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Evaluation of the rearrangement of taxonomic position of *Peronophythora litchii* based on partial DNA sequences

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ABSTRACT. Similarity levels of ribosomal ITS sequences and 28S ribosomal DNA sequences among members of the Oomycota were analyzed to determine the validity of the suggestion of transferring *Peronophythora litchii* to *Phytophthora* based on their close phylogenetic relationship. The validity of the suggestion is dependent on the accuracy of the assumption of the existence of a negative correlation between the order of the taxonomic ranks and the level of the sequence similarity of these two DNA fragments. Included in this report are sequences obtained in this study and sequences retrieved from the GenBank. *Phytophthora sojae* and *Plasmopara viticola* showed a higher level of ITS sequence similarity than all the species pairs of *Phytophthora* and several isolate pairs of *Peronospora parasitica* tested, indicating the lack of validity of transferring the taxonomic rank based on the ITS sequence relationships. The isolates AR 246 and HV 656 of *Bremia lactucae* showed a lower level of 28S sequence similarity than some species pairs of the same genus of *Peronospora*, *Phytophthora*, *Pythium, Albugo* and *Achlya*, and most species pairs of the different genera tested indicated also the lack of validity of transferring the taxonomic rank based on the 28S sequence relationships. The taxonomic statuses of *Peronophythora* as a distinct genus transitional between *Peronospora* and *Phytophthora* and of Peronophythoraceae as a distinct family transitional between Peronosporaceae and Pythiaceae, therefore, remain valid.

Keywords: 28S rDNA; ITS rDNA; *Peronophythora litchii*; *Phytophthora*; Sequence similarity.

INTRODUCTION

Peronophythora litchii Chen ex Ko et al. is unique in its ability to produce *Peronospora*-like sporangiophores on artificial media (Chen, 1961; Ko et al., 1978). This causal organism of litchi fruit rot produced differentiated sporangiophores with dichotomous branchlets (Figure 1A). The growth of sporangiophores was terminated at maturation by the synchronous formation of sporangia on the tapering tips of branchlets (Figure 1B). The sporangia on each sporangiophore enlarged and matured at approximately the same rates (Figure 1C, D). Although *Pp. litchii* produced sporangiophores characteristic of the species of the Peronosporaceae, its sex organs and ease of culture on artificial media resembled those in the Pythiaceae. A new family Peronophythoraceae was, therefore, established to accommodate the species with characteristics of both Pythiaceae and Peronosporaceae (Ko et al., 1978). The transitional nature of *Pp. litchii* was further supported by the observation of the characteristics of both *Peronospora* and *Phytophthora* in its oospore germination (Ann and Ko, 1980).

In 1982, Chi et al. (1982) noted the growth renewal of sporangiophores of *Pp. litchii* and re-named the organism *Phytophthora litchii*. Subsequently, Huang et al. (1983) and Ho et al. (1984) re-examined the asexual reproduction of the organism to ascertain its taxonomic status. Their studies substantiated the findings of Chi et al. (1982) that occasionally, the sporangiophore was capable of growth renewal, but also showed that in general, the sporangisphore of *Peronophythora* is basically determinate and sometimes indeterminate. Since the asexual reproduction of *Peronophythora* had the characteristics of both *Peronospora* and *Phytophthora*, Huang et al. and Ho et al. considered it unique enough to justify *Peronophythora's* retention as a distinct genus, transitional between *Phytophthora* and *Peronospora* (Ann and Ko, 1980; Chen, 1961; Ko et al., 1978). It is also not known whether the growth renewal of conidiophores on artificial media will also occur in members of Peronosporaceae when their culture on media becomes possible in the future. In recent years, partial DNA sequences have been shown to be useful in the

