Morphological and molecular discrimination among *Albugo candida* materials infecting *Capsella bursa-pastoris* world-wide

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Choi, Y.J., Shin, H.D., Hong, S.B. and Thines, M. (2007). Morphological and molecular discrimination among *Albugo candida* materials infecting *Capsella bursa-pastoris* world-wide. Fungal Diversity 27: 11-34.

The genus *Albugo* with *A. candida* as the type species causes white blister rust disease in economically important crops. Recently, molecular approaches revealed the high degree of genetic diversity exhibited within *A. candida* complex. However, before any taxonomic division of the complex at the species level, the correct status of *A. candida* on *Capsella bursa-pastoris*, from which it was originally described, should be determined. A worldwide study of white rust pathogens on *C. bursa-pastoris* was performed on 36 specimens, using morphological analysis and sequence analysis from the *cytochrome c oxidase* subunit II (COX2) region of mtDNA, the internal transcribed spacer (ITS) region of the rDNA, and D1 and D2 regions of the nrDNA. Specimens were obtained from Asia (India, Korea, Palestine), Europe (England, Finland, Germany, Hungary, Ireland, Latvia, Netherlands, Romania, Russia, Sweden, Switzerland), North and South America (Argentina, Canada, USA), and Oceania (Australia). The molecular data indicated strong support for a species partition separating Korean and other continental specimens. There were 7.25, 17.4, and 4.8% sequence dissimilarity between the two groups in COX2, ITS, and 28S regions, respectively. The surface ornamentation of oospores of Korean specimens was morphologically distinguished from previously known characteristics of *A. candida*. Therefore, *Albugo koreana* sp. nov., collected from *C. bursa-pastoris* in Korea, is described here. The concept of *A. candida sensu stricto* is discussed and a type specimen determined for this species.

Key words: *Albugo koreana* sp. nov., COX2 mtDNA, ITS rDNA, taxonomy, white blister rust

Introduction

Albugo candida (Pers.) Roussel (*Albuginales*, Peronosporomycetes), among about 50 species of the genus *Albugo* (Biga, 1955; Choi and Priest,

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1995), is an obligate parasitic fungus responsible for white rusts in brassicaceous hosts over widely different geographical areas of the world. The pathogen causes significant damage in economically important agricultural crops and common weeds (Farr *et al*., 1989), and its hosts have been reported to include as many as 63 genera and 241 species (Biga, 1955; Saharan and Verma, 1992), of which *Brassica* and *Raphanus* species are the most important cultivated hosts. *Albugo* has been typified by Kuntze (1891), who gave '*Uredo candida* (Pers.) Pers.' as the type species, which nomenclature shall be discussed later. *Albugo candida* has been separated into different forms by Săvulescu and Rayss (1930), and divided into two varieties by Biga (1955) based on the sporangial size and host ranges, *viz*. var. *candida* and var. *macrospora*. Although *A*. *macrospora* (Togashi) S. Ito has been described, based on the variety *A*. *candida* var. *macrospora* (Togashi, 1935), which has been reported from some species of the genera *Brassica* and *Raphanus* in the *Brassicaceae* (Zhang and Wang, 1981; Yu *et al*., 1998), neither the species nor the variety is widely accepted. All other species from *Brassicaceae* and *Capparaceae* (e.g. *A*. *capparis* (de Bary) Kuntze, *A*. *chardonii* W. Weston, *A*. *lepidii* A.N.S. Rao, *A*. *wasabiae* Hara) are also not widely accepted. Therefore, the name *A*. *candida* is mostly used to refer to the fungus causing white rusts on all brassicaceous hosts as a single species.

A recent molecular study (Choi *et al*., 2006) has revealed that the high degree of genetic diversity exhibited within the *A*. *candida* complex warrants its division into several distinct species. Also in the study of Voglmayr and Riethmüller (2006), *A*. *candida* was shown to consist of at least two distinct linages. To resolve the taxonomic problems of the complex, however, the precise status of the type species of the genus, *A*. *candida*, is first to be determined. The pathogen was originally described from a variety of hosts, but the type specimen is selected on *Capsella bursa-pastoris* (L.) Medik. in Europe in this study. This plant has a worldwide distribution and is also an important vegetable in Korea. Many monographic studies have recognized *A*. *candida* as the sole species infecting *C*. *bursa-pastoris*, but in our previous study (Choi *et al*., 2006), *A*. *candida* on this host collected in Korea was differentiated from those in other geographical regions on molecular grounds. However, the study included only a small number of specimens from a limited geographical area. Thus, a comprehensive study based on morphological and molecular data was needed. In the present study, 36 *Albugo* collections on *Capsella* specimens comprising 8 from North and South America (Argentina, Canada, USA), 8 from Asia (India, Korea, Palestine), 18 from Europe (England, Finland, Germany, Hungary, Ireland, Latvia, Netherlands, Romania, Russia, Sweden, Switzerland), and 2 from Oceania (Australia), were used for phylogenetic and morphological

analyses. Several studies have already proven that the sequence analyses of the ITS region of nrDNA (Cooke *et al*., 2000; Constantinescu and Fatehi, 2002; Choi *et al*., 2003; Voglmayr, 2003; Göker *et al*., 2004; Thines and Spring, 2005; Spring *et al*., 2006) and the mitochondrial COX2 gene, which encodes subunit II of the cytochrome c oxidase complex (Hudspeth *et al*., 2003; Martin, 2000; Martin and Tooley, 2003) are useful to resolve closely related species within the *Oomycota*. Molecular analysis based on LSU nrDNA was recently used to uncover the phylogenetic relationship among several species of the *Albuginaceae* (Voglmayr and Riethmüller, 2006). In morphological analysis characteristics of the oospore wall, which were previously suggested as useful to identify and compare species of *Albugo* (Choi and Priest, 1995; Voglmayr and Riethmüller, 2006), were found to be more helpful in distinguishing *Albugo* specimens on *C. bursa-pastoris*, than characters of the sporangiophores and sporangia. The present work combines the use of morphological and molecular tools to investigate and to correctly identify *Albugo* specimens infecting *C. bursa-pastoris* throughout the world and to help solving the systematic problem of the *A. candida* complex by stabilising the taxonomy of this pathogen by selecting an appropriate type specimen.

Materials and methods

Fungal specimens

Phylogenetic and morphological analyses were carried out with 81 *Albugo* specimens (Table 1). Of these, 36 dried herbarium specimens of *Albugo* on *Capsella* were sequenced. For comparison, 30 sequences of other *Albugo* species were also analyzed. Voucher specimens of the newly collected materials are preserved in SMK (Herbarium of Systematic Mycology of Korea, Korea University, Seoul, Korea), and some were also deposited in BPI (U.S. National Fungus Collections, Beltsville, Maryland, USA). Herbarium abbreviations follow those given in Holmgren and Holmgren (1998).

Morphological analysis

Herbarium specimens were moistened with 70% alcohol and fungi were transferred to 60% lactic acid on a slide. The microscope preparations were warmed up covered with coverslips and examined in brightfield- and DIC- light microscopy. Measurements were performed at c. 1000× for sporangia and 100-1000× for other organs.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using sporangiophores and sporangia formed on the lower or upper surface of the infected leaves, or using the infected host tissue of herbarium specimens. DNA extraction was performed by the methodology described in Lee and Taylor (1990). The rDNA region containing the partial 18S gene, both internal transcribed spacers (ITS1 and ITS2) and the 5.8S gene of these specimens was amplified using primers DC6 (5'-GAG-GGA-CTT-TTG-GGT-AAT-CA-3') and LR-0 (5'-GCT-TAA-GTT-CAG-CGG-GT-3'). PCR reactions were conducted in 50 µl reaction volumes, with each reaction tube containing 1.2 µl of template DNA solution (approximately 100 ng), as prepared above, 5 µl of 10× buffer [50 mM KCl, 100 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 15 mM $MgCl_2$], 3 µl of 2.5 mM dNTP, 0.4 µl (each) of 100 pM primers, 0.4 µl of *Taq* polymerase (5 unit μl^{-1}), and 39.6 µl of ddH_2O. The thermal cycling parameters were: denaturation for 1 min at 95ºC, annealing for 1 min at 58ºC, and extension for 2 min at 72ºC. Thirty-five cycles were performed, with the first denaturation and last extension times extended to 5 and 10 min, respectively. For COX2 amplification, the forward (5'-GGC-AAA-TGG-GTT-TTC-AAG-ATC-C-3') and reverse (5'-CCA-TGA-TTA-ATA-CCA-CAA-ATT-TCA-CTA-G-3') primers designed by Hudspeth *et al*. (2000) were employed. Reactions identical to that for the amplification of the ITS region were performed using the following cycling conditions; denaturation for 30 s at 96ºC, annealing for 30 s at 50ºC, and extension for 1 min at 72ºC. Thirty cycles were performed, with both the first denaturation and last extension times extended to 4 min. The PCR products were purified using a QIAquick gel extraction kit (Qiagene, Hilden, Germany). Purified DNAs were directly sequenced on an automatic sequencer (ABI Prism TM 377 DNA Sequencer), with the primers ITS1, ITS2, and ITS3 (White *et al*., 1990) for ITS rDNA and the primers identical to those used to amplify for the COX2 mtDNA. PCR and sequencing of the nuclear large subunit D1/D2 region of four specimens were performed as described in Riethmüller *et al*. (2002).

