Phylogenetic relationships of *Phytophthora* species based on ribosomal ITS I DNA sequence analysis with emphasis on Waterhouse groups V and VI

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Phylogenetic relationships among *Phytophthora* species were investigated by sequence analysis of the internal transcribed spacer region I of the ribosomal DNA repeat unit. The extensive collection of isolates included taxa from all six morphological groups recognized by Waterhouse (1963) including molecular groups previously identified using isozymes and mtDNA restriction fragment length polymorphisms. Similar to previous studies, the inferred relationships indicated that molecular groups of *P. cryptogea/drechsleri*-like and *P. megasperma*-like taxa are polyphyletic. Morphological groups V and VI, which are differentiated by the presence of amphigynous or paragynous antheridia, are not monophyletic: species of the two groups are interspersed in the tree. Species with papillate and semi-papillate sporangia (groups I–IV) clustered together and this cluster was distinct from those of species with non-papillate sporangia. There was no congruence between the mode of antheridial attachment, sporangial caducity, or homo- or heterothallic habit and the molecular grouping of the species. Our study provides evidence that the antheridial position together with homo- or heterothallic habit does not reflect phylogenetic relationships within *Phytophthora*. Consequently, confirming studies done previously (Cooke & Duncan 1997), this study provides evidence that the morphological characters used in *Phytophthora* taxonomy are of limited value for deducing phylogenetic relationships, because they exhibit convergent evolution.

INTRODUCTION

The most commonly used taxonomic scheme for the genus *Phytophthora* (Waterhouse 1963, Stamps *et al*. 1990) is based on differences in sporangium and oospore morphology. Key characters used include the degree of papillation of the sporangia and the nature of the antheridial attachment to the oogonium. The six morphological groups described by these characters provide a basis for the identification of many species. However, this scheme was never meant to reflect phylogeny (Waterhouse 1963). Advancements in molecular methods have permitted a more rational study of phylogenetic relationships within the genus *Phytophthora*. Species with various degrees of intraspecific diversity have been identified using isozyme and mitochondrial (mt) DNA or nuclear DNA RFLP analyses (Förster, Oudemans & Coffey 1990a, Oudemans & Coffey 1991a, b). However, investigation of relationships within and between certain taxa has lagged behind due to the large genetic distances encountered. Methods involving other genetic markers such as sequence analysis of various regions of the ribosomal DNA repeat (White *et al*. 1990, Hibbett 1992) have improved our understanding of species relationships. Sequence analysis of the slowly evolving small subunit ribosomal RNA separated the oomycetes together with chrysophytes and diatoms from chytridiomycetes and 'higher' fungi (Förster *et al*. 1990b). Lee & Taylor (1992) were the first to study the taxonomy of the genus *Phytophthora* by comparing the more rapidly evolving internal transcribed spacer (ITS) I and II sequences of five species, *P. capsici*, *P. citrophthora*, *P. palmivora*, *P. megakarya*, and *P. cinnamomi*. More recently, the relationships among species of *Phytophthora* have been examined in more detail using ITS sequence data (Crawford *et al*. 1996a,b, Cooke & Duncan 1997, Cooke *et al*. 1999). In these latter studies the resultant grouping of species agreed to some degree with the classical morphological groupings based on sporangial papillation. There was no separation, however, of semi-papillate and papillate species. Antheridial attachment and homo- and heterothallism were found not to be indicative of close phylogenetic relationships (Cooke & Duncan 1997, Cooke *et al*. 1999). It was suggested that these latter characters may be under relatively simple genetic control or may have evolved more than once. The studies by Crawford *et al*. (1996a, b) and Cooke & Duncan (1997) suggested a broad framework for the phylogeny of the genus. The present study expanded upon this past work by examining a more comprehensive collection of well-characterized isolates of the genus, including a large number of isolates of problem taxa

such as *Phytophthora cryptogea*, *P. drechsleri* and *P. megasperma*. These isolates have been extensively characterized using isozyme and mtDNA RFLP analyses (e.g. Mills, Förster & Coffey 1991, Förster & Coffey 1993, Oudemans & Coffey 1991b) and represent the known molecular variability of the respective species.