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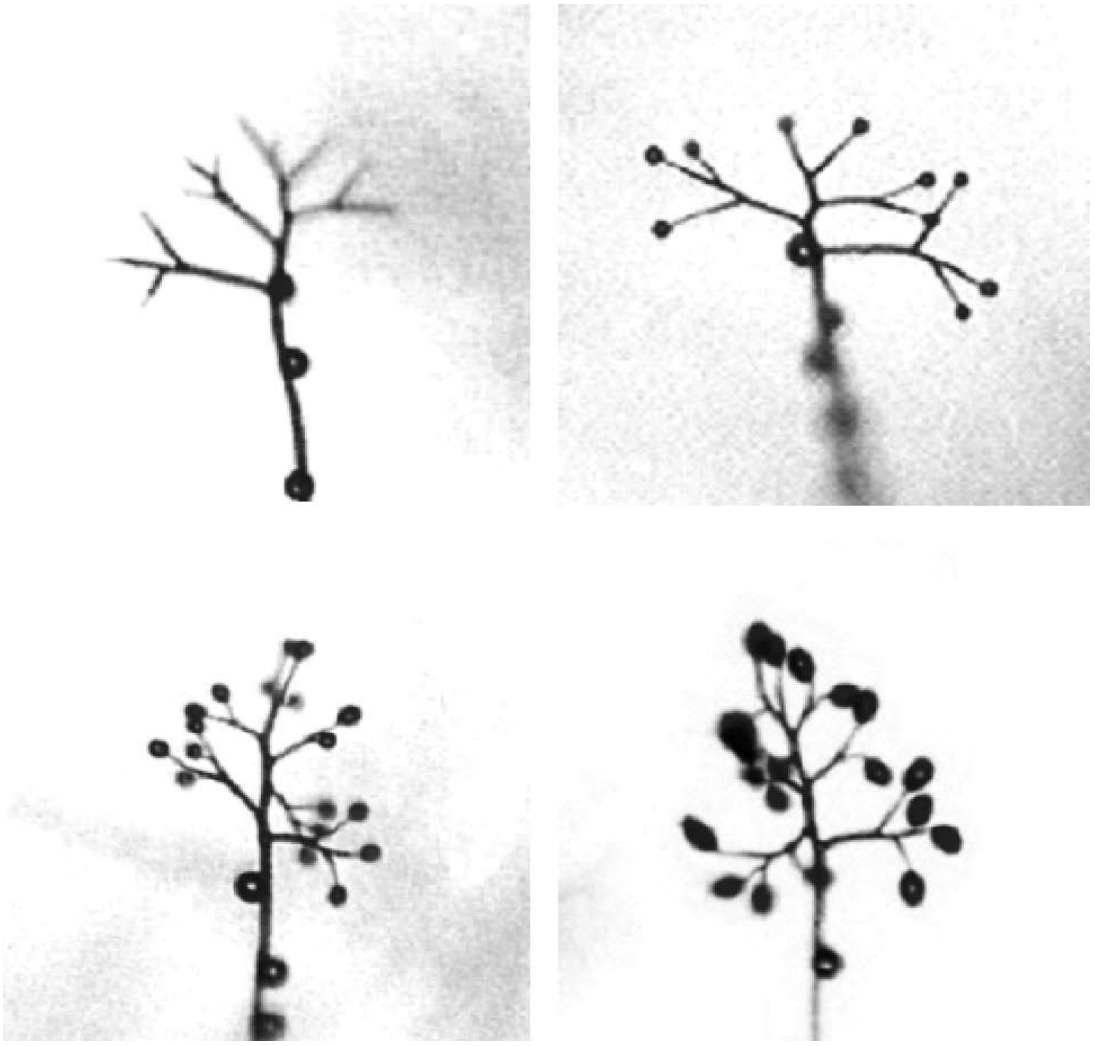

Figure 1. Different stages of development of sporangia on differentiated sporangiophores of *Peronophythora litchii*. A, sporangiophore with dichotomous branchlets; B, initiation of synchronous formation of sporangia; C, the early stage of sporangial development; D, the mature stage of sporangia.

phylogenetic studies of Oomycota (Lee and Taylor, 1992; Crawford et al., 1996; Matsumoto et al., 1999; Hudspeth et al., 2000; Forster et al, 2000; Cooke et al, 2000; Petersen and Rosendahl, 2000; Riethmuller et al., 2002; Martin and Tooley, 2003; Voglmayr, 2003; Ko et al., 2006). When Riethmuller et al. (2002) and Voglmayr (2003) reported the phylogenetic relationships of members in Oomycota using 28S ribosomal DNA sequences and ribosomal ITS sequences, respectively, the authors also suggested the transfer of *Peronophythora litchii* to *Phytophthora* because of its close phylogenetic relationship to *Phytophthora palmivora* and *Ph. arecae*. *Pernophythora litchii*, with well differentiated sporangiophore and synchronous formation of sporangia at maturity (Figure 1A-D), is morphologically and physiologically distinct from species of *Phytophthora*, which usually form undifferentiated sporangiophore producing sporangia as they grow (Figure 2A-D). We therefore undertook to evaluate the suitability of using these two types of phylogenic relationships to change the traditional taxonomic status, which was based on morphological and physiological characteristics.

The use of DNA-fragment-based phylogenetic relationships to determine the taxonomic status of an organism is dependent on the assumption that for that fragment of DNA, different isolates of the same species have higher levels of DNA homology than different species of the same genus, which in turn have higher levels of DNA homology than different species from different genera. The accuracy of this assumption has been tested for neither 28S nor ITS DNA. We, therefore, sequenced the ribosomal ITS DNA of 20 isolates belonging to thirteen species from six genera in Peronosporaceae, Peronophythoraceae and Pythiaceae, and analyzed their sequence homology in this study. The ITS DNA sequences retrieved from GenBank or published by Vaglmayr (2003) and the 28S DNA sequences published by Riethmuller et al. (2002) were also included in the analysis to test the accuracy of the assumption for these two DNA fragments.

