Genetic Variation and Phylogeny of *Spongospora subterranea* f.sp. *subterranea* Based on Ribosomal DNA Sequence Analysis

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ABSTRACT

The nuclear rDNA regions of the two internal transcribed spacers (ITS1 and ITS2) and 5.8S rRNA gene from 52 field isolates of *Spongospora subterranea* f.sp. *subterranea* obtained from the British Isles and North America were polymerase chain reaction-amplified, sequenced, and assessed for genetic variation. Two genetically distinct groups (I and II) were identified based on the ITS sequence diversity among the isolates, representing 34.6% and 65.4% of the isolates, respectively. British Isles isolates occurred in groups I and II, whereas North American isolates belonged only to group II. The British Isles groups of *S. subterranea* were associated with particular potato cultivars. The full-length small-subunit rRNA gene of *S. subterranea* was sequenced and analyzed by both neighbor-joining and parsimony methods to clarify the taxonomic position of this pathogen. The results of phylogenetic analysis showed that *S. subterranea* grouped together with other species of plasmodiphorids, and this group clustered with the phylum Cercozoa, an assemblage of filose and reticulose amoebae and phylogenetically related zooflagellates. The recognition of the existence of different genetic groups within *S. subterranea* will be important for the design of plant-breeding programs and in testing for plant resistance.

RESUMEN

Las regiones rADN nuclear de los dos espaciadores transcritos internos (ITS1 e ITS2) y el gen 5.8S rARN de 52 aislamientos de campo de *Spongospora subterranea* f.sp. *subterranea* obtenidos de las Islas Británicas y de Norteamérica fueron amplificadas por reacción en cadena de la polimerasa, secuenciadas y evaluadas para variación genética. Se identificaron dos grupos genéticamente diferentes (I y II) en base de la diversidad de frecuencia ITS entre los aislamientos, lo que representa el 34.6% y el 65.4% de los aislamientos respectivamente. En los aislamientos de las Islas Británicas se encontró los grupos I y II, mientras que los aislamientos de Norteamérica pertenecían sólo al grupo II. Los grupos de *S. subterranea* de las Islas Británicas estuvieron asociados con cultivares especiales de papa. El tamaño completo de la sub-unidad del gen rARN de *S. subterranea* fue secuenciado y analizado por los métodos "neighbor-joining y parsimony," con el objeto de aclarar la posición taxonómica de este patógeno. El resultado del análisis filogenético demostró que *S. subterranea* junto con otras especie de plasmodiophoridos está relacionado con el phylum Cercozoa que incluye un conjunto de amebas filiformes y zooflagelados filogenéticamente relacionados. El reconocimiento de la existencia de diferentes grupos genéticos dentro de *S. subterranea* será importante para el diseño de programas de mejoramiento y pruebas de resistencia de la planta.

INTRODUCTION

Spongospora subterranea (Wallr.) Lagerh f.sp. *subterranea* Tomlinson, a soil-borne obligate biotroph, causes powdery scab in potatoes. Powdery scab seriously reduces tuber

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quality and marketability and is a major concern to potato growers worldwide. In some potato-production regions, *S. subterranea* is also important as the vector of potato mop-top virus (Jones and Harrison 1969), which causes a reduction in growth and internal tuber necroses and has recently been found in the United States (Lambert et al. 2003). Control of powdery scab of potato is difficult because of the persistence of the spore balls in soil or on tubers. There is no effective chemical or cultural treatment and all cultivars are susceptible to some degree (Harrison et al. 1997). Therefore, the development of resistant cultivars is now considered the most economical and efficient method for the control of powdery scab.

Successful breeding and effective deployment of durable plant resistance requires an understanding of pathogen diversity and the ways virulence evolves in pathogen populations. However, little information is available on the existence of physiological races, pathotypes, or genetic variation in populations of *S. subterranea*. Khrobrykh (1938) suggested that viruliferous and non-viruliferous "forms" of *S. subterranea* exist but failed to find any conclusive evidence of specialization. Böning and Wallner (1938) claimed to have found a virulent form of the pathogen in Germany, but there was no evidence that it was particularly adapted to any variety of potato. The possibility of the existence of *S. subterranea* pathotypes was studied more intensively by Würzer (1964) using 17 isolates from America and Europe. He noted that 17 isolates could be divided into two groups according to the diameter of resting spores, but found no conclusive evidence of biological specialization. In a review of powdery scab, Harrison et al. (1997) concluded that the existence of physiological races, or pathotypes, of *S. subterranea* has not yet been demonstrated and that this requires further investigation. The differences in the susceptibility of potato cultivars to powdery scab have been demonstrated in many trials (Christ 2000; Christ and Petrunak 1997; Christ and Weidner 1988; Gans et al. 1987; Sica and Christ 2000a, 2000b), but it is not known whether the level of susceptibility is affected by different populations or pathotypes of *S. subterranea*. Bulman and Marshall (1998) assessed the genetic variation in ribosomal internal transcribed spacer (ITS1 and 2) sequences of Australasian and European *S. subterranea* isolates and noted that no sequence variation was detected between any of the Australasian or European isolates with the exception of one from Inverness (Scotland) which was identical to two Peruvian samples. Considering the worldwide distribution of *S. subterranea*, more samples from different geographic locations are clearly required to assess overall *S. subterranea* variation.