Phytophthora cryptogea, *P. drechsleri* and *P. megasperma* have a long history of taxonomic controversy involving their classification (Hamm & Hansen 1982, Hansen *et al*. 1986, Mills *et al*. 1991, Förster & Coffey 1993). *P. cryptogea* and *P. drechsleri*, which are characterized by non-papillate sporangia and amphigynous antheridia, are placed into group VI (Waterhouse 1963, 1970, Stamps *et al*. 1990) whereas *P. megasperma* with non-papillate sporangia and predominantly paragynous antheridia is in group V. In addition to subtle morphological differences, *P. drechsleri* is thought to be distinct from *P. cryptogea* by its capability to grow at 35 °C. However, in an analysis based on isozymes and mtDNA RFLPs (Mills *et al*. 1991) these two species could not be differentiated. Nine distinct molecular groups with little genetic similarity could be identified among the species and the phenetic analysis suggested that these species were not monophyletic. A similar structure was found for *P. megasperma*-like taxa in a mtDNA RFLP analysis of over 200 isolates of worldwide origin (Förster & Coffey 1993). Again, nine molecular groups were identified most of which were not closely related to each other. This extensive diversity within *P. cryptogea/drechsleri* and *P. megasperma* raised the question whether some of the molecular groups might represent separate species since intraspecies diversity within other *Phytophthora* species was found to be much more limited (Förster *et al*. 1990a, Oudemans & Coffey 1991a, b). This hypothesis was supported by the fact that two molecular groups of *P. cryptogea/drechsleri* were interspersed among the *P. megasperma* groups (Förster & Coffey 1993). Moreover, when additional species of group VI were included, *P. erythroseptica*, *P. richardiae* and *P. lateralis* were found to be interspersed among the *P. cryptogea/drechsleri* groups (Mills *et al*. 1991). It was speculated that *P. cryptogea/drechsleri*-like and *P. megasperma*-like taxa might be polyphyletic in which members evolved similar morphological features, but are only distantly related to each other. Recently, the host-specific groups of *P. megasperma* from soybean, alfalfa, and clover, previously assigned to different *formae speciales* (Kuan & Erwin 1980, Pratt 1981) were classified as separate species: *P. sojae*, *P. medicaginis* and *P. trifolii* (Hansen & Maxwell 1991). In addition, two molecular groups of *P. cryptogea/drechsleri* (groups K and J) were assigned to *P. gonapodyides* (Brasier, Hamm & Hansen 1993). Our intent was to further investigate the relationships between the various molecular groups within and between the polyphyletic species groups and to test the validity of retaining these species in future taxonomic schemes. Additional species of Waterhouse's groups V and VI were included to study the relationships to other species that are characterized by similar morphological features.

A second goal of our study was to elucidate evolutionary relationships within the whole genus. Therefore, in addition to the species with non-papillate sporangia we included representatives from morphological groups I–IV which produce papillate (groups I and II) or semi-papillate (groups III and IV) sporangia and paragynous (groups I and III) or amphigynous (groups II and IV) antheridia. This selection of isolates covers a wide range of morphological and physiological characteristics and we anticipated that the phylogenetic analysis might provide answers about the evolution of these features.

MATERIALS AND METHODS

Fungal isolates

The isolates of *Phytophthora* used in this study are from the *Phytophthora* species collection at the University of California, Riverside, and are listed in Table I. When more than one isolate of a species or molecular group was included in the analysis, the most divergent representatives of the species or group were chosen. Working stocks of the cultures were maintained on clarified or non-clarified V8 agar (Ribeiro 1978).

DNA manipulations

DNA preparations generated in previous studies (Mills *et al*. 1991, Förster & Coffey 1992, 1993) were used when available or crude DNAs were isolated in a rapid extraction procedure (Judelson 1996). In this procedure approx. 5 mm^3 of mycelium was placed in microfuge tubes and 0.3 ml of extraction buffer (0.2 M Tris–HCl pH 8.5, 0.25 M NaCl, 0.025 M Na_2EDTA) was added. The samples were boiled for 5 min, vortexed for 5 min with 0.2 ml phenol and 0.2 ml chloroform and spun for 10 min. DNA was precipitated by adding 0.2 ml of isopropanol to 0.25 ml of the supernatant and centrifugation for 15 min. The DNA pellet was washed with 95% ethanol and resuspended in 0.1 ml of 10 mM Tris–HCl pH 7.5, 0.1 mM EDTA. 3 µl of this extract was used for PCR amplifications.

