Molecular taxonomy of recently described *Phytophthora* and *Pythium*

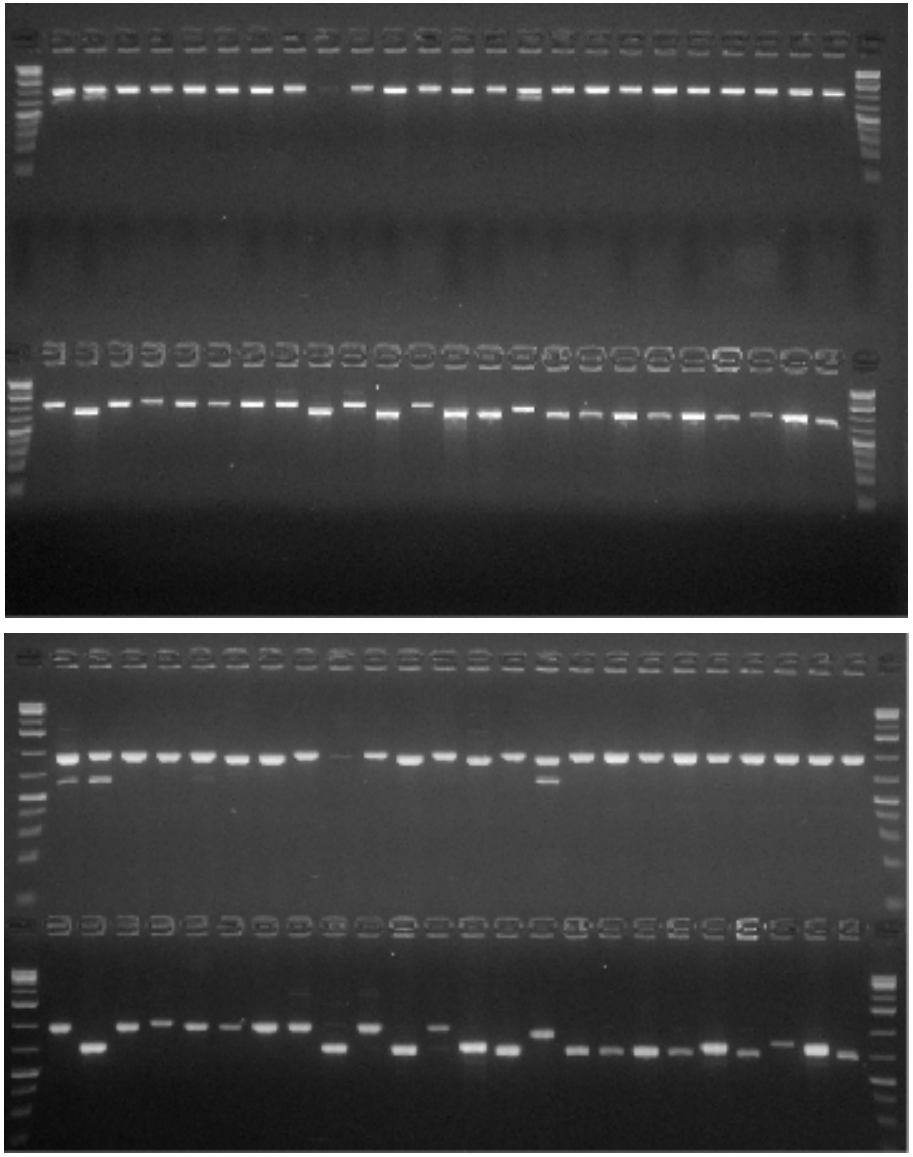
Dr. Lassaad Belbahri

Declining Polish forests

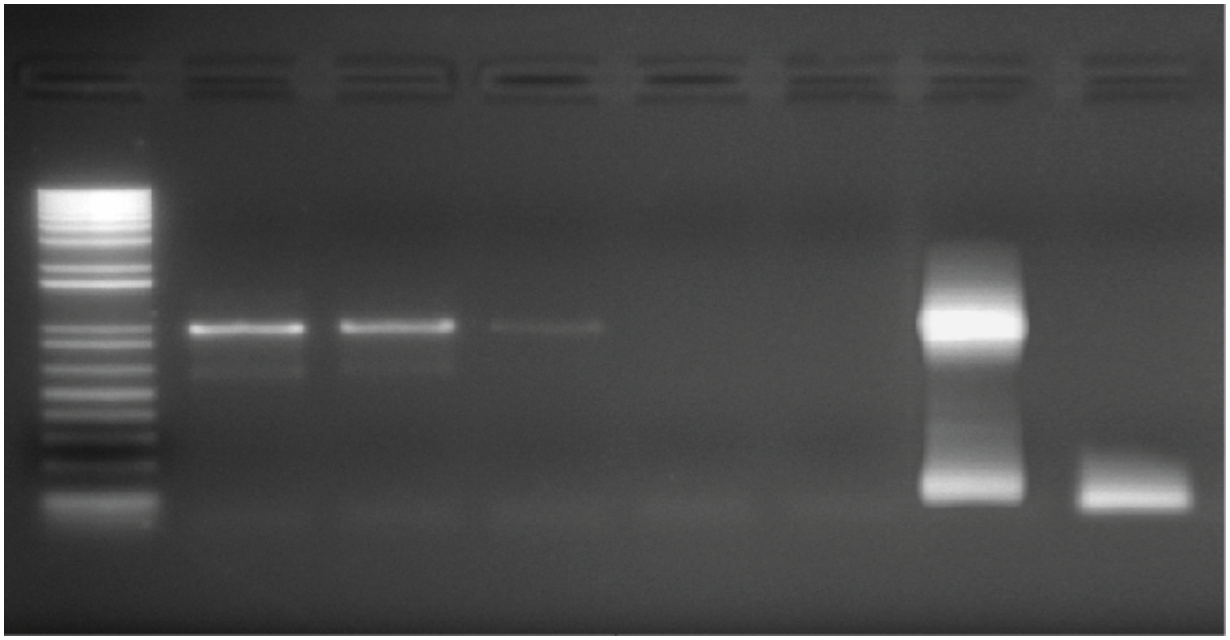
***Alnus glutinosa*, Kolo, Poland.**

Direct PCR, DNA barcoding

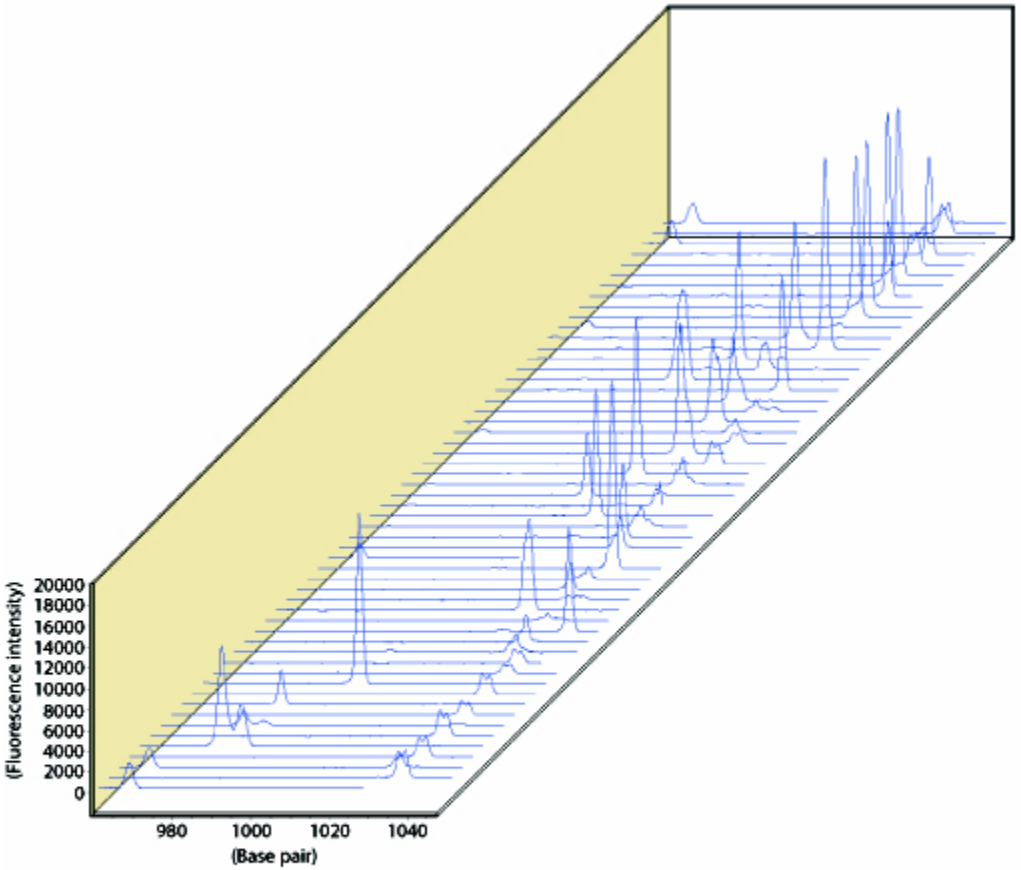
Collaboration with FASTERIS SA (Geneva)