Spongospora subterranea is considered a member of the order Plasmodiophorida (Karling 1968), which are characterized as having cruciform nuclear division, multinucleate plasmodia, biflagellate zoospores and resting spores. Other economically important members of plasmodiophorids include *Plasmodiophora brassicae* Woronin, the cause of club root disease of brassicas; *Polymyxa* species, the vectors of several plant viruses (Adams 1991); and *S. subterranea* (Wallr.) Lagerh f.sp. *nasturtii* Tomlinson, the cause of crook root disease of watercress (Tomlinson 1958). The taxonomic affinities of the Plasmodiophorida have been unclear for a long time, and traditionally mycologists have placed these organisms within fungi (Alexopoulos and Mims 1979; Sparrow 1958), whereas, Barr (1992) and Alexopoulos et al. (1996) considered that the plasmodiophorids may have had their origin in the protozoans. Recently, DNA sequences obtained from small-subunit rRNA gene (SSU rRNA) have been used to predict phylogeny for species in the Plasmodiophorida, but the results were conflicting. Based on the phylogenetic analysis of SSU rDNA sequences of *P. brassicae* and *Polymyxa* spp., Castlebury and Domier (1998), Ward and Adams (1998), and Kühn et al. (2000) reported that the plasmodiophorids are a distinct group and are not closely related to any other eukaryotes. Down et al. (2002) examined the SSU rDNA sequences of *P. brassicae* and *S. subterranea* f.sp. *nasturtii*, and concluded that they are not closely related to a range of protists and fungi. However, Cavalier-Smith and Chao (1997) and Bulman et al. (2001) found that the plasmodiophorids are related to the Rhizopoda based on phylogenetic analyses of SSU rDNA sequences. Since the number of available SSU rDNA sequences from a variety of other eukaryotes has increased quickly during the last few years, further studies on the phylogeny of *S. subterranea* and other species in the Plasmodiophorida using SSU rDNA sequences may therefore contribute new information on its taxonomic position and relationships with other eukaryotes.

Our objective was to investigate genetic variation in isolates of *S. subterranea* from the British Isles and North America using ITS1/2 including 5.8S rDNA sequences and to confirm phylogenetic relationships of *S. subterranea* using SSU rDNA sequences.

TABLE 1—*List of* S. subterranea *isolates and internal transcribed spacer (ITS) DNA sequence types.*