Primer selection (ITS 1 and ITS 2) and PCR amplification protocols of the ITS I region were based on methods by White *et al*. (1990). For sequencing, PCR products were column-purified using Wizard PCR Preps (Promega Corporation). Double-stranded DNA templates were sequenced completely on both strands using the *fmol* DNA sequencing system (Promega Corporation) and ^{33}P end-labelled primers ITS 1 and ITS 2.

DNA sequence analysis

ITS I sequences were aligned for phylogenetic analysis using the program CLUSTAL W version 1.60 (Thompson, Higgins & Gibson 1994) and adjusted manually. Phylogenetic relationships were determined by neighbour-joining analyses (Saitou & Nei 1987) based on two-parameter distances (Kimura 1980) and bootstrap analyses (Felsenstein 1985) with 1000 replicates using CLUSTAL W.

RESULTS

ITS I sequences from *Phytophthora* species examined in this study ranged from 174 to 235 bp and the concensus length for the sequence alignment was 259 bp. All sequences were deposited in GenBank (Accession Numbers AF242778-

Table 1. *Phytophthora* isolates used in the study.

Isolate	Species	WG[a]	MG	Host	Origin	Alternative sources
P1235	*P. cactorum*	I		*Raphiolepsis indica*	California	
P8497	*P. tentaculata*	I		*Chrysanthemum leucanthemum*	Germany	65520 (Kröber)
P630	*P. capsici*	II	CAP B	*Theobroma cacao*	Brazil	ATCC 46705
P1249	*P. capsici*	II	CAP B	*Spondias purpurea*	Costa Rica	007 (Umana)
P6539	*P. capsici*	II	CAP A	*Capsicum annuum*	New Mexico	H874 (Uchida)
P1200	*P. citrophthora*	II	CTR 2	*Theobroma cacao*	Brazil	ATCC 64802
P1324	*P. citrophthora*	II	CTR 1	*Citrus* sp.	California	ATCC 64854
P1182	*P. palmivora*	II		*Morrenia odorata*	Florida	ATCC 52158
P1321	*P. citricola*	III	CIT 2	*Rubus idaeus*	California	ATCC 64809
P1805	*P. citricola*	III	CIT 1	*Humulus lupulus*	California	13-3-5 (Mircetich)
P3049	*P. citricola*	III	CIT 5	*Persea americana*	California	Coffey
P1391	*P. infestans*	IV		*Solanum lycopersicum*	California	P177 (Vartanian)
P1847	*P. infestans*	IV		*Solanum tuberosum*	Great Britain	
P3359	*P. fragariae*	V		*Fragaria* sp.	Oregon	H2FSC (Converse), ATCC 16678
P3359	*P. fragariae* var. *rubi*	V		*Rubus idaeus*	Germany	M4 (Seemüller)
P6701	*P. humicola*	V		*Citrus* sp.	Taiwan	(Ann)
P6195T	*P. insolita*	V		Soil	Taiwan	Pmc5-1 (Ko), ATCC 38789
P3888	*P. lateralis*	V		*Chamaecyparis lawsoniana*	Oregon	262 (Hamm)
P127	*P. medicaginis*	V	M H	*Medicago sativa*	Australia	
P7029	*P. medicaginis*	V	M H	*M. sativa*	California	1129-5 (Erwin)
P3547	*P. megasperma*	V	M A	*Malus sylvestris*	New Zealand	IMI 133317, 400 (Hansen)
P6979	*P. megasperma*	V	M A	*Actinidia deliciosa*	France	190.87 (Robin)
P6204	*P. megasperma*	V	M B	*Prunus avium*	Switzerland	77197 (Bolay)
P6720	*P. megasperma*	V	M B	*Prunus persica*	California	22-2-3 (Mircetich)