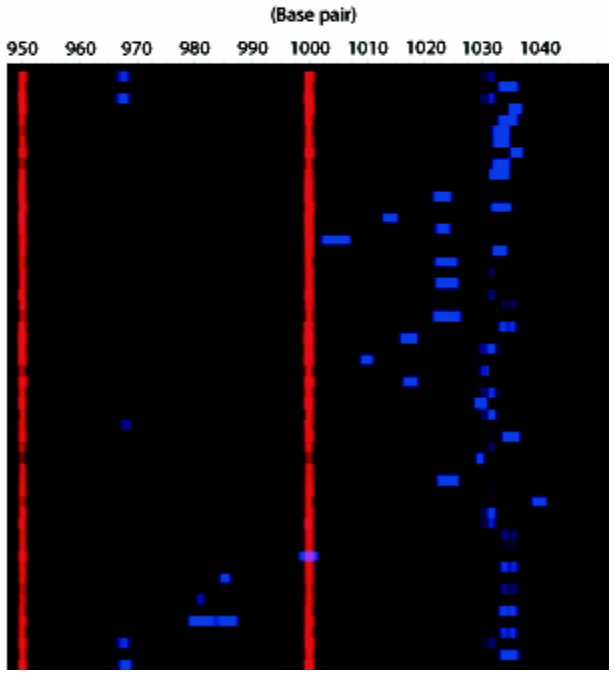


Single tube nested PCR for sensitive detection of oomycetes

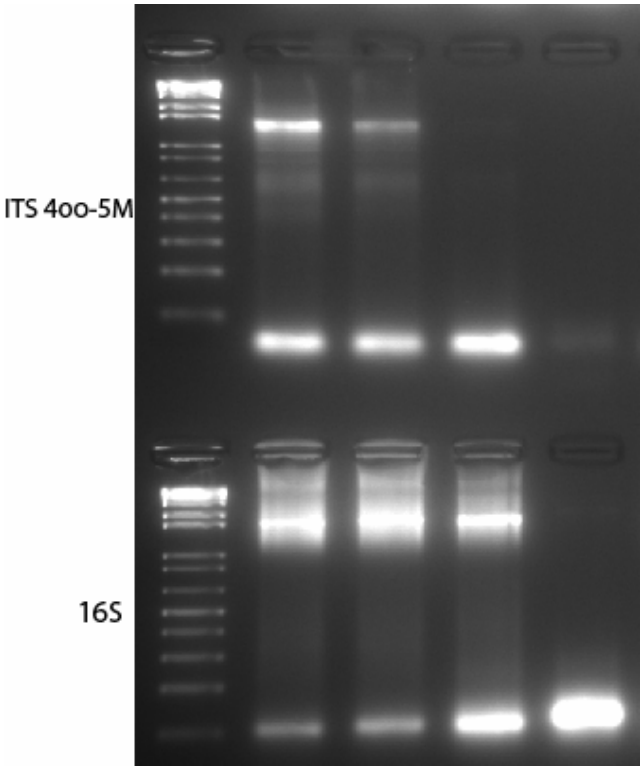


ARISA Analysis of oomycete infected *Rhododendron* leaves

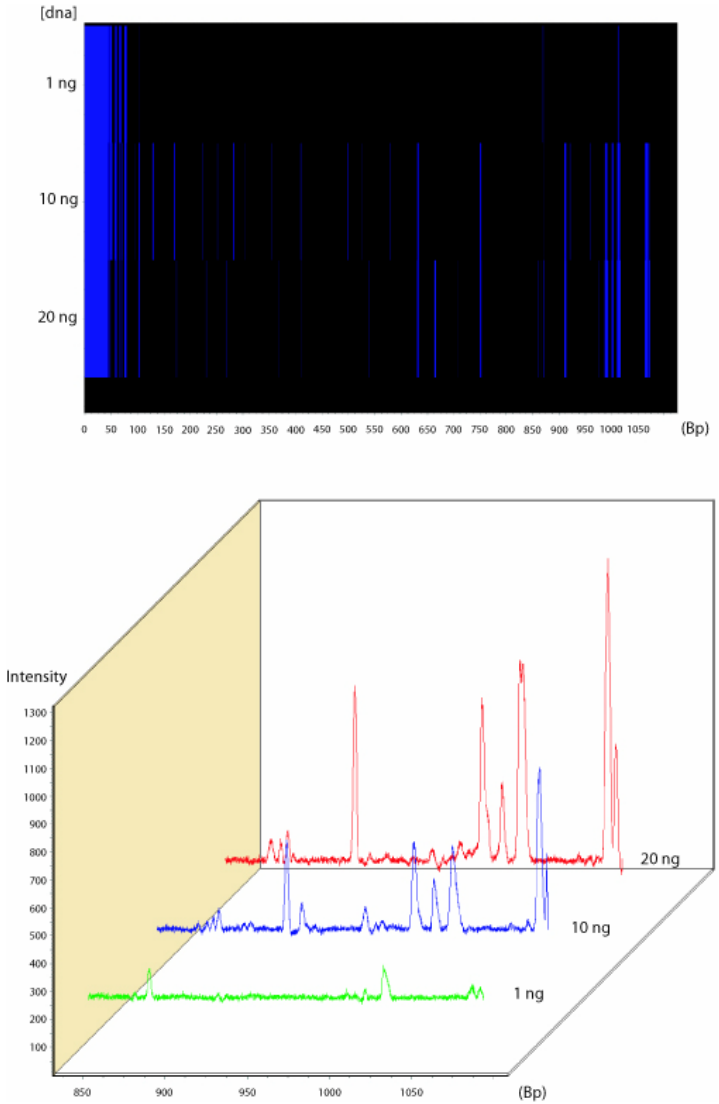




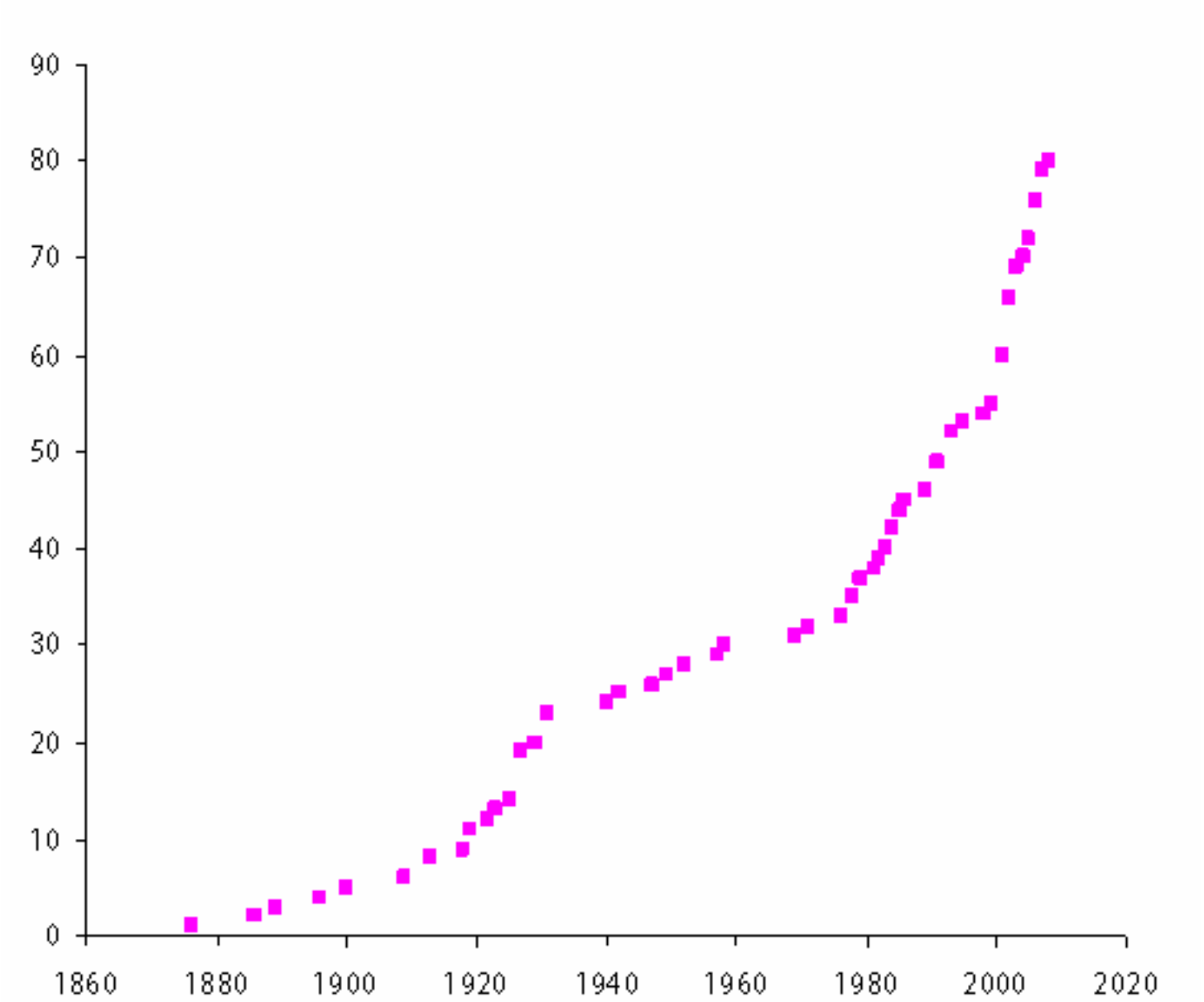