Isolation No.	Potato Cultivar	Geographic origin	Year of isolation	ITS type
1	Saturna	Co. Donegal, Ireland	1996	I
2	Saturna	Co. Donegal, Ireland	1996	I
3	Saturna	Co. Donegal, Ireland	1996	I
4	Saturna	Co. Donegal, Ireland	1997	I
5	Saturna	Co. Donegal, Ireland	1997	I
6	Saturna	Co. Donegal, Ireland	1997	I
7	Kerr's Pink	Co. Donegal, Ireland	1996	II
8	Kerr's Pink	Co. Donegal, Ireland	1996	II
9	Kerr's Pink	Co. Donegal, Ireland	1996	II
10	Kerr's Pink	Co. Donegal, Ireland	1997	II
11	Kerr's Pink	Co. Donegal, Ireland	1997	II
12	Kerr's Pink	Co. Donegal, Ireland	1997	II
13	Cara	Co. Donegal, Ireland	1996	II
14	Cara	Co. Donegal, Ireland	1997	II
15	British Queen	Co. Donegal, Ireland	1997	I
16	Banner	Co. Donegal, Ireland	1997	I
17	Navan	Belfast, Northern Ireland	1997	I
18	Navan	Belfast, Northern Ireland	1997	I
29	Saturna	Aberdeen, Scotland	1997	I
20	Saturna	Aberdeen, Scotland	1997	I
21	Saturna	Aberdeen, Scotland	1997	I
22	Pentland Squire	Aberdeen, Scotland	1997	II
23	Pentland Squire	Aberdeen, Scotland	1997	II
24	Pentland Squire	Aberdeen, Scotland	1997	II
25	Wilja	Aberdeen, Scotland	1997	I
26	Wilja	Aberdeen, Scotland	1997	I
27	Wilja	Aberdeen, Scotland	1997	I
28	Maris Bard	Aberdeen, Scotland	1997	I
29	Maris Bard	Aberdeen, Scotland	1997	I
30	Unknown	Maine, USA	1996	II
31	Unknown	Maine, USA	1996	II
32	Unknown	Maine, USA	1996	II
33	Unknown	Colorado, USA	1997	II
34	Unknown	Colorado, USA	1999	II
35	Unknown	Pennsylvania, USA	1992	II
36	Unknown	Pennsylvania, USA	1993	II
37	Unknown	Pennsylvania, USA	1994	II
38	Unknown	Pennsylvania, USA	1995	II
39	Unknown	Pennsylvania, USA	1996	II
40	Unknown	Pennsylvania, USA	1997	II
41	Unknown	Pennsylvania, USA	2000	II
42	Unknown	Idaho, USA	1996	II
43	Unknown	Idaho, USA	1998	II
44	Unknown	Idaho, USA	1999	II
45	Unknown	Idaho, USA	2000	II
46	Unknown	Colorado, USA	1997	II
47	Unknown	Colorado, USA	1999	II
48	Unknown	New York, USA	1998	II
49	Unknown	New York, USA	2000	II
50	Unknown	California, USA	2000	II
51	Unknown	Washington, USA	2000	II
52	Unknown	New Brunswick, Canada	2002	II

MATERIALS AND METHODS

Sources of S. subterranea

Fifty-two field isolates of *S. subterranea* were obtained from the Republic of Ireland, Northern Ireland, Scotland, Canada, and USA (Table 1) either from different fields or from the same field with samples taken over several years. These locations were chosen because of access to potato production areas with powdery scab and because this work had not been carried out previously in these areas. Each isolate was prepared by the removal of single spore balls from powdery scab lesions of naturally infected potato tubers by the method of Qu et al. (2001).

DNA Extraction

Genomic DNA of *S. subterranea* was extracted from spore balls using the modified method of Möller and Harling (1996). A single spore ball pellet was crushed between a sterile microscope slide and a cover slip that had been coated with Sigmacote (Sigma). Spore rupture was monitored with the aid of a compound microscope. After removal of the cover slip, squashed spore balls or cystosori (resting spores) were washed directly into 1.5-mL microcentrifuge tubes with 10 µL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). This procedure was repeated five times for each collection and extracts were transferred to a 1.5-mL microcentrifuge tube, centrifuged at 10,000 g for 2 min and the supernatant subjected to PCR amplification immediately, or stored at -20 C.

PCR Amplification

PCR amplifications were performed in 50-µL reaction mixtures containing 1 x PCR buffer (Applied Biosystems, Foster City, CA), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 1 unit AmpliTaq polymerase (Applied Biosystems), and 20-30 ng DNA template. PCR amplifications were carried out on a PTC-100-60 programmable Thermal Controller (MJ Research, Waltertown, MA) with an initial cycle at 95 C for 2 min, 55 C for 0.5 min, and 72 C for 1 min, followed by 35 cycles of 95 C for 0.5 min, 55 C for 0.5 min and 72 C for 1 min, and a final cycle of 72 C for 7 min. The universal primers ITS4 and ITS5 (White et al. 1990) were used to amplify ITS regions including 5.8S rDNA of *S. subterranea*. The universal primers A (Medlin et al. 1988) and NS3 (White et al. 1990) based on conserved SSU rDNA sequences and *S. subterranea*-specific primers SsA (5'-CGATCAACCGAATGACAGCG-3') and SsB (5'-CCTTCAACAGACAATCGCACC-3') were used to amplify *S. subterranea* SSU rDNA. Primers SsA and SsB were designed in this study. Amplified PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA).

DNA Sequencing

Both strands of each PCR product were sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA). Primers ITS4 and ITS5 were used for ITS DNA sequencing. Primers A, NS3, NS5-6 (White et al. 1990), SsA, and SsB were used for SSU rDNA sequencing. The DNA sequences were determined by an automated ABI 373 DNA Sequencer (PE Applied Biosystems). Consensus sequences of PCR products were obtained by comparison of complementary strands using SeqMan program in DNAStar (DNAStar Inc., Madison, WI). The sequences have been deposited in the GenBank with the accession numbers AY604171, AY604172, and AY604173.

