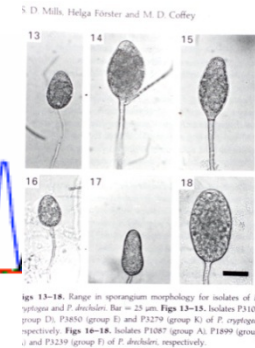
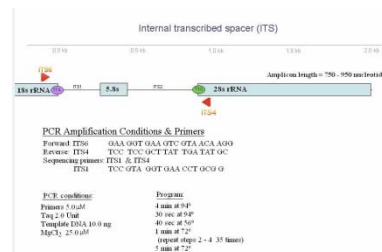
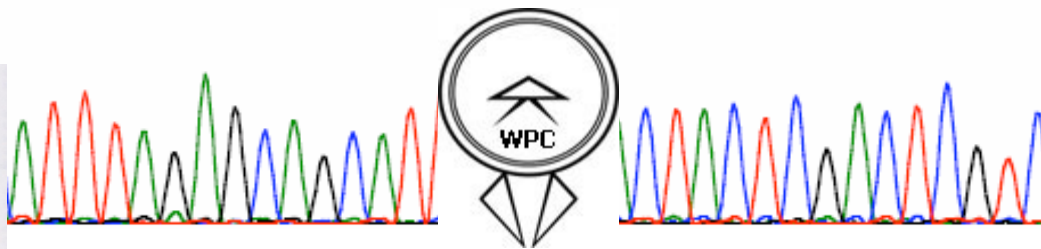
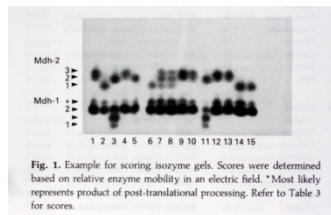


Past and Current Taxonomic Status of *Phytophthora cryptogea* and *P. drechsleri* and Associated Species

A Work in Progress



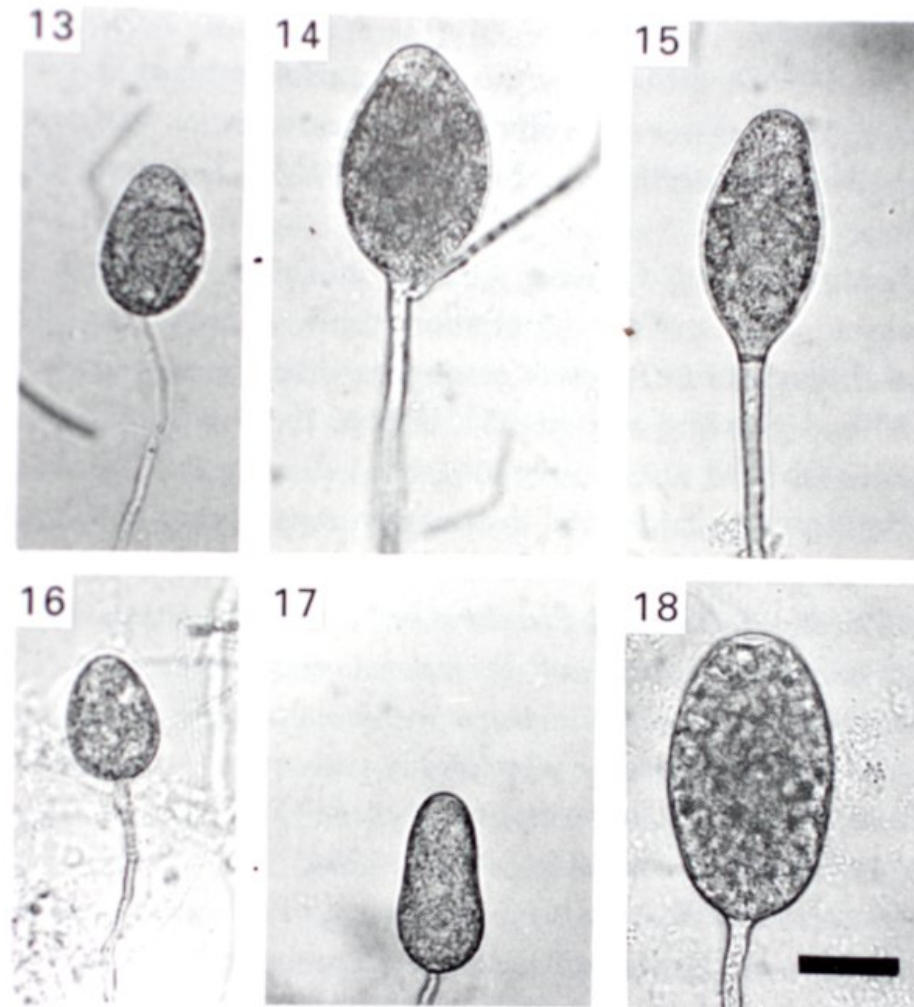
**Michael D. Coffey , Shirley Tu
& Masoomeh Peiman**
University of California, Riverside

Taxonomic structure of *Phytophthora cryptogea* and *P. drechsleri* based on isozyme and mitochondrial DNA analyses

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Intra- and interspecific isozyme variation was evaluated for 123 isolates assigned to either *Phytophthora cryptogea* or *P. drechsleri*, and compared with that of 15 isolates of *P. erythroseptica* and 11 isolates of *P. lateralis*. Isolates of *P. cryptogea* and *P. drechsleri* were from worldwide sources and displayed a high degree of variability. The majority of these isolates were subsequently divided into ten distinct groups based on numerical analysis of 24 putative enzyme loci. None of the enzyme loci were monomorphic for all ten groups. Analysis of mitochondrial (mt) DNA restriction fragment length polymorphisms of selected isolates from each isozyme group supported the isozyme data. Differences in morphological features of the ten isozyme groups of *P. cryptogea* and *P. drechsleri* were not sufficiently distinct to readily distinguish between them. Isozyme analysis of *P. erythroseptica* revealed that it is a uniform and distinct taxon. The isolates of *P. lateralis* also formed a homogeneous and discrete group. An interspecific comparison revealed that the variation among the ten isozyme groups of *P. cryptogea* and *P. drechsleri* was as great as that observed among *P. cinnamomi*, *P. cambivora*, *P. lateralis*, *P. erythroseptica* and *P. richardiae*. The combined results of isozyme and mtDNA analysis indicate that there are at least seven distinct molecular groups within the *P. cryptogea* and *P. drechsleri* complex.



figs 13–18. Range in sporangium morphology for isolates of *P. cryptogea* and *P. drechsleri*. Bar = 25 μ m. **Figs 13–15.** Isolates P3104 (group D), P3850 (group E) and P3279 (group K) of *P. cryptogea* respectively. **Figs 16–18.** Isolates P1087 (group A), P1899 (group J) and P3239 (group F) of *P. drechsleri*, respectively.

