	DQ007489		Voglmayr and Riethmüller (2006)
<i>A. occidentalis</i>			AY286220	Hudspeth et al. (2003)
<i>A. tragopogonis</i>			AY286221	Hudspeth et al. (2003)
Peronosporales				
Peronosporaceae				
Genus <i>Basidiophora</i>				
<i>B. entospora</i>	HV 123	AY035513		Riethmüller et al. (2002)
	HV 123		DQ365699	Göker et al. (2007)
Genus <i>Bremia</i>				
<i>B. lactucae</i>	HV 704	AY035507		Riethmüller et al. (2002)
	HV 759		DQ365701	Göker et al. (2007)
Genus <i>Halophytophthora</i>				
<i>H. avicenniae</i>	CBS 188.85	AY598668		Lévesque and de Cock (2004)
<i>H. polymorphica</i>	CBS 680.84	AY598669		Lévesque and de Cock (2004)
Genus <i>Hyaloperonospora</i>				
<i>H. barbareae</i>	MG 13-6	AY035499		Riethmüller et al. (2002)
<i>H. brassicae</i>	MG 12-10	AY035503		Riethmüller et al. (2002)
<i>H. dentariae</i>	MG 5-8	AY035505		Riethmüller et al. (2002)
<i>H. erophilae</i>	MG 19-4		DQ365705	Göker et al. (2007)
<i>H. niessliana</i>	MG 4-1	AY035498		Riethmüller et al. (2002)
<i>H. parasitica</i>	AR 154	AY035501		Riethmüller et al. (2002)
	MG 5-8		DQ365708	Göker et al. (2007)
<i>H. thlaspeos-arvensis</i>	MG 15-2	AY035502		Riethmüller et al. (2002)
Genus <i>Paraperonospora</i>				
<i>P. leptosperma</i>	HV 383	AY035515		Riethmüller et al. (2002)
	HV 383		DQ365712	Göker et al. (2007)
Genus <i>Peronospora</i>				
<i>P. aestivalis</i>	HV 167	AY035482		Riethmüller et al. (2002)
	MG 18-4		DQ365714	Göker et al. (2007)
<i>P. alsinearum</i>	HV 6a	AY035472		Riethmüller et al. (2002)
<i>P. aparines</i>	MG 4-5	AY035484		Riethmüller et al. (2002)
	MG 4-5		DQ365717	Göker et al. (2007)
<i>P. arvensis</i>	MG 15-10	AY035491		Riethmüller et al. (2002)
	MG 15-9		DQ365719	Göker et al. (2007)
<i>P. boni-henrici</i>	AR 167	AY035475		Riethmüller et al. (2002)
	MG 7-4		DQ365720	Göker et al. (2007)
<i>P. bulbocapni</i>		AF119599		Riethmüller et al. (1999)
<i>P. calotheca</i>	HV 83	AY035483		Riethmüller et al. (2002)
	MG 6-2		DQ365721	Göker et al. (2007)
<i>P. conglomerata</i>	HV 27	AY035489		Riethmüller et al. (2002)
	MG 18-11		DQ365723	Göker et al. (2007)
<i>P. ficariae</i>		AF119600		Riethmüller et al. (1999)

**Table 2** continued

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	CoxII	
<i>P. hiemalis</i>	HV 5a	AY035469		Riethmüller et al. (2002)
	MG 4-4		DQ365724	Göker et al. (2007)
<i>P. lamii</i>	MG 14-1	AY035494		Riethmüller et al. (2002)
	MG 14-2		DQ365725	Göker et al. (2007)
<i>P. myosotidis</i>	MG 1-4	AY035473		Riethmüller et al. (2002)
<i>P. potentillae-sterilis</i>	MG 14-5	AY035486		Riethmüller et al. (2002)
<i>P. pulveracea</i>	MG 9-5	AY035470		Riethmüller et al. (2002)
<i>P. rumicis</i>	HV 300	AY035476		Riethmüller et al. (2002)
<i>P. sanguisobae</i>	MG 12-6	AY035487		Riethmüller et al. (2002)
	MG 12-2		DQ365729	Göker et al. (2007)
<i>P. silvestris</i>	AR 194	AY035490		Riethmüller et al. (2002)
<i>P. sparsa</i>	MG 14-9	AY035488		Riethmüller et al. (2002)
<i>P. trifolii-alpestris</i>	MG 9-10	AY035481		Riethmüller et al. (2002)
<i>P. trifolii-hybridi</i>	MG 13-8	AY035480		Riethmüller et al. (2002)
<i>P. trifolii-minoris</i>	MG 11-9	AY035479		Riethmüller et al. (2002)
<i>P. trivialis</i>	MG 6-4	AY035471		Riethmüller et al. (2002)
	MG 6-4		DQ365733	Göker et al. (2007)
<i>P. variabilis</i>	MG 8-6	AY035477		Riethmüller et al. (2002)
	MG 8-6		DQ365734	Göker et al. (2007)
Genus <i>Phytophthora</i>				
<i>P. boehmeriae</i>	PD_00181	EU080166		Blair et al. (2008)
	325		AY129177	Martin and Tooley (2003)
<i>P. cactorum</i>	PD_00929	EU080282		Blair et al. (2008)
	MG 34-2		DQ365737	Göker et al. (2007)
<i>P. capsici</i>	PD_00009	EU080856		Blair et al. (2008)
	AR 244		DQ365739	Göker et al. (2007)
<i>P. cinnamomi</i>	PD_00394	EU080457		Blair et al. (2008)
	Cn-2		AY129182	Martin and Tooley (2003)
<i>P. citricola</i>	SB2084		AY129184	Martin and Tooley (2003)
<i>P. citrophthora</i>	PD_00041	EU080542		Blair et al. (2008)
<i>P. clandestina</i>	PD_00134	EU079871		Blair et al. (2008)
<i>P. erythroseptica</i>	PD_00014	EU079832		Blair et al. (2008)
	ATCC 36302		AY129191	Martin and Tooley (2003)
<i>P. europaea</i>	PD_00082	EU079486		Blair et al. (2008)
<i>P. gonapodyides</i>	PD_00040	EU080535		Blair et al. (2008)
	393		AY129197	Martin and Tooley (2003)
<i>P. heveae</i>	PD_00073	EU080800		Blair et al. (2008)
	MG 25-8		DQ365742	Göker et al. (2007)
<i>P. ilicis</i>	PD_00133	EU079864		Blair et al. (2008)
	343		AY129202	Martin and Tooley (2003)
<i>P. insolita</i>	PD_00175	EU080180		Blair et al. (2008)
	MG 33-8		DQ365744	Göker et al. (2007)
<i>P. multivesiculata</i>	PD_00001	EU080070		Blair et al. (2008)
	MG 33-6		DQ365748	Göker et al. (2007)
<i>P. nicotianae</i>	PD_01305	EU080889		Blair et al. (2008)
	Pn-17		AY129215	Martin and Tooley (2003)
<i>P. palmivora</i>	329		AY129217	Martin and Tooley (2003)

**Table 2** continued

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	<i>CoxII</i>	
<i>P. quercina</i>	PD_00035	EU080489		Blair et al. (2008)
	MG 34-3		DQ365751	Göker et al. (2007)
<i>P. ramorum</i>	PD_00058	EU080688		Blair et al. (2008)
	CBS 101553		EU427471	Martin (2008)
<i>P. syringae</i>	PD_00044	EU080562		Blair et al. (2008)
	IMI 296829		AY129224	Martin and Tooley (2003)
Genus <i>Plasmopara</i>				
<i>P. baudysii</i>	HV 571	AY035517		Riethmüller et al. (2002)
<i>P. densa</i>	MG 6-1	AY035525		Riethmüller et al. (2002)
	MG 1-6		DQ365754	Göker et al. (2007)
<i>P. geranii</i>	HV 6.4.P.P	AY035520		Riethmüller et al. (2002)
<i>P. halstedii</i>	AR 151	AY035523		Riethmüller et al. (2002)
<i>P. megasperma</i>	HV B.M.4.4	AY035516		Riethmüller et al. (2002)
	MG 39-4		DQ365755	Göker et al. (2007)
<i>P. obducens</i>	HV 5.4.P.O	AY035522		Riethmüller et al. (2002)
	HV 306		DQ365757	Göker et al. (2007)
<i>P. pimpinellae</i>	HV 634	AY035519		Riethmüller et al. (2002)
<i>P. pusilla</i>	MG 8-10	AY035521		Riethmüller et al. (2002)
	MG 8-10		DQ365759	Göker et al. (2007)
<i>P. sii</i>	HV 550	AY035518		Riethmüller et al. (2002)
<i>P. viticola</i>	AR 150	AY035524		Riethmüller et al. (2002)
	MG 11-5		DQ365760	Göker et al. (2007)
Genus <i>Plasmoverna</i>				
<i>P. isopyri-thalictroidis</i>	HV 266	AY035526		Riethmüller et al. (2002)
<i>P. pygmaea</i>		AF119605		Riethmüller et al. (1999)
Genus <i>Pseudoperonospora</i>				
<i>P. cubensis</i>	HV 221 h	AY035496		Riethmüller et al. (2002)
<i>P. humuli</i>	HV 129	AY035497		Riethmüller et al. (2002)
	HV 129		DQ365763	Göker et al. (2007)
<i>P. urticae</i>	HV 713		DQ365764	Göker et al. (2007)
Genus <i>Viennotia</i>				
<i>V. oplismeni</i>	HV isol. 11	AY035527		Riethmüller et al. (2002)
Pythiales				
Pythiaceae				
Genus <i>Lagenidium</i>				
<i>L. callinectes</i>	ATCC 24973	AB285217		–
	ATCC 200337		AF290308	Cooke et al. (2000)
<i>L. chthamalophilum</i>		AF235946		Petersen and Rosendahl (2000)
<i>L. giganteum</i>	ATCC 36492		AF086697	Hudspeth et al. (2000)
<i>L. myophilum</i>	ATCC 66280	AB285220		–
<i>L. humanum</i>	ATCC 76726		AF290310	Cooke et al. (2000)
<i>L. thermophilum</i>	ATCC 200318	AB285219		–
	ATCC 200318		AF290304	Cooke et al. (2000)
Genus <i>Pythium</i>				
<i>P. acrogynum</i>	CBS 549.88	AY598638		Lévesque and de Cock (2004)
<i>P. adhaerens</i>	CBS 520.74	AY598619		Lévesque and de Cock (2004)
<i>P. anandrum</i>	CBS 285.31	AY598650		Lévesque and de Cock (2004)



**Table 2** continued

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	<i>CoxII</i>	
<i>P. angustatum</i>	CBS 522.74	AY598623		Lévesque and de Cock (2004)
<i>P. aploeroticum</i>	CBS 772.81	AY598631		Lévesque and de Cock (2004)
<i>P. arrhenomanes</i>	CBS 324.62	AY598628		Lévesque and de Cock (2004)
	1993-39		AF196586	–
<i>P. boreale</i>	CBS 551.88	AY598662		Lévesque and de Cock (2004)
	CBS 551.88		EF408876	–
<i>P. capillosum</i>	CBS 222.94	AY598635		Lévesque and de Cock (2004)
<i>P. caudatum</i>	ATCC 58383		AF290309	Cooke et al. (2000)
<i>P. conidiophorum</i>	CBS 223.88	AY598629		Lévesque and de Cock (2004)
<i>P. cucurbitacearum</i>	CBS 748.96	AY598667		Lévesque and de Cock (2004)
<i>P. cylindrosporium</i>	CBS 218.94	AY598643		Lévesque and de Cock (2004)
<i>P. deliense</i>	1989-19		AF196589	–
<i>P. dimorphum</i>	CBS 406.72	AY598651		Lévesque and de Cock (2004)
<i>P. echinulatum</i>	CBS 281.64	AY598639		Lévesque and de Cock (2004)
<i>P. graminicola</i>	CBS 327.62	AY598625		Lévesque and de Cock (2004)
	ATCC 96234		AB160849	–
<i>P. helicandrum</i>	CBS 393.54	AY598653		Lévesque and de Cock (2004)
<i>P. helicoides</i>	CBS 286.31	AY598665		Lévesque and de Cock (2004)
	CBS 167.68		AB257273	Kageyama et al. (2007)
<i>P. heterothallicum</i>	CBS 450.67	AY598654		Lévesque and de Cock (2004)
	ATCC 18198		AF196595	–
<i>P. inflatum</i>	CBS 168.68	AY598626		Lévesque and de Cock (2004)
	MAFF 305863		DQ071379	Villa et al. (2006)
<i>P. insidiosum</i>	CBS 574.85	AY598637		Lévesque and de Cock (2004)
	ATCC 58643		AF196597	–
<i>P. intermedium</i>	CBS 266.38	AY598647		Lévesque and de Cock (2004)
	MAFF 305570		DQ071380	Villa et al. (2006)
<i>P. mastophorum</i>	CBS 375.72	AY598661		Lévesque and de Cock (2004)
<i>P. monospermum</i>	CBS 158.73	AY598621		Lévesque and de Cock (2004)
	AR 213		DQ365765	Göker et al. (2007)
<i>P. multisporum</i>	CBS 470.50	AY598641		Lévesque and de Cock (2004)
<i>P. myriotylum</i>	1993-43		AF196608	–
<i>P. nodosum</i>	MAFF 305905		DQ071399	Villa et al. (2006)
<i>P. oedochilum</i>	CBS 292.37	AY598664		Lévesque and de Cock (2004)
	CBS 252.70		AB108012	
<i>P. oligandrum</i>	CBS 382.34	AY598618		Lévesque and de Cock (2004)
	81-10		AF196610	–
<i>P. ostracodes</i>	CBS 768.73	AY598663		Lévesque and de Cock (2004)
	CBS 768.73		AB108013	Kageyama et al. (2007)
<i>P. paroecandrum</i>	CBS 157.64		DQ071391	Villa et al. (2006)
<i>P. perplexum</i>	CBS 674.85	AY598658		Lévesque and de Cock (2004)
<i>P. pleroticum</i>	CBS 776.81	AY598642		Lévesque and de Cock (2004)
<i>P. polymastum</i>	CBS 881.70	AY598660		Lévesque and de Cock (2004)
<i>P. prolatum</i>	CBS 845.68	AY598652		Lévesque and de Cock (2004)
<i>P. sulcatum</i>	ATCC 24735		AF196620	–
<i>P. undulatum</i>	AR 55	AF119603		Riethmüller et al. (1999)
	MG 33-2		DQ365766	Göker et al. (2007)