Sequence Analysis

The SSU rDNA sequences of 61 other organisms representing Eumycota, Protozoa, Stramenopila, Plantae, Animalia, and Archaea, were obtained from the Genbank databases and have the following accession numbers: *Achlya bisexualis* Coker and Couch M32705, *Adiantum raddianum* Presl X78889, *Allogromia* sp. X86093, *Alternaria alternata* (Fr.) Keissl. U05194, *Amphidinium corpulentum* Kofoid and Swezy AF274252, *Amphisorus* sp. AJ404313, *Ascosphaera apis* (Maasen ex Claussen) Olive and Spiltoir M83264, *Assulina muscorum* Greeff AJ418791, *Babesia bigemina* Smith and Kilborne X59605, *Blastocrithidia culicis* Novy et al. L29266, *Boletus satanas* Lenz M94337, *Bryum argenteum* Hedw. U18529, *Bullera unica* Hamam and Nakase D78330, *Cercomonas longicauda* Dujardin AF101052, *Chaunacanthid* sp. AF018158, *Chlamydomonas dysosmos* Moewus U13985, *Chlorarachnion reptans* Geitler X70809, *Chlorella vulgaris* Beij. X13688, *Chromulina chromophila* Stein M87332, *Chytridium confervae* (Wille) Minden M59758, *Collozoum serpentinum* Haeckel AF018162, *Cronartium ribicola* Dietr. M94338, *Cryothecomonas aestivalis* Drebes at al. AF290541, *Crypthecodinium cohnii* Biecheler M64245, *Cyclorbiculina compressa* d'Orbigny AJ404303, *Cylindrotheca closterium* (Her.) Reimer and Lewin M87326, *Dictyostelium discoideum* Raper K02641, *Ditylum brightwellii* (West) Grunow X85386, *Entamoeba dispar* Brumpt Z49256, *Euplotes aediculatus* Pierson M14590, *Fragilaria striatula* Lyngbye X77704, *Fucus distichus* L. M97959, *Glycine max* (L.) Merr. X02623, *Gymnodinium fuscum* (Her.) Stein AF022194, *Gyrodinium impudicum* Fraga and Bravo AF022197, *Haliommatidium* sp. AF018159, *Haloferax alexandrinus* Asker and Ohta AB037474, *Heteromita globosa* Dujardin U42447, *Kluyveromyces lactis* (Boidin et al.) Van der Walt X51830, *Lagenidium giganteum* Couch X54266, *Lepidodinium viride* Watanabe et al. AF022199, *Lotharella vacuolata* AF054890, *Neocallimastix frontalis* (Braune) Vavra and Joyon ex Heath X80341, *Oxytricha nova* Klobutcher et al. M14601, *Padina crassa* Yamada AF350240, *Paramecium tetraurelia* Sonneborn X03772, *Paraphysomonas vestita* (Stokes) de Saedeleer AF109325, *Physarum polycephalum* Schwein. X136160, *Phytophthora megasperma* Drechsler X54265, *Placopecten magellanicus* Gmelin X53899, *Plasmodiophora brassicae* Woronin U18981, *Polymyxa betae* Keskin AF310902, *Polymyxa graminis* Ledingham AF310898, *Sarcocystis muris* Blanchard M64244, *Sphaerozoum punctatum* Müller AF018161, *Spongospara. subterranea* (Wallr.) Lagerh f.sp. *nasturtii* Tomlinson AF25744, *Sorites orbiculus* Forskal AJ404310, *Sorites* sp. AJ404311, *Tetrahymena thermophila* M10932, *Toxoplasma gondii* Nicolle and Manceaux M97703, *Trinema enchelys* Ehrenberg AJ418792 and *Xenopus laevis* Daudin K01373.

DNA sequences were aligned using Clustal W version 1.6 (Thompson et al. 1994) and the alignment was then refined manually. Since archaeans have been previously used as outgroup species in phylogenetic analyses of a large number of highly divergent sequences of eukaryotes (Baldauf and Doolit-

tle 1997), the SSU rDNA sequence of the Archaea *Haloferax alexandrinus* was used as an outgroup in this study. The sequence alignment has been deposited in TreeBASE with the accession numbers S1074 and M1831. Ambiguously aligned sequence regions were excluded from the data matrix before analysis. Phylogenetic analyses were conducted using searches based on distance and maximum parsimony in PAUP version 4.0b10 (Swofford 2002). In the distance method, a neighbor-joining tree was obtained using Kimura's two-parameter distances. The maximum parsimonious trees were inferred with a heuristic search using stepwise addition with 1000 random addition sequences. All nucleotide substitutions were equally weighted and alignment gaps were treated as missing information. The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis from 1000 replications (Felsenstein 1985).

