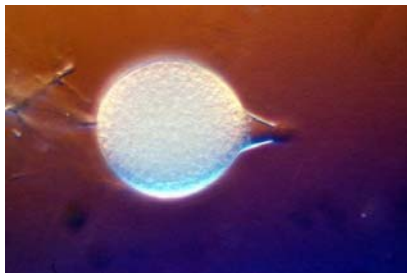
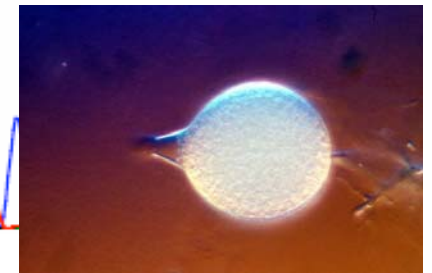
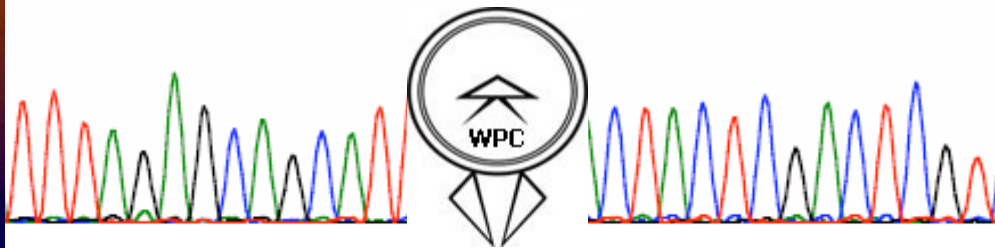


Molecular Phylogeny of the Marine *Halophytophthora* Species *A Work in Progress*