MATERIALS AND METHODS

Fungal material

The organisms with ITS sequenced in this study are listed in Table 1 Each isolate used was derived from a single zoospore (Ho and Ko, 1997; Zheng and Ko, 1997; Ann and Ko, 1989) and maintaimed on 10% V-8 juice agar (Wu et al., 2003). Those organisms with ITS sequences available in GenBank for comparison are listed in Table 2. To obtain mycelia for genomic DNA extraction, 20 pieces of agar culture (approximately 1×1×2 mm) cut from the advancing margin of a 3-day-old colony growing on a plate of lima bean agar for species of *Phytophthora* and *Peronophythora*, or on potato dextrose agar for species of *Pythium*, were placed in a 250-ml flask containing 100 ml Plich's liquid medium (Zhang et al., 2004). After incubation at 25°C in darkness on a shaker for 6 days, mycelia were collected on a filter paper and stored at -20°C until use. Sporangia of *Peronospora parasitica*, *Pseudoperonospora cubensis*, and *Plasmopara viticola* obtained directly from infected leaves of their respective hosts (Table 1) were also stored at -20°C.

DNA preparation

DNA was prepared according to Panabieres et al. (1989) with the following modification. Approximately 10 mg of frozen mycelia or sporangia were ground in liquid nitrogen, and the resulting powder was suspended in 0.5 ml of NIB buffer consisting of 100 mM NaCl, 10 mM EDTA, 10 mM β-mercaptoethanol, 0.5% NP-40, and 30 mM Tris-HCl, pH 8.0. The suspension was centrifuged at 12,000 *g* for 1 min. After removal of the supernatant, the pellet was resuspended in NIB buffer, and the suspension was centrifuged as above. The resulting pellet was then resuspended in 0.8 ml of homogenization buffer consisting of 0.1 M NaCl, 0.2 M sucrose and 10 mM EDTA before adding 0.2 ml of lysis buffer containing 2.5% sodium dodeyl sulphate, 0.25 M EDTA, and 0.5 M Tris, pH 9.2.

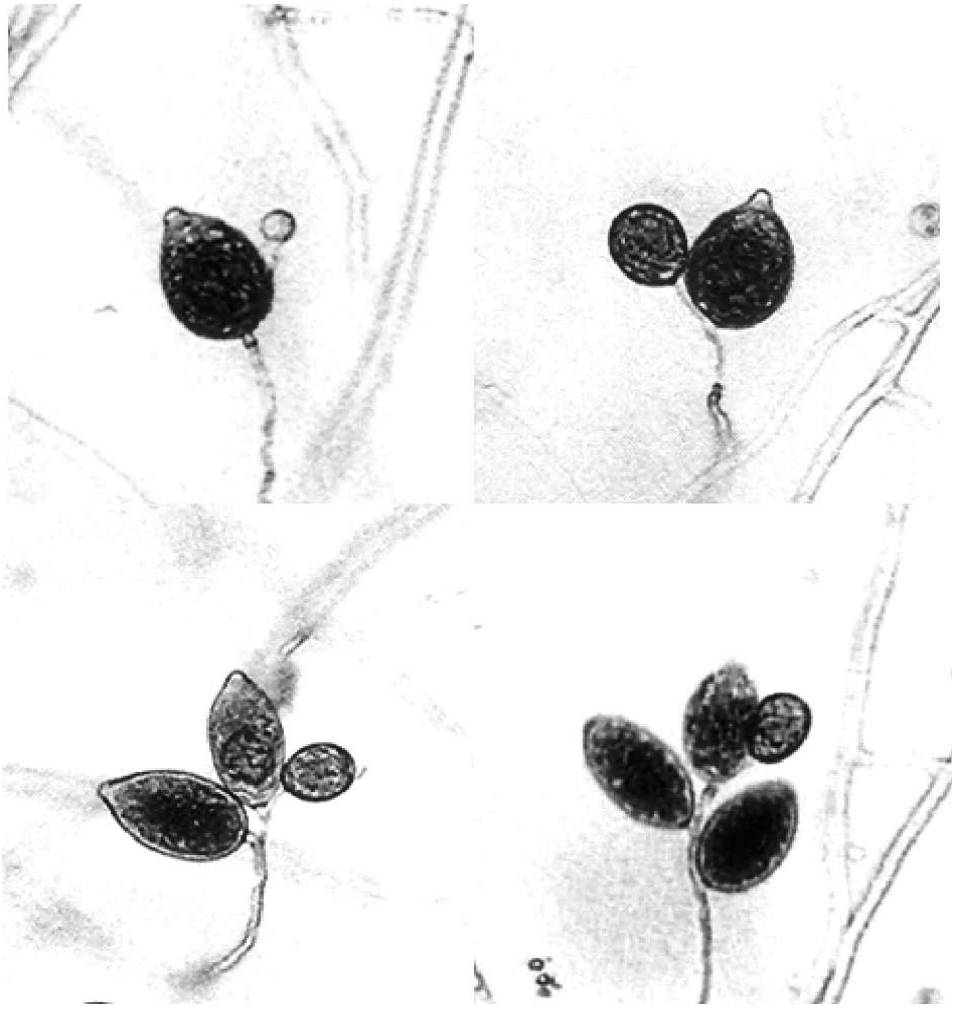

Figure 2. Development of sporangia on undifferentiated sporangiophores of *Phytophthora palmivora*. A, re-growth of sporangiophore and initiation of a new sporangium on its tip after maturation of the first sporangium; B, enlargement of the second sporangium; C, re-growth of sporangiophore and formation of a new sporangium on its tip after; maturation of the second sporangium; D, re-growth of sporangiophore and formation of a new sporangium on its tip after maturation of the third sporangium.