ITS rDNA PCR Amplification of oomycete community from soil DNA



ARISA analysis of oomycete community from soil DNA

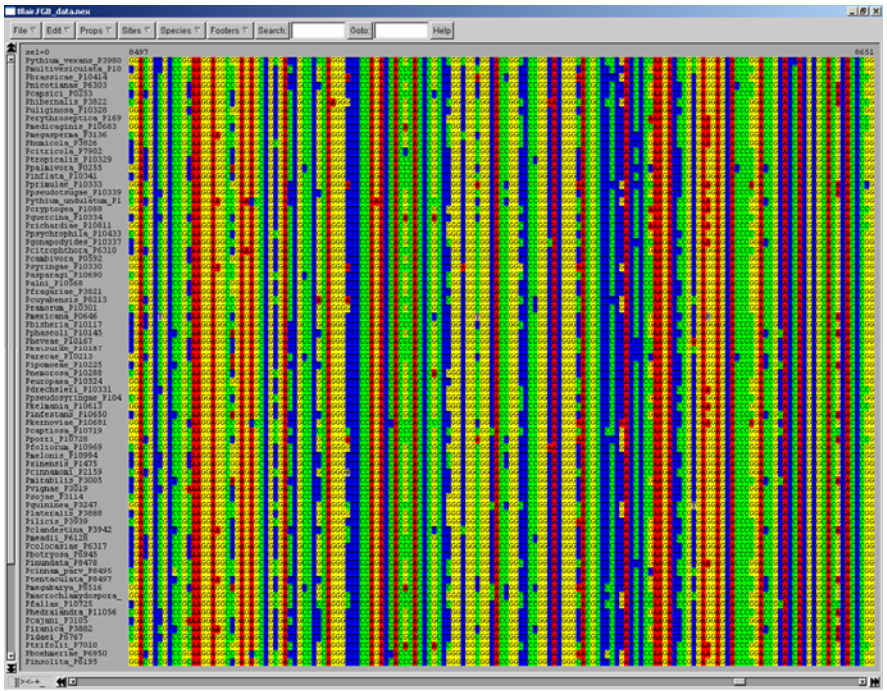


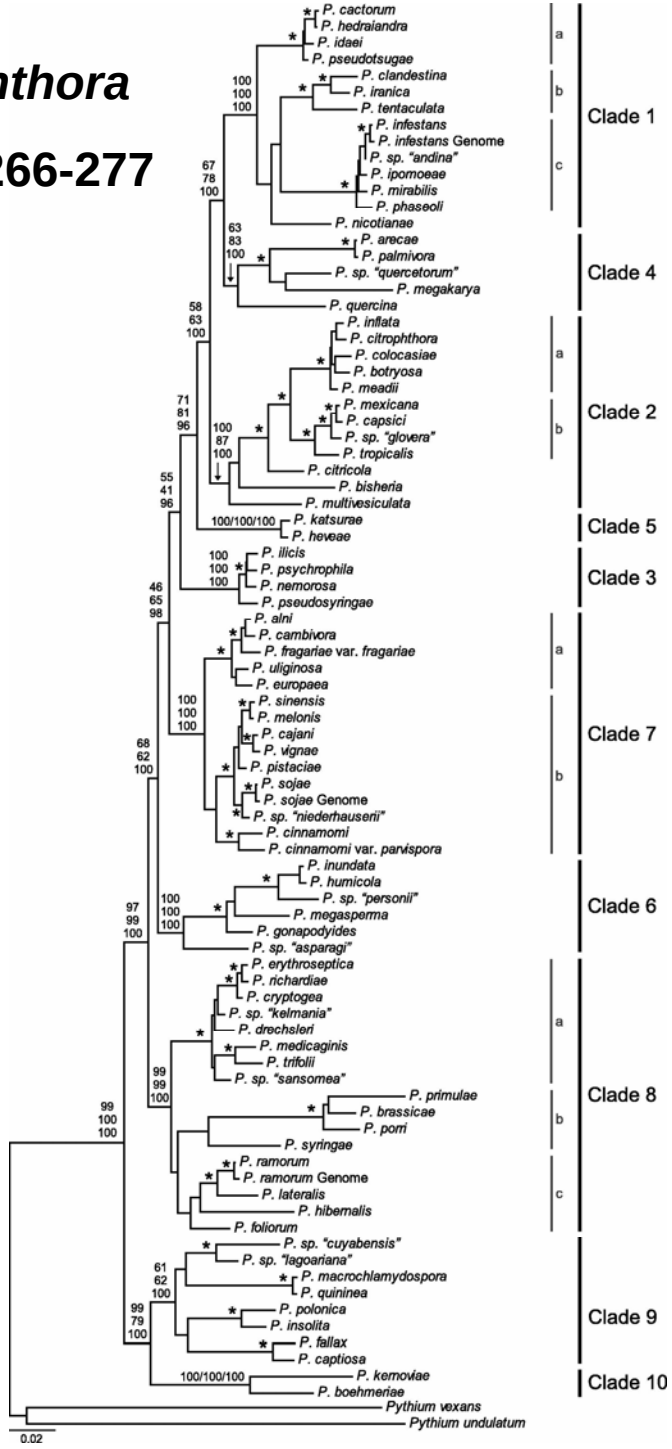
Evolution rate of new species discovery during time