**Table 2** continued

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	CoxII	
<b>Ripidiales</b>				
<b>Rhipidiaceae</b>				
Genus <i>Sapromyces</i>				
<i>S. elongatus</i>	AR 9	AF119618	AF086700	Riethmüller et al. (2002) Hudspeth et al. (2000)
<b>Leptomitales</b>				
<b>Leptomitaceae</b>				
Genus <i>Apodachlya</i>				
<i>A. brachynema</i>	AR 93	AF119590		Riethmüller et al. (2002)
<i>A. pyrifer</i>			AF086695	Hudspeth et al. (2000)
Genus <i>Leptomit</i>				
<i>L. lacteus</i>	AR 80 ATCC 38076	AF119597	AF086696	Riethmüller et al. (2002) Hudspeth et al. (2000)
<b>Saprolegniales</b>				
<b>Leptolegniaceae</b>				
Genus <i>Aphanomyces</i>				
<i>A. laevis</i>	AR 47	AF119586		Riethmüller et al. (2002)
<i>A. stellatus</i>	AR 51	AF119587		Riethmüller et al. (2002)
Genus <i>Leptolegnia</i>				
<i>L. caudata</i>		AF218176		Leclerc et al. (2000)
Genus <i>Pachymetra</i>				
<i>P. chaunorhiza</i>	CBS 960.87	AF119598		Riethmüller et al. (2002)
Genus <i>Plectospira</i>				
<i>P. myriandra</i>	CBS 523.87	AF119606		Riethmüller et al. (1999)
<b>Saprolegniaceae</b>				
Genus <i>Achlya</i>				
<i>A. americana</i>	AR 26	AF119574		Riethmüller et al. (2002)
<i>A. caroliniana</i>	AR 97	AF119576		Riethmüller et al. (2002)
<i>A. colorata</i>	CBS 545.67	AF119577		Riethmüller et al. (2002)
<i>A. dubia</i>	CBS 546.67	AF119578		Riethmüller et al. (2002)
<i>A. klebsiana</i>	CBS 101.49	AF119579		Riethmüller et al. (2002)
<i>A. papillosa</i>	CBS 101.52	AF119580		Riethmüller et al. (2002)
<i>A. racemosa</i>	AR 48	AF119581		Riethmüller et al. (2002)
<i>A. radiosa</i>	AR 2	AF119582		Riethmüller et al. (2002)
<i>A. spinosa</i>	AR 95	AF119583		Riethmüller et al. (2002)
<i>A. treleaseana</i>	CBS 575.67	AF119584		Riethmüller et al. (2002)
Genus <i>Aplanes</i>				
<i>A. androgynus</i>		AF119588		Riethmüller et al. (2002)
Genus <i>Aplanopsis</i>				
<i>A. spinosa</i>	CBS 112.61	AF119589		Riethmüller et al. (2002)
Genus <i>Brevilegnia</i>				
<i>B. bisporea</i>	CBS 569.67	AF119591		Riethmüller et al. (2002)
<i>B. megasperma</i>	AR 4	AF119592		Riethmüller et al. (2002)
Genus <i>Calyptralegnia</i>				
<i>C. achlyoides</i>	AR 5	AF119593		Riethmüller et al. (2002)
Genus <i>Dictyuchus</i>				
<i>D. monosporus</i>	CBS 467.81	AF119595		Riethmüller et al. (2002)

**Table 2** continued

Taxon	Isolate no.	GenBank accession no.		Reference
		D1/D2	CoxII	
<i>D. sterilis</i>	ATCC 44890		AF086691	Hudspeth et al. (2000)
Genus <i>Isoachlya</i>				
<i>I. toruloides</i>		AF235947		Petersen and Rosendahl (2000)
Genus <i>Protoachlya</i>				
<i>P. paradoxa</i>	ATCC 44892	DQ393493		Hulvey et al. (2007)
<i>P. Polyspora</i>	ATCC 28092	DQ393492		Hulvey et al. (2007)
Genus <i>Pythiopsis</i>				
<i>P. cymosa</i>		AF218172		Leclerc et al. (2000)
	ATCC 26880		AF086689	Hudspeth et al. (2000)
Genus <i>Saprolegnia</i>				
<i>S. anisospora</i>	CBS 537.67	AF119609		Riethmüller et al. (2002)
<i>S. diclina</i>	AR 12	AF119610		Riethmüller et al. (2002)
<i>S. eccentrica</i>	CBS 551.67	AF119611		Riethmüller et al. (2002)
<i>S. ferax</i>	AR 16	AF119612		Riethmüller et al. (2002)
	ATCC 36051		AF086690	Hudspeth et al. (2000)
<i>S. hypogyna</i>	CBS 869.72	AF119613		Riethmüller et al. (2002)
<i>S. litoralis</i>	CBS 535.67	AF119614		Riethmüller et al. (2002)
<i>S. monilifera</i>	CBS 558.67	AF119615		Riethmüller et al. (2002)
<i>S. parasitica</i>	IFO 32780		DQ071421	Villa et al. (2006)
Genus <i>Scoliolegnia</i>				
<i>S. asterophora</i>	AR 94	AF119619		Riethmüller et al. (2002)
Genus <i>Thraustotheca</i>				
<i>T. clavata</i>	AR 10	AF119620		Riethmüller et al. (2002)

of the best ML tree recovered by RAxML was –13011.219625. The base frequencies were A 0.314994, C 0.111223, G 0.166925, and T 0.406857.

#### Phylogenetic position of *Pythium*

All phylogenetic trees constructed in this study based on two different regions, rDNA D1/D2 and *coxII*, and two different methods, ML and NJ, showed a basal division of Oomycetes into two lineages with strong or moderate support (Figs. 1, 2). One lineage was composed of the Albuginales, Peronosporales, and Pythiales, including the genus *Pythium*. Another was composed of the Leptomitales and Saprolegniales. The same phylogenetic relationship between two subclasses was reported by several previous investigations based on LSU rDNA (Petersen and Rosendahl 2000; Riethmüller et al. 2002), small subunit (SSU) rDNA (Dick 1999), and *coxII* (Hudspeth et al. 2000).

Within the lineage including *Pythium*, the genus *Pythium* was clearly differentiated from the other genera analyzed, but it appeared to be a nonmonophyletic group including several monophyletic groups. The genus includes very divergent members phylogenetically, which is clear

when compared with those of the other genera. The genus *Pythium* is placed in an intermediate position between *Lagenidium*, and *Phytophthora* or *Halophytophthora* in D1/D2 phylogenetic tree. Thus, it is suggested that the genus is an ancestor of *Phytophthora* or *Halophytophthora* (Fig. 1). A similar phylogenetic position of *Pythium* was not shown in the *coxII* analyses, but the phylogenetic position of *Pythium* in *coxII* trees was not resolved because most of the basal branches were not supported significantly (Fig. 2). In contrast to the relationships between genera of Pythiales, Peronosporales appeared to be a monophyletic group, with significant support in D1/D2 analyses (Fig. 1).

#### Phylogeny of *Pythium*

Previous phylogenetic analysis based on most sequence data of *Pythium* (116 species) was reported by Lévesque and de Cock (2004). This study divided *Pythium* into 11 clades, A–K. Because at least one species was chosen from each clade and was analyzed with many isolates obtained from soils in this study, it was suggested that the most morphologically or phylogenetically divergent *Pythium* species were analyzed. Thus, it was expected that the

phylogeny of *Pythium* was more appropriately evaluated in this study than in previous studies. As a result, *Pythium* was divided into five well- or moderately supported clades (1–5) common to all phylogenetic trees, although tree topologies among clades were slightly different among trees. Each of the five clades corresponded to one clade or one group clustered of several clades of 11 clades (A–K) in a previous study (Lévesque and de Cock 2004). Detail relationships between the five clades in this study (1–5) and 11 clades in the previous study (A–K; Lévesque and de Cock 2004) are also discussed.

Clade 1 was composed of six species and five unidentified *Pythium* isolates in D1/D2 analyses. This clade was strongly supported by bootstrap values (BV) of 96% in ML and 98% in NJ analyses (Fig. 1). A single clade comparable with clade 1 was also detected in the *coxII* trees, and it was supported by 98% BV (ML) and 100% BV (NJ; Fig. 2). Considering the species included in this clade, it was shown that this clade was comparable with clade K of the previous study (Lévesque and de Cock 2004). According to the previous study, two species, *P. chamaeophyon* Sideris and *P. indigoferae* E. J. Butler, are also included in this clade.

Clade 2 was composed of only one species, *P. apinafurcum* Uzuhashi & Tojo, and was only distantly related to the other clades, sufficient to be an independent phylogenetic group. Among eight *P. apinafurcum* isolates obtained in this study, D1/D2 sequences of all isolates were identical, whereas *coxII* showed two sequence patterns among the isolates regardless of their origins. Therefore, two isolates with different *coxII* sequences were analyzed. As a result, a monophyletic group composed of only two isolates was detected, with significant support of 96% BV (ML) and 100% BV (NJ). As in the D1/D2 analyses, this clade was distantly related to the other clades of *Pythium*. The phylogenetic position of clade 2 had not been detected in any previous studies, including that of Lévesque and de Cock (2004). Therefore, *P. apinafurcum* was phylogenetically unique within *Pythium*.

Clade 3 consisted of 21 species and nine unidentified *Pythium* isolates in the D1/D2 analyses. This clade was supported by 93% BV (ML) and 99% BV (NJ), although one species of *Lagenidium*, *La. myophilum* Hatai & Lawhav., was also included in this clade because the D1/D2 sequence of this species (AB285220) was identical to that of *P. capillosum* B. Paul (AY598635). However, the other species of *Lagenidium* analyzed in this study, *La. chthamalophilum* T.W. Johnson, *La. callinectes* Couch, *La. thermophilum* K. Nakam., Miho Nakam., Hatai & Zafran, formed a well-supported clade, which was located in a distant position from *La. myophilum*. Therefore, it is doubtful that the sequence data for *La. myophilum* are of a *Lagenidium* species. A single clade comparable with clade 3 was also detected in the *coxII* trees and supported by 86%