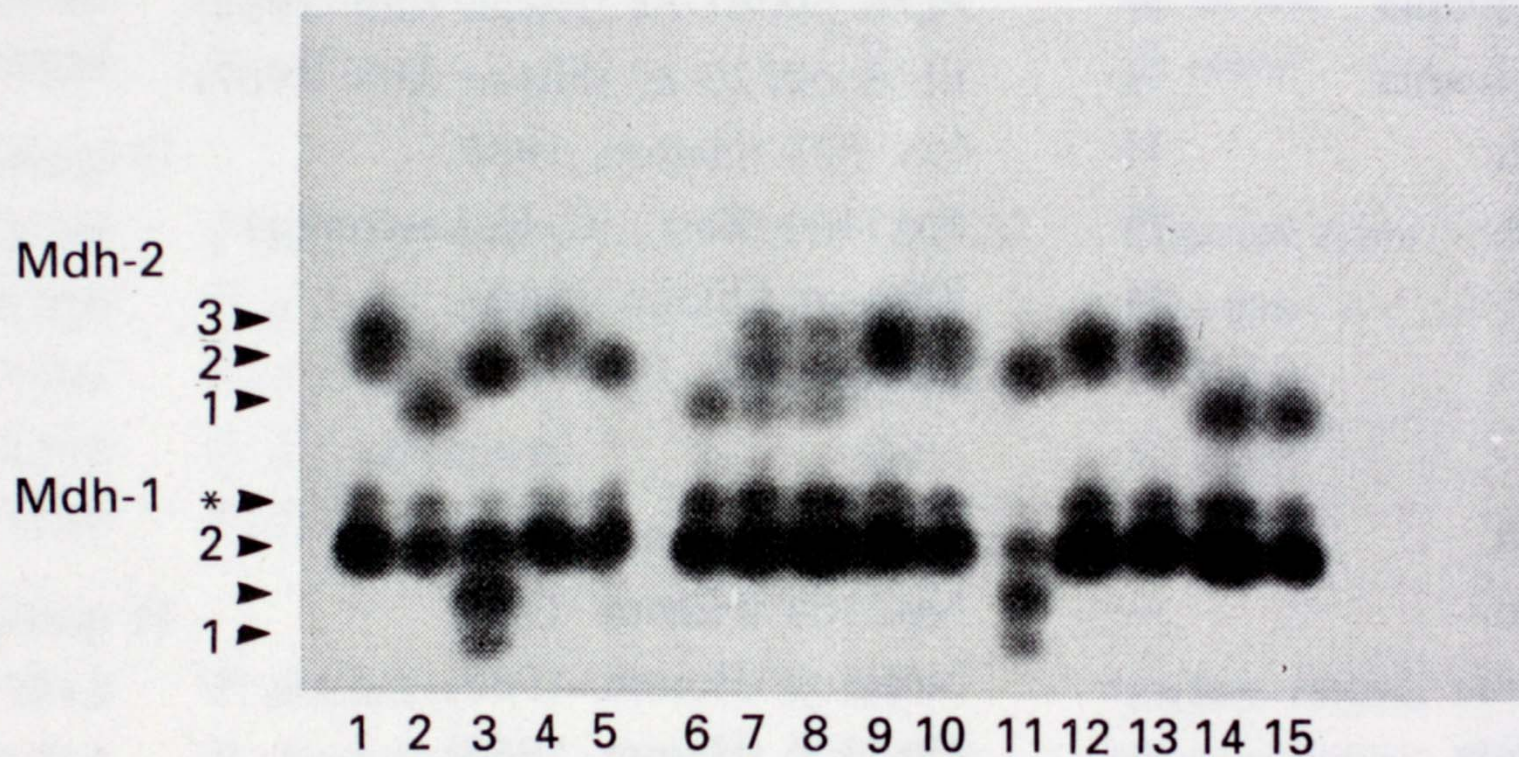
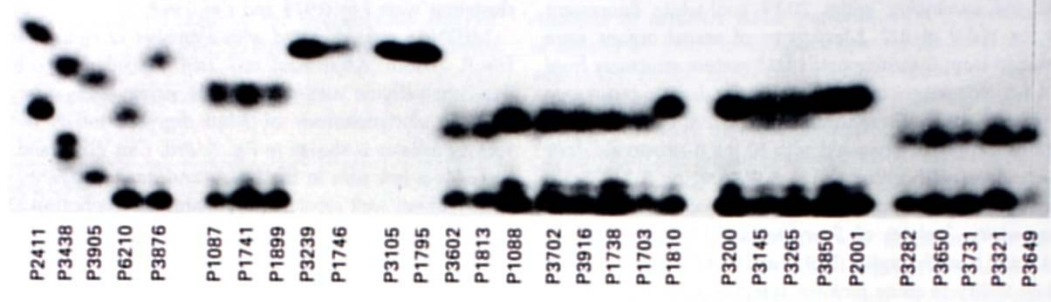
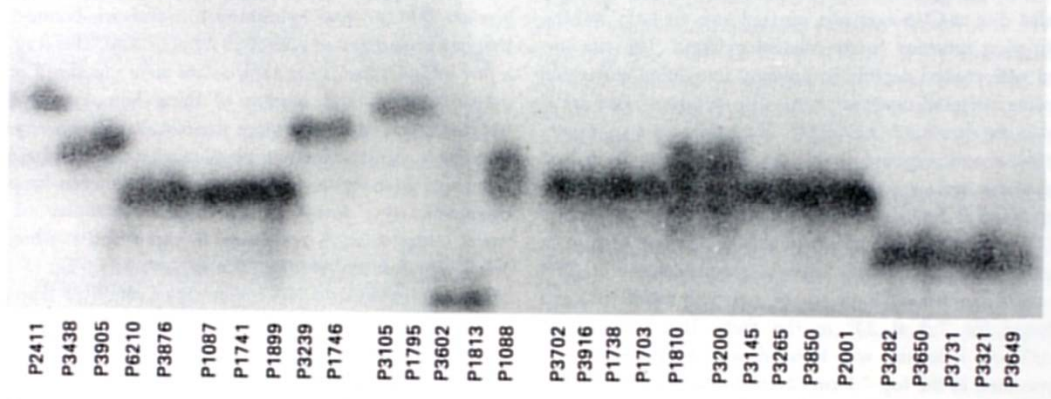


Fig. 1. Example for scoring isozyme gels. Scores were determined based on relative enzyme mobility in an electric field. * Most likely represents product of post-translational processing. Refer to Table 3 for scores.