Sequence alignment and phylogenetic analysis

Sequences were edited with DNASTAR computer package, with alignment of the sequences performed using CLUSTAL W (Thompson *et al*., 1994) program. Bayesian analysis was performed using the computer program MRBAYES version 2.01 (Huelsenbeck and Ronquist, 2001). This program performs a Bayesian inference of the phylogeny, using Metropolis-coupled Markov chain Monte Carlo (MC^3; Geyer, 1991) analyses. The general time

Table 1. Summary of information about *Albuginaceae* specimens used in this study

Species	Host	Geographical origin	Herbarium number	Year	GenBank	
					ITS	COX2
Albugo candida	*Arabis turrita*	Bulgaria	SOMF 00337	1955	AY929825	AY913803
	Aubrieta deltoidea	Germany, Oberhessen	BPI 184659	1953	DQ418500	DQ418511
	Berteroa incana	Austria, Krems	BPI 184200	1987	DQ418495	DQ418508
	Biscutella laevigata	Switzerland, Canton de Valais	BPI 184686	1903	DQ418494	DQ418506
	Brassica juncea	Korea, Namyangju	SMK 15570	1998	AY929826	AY927046
	Capsella bursa-pastoris	-	-	-	-	AY286229
	C. bursa-pastoris	Argentina, Ushuaia	BPI 796115	1969	-	DQ643923
	C. bursa-pastoris	Australia, New South Wales	BPI 199978	1983	DQ643906	DQ643924
	C. bursa-pastoris	Australia, New South Wales	PREM 48795		-	DQ643925
	C. bursa-pastoris	Canada, Vineland	BPI 184485	1929	DQ643907	DQ643926
	C. bursa-pastoris	England, Kings Lanagley Herts	BPI 184477	1953	-	DQ643927
	C. bursa-pastoris	Finland, Monnonen	BPI 184490	1936	DQ643908	DQ643928
	C. bursa-pastoris	Finland, Helsinki	BPI 796116	1966	DQ643909	DQ643929
	C. bursa-pastoris	Germany	BPI 184368	1928	-	DQ643930
	C. bursa-pastoris	Germany, Munich	BPI 184789	1950	-	DQ643931
	C. bursa-pastoris	Germany, Munich	BPI 184796	1950	-	DQ643932
	C. bursa-pastoris	Germany, Hersbruck	BPI 184801	1947	-	DQ643933
	C. bursa-pastoris	Germany	BPI 790098	1948	DQ643910	DQ643934
	C. bursa-pastoris	Germany, Hersbruck	BPI 790100	1946	-	DQ643935
	C. bursa-pastoris	Hungary, Posonii	BPI 184802	1890	-	DQ643936
	C. bursa-pastoris	India, Ladoga	BPI 184783	1908	-	DQ643937
	C. bursa-pastoris	Ireland, Dublin	BPI 184476	1952	DQ643911	DQ643938
	C. bursa-pastoris	Ireland, Tipperary	BPI 184478	1935	-	DQ643939
	C. bursa-pastoris	Korea, Seoul	BPI 871286 (=SMK 15670)	1999	AY929830	AY927049/ EF655652 [a]
	C. bursa-pastoris	Korea, Wonju	BPI 871288 (=SMK 21090)	2005	DQ643912	DQ643940

Table 1 Continued. Summary of information about *Albuginaceae* specimens used in this study

Species	Host	Geographical origin	Herbarium number	Year	GenBank	
					ITS	COX2
A. candida	*C. bursa-pastoris*	Korea, Yongin	BPI 871287 (=SMK 17254)	2000	AY929831	AY927050
	C. bursa-pastoris	Korea, Chunchon	BPI 871289 (=SMK 21128)	2005	DQ643913	DQ643941
	C. bursa-pastoris	Korea, Namyangju	SMK 13752	1997	AY929829	AY927048/ EF655653 [a]
	C. bursa-pastoris	Korea, Chunchon	SMK 15802	1999	DQ643914	DQ643942
	C. bursa-pastoris	Latvia, Riga	BPI 184493	1936	DQ643915	DQ643943
	C. bursa-pastoris	Netherlands, Zuid-Holland	BPI 184451	1958	DQ643916	DQ643944
	C. bursa-pastoris	Palestine, Jerusalem	BPI 184491	1935	DQ643917	DQ643945
	C. bursa-pastoris	Romania, Transylvania	BPI 184429	1923	-	DQ643946
	C. bursa-pastoris	Russia	- [b]	2004	DQ643918	DQ643947
	C. bursa-pastoris	Sweden	BPI 184357	1927	-	DQ643948
	C. bursa-pastoris	Switzerland, Canton de Vaud	BPI 184460	1917	-	DQ643949
	C. bursa-pastoris	USA, New York	BPI 184430	1888	-	DQ643950
	C. bursa-pastoris	USA, Wisconsin	BPI 184457	1947	-	DQ643951
	C. bursa-pastoris	USA, Washington	BPI 184791	1915	DQ643919	DQ643952
	C. bursa-pastoris	USA, Virginia	BPI 184793	1935	-	DQ643953
	C. bursa-pastoris	USA, Tennessee	BPI 184795	1936	-	DQ643954
	C. bursa-pastoris	USA, Tennessee	BPI 184798	1934	-	DQ643955
	Cardaminopsis halleri subsp. *ovirensis*	Romania, Suceava	BPI 199991	1980	DQ418502	DQ418513
	Descurainia sophia	URSS	SOMF 19655	1977	AY929832	AY927051
	Diplotaxis erucoides	Palestine, Kiriat-Anabim	BPI 184862	1935	DQ418496	DQ418517
	Diptychocarpus strictus	URSS	SOMF 19659	1978	AY929833	AY927052
	Draba nemorosa	Korea, Gapyong	SMK15732	1999	AY929834	AY927053
	Eruca sativa	Pakistan, Daudkhel	BPI 184870	1968	DQ418503	DQ418514

Table 1 Continued. Summary of information about *Albuginaceae* specimens used in this study