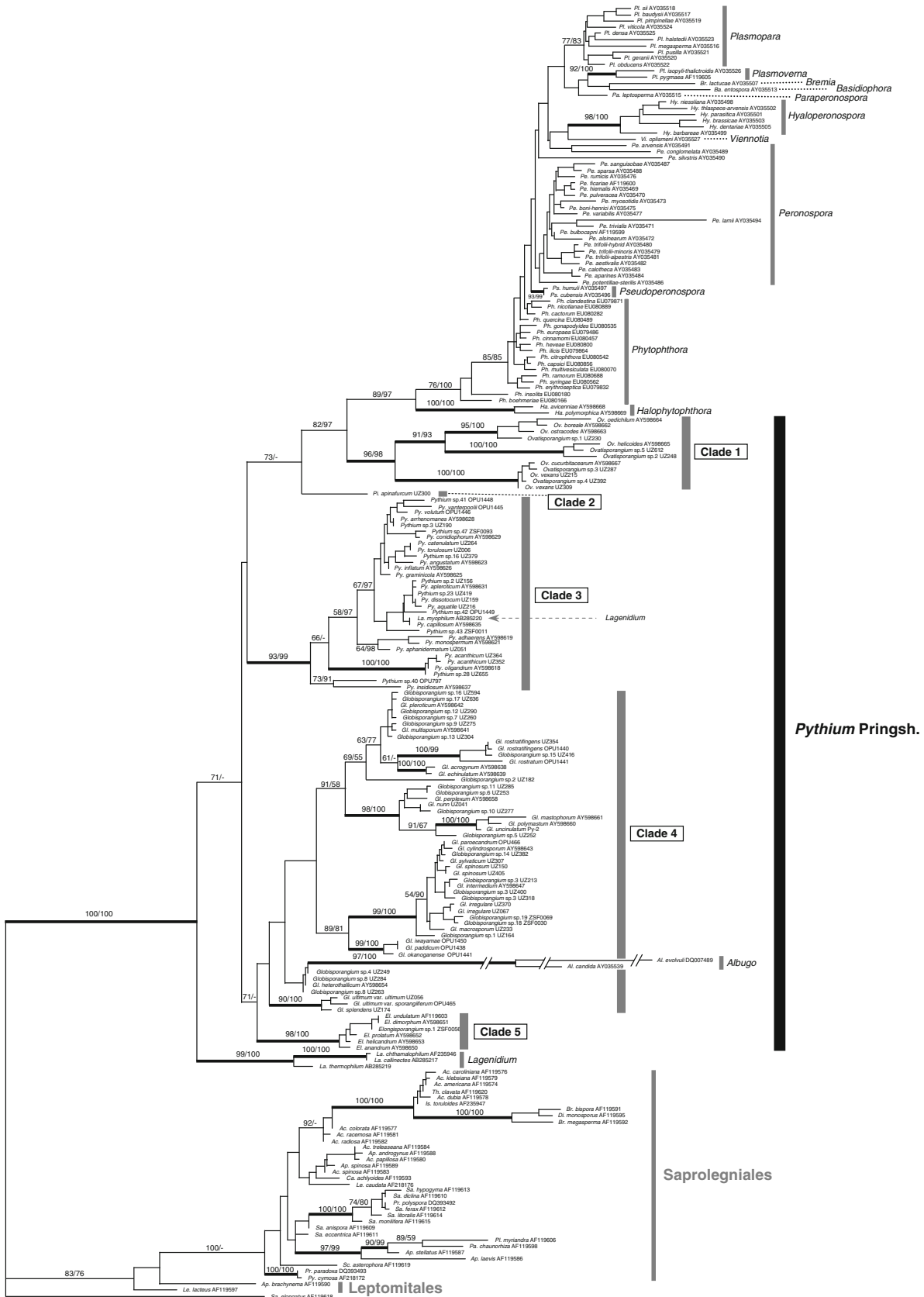
**Fig. 1** Phylogenetic tree of *Pythium* and related genera belonging to the Peronosporomycetes based on rDNA LSU D1/D2 sequences. Branch lengths were estimated with RAxML under maximum likelihood. Numbers on branches represent bootstrap values (BV) greater than 50%. Maximum likelihood (ML) BV from 100 replicates conducted with RAxML (left) and neighbor-joining (NJ) BV from 100 replicates conducted with PAUP\* (right) are shown. Numbers following the species name indicate GenBank accession numbers

BV in the NJ analysis, but it was not supported in the ML tree. In the *coxII* analyses, three *Pythium* species, *P. deliense* Meurs, *P. myriotylum* Drechsler, and *P. sulcatum* R. G. Pratt & J. E. Mitch., were also included in this clade.

Clade 3 consisted of species classified into clades A, B, C, and D in the previous study (Lévesque and de Cock 2004). Each subclade equaling four clades (A–D) was also detected in D1/D2 phylogenetic trees in this study. Furthermore, these four clades (A–D) were also detected as a monophyletic group with significant support in the previous study, which is comparable with clade 3 (Lévesque and de Cock 2004). Therefore, there is little doubt that the members of clade 3 represent a monophyletic group. According to the previous study (Lévesque and de Cock 2004), *P. amasculinum* Y. N. Yu, *P. aristosporum* Vanterp., *P. chondricola* De Cock, *P. coloratum* Vaartaja, *P. conidiophorum* Jokl, *P. diclinum* Tokun., *P. dissimile* Vaartaja, *P. flevoense* Plaäts-Nit., *P. folliculosum* B. Paul, *P. grandisporangium* Fell & Master, *P. hydnosporum* (Mont.) J. Schröt., *P. lutarium* Ali-Shtayeh, *P. marinum* Sparrow, *P. pachycaule* Ali-Shtayeh, *P. periillum* Drechsler, *P. periplocum* Drechsler, *P. plurisporium* Abad, Shew, Grand & L. T. Lucas, *P. porphyrae*, *P. pyrilobum* Vaartaja, *P. scleroteichum* Drechsler, *P. sulcatum* R. G. Pratt & J. E. Mitch., *P. tracheiphilum* Matta, and *P. zingiberis* M. Takah. were also included in this clade.

Clade 4 consisted of 28 *Pythium* species and 22 unidentified *Pythium* spp. isolates in the D1/D2 analyses. The *Albugo* clade was also included in clade 4 in the ML tree. However, clade 4 was not closely related to *Albugo* because the phylogenetic position of *Albugo* was not resolved due to long branches. A single clade comparable with clade 4 was also detected in the *coxII* analyses, although significant support was not obtained. A BV greater than 50% was shown in the NJ tree (53%).

Clade 4 included species belonging to five clades (E, F, G, I, and J) of the previous study (Lévesque and de Cock 2004). A subclade identical to the five clades (clade E, F, G, I, and J) was detected in clade 4, with strong to moderate support in the D1/D2 trees. One exception was shown in clade I. However, most subclades identical to the five clades were not detected in *coxII* analyses. Furthermore, members of clade 4 were not detected as a monophyletic group in the previous study (Lévesque and de Cock 2004), although all members were closely related. As the tree topology within clade 4



largely differed among the trees constructed in this and the previous study, the relationships among members of the clade were not resolved. Considering the low support for this clade and unclear relationships among isolates in this clade, it is unclear whether members of clade 4 form a monophyletic group. Further phylogenetic analyses, including more isolates, are needed to resolve the phylogeny of the clade and relationships among clades. First, the isolation of various isolates, including new species, is necessary. According to the previous study (Lévesque and de Cock 2004), *P. acanthophoron* Sideris, *P. buismaniae* Plaäts-Nit., *P. debaryanum* R. Hesse, *P. erinaceum* J. A. Robertson, *P. hypogynum* Middleton, *P. kunmingense* Y. N. Yu, *P. mammillatum* Meurs, *P. marsipium* Drechsler, *P. middletonii* Sparrow, *P. minor* Ali-Shtayeh, *P. nagaii* S. Ito & Tokun., *P. orthogonon* Ahrens, *P. radiosum* B. Paul, and *P. violae* Chesters & Hickman were also included in this clade.

Clade 5 consisted of five species and one *Pythium* sp. isolate in the D1/D2 analyses. A single clade comparable with clade 5 was also detected in the *coxII* phylogenetic trees, although only two isolates were analyzed. This clade was supported by high BV greater than 98% in all of phylogenetic trees. This clade was comparable with clade H in the previous study (Lévesque and de Cock 2004). As in the previous study (Lévesque and de Cock 2004), clade 5 was closely related to clade 4 and clustered with clade as monophyletic group in the D1/D2 analyses.

#### Relationships between phylogeny and morphology in *Pythium*

Principal relationships between molecular phylogeny and morphological relationships within *Pythium* are shown in Fig. 3. This figure was developed from the phylogeny based on the D1/D2 ML tree and morphological characteristics. The shape of sporangium, formation of papilla, proliferation and zoospores, structure of oogonium, extent of oospores within the oogonium, and the number of antheridia per oogonium are shown. In addition to all isolates analyzed here, the morphological characteristics of other species in each clade suggested by results of the previous study (Lévesque and de Cock 2004) were also considered in the examination.

#### Clade 1

The isolates of clade 1 formed sporangia of various shapes, such as (ob-)ovoid, (sub-)globose, lemon-shaped, or clavate. However, ovoid was the most common shape among the isolates and was frequently formed in each isolate. Sporangia with apical or lateral papilla and/or one to three or more germ tubes were observed in most species in this clade (Fig. 4a–f). Zoospores were formed in all isolates,

**Fig. 2** Phylogenetic tree of *Pythium* and related genera belonging to the Peronosporomycetes based on partial cytochrome oxidase II gene sequences. Branch lengths were estimated with RAxML under maximum likelihood (ML). Numbers on the branches represent bootstrap values (BV) greater than 50%. ML BV from 100 replicates conducted with RAxML (left) and neighbor-joining (NJ) BV from 100 replicates conducted with PAUP\* (right) are shown. Numbers following the species name indicate GenBank accession numbers

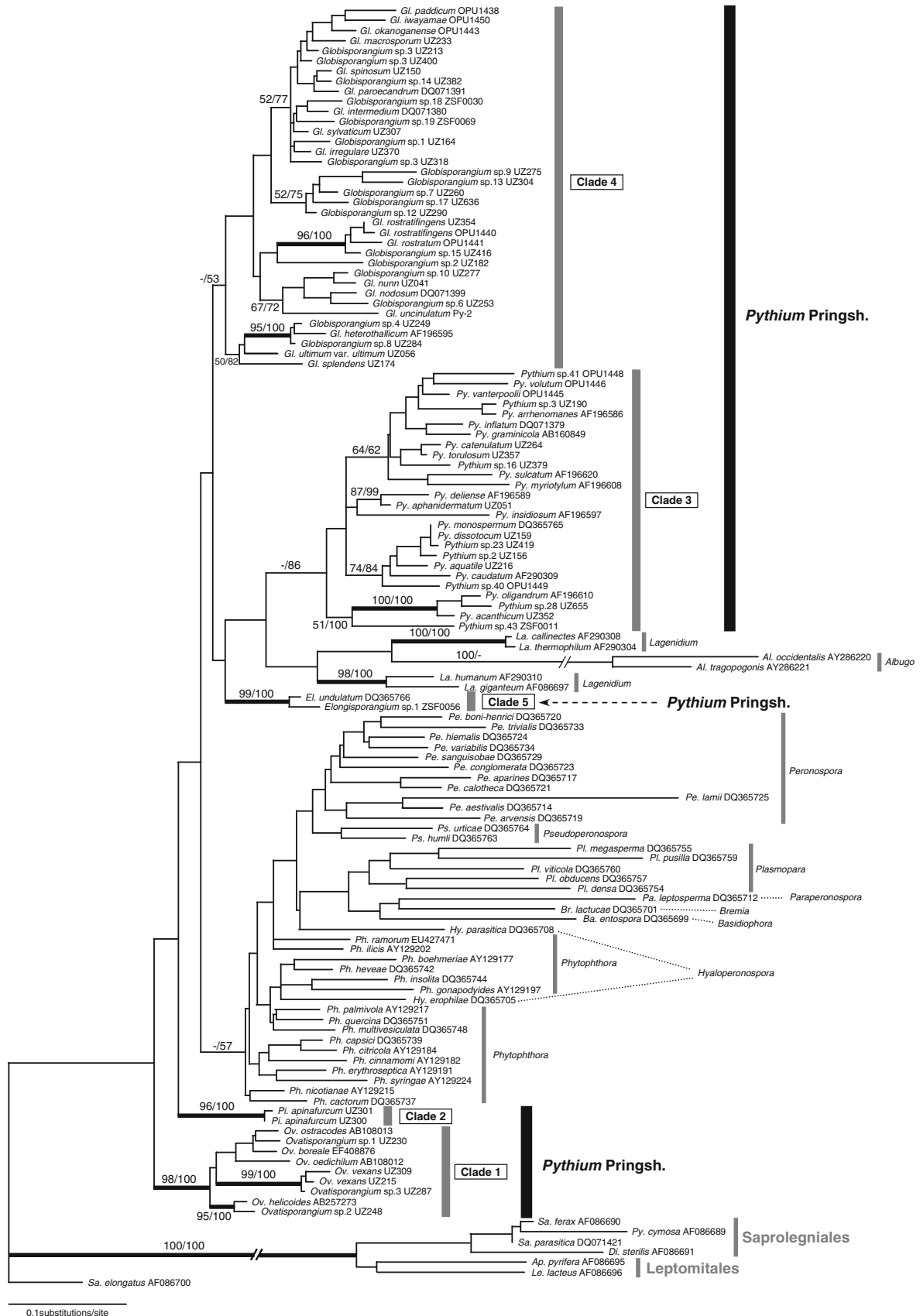
and internal proliferations were also observed in most species (Fig. 4g–i).