Table 1. Collection data and GenBank accession number for ITS sequenced in the present study.

Species and present study	Associated habitat	Location	Source	GenBank accession no.
Peronospora parasitica	*Brassica oleracea*	China	NAU[a]	AY269996
Pseudoperonospora cubensis	*Cucumis sativus*	China	NAU	AY744946
Plasmopara viticola	*Vitis vinifera*	China	NAU	AY742739
Peronophythora litchii	*Litchi chinensis*	Taiwan	P. J. Ann	AY269997
Phytophthora capsici				
98110 (A1)*	*Capsicum frutescens*	Taiwan	P. J. Ann	AY742735
20081 (A1)	*Capsicum frutescens*	Taiwan	P. J. Ann	AY742736
Ph. infestans				
TD1 (A1)*	*Solanum tuberosum*	China	Q. H. Chen	AY269995
TD2 (A2)	*Solanum tuberosum*	China	Q. H. Chen	AY922974
Ph. nicotianae				
991 (A1)*	Soil	Taiwan	G. A. Zentmyer	AY208131
6134 (A2)	*Solanum melongena*	Taiwan	P. J. Ann	AY208128
Ph. palmivora				
8829*	*Citrus* sp.	Taiwan	P. J. Ann	AY744949
88108 (A1)	*Carica papaya*	Taiwan	P. J. Ann	AY742734
Ph. sojae, S317	*Glycine max*	China	C. Y. Shen	AY245092
Ph. tropicalis				
23047 (A1)*	*Prunus persica*	Taiwan	P. J. Ann	AY742737
129F-1 (A0)	*Dianthus caryphyllus*	Taiwan	W. H. Ko	AY742738
Pythium aphanidermatum	*Cucumis sativus*	China	NAU	AY269999
Py. splendens				
117 (-)*	Soil	Taiwan	M. Aragaki	AY269993
461 (+)	Soil	Taiwan	M. Aragaki	AY269994
Py. vexans				
Pyv 6-1*	Soil	Taiwan	W. H. Ko	AY269998
Pyv 6-2	Soil	Taiwan	W. H. Ko	AY922975

*Used in the comparison with those available in GenBank.
[a]NAU=Nanjing Agricultural University.

After incubation at 55°C for 30 min, the homogenate was extracted twice with the same volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged at 12,000 *g* for 15 min. The aqueous phase was extracted with an equal volume of chloroform-isoamyl alcohol (24:1), precipitated with two volumes of cold absolute alcohol for 10 min, and centrifuged at 12,000 *g* for 15 min. The pellet was washed with 70% ethanol, air-dried, and resuspended in 50 µl TE buffer consisting of 1 mM EDTA and 10 mM Tris-HCl, pH 7.4. The A260 nm / A280 nm ratios of these DNA preparations were between 1.7 and 2.0, and their A260 nm / A230 nm ratios were between 1.6 and 2.0, suggesting that they were essentially free of proteins and carbohydrates. All DNA preparations were kept at -20°C.

DNA amplification and sequencing

The 5.8S rRNA gene and the two flanking internal transcribed spacers (ITS1 and ITS2) were amplified with primers ITS1 and ITS4 (White et al., 1990). The 50 µl reaction mixture—consisting of 10 ng template DNA, 1 µM each primer, 100 µM each dNTP, 5 µl 10 X polymerase chain reaction (PCR) buffer, 1.5 mM $MgCl_2$, and 2.5 units Taq DNA polymerase (Promega Corp., Madison, WI, USA)—was subjected to thermal cycling in a PTC-200 DNA Engine Cycler (MJ Research, MA, USA). The thermal cycling parameters were as follows: initial denaturation for 5 min at 94°C, then 1 min at 55°C, 3 min at 72°C, and 1 min at 94°C for 30 cycles, and a final elongation at 72°C for 10 min. Amplification products were run on 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination in order to determine the number and size of DNA products amplified in the PCR. PCR products were subcloned using the pGEM-T Easy Vector System of Promega, and these clones from each isolate were sequenced using the

Table 2. Similarity of ITS sequenced in this study with those available in GenBanka.