Schulz



**Michael D. Coffey, Julie Huss
& Masoomeh Peiman**
University of California, Riverside

Zoosporangium of *Halophytophthora tartarea*



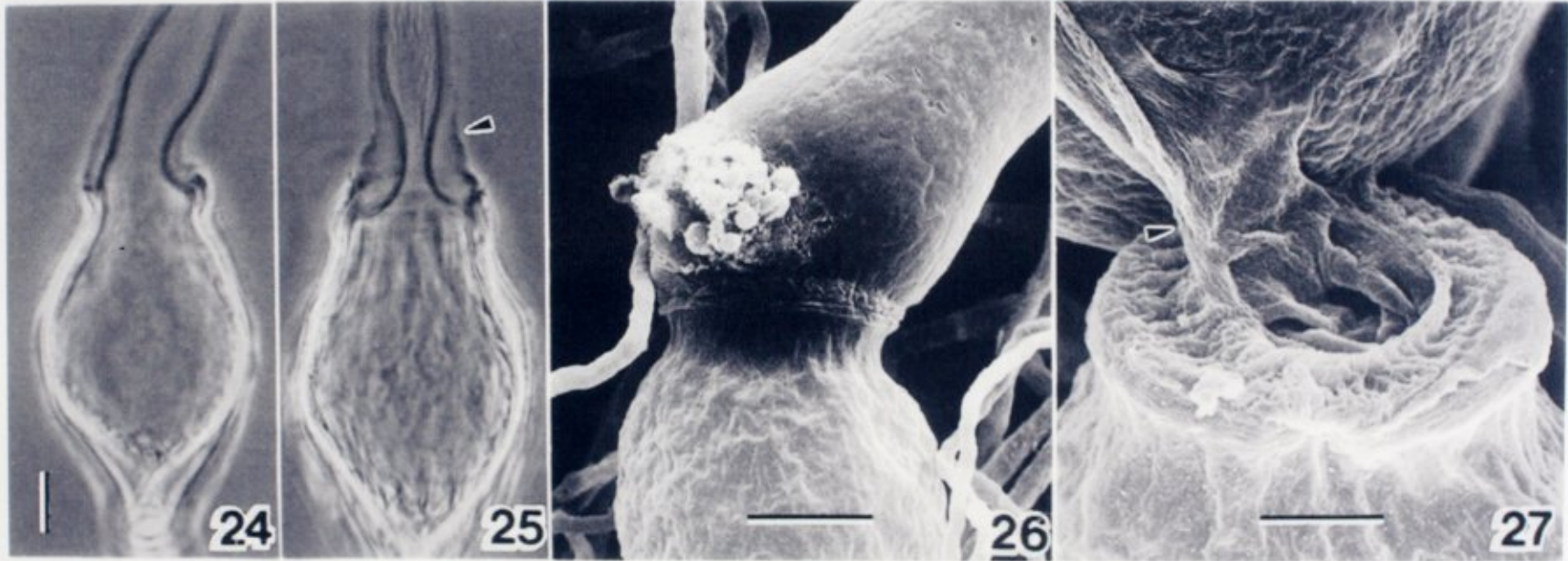
Source: Schulz

Zoosporangium of *Halophytophthora vesicula*



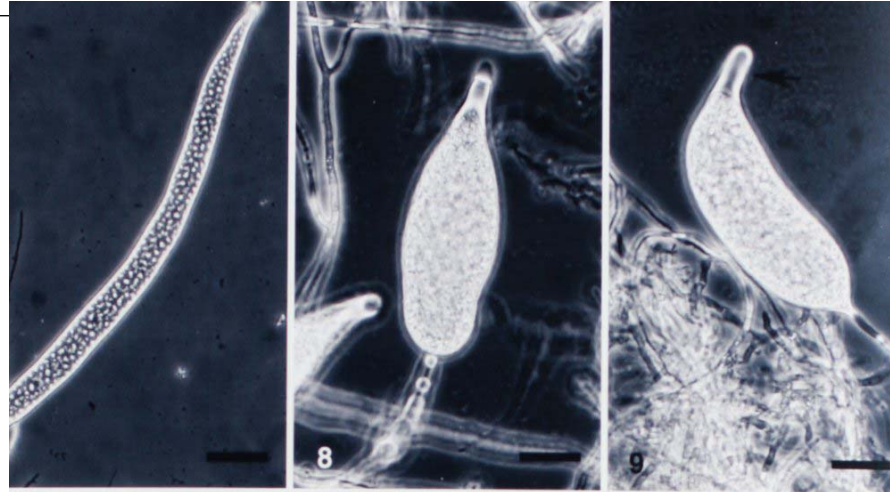
Source: S.Y. Newell

Zoosporangium of *Halophytophthora porrigovesica*



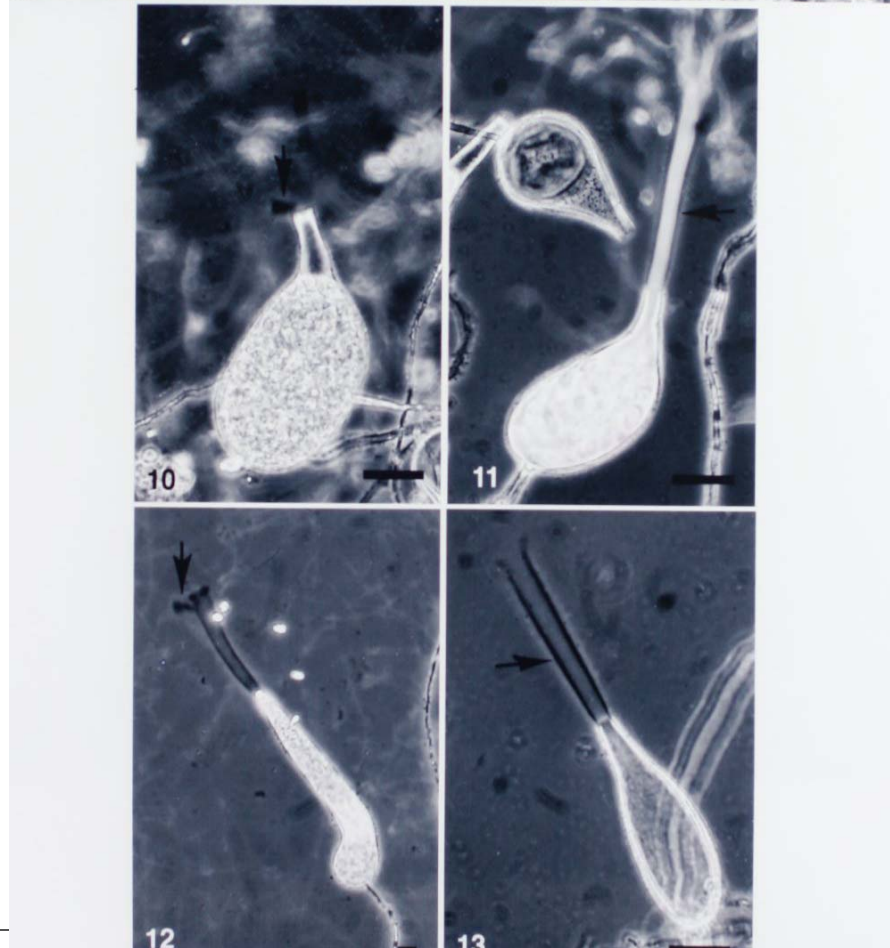
Figs. 24–27. Light and scanning electron micrographs of *H. porrigovesica*. 24. Vacant zoosporangium just after zoospore release, showing expanded vesicle. 25. Zoospore-discharged, old zoosporangium with wrinkled inner wall and shrunken vesicle. Note mucilaginous material (arrowhead) deposited around the opening of zoosporangium and along the vesicle membrane. 26. An expanded vesicle containing zoospores. 27. Constricted opening of zoospore-discharged zoosporangium. Note a vesicle membrane (arrowhead) remaining at the opening. Bars: 24 (= 25), 26 = 10 μm ; 27 = 5 μm .