In the sexual stage, the surface of oogonia was smooth in all isolates. One exception is known in *P. carbonicum* B. Paul, which is shown to be included in clade K corresponding to clade 1 (de Cock et al. 2008). This species formed both smooth and ornamented oogonia with small projections (Paul 2003). Although oogonia and oospores were generally colorless in most species in this clade, yellowish oogonia or oospores were observed in two unidentified isolates, UZ287, UZ392, and two isolates of *P. vexans* de Bary and were known in *P. helicoides* Drechsler (van der Plaäts-Niterink 1981). Antheridia were monoclinal or diclinal and were mainly divided into two types in terms of both their shape and attachment to the oogonium. One type of antheridia was bell-shaped and had broad contact with the oogonium (Fig. 4j). Another was small and had apical contact with the oogonium (Fig. 4k). One exception was observed in the UZ230 isolate, in which an antheridium was absent, or many antheridia were produced per oogonium, showing undefined shapes or encircling an oogonium. Oospores were aplerotic or plerotic, and both types were observed among or within an isolate. Because morphological characteristics of the sexual stage largely varied not only among isolates but also within single isolates, this clade was characterized by the ovoid sporangia with or without papillae and the frequent formation of zoospores.

One remarkable exception of the morphological characteristics in clade 1 was known in *P. indigoferae*. Although this species was included in clade K by a previous study (Lévesque and de Cock 2004), it was known to form filamentous inflated sporangia (van der Plaäts-Niterink 1981). This morphology, which is inconsistent with the characteristics of this clade, was also pointed out in a previous study (Lévesque and de Cock 2004). However, the previous study concluded that further examination for the species was impossible because the strain of the species (CBS 261.30) no longer forms sporangia. Therefore, the inconsistent morphological characteristics of the species could not be resolved here.

#### Clade 2

Clade 2 comprises only one species, *P. apinafurcum*. Thus, the morphological characteristics of the clade are identical



to those of the species. This species was characterized by complexly branched secondary hyphae (Fig. 5b) and oogonium, within which two oospores were frequently observed (Fig. 5f). The other morphological characteristics were sickle-shaped appressoria, globose nonproliferating sporangia, smooth oogonia, and plerotic or aplerotic oospores (Fig. 5a, c–e). Zoospores were rarely observed.

### Clade 3

Most species in clade 3 formed one characteristic, defined as filamentous sporangia, although various shapes were observed; that is, sporangia that did not differ from vegetative hyphae (noninflated), consisting of a lobate or toruloid inflated element, or catenulate globose elements, were observed among isolates (Fig. 6a–f). A few exceptions were shown in OPU797 and ZSF0093 unidentified isolates in this study and were known in *P. tracheiphilum*, *P. salpingophorum* Drechsler and *P. conidiophorum* (van der Plaäts-Niterink 1981). The OPU797 isolate formed unique ovoid- or pyriform-shaped sporangia. The tip of the sporangia intensively elongated and often reached 150 µm or more (Fig. 6g). This feature was rarely observed in other *Pythium* species. This isolate also sometimes formed sporangia-like filamentous shapes on the same agar medium. The ZSF0093 isolate formed subglobose sporangia, which were often contiguous as a chain (Fig. 6f). Unlike the catenulate sporangia shown in several species within the clade, the shapes were generally ellipsoid rather than globose. A similar shape of sporangia was also known in *P. tracheiphilum*, although the catenulate feature was not shown in this species (van der Plaäts-Niterink 1981). Two species, *P. conidiophorum* and *P. salpingophorum*, show similar morphological characteristics of globose sporangia (van der Plaäts-Niterink 1981). According to our and previous studies, it was suggested that the three species, *P. tracheiphilum*, *P. conidiophorum*, and *P. salpingophorum*, and an isolate, ZSF0093, having (sub-)globose sporangia, were phylogenetically closely related (Lévesque and de Cock 2004). Zoospores were observed in most species in clade 3 regardless of the shape of sporangia. When zoospores were observed, discharge tubes originating from sporangia tended to be longer than those of globose or ovoid sporangia and were 300 µm or more (Fig. 6h). The shapes or sizes of filamentous sporangia varied largely among or within isolates. Because the number of zoospores formed in a vesicle reflected the amount of protoplasm in the original sporangium, the number of zoospores in a vesicle was more variable than in globose sporangia, varying from two to about 40 or more.

A sexual stage was not observed in two isolates, UZ156 and UZ190. In the other isolates, oogonia of most species had a smooth surface, but a few isolates, *P. acanthicum*

**Fig. 3** Relationships between phylogeny and morphology of *Pythium* based on the maximum likelihood (ML) phylogenetic tree using D1/D2 sequences. *Sp* sporangium morphology (*O* ovoid, obovoid or pyriform. *G* globose, subglobose, ellipsoid or cylindrical. *V* amorphous. *E* elongated shapes. *Fi* filamentous inflated. *Fn* filamentous noninflated. *C* catenulate). *Pa* papilla (+produced). *Pr* internal proliferation (+produced). *Zo* zoospores (+produced). *Og* surface wall of oogonia (*S* smooth. *O* ornamented). *Os* oospores (*A* aplerotic, *P* plerotic, *NP* nearly plerotic). *An* number of the antheridium per oogonium (*M* many antheridia undefined)

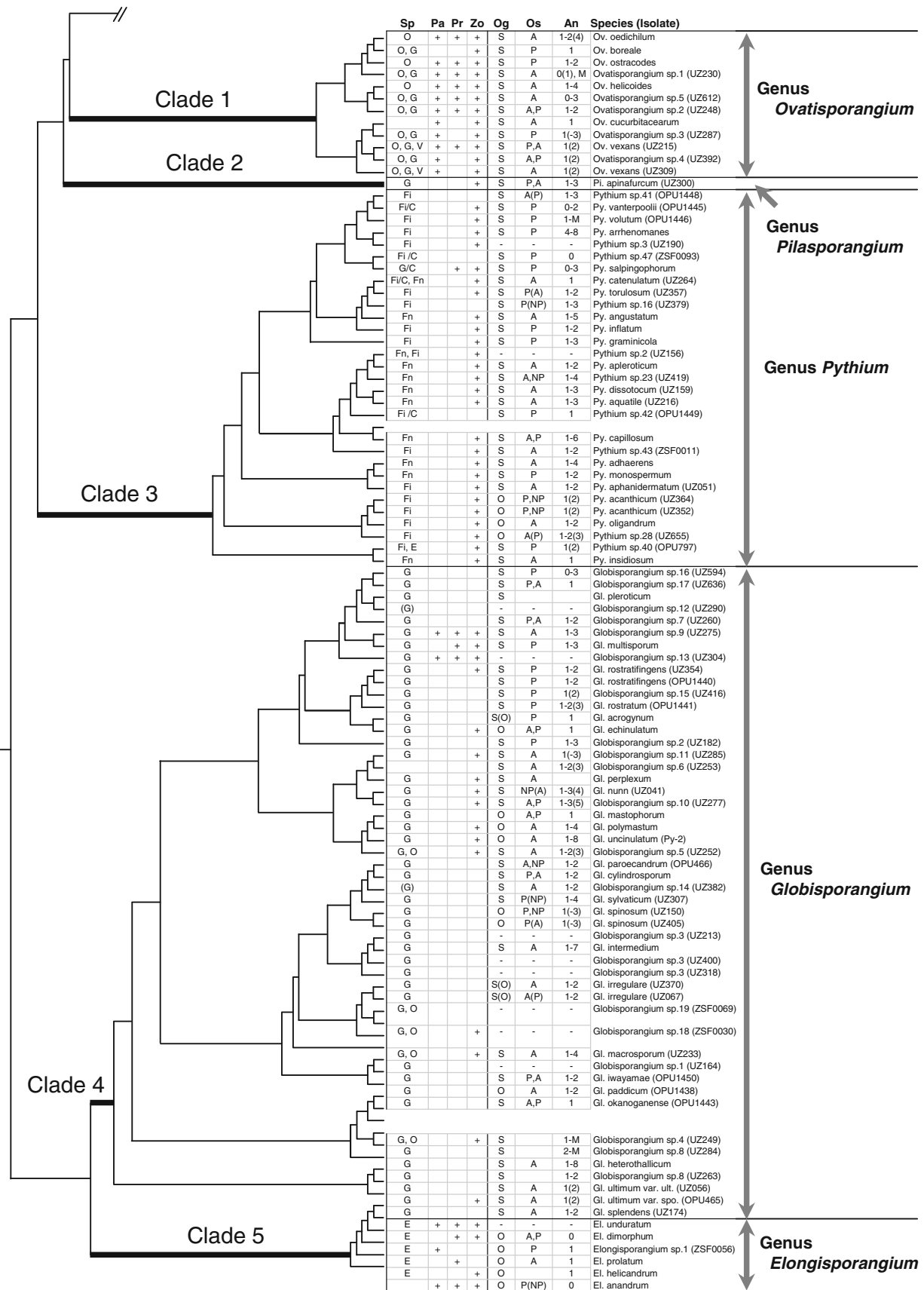
Drechsler, *P. oligandrum* Drechsler, and UZ655, formed oogonia with ornamented walls with acute spines (Fig. 6i). All isolates with ornamented oogonia clustered in a monophyletic group within clade 3, which was comparable with clade D in the previous study (Lévesque and de Cock 2004), revealing that species with ornamented oogonia were phylogenetically closely related. The extent of oospores within an oogonium, such as plerotic or aplerotic, and the number of antheridia per oogonium varied among or within isolates in this clade. Therefore, this clade was clearly characterized by filamentous sporangia, although one subclade was characterized by ornamented oogonia.

### Clade 4

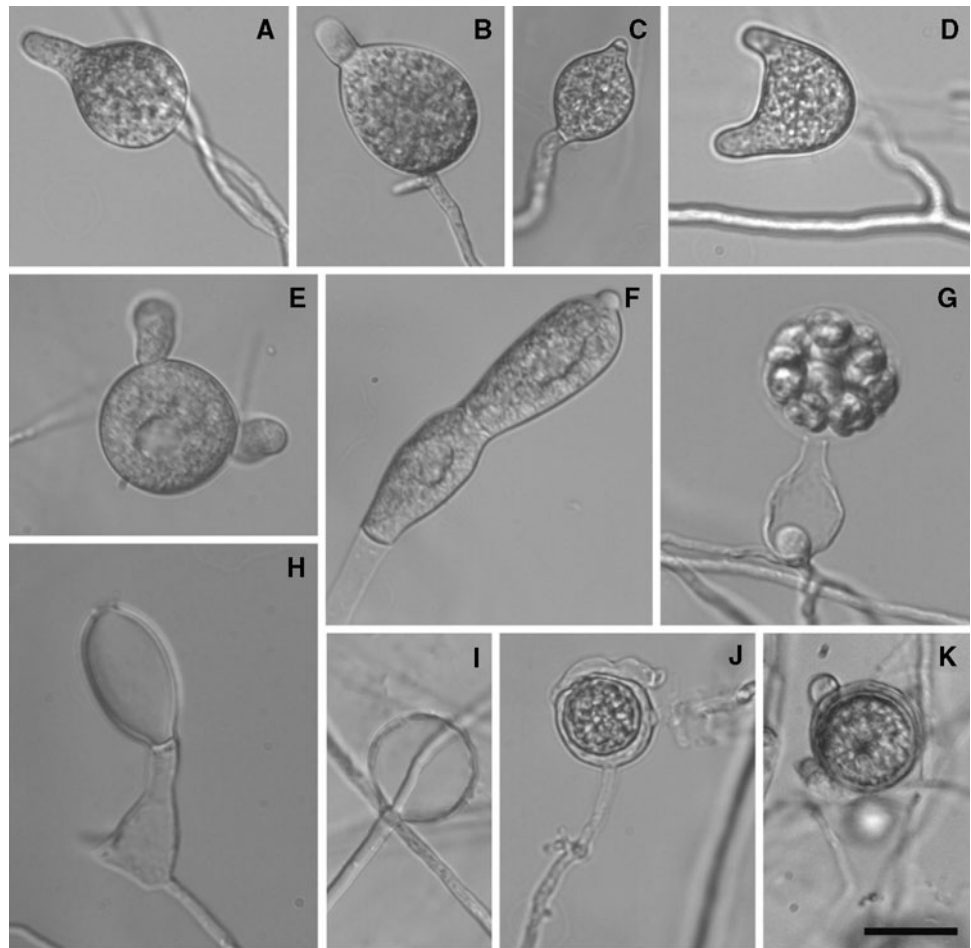
Most species within clade 4 formed globose sporangia similar to those of clade 2 (Fig. 7a, b), although no sporangia were observed in two isolates, UZ253 and UZ290. Other shapes, such as ovoid, pyriform, ellipsoid, and cylindrical, were also observed in many isolates. Sporangia with papilla were only observed in UZ275 and UZ304 isolates and also known in *P. marsipium* (van der Plaäts-Niterink 1981). Although the structure was also known in *P. rostratifyingens* De Cock & Lévesque (de Cock and Lévesque 2004), it was not observed in two isolates identified as this species, UZ354 and OPU1440. Zoospores were observed or known in less than half of all isolates within the clade, much fewer than in other clades. When zoospores were produced, the discharge tube was generally shorter than that of filamentous sporangia (Fig. 7c). Proliferating sporangia were formed in UZ275 and UZ304 isolates (Fig. 7d) and described in the reports in *P. multi-sporum* Poitras and *P. middletonii* (van der Plaäts-Niterink 1981). Unlike in the monograph by van der Plaäts-Niterink (1981), the OPU1443 isolate of *P. okanoganense* did not form this structure.