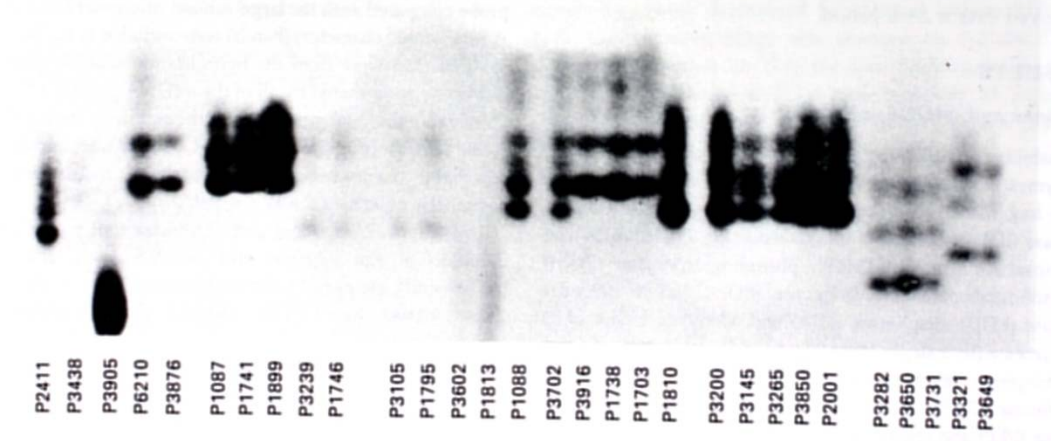
2 Mdh



3 G-6-pi



4 G-6-pdh



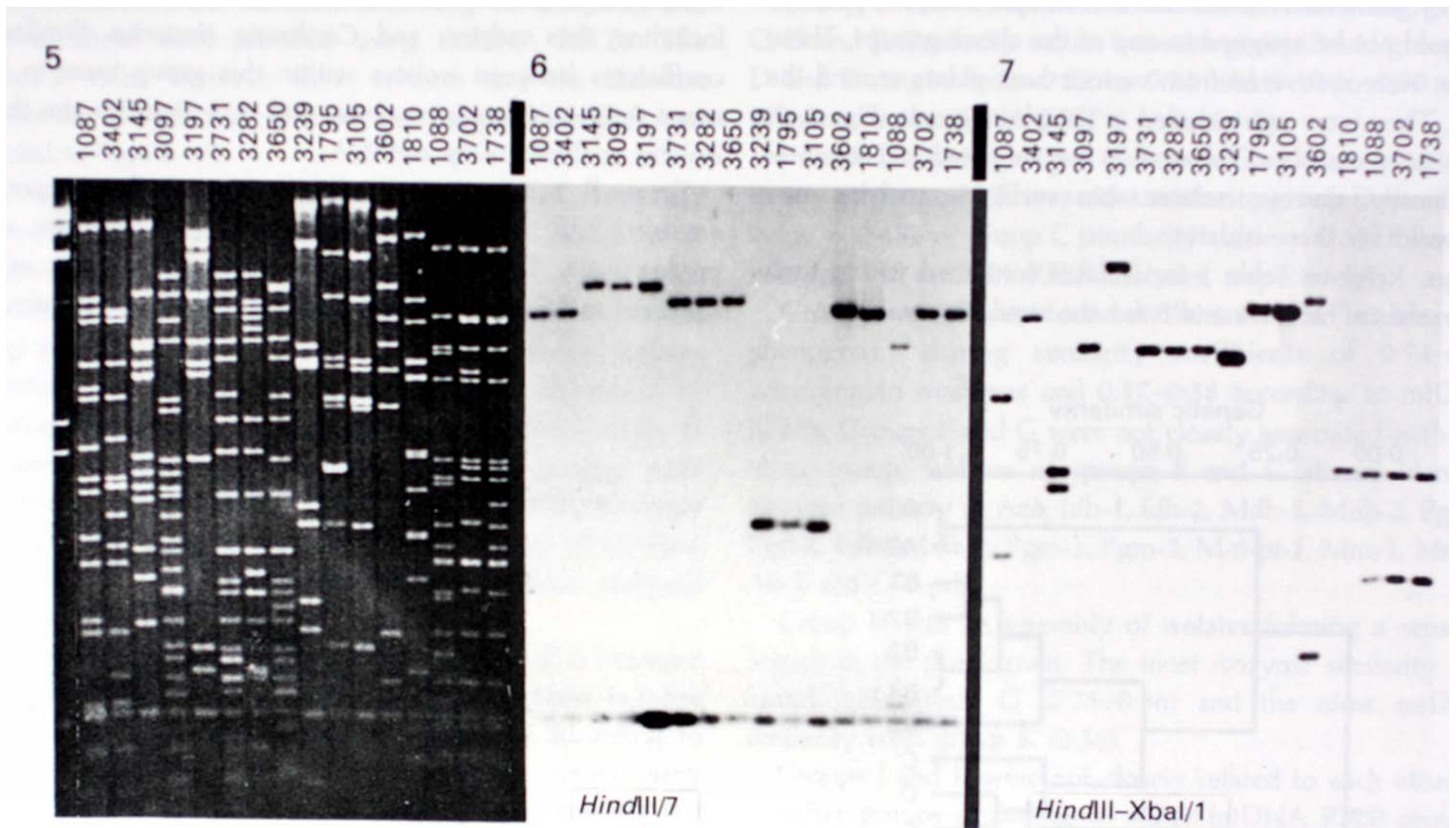


Fig. 5-7. Analysis of restriction fragment length polymorphisms in mitochondrial (mt) DNAs using hybridization techniques. Fig. 5 shows agarose gel electrophoresis of *Msp*I digested mtDNAs from various isolates of *P. cryptogea* and *P. drechsleri*. *Hind*III digested lambda DNA was used as a size marker (first lane). A high degree of mtDNA diversity was evident among the isolates. Fig. 6 and 7 show