RESULTS

PCR Amplification and Sequencing of S. subterranea *ITS Regions*

DNA was extracted from the spore balls of 52 *S. subterranea* isolates and PCR amplifications were performed using universal primers ITS4 and ITS5. A single product of approximately 580 bp was generated from each isolate. The products from each isolate were then sequenced, and it was shown that *S. subterranea* ITS1, ITS2 regions and 5.8S rDNA consist of 151-156 bp, 154-152 bp and 159 bp, respectively.

S. subterranea *ITS Sequence Analysis*

Multiple alignments of the ITS1 and ITS2 sequences of the 52 *S. subterranea* isolates revealed that there were two distinct ITS types, I and II (Figure 1). The ITS sequence type I dif-

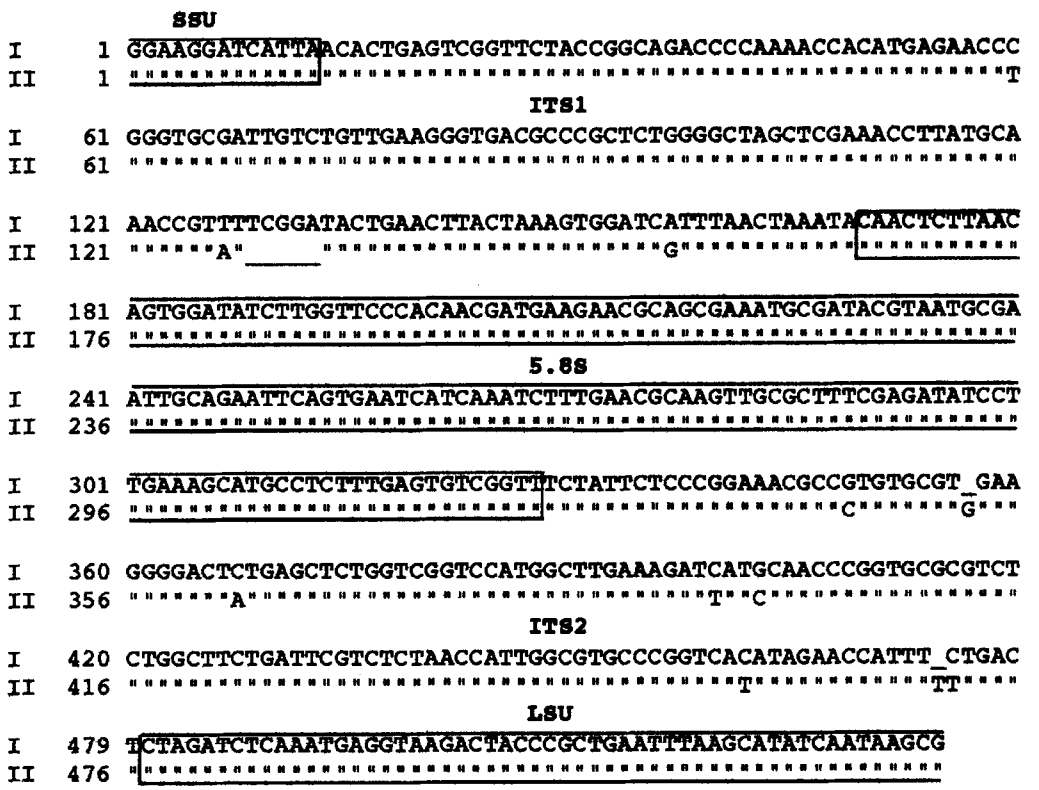

FIGURE 1.
Alignment of two types (I and II) of *S. subterranea* internal transcribed spacer (ITS) and 5.8S rDNA sequences. Double dots represent a base identical to that in the top sequence. A dash represents a gap. Large blocks delineate three rRNA genes: small-subunit (SSU) rDNA, 5.8S rDNA and large-subunit (LSU) rDNA.

fered from type II by having three nucleotide differences (positions 60, 127, and 157) and an insertion of five nucleotides (positions 129-133) in ITS1 regions, and six nucleotide differences (positions 348, 367, 399, 402, 461, and 475) and two positions where single base deletions occurred (positions 356 and 474) in ITS2 regions. A total of 2.9% divergence was found between the two ITS types, and the degree of divergence differed for the ITS1 and ITS2 regions. The degree of divergence within the ITS2 region (3.9%) was double that of the ITS1 region (1.9%). The 5.8S rDNA sequence was identical in all 52 *S. subterranea* isolates.