A sexual stage was not observed in eight isolates, UZ164, UZ213, UZ290, UZ304, UZ318, UZ400, ZSF0030, and ZSF0069. Among species in this clade, three, *P. heterothallicum* W. A. Campb. & F. F. Hendrix, *P. intermedium* de Bary, and *P. splendens*, are known to be heterothallic (van der Plaäts-Niterink 1981). As in the previous study, the UZ174 isolate identified as *P. splendens* did not form sexual reproductive organs in culture,





**Fig. 4** Morphology of species in the genus *Ovatisporangium*. Ovoid sporangia with germ tubes (a *Ovatisporangium* sp. 1 UZ230, b *Ovatisporangium* sp. 2 UZ248). c Ovoid sporangium with a papilla of *O. vexans*. d Unique shape of sporangia of *P. vexans*. e Globose sporangium with two germ tubes of *Ovatisporangium* sp.2 UZ248. f Clavate sporangium with papilla of *O. vexans*. g Vesicle with zoospores of *Ovatisporangium* sp. 1 UZ230. h Empty sporangium after zoosporogenesis of *O. vexans*. i Internally proliferating sporangium of *Ovatisporangium* sp. 2 UZ248. j Smooth oogonium with broadly connected antheridium of *O. vexans*. k Smooth oogonium contacted by antheridium of *Ovatisporangium* sp.2 UZ248. Bar 20  $\mu$ m



suggesting this isolate may be heterothallic. This isolate formed sexual organs in dual culture with a female isolate of *P. splendens* (CBS 266.69) but not in a dual culture with a male isolates of the species (CBS 462.48), indicating that the UZ174 isolate was a male isolate of this species. Both homothallic and heterothallic isolates are known in *P. sylvaticum*. The UZ307 isolate identified as *P. sylvaticum* formed sexual organs in single culture, i.e., the isolate is homothallic. Ornamented oogonia were known or observed in several species. The number and shape of the projections of their ornamented walls largely varied among species. For example, it was spine-like with a blunt tip (*P. spinosum* Sawada), conical with an acute tip (*P. uncinulatum* Plaäts-Nit. & I. Blok) or with a blunt tip, with occasionally branching dichotomously (*P. paddicum* Hirane) (Fig. 7f, g, i, j). Unlike the monophyly in clade 3, the species with ornamented oogonia were located in scattered positions within the clade.

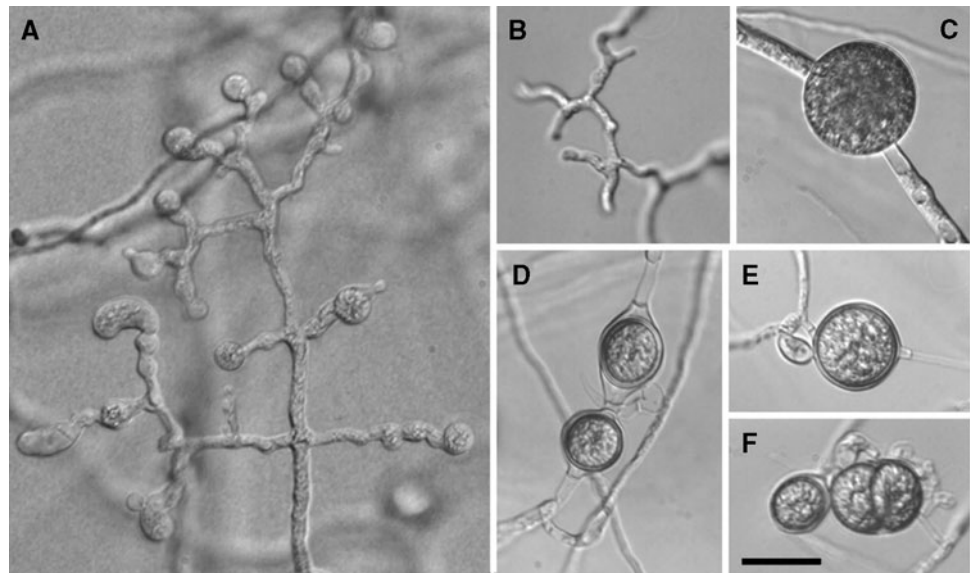
Various characteristics of antheridial stalks or cells were also observed among isolates. The three isolates, UZ252, UZ253, and UZ285, formed swollen antheridial stalks similar to each other (Fig. 7h). When the antheridium did not contact the oogonium, its stalk was similar to a

filamentous inflated sporangium. The other three isolates, UZ249, UZ263, and UZ284, produced many antheridia or slender antheridial stalks per oogonium surrounding the oogonium (Fig. 7k). All three isolates were closely related and formed a single subclade together with *P. heterothallicum*. Although *P. heterothallicum* is a heterothallic species, it produced many antheridia, often forming a complicated knot around the oogonium in dual culture. Therefore, this unique feature of the antheridium was also supported phylogenetically. Oospores were plerotic or aplerotic, and the features varied among or within isolates (Fig. 7f–l). Although a few morphological features characterized small subclades, there were no morphological characteristics of sexual organs common to all isolates within the clade. Therefore, clade 4 was only characterized by globose sporangia. However, the number of species forming zoospores was obviously small in this clade compared with other clades.

#### Clade 5

Clade 5 was clearly characterized by very large elongated clavate sporangia (Fig. 8a, b). These characteristic are not

**Fig. 5** Morphology of the genus *Pilasporangium*. **a** Sickle-shape appressoria. **b** Complexly branched secondary hyphae. **c** Intercalary globose sporangium. **d** Intercalary smooth oogonia in chain with monoclinal antheridia and nearly plerotic oospores. **e** Smooth oogonium with declinous antheridium and plerotic oospore. **f** Smooth oogonium with two oospores. Bar 20  $\mu$ m



known in any *Pythium* species. Sporangia with papilla, internal proliferating sporangia, and zoospores are sometimes observed in most species. Although a sexual stage is not known in *P. undulatum* H. E. Petersen, all other species produced ornamented oogonia (Fig. 8c). There are fewer antheridia per oogonium than in other clades, which usually have one or none. Most morphological characteristics of the asexual and sexual stages are similar within the clade.

Based on relationships between morphology and phylogeny, it was shown that the sporangial morphology correlated with the phylogeny of *Pythium*. Although the same relationship between phylogeny and sporangial morphology had also been suggested in several previous studies (Briard et al. 1995; Matsumoto et al. 1999; Lévesque and de Cock 2004), more detailed variations among shapes were shown in this study. Previous studies had reported on two morphological shapes, filamentous and globose. However, the globose sporangia were further divided into three morphological types, globose, ovoid, and elongated shapes in this study based on the examination of more species. As a result, three clades, clades 1, 3, and 5, were clearly differentiated by sporangial morphology: ovoid, filamentous, and elongate clavate sporangia, respectively. Clades 2 and 4 were commonly characterized by globose sporangia, although they were distinguished from each other phylogenetically.

#### Relationships between *Pythium* and related genera

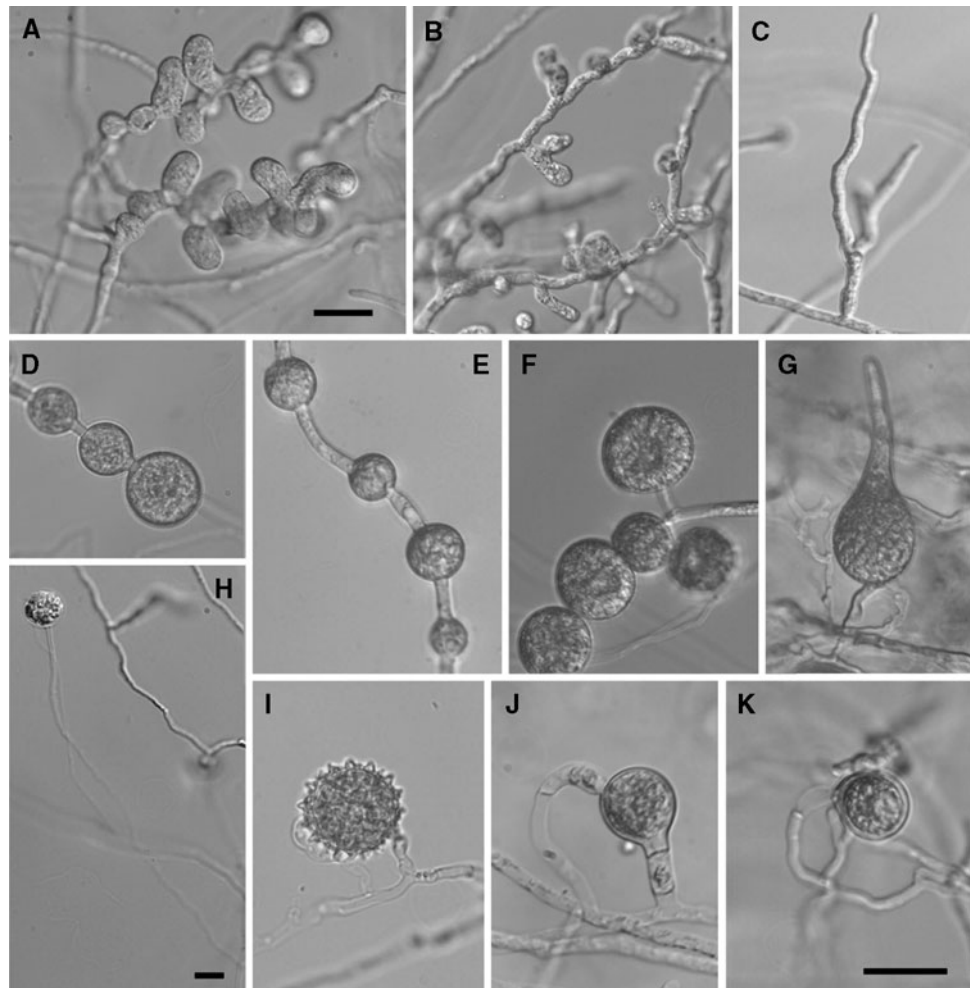
Clade 1 was closely related to genera of Peronosporales (*Phytophthora* and *Halophytophthora*) in D1/D2 analyses. A similar phylogenetic relationship was indicated in several previous phylogenetic analyses based on the LSU

rDNA (Briard et al. 1995), rDNA ITS (Villa et al. 2006), and  $\beta$ -tubulin (Villa et al. 2006; Belbahri et al. 2008). Furthermore, a sister group between clade 1 and a monophyletic group of all genera of Peronosporales, including *Phytophthora* and *Halophytophthora*, was shown in this study and highly supported by 82% BV (ML) and 97% BV (NJ) (Fig. 1). Similarities between *Pythium* species within clade 1 and *Phytophthora* have been recognized in various studies. Ovoid-shaped sporangia with or without papilla were often formed in *Pythium* species of clade 1 and several *Phytophthora* species. In molecular characteristics, the 5S rDNA of the *Pythium* species of clade 1 were linked on the same strand within the intergenic spacer (IGS) region (Belkhiri et al. 1992). Although this position has not been seen in other *Pythium* species, it is commonly shown in *Phytophthora* species (Bedard et al. 2006). This evidence indicates that the members of clade 1 are clearly differentiated from *Pythium* species within the other clades and more closely related to *Phytophthora* than the other *Pythium* species.

On the other hand, the species of clade 1 were clearly differentiated from other genera in the lineage by the formation of zoospores within a vesicle. Clade 1 was placed on the more basal line in the lineage of Peronosporales. This hypothesis was also supported by other molecular phylogenetic analyses (Riethmüller et al. 1999; Cooke et al. 2000; Petersen and Rosendahl 2000). Therefore, it is suggested that the features of the formation of zoospore of clade 1 are ancestral features in this lineage, i.e., the formation of a vesicle may have disappeared in the evolution in the lineage.