Source: Nakagiri *et al.* 2001



*Halophytophthora
elongata*

Source: H.H.Ho *et al.*
2003



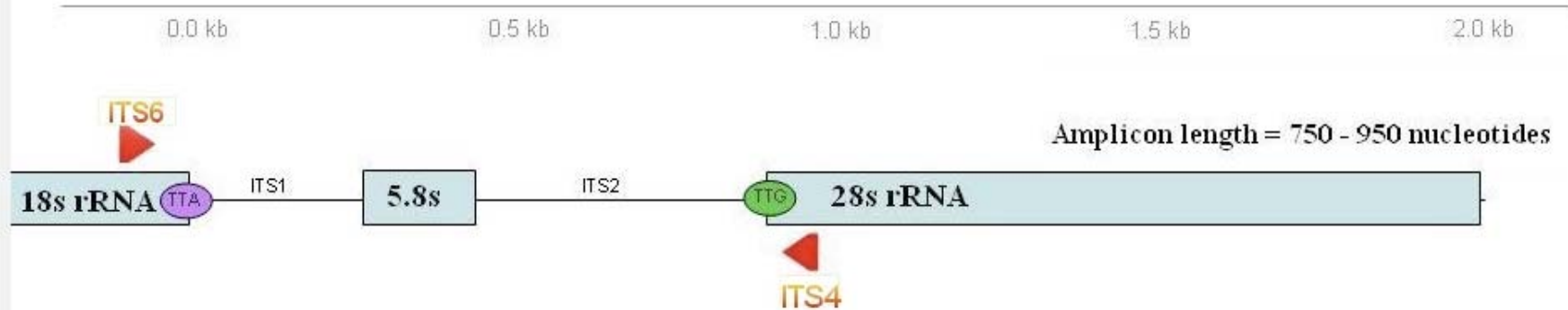
KEY TO SPECIES OF *HALOPHYTOPHTHORA*Source: Nakagiri *et al.* 2001

1. Sporangium papillate 2
 1'. Sporangium epapillate 11
 2. Sporangium with spines 3
 2'. Sporangium smooth-walled 5
 3. Spines short, 1–5 μm , clustering in groups and angled toward the center of the group; mycoparasitic
 *H. mycoparasitica* (Fell & Master) H. H. Ho & S. C. Jong
 3'. Spines long, 10–30 μm , distributed over whole surface of the zoosporangia or around papilla; saprophytic 4
 4. Zoosporangium globose to ovate or obovate *H. spinosa* (Fell & Master) H. H. Ho & S. C. Jong var. *spinosa*
 4'. Zoosporangium multi-lobed or ovate *H. spinosa* var. *lobata* (Fell & Master) H. H. Ho & S. C. Jong
 5. Vesicle present 6
 5'. Vesicle absent 9
 6. Sporangial closure material including a solid plug (dehiscence plug) visible after ejection and persistent longer than 3 h *H. masteri* Nakagiri & S. Y. Newell
 6'. Sporangial closure material not including a solid plug (dehiscence plug-like material) vanishing at ejection 7
 7. Zoosporangiophore proliferating sympodially *H. vesicula* (Anastasiou & Churchl.) H. H. Ho & S. C. Jong
 7'. Zoosporangiophore branching irregularly 8
 8. Zoosporangium broadly elliptical to limoniform
 *H. batemanensis* (Gerr.-Corn. & J. A. Simpson) H. H. Ho & S. C. Jong
 8'. Zoosporangium polymorphic, frequently with a hollow lateral process
 *H. polymorphica* (Gerr.-Corn. & J. A. Simpson) H. H. Ho & S. C. Jong
 9. Dehiscence plug visible after ejection 10
 9'. Dehiscence plug-like material liquescent, vanishing at ejection; zoosporangium sometimes externally proliferating
 *H. exoprolifera* H. H. Ho, Nakagiri & S. Y. Newell
 10. Dehiscence plug short, 3–7 μm , extruding through exit pore and protruded end ragged in appearance, semi-persistent less than 1 h after ejection *H. bahamensis* (Fell & Master) H. H. Ho & S. C. Jong
 10'. Dehiscence plug long, 10–50 μm , extruding through exit pore and protruded end convex, persistent at least several days *H. epistomium* (Fell & Master) H. H. Ho & S. C. Jong
 10". Dehiscence plug intermediate, 10–22 μm , extending through exit pore and protruded end disintegrating or "cigarette-ash" shape, evanescent 1–5 min after ejection *H. tartarea* Nakagiri & S. Y. Newell
-
11. Vesicle present 12
 11'. Vesicle absent; zoosporangium operculate *H. operculata* (Pegg & Alcorn) H. H. Ho & S. C. Jong
 12. Vesicle spherical, retaining zoospores inside for a while before release; zoosporangium operculate
 *H. kandeliae* H. H. Ho, H. S. Chang & S. Y. Hsieh
 12'. Vesicle cylindrical, extending at once for zoospore release; zoosporangium inoperculate 13
 13. Vesicle with a small, spherical annexed vesicle at the apex; dehiscence plug-like material cupulate
 *H. avicenniae* (Gerr.-Corn. & J. A. Simpson) H. H. Ho & S. C. Jong
 13'. Vesicle without an annexed vesicle; dehiscence plug-like material lens-shaped
 *H. porrigovesica* Nakagiri, Tad. Ito, Manoch & Tanticharoen

After alignment of the edited sequences with ClustalW and MacClade, phylogenetic analyses were conducted in MEGA4, Neighbor-Joining method (1) the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (2)

- 1. Saitou N & Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
- 2. 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°