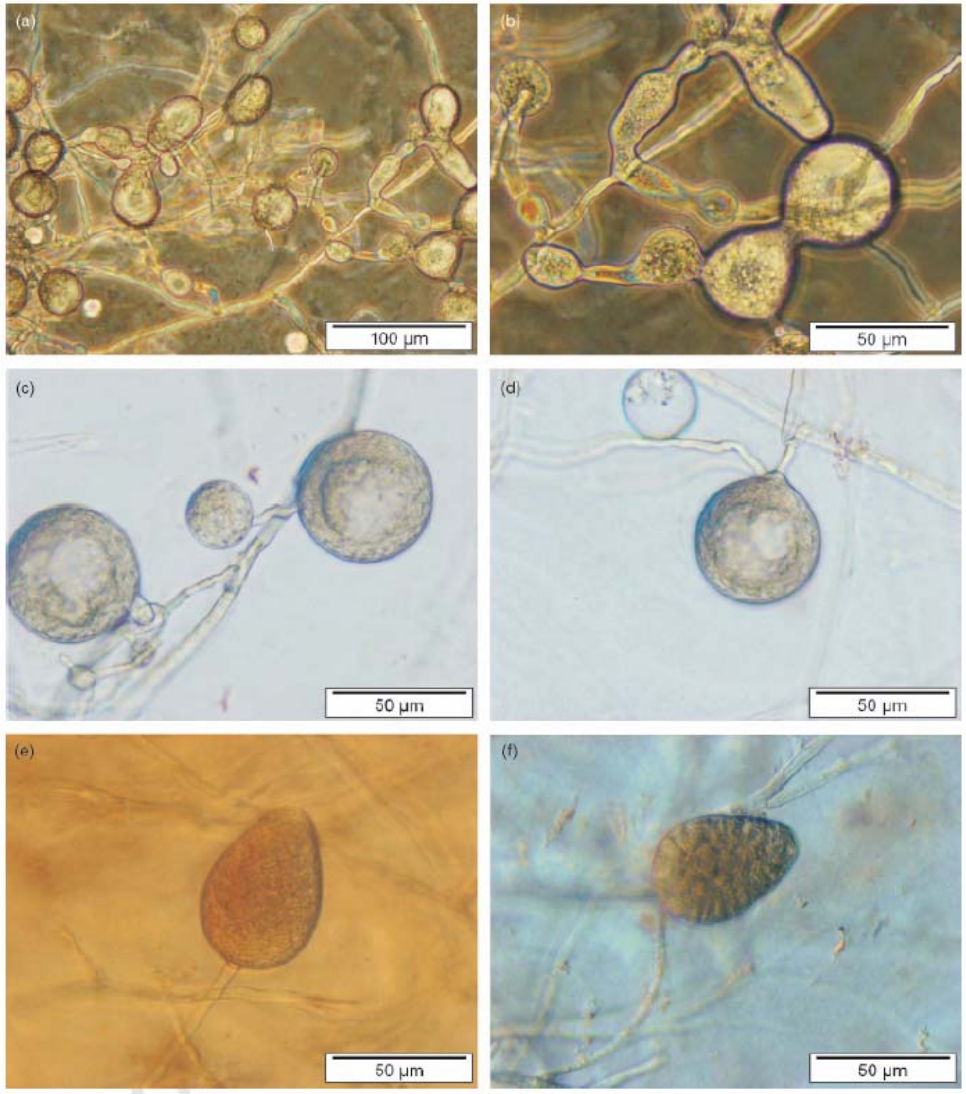
Genomic assisted Phylogeny in the genus *Phytophthora*

Blair *et al.* (2007) *Fungal Genetics and Biology* 45 266-277



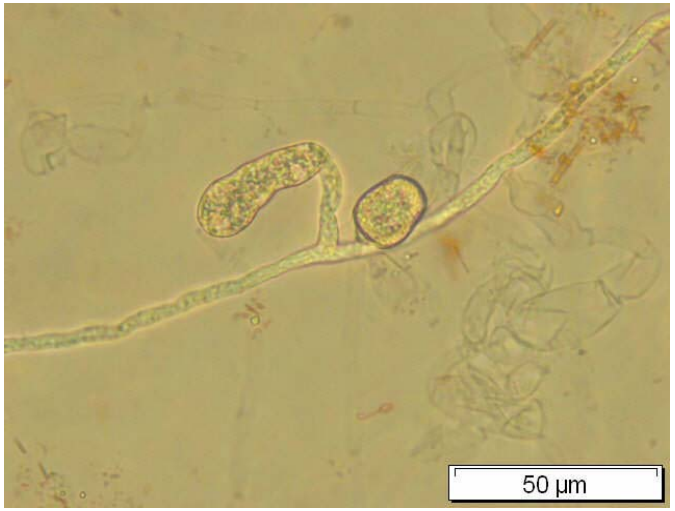


Morphological description : *Ph. polonica*



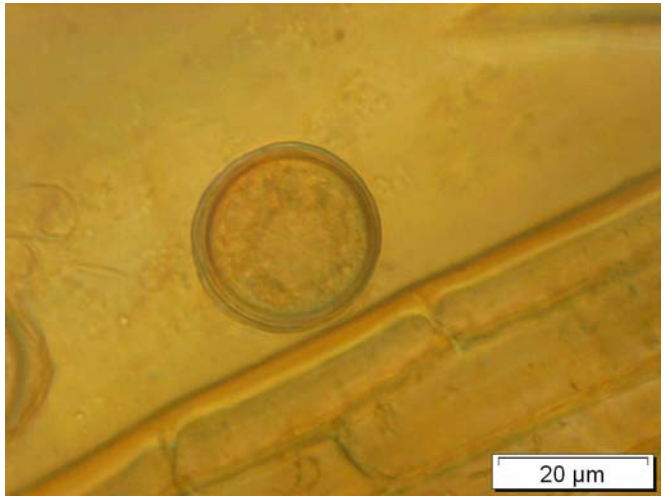
Morphological description: *Py. recalcitrans*

Hypha and hyphal swellings



Appressoria

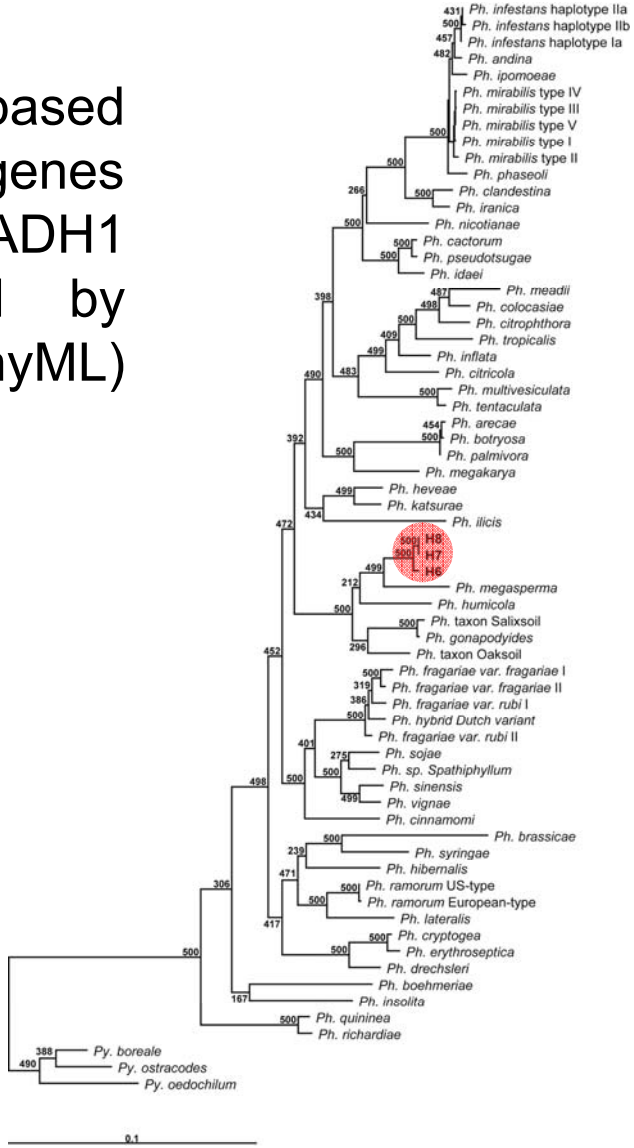
Antheridia



Oospore

Phylogenetic analysis of *Phytophthora* spp.