Isolate	Species	IG(DG)*	Host	Origin	Mating type†	Alternative sources
Group A						
P1087A	<i>P. drechsleri</i>	A1(A1)	<i>Beta vulgaris</i>	Idaho	A2	ATCC 46724, CBS 292-35 (Tucker)
P1904	<i>P. drechsleri</i>	A1	<i>B. vulgaris</i>	California	A1	EP1265 (Erwin)
P3858	<i>P. cryptogea</i>	A1	<i>Carthamus tinctorius</i>	California	A2	9-1-5 (Conn, 1988), JK205 (Klisiewicz)
P6139	<i>P. cryptogea</i>	A1	<i>C. tinctorius</i>	Arizona	A2	P201 (Klisiewicz), PcR1 Duniway, 1989
P6140	<i>P. cryptogea</i>	A1	<i>C. tinctorius</i>	California	A1	P174 (Klisiewicz), CSF74 Duniway, 1989
P3901	<i>P. erythroseptica</i>	A1	<i>Solanum tuberosum</i>	?	A2	ATCC 10924, no. 406 (Hamm, 1988)
P1741	<i>P. drechsleri</i>	A1(A2)	<i>Lycopersicon esculentum</i>	?	A2	IMI 40500, CBS 359.52
P3857	<i>P. drechsleri</i>	A1	<i>Capsicum annuum</i>	Mexico	A2	ATCC 60786, P209 (Erwin)
P1899	<i>P. drechsleri</i>	A2	<i>Beta vulgaris</i>	California	A1	EP389 (Erwin)
P3402	<i>P. drechsleri</i>	A2(A3)	<i>B. vulgaris</i>	California	A1	EP1334-26 (Erwin)
Group B						
P1088A	<i>P. cryptogea</i>	B1(B1)	<i>Aster</i> sp.	?	A1	CBS 290.35 (Tucker), ATCC 46721
P3085	<i>P. cryptogea</i>	B1	<i>Aster</i> sp.	California	A1	ATCC 15402, N57 (Gallegly)
P3449	<i>P. cryptogea</i>	B1	<i>Callistephus chinensis</i>	California	A1	ATCC 21278
P3700	<i>P. cryptogea</i>	B2(B2)	<i>Asparagus officinalis</i>	California	A1	PmACA 004 (Falloon, 1988)
P3702	<i>P. cryptogea</i>	B2(B2)	<i>A. officinalis</i>	California	A1	PmACA 060 (Falloon, 1988)
P6048	<i>P. cryptogea</i>	B2	<i>A. officinalis</i>	California	A1	PmACA 108 (Falloon, 1989)
P3916T	<i>P. erythroseptica</i>	B3	<i>Solanum tuberosum</i>	Ireland	A1	VKMF-1835 (Soviet Union)
P3453T	<i>P. erythroseptica</i>	B3(B3)	<i>S. tuberosum</i>	Ireland	A1	CBS 129.23
P1693T	<i>P. erythroseptica</i>	B3	<i>S. tuberosum</i>	Ireland	A1	IMI 34684
P1738T	<i>P. cryptogea</i>	B4(B3)	<i>Lycopersicon esculentum</i>	Ireland	A1	CBS 113.19
P3447	<i>P. cryptogea</i>	B4(B3)	<i>L. esculentum</i>	Channel Is.	H	IMI 69664
P3448T	<i>P. cryptogea</i>	B4	<i>L. esculentum</i>	Ireland	A1	IMI 180615
P3584T	<i>P. cryptogea</i>	B4	<i>L. esculentum</i>	Ireland	A1	ATCC 56962, H12.1 (Ho)
P1703	<i>P. cryptogea</i>	B5	<i>Solanum tuberosum</i>	Ohio	A2	ATCC 36301, no. 116 (Rowe)
P1739	<i>P. cryptogea</i>	B5	<i>Lycopersicon esculentum</i>	New Zealand	A1‡	IMI 45168, no. 521 (Barr, 1988)
P3100	<i>P. drechsleri</i>	B5	<i>L. esculentum</i>	Japan	A1	ATCC 58425, H13.2 (Ho)
P3806	<i>P. cryptogea</i>	B5	<i>Senecio</i> sp.	California	A2	P172 (Erwin), no. 522 (Barr, 1988)
Group C						
P1076	<i>P. cryptogea</i>	C	<i>Pinus radiata</i>	California	A2‡	ATCC 46723, (Zentmyer)

Group J						
P3280	<i>P. cryptogea</i>	J1	<i>Malus pumila</i>	Kentucky	S	NY 220 (Jeffers)
P3282	<i>P. cryptogea</i>	J1(J3)	<i>Rubus</i> sp.	New York	S	NY 315 (Jeffers)
P3302	<i>P. cryptogea</i>	J1	<i>Malus pumila</i>	New York	S	NY 353 (Jeffers)
P3303	<i>P. cryptogea</i>	J1	<i>Prunus persica</i>	New York	S	NY 361 (Jeffers)
P3650	<i>P. cryptogea</i>	J1(J1)	<i>Malus pumila</i>	New York	A1†	NY 082 (Jeffers, 1978)
P3731	<i>P. cryptogea</i>	J2(J2)	<i>Prunus cerasus</i>	Michigan	S	M441 (Jones, 1987)
P3097	<i>P. cryptogea</i>	J3(J4)	<i>Pseudotsuga menziesii</i>	Oregon	S	ATCC 34302, no. 47 (Roth)
P3199	<i>Phytophthora</i> sp.	J3	<i>P. menziesii</i>	Oregon	S	No. 266 (Hansen)
Group K						
P3196	<i>Phytophthora</i> sp.	K1(K2)	<i>Pseudotsuga menziesii</i>	Canada (B.C.)	S	No. 133 (Hansen)
P3197	<i>Phytophthora</i> sp.	K1(K2)	<i>Abies nobilis</i>	Oregon	S	No. 139 (Hansen)
P3279	<i>P. cryptogea</i>	K1	<i>Prunus cerasus</i>	New York	A1†	NY 154 (Jeffers, 1980)
P3317	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 413, Pch 18 (Jeffers)
P3318	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 416, Pch 19 R-2 (Jeffers)
P3320	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 414, Pch 20C (Jeffers)
P3321	<i>P. cryptogea</i>	K1(K1)	<i>P. persica</i>	New York	S	Pch 21 R-2 (Jeffers)
P3649	<i>P. cryptogea</i>	K1(K1)	<i>Malus pumila</i>	New York	A1†	NY 001 (Jeffers, 1978)
P3673	<i>P. cryptogea</i>	K1	<i>Prunus cerasus</i>	Michigan	A1†	M417 (Jones)
P3713	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	S	C-1 (Jones)
P3716	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	S	M103 (Jones, 1986)
P3722	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	A1†	M172 (Jones, 1986)

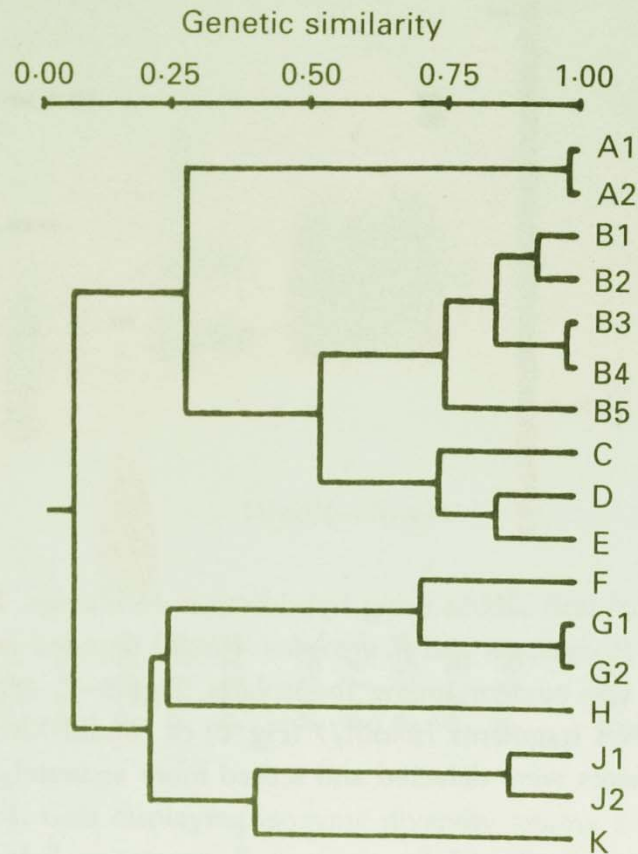


Fig. 8. Phenogram based on isozyme analysis showing the groupings of isolates of *P. cryptogea*, *P. drechsleri*, *P. melonis* and *P. sinensis* of worldwide origin. The phenogram was constructed with the NTSYS program using the unweighted pair-group method with arithmetic averaging (UPGMA) from Roger's similarity values. Refer to Table 1 for isolates corresponding to each isozyme group (IG).