Species	Host	Geographical origin	Herbarium number	Year	GenBank	
					ITS	COX2
A. candida	*Erysimum cuspidatum*	Romania, Mehedinti	BPI 199988	1979	DQ418498	DQ418519
	Heliophila meyeri	S. Africa, Vanrhynsdorp	BPI 184888	1896	DQ418493	DQ418515
	Iberis amara	USA, California	BPI 184897	1938	DQ418499	DQ418522
	Lepidium campestre	Korea, Seoul	SMK 13747	1997	AY929835	AY927054 EF655651 [a]
	L. virginicum	Korea, Seoul	SMK 17251	2000	AY929838	AY927057/ EF655650 [a]
	Lunaria sp.	USA, Oregon	CUP 065639	2000	AY929840	AY913797
	Raphanus sativus	Korea, Seoul	SMK 10614	1990	AY929841	AY927059
	Sisymbrium luteum	Korea, Pyeongchang	SMK 19086	2002	AY929844	AY913808
	Thlaspi arvense	USA, New York	CUP 065777	2002	AY929847	AY913809
A. candida (as *Uredo alpina*)	*Arabis alpina*	Norh West Europe	L 910262-933	-	-	-
A. candida (as *U. armoraciae*)	*Armoracia rusticana* (as '*Amoricana rusticana*')	North West Europe	L 910264-939	-	-	-
A. candida (as *U. candida* var. *alyssi*)	*Alyssum* sp.	North West Europe	L 910263-159	-	-	-
A. candida (as *U. candida* var. *alyssi*)	*Alyssum* sp.	North West Europe	L 910263-181	-	-	-
A. candida (as *U. candida*)	*C. bursa-pastoris*, *Arabis alpina*	North West Europe	L 910263-177	-	-	-
A. candida (as *U. candida*)	*Raphanus raphanistrum*	North West Europe	L 910263-176	-	-	-
A. candida (as *U. candida*)	*Raphanus raphanistrum*	North West Europe	L 910263-155	-	-	-
A. candida (as *U. candida*)	*Sinapis arvensis*	North West Europe	L 910263-184	-	-	-
A. candida (as *U. candida*)	*Sinapis arvensis*	North West Europe	L 910263-188	-	-	-

Table 1 Continued. Summary of information about *Albuginaceae* specimens used in this study

Species	Host	Geographical origin	Herbarium number	Year	GenBank	
					ITS	COX2
A. candida (as *U. cruciferarum*)	*Cardamine* sp.	North West Europe	L 910264-384	-	-	-
A. candida (as *U. lactea* var. *cheiranthi incani*)	*Cheiranthus incanus*	North West Europe	L 910263-325	-	-	-
A. candida (as *U. thlaspeos bursae pastoris*)	*C. bursa-pastoris*	North West Europe	L 910264-213 **Lectotypus**	-	-	-
A. capparis	*Capparis rupestris*	Italy	BR 75128-51	1887	-	EF655654
A. chardonii	*Cleome anomala*[T]	Colombia	CUP 000668	1929	-	AY913799
A. ipomoeae-panduratae	*Ipomoea hederacea*	Korea, Yangpyong	SMK 19628	2003	DQ643920	AY913804
A. occidentalis	*Spinacia oleracea*	USA, Texas	-	-	-	AY286220
A. trianthemae	*Lampranthus* sp. cult.	Australia, South Australia	DAR 72457	1996	DQ643922	AY913800
Pustula tragopogonis	*Helianthus annuus*	South Africa	-	-	-	AY286221
P. tragopogonis (as *Uredo alba*)	fragment of an *Asteraceae*	North West Europe	L 910264-939	--	--	--
P. tragopogonis (as *U. candida* var. *tragopogi*)	*Tragopogon* sp.	North West Europe	L 910263-156	-	-	-
P. tragopogonis (as *U. candida* var. *tragopogi*)	*Tragopogon* sp.	North West Europe	L 910263-157	-	-	-
Wilsoniana achyranthis	*Achyranthes japonica*	Korea, Namyangju	SMK 19955	2003	DQ643905	AY913807
W. bliti	*Amaranthus spinosus*	Korea, Chunchon	SMK 19835	2003	AY929824	AY913805
W. portulacae	*Portulaca oleracea*	Korea, Jeju	SMK 18991	2002	DQ643921	AY913806

[a] Accession number of GenBank Database for 28S partial sequences.
[b] Leg. & det. by V.A. Mel'nik (Komarov Institute of Botany, Russian Academy of Sciences, Russia).

reversible model (GTR), with gamma-distributed substitution rates for COX2 alignment and without gamma-distributed substitution rates for ITS and 28S alignments, was respectively selected using Modeltest 3.06 (Posada and Crandall, 1998) and PAUP* version 4b10 (Swofford, 2002). Four incrementally heated simultaneous Markov chains were run for one-million generation, saving a tree every 100th generation. Among these, the first 1000 trees were discarded. MRBAYES was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities of the groups. Branch lengths were computed as the mean values over the sampled trees. To test the reproducibility of results, the analysis was repeated four times, starting with random trees and default parameter values. The complete alignment together with the trees obtained has been deposited in TreeBase (http://www.treebase.org/) as SN3415.

Results

Morphological analysis

Thirty-six morphologically similar specimens of *A. candida* from *C. bursa-pastoris* were collected in Korea or borrowed from international herbaria. In Korean specimens, the sporangiophores were cylindrical or clavate (Figs 1A, B), and the sporangia were arranged in a basipetal chain, hyaline, globose or subglobose, with equal thin wall, base rounded or subtruncate (Figs 1C, D). Haustoria were globose to knob-like (Fig. 1E). Oogonia were broadly globose or irregular, and oospores were verrucose or tuberculate without blunt ridges, with warts which were neither confluent nor branched (Figs 1F, G).

Based on characteristics of sporangiophores and sporangia, the Korean specimens were morphologically similar to those from other countries. However, they could be easily distinguished on oospore characteristics. The surface of oospores in Korean specimens were clearly verruculate or tuberculate without ridges (Figs 1F, G), whereas those from other countries were tuberculate with ridges that are often confluent or branched (Figs 1H, I). The oospore ornamentation of *Albugo* specimens from *Draba* and *Lepidium* was exhibiting some similarity compared to Korean materials from *Capsella*, while those from *Eruca* and *Heliophila* were identical to those of *A. candida* on *C. bursa-pastoris* from all countries, except for Korea (Figs 1J-M). The oospores from *Eruca* are mostly observed in the leaves of the host plants, while those from *Heliophila* are usually confined to the stems and fruits, and are rarely found in the leaves.