Phylogenetic analysis based on four concatenated genes (ß-tubuline, EF1-α, NADH1 and COX1), induced by Maximum Likelihood (PhyML)

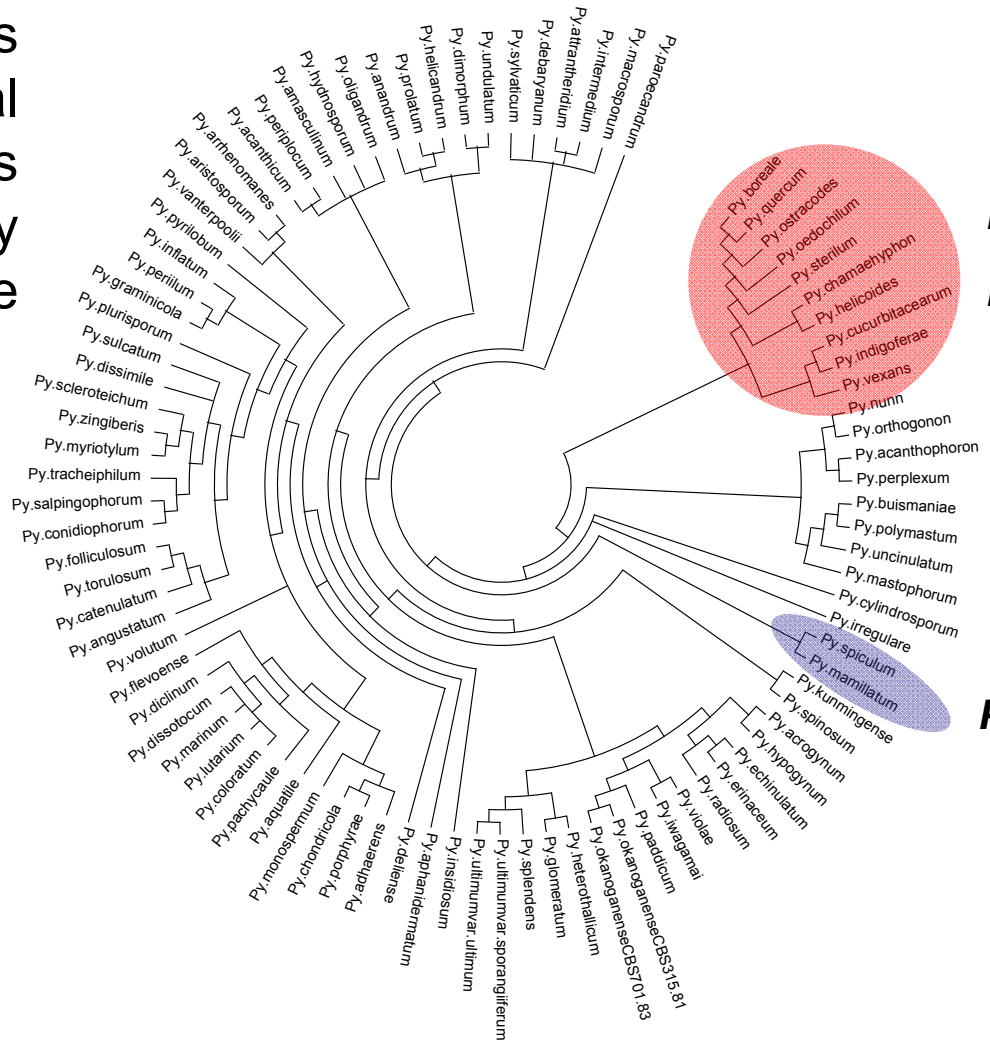


***Ph. sylvatica* sp. nov**

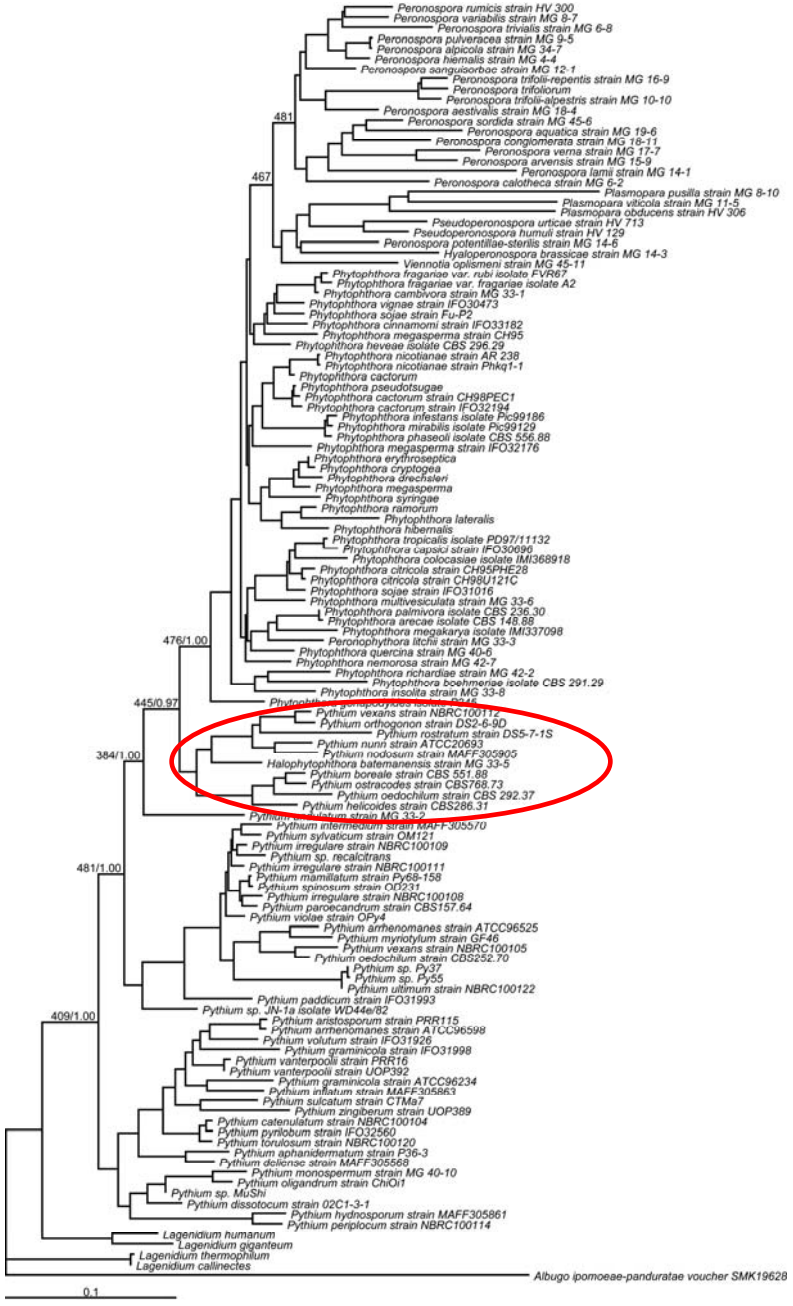
***Ph. hungarica* sp. nov**

Phylogenetic analysis of *Pythium* spp.

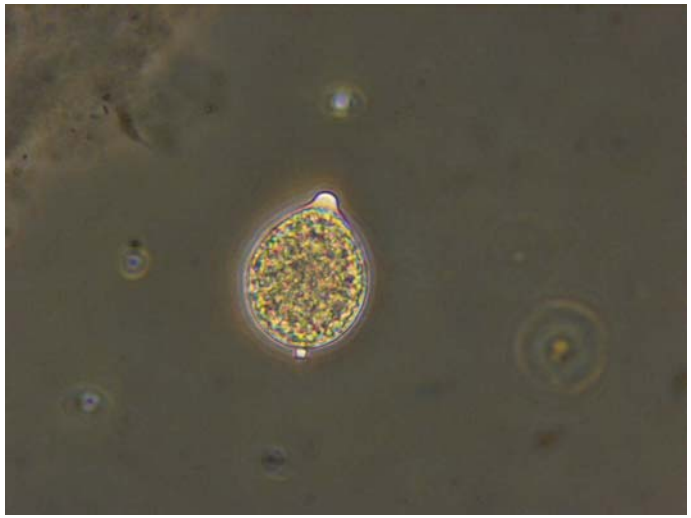
Unrooted phylogenetic analysis based on Internal Transcribed Spacers 1 and 2 induced by Bayesian Inference (MrBayes v.3.1.2)



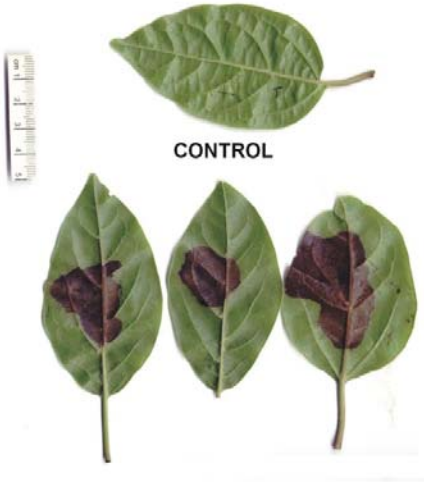
Towards a redefinition of oomycete genera