P6957T	*P. megasperma*	V	M B	*Althaea rosea*	Washington, DC	CBS 402.72, IMI 32035
P3415	*P. megasperma*	V	M C	*Medicago sativa*	Canada	DW17B, 398 (Barr)
P3112	*P. megasperma*	V	M D	*Malus* sp.	California	52 (Hansen), 20-3-9 (Mircetich)
P3698	*P. megasperma*	V	M E	*Asparagus officinalis*	Switzerland	83140 (Falloon)
P6268	*P. megasperma*	V	M E	*A. officinalis*	Netherlands	PD88421 (Falloon)
P6616	*P. megasperma*	V	M E	*A. officinalis*	France	240.89 (Baudry)
P3163	*P. megasperma*	V	M F	*Lychnis alba*	New York	72 (Hansen)
P8488T	*P. quininea*	V		*Cinchona officinalis*	Peru	CBS 407.48
P3114	*P. sojae*[b]	V	M I	*Glycine max*	Wisconsin	1-16 (Maxwell)
P8213	*P.* sp. nov.	V		Rainforest soil	Ecuador	103 (Coffey)
P7010	*P. trifolii*[b]	V	M G	*Trifolium* sp.	Mississippi	33 (Hansen), 107 (Pratt)
P1995	*P. cambivora*	VI		*Malus* sp.	Australia	87 (Wallace)
P6358	*P. cambivora*	VI		*Prunus dulcis*	Australia	5 (Wicks)
P2428	*P. cinnamomi*	VI		*Persea americana*	California	Pc428
P6379	*P. cinnamomi*	VI		*Ananas comosus*	Taiwan	Pcip 1-2 (Ann)
P8495	*P. cinnamomi* var. *parvispora*	VI		*Beaucarnea* sp.	Germany	65425 (Kröber)
P1087T	*P. cryptogea/drechsleri*	VI	C/D A	*Beta vulgaris*	Idaho	ATCC 46724, CBS 292.35 (Tucker)
P1741	*P. cryptogea/drechsleri*	VI	C/D A	*Solanum lycopersicum*		IMI 40500, CBS 359.52
P3402	*P. cryptogea/drechsleri*	VI	C/D A	*Beta vulgaris*	California	EP1334-26 (Erwin)
P1703	*P. cryptogea/drechsleri*	VI	C/D B	*Solanum tuberosum*	Ohio	ATCC 36301, no. 116 (Rowe)
P3700	*P. cryptogea/drechsleri*	VI	C/D B	*Asparagus officinalis*	California	PmACA 004 (Falloon)
P1702	*P. cryptogea/drechsleri*	VI	C/D C	*Pseudotsuga menziesii*	Oregon	ATCC 34301, no. 37 (Pratt)
P3145	*P. cryptogea/drechsleri*	VI	C/D D	*Begonia elatior*	Germany	64132 (Kröber)
P3850	*P. cryptogea/drechsleri*	VI	C/D E	*Actinidia deliciosa*	California	15C (Conn)
P3239	*P. cryptogea/drechsleri*	VI	C/D F	*Cucumis sativa*	China	PT-39, B-35B (Tsao)
P3105	*P. cryptogea/drechsleri*	VI	C/D G	*Cajanus cajan*	India	ATCC 44388, P2 (Erwin)
P3602	*P. cryptogea/drechsleri*	VI	C/D H	*Malus pumila*	Arizona	A35R (Matheron)
P3650	*P. cryptogea/drechsleri*	VI	C/D J	*M. pumila*	New York	NY 082 (Jeffers)
P3197	*P. cryptogea/drechsleri*	VI	C/D K	*Abies nobilis*	Oregon	No. 139 (Hansen)
P7377	*P. cryptogea/drechsleri*	VI	C/D L	*Spathiphyllum* sp.	Netherlands	PD90/418 (van Kesteren)
P340	*P. erythroseptica*			*Solanum tuberosum*	Australia	T-2 (Zentmyer)
P3876	*P. richardiae*	VI		*Zantedeschia aethiopia*		ATCC 46734
P7471	*P. vignae*	VI		*Vigna unguiculata*	Australia	UQ168P2

[a] WG = Morphological groups (Waterhouse, 1963); MG = Molecular groups for *P. megasperma* (M) or *P. cryptogea/drechsleri* (C/D) based on isozyme or mtDNA RFLP analysis as defined by Mills *et al.* (1991), Förster & Coffey (1993), Mchau & Coffey (1994, 1995), Oudemans *et al.* (1994).