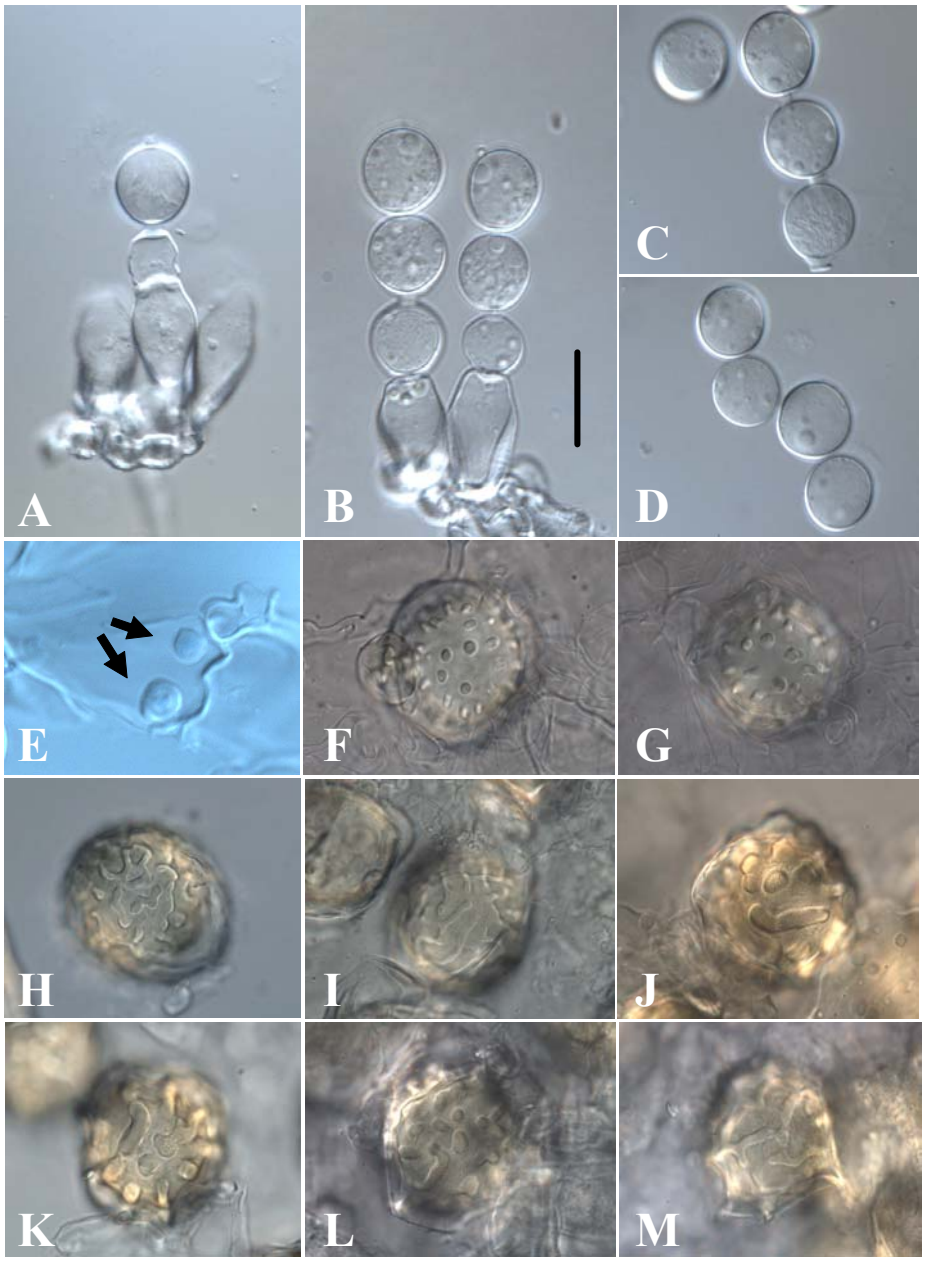


Fig. 1. *Albugo koreana* (A-G) and *A. candida* (H-M). **A-B.** Sporangiophores. **C-D.** Sporangia. **E.** Haustoria mounted in cotton blue in lactic acid. **F-G.** Oospores of *A. koreana* on *Capsella bursa-pastoris* in Korea. **H-I.** Oospores of *A. candida* on *C. bursa-pastoris* in other countries. **J-K.** Oospores of *A. candida* on *Eruca*. **L-M.** Oospores of *A. candida* on *Heliophila*. Bars: A-D: 20 μm; E: 10 μm; F-M: 30 μm.

Phylogenetic analysis

ITS of *A. candida* specimens on *C. bursa-pastoris* was of two different sizes; materials from Korea were 845 bp in length, while those from other countries ranged from 835 to 840 bp. No indels were found in COX2 mtDNA sequences. The phylogenetic relationship between *Albugo* specimens on *C. bursa-pastoris* from Korea and other countries was inferred from the Bayesian (MCMC) analysis of the aligned sequences of the COX2 mtDNA, ITS rDNA, and 28S rDNA. In all alignments all analyses showed the same tree topology, and almost identical posterior probability values. After running one-million generations, a 50% majority rule consensus tree was constructed from all the trees (excluding the first 1000) as shown in Fig. 2 for COX2 mtDNA, Fig. 3 for ITS rDNA, and Fig. 4 for 28S rDNA.

Albugo candida materials from the *Brassicaceae* formed a well-supported group with high posterior probability (100% in COX2, ITS, and 28S sequences). All trees identified three distinct clades within *A. candida s.l.* The first clade (group 1 in Figs 2-4) contains *A. candida* from *Capsella bursa-pastoris* worldwide, except for Korea, and from several other genera and countries and exhibited only little nucleotide differences. This clade was supported by high posterior probability values, ranging from 83% (ITS) to 100% (COX2 and LSU), in all phylogenetic reconstructions. The second clade (group 2 in Figs 2-4) includes *A. lepidii* and several other distinct lineages. These include *A. candida s.l.* from *Descuriana*, *Diptychocarpus* and *Draba* in the COX2 and ITS datasets and *Sisymbrium*, *Erysimum*, *Cardamine*, *Cleome* and *Arabidopsis*. The whole clade is strongly supported in ITS and LSU, but only weakly supported in the COX2 phylogenetic reconstruction. The third clade (group 3 in Figs 2-4) includes the *Albugo* specimens from *Capsella* found in Korea. Korean materials were grouped with posterior probability of 100% in COX2, ITS, and 28S analyses. Groups 2 and 3 were placed sister to each other with 100% and 96% posterior probability in ITS and LSU analyses, respectively. In the COX2 analysis, significant support for this arrangement is lacking. Nucleotide distances between two groups infective to *Capsella* were 7.25% in the COX2 mtDNA, 17.4% in ITS rDNA, and 4.8% in 28S rDNA sequences.

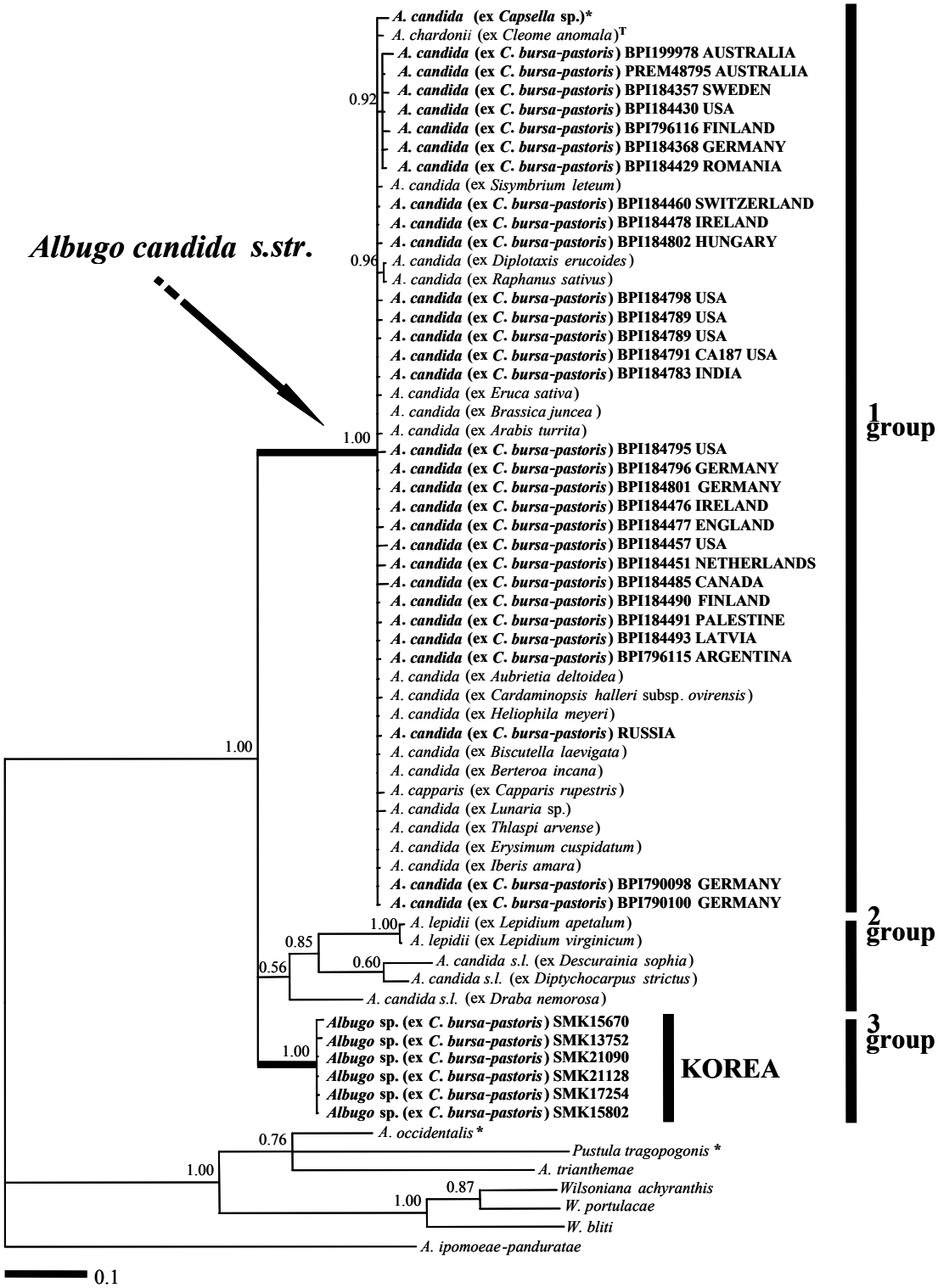
Discussion

The sequence analysis of *A. candida s.l.*, based on the COX2 mtDNA, ITS rDNA and 28S rDNA genes, was used to elucidate the phylogenetic structure within this complex. Although *A. candida* is commonly regarded as the sole species causing white rusts on various plants of the *Brassicaceae*,

including *Capsella*, this study showed that *A. candida* from *Capsella* was clearly divided into two clades and that several distinct lineages are present in *A. candida s.l.* The phylogenetic partition between Korean and other continental specimens indicated by two nuclear and one mitochondrial locus suggests that there is a reproductive barrier between these groups, meeting the criteria of GCPSR (genealogical concordance phylogenetic species recognition, Taylor *et al*., 2000). Therefore, Korean specimens warrant species rank, based on morphological and molecular data. White rust pathogens from various brassicaceous plants, including *Capsella* of other continental regions, formed a well-supported group in analysis of both COX2, ITS and 28S rDNA. The present study revealed that the majority specimens from the *Brassicaceae* are positioned within this large group (group 1 in Figs 2-4).