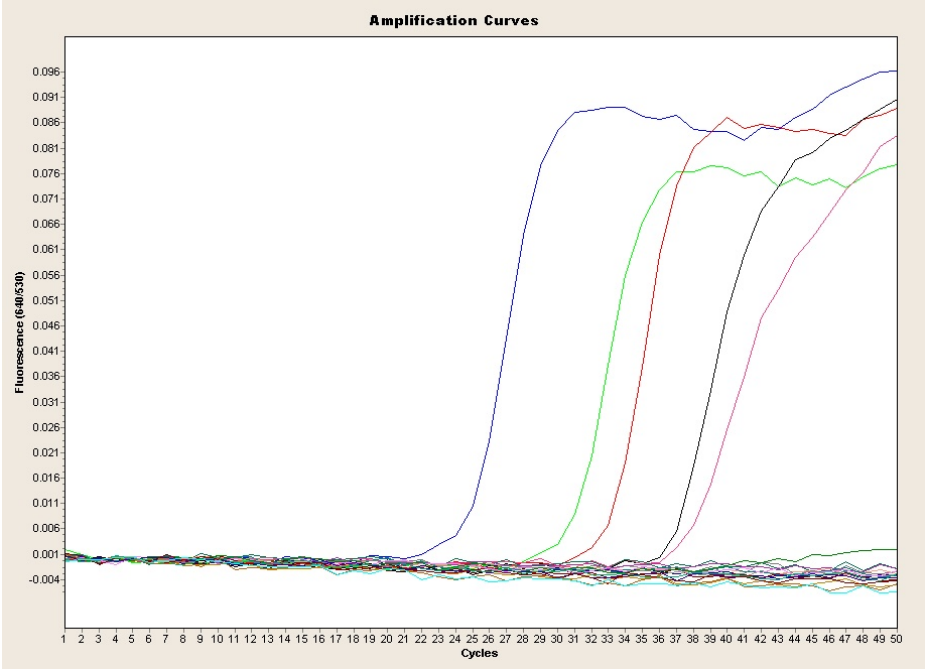
Ph. hedraiandra

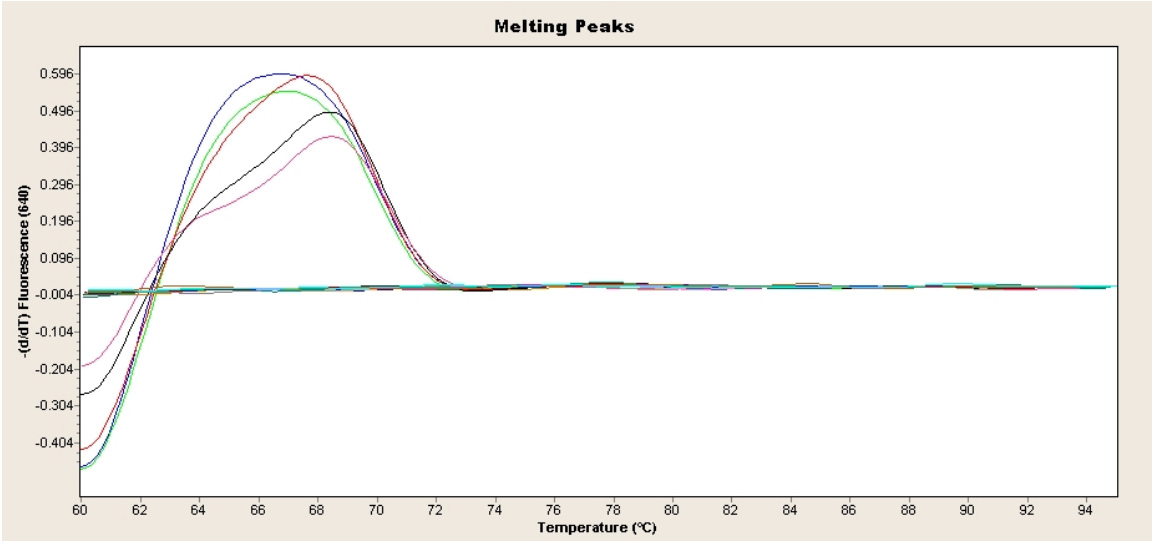


Ph. hedraiandra, a species with high hybridization potential spreading on multiple hosts.