[b] *P. medicaginis*, *P. sojae*, and *P. trifolii* are the former *P. megasperma* groups H, I, and G, respectively.

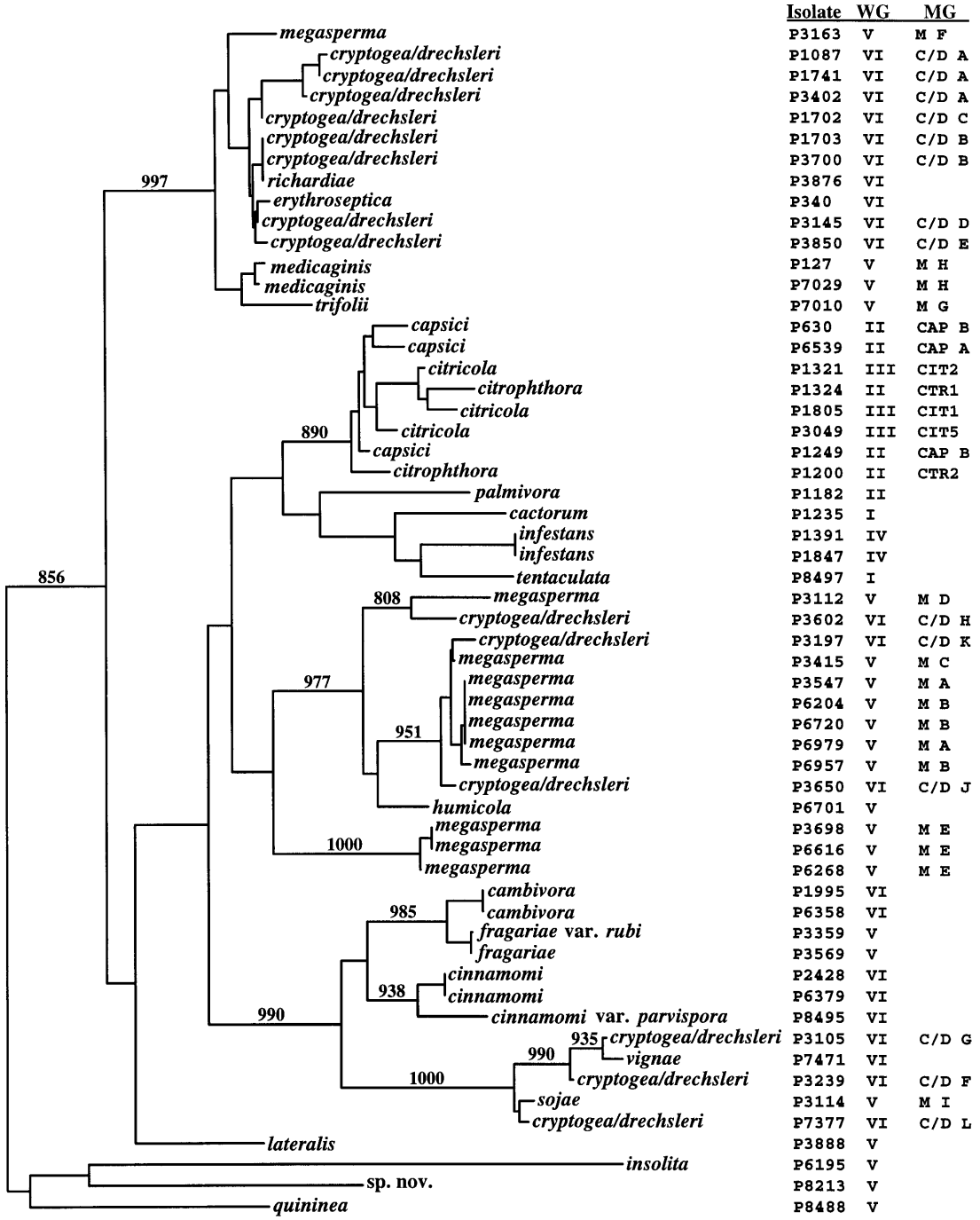

Fig. 1. Phylogenetic tree for *Phytophthora* species based on neighbour-joining analysis of ITS I. Numerals adjacent branches denote the number of bootstrap replicates out of 1000 and greater than 800 supporting a major group. The tree is mid-point rooted. Isolate code, Waterhouse morphological group (WG) and molecular group (MG) designations are given (see Table 1 and main text).

AF242834). Fig. 1 presents the results of a neighbour-joining analysis of the ITS I sequence data. Several evolutionary lineages can be identified in the tree.

The *P. cryptogea/drechsleri*-like and *P. megasperma*-like taxa are not monophyletic and, in addition, they cannot be separated. *P. megasperma*-like taxa with predominantly host-specific isolates from Douglas fir (group F), clover (*P. trifolii*; former *P. megasperma* group G) and alfalfa (*P. medicaginis*; former *P. megasperma* group H) cluster together. There is a close relationship between five groups of *P. cryptogea/drechsleri* (groups A–E) which originated from various host plants. Similarly close are groups A, B, and C of *P. megasperma* with isolates from diverse hosts and *P. cryptogea/drechsleri* groups J and K, both containing isolates from deciduous fruit trees and conifers. Three representatives of *P. megasperma* group E with isolates from asparagus are quite distinct, there are no close relationships to other groups evident. An evolutionary distinct line contains *P. sojae* (former *P. megasperma* group I) and three groups of *P. cryptogea/drechsleri*: group F with isolates from cucumber; group G with isolates of *P. drechsleri* f. sp. *cajani*; and group L, a newly identified group (Förster & Coffey unpublished) containing isolates from various ornamentals.