In a study of Voglmayr and Riethmüller (2006), two clades are apparent in *A. candida s.l*., which are both parasitic to a variety of *Brassicaceae*, and are supported with high bootstrap values. To this dataset, *Albugo* specimens from *Capsella* in Korea were added, so several interesting points can be addressed. Usually *Albugo* specimens from the same host genus are grouped closely together, with the exception of *Capsella* in the present study and that of Choi *et al*. (2006) and *Sisymbrium* in the dataset of Voglmayr and Riethmüller (2006). In the corresponding phylogenetic reconstruction, *Albugo* from *Sisymbrium loeselii* is identical in sequence with *Albugo* from *Erysimum cheiranthoides*. This might possibly be explainable by a misidentification of the host, which is not highly unlikely, as *E. cheiranthoides* and *S. loeselii* are quite similar in appearance. In the dataset of Voglmayr and Riethmüller (2006), *Albugo* specimens from *Lunaria*, *Capsella*, *Raphanus* and *Berteroa* are grouped closely together, which is in accordance with the study of Choi *et al*. (2006), who also found the Korean specimens from *Brassica* and *Raphanus*, who have been reported to host *A. macrospora*, nested in the large, homogenous group of *A. candida s.str*. It is notable that the specimen from *Eutrema* originating from Taiwan, included in the first study, is also placed within this group. From this host species *A. wasabiae* has been described from Japan, a species ignored by subsequent authors. The specimens from *Arabidopsis* are placed in the more heterogenous, smaller group in Voglmayr and Riethmüller (2006) and in a similar distinct, heterogenous group in Choi *et al*. (2006), in the latter study,

Fig. 2. Phylogenetic tree for *Albuginaceae* species from various hosts based on the partial COX2 mtDNA. Bayesian analysis showing mean branch lengths of a 50% majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. Numbers above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in bold. An asterisk (*) shows taxa obtained from GenBank.


Albugo candida s.str.
A. candida (ex Capsella sp.)*
A. chardonii (ex Cleome anomala)T
A. candida (ex C. bursa-pastoris) BPI199978 AUSTRALIA
A. candida (ex C. bursa-pastoris) PREM48795 AUSTRALIA
A. candida (ex C. bursa-pastoris) BPI184357 SWEDEN
A. candida (ex C. bursa-pastoris) BPI184430 USA
A. candida (ex C. bursa-pastoris) BPI796116 FINLAND
A. candida (ex C. bursa-pastoris) BPI184368 GERMANY
A. candida (ex C. bursa-pastoris) BPI184429 ROMANIA
A. candida (ex Sisymbrium leteum)
A. candida (ex C. bursa-pastoris) BPI184460 SWITZERLAND
A. candida (ex C. bursa-pastoris) BPI184478 IRELAND
A. candida (ex C. bursa-pastoris) BPI184802 HUNGARY
A. candida (ex Diplotaxis erucoides)
A. candida (ex Raphanus sativus)
A. candida (ex C. bursa-pastoris) BPI184798 USA
A. candida (ex C. bursa-pastoris) BPI184789 USA
A. candida (ex C. bursa-pastoris) BPI184789 USA
A. candida (ex C. bursa-pastoris) BPI184791 CA187 USA
A. candida (ex C. bursa-pastoris) BPI184783 INDIA
A. candida (ex Eruca sativa)
A. candida (ex Brassica juncea)
A. candida (ex Arabis turrita)
A. candida (ex C. bursa-pastoris) BPI184795 USA
A. candida (ex C. bursa-pastoris) BPI184796 GERMANY
A. candida (ex C. bursa-pastoris) BPI184801 GERMANY
A. candida (ex C. bursa-pastoris) BPI184476 IRELAND
A. candida (ex C. bursa-pastoris) BPI184477 ENGLAND
A. candida (ex C. bursa-pastoris) BPI184457 USA
A. candida (ex C. bursa-pastoris) BPI184451 NETHERLANDS
A. candida (ex C. bursa-pastoris) BPI184485 CANADA
A. candida (ex C. bursa-pastoris) BPI184490 FINLAND
A. candida (ex C. bursa-pastoris) BPI184491 PALESTINE
A. candida (ex C. bursa-pastoris) BPI184493 LATVIA
A. candida (ex C. bursa-pastoris) BPI796115 ARGENTINA
A. candida (ex Aubrietia deltoidea)
A. candida (ex Cardaminopsis halleri subsp. ovirensis)
A. candida (ex Heliophila meyeri)
A. candida (ex C. bursa-pastoris) RUSSIA
A. candida (ex Biscutella laevigata)
A. candida (ex Berteroa incana)
A. capparis (ex Capparis rupestris)
A. candida (ex Lunaria sp.)
A. candida (ex Thlaspi arvense)
A. candida (ex Erysimum cuspidatum)
A. candida (ex Iberis amara)
A. candida (ex C. bursa-pastoris) BPI790098 GERMANY
A. candida (ex C. bursa-pastoris) BPI790100 GERMANY
A. lepidii (ex Lepidium apetalum)
A. lepidii (ex Lepidium virginicum)
A. candida s.l. (ex Descurainia sophia)
A. candida s.l. (ex Diptychocarpus strictus)
A. candida s.l. (ex Draba nemorosa)
Albugo sp. (ex C. bursa-pastoris) SMK15670
Albugo sp. (ex C. bursa-pastoris) SMK13752
Albugo sp. (ex C. bursa-pastoris) SMK21090
Albugo sp. (ex C. bursa-pastoris) SMK21128
Albugo sp. (ex C. bursa-pastoris) SMK17254
Albugo sp. (ex C. bursa-pastoris) SMK15802
KOREA
A. occidentalis *
Pustula tragopogonis *
A. trianthemae
Wilsoniana achyranthis
W. portulacae
W. bliti
A. ipomoeae-panduratae
0.92
0.96
1.00
1.00
1.00
0.85
0.56
0.60
1.00
0.76
1.00
0.87
1.00
1 group
2 group
3 group
0.1