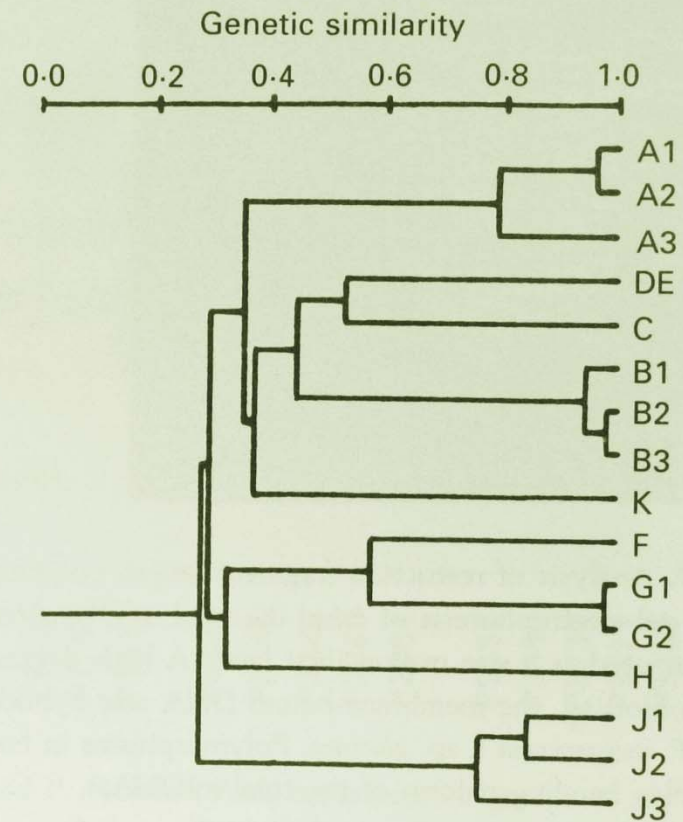


Fig. 9. Phenogram based on mtDNA RFLP analysis showing grouping of isolates of *P. cryptogea*, *P. drechsleri* and *P. melonis* of worldwide origin. The phenogram was constructed with the NTSYS program using the unweighted pair-group method with arithmetic averaging (UPGMA) from Dice similarity values. Refer to Table 1 for isolates corresponding to each DNA group (DG).

Isolate	Species	IG(DG)*	Host	Origin	Mating type†	Alternative sources
Group A						
P1087A	<i>P. drechsleri</i>	A1(A1)	<i>Beta vulgaris</i>	Idaho	A2	ATCC 46724, CBS 292-35 (Tucker)
P1904	<i>P. drechsleri</i>	A1	<i>B. vulgaris</i>	California	A1	EP1265 (Erwin)
P3858	<i>P. cryptogea</i>	A1	<i>Carthamus tinctorius</i>	California	A2	9-1-5 (Conn, 1988), JK205 (Klisiewicz)
P6139	<i>P. cryptogea</i>	A1	<i>C. tinctorius</i>	Arizona	A2	P201 (Klisiewicz), PcR1 Duniway, 1989
P6140	<i>P. cryptogea</i>	A1	<i>C. tinctorius</i>	California	A1	P174 (Klisiewicz), CSF74 Duniway, 1989
P3901	<i>P. erythroseptica</i>	A1	<i>Solanum tuberosum</i>	?	A2	ATCC 10924, no. 406 (Hamm, 1988)
P1741	<i>P. drechsleri</i>	A1(A2)	<i>Lycopersicon esculentum</i>	?	A2	IMI 40500, CBS 359.52
P3857	<i>P. drechsleri</i>	A1	<i>Capsicum annuum</i>	Mexico	A2	ATCC 60786, P209 (Erwin)
P1899	<i>P. drechsleri</i>	A2	<i>Beta vulgaris</i>	California	A1	EP389 (Erwin)
P3402	<i>P. drechsleri</i>	A2(A3)	<i>B. vulgaris</i>	California	A1	EP1334-26 (Erwin)
Group B						
P1088A	<i>P. cryptogea</i>	B1(B1)	<i>Aster</i> sp.	?	A1	CBS 290.35 (Tucker), ATCC 46721
P3085	<i>P. cryptogea</i>	B1	<i>Aster</i> sp.	California	A1	ATCC 15402, N57 (Gallegly)
P3449	<i>P. cryptogea</i>	B1	<i>Callistephus chinensis</i>	California	A1	ATCC 21278
P3700	<i>P. cryptogea</i>	B2(B2)	<i>Asparagus officinalis</i>	California	A1	PmACA 004 (Falloon, 1988)
P3702	<i>P. cryptogea</i>	B2(B2)	<i>A. officinalis</i>	California	A1	PmACA 060 (Falloon, 1988)
P6048	<i>P. cryptogea</i>	B2	<i>A. officinalis</i>	California	A1	PmACA 108 (Falloon, 1989)
P3916T	<i>P. erythroseptica</i>	B3	<i>Solanum tuberosum</i>	Ireland	A1	VKMF-1835 (Soviet Union)
P3453T	<i>P. erythroseptica</i>	B3(B3)	<i>S. tuberosum</i>	Ireland	A1	CBS 129.23
P1693T	<i>P. erythroseptica</i>	B3	<i>S. tuberosum</i>	Ireland	A1	IMI 34684
P1738T	<i>P. cryptogea</i>	B4(B3)	<i>Lycopersicon esculentum</i>	Ireland	A1	CBS 113.19
P3447	<i>P. cryptogea</i>	B4(B3)	<i>L. esculentum</i>	Channel Is.	H	IMI 69664
P3448T	<i>P. cryptogea</i>	B4	<i>L. esculentum</i>	Ireland	A1	IMI 180615
P3584T	<i>P. cryptogea</i>	B4	<i>L. esculentum</i>	Ireland	A1	ATCC 56962, H12.1 (Ho)
P1703	<i>P. cryptogea</i>	B5	<i>Solanum tuberosum</i>	Ohio	A2	ATCC 36301, no. 116 (Rowe)
P1739	<i>P. cryptogea</i>	B5	<i>Lycopersicon esculentum</i>	New Zealand	A1‡	IMI 45168, no. 521 (Barr, 1988)
P3100	<i>P. drechsleri</i>	B5	<i>L. esculentum</i>	Japan	A1	ATCC 58425, H13.2 (Ho)
P3806	<i>P. cryptogea</i>	B5	<i>Senecio</i> sp.	California	A2	P172 (Erwin), no. 522 (Barr, 1988)
Group C						
P1076	<i>P. cryptogea</i>	C	<i>Pinus radiata</i>	California	A2‡	ATCC 46723, (Zentmyer)