Some interesting relationships between additional species of Waterhouse's groups V and VI are evident. The ITS I

sequence of *P. richardiae* was identical to the one of *P. cryptogea/drechsleri* group B. *P. richardiae* and *P. erythroseptica* (represented by the common potato group, Mao & Coffey unpublished) are found within a tight cluster containing molecular groups of *P. cryptogea/drechsleri* and *P. megasperma*. *P. vignae* is within a cluster together with *P. cryptogea/drechsleri* groups F, G, and L, and *P. sojae*. There is a close affiliation between *P. cambivora* and *P. fragariae*, and *P. cinnamomi* is a sister species of *P. cambivora* and *P. fragariae*. *P. humicola* is related to various molecular groups of *P. cryptogea/drechsleri* and *P. megasperma*. In contrast, some other group V and VI species appear to be quite distinct such as *P. quininea*, *P. insolita* and a *Phytophthora* isolate with non-papillate sporangia from an Ecuadorian rain forest, *Phytophthora* sp. nov. In addition, this sequence analysis confirmed the taxonomic status of two recently described *Phytophthora* varieties: *P. fragariae* var. *rubi* (Förster & Coffey 1992, Wilcox *et al*. 1993) is found very close to *P. fragariae*, and *P. cinnamomi* var. *parvispora* (Kröber & Marwitz 1993) is grouped close to *P. cinnamomi*.

Species representing Waterhouse's morphological groups I–IV form a separate cluster. *P. capsici* and *P. citrophthora* (both group II), and *P. citricola* (group III) are not monophyletic and there is a close relationship among these three species. *P. cactorum* and *P. tentaculata* (both group I), *P. palmivora* (group II) and *P. infestans* (group IV) are more distantly related.