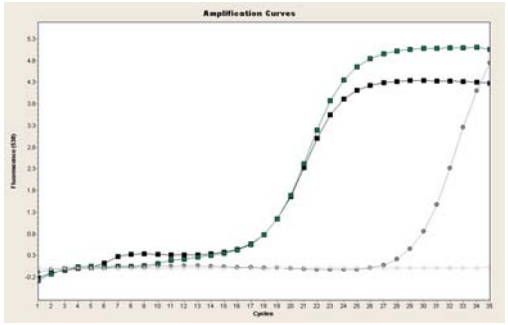


Ph. ramorum detection by real time PCR





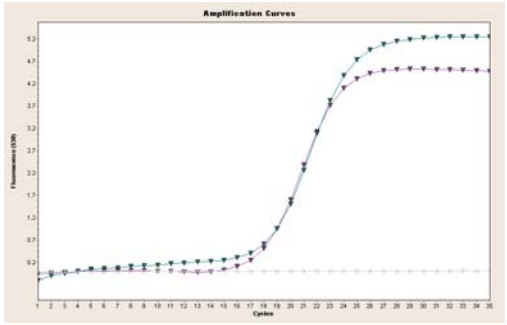
Ph. alni detection by real time PCR



Ph. alni subsp. alni

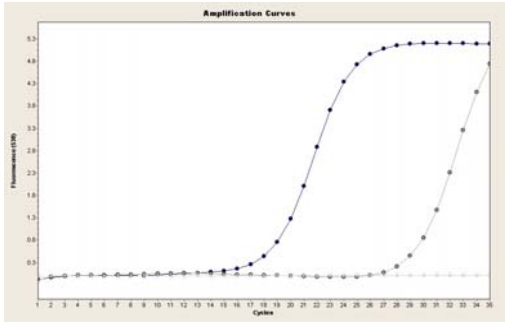
Ph. alni subsp. multiformis

Ph. alni subsp. uniformis



Ph. alni subsp. alni

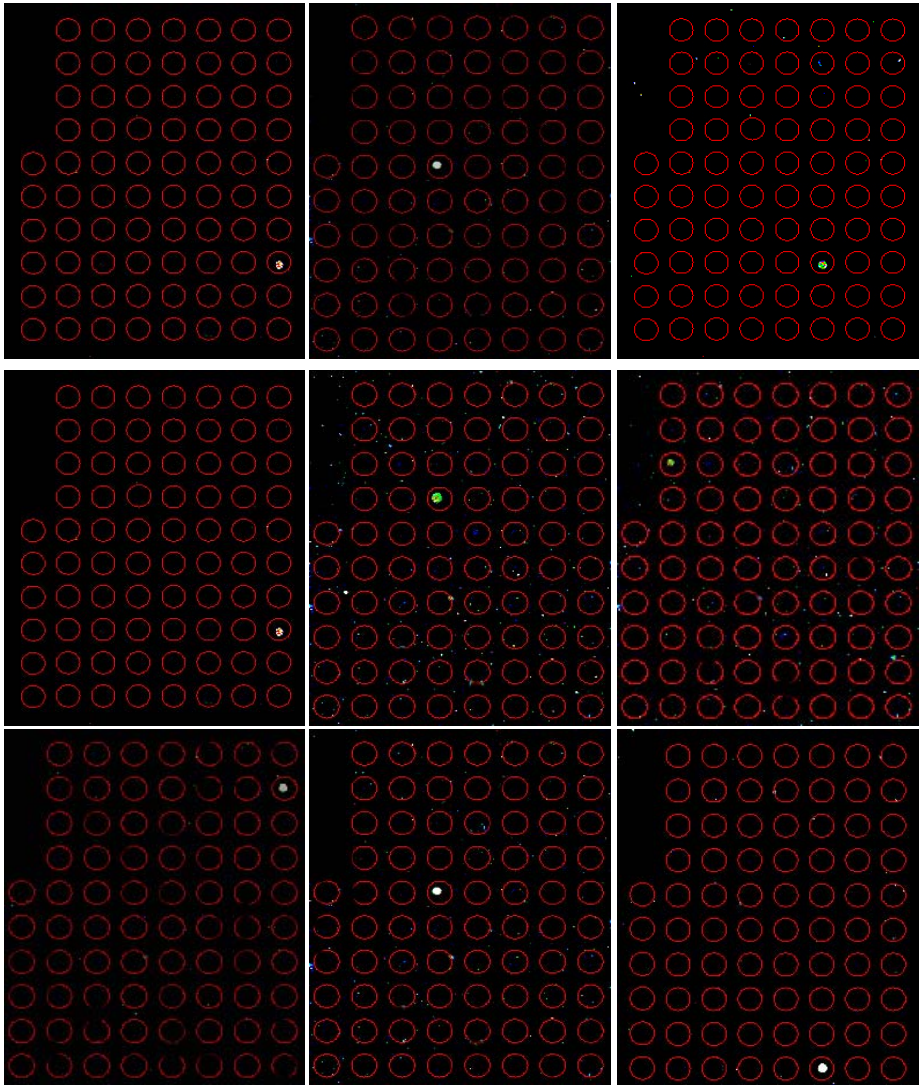
Ph. alni subsp. multiformis



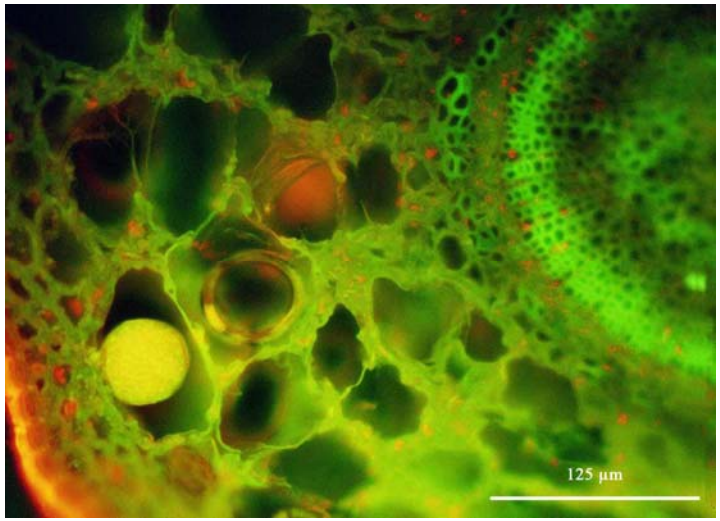
Ph. alni subsp. alni

Ph. alni subsp. uniformis

Detection of Py. spp. and *Ph.* spp. by microarray

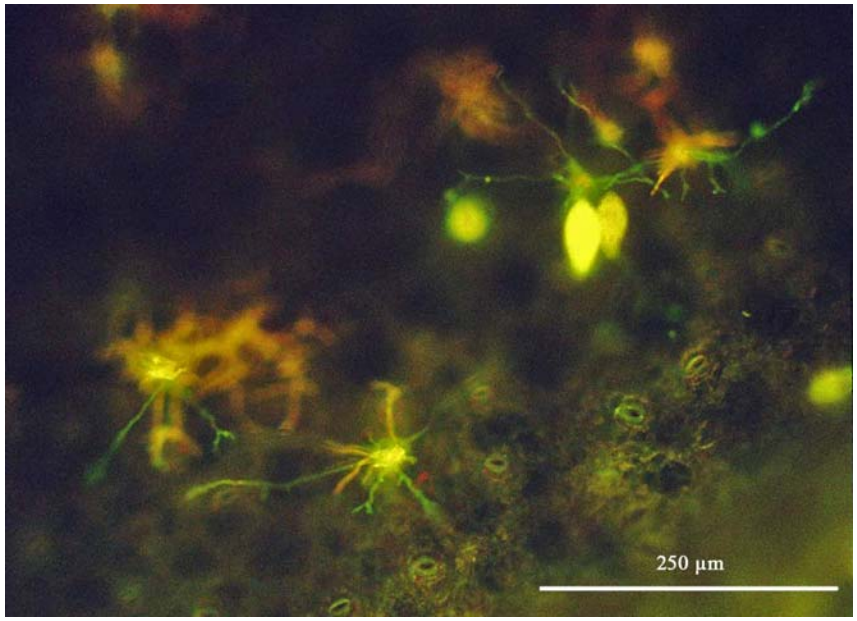


Ph. ramorum, tissue colonization

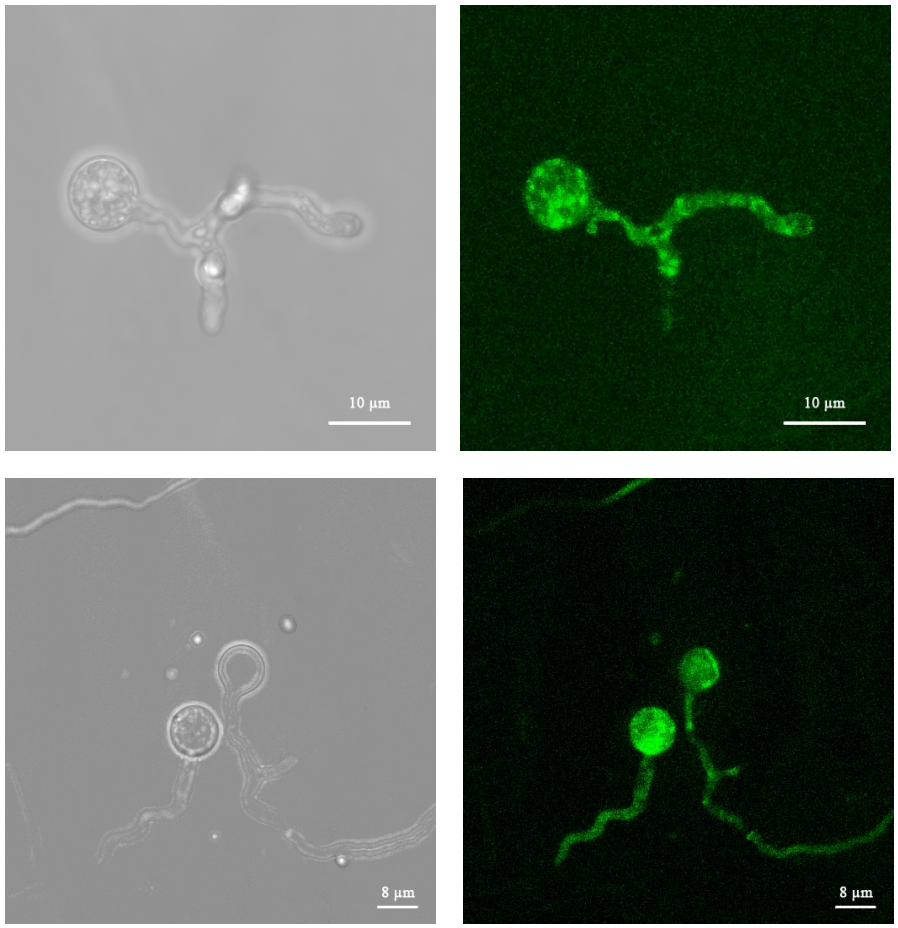


P. ramorum in *Rhododendron* cortex tissue, chlamydospores with white-yellow autofluorescence in cortex of necrotic *Rhododendron* stem

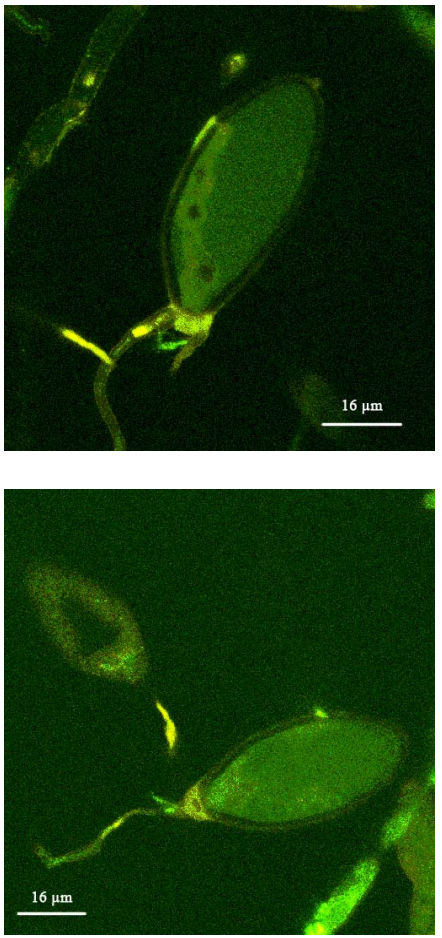
Tissue of infected *Rhododendron* leaves with *Ph. ramorum* structures, hyphae with zoosporangia growing out of stomata on discolored
leaf surface (vital staining with FUN® 1, Molecular Probes), zoospore production is induced, when infected
leaves were moistened and kept at low temperatures (7°C)