Clade 2 was placed on a basal line in a monophyletic group, including clade 1 and genera of Peronosporales in the D1/D2 analysis. A species of clade 2, *P. apinafurcum*,

**Fig. 6** Morphology of the genus *Pythium*. Filamentous inflated sporangium (a *Pythium* sp. 16 UZ379, b *Pythium* sp. 43 ZSF0011). c Filamentous noninflated sporangium of *Pythium* sp. 2 UZ156. d Adjacent globose sporangia of *P. catenulatum*. e Catenulate globose sporangia of *Pythium* sp. 42 OPU1449. f Adjacent subglobose sporangia of *Pythium* sp. 47 ZSF0093. g Pyriform sporangium with elongated tip of *Pythium* sp. 40 OPU797. h Vesicle with long discharge tube and zoospores of *Pythium* sp. 2 UZ156. i Ornamented oogonium with monoclinal antheridium of *P. acanthicum*. j Smooth oogonium with monoclinal antheridium of *Pythium* sp. 23 UZ419. k Smooth oogonium with several antheridia and plerotic oospore of *P. torulosum*. Bars 20  $\mu$ m (Bar A for A–G, Bar K for I–K)



may be an ancestral species of the lineage, because it is located in a more basal position (Fig. 1). This species is characterized by complexly branched secondary hyphae, forms globose sporangia without papilla, and proliferates (Uzhashi et al. 2009). These morphological characteristics were more similar to those of members of clade 4 than clade 1. The phylogenetic position of clade 2 in the lineage indicates that members of this lineage may have evolved from a *P. apinafurcum*-like species with globose sporangia. However, there is some doubt regarding the phylogenetic position, because clade 2 is composed of only one species. Therefore, the question of how ovoid sporangia evolved was not elucidated in this study. Further examination of the distribution in *Pythium* species, including clade 2, is necessary to clarify not only the phylogenetic position of clade 2 but also the evolution of several morphological characteristics, such as sporangia.

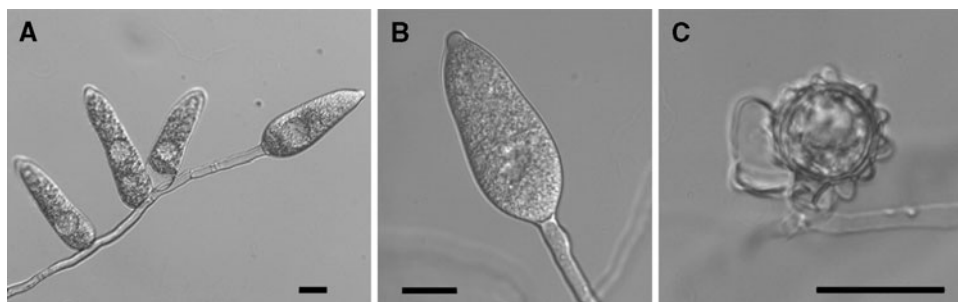
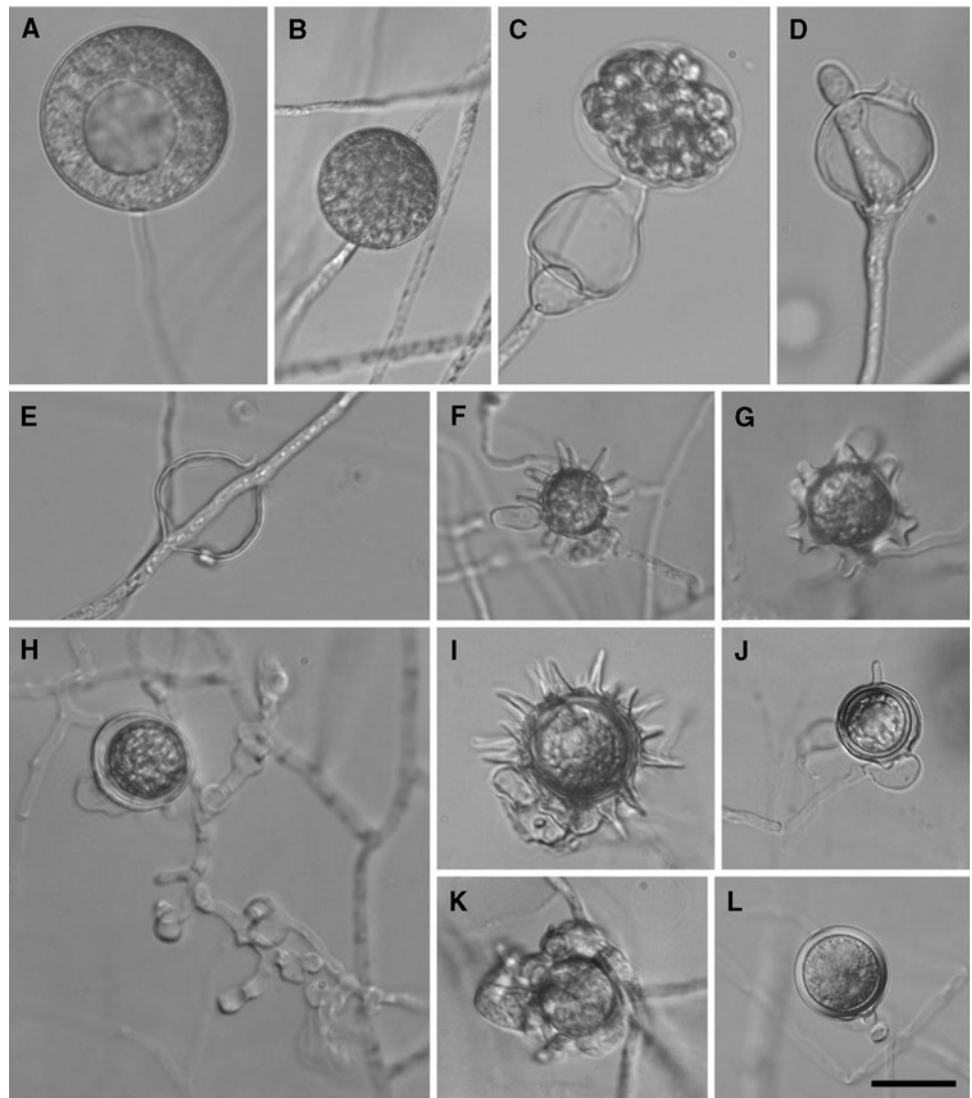
The shape of filamentous sporangia in clade 3 was clearly different from those of *Pythium* species in other clades. This difference of sporangial morphology in *Pythium* species has been noted by previous research (Fischer

1892; Schröter 1897; Sideris 1931) in which species with filamentous sporangia were differentiated at the generic level. Although it was clear that clade 3 was phylogenetically distantly related to other clades, the phylogenetic position of clade 3 among *Pythium* species has not yet been elucidated because it differed among trees. Likewise, the question of how filamentous sporangia evolved within *Pythium* has not been resolved.

### Taxonomy

The results of this phylogenetic analyses based on comprehensive sequence data of the nuclear rDNA D1/D2 region and mitochondrial gene (*coxII*) show that the genus *Pythium* is a nonmonophyletic group, and the members include phylogenetically diverse organisms. These results indicate that taxonomic revisions are necessary in the genus *Pythium*. In all phylogenetic trees, *Pythium* species were divided into five strongly or moderately supported clades. Each clade was characterized by sporangial

**Fig. 7** Morphology of the genus *Globisporangium*. **a** Terminal globose sporangium of *Globisporangium* sp. 9 (UZ275). **b** Intercalary globose sporangium of *Pythium* sp. ZSF0069. **c** Vesicle with zoospores of *Globisporangium* sp. 9 (UZ275). **d** Internally proliferating sporangium of *Globisporangium* sp. 9 (UZ275). **e** Internally proliferating sporangium of *Globisporangium* sp. 13 (UZ304). **f** Ornamented oogonium with finger-like projection and declinuous antheridia of *P. spinosum*. **g** Ornamented oogonium with bold and dichotomous projections of *G. paddicum*. **h** Smooth oogonium, antheridium with stalk complexly lobed, and aplerotic oospore of *Globisporangium* sp. 5 UZ252. **i** Ornamented oogonium with conical and acute projections of *G. uncinulatum*. **j** Ornamented oogonium with finger-like projection, monoclincous antheridia and aplerotic oospore of *G. irregulare*. **k** Smooth oogonium with many antheridia of *Globisporangium* sp. 8 UZ284. **l** Smooth oogonium with plerotic oospore of *G. rostratum*. Bar 20  $\mu$ m



**Fig. 8** Morphology of the genus *Elongisporangium*. **a** Elongated clavate sporangia of *Elongisporangium* sp. 1 ZSF0056. **b** Terminal elongated clavate sporangium with a papilla of *Elongisporangium*

sp.1 ZSF0056. **c** Ornamented oogonium with ebetate projections of *Elongisporangium* sp.1 ZSF0056. Bars 20  $\mu$ m

morphology. Thus, the sporangial shape is regarded as an important taxonomic criterion. It was concluded that morphological and phylogenetic differences among clades deserve recognition of the generic level according to a comparison of taxonomic criteria used for related genera.

Among the five clades, three (1, 3, and 5) were differentiated by their sporangial shapes and were strongly supported by most phylogenetic trees. Based on these results, each of their three clades was identified as a separate genus. On the other hand, clades 2 and 4 were

characterized by globose sporangia and were difficult to differentiate by morphological characteristics. Although the monophyly of clade 4 was not resolved, it is clear that the members of clade 4 are closely related and can be clearly differentiated from clade 2 and the others phylogenetically. Therefore, it was concluded that clade 4 should be considered a single genus. Further phylogenetic analyses, including more isolates, are needed to resolve the phylogeny of the clade and relationships among clades. Likewise, clade 2 is phylogenetically differentiated from all other clades, including clade 4, having similar globose sporangia. Thus, it is considered as a separate genus. As a result, the genus *Pythium* Pringsh. was divided into five genera, four of which are new.

The type species of *Pythium* defined by Pringsheim (1858), *P. monospermum*, was included in clade 3. Therefore, the genus *Pythium* was restricted to species producing filamentous sporangia clustered as in clade 3. According to the morphology and phylogeny in this and previous studies, 57 species were redefined as members of the genus *Pythium*.

The first new genus, named *Ovatisporangium*, is characterized by ovoid sporangia (clade 1). It has been debated whether species belonging to the genus are appropriately classified as *Pythium* (Briard et al. 1995; Panabieres et al. 1997; Dick 2001b; Villa et al. 2006; Belbahri et al. 2008). Our study resolved this debate by establishing clade 1 as an independent genus. It is occasionally difficult to differentiate *Ovatisporangium* species from other species of two new genera, *Globisporangium* (clade 4; mentioned below) and *Pilasporangium* (clade 2; mentioned below), because globose sporangia formed in many species of the *Ovatisporangium*, are sometimes formed in species of the two genera. Therefore, molecular characteristics are a useful tool for classification of species in each genus. Based on phylogeny and morphology, 15 species were transferred to *Ovatisporangium* from *Pythium* Pringsh.

The second new genus, named *Elongisporangium*, was erected for species with elongated clavate sporangia (clade 5). Based on the morphology and phylogeny, five species were transferred to this genus from *Pythium* Pringsh.

The third new genus, named *Globisporangium*, is characterized by globose sporangia (clade 4). Because this shape is similar to those of *Ovatisporangium* and *Pilasporangium* (clade 2; mentioned below), molecular phylogenetic analyses are often needed to classify each genus. In contrast to other genera, the monophyly of this new genus was not strongly supported. Thus, it is suggested that the genus was composed of phylogenetically various species. Therefore, further examination of the taxonomy of *Globisporangium* may be needed based on morphology or phylogeny of other species, such as the new species

included in this genus. According to the morphological and phylogenetic characteristics, 68 species were transferred to this genus from *Pythium* Pringsh.

The last new genus, named *Pilasporangium*, is composed of only one species, *P. apinafurcum* (clade 2). Isolation and examination of the morphology and phylogeny of many species included in this genus are needed to clarify the characterization of this genus. Likewise, in *Ovatisporangium* and *Globisporangium*, molecular phylogenetic analyses are needed to classify this genus.

### Key to genera

1	Sporangia filamentous, inflated or non-inflated	<i>Pythium</i>
	Sporangia not filamentous	
2	Sporangia usually globose	
2	Sporangia mainly ovoid to pyriform, sometimes irregularly shape	<i>Ovatisporangium</i>
3	Sporangia clavate to elongate	<i>Elongisporangium</i>
3	Sporangia sometimes proliferating	<i>Globisporangium</i>
	Sporangia not proliferating, secondary hyphae branched complexly	<i>Pilasporangium</i>

### *Pythium* Pringsh. emend. Uzuhashi, Tojo & Kakish. Fig. 6

Mycelium well developed, often with appressoria. Hyphae hyaline, aseptate. Sporangia either filamentous, not differentiated from the vegetative hyphae, or consisting of lobate or toruloid inflated elements, or occasionally globose in a chain. Sporangial contents move and form a vesicle at the tip with an undifferentiated mass of protoplasm; this mass then differentiates into a number of biflagellate zoospores. Oogonia (sub-)globose, terminal or intercalary, with a smooth or ornamented wall. Antheridia 1 to several per oogonium, sometimes absent, monoclinal, diclinous or hypogynous, stalked or sessile, of various shapes. Oospores usually single, rarely two or more in an oogonium, plerotic or applerotic with a thin or thick wall.

*Pythium* species occur as saprophytes or parasites in soils, water, or on plant or animal substrates.