Of the 16 *S. subterranea* isolates obtained from the Republic of Ireland, eight isolates (50%) had ITS I type sequences and eight isolates (50%) had ITS II type sequences. Of the 11 isolates obtained from Scotland, eight isolates (72.7%) had ITS I type sequences and three isolates (27.3%) had ITS II type sequences. Both isolates obtained from Northern Ireland had ITS I type sequences. All of the 23 North American isolates had ITS II type sequences.

In this investigation, it appeared that the ITS types of British Isles *S. subterranea* isolates were related to the potato cultivars infected. For example, all the *S. subterranea* collections from cv Saturna originating from the Republic of Ireland and Scotland had ITS I type sequences and all the collections from cv Kerr's Pink from the Republic of Ireland had ITS II type sequences. In North America, however, although cultivar is not listed, most samples were from unique cultivars.

PCR Amplification and Sequencing of S. subterranea *SSU rDNA*

The primer pair NS3/SsB was initially used for PCR amplification of *S. subterranea* SSU rDNA. The *S. subterranea*-specific primer SsB was designed from the ITS1 sequence of *S. subterranea*. After PCR amplification of DNA from two *S. subterranea* isolates No. 1 and No. 7 (represented two ITS groups), a single fragment of approximately 1,300 bp was generated from each collection. After the NS3-SsB region was sequenced, another *S. subterranea*-specific primer SsA was designed from this sequence data, and a single fragment of approximately 800 bp was generated following PCR amplification of DNA of the two *S. subterranea* isolates with the primer pair A/SsA. These fragments from each isolate were also sequenced. When the sequences of the two PCR products, *c.* 800 bp and 1,300 bp, from the two *S. subterranea* collections were aligned, it was found that the full *S. subterranea* SSU rDNA region consisted of 1,837 bp, and that the SSU rDNA sequences in both *S. subterranea* isolates were identical.

Phylogenetic Analysis of S. subterranea *SSU rDNA Sequences*

The complete *S. subterranea* SSU rDNA sequences were aligned with 61 SSU rDNA sequences from a very broad diversity of eukaryotes. The data set consisted of 2244 characters, but 651 ambiguously aligned characters were excluded from further phylogenetic analysis. Of the 1593 included characters, 313 were constant, 233 were variable uninformative, and 1047 were parsimony informative.

The heuristic search of the sequence data with the Archaea *Haloferax alexandrinus* as an outgroup species yielded three equally parsimonious trees with a length of 7602, consistency index = 0.348, and retention index = 0.652. One arbitrarily chosen tree is shown as a phylogram in Figure 2. The neighbour-joining analysis using Kimura's two-parameter distance produced similar topologies to those seen in the most parsimonious trees. The neighbour-joining distance and maximum parsimony analyses show the following phylogenetic patterns: *S. subterranea* forms a monophyletic group with other species from Plasmodiophorida with 100% bootstrap in both neighbor joining distance and maximum parsimony analysis. *Spongospora subterranea* f.sp. *subterranea* is distinct from *S. subterranea* f.sp. *nasturtii*. The plasmodiophorid clade forms a sister group to a clade consisting of chlorarachneans, euglyphids, and sarcomonads with a high bootstrap support, 98% in neighbour-joining distance and 96% in maximum parsimony analysis, respectively. Euglyphids and sarcomonads clustered in a group as a sister taxon to chlorarachneans.

The resulting phylogenetic tree also shows strong bootstrap support for most major groups: plants form a group; animals and fungi are closely related; stramenopiles are in a group; apicomplexans, ciliates, and dinozoans group together. Mycetozoa and kinetoplastida fall within the crown group.

DISCUSSION

Spongospora subterranea is a member of the plasmodiophorids, and the taxonomy of the Plasmodiophorida has been debated for a long time. The relationships between plasmodiophorids and other eukaryote groups have been reported in several recent SSU rDNA phylogenetic analyses. Cavalier-