Species and isolate	Associatedd habitat	Location	Source	GenBank accession no.	Similarity (%)
Peronospora parasitica					
SMK 13558	*Arabis glabra*	Korea	H. D. Shin	AY 210983	94.6
SMK 17785	*Barrarea orthoceras*	Korea	H. D. Shin	AY 210984	95.1
SMK 16040	*Brassica campestris*	Korea	H. D. Shin	AY 210986	99.4
SMK 13731	*Brassica campestris*	Korea	H. D. Shin	AY 210985	99.6
SMK 15691	*Capsella bursa-paestoris*	Korea	H. D. Shin	AY 210987	84.8
SMK 18835	*Capsella bursa-paestoris*	Korea	H. D. Shin	AY 210988	84.6
HV 746	*Capsella bursa-paestori*	ND	ND	AY 198254	82.8
SMK 18819	*Cardamine flexuosa*	Korea	H. D. Shin	AY 210989	75.9
SMK 17273	*Cardamine leucaretha*	Korea	H. D. Shin	AY 210990	95.1
SMK 17298	*Cardamine leucaretha*	Korea	H. D. Shin	AY 210991	95.1
SMK 17322	*Cardamine leucaretha*	Korea	H. D. Shin	AY 210992	95.2
SMK 18539	*Cardamine leucaretha*	Korea	H. D. Shin	AY 210993	95.2
SMK 18833	*Cardamine scutata*	Korea	H. D. Shin	AY 210994	76.7
SMK 17319	*Darba nemorosa*	Korea	H. D. Shin	AY 210995	81.9
SMK 17270	*Darba nemorosa*	ND	H. D. Shin	AY 210996	81.9
SMK 18842	*Darba nemorosa*	Korea	ND	AY 210999	81.9
SMK 18831	*Darba nemorosa*	Korea	H. D. Shin	AY 210998	81.6
SMK 18811	*Darba nemorosa*	Korea	H. D. Shin	AY 210997	81.8
Maks 9	ND	ND	H. D. Shin	AY 578093	84.2
SMK 15604	*Raphanus sativus*	Korea	H. D. Shin	AY 211002	90.2
SMK 15363	*Raphanus sativus*	Korea	H. D. Shin	AY 211001	89.3
SMK 14315	*Raphanus sativus*	Korea	H. D. Shin	AY 211000	90.2
SMK 11409	*Rorippa islandica*	Korea	H. D. Shin	AY 211003	76.6
SMK 12789	*Rorippa islandica*	Korea	H. D. Shin	AY 211005	75.5
SMK 18713	*Rorippa islandica*	Korea	H. D. Shin	AY 211004	76.6
SMK 18859	*Rorippa islandica*	Korea	H. D. Shin	AY 211007	75.6
SMK 18834	*Rorippa islandica*	Korea	H. D. Shin	AY 211006	75.4
SMK 18194	*Sisymbrium letewm*	Korea	H. D. Shin	AY 211008	84.9
ND[b]	*Thlaspi arvense*	Sweden	O. Constantinescu	AF 465759	76.8
SMK 17271	*Thlaspi arvense*	Korea	H. D. Shin	AY 211009	83.5
SMK 18832	*Thlaspi arvense*	Korea	H. D. Shin	AY 211010	83.6
Pseudoperonospora cubensis			H. D. Shin		
HV 222	*Cucumis sativus*	Austria	H. Voglmayr	AY 198306	99.8
Plasmopara viticola				ND	
Peronophythora litchii					
90113	*Litchi chinensis*	Taiwan	L. C. Huang	AY 251666	100
CBS 100.81	*Litchi chinensis*	ND	H. Voglmayr	AY 198308	100
Phytophthora capsici					
H599	*Capsicum annuum*	New Mexico	M. Aragaki	AY 208129	99.9
21170	*Capsicum annuum*	Taiwan	L. C. Huang	AY 251662	99.9
KACC 40157	*Capsicum annuum*	Korea	S. B. Hong	AF 228078	92.2
H210	*Lycopersicon esculentum*	Hawaii	M. Aragaki	AY 208130	100
KACC 40177	*Lycopersicon esculentum*	Korea	S. B. Hong	AF 228079	99.1
P 141	*Lycopersicon esculentum*	ND	K. L. Ivors	AY 423292	99.9

Table 2. (Continued)