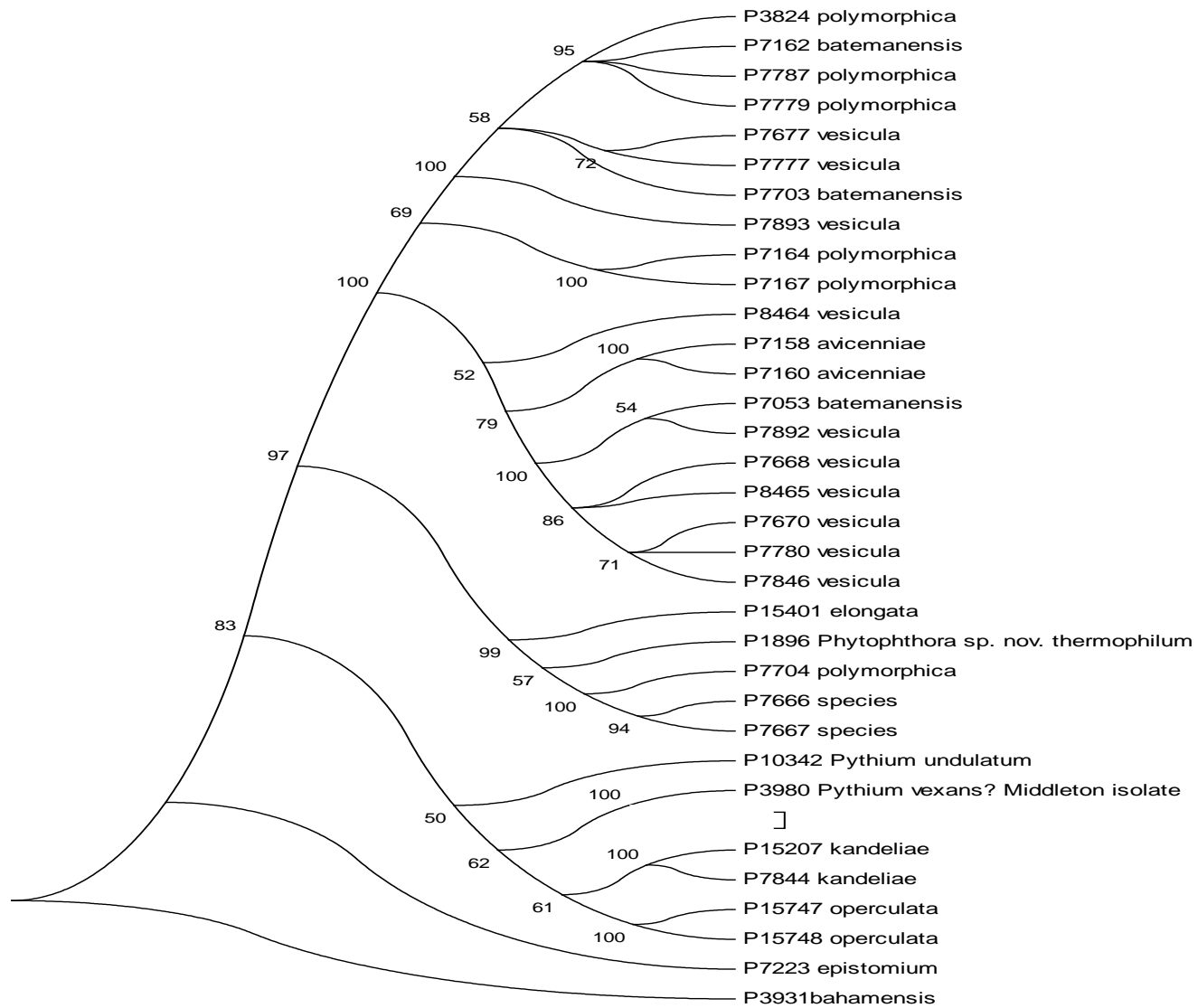
30 sec at 94°

40 sec at 56°

1 min at 72°

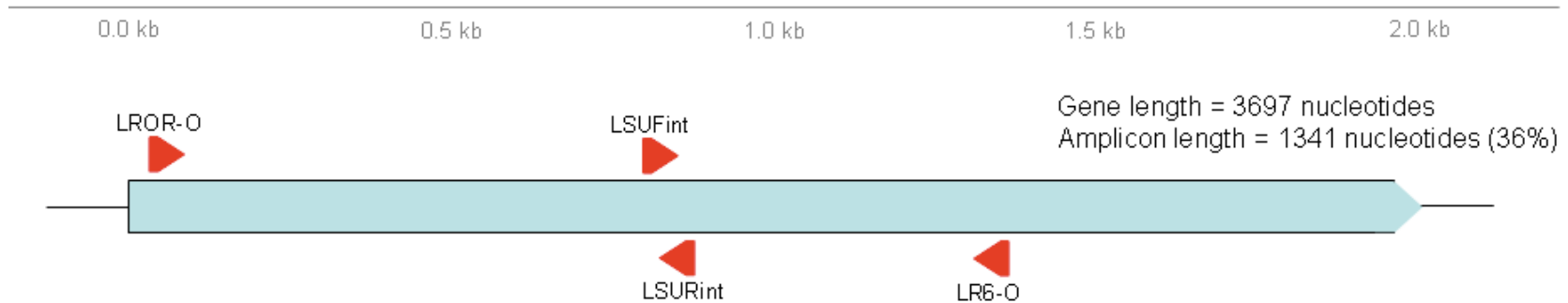
(repeat steps 2 - 4 35 times)