Type species: *Pythium monospermum* Pringsh., Jb. Wiss. Bot. 1: 288, 1858.

Additional species of the *Pythium*.

*Pythium acanthicum* Drechsler, J. Wash. Acad. Sci. 20: 408, 1930.

*Pythium adhaerens* Sparrow, Ann. Bot., Lond. 45: 258, 1931.

- Pythium amasculinum* Y. N. Yu, Acta microbiol. sin. 13: 118, 1973.
- Pythium angustatum* Sparrow, Ann. Bot., Lond. 45: 272, 1931.
- Pythium aphanidermatum* (Edson) Fitzp., Mycologia 15: 168, 1923.
- Pythium apleroticum* Tokun., in Ito & Tokunaga, Trans. Sapporo nat. Hist. Soc. 14: 12, 1935.
- Pythium aquatile* Höhnk, Veröff. Inst. Meeresf. Bremerhaven 2: 94, 1953.
- Pythium aristosporum* Vanterp., Ann. appl. Biol. 25: 537, 1938.
- Pythium arrhenomanes* Drechsler, Phytopathology 18: 874, 1928.
- Pythium capillosum* B. Paul, Trans. Br. mycol. Soc. 89: 195, 1987.
- Pythium catenulatum* V. D. Matthews, Stud. Genus *Pythium*: 47, 1931.
- Pythium caudatum* (G.L. Barron) M.W. Dick, Straminipilous Fungi, Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protists, the Plasmodiophorids and Similar Organisms (Dordrecht): 294, 2001.
- Pythium chondricola* De Cock, Mycotaxon 25: 102, 1986.
- Pythium coloratum* Vaartaja, Mycologia 57: 417, 1965.
- Pythium conidiophorum* Jokl, Oesterr. Bot. Ztschr. 67: 33, 1918.
- Pythium contiguanum* B. Paul, FEMS Microbiol. Lett. 183: 108, 2000.
- Pythium deliense* Meurs, Phytopath. Z. 7: 176, 1934.
- Pythium declinum* Tokun., in Ito & Tokunaga, Trans. Sapporo nat. Hist. Soc. 14: 12, 1935.
- Pythium dissimile* Vaartaja, Mycologia 57: 421, 1965.
- Pythium dissotocum* Drechsler, J. Wash. Acad. Sci. 20: 402, 1930.
- Pythium flevoense* Plaäts-Nit., Acta bot. neerl. 21: 636, 1972.
- Pythium folliculosum* B. Paul, Mycol. helv. 4: 204, 1991.
- Pythium graminicola* Subramaniam, Bull. Agric. Res. Inst. Pus. 177: 1, 1928 [as '*graminicolum*'].
- Pythium grandisporangium* Fell & Master, Can. J. Bot. 53: 2920, 1975.
- Pythium hydnosporum* (Mont.) J. Schröt., in de Bary, Abh. senckenb. naturforsch. Ges. 12: 19, 1879.
- Pythium inflatum* V. D. Matthews, Stud. Genus *Pythium*: 45, 1931.
- Pythium insidiosum* De Cock, L. Mend., A. A. Padhye, Ajello & Kaufman, J. Clin. Microbiol. 25: 345, 1987.
- Pythium kasuhmirensense* B. Paul, FEMS Microbiol. Lett. 282: 253, 2008.
- Pythium lutarium* Ali-Shtayeh, in Ali-Shtayeh & Dick, J. Linn. Soc., Bot. 91: 309, 1985.
- Pythium lycopersicum* G. Karaca, G. Tepedelen, & B. Paul, FEMS Microbiol. Lett. 288: 165, 2008.
- Pythium marinum* Sparrow, Dansk bot. Ark. 8: 5, 1934.
- Pythium myriotylum* Drechsler, J. Wash. Acad. Sci. 20: 404, 1930.
- Pythium oligandrum* Drechsler, J. Wash. Acad. Sci. 20: 409, 1930.
- Pythium pachycaule* Ali-Shtayeh, in Ali-Shtayeh & Dick, J. Linn. Soc., Bot. 91: 313, 1985.
- Pythium papillatum* V. D. Matthews, J. Elisha Mitchell scient. Soc. 43: 231, 1928.
- Pythium pectinolyticum* B. Paul, FEMS Microbiol. Lett. 199: 56, 2001.
- Pythium periilum* Drechsler, J. Wash. Acad. Sci. 20: 403, 1931.
- Pythium periplocum* Drechsler, J. Wash. Acad. Sci. 20: 405, 1930.
- Pythium perniciosum* Serbinow, Scripta Bot. Horti Univ. Imper. Petrop. 28: 29, 1912.
- Pythium phragmitis* Nechw., in Nechwatal, Wielgoss & Mendgen, Mycol. Res. 109: 1343, 2005.
- Pythium plurisporium* Abad, Shew, Grand & L.T. Lucas, Mycologia 87: 897, 1996.
- Pythium polycarpum* B. Paul, Hydrobiologia 131: 31, 1986.
- Pythium porphyrae* M. Takah. & M. Sasaki, Trans. Mycol. Soc. Japan 18: 280, 1977.
- Pythium pyrilibum* Vaartaja, Mycologia 57: 425, 1965.
- Pythium rhizo-oryzae* B. Paul, in Bala, Gautam & Paul, Curr. Microbiol. 52: 104, 2006.
- Pythium salpingophorum* Drechsler, J. Wash. Acad. Sci. 20: 407, 1931.
- Pythium scleroteichum* Drechsler, J. Agric. Res., Washington 49: 881, 1934.
- Pythium sukuiense* W. H. Ko, Shin Y. Wang & Ann, Mycologia 96: 647, 2004.
- Pythium sulcatum* R. G. Pratt & J. E. Mitch., Can. J. Bot. 51: 334, 1973.
- Pythium tardicrescens* Vanterp, Ann. appl. Biol. 25: 533, 1938.
- Pythium tenue* Gobi, Script. Bot. Hort. Petr. Fasc. 15: 211, 1899.
- Pythium torulosum* Coker & P. Patt., J. Elisha Mitchell scient. Soc. 42: 247, 1927.
- Pythium tracheiphilum* Matta, Phytopath. Mediterr. 4: 51, 1965.
- Pythium vanterpoolii* V. Kouyeas & H. Kouyeas, Annals Inst. Phytopath. Benaki, N.S. 5: 210, 1963.
- Pythium volutum* Vanterp. & Truscott, Can. J. Res. 6: 77, 1932.

*Pythium zingiberis* M. Takah., Ann. Phytopath. Soc. Japan 18: 115, 1954 [as 'zingiberum'].

***Ovatisporangium*** Uzuhashi, Tojo & Kakish., gen. nov. Fig. 4.

Mycelium bene evolvens, ex hyphis principalibus hyalinis, ramosis, nonseptatis, demum raro septatis compositum. Appressoria saepe efferentia. Sporangia terminalia, intercalaria vel latetaliter sessilia in hyphis, ovoidea, obovoidea, globosa, subglobosa, pyriformia vel obpyriformia, saepe papillata et interne prolifera. Zoosporae biflagellatae in vesicula protoplasmatis sporangii formantes. Oogonia terminalia vel intercalaria, globosa vel subglobosa, pariete laevi vel ornato. Antheridia monoclina, diclina vel hypogyna. Oosporae globosae, vulgo una in oogonio, pleroticae vel appleroticae.

Mycelium well developed, often with appressoria. Hyphae hyaline, aseptate, rarely septate in old. Sporangia terminal, intercalary or laterally sessile on hyphae, (ob-)ovoid, (sub-)globose, lemon-shaped, clavate or various shapes, sometimes papillate and internally proliferating. Zoospores biflagellate, formed in a vesicle of sporangial protoplasm. Oogonia terminal or intercalary, (sub-)globose, with a smooth or ornamented wall. Antheridia variable in the shape, one to several per oogonium, sometimes absent, monoclinal, diclinal or hypogynous, stalked or sessile. Oospores globose, usually single in an oogonium, plerotic or applerotic with a thin or thick wall.

*Ovatisporangium* species occur as saprophytes or parasite in soils, water, or on plant substrates.

Type species: *Ovatisporangium helicoides* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium helicoides* Drechsler, J. Wash. Acad. Sci. 20: 413, 1931.

Additional species of *Ovatisporangium*.

*Ovatisporangium boreale* (R. L. Duan) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium boreale* R. L. Duan, Acta Mycol. Sin. 4: 1, 1985 [as 'borealis'].

*Ovatisporangium carbonicum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium carbonicum* B. Paul, FEMS Microbiol. Lett. 219: 270, 2003.

*Ovatisporangium chamaehyphon* (Sideris) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium chamaehyphon* Sideris, Mycologia 24: 33, 1932.

*Ovatisporangium citrinum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium citrinum* B. Paul, FEMS Microbiol. Lett. 234: 273, 2004.

*Ovatisporangium cucurbitacearum* (S. Takim.) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium cucurbitacearum* S. Takim., Ann. Phytopath. Soc. Japan 11: 91, 1941.

*Ovatisporangium indigoferae* (E. J. Butler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium indigoferae* E. J. Butler, Mem. Dep. Agric. India 1: 73, 1907.

*Ovatisporangium litorale* (Nechw.) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium litorale* Nechw., in Nechwatal & Mendgen, FEMS Microbiol. Lett. 255: 99, 2006.

*Ovatisporangium megacarpum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium megacarpum* B. Paul, FEMS Microbiol. Lett. 186: 231, 2000.

*Ovatisporangium mercuriale* (Belbahri, B. Paul & Lefort) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium mercuriale* Belbahri, B. Paul & Lefort, FEMS Microbiol. Lett. 284: 20, 2008.

*Ovatisporangium montanum* (Nechw.) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium montanum* Nechw., in Nechwatal & Wsswald, Mycol. Prog. 2: 79, 2003.

*Ovatisporangium oedichilum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium oedichilum* Drechsler, J. Wash. Acad. Sci. 20: 414, 1931.

*Ovatisporangium ostracodes* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium ostracodes* Drechsler, Phytopathology 33: 286, 1943.

*Ovatisporangium sterile* (Belbahri & Lefort) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium sterile* Belbahri & Lefort, in Belbahri, Calmin, Sanchez-Hernandez, Oszako & Lefort, FEMS Microbiol. Lett. 255: 210, 2006 [as 'sterilum'].

*Ovatisporangium vexans* (de Bary) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium vexans* de Bary, J. Bot. Paris 14: 105, 1896.

***Globisporangium*** Uzuhashi, Tojo & Kakish., gen. nov. Fig. 7.

Mycelium bene evolvens, ex hyphis principalibus hyalinis, ramosis, nonseptatis, demum raro septatis compositum. Appressoria saepe efferentia. Sporangia terminalia, intercalaria vel latetaliter sessilia in hyphis, globosa vel clavata, saepe papillata et interne prolifera. Zoosporae biflagellatae in vesicula protoplasmatis sporangii formantes. Oogonia terminalia vel intercalaria, globosa, subglobosa, pariete laevi vel ornato. Antheridia monoclina, diclina vel hypogyna. Oosporae globosae, vulgo una in oogonio, pleroticae vel appleroticae.



Mycelium well developed, often with appressoria. Hyphae hyaline, aseptate, rarely septate in old. Sporangia terminal, intercalary or laterally sessile on hyphae, (sub-)globose, lemon-shaped or clavate, sometimes internally proliferating. Zoospores biflagellate, formed in a vesicle of sporangial protoplasm. Oogonia terminal or intercalary, (sub-)globose, with a smooth or ornamented wall. Antheridia variable in the shape, one to several per oogonium, sometimes absent, monoclinal, declinal or hypogynous, stalked or sessile. Oospores globose, usually single in an oogonium, plerotic or aplerotic with a thin or thick wall.

*Globisporangium* species occur as saprophytes or parasites in soils, water, or on plant or animal substrates.

Type species: *Globisporangium paroecandrum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium paroecandrum* Drechsler, J. Wash. Acad. Sci. 20: 406, 1930.

Additional species of *Globisporangium*.

*Globisporangium abappressorium* (Paulitz & M. Mazzola) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium abappressorium* Paulitz & M. Mazzola, in Paulitz, Admas & Mazzola, Mycologia 95: 81, 2003.

*Globisporangium acrogynum* (Y. N. Yu) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium acrogynum* Y. N. Yu, Acta microbiol. sin. 13: 117, 1973.

*Globisporangium acanthophoron* (Sideris) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium acanthophoron* Sideris, Mycologia 24: 36, 1932.

*Globisporangium apiculatum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium apiculatum* B. Paul, FEMS Microbiol. Lett. 263: 195, 2006.

*Globisporangium attrantheridium* (Allain-Boulé & Lévesque) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium attrantheridium* Allain-Boulé & Lévesque, in Allain-Boulé, Tweddell, Mazzola, Bélanger & Lévesque, Mycol. Res. 108: 798, 2004.