Species and isolate	Associatedd habitat	Location	Source	GenBank accession no.	Similarity (%)
ND	Pepper	Italy	I. Camele	AJ 555612	99.6
IMI 352321	*Piper nigrum*	India	D. E. Cooke	AF 266787	99.3
IMI 325900	*Theobroma cacao*	Brazil	A. A. Appiah	AF 167083	97.6
IND 44	*Theobroma cacao*	India	A. A. Appiah	AF 467085	97.7
IMI 304412	*Theobroma cacao*	Cote d'Ivoire	A. A. Appiah	AF 467084	97.5
Ph. infestans					
KACC 40706	*Lycopersicon esculentum*	Korea	S. B. Hong	AF 228084	100
P6.4CIP-C	ND	ND	G. Z. Abad	AF 489701	100
KACC 40707	*Solanum tuberosum*	Korea	S. B. Hong	AF 228083	100
IMI 66006	*Solanum tuberosum*	The Netherlands	D. E. Cooke	AF 266779	99.5
Ph. nicotianae					
KACC 40403	*Epiphyllum trucatum*	Korea	S. B. Hong	AF 228085	99.8
KACC 40407	*Lilium lonegiflorum*	Korea	S. B. Hong	AF 228086	99.9
UQ 848	ND	Australia	D. E. Cooke	AF 266776	99.9
IMI 325395	ND	ND	A. R. Crawford	L 41383	97.3
331	*Nicotiana tabacum*	USA	K. L. Ivors	AY 423299	100
IMI 354462	*Theobroma cacao*	Malaysia	A. A. Appiah	AF 467087	99.9
Ph. palmivora					
21162	*Carica papaya*	Taiwan	P. J. Ann	AY 208126	99.9
93105	*Cattleya* spp. (orchid)	Taiwan	L. C. Huang	AY 251647	99.7
9257	*Cattleya* spp. (orchid)	Taiwan	L. C. Huang	AY 251648	99.9
KACC 40167	*Chrysalidocarpus lutescens*	ND	S. B. Hong	AF228087	99.7
KACC 40409	*Cymbidium* sp.	Korea	S. B. Hong	AF 228088	98.7
UQ 1294	ND	Papus New Guinea	D. E. Cooke	AF 266780	99.7
P 80	ND	ND	A. R. Crawford	L 41384	99.2
VR 13	ND	Ghana	A. A. Appiah	AF 467091	97.8
20230	*Persea americana*	Taiwan	P. J. Ann	AY 208127	100
94P30	*Theobroma cacao*	Indonesia	M. Ducamo	AJ 517463	99.9
PPaP 33	*Theobroma cacao*	Taiwan	A. A. Appiah	AF 467093	99.7
TRI 1	*Theobroma cacao*	Trinidad	M. Ducamp	AJ 517462	99.7
GHWR 48	*Theobroma cacao*	Ghana	A. A. Appiah	AF 467090	99.9
PNG 14	*Theobroma cacao*	Papus New Guinea	M. Ducamp	AJ 517464	99.5
4C7	*Theobroma cacao*	Cuba:Baracao	M. Ducamp	AJ 517465	99.7
PPaC 55	*Theobroma cacao*	Taiwan	A. A. Appiah	AP 467094	99.4
TP 1	*Theobroma cacao*	Ghana	A. A. Appiah	AP 467092	99.9
PI-10	*Theobroma cacao*	Costa Rica	K. L. Ivors	AY 423300	99.9
IMI 325923	*Theobroma cacao*	Costa Rica	A. A. Appiah	AF 467088	99.6
94P43	*Theobroma cacao*	Indonesia	A. A. Appiah	AF 467089	99.6
Ph. sojae					
R 25	*Glycine max*	USA	M. Catal	AY 590277	99.8
R 1	*Glycine max*	USA	M. Catal	AY 590274	99.8
R 7	*Glycine max*	USA	M. Catal	AY 590276	99.8
MSU 85	*Glycine max*	USA	M. Catal	AY 590271	99.8
MSU 88	*Glycine max*	USA	M. Catal	AY 590273	99.6
MSU 84	*Glycine max*	USA	M. Catal	AY 590270	99.6

Table 2. (Continued)

Species and isolate	Associatedd habitat	Location	Source	GenBank accession no.	Similarity (%)
KACC 40412	*Glycine max*	ND	S. B. Hong	AF 228089	99.6
MSU 72	*Glycine max*	USA	M. Catal	AY 590266	99.6
UQ 1200	*Glycine max*	Australia	D. E. Cooke	AF 266769	99.6
SOY 324	*Glycine max*	ND	D. E. Cooke	AF 403501	99.6
MSU 86	*Glycine max*	USA	M. Catal	AY 590272	99.6
R4	*Glycine max*	USA	M. Catal	AY 590275	99.8
ATCC 48068	*Glycine max*	ND	K. L. Ivors	AY 423301	99.8
MSU 76	ND	USA	M. Catal	AY 590268	99.8
MSU 75	ND	USA	M. Catal	AY 590267	99.8
MSU 83	ND	USA	M. Catal	AY 590269	99.8
ND	ND	ND	Q. Chen	AY 256844	99.6
P 2-3	ND	ND	Q. Weng	AY 277278	99.6
Pg 2	Soil	USA	Z. Zhang	AY 242606	99.8
Pg 1	Soil	USA	Z. Zhang	AY 242605	99.6
Pg 3	Soil	USA	Z. Zhang	AY 242607	99.8
Ph. tropicalis					
066	*Cyclamen persicum*	ND	R. Schubert	AJ 299733	99.2
H 778-1	*Dianthus caryophyllus*	ND	R. Schubert	AJ 299734	99.2
H 213	*Leucosperinum* sp.	Hawaii	M. Aragaki	AY 207010	99.3
H 352	*Theobroma cacao*	New Guinea	M. Aragaki	AY 208125	98.3
Pythium aphanidermatum					
100439R	ND	ND	G. W. Moorman	AF 452149	100
346952	ND	ND	G. W. Moorman	AF 452151	100
340458	ND	ND	G. W. Moorman	AF 452148	100
ND	ND	Egypt	Y. H. Gherbawy	AJ 628984	100
141749R	ND	ND	G. W. Moorman	AF 452146	100
135	ND	France	A. M. Schurko	AY 151180	100
P 36-3	ND	ND	K. Kageyama	AB 095052	100
UQ 2071	Soil	Australia	D. E. Cooke	AF 271227	100
Zen 97-378-U	ND	ND	G. W. Moorman	AF 452152	100
TOc 159	ND	ND	G. W. Moorman	AJ 233438	100
363669R	ND	ND	G. W. Moorman	AF 452150	100
P 12	ND	ND	G. W. Moorman	AF 452153	99.6
DC 16	ND	ND	B. G. Lou	AY 278109	99.2
F-1245	ND	India	B. Paul	AY 207380	98.7
Py. splendens					
OPU 591	*Pachiva aguatica*	Japan	M. Tojo	AY 375242	99.5
Py. vexans					
80936-95	ND	ND	G. W. Moorman	AF 452137	96.8
81708-98	ND	ND	G. W. Moorman	AF 452136	96.8
UQ 2074	Soil	Australia	D. E. Cooke	AF 271224	94.1