DISCUSSION

Relationships among and between molecular groups within P. cryptogea/drechsleri *and* P. megasperma

The high molecular diversity within *P. cryptogea/drechsleri*-like and *P. megasperma*-like taxa, previously interpreted as evidence of separate taxa (Mills *et al*. 1991, Förster & Coffey 1993), was confirmed in the present study. In the tree that was constructed from the ITS I sequence data the various molecular groups of *P. cryptogea/drechsleri* and *P. megasperma* formed several distinct lineages. When compared with the phenetic analysis of isozyme or mtDNA RFLP data (Mills *et al*. 1991, Förster & Coffey 1993) very similar relationships between the molecular groups within *P. cryptogea/drechsleri* or *P. megasperma* were found. However, some of the molecular groups clustered very closely suggesting the ultimate merging of these groups, e.g. *P. cryptogea/drechsleri* groups A–E or *P. megasperma* groups A, B, and C. In addition to the host-specific *P. megasperma*-like taxa that have been recently separated from *P. megasperma sensu stricto* (e.g., *P. medicaginis*, *P. trifolii*, *P. sojae*; Hansen & Maxwell, 1991), another distinct clade containing host-specific isolates from asparagus was identified. As previously indicated in the mtDNA RFLP analysis of *P. megasperma* (Förster & Coffey 1993), some very close relationships between molecular groups of the species were evident, and *P. cryptogea/drechsleri*-like and *P. megasperma*-like taxa could not be separated into monophyletic groups. This was supported by high bootstrap values shown in Fig. 1. Particularly close relationships were found between *P. megasperma* groups A, B, C, and *P. cryptogea/drechsleri* groups J and K, which have been designated as *P. gonapodyides* (Brasier *et al*. 1993). Two additional clusters contain molecular groups from both *P. megasperma*-like and *P. cryptogea/drechsleri*-like taxa.

From these data it appears that the antheridial position (predominantly paragynous in *P. megasperma*, amphigynous in *P. cryptogea/drechsleri*) together with homo- or heterothallism does not justify the taxonomic separation of the two species groups. We agree, however, with the study by Mills *et al*. (1991) that *P. cryptogea*-like and *P. drechsleri*-like taxa should not be merged into a single species, neither is a general merging with *P. megasperma*-like taxa supported. In contrast, in future taxonomic schemes the distinct clusters of molecular groups should be recognized.

Relationships among species with non-papillate sporangia (morphological groups V and VI)

Additional species of morphological groups V and VI were included to further evaluate relationships among species with similar morphological features. The analysis confirmed that groups V and VI, which are differentiated by the presence of amphigynous or paragynous antheridia, do not compose monophyletic groups: species of the two groups are found interspersed in the tree. Particularly interesting is a cluster consisting of *P. cinnamomi* and *P. cambivora* (both group IV) and *P. fragariae* (group V), which have been previously shown to be related (Cooke & Duncan 1997). Another cluster contains three molecular groups of *P. cryptogea/drechsleri*, *P. vignae* (both group VI) and *P. sojae* (group V). Other studies based on nuclear DNA RFLP data (Whisson *et al*. 1993) or on ITS sequence data (Crawford *et al*. 1996b) also indicated a close relationship between *P. sojae* and *P. vignae*.

The very close association of molecular groups of *P. cryptogea/drechsleri* and *P. megasperma* with the morphological group VI species *P. erythroseptica* and *P. richardiae* calls into question the validity of retaining the latter two species as separate taxonomic entities. They may simply represent morphological variants differing in the size range of spore structures.

Our study provides evidence that the antheridial position together with homo- or heterothallic habit does not reflect phylogenetic relationships within *Phytophthora* and confirms previous work by Cooke & Duncan (1997). These characters seem to have evolved independently numerous times and may therefore be under quite simple genetic control. The genetic distinctiveness of some group V and VI species such as *P. quininea*, and even more so of *P. insolita* and an isolate from an Ecuadorian rain forest (*Phytophthora* sp. nov.), indicates that organisms with similar morphological features may be genetically very diverse.

