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anses

Use of DNA HTS to assist in the detection of plant pathogenic fungi

Renaud IOOS

Plant health laboratory, mycology unit



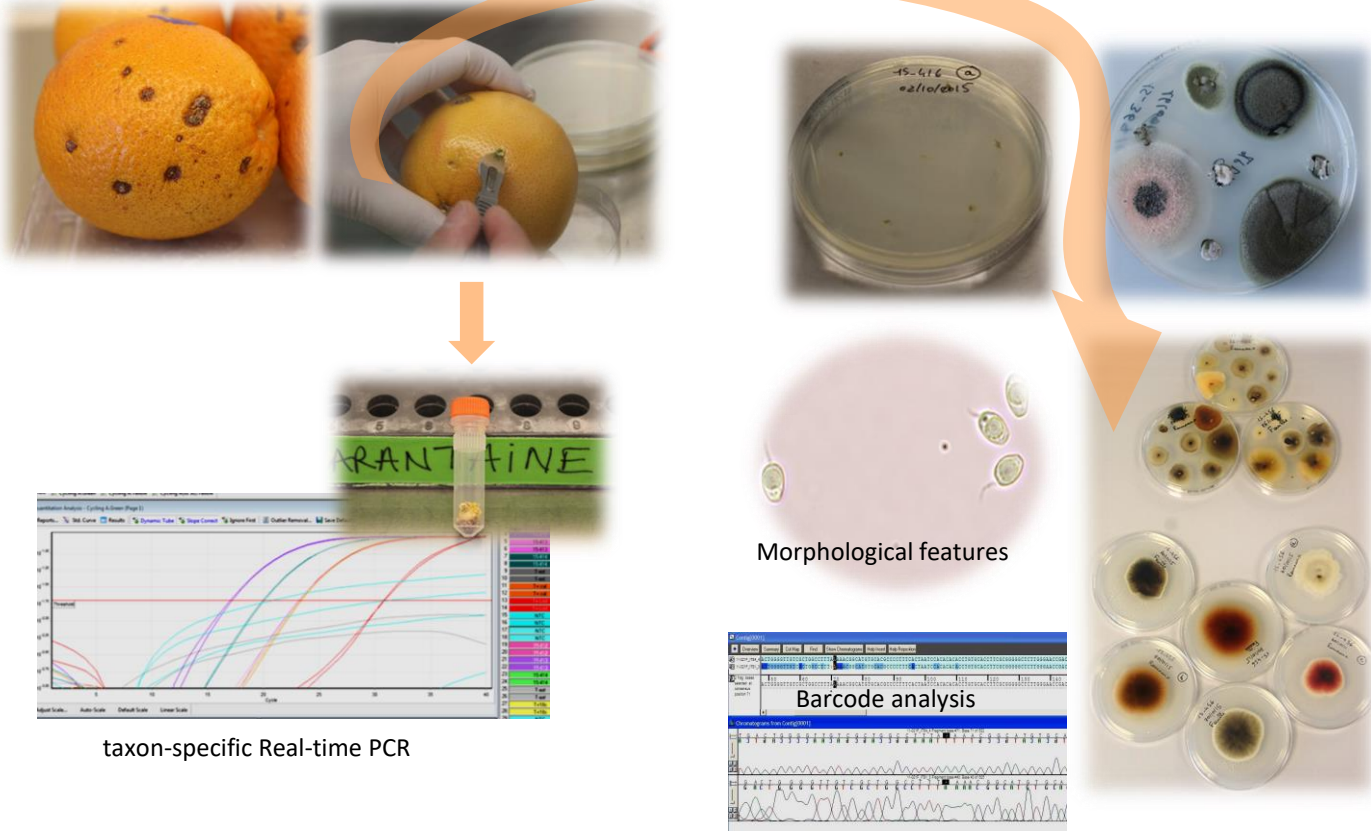
EURL for plant pathogenic fungi and oomycetes

Nancy, France

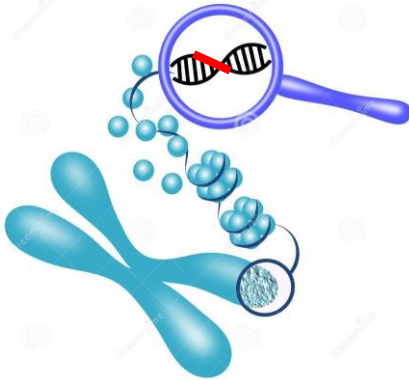


HTS? Handle, Taste, Smell
A “low-tech” way to assess the fungal community...

How to accurately detect a fungal plant pathogen?



Development of specific detection assays targeting DNA



Principle: to detect exclusive DNA traces of a pest () in a complex mixture and make them observable by PCR, qPCR, LAMP, ...



Search for hallmarks: targeted or random

- Phylogenetic markers (housekeeping genes, rDNA, etc.)
- Fingerprints (microsat., SCAR, ...)
- Genes related to pathogenicity

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5' Sequences                               3' Sequences
CCACGACTCCAAGTGCACCCGAT//TGCACCTTCCACTGGTGGTGGC Wnt1
ACAGAGTGCAGTGCACCCGAT//TGTAACTTCCACTGGTGGTGGC