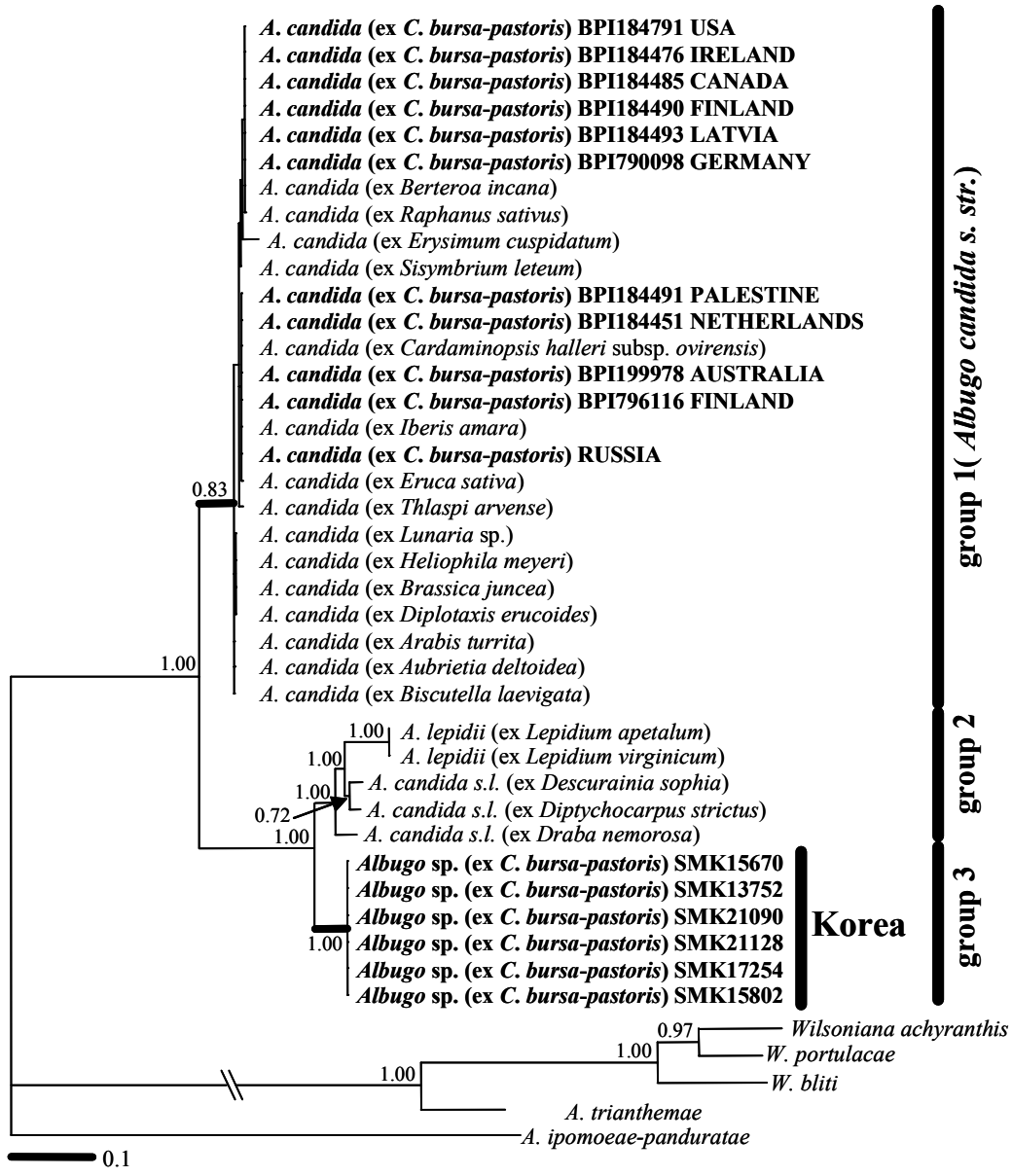


Fig. 3. Phylogenetic tree for *Albuginaceae* species from various hosts based on the complete ITS nrDNA. Bayesian analysis showing mean branch lengths of a 50% majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. Numbers above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in bold.

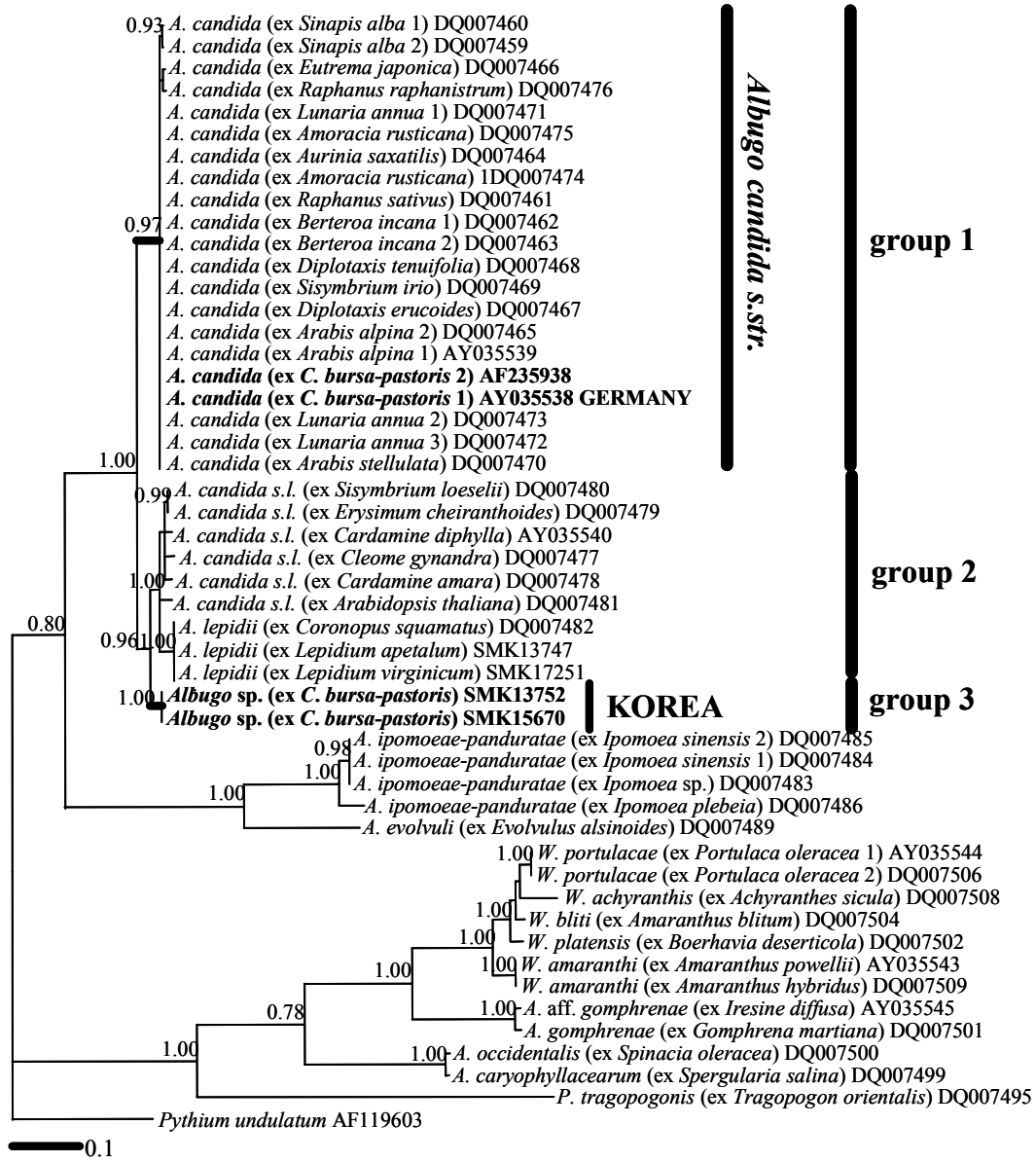


Fig. 4. Phylogenetic tree for *Albuginaceae* species from various hosts based on the partial 28S nrDNA sequences alignment of Voglmayr and Riethmüller (2006). Bayesian analysis showing mean branch lengths of a 50% majority-rule consensus tree calculated from trees revealed during MCMC analysis of one-million generations. Numbers above the branches are the posterior probability values. The number of nucleotide changes between taxa is represented by branch length and the scale bar equals the number of nucleotide substitution per site. *Albugo* specimens from *Capsella bursa-pastoris* are in bold.

this clade also contained *Albugo* from *Lepidium*, and *Albugo* from the Korean specimens from *C. bursa-pastoris*, which form a homogenous group. In the reanalysis of the dataset of Voglmayr and Riethmüller (2006), to which two *Albugo* specimens from *Capsella* similar results could be obtained, which show a placement of the Korean specimens from *Capsella* in a third distinct clade (group 3 in Fig. 4), which is placed sister to the clade containing *A. lepidii* and several further distinct lineages. Based on the molecular and morphological data available, we believe that this phylogenetic group is to be regarded as new species, *A. koreana*. It seems also likely that other lineages in *A. candida s.l.* are distinct species. However, a more detailed phylogenetic study, in combination with thorough morphological investigations, need to be conducted before further conclusions can be drawn.