5 min at 72°



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

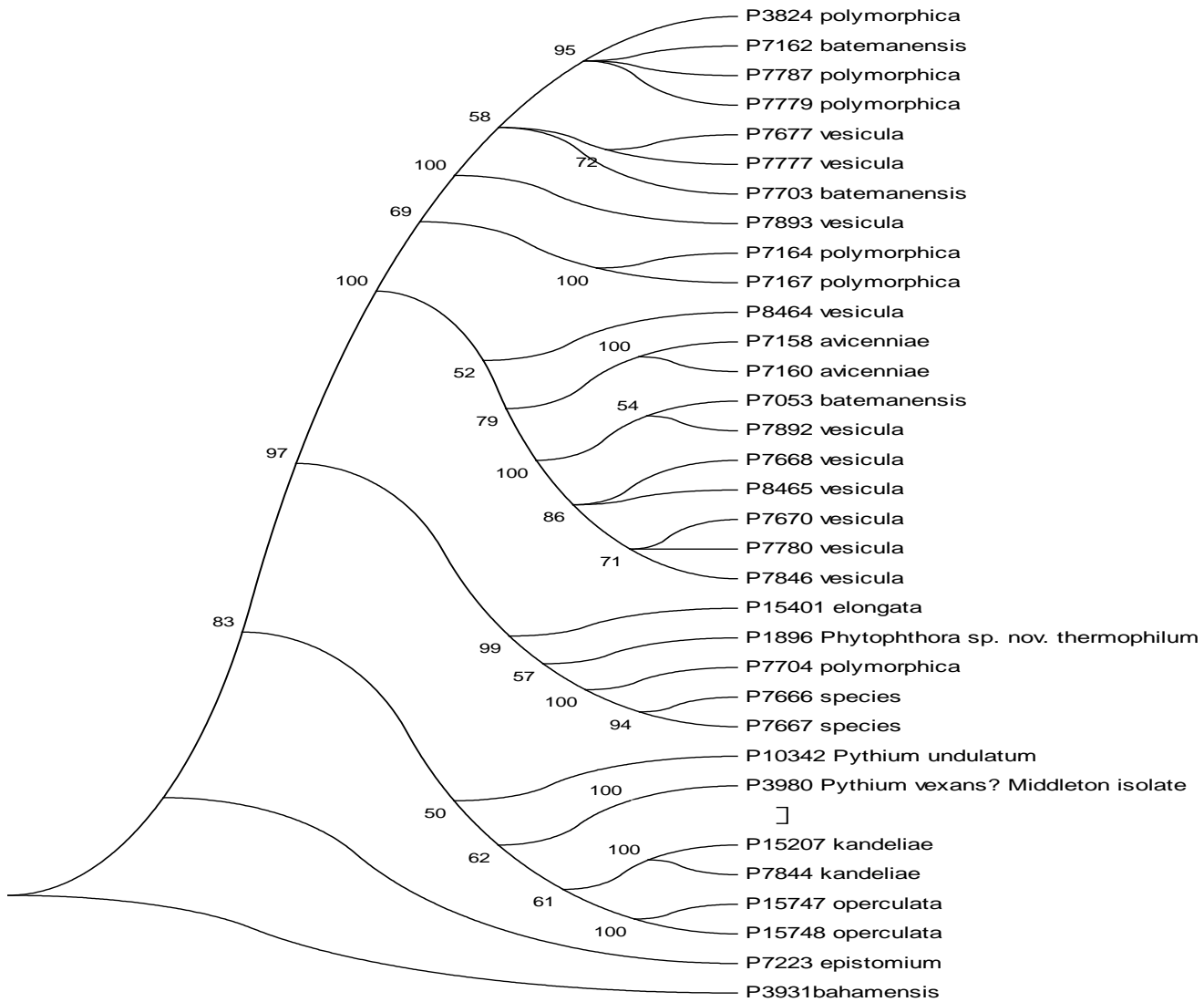
*Primer position relative to the sequence of *P. megasperma* X75631 from NCBI