Group J						
P3280	<i>P. cryptogea</i>	J1	<i>Malus pumila</i>	Kentucky	S	NY 220 (Jeffers)
P3282	<i>P. cryptogea</i>	J1(J3)	<i>Rubus</i> sp.	New York	S	NY 315 (Jeffers)
P3302	<i>P. cryptogea</i>	J1	<i>Malus pumila</i>	New York	S	NY 353 (Jeffers)
P3303	<i>P. cryptogea</i>	J1	<i>Prunus persica</i>	New York	S	NY 361 (Jeffers)
P3650	<i>P. cryptogea</i>	J1(J1)	<i>Malus pumila</i>	New York	A1†	NY 082 (Jeffers, 1978)
P3731	<i>P. cryptogea</i>	J2(J2)	<i>Prunus cerasus</i>	Michigan	S	M441 (Jones, 1987)
P3097	<i>P. cryptogea</i>	J3(J4)	<i>Pseudotsuga menziesii</i>	Oregon	S	ATCC 34302, no. 47 (Roth)
P3199	<i>Phytophthora</i> sp.	J3	<i>P. menziesii</i>	Oregon	S	No. 266 (Hansen)
Group K						
P3196	<i>Phytophthora</i> sp.	K1(K2)	<i>Pseudotsuga menziesii</i>	Canada (B.C.)	S	No. 133 (Hansen)
P3197	<i>Phytophthora</i> sp.	K1(K2)	<i>Abies nobilis</i>	Oregon	S	No. 139 (Hansen)
P3279	<i>P. cryptogea</i>	K1	<i>Prunus cerasus</i>	New York	A1†	NY 154 (Jeffers, 1980)
P3317	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 413, Pch 18 (Jeffers)
P3318	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 416, Pch 19 R-2 (Jeffers)
P3320	<i>P. cryptogea</i>	K1	<i>P. persica</i>	New York	S	NY 414, Pch 20C (Jeffers)
P3321	<i>P. cryptogea</i>	K1(K1)	<i>P. persica</i>	New York	S	Pch 21 R-2 (Jeffers)
P3649	<i>P. cryptogea</i>	K1(K1)	<i>Malus pumila</i>	New York	A1†	NY 001 (Jeffers, 1978)
P3673	<i>P. cryptogea</i>	K1	<i>Prunus cerasus</i>	Michigan	A1†	M417 (Jones)
P3713	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	S	C-1 (Jones)
P3716	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	S	M103 (Jones, 1986)
P3722	<i>P. cryptogea</i>	K1	<i>P. cerasus</i>	Michigan	A1†	M172 (Jones, 1986)

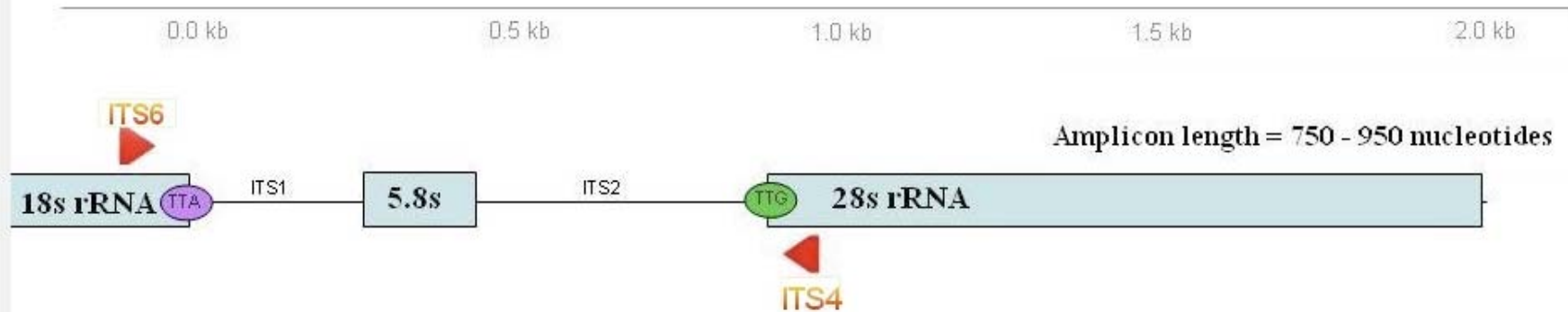
P. erythroseptica

P340	<i>P. erythroseptica</i>	Per	<i>Solanum tuberosum</i>	Australia	H	T-2 (Zentmyer)
P1698	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	England	H	ATCC 28766, no. 38 (Pitt)
P1699	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Ohio	H	ATCC 36302
P3356	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Scotland	H	IMI 303923, R38 (Kennedy)
P3451	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	England	H	IMI 146453 (Lonsdale, 1971)
P3452	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	England	H	IMI 21277 (ex CBS, 1948)
P3454	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	England	H	IMI 181716 (Lonsdale, 1971)
P3937	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Pennsylvania	H	PDA 69061-84 (Hwan Kim, 1984)
P3938	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Pennsylvania	H	PDA 69720-87 (Hwan Kim, 1987)
P6122	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Oregon	H	No. 575 (Hamm, 1988)
P6142	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Canada	H	No. 464 (Barr), (C. H. Lawrence)
P6180	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Ireland	H	Bannon, Dublin 1989
P6210	<i>P. erythroseptica</i>	Per	<i>S. tuberosum</i>	Peru	H	Torres, IPC, 1989

After alignment of the edited sequences with **ClustalW** and **MacClade**, phylogenetic analyses were conducted in **MEGA 4** (1), Neighbor-Joining method (2) the percentage of replicate tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to branches (3)

- 1. Tamura K, Dudley J, Nei M & Kumar S (2007) *MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution* 24:1596-1599.
- 2. Saitou N & Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
- 3. Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783 – 791.

Internal transcribed spacer (ITS)



PCR Amplification Conditions & Primers

Forward: ITS6 GAA GGT GAA GTC GTA ACA AGG

Reverse: ITS4 TCC TCC GCT TAT TGA TAT GC

Sequencing primers: ITS1 & ITS4

ITS1 TCC GTA GGT GAA CCT GCG G

PCR conditions:

Primers 5.0 μ M
Taq 2.0 Unit
Template DNA 10.0 ng
MgCl₂ 25.0 μ M

Program:

4 min at 94°
30 sec at 94°
40 sec at 56°
1 min at 72°
(repeat steps 2 - 4 35 times)
5 min at 72°

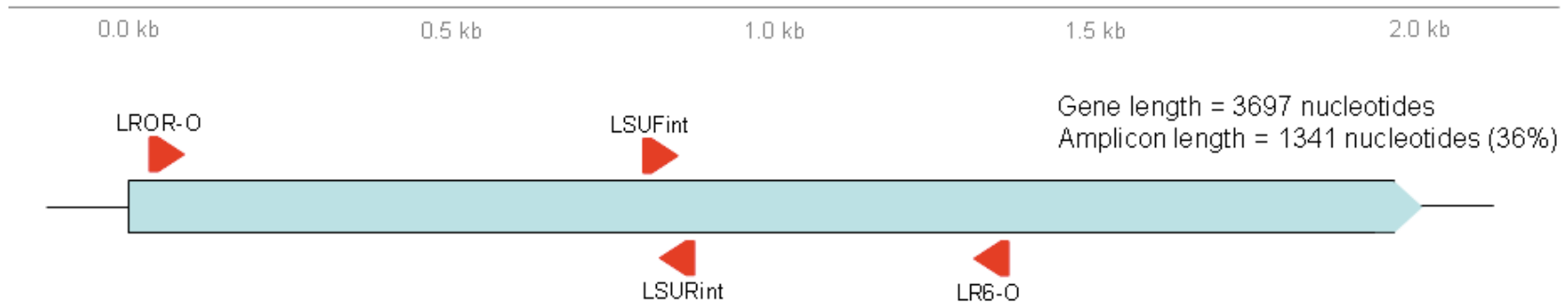
242 accessions used to generate
ITS sequences

See the 2,5 meter
ITS Tree!!!



48-ITS

Large Subunit Ribosomal RNA



PCR Amplification Conditions & Primers

Forward: LROR-O ACC CGC TGA ACT YAA GC Position 25 - 41
Reverse: LR6-O CGC CAG ACG AGC TTA CC Position 1349 - 1365

(derived from Riethmuller A, Voglmayr H, Goker M, Weib M, Oberwinkler F (2002) Mycologia 94(5):834-849.

Additional Sequencing Primers

Forward: LSUFint CKT TGA CGA AAT GGA GCG AT Position 797 - 816
Reverse: LSURint TTT CCA CAC CCT AAC ACT TGC Position 859 - 879

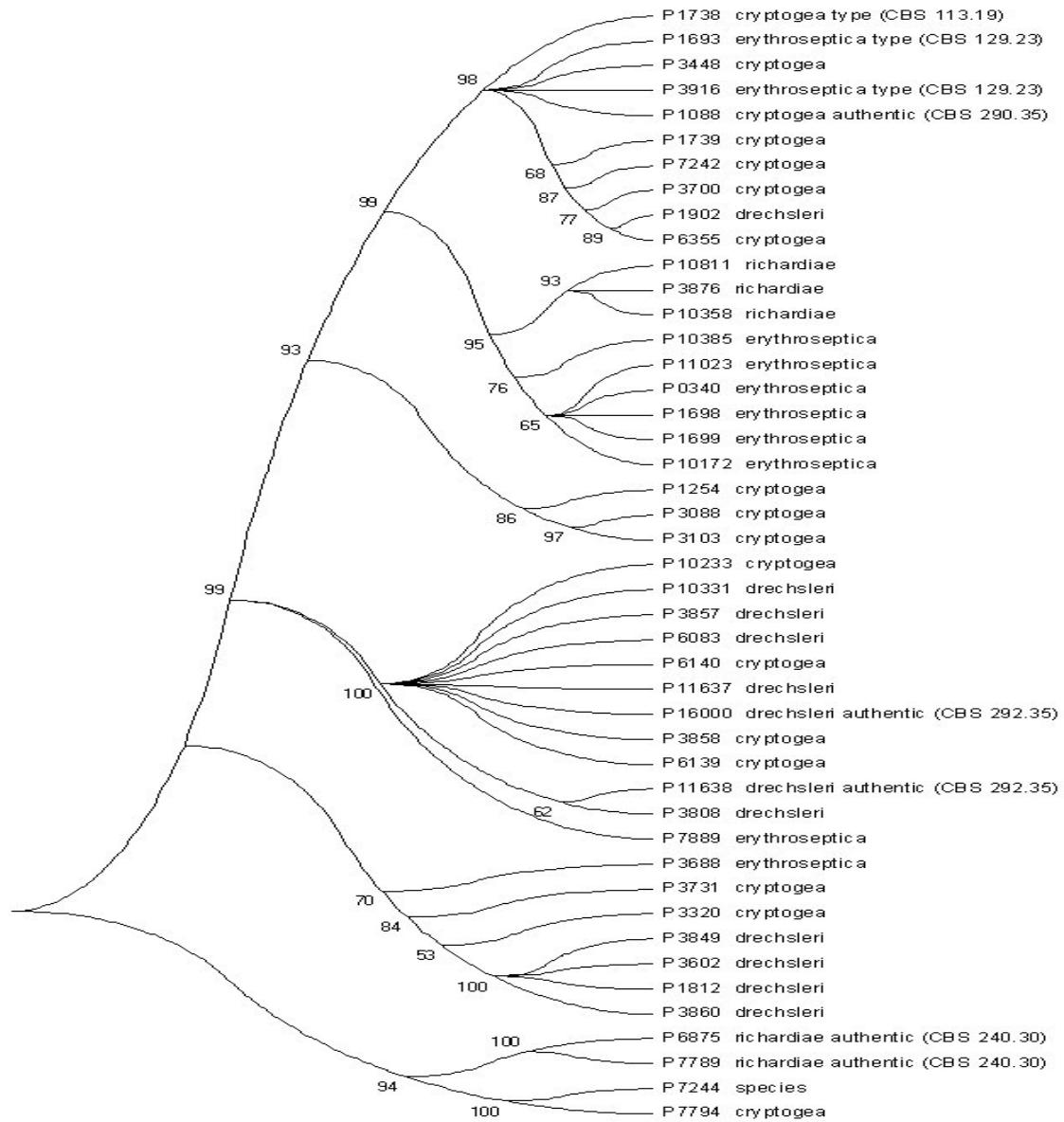
*Primer position relative to the sequence of *P. megasperma* X75631 from NCBI

PCR conditions:

2.5 mM Mg (AmpliTaq)
dNTPS 200 μ M
Primers 0.2 - 1.0 μ M
(varies depending on degeneracy of primers)
Taq 1 Unit
Template DNA 5 ng

Program:

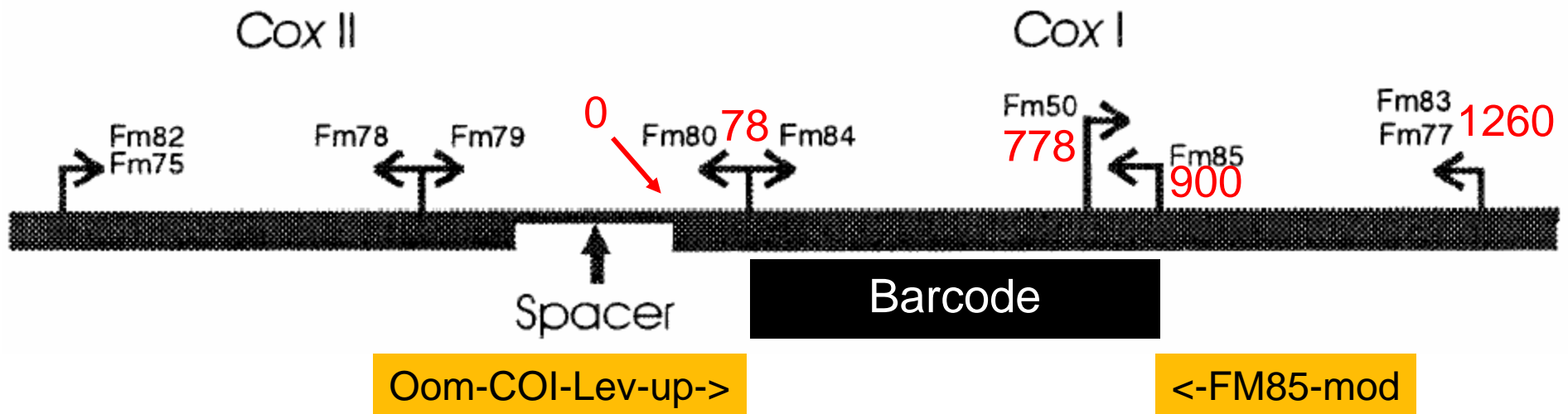
2 min 94°
30 sec 94° |
30 sec 53° | 35 cycles
2 min 72° |
5 min 72°



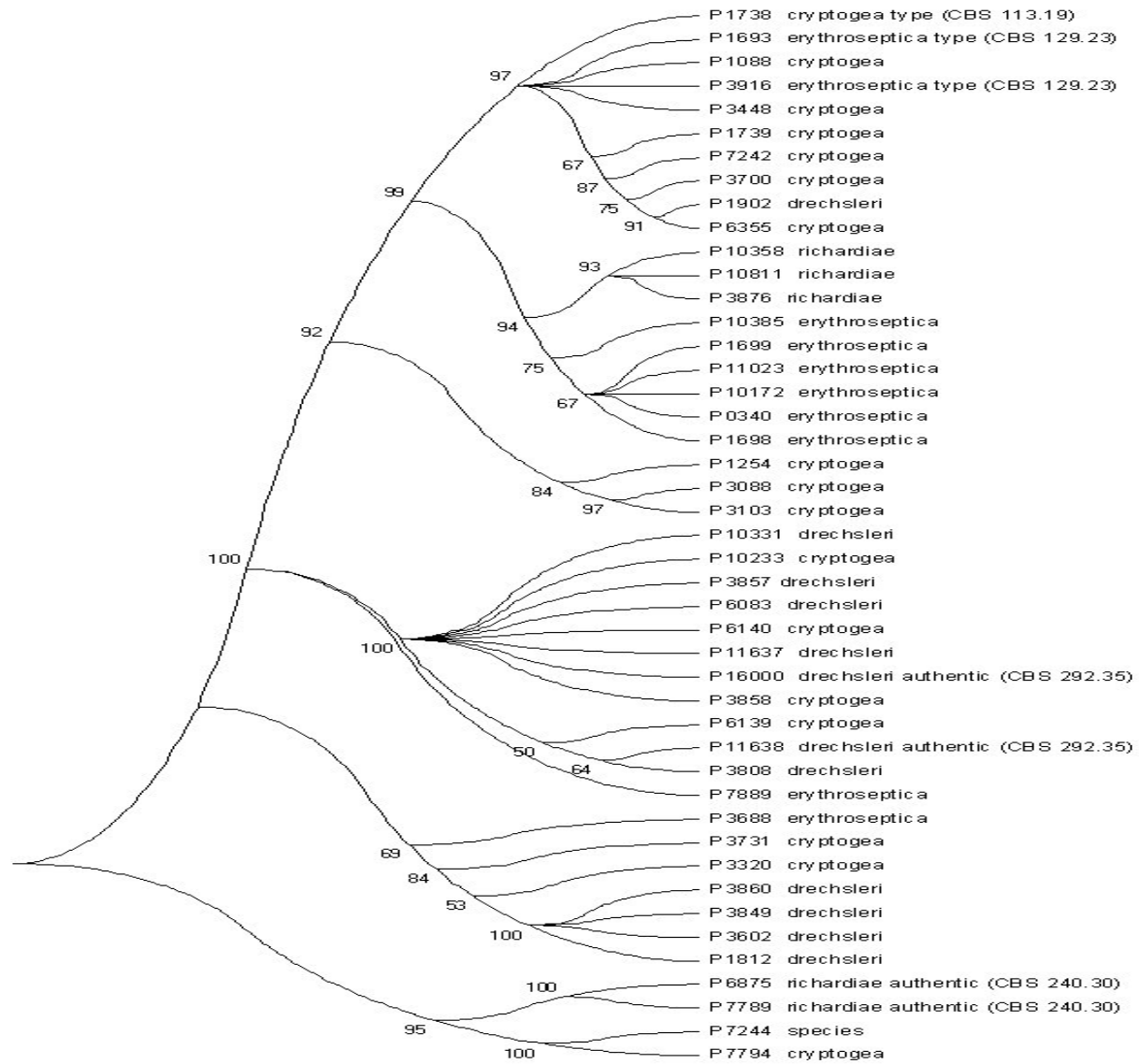
LSU

COXI barcoding

- Cytochrome Oxidase I (COXI)
- Good primers designed that amplify a 727bp region of COXI
- No introns in oomycete COXI (as opposed to true fungi)
- From G.P. Robideau, A.W.A.M. de Cock, M. Peiman, K. Bala, M. D. Coffey, and C.A. Lévesque. 2008



Martin, F. N., and P. W. Tooley. 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* **95**:269-284.



COX 1

Results obtained so far with
ITS, LSU and COXI validate
the separation
of *P. cryptogea* and *P. drechsleri*
into two distinct and closely related
phylogenetic species

Based on preliminary results with ITS sequencing of 242 accessions from the WOGRC there are probably 6-10 additional phylogenetic species many originally described as either *P. cryptogea* or *P. drechsleri*

Acknowledgements

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