Ph. ramorum, Genetic transformation

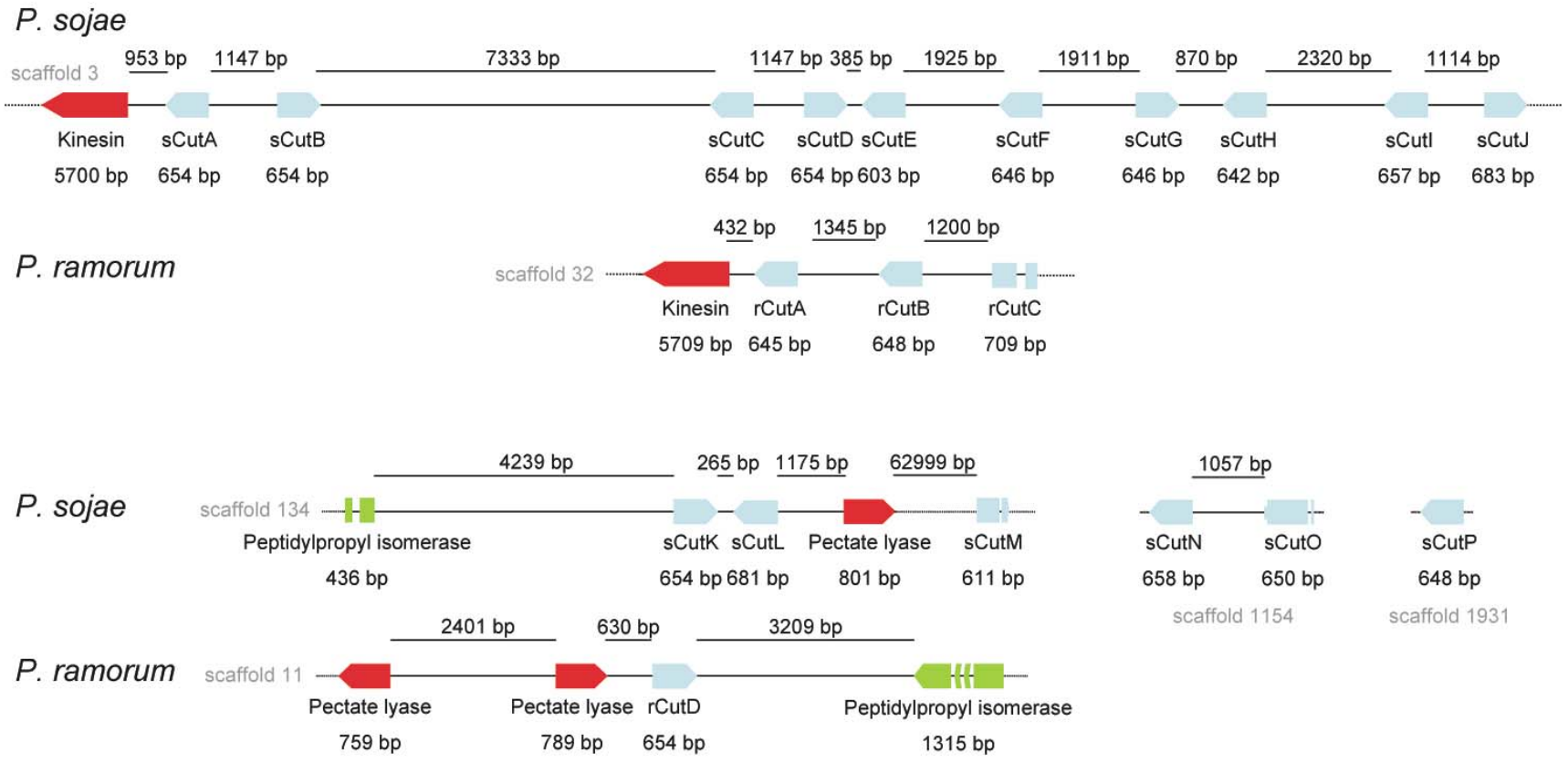


Transgenic *Ph. ramorum* strain BBA26/02-4: CLSM micrographs indicating GFP expression in germinating cysts.
Left: transmission image
Right: GFP signals

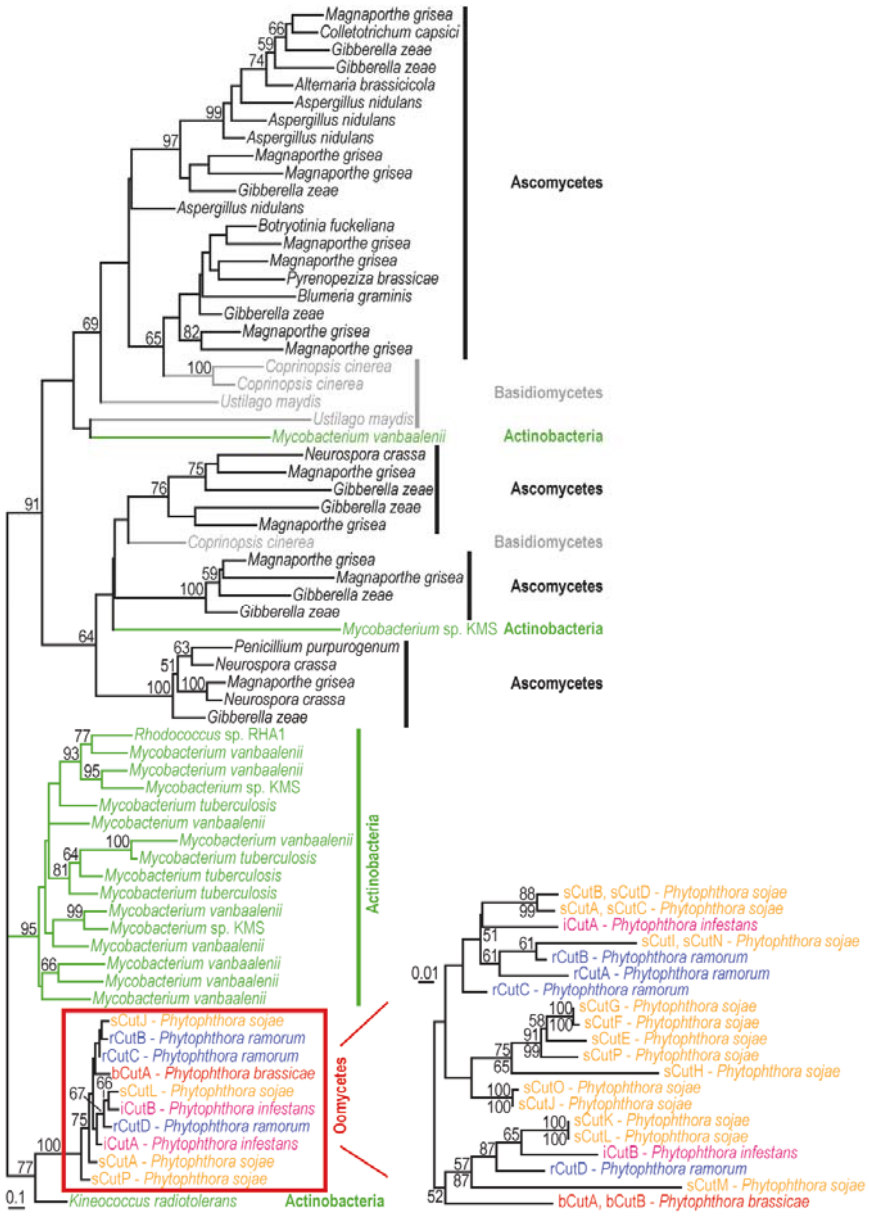


Transgenic *Ph. ramorum* strain BBA9/95_6G: CLSM micrographs showing GFP expression in protoplasm of zoosporangia and hyphae, yellow signals: natural *Ph. ramorum* autofluorescence in some hyphae, sporangium wall, base and pedicel, GFP signals were amplified with Alexa Fluor 488®labelled anti GFP antibodies (Molecular Probes)

Genomic organisation of virulence factors in oomycete genomes



Evolutionary lines of oomycete virulence factors



Outlook

Diversity of oomycete species using non culture based techniques

Reinforcing environmental monitoring tools (Molecular toolbox)

Evolutionary history of main virulence factors

Linking genetic phylogeny to morphology

Special Thanks To:

Steve Woodward

Eduardo Moralejo

Enrique Descals

Jozef Bakonyi

Thanks for your attention