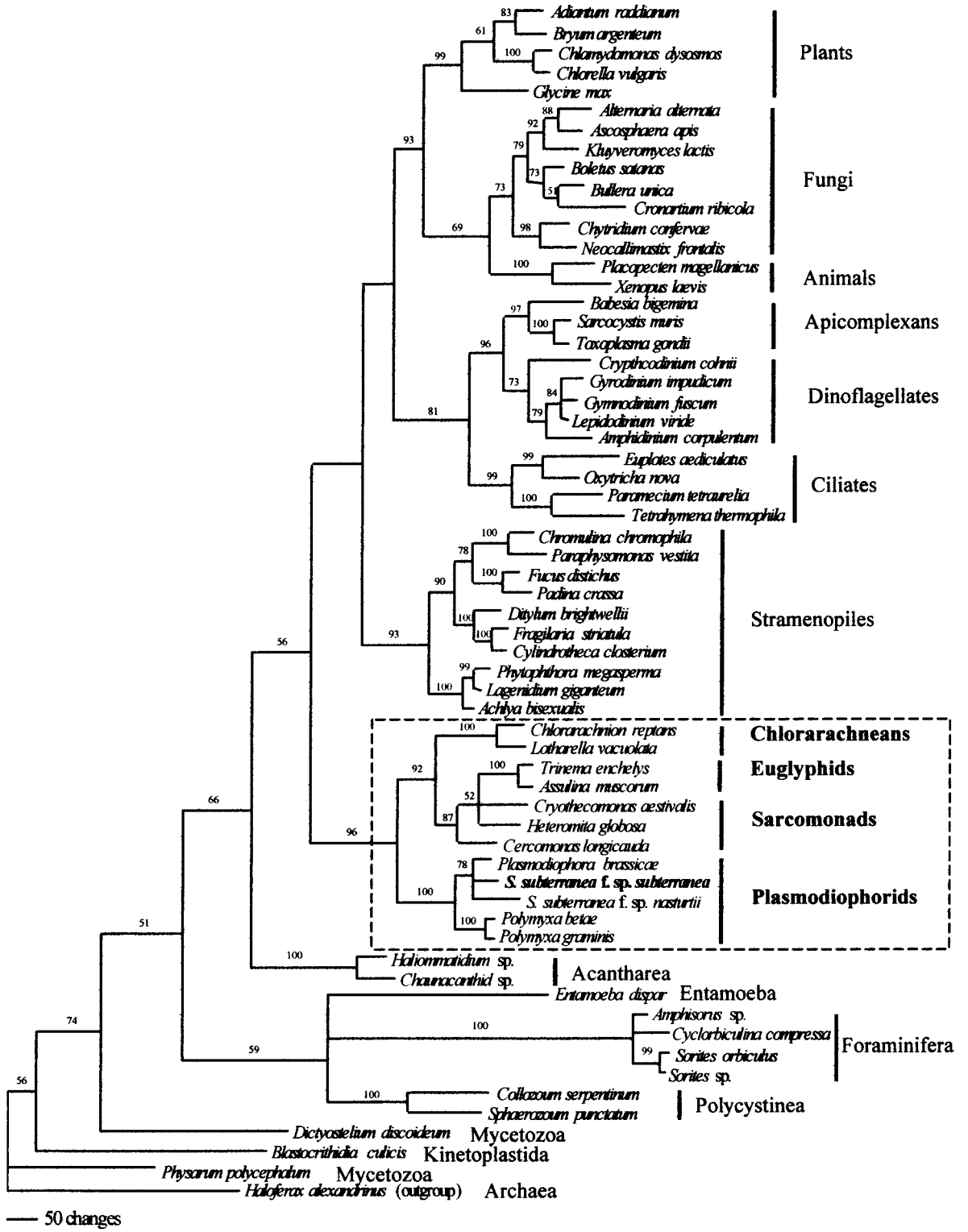

FIGURE 2.
One of three equally parsimonious trees generated following a heuristic analysis of SSU rDNA sequences of *S. subterranea* and 61 organisms obtained from Genbank. *Haloferax alexandrinus* was used as outgroup to root the tree. Each number indicates the percentage of bootstrap samplings, derived from 1000 replications. The groups located within the box belong to Cercozoa.

Smith and Chao (1997) found that *P. brassicae* is a sister to the chlorarachneans, euglyphids and sarcomonads clade. Bulman et al. (2001) reported that several plasmodiophorid species are related to sarcomonads and chlorarachneans. However, Castlebury and Domier (1998), Ward and Adams (1998), Kühn et al. (2000), and Down et al. (2002) reported that that the plasmodiophorids are not related to any other eukaryotes and suggested that the plasmodiophorids are a distinct group. In this investigation *S. subterranea* formed a monophyletic group with other species of plasmodiophorids, and this group was related to chlorarachneans, euglyphids, and sarcomonads based on SSU rDNA sequence analysis. This result is in agreement with the studies of Cavalier-Smith and Chao (1997) and Bulman et al. (2001).