*Globisporangium bifurcatum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium bifurcatum* B. Paul, FEMS Microbiol. Lett. 224: 217, 2003.

*Globisporangium buismaniae* (Plaäts-Nit.) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium buismaniae* Plaäts-Nit., Stud. Mycol. 21: 44, 1981.

*Globisporangium carolinianum* (V. D. Matthews) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium carolinianum* V. D. Matthews, Stud. Genus *Pythium*: 71, 1931.

*Globisporangium campanulatum* (R. Mathew, K. K. Singh & B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium campanulatum* R. Mathew, K. K. Singh & B. Paul, FEMS Microbiol. Lett. 226: 10, 2003.

*Globisporangium canariense* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium canariense* B. Paul, FEMS Microbiol. Lett. 208: 136, 2002.

*Globisporangium cryptoirregularare* (Garzón, Yáñez & Moorman) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium cryptoirregularare* Garzón, Yáñez & Moorman, Mycologia 99: 300, 2007.

*Globisporangium cylindrosporum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium cylindrosporum* B. Paul, Int. J. Mycol. Lichenol. 4: 339, 1992.

*Globisporangium cystogenes* (De Cock & Lévesque) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium cystogenes* De Cock & Lévesque, Stud. Mycol. 50: 484, 2004.

*Globisporangium debaryanum* (R. Hesse) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium debaryanum* R. Hesse, Diss. Halle.: 34, 1874 [as ‘*de-baryanum*’].

*Globisporangium echinulatum* (V. D. Matthews) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium echinulatum* V. D. Matthews, Stud. Genus *Pythium*: 101, 1931.

*Globisporangium erinaceum* (J. A. Robertson) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium erinaceum* J. A. Robertson, N. Z. J. Bot. 17: 283, 1977.

*Globisporangium glomeratum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium glomeratum* B. Paul, FEMS Microbiol. Lett. 225: 49, 2003.

*Globisporangium heterothallicum* (W. A. Campb. & F. F. Hendrix) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium heterothallicum* W. A. Campb. & F. F. Hendrix, Mycologia 60: 803, 1968.

*Globisporangium hypogynum* (Middleton) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium hypogynum* Middleton, Phytopathology 31: 863, 1941.

*Globisporangium intermedium* (de Bary) Uzuahshi & Tojo, comb. nov.

Basionym: *Pythium intermedium* de Bary, Bot. Ztg. 39: 554, 1881.

*Globisporangium irregulare* (Buisman) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium irregulare* Buisman, Meded. Phytopath. Labor. Willie Commelin Scholten Baarn 11: 38, 1927.

- Globisporangium iwayamae* (S. Ito) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium iwayamae* S. Ito, in Ito & Tokunaga, Trans. Sapporo nat. Hist. Soc. 14: 13, 1935.
- Globisporangium kunmingense* (Y. N. Yu) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium kunmingense* Y. N. Yu, Acta microbial. sin. 13: 119, 1973.
- Globisporangium longandrum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium longandrum* B. Paul, FEMS Microbiol. Lett. 202: 240, 2001.
- Globisporangium longisporangium* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium longisporangium* B. Paul, FEMS Microbiol. Lett. 246: 208, 2005.
- Globisporangium lucens* (Ali-Shtayeh) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium lucens* Ali-Shtayeh, in Ali-Shtayeh & Dick, J. Linn. Soc., Bot. 91: 303, 1985.
- Globisporangium macrosporium* (Vaartaja & Plaäts-Nit.) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium macrosporium* Vaartaja & Plaäts-Nit., in van der Plaäts-Niterink, Stud. Mycol. 21: 89, 1981.
- Globisporangium mamillatum* (Meurs) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium mamillatum* Meurs, Wortelrot veroorzaakt door Schimmels uit de Gesl *Pythium* en *Aphanomyces* Proefschr Univ Utrecht: 39, 1928.
- Globisporangium marsipium* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium marsipium* Drechsler, Phytopathology 31: 505, 1941.
- Globisporangium mastophorum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium mastophorum* Drechsler, J. Wash. Acad. Sci. 20: 411, 1930.
- Globisporangium megalacanthum* (de Bary) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium megalacanthum* de Bary, Abh Senckenb Naturforsch Ges 12: 242, 1981.
- Globisporangium middletonii* (Sparrow) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium middletonii* Sparrow, Aquatic Phycomycetes Edn 2 (Ann Arbor): 1038, 1960.
- Globisporangium minor* (Ali-Shtayeh) Uzuhashi, Tojo & Kakish., comb. nov.  
Basionym: *Pythium minor* Ali-Shtayeh, in Ali-Shtayeh & Dick, J. Linn. Soc., Bot. 91: 299, 1985.
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Basionym: *Pythium multisporum* Poitras, Mycologia 41: 171, 1949.
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Basionym: *Pythium nagaii* S. Ito & Tokun., J. Fac. Agric., Hokkaido Imp. Univ. Sapporo 32: 209, 1933 [as 'nagae'].
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Basionym: *Pythium nodosum* B. Paul, D Galland, T. Bhatn. & Dulieu, FEMS Microbiol. Lett. 158: 209, 1998.
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Basionym: *Pythium nunn* Lifsh., Stangh. & R. E. D. Baker, Mycotaxon 20: 374, 1984.
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Basionym: *Pythium ornacarpum* B. Paul, FEMS Microbiol. Lett. 180: 340, 1999.
- Globisporangium orthogonon* (Ahrens) Uzuhashi, Tojo & Kakish., comb. nov.  
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Basionym: *Pythium parvum* Ali-Shtayeh, in Ali-Shtayeh & Dick, J. Linn. Soc., Bot. 91: 303, 1985.
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Basionym: *Pythium polymastum* Drechsler, J. Wash. Acad. Sci. 20: 412, 1930.
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Basionym: *Pythium proliferatum* B. Paul, FEMS Microbiol. Lett. 206: 193, 2002.
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Basionym: *Pythium pulchrum* Minden, in Falck, Falck. Mykol. Unters. 2: 224, 1916.

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Basionym: *Pythium radiosum* B. Paul, Mycol. Helv. 5: 2, 1992.

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Basionym: *Pythium ramificatum* B. Paul, Hydrobiologia 140: 235, 1986.

*Globisporangium recalitrans* (Belbahri & Maralejo) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium recalitrans* Belbahri & Maralejo, Mycologia 100: 312, 2008.

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Basionym: *Pythium regulare* B. Paul, Curr. Microbiol. 47: 310, 2003.

*Globisporangium rhizosaccharum* (K. K. Singh, R. Mathew, Masih & Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium rhizosaccharum* K. K. Singh, R. Mathew, Masih & Paul, FEMS Microbiol. Lett. 221: 234, 2003.

*Globosum rostratiformans* (De Cock & Lévesque) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium rostratiformans* De Cock & Lévesque, Stud. Mycol. 50: 485, 2004.

*Globisporangium rostratum* (E. J. Butler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium rostratum* E. J. Butler, Mem. Dep. Agric. India, Bot. Ser. 1: 84, 1907.

*Globisporangium salinum* (Höhnk) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium salinum* Höhnk, Veröff. Inst. Meeresf. Bremerhaven 2: 89, 1953.

*Globisporangium segnitium* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium segnitium* B. Paul, FEMS Microbiol. Lett. 217: 210, 2002.

*Globisporangium solare* (De Cock, Melero-Vara, Y. Serrano & Julio Gómez) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium solare* De Cock, Melero-Vara, Y. Serrano & Julio Gómez, Mycol. Res. 112: 1117, 2008.

*Globisporangium spiculum* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium spiculum* B. Paul, in Lassaad, Calmin, Sanchez-Hernandez & Lefort, FEMS Microbiol. Lett. 254: 319, 2006.

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Basionym: *Pythium spinosum* Sawada, J. Nat. Hist. Soc. Formosa 16: 199, 1926.

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*Globisporangium sylvaticum* (W. A. Campb. & F. F. Hendrix) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium sylvaticum* W. A. Campb. & F. F. Hendrix, Mycologia 59: 274, 1967.

*Globisporangium terrestre* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium terrestre* B. Paul, FEMS Microbiol. Lett. 212: 256, 2002 [as ‘terrestris’].

*Globisporangium toruloides* (B. Paul) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium toruloides* B. Paul, Trans. Br. mycol. Soc. 86: 331, 1986.

*Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish., comb. nov. var. *ultimum*.

Basionym: *Pythium ultimum* Trow, Ann Bot 15: 300, 1901. var. *ultimum*.

*Globisporangium ultimum* var. *sporangiiiferum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium ultimum* var. *sporangiiiferum* Drechsler, Sydowia 14: 107, 1960 [as ‘*sporangiferum*’].

*Globisporangium uncinulatum* (Plaäts-Nit. & I. Blok) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium uncinulatum* Plaäts-Nit. & I. Blok, Neth. JI Pl. Path. 84: 135, 1978.

*Globisporangium violae* (Chesters & Hickman) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium violae* Chesters & Hickman, Trans. Br. mycol. Soc. 27: 60, 1944.

***Elongisporangium*** Uzuhashi, Tojo & Kakish., gen. nov. Fig. 8.

Mycelium bene evolvens, ex hyphis principalibus hyalinis, ramosis, nonseptatis, demum raro septatis compositum. Appressoria saepe efferentia. Chlamydosporae raro formantes. Sporangia vulgo clavata usque elongata, saepe papillata et interne prolifera. Zoosporae biflagellatae in vesicula protoplasmatis sporangii formantes. Oogonia terminalia vel intercalaria, globosa vel subglobosa, pariete ornato. Antheridia monoclina, diclina vel hypogyna. Oosporae globosae, vulgo una in oogonio, pleroticae vel apleroticae.

Mycelium well developed, often with appressoria, rarely with chlamydo-spores. Hyphae hyaline, aseptate, rarely septate in old. Sporangia mostly terminal, clavate to elongate, sometimes papillate and internally proliferating. Zoospores biflagellate, formed in a vesicle of sporangial protoplasm. Oogonia terminal or intercalary, (sub-)globose, with a ornamented wall. Antheridia variable in the shape, 1–2 per oogonium, sometimes absent, monoclinal, diclinous or hypogynous, stalked or sessile. Oospores globose, usually single in an oogonium, plerotic or aplerotic with a thin or thick wall.

*Elongisporangium* species occur as saprophytes or parasite in soils, water, or on plant substrates.

Type species: *Elongisporangium anandrum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium anandrum* Drechsler, J. Wash. Acad. Sci. 20: 410, 1930.

Additional species of *Elongisporangium*.

*Elongisporangium dimorphum* (F. F. Hendrix & W. A. Campb.) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium dimorphum* F. F. Hendrix & W. A. Campb, Mycologia 63: 979, 1971.

*Elongisporangium helicandrum* (Drechsler) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium helicandrum* Drechsler, Bull. Torrey bot. Club 77: 255, 1950.

*Elongisporangium prolatum* (W. A. Campb. & F. F. Hendrix) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium prolatum* W. A. Campb. & F. F. Hendrix, in Hendrix & Campbell, Mycologia 61: 387, 1969.

*Elongisporangium undulatum* (H. E. Petersen) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium undulatum* H. E. Petersen, Annls mycol. 8: 531, 1910.

***Pilasporangium*** Uzuhashi, Tojo & Kakish., gen. nov.

Fig. 5.

Mycelium bene evolvens, ex hyphis principalibus hyalinis, ramosis, nonseptatis, demum raro septatis compositum. Appressoria saepe efferentia. Sporangia terminalia vel intercalaria, globosa vel subglobosa. Zoosporae biflagellatae in vesicula protoplasmatis sporangii formantes. Oogonia terminalia vel intercalaria globosa vel subglobosa, pariete laevi. Antheridia monoclina, diclina vel hypogyna. Oosporae globosae, una vel duae in oogonio, pleroticae vel apleroticae.

Mycelium well developed, often with appressoria. Hyphae hyaline, aseptate, rarely septate in old. Sporangia terminal or intercalary, globose. Zoospores biflagellate, formed in a vesicle of sporangial protoplasm. Oogonia terminal or intercalary, (sub-)globose, with a smooth wall. Antheridia variable in the shape, one to several per oogonium, sometimes absent, monoclinal, diclinal or hypogynous, stalked or sessile. Oospores globose, one to two in an oogonium, plerotic or aplerotic with a thin or thick wall.

*Pilasporangium* species occur as saprophyte or parasite in soils, water, or on plant substrates.

Type species: *Pilasporangium apinafurcum* (Uzuhashi & Tojo) Uzuhashi, Tojo & Kakish., comb. nov.

Basionym: *Pythium apinafurcum* Uzuhashi & Tojo, Mycoscience 50: 283, 2009.

The genus *Pilasporangium* includes only one species.

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