Relationships among species from all six morphological groups

When we included representatives of morphological groups I, II, III, and IV, there also was no strict congruence between morphological characterization and molecular grouping. In our phylogeny, however, non-papillate species did not cluster with papillate or semi-papillate species; semi-papillate and papillate species formed a separate cluster. Although this cluster was not well supported statistically (bootstrap value 452/1000), the presence of papillae appears to have

phylogenetic significance. Therefore, it is evident that among the main discriminating characters used in *Phytophthora* taxonomy, only the degree of papillation of the sporangium shows some congruence with relationships based on molecular data. Other characteristics, such as amphigynous or paragynous antheridial attachment, homo- or heterothallism, and the additional ''primitive'' or ''advanced'' characters as defined by Brasier (1983) and Brasier & Hansen (1992), such as sporangial caducity and host range are not confined to a single lineage. Our results support a recent ITS I and ITS II sequence analysis (Cooke & Duncan 1997). In contrast, in the study by Crawford *et al*. (1996a,b) species with papillate, semi-papillate or non-papillate sporangia were found in different clusters. This difference might be explained by the selection of isolates and species used. Relationships between species that were common in the study of Crawford *et al*. (1996a, b) and the present study were quite similar, e.g. *P. cactorum* and *P. palmivora* were related, as were *P. medicaginis* and *P. trifolii*, and more distantly, *P. vignae* and *P. cinnamomi*. The taxonomic significance of the antheridial position was also questioned in two recent studies. In the study by Hüberli, Tommerup & St Hardy (1997) paragynous antheridia were found to be widespread among Australian and Papua New Guinean isolates of the heterothallic species *P. cinnamomi*. In addition, in *P. boehmeriae*, which was originally described as having only amphigynous antheridia, paragynous antheridia were produced predominantly on certain culture media (Gao *et al*. 1998).

Species with papillate and semi-papillate sporangia exhibit a wide range of physiological diversity; there are narrow host range (e.g. *P. infestans*) and wide host range species (e.g. *P. cactorum* and *P. palmivora*), and species with low (*P. infestans*) or high (*P. palmivora*) temperature requirements. These various morphological and physiological characters appear to have evolved several times independently through convergence producing nonhomologous characters that look alike with the same evolutionary change occurring at least twice, and thus making them homoplasies (Abbott, Bisby & Rogers 1985). This may be an indication that these characters may be under relatively simple genetic control.

A close relationship between *P. capsici*, *P. citrophthora* and *P. citricola* was previously evident in an isozyme and mtDNA RFLP study (Oudemans, Förster & Coffey 1994), and in work based on ITS sequence data (Cooke & Duncan 1997). In the isozyme and mtDNA study, *P. citricola* subgroup CIT5 appeared to be more closely related to *P. capsici* than it was to the other molecular groups of *P. citricola*. This finding is not supported in the present ITS I analysis due to high sequence similarity between the three species. However, *P. citricola* is not monophyletic.

Taxonomic implications

The presented phylogeny is a gene tree that reflects the evolution of a single short sequence. However, it has to be emphasized that whenever other molecular data (isozyme or RFLP data) were available, the inferred relationships are congruent. Thus, in addition to the relationships between *P. citricola*, *P. citrophthora* and *P. capsici*, previously suggested, relationships within *P. megasperma*-like (Förster & Coffey 1993) and *P. cryptogea/drechsleri*-like taxa (Mills *et al*. 1991) could be confirmed by the ITS I sequence study. Therefore, there seems to be considerable support for the relationships presented. Still, the limitations of ITS I sequencing need to be emphasized: the sequence is relatively short and, when more diverse organisms are analyzed, it is increasingly difficult to align the sequences. It is unlikely that addition of ITS II sequence data would resolve the phylogenetic relationships, because it has been previously shown for species of *Phytophthora* that ITS II is less variable than ITS I and analyses result in very similar phylogenetic relationships for the two regions (Cooke & Duncan 1997). Longer sequences from various genomic regions will be required to further investigate some of these relationships. In addition, taxon sampling may play a role in resolving phylogenetic relationships within *Phytophthora*.

Clearly, a revised taxonomy of *Phytophthora* that includes molecular genetic markers would be different from the current one. The results of our study, however, did not facilitate our understanding of the evolution of species within the genus. Within clusters that we identified in this study there was no clear correlation with a geographical origin, with a particular host group, or to other identifiable ecological factors. Although we gained more insight into genetic relationships, especially morphological groups V and VI, an improved definition of species delineation for the genus *Phytophthora* did not materialize.

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