[a]Data were retrieved from GenBank on November 2, 2004.
[b]ND=No data.

dideoxy method of Sanger (Sanger and Coulson, 1975) by Takara Biotechnology Co., Kyoto, Japan. Three clones for each isolate were sequenced.

Data analysis

The nucleotide alignments were carried out using the optimal alignment method of DNAMAN software (Version 4.0, Lynnon BioSoft, Quebec, Canada).

RESULTS AND DISCUSSION

The similarity of the overall ITS sequences between two isolates of the same species sequenced in this study was all very high, ranging from 98.9 to 100% (data not shown). Therefore, only one isolate from each species (Table 1) was used for comparison with isolates of the same species with ITS sequences available in GenBank in the homology tests. The similarity of the ITS sequence of *Peronospora parasitica* on *Brassica oleracea* sequenced in this study with the 31 isolates of the same species on different hosts in the Brassicaceae retrieved from the GenBank ranged from 75.4 to 99.6% (Table 2). Those with more than 95% ITS sequence similarity were on *Brassica campestris*, *Barbarea orthoceras*, and *Cardamine leucaretha* while those with less than 77% similarity were on *Cardamine flexuosa*, *Cardamine scutata*, *Rorippa islandica*, and *Thlaspi arvense*. The ITS sequence similarity of *Phytophthora capsici* and *Pythium vexans* sequenced in this study with those available in GenBank was 92.2 to 100% and 94.1 to 96.8%, respectively. The similarity of ITS sequences of all other species sequenced in this study with those of the same species retrieved from GenBank was more than 97% (Table 2).

Voglmayr (2003) suggested transferring *Pp. litchii* to *Phytophthora* because of its close ITS sequence relationship with *Ph. palmivora* and *Ph. arecae*. The validity of the suggestion is dependent on the assumption of a negative correlation between the order of the taxonomic ranks and the level of ITS sequence similarity. To test the assumption using the isolates sequenced in this study, the ITS sequences of *Phytophthora sojae* and *Pseudoperonospora cubensis* were compared with species of the same or different genus. The similarity levels of 99.3% and 89.5% between *Ph. sojae* and *Plasmopara viticola* and *Ph. sojae* and *Ph. tropicalis*, respectively, (Table 3) are inconsistent with the assumption. The accuracy of the assumption further diminished when these data were compared with the 75.4% similarity between *Pe. parasitica* on *B. oleracea* and the same species on *R. islandica* (SMK 18834) (Table 2), thus invalidating the suggestion to transfer *Pp. litchii* to *Phytophthora* based on the ITS sequence relationship. The result of the 88.1% similarity between *Ps. cubenis* and *Pp. litchii* in comparison with 72.8% between *Ps. cubensis* and *Pe. parasitica*, 83.5% between *Ps. cubensis* and *Pl. viticola*, and 83.8% between *Ps. cubensis* and *Ph. sojae* (Table 3) also invalidated the transfer of *Pp. litchii* to *Phytophthora*. The ITS sequence analysis of data published by Voglmayr (2003) also failed to support the transfer of taxonomic ranks in Oomycota based on the sequence relationship of this DNA fragment. Among the four species pairs of the same genus compared, three have lower levels of sequence similarity than the four species pairs of different genera tested (Table 4).

Riethmuller et al. (2002) suggested that *Pp. litchii* should be transferred to *Phytophthora* because of its close 28S sequence relationship with *Ph. arecae*. The validity of the suggestion is also dependent on the assumption that different isolates of the same species have higher levels of 28S sequence similarity than different species of the same genus, which in turn have higher levels of

Table 3. Similarity of ITS sequences of *Phytophthora sojae* and *Pseudoperonospora cubensis* to those of other species used in this study.

Species compared	Similarity (%)	
	Ph. sojae	*Ps. cubensis*
Peronospora parasitica	70.4	72.8
Pseudoperonospora cubensis	83.8	100
Plasmopara viticola	99.3	83.5
Peronophythora litchii	86.9	88.1
Phytophthora capsici	88.8	88.4
Ph. infestans	84.6	87.3
Ph. nicotianae	85.0	87.1
Ph. palmivora	86.5	88.0
Ph. sojae	100	83.8
Ph. tropicalis	89.5	88.8
Pythium aphanidermatum	59.5	58.9
Py. splendens	61.8	61.3
Py. vexans	64.9	66.0

similarity than different species from different genera. Comparison of 28S sequence similarity between isolates of the same species, between species of the same genus, and between species of different genera based on the data published by Riethmuller et al. (2002) fails to support this assumption. The 92.1% 28S sequence similarity level between isolates AR246 and HV656 of *Bremia lactucae* was lower than some different species pairs of *Peronorpora*, *Phytophthora*, *Pythium*, *Albugo*, and *Achlya* (Table 5). This is inconsistent with the assumption. Also undermining the assumption is the lower similarity levels between *Peronospora avensis* and *Pe. niessleana* (75.0%),

Table 4. The ITS sequences similarity between species of the same genus and different genus based on the data published by Voglmayr (2003).