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

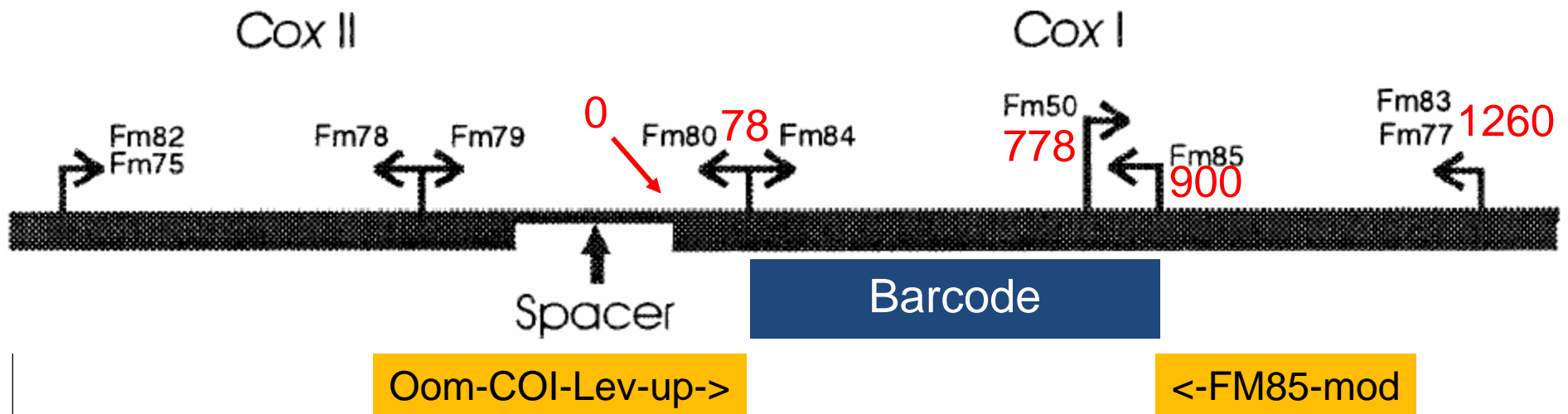
Program:

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

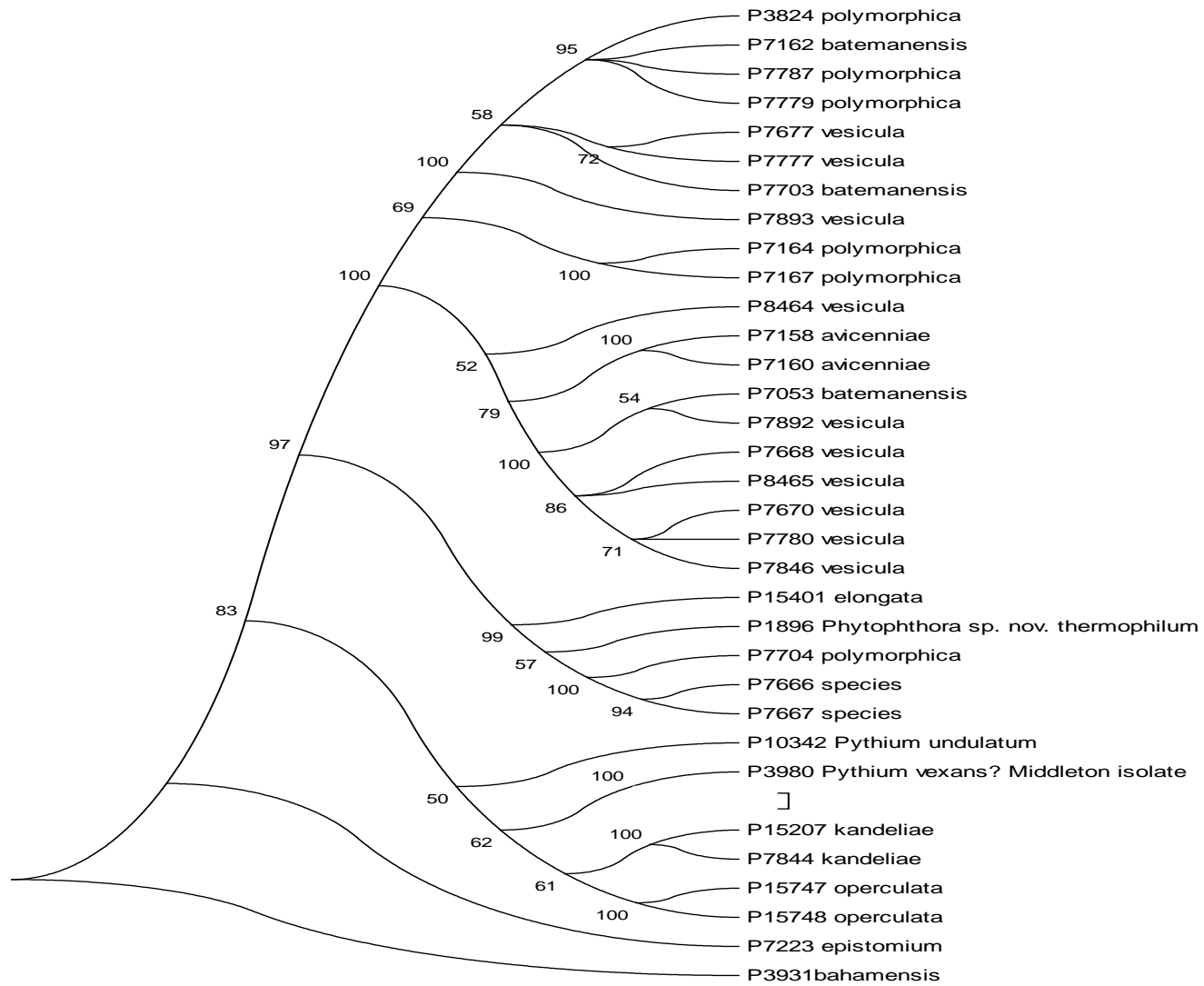


COXI barcoding

- 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

BLAST SEARCHES

with some ITS sequences

WPC11614B1171kandeliae ITS

Pythium mercuriale strain STE-U 6124 clone 4 internal transcribed spacer 1, 65%

Pythium mercuriale strain STE-U 6204 clone 0 internal transcribed spacer 1, 68%

Pythium sp. Py292 internal transcribed spacer 1, 68%

```
CCACACCTAAAATCTTTCCACGTGAATTGTTTGGACAACGTTGGGCTTCGTTGGCGGCTTCCCTTGTGGTTGTCTG
TCGGCTTGAGGCTATCAGATGGCTTTGTTGCTTGGTTGGTTGATTGGTTGATTGGTTGCTCCGTCAATCGGTCGTT
CTCGAACGAGTGATAAAGCGGTCTTTTTGTAAACCCATTGTGATATTGAAACTGATTGTACTGTGGGGACGAAAGT
CTCTGCTTTGAACTAGATAGCAACTTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTG
CGATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTGAACGCATATTGCACTTTTCGGGTTTTGCCTG
GAAGTATGTCTGTATCAGTGTCCGTAGACTAAAGTTGCCTTTCTTGCGTTCGTGTAGTCGTTCGGTTGGAAAGAGCAG
ATGTGAGGTGTCTTGCGCGGTCTGCATGGCTTGGCTCCGGTCGGGTCTGTTTGTTCGCAAGTCCCTTTAAAG
TCGGACGTGTTTCTCTGTGTGGTGCAGCGGTGACGGATCTGTGGACGTGCGTGCCTGCGGGACGACAATGTTGTG
CGCGGACGAGTCTGGCGGTCTTTGGTGCTTGCCTGTTGGGATTCCTCGATTGGCGGTATGTTTGGCCTCGGCTT
TGACAATGCAGCTTATTGGGTGTTTCTCTGCGCGGCTTGTGCTGTATGAAGACGAACCGGATGGTCTGTGTGTGTG
CGTTGTGTGTGTCTGTGCGTTTCGTTTCGTTTGCAGAGCCGTTTGTATGCGTGTGTCATGCATGGAGCGTGGTATA
TTTGGGAAGTTGTGCTGGTGTTCGGCTTTTCGGGTTCGGTGTGAGCATCTCAA
```

Pythium mercuriale sp. nov. (Pythiaceae) is characterized by forming thin-walled chlamydospores, subglobose to obovoid, papillate sporangia proliferating internally and smooth-walled oogonia surrounded by multiple antheridia. Maximum likelihood phylogenetic analyses based on both ITS and beta-tubulin sequence data place *P. mercuriale* in a clade between *Pythium* and *Phytophthora*.

FEMS Microbiol Lett. 2008 Jul;284(1):17-27.

Intraspecific and within-isolate sequence variation in the ITS rRNA gene region of *Pythium mercuriale* sp. nov. (Pythiaceae).

[Belbahri L](#), [McLeod A](#), [Paul B](#), [Calmin G](#), [Moralejo E](#), [Spies CF](#), [Botha WJ](#), [Clemente A](#), [Descals E](#), [Sánchez-Hernández E](#), [Lefort F](#).

WPC15750C1315mycoparasitica_ITS

Pythium sp. F-1326 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, 24%

Pythium erinaceum strain F1502.1 internal transcribed spacer 1, 24%

Pythium sp. F-42.1 18S ribosomal RNA gene, partial sequence; Internal transcribed spacer 1, 24%

```
CCACACCTAAAAAATACCTTTCCACGTGAACCGTTTGTTTTACAGTTGTTTCATTGTCTGAGGAATGGGCCGATCTCTG
TCATGGAGTGACGCCTGCCTCGGATAGAGCGAAGGTTTTCTGCGTCTCTGTTGCTTTATTGGAACGGAGTGCGGAG
AGCTGATGTATACTTTTCAAACCCATTTTTACTATACCTGATATTTACTGTGAAGGAGAAATCCTTTGCTTTTACTAGATA
TACAACCTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTG
CAGAATTCAGTGAGTCATCGAGATTTTGAACGCACATTGCACTTTCGGGTTATGCCTGGGAGTATGTCTGTATCAGTG
TCCGTACCTCAAACCTCGCCTGTTTTTTCTTTTTTGAAGAGAAGCACGGCAAATGTGAAGTCTCTCATGCTTTTGGCC
TAGCCAAGGTGTGAGTGCTTTGAAATGGACACGATCTCTTTTGTGTGTGGAGAGTGATTTAGTGTGATTAGGCGTGT
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AAGCATATGGAGGAGGTTTCTAATCAAGTGGACTGCTTGTATTCGCGGTATGTTTGGTTCTTCACTGAACTTGACAGA
GTAGCGTTTTTGCAGCATCATAGTCTGCCTGTGACGGATCTTTTTCGCTTGTTCCTTGCAGAGCTGAGACGTGAATTG
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TGCTTGTGATGCATACGATTGTGGTCTCCAATTTTGAATCTTCTCTTTTTGTGAAGATCAACCCTTTTTTGGACCTG
ATATCAGACAAGATTACCCGCTGAATTTAAGCATATCATAANNCNGNAGGAA
```


***Pythium erinaceum* sp. nov. from wheatfields
in Canterbury, New Zealand
G. I. Robertson**

Abstract

Pythium erinaceum sp. nov., a species with spherical sporangia and echinulate oogonia, is described from soil from Canterbury, New Zealand. It is distinguished from similar *Pythium* species with echinulate oogonia by a slow growth rate, a distinctive rosette pattern of growth, and the production of oogonia bearing long, tapering acuminate spines.