In recent taxonomic treatments, Cavalier-Smith (1998) created a new phylum Cercozoa under the Kingdom Protozoa by renaming a radically modified phylum Rhizopoda. The new phylum Cercozoa comprises several diverse groups previously of uncertain affinity, including chlorarachneans, euglyphids, sarcomonads, and plasmodiophorids (Cavalier-Smith 1998). In the present SSU rDNA phylogenetic analysis, *S. subterranea* and other species of plasmodiophorids formed a deep branch within the Cercozoa clade, and this result supplies further evidence to support Cavalier-Smith's proposal (Cavalier-Smith 1998) to place the Plasmodiophorida within the phylum Cercozoa. Species of plasmodiophorids represent an important group of plant pathogens that cause serious losses to crops. The classification of this group within the phylum Cercozoa represents a significant step in our understanding of plasmodiophorid pathogens.

There are two forma speciales in *S. subterranea: S. subterranea* f.sp. *subterranea*, the cause of powdery scab of potato, and *S. subterranea* f.sp. *nasturtii*, the cause of crook root of watercress (Tomlinson 1958). Considering the host specificity and differences in morphological characters, Dick (2001) has recently raised this taxon to specific rank. In this study, comparison of ITS rDNA sequences and phylogenetic analyses of SSU rDNA sequences have shown that *S. subterranea* f.sp. *subterranea* is distinct from *S. subterranea* f.sp. *nasturtii*. These molecular data support the view of Dick (2001) that *S. subterranea* f.sp. *subterranea* and *S. subterranea* f.sp. *nasturtii* should be separated into distinct species.

In this study genetic variation within 52 isolates of *S. subterranea* obtained from the Republic of Ireland, Northern Ireland, Scotland, Canada, and USA was investigated and two distinct genetic groups (I and II) were identified based on the ITS sequence diversity among the isolates. The two types of ITS sequences identified in this investigation were previously reported by Bulman and Marshall (1998) in 20 isolates of *S. subterranea* obtained from Australia, New Zealand, Scotland, Peru, the Netherlands, and Switzerland. In their study, Bulman and Marshall (1998) found that no sequence variation was detected among any of the Australasian or European isolates with the exception of one from Inverness (Scotland), which was identical to the two Peruvian samples. In this investigation, however, isolates from both Ireland and Scotland have the two types of ITS sequences indicating that the genetic variations of *S. subterranea* exist in these countries. However, no variation was found in North American samples and all isolates from different locations in Canada and USA have the same type (II) of ITS sequences.

From the results of this investigation and the Bulman and Marshall (1998) study, it is interesting to note that all Australasian and North American isolates consisted of only one genetic group (II) and South American isolates consisted of only one genetic group (I), whereas European isolates consisted of both genetic groups (I and II). Karling (1968) suggested that powdery scab originated from the highlands of South America (Peru), was introduced with potato to Europe in post-Colombian times, and then back to North America and other parts of the world by the shipment and importations of infected tubers for food and seed. It appears that genetic group I might originate from South America and was then introduced to Europe, but it is not clear where and when the genetic group II originated. If European populations of *S. subterranea* are the source of Australasian and North American populations as suggested by Karling (1968), the genetic group I should also exist in Australia and North America.

Another interesting finding in this study is that the British Isles genetic groups of *S. subterranea* are associated with particular potato cultivars with no exception. For example, all isolates from the cv Saturna from different locations in Ireland and Scotland over different years were in one genetic group (I) and all isolates from the cv Kerr's Pink from Ireland over different years were in another genetic group (II), even though some isolates from Saturna and Kerr's Pink were collected from the same area by the same growers. These results suggest that the occurrence of genetic groups may be cultivar related. However, the numbers and sources of *S. subterranea* isolates are limited in this investigation and therefore further clarifica-

tion of this relationship using more isolates is required before arriving at definite conclusions.

While it is widely known that subgroups/pathotypes do occur in plasmodiophorids including *P. graminis* (Ward and Adams 1996) and *P. brassicae* (Some et al. 1996; Williams 1966) and that molecular variation is also found within subgroups of *P. graminis* (Ward and Adams 1996) and pathotypes of *P. brassicae* (Möller and Harling 1996), there is no information about physiological specialization in *S. subterranea*. It is possible that the *S. subterranea* genetic groups found in this study may be associated with different pathotypes of *S. subterranea*, but relationships between genetic variation and subgroups/pathotypes have yet to be established. Further research is needed to clarify the significance of these groups and if they represent physiological races of *S. subterranea*. This may have important implications for future potato breeding programs and in screening for cultivar resistance to powdery scab.

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