Same genus		Different genus	
Species compared	Similarity (%)	Species compared	Similarity (%)
1. *Pe. sparsa*		1. *Pe. sparsa*	
Pe. alta	82.5	*Ph. palmivora*	92.1
2. *Pe. aestivalis*		2. *Ps. cubensis*	
Pe. lepidii-sativi	72.9	*Pe. sherardiae*	92.4
3. *Ph. nicotianae*		3. *Ps. cubensis*	
Ph. cactorum	94.6	*Ph. nicotianae*	88.2
4. *Py. irregulare*		4. *Pe. sparsa*	
Py. ultimum	72.2	*Pp. litchii*	91.9

Table 5. The 28S sequence similarity between isolates of the same species, between species of the same genus, and between species of different genus based on the data published by Riethmuller et al. (2002).

Isolate/species compared	No. of data available	Similarity (%)
Between different isolates of the same species		
Bremia lactucae	6	92.1-99.4
Between different species of the same genus		
Peronospora	37	75.0-99.4
Phytophthora	8	96.0-100
Pythium	8	82.6-98.4
Albugo	8	65.2-94.4
Achlya	2	92.6
Between species of different genus		
1. *Saprolegnia ferax*		
Scoliolegnia asterophora		94.2
2. *Calyptralegnia achlyoides*		
Aplanes androgynus		96.2
3. *Pachymetra chaunorhiza*		
Aphanomyces stellatus		95.4
4. *Dictyuchus monosporus*		
Brevilegnia megasperma		95.7
5. *Bremiella baudysii*		
Plasmopora sii		98.4
6. *Peronophythora litchii*		
Phytophthora multivesticulata		96.9
7. *Peronospora potertillae-sterilis*		
Phytophthora nicotianae		95.8
9. *Pythium middletonii*		
Lagenidium chthamalophilum		87.0

Pythium undulatum and *Py. monospermum* (82.6%), and *Albugo portulacae* and *Al. tragoponis* (MG9-4) (65.2%) in comparison with the similarity levels of all the eight species pairs of different genera tested (87.0 to 98.4 %) (Table 5). The inaccuracy of the assumption invalidates the suggestion to transfer *Pp. litchii* to *Phytophthora* based on the 28S sequence relationship.

Results from this study show that although ITS and 28S sequences are useful in phylogenetic studies, they are not valid in the determination of the taxonomic status of Oomycota. Therefore, the taxonomic status of *Peronophythora* as a distinct genus transitional between *Peronospora* and *Phytophthora*, and that of Peronophythoraceae as a distinct family transitional between Peronosporaceae and Pythiaceae (Ann and Ko, 1980; Chen, 1961; Ho et al., 1984; Huang et al., 1983) remain valid. The reason for the lack of correlation between the taxonomic ranks and sequence similarity of ITS and 28S DNA in Oomycota is not known. It is possible that these two DNA fragments are not related to the morphological and physiological traits and the nucleotide substitutions in these regions are, therefore, independent of the taxonomical characteristic changes in this group of organisms. Further study is needed to test this hypothesis.

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根據部份 DNA 序列重定 *Peronophythora litchii* 分類的評估

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本文利用卵菌門內組成員，互相之間的 ribosomal ITS 序列及 28S ribosomal DNA 序列的相似度，來決定根據近系統關係而建議將 *Peronophythora litchii* 移到 *Phytophthora* 的正當性。此建議的正當性，全建立在分類階級的高低與此兩 DNA 片段的相似度成反比的這一假設的正確性。本文所用的材料包括此研究所得的序列及由 GenBank 所獲得的序列。*Phytophthora sojae* 同 *Plasmopara viticol*a 之間的序列相似度，高於 *Phytophthora* 屬內不同種之間的序列相似度，也高於 *Peronospora parasitica* 種內幾個不同分離株之間的序列相似度。由此可見根據 ITS 序列的關係來改變分類階級缺乏正當性。*Bremia lactucae* 種內 AR246 與 HV656 之間的 28S 序列相似度低於 *Peronospora*, *Phytophthora*, *Pythium, Albugo* 及 *Achlya* 同屬內有些不同種之間的序列相似度，也低於很多不同屬之間的序列相似度，這些証據都顯示根據 28S 序列的關係來改變分類階級是沒有正當性的。在分類地位上，*Peronophythora* 當特異的屬，介於 *Peronospora* 及 *Phytophthora* 之間，而 *Peronophythoraceae* 當特異的科，介於 *Peronosporaceae* 及 *Pythiaceae* 因此保持有效。

關鍵詞：ITS rDNA；*Peronophythora litchii*；*Phytophthora*；序列相似度；28S rDNA。