The taxonomy of species within the *Albuginaceae* has been based primarily upon differences in the host range (Kochman and Majewski, 1970; Vanev *et al*., 1993), particularly host families. The size of oospores, sporangiophores and sporangia of the *Albuginaceae* may vary according to host plant and climatic factors (Togashi *et al*., 1931; Makinen and Hietajarvi, 1965). However, ornamentation of the oospore wall remains the critically important morphological characteristic for distinguishing species of *Albugo* (Biga, 1955; Choi and Priest, 1995; Voglmayr and Riethmüller, 2006). The most useful characteristic for distinguishing *A. koreana* from *A. candida s.str.* is the surface ornamentation of the oospores, which are verrucose or tuberculate without ridges in the first and with confluent ridges in the latter species. As described and illustrated by Wilson (1907), oospores in *A. candida s.l.* can be "verrucose, or with low blunt ridges which are often confluent and irregularly branched", judging from his drawings, his statement refers to characters which can be found on the same oospore (Wilson, 1907, Fig. 1). Corresponding to results of the phylogenetic analysis, white rust pathogens that infect *Eruca* and *Heliophila* also had the same oospore ornamentation as *A. candida s.str.* Besides *A. candida s.str.* from *C. bursa-pastoris*, the fungus from *Armoracia rusticana* (Voglmayr and Riethmüller, 2006), *Raphanus sativus* (Wilson, 1907), *Brassica campestris* (Tewari and Skoropad, 1977), and *B. juncea* (Nath *et al*., 2000) have been previously observed to have identical oospore characteristics. Therefore, oospores with low blunt ridges that are often confluent and irregularly branched might be seen as a morphological characteristic of *A. candida s.str.* infecting various brassicaceous plants, including *Capsella* from countries other than Korea, and oospores without blunt ridges as a characteristic of Korean specimens of *Albugo* from *Capsella*. *Albugo* materials from five genera, *Capsella*, *Draba*, *Eruca*, *Heliophila*, and *Lepidium*, from which oospores were successfully observed, were divided into two types on the basis of wall

ornamentation; *Albugo* specimens from *Draba* and *Lepidium* were exhibiting some similarity compared to *A. koreana*, while those from *Eruca* and *Heliophila* were identical to those of *A. candida s.str.* Therefore, the three molecular groups of *A. candida s.l.* found by Choi *et al.* (2006) and in the amended dataset of Voglmayr and Riethmüller (2006) as well as ITS and COX2 molecular phylogenetic reconstructions in this study, may be morphologically also differentiated by oospore characteristics, with groups 2 and 3, which are grouped with high support in LSU and ITS trees, exhibiting similar oospore ornamentation. Group 1 most likely contains a single species, *A. candida s.str.*, group 2 comprises several species, amongst which are *A. lepidii* and several other lineages, which might also warrant species rank, and its sister group (group 3) contains *Albugo* specimens from *Capsella* from Korea, which are assigned to the new species *A. koreana* below.

Molecular data in this study and Choi *et al.* (2006) revealed that *Lepidium* white rusts are differentiated from *A. candida s.str.* and *Albugo* from *Capsella* in Korea. This result supports the conclusion of Rao (1979), who described *Albugo* specimens from *Lepidium* as *A. lepidii* A. N. S. Rao. The presence of several species on the same host genus, based on molecular and morphological properties, was also shown by Voglmayr and Riethmüller (2006), who found that two distinct species were parasitic to *Amaranthus*, based on nrLSU-DNA sequences and oospore ornamentation. As this species is clearly embedded within *Wilsoniana* Thines, both morphologically and based on molecular phylogeny, as also shown by Voglmayr and Riethmüller (2006) and a reinvestigation of the dataset in the present study, *A. amaranthi* (Schwein.) Kuntze is combined into that genus below, to avoid nomenclatural confusion. *A. gomphrenae*, which is most likely also a member of that genus, as molecular data suggests (Voglmayr and Riethmüller, 2006), is not transferred to *Wilsoniana* in this study, because no specimen was obtained to critically study the morphology of this species.

Taxonomy

Species concept in *Albugo* parasitic to *Brassicaceae* s.l.

Traditionally, species in the *Albuginaceae* were thought to be host family specific, especially in the *Brassicaceae* and *Asteraceae*. This view is, however, asserted by recent molecular phylogenetic studies (Thines and Spring, 2005; Voglmayr and Riethmüller, 2006; Choi *et al.*, 2006), which revealed that several distinct lineages are present on the same host family and that at least some species are possibly host genus specific.

Several species of *Albugo* parasitic to *Brassicaceae* have been described, but are mostly treated as synonyms of *A. candida*, amongst which the most widely known binominals are *U. cruciferarum* DC., *Cystopus sphaericus* Bonord., *Caeoma candidum* Schltdl., *A. wasabiae* Hara, *A. macrospora* (Togashi) S. Ito, *A. lepidii* N.A.S. Rao, *U. cheiranthi* Pers. and *U. thlaspi* Sowerby. Listing all binominals, including combinations and varieties would fill a manuscript of its own. This is due to the fact that *Albugo candida s.l.* is parasitic to a wide range of *Brassicaceae* and that white rust incidence in very common on some of the most widely distributed *Brassicaceae*. As already Fischer (1892), after listing a few synonyms, mentioned, there are numerous synonyms of this "*vielbenannten Pilzes*" [fungus named many times]. Also in the brassicaceous hosts formerly assigned to the *Capparaceae*, two species of *Albugo* have been described. The type material of one of these, *A. chardonii* W. Weston, is indistinguishable from *A. candida* in molecular phylogenetic reconstructions and therefore needs also to be considered a synonym of or at least a species closely related to *A. candida s.str*. The slight wall thickening at the base and sides given in the key of Choi and Priest (1995) is a feature present in some sporangia of almost every collection of *A. candida* (Thines and Spring, 2005). The other species described in *Capparaceae* is *A. capparis* (de Bary) Kuntze, which has been doubted to be a distinct species almost since its description. De Bary (1863), who formally described the species, which was given as a variety of *A. candida* by Rabenhorst (1844), noted this species to be "*in speciminibus C. candida omnino similia*". Later also Pirotta (1884, as cited in Fischer, 1892) was not able to distinguish between *A. candida* and *A. capparis*. This finding was also confirmed in Saccardo's Sylloge Fungorum (Berlese and DeToni, 1888), who stated this species to be "*A C. candida non v. vix distinguendus*". Biga (1955) included this species in his key, which is starting at the host family level, but the features given by him are identical to *A. candida* var. *macrospora*, comparing both sporangia and oospore features. Therefore, it is interesting that Choi and Priest (1995), who unfortunately did not mention any material they might have studied, cited the two references mentioned above (Biga, 1955; Saccardo, 1888) to give the oospore ornamentation as "tuberculate", a feature that would indicate that this species might be related to *A. lepidii* and also not to be included in *A. candida s.str*. Specimens of *Albugo* from *Capparaceae* sequenced so far are either placed within *A. candida s.str*. or in a sister clade to *A. lepidii*, amongst other specimens from *Brassicaceae* (Figs 2, 4). Whether this clade contains several distinct species, which could include *A. capparis* and *Uredo cheiranthi*, or not, has to be elucidated by thorough investigation of Rabenhorst's material. *Albugo* from *Capsella* in Korea is placed sister to the clade comprising *A. lepidii* and

several other distinct lineages, indicating that describing a new species for the pathogen on *Capsella* from Korea, which is distinct from the *Capsella* infecting *Albugo candida s.str*., is highly warranted.