New Zealand Journal of Botany, 1979, Vol. 17:283-6

WPC15747C1319operculata ITS

Pythium vexans isolate Pyv6-2 internal transcribed spacer 1, 66%

Pythium vexans isolate Pyv6-1 internal transcribed spacer 1, 66%

Pythium vexans ITS1, 5.8S rRNA and ITS2, isolate IFAPA-CH834, 66%

CCACACCTAAAATCTTTCCACGTGAACCGTTTTGTGTAATAGTTGGGCTTCGCTGCCTGGCAGTCTGCGCTCGCAGTCT
GTTGGAACGCTTGAGGCTATCAAGTGGGTGTGTGGTCTGTCGCTCTCGGGCGCGGGTCGCACTTCCGCTTCTGTTTGA
ACCCATACTTAACTTCTGAACATACTGTGGGGACGAAAGTCCTTGCTTTGAACTAGATAGCAACTTTCAGCAGTGGATG
TCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGGATTGAGTGCATCGAAC
TTTTGAACGCATATTGCACTTTCGGGTTATGCCTGGAAGTATGTCTGTATCAGTGTCCGTAACTCAAACCTTGCCTTTGTTT
TGTCGTGTAGTCGACGGAATGGACGGCAGACGTGAAGTGTCTTGCGTGGTGGCCGCTTGTGCGGCACGCTGTGCAAGTC
CTTTTAAATGTTGGACGTGATATCTGTATGTGTCGTTTGCTGTTTGTGTCTGGCGTGTCTGCGGGCGTGTGCGGGTGACCT
TTGGCGATGACATTGGATATATGCTCAATAGGCGGTATGTTAGGCTTCGGCTTTGACAGAGTAGCTTGTTGTGGCGTGTG
TCTGGTTGTTGCTGTATGGGTGAGCTCGTGGTGGTGGATGTCGTTAGTGTGTGAACGGTGATTGTCGCATGTAGTGTGT
GTCCGTTGAATTGGGAAGTTGTGCTTTGCTCTTGTGAGCTAGCATCTCAA

WPC15165B1830porrigovesica ITS

Phytophthora richardiae genes for ITS1, 5.8S rRNA, ITS2, complete sequence, 67%

Phytophthora richardiae genes for ITS1, 5.8S rRNA, ITS2, complete sequence, 67%

Phytophthora richardiae internal transcribed spacer 1, 67%

```
CCACACCTAAAACTTTCCACGTGAACTGTGTTTTGTTGTTTGGGGCGTGCCGCTTCGGCGGGTGGCCCTTG
GCGGGAGGCTAGTCCTCCTGCTTGTTGTAAACCCTTTTGAATTTGTCTGAACGTA CTGCGGGACGAAAGTCTC
TGCTTTGAACTAGATAGCAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGAAGAACGCTGCGAACTGC
GATACGTAATGCGAATTGCAGGATTCAGTGAGTCATCGAAATTTTGAACGCATATTGCACTTCCGGGTTATGCCTG
GGAGTATGCCTGTATCAGTGTCCGTACATCACTCTTGGCCGCCTTGCTCCCGTGTAGTCGGGTGTGGGTGGTGC
CAGATGTGGGGCGTCTTGTGTGCGTGCGCGCGCTTTGCGCGTGCATGCGGGCGAGTCCCCTGAAATGTCCG
ACCTGTGTGTCTCTTTGCCTGAAAAGCTGTGGCGGATGCTGTTGTGGAGGCTGCCTGTGTGGGCGGTGAGCGA
CGTATCTCGTGTTCGGGGCGCTGGCGAAGGAGTGGTCTATTCGCGGTATGGTTGGCTTCGGCTGAACAATCTGCT
TATGGGTCGTTTCTTGGCTGTCCTGGCGTTATGCGGGGTGTGTGGCTTGCCTGCGCGTTGGCGTGGCTTCTG
AGCGGTTTGTTCGCGTGAGCGTAGTAGGGCGGTCTCGTGGCCGAGCACGGTCTCCA ACTTTGGGAAGCTTGT
GTGCCTCGTGTGCGCATCTCAA
```

Phylogenetic trees generated with MEGA 4
for ITS, LSU and COX1
with 30 accessions of 9 *Halophytophthora*
species produced similar preliminary
results

Different *Halophytophthora* species
showed close phylogenetic relationships
to either *Phytophthora*, *Pythium* or
Phytopythium species

Work in progress

ITS, β -TUBULIN, LSU and COX1
barcode sequences
are being generated for
the 15 species of *Halophytophthora*
represented by 65 accessions

Representative species of
Phytophthora, *Pythium* and
Phytopythium are being included