Lectotypification of *Albugo candida*

Albugo candida has first been described as an independent species by Persoon in Gmelin (1792) as *Aecidium candidum* Pers. Later, several species of *Aecidium* where transferred to *Uredo* to which numerous species were added by Persoon (1796, 1797, 1800). However, *Aecidium candidum* is first mentioned again in 1801 in the *Synopsis Fungorum* (Persoon, 1801), where it is transferred to *Uredo* as *Uredo candida* (Pers.) Pers. It is noteworthy that an elusion is made to an earlier publication (Persoon, 1796), where *Botrytis parasitica* [*Hyaloperonospora parasitica* (Pers.) Constant.] is described to occur on *C. bursa-pastoris* also exhibiting symptoms of infection with *A. candida*. In the *Synopsis Fungorum*, Persoon (1801) gives three varieties of the species. The first variety, *Uredo candida* α *thlaspeos*, the third variety, *U. candida* γ *alyssi* and also a separate species, *U. cheiranthi*, are undoubtedly members of the *A. candida* complex. The second variety, *U. candida* β *tragopogi* is now placed in *Pustula* [*Pustula tragopogonis* (Pers.) Thines]. In 1806, Roussel described *Albugo* as genus separate from *Uredo* (Roussel, 1806) and combined *Uredo candida* (Pers.) Pers. into this genus, also adopting the broad host range given by Persoon (1801) for this pathogen. Later, Kuntze (1891) listed the *Albugo* species known to him; amongst them were *A. candida* and *A. tragopogonis*. Kuntze (1891) typified the genus *Albugo* with *A. candida*, based on *U. candida*, and gave, among others, *A. cruciferarum* (DC.) Gray as a synonym, which, in the sense of Gray (1821) and the authors of the basionym, *U. cruciferarum* DC. (Lamarck and Candolle, 1805), is confined to hosts in the *Brassicaceae*.

To avoid nomenclatural confusion, *Ae. candidum* Pers. needs to be typified with a specimen that agrees with the original description, stabilises the current taxonomy and makes further name-changes in *A. candida s.str*. unnecessary. In the National Herbarium of the Netherlands, section Leiden (L), Persoon's herbarium is deposited. However, no specimen filed under *Ae. candidum* without giving a variety is preserved. This is most likely due to later re-labeling of the specimens. Several specimens of *U. candida* are preserved, but no collection details are given, which makes it impossible to determine with certainty, which specimen is the oldest or was considered to be typical for *Ae. candidum* by Persoon. In addition to the specimens filed under *U. candida*, several other specimens of white rust on various *Brassicaceae* are preserved. Among these are one labeled *U. lactea* var. *cheiranthi incani* on *Cheiranthus*

incanus (L 910263-325), with a second label as *Ae*. *candidum* β *cheiranthi*, possibly the specimen upon which Persoon has described *U*. *cheiranthi* Pers.; *U*. *thlaspeos bursae pastoris* (L 910264-213), which is possibly the specimen on which *U*. *candida* γ *thlaspeos* Pers. was based, and a specimen labeled *U*. *amoraciae* (L 910264-938) on *Armoracia rusticana*. In addition, one specimen labeled *U*. *candida*, showing white rust on both *Arabis* sp. and *C*. *bursa-pastoris* (L 910263-177) and two specimens labeled *U*. *candida* γ *alyssi* (L 910263-159, L 910263-161), as well as two labeled *U*. *candida* β *tragopogi* (L 910263-156, L 910263-157 – the first one being a doublet of the second one) are preserved.

The type specimen for *Ae*. *candidum* is chosen among the two exsiccati which contain the most widely known and distributed host-pathogen association, i.e. white blister rust on *C*. *bursa-pastoris*. As a definite solution, the specimen L 910264-213, *lectotypus hic designatus*, containing only *Capsella* as host plant is chosen as lectotype for *Ae*. *candidum* Pers., which is now known as *A*. *candida* (Pers.) Roussel.

In the material allowed to take for oospore investigations in the National Herbarium of the Netherlands, only two immature oospores of about 50 µm in diameter were found. Therefore, although oospore ornamentation was found to be similar to recent collections from *Capsella* in Europe, no explicit statements can be made about the oospore ornamentation in this specimen. However, the size of the oospores renders it highly likely that the species on the specimen examined is indeed *A*. *candida* and not the species now discovered in *C*. *bursa-pastoris* from Korea.

Albugo koreana Y.J. Choi, Thines & H.D. Shin, **sp. nov.** (Fig. 1)
MycoBank: 510868.

Etymology: 'koreana' refers to the country in which the fungus was first collected.

Chromista, *Albuginales*. *Mycelia* intercellularia, haustoria intracellularia, vesicularia. *Sorus* hypophyllus, distinctus, rotundibus vel irregularibus, saepe confluentibus, albus vel raro flavus, 0.5-5 mm diam. *Sporangiophora* hyalina, clavata vel cylindracea, 20-40 × 10-15(-18) µm. *Sporangia* hyalina, globosa vel subglobosa, (13.5-)15-22 × 12.5-17(-19) µm, parietibus aequalibus. *Oogonia* in folia, globosa vel irregulares, flavida, 37-51(-63) µm diam. *Oospora* luteola vel brunnea, globosa, verruculosa vel tuberculata, 31-46 µm diam., verrucis singularis, haud ramosis, 2-5 × 3-4 µm.

Mycelium intercellular with small globose to knob-like haustoria with short stalk, one to several in each host cell. *Sori* hypophyllous, distinct, rounded or irregular, often confluent, 0.5-5 mm diam., white or rarely pale yellow, covering mostly large areas of the lower and rarely upper side of the leaves, stems and inflorescences. *Sporangiophores* hyaline, clavate or cylindrical, straight to slightly curved, 20-40 × 10-15(-18) µm, mostly grouped or sometimes single, thick-walled, especially towards the base up to 5 µm.

Sporangia arranged in basipetal chains, hyaline, globose or subglobose, with equal thin wall, (13.5-)15-22 × 12.5-17(-19) µm, l/w ratio = 0.93-1.27 (n = 100), tip round, base rounded or subtruncate, verrucose, pedicel mostly absent, often a minute protuberance visible at the point of attachment to the sporangiophores or other sporangia, primary sporangia similar to the secondary sporangia, although the first exhibit a slightly thicker wall. *Oogonia* in leaves, broadly globose or irregular, yellowish to brownish, 37-51(-63) µm diam (n = 100), wall smooth. *Oospores* plerotic, globose, yellowish, 31-46 µm diam (n = 100), wall thick, verruculate or tuberculate without blunt ridges, warts single, not confluent or branched, 2-5 × 3-4 µm.

Typus: Korea, Seoul, Korea University, on leaves of *Capsella bursa-pastoris* affected by white rust disease, 2 April 1999, H.D. Shin (BPI871286, **holotypus**; SMK15670, **isotypus**).

Habitat: On living leaves, stems and fruits of *Capsella bursa-pastoris*.

Known distribution: Korea.

Other materials examined: KOREA, Namyangju, Deokso, 4 May 1997, H.D. Shin (SMK13752); KOREA, Chunchon, Gangwon-do Forest Experiment Station, 14 May 1999, H.D. Shin (SMK15802); KOREA, Yongin, Mt. Kwanak, 28 April 2000, H.D. Shin (BPI871287; SMK17254); KOREA, Hongchon, Experimental Forest of Kangwon National University, 21 May 2005, H.D. Shin and Y.J. Choi (BPI871289; SMK21128); KOREA, Wonju, Mt. Chiak, 6 May 2005, H.D. Shin and Y.J. Choi (BPI871288; SMK21090).

Wilsoniana amaranthi (Schwein.) Y.J. Choi, Thines & H.D. Shin, **comb. nov.**

Basionym: *Caeoma amaranthi* Schwein. (Syn. Fung. Amer. Bor.: 292, 1832).

Acknowledgements

The authors are grateful to Dr. E.H.C. McKenzie (Landcare Research, Auckland, New Zealand) for helpful comments and suggestions, to Drs. Uwe Braun and Hermann Voglmayr for helpful comments regarding nomenclature, to the curators of L (National Herbarium of the Netherlands, Leiden Branch), BPI (U.S. National Fungus Collections, Beltsville, Maryland, USA), CUP (Plant Pathology Herbarium, Cornell University, New York, USA), DAR (Plant Pathology Herbarium, Orange Agricultural Institute, New South Wales, Australia), PREM (National Fungal Herbarium, Pretoria, South Africa), and SOMF (Mycological Collection Herbarium, Institute of Botany, Sofia, Bulgaria) for providing the *Albugo* specimens, and to the staff at KACC (Korean Agricultural Culture Collection) for their technical assistance in DNA sequencing and phylogenetic analysis. This work was financially supported by a research grant from the Korea Research Foundation (KRF-2003-015-C00611) and the Deutsche Forschungsgemeinschaft (Th 1361/2-1).

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(Received 14 August 2006; accepted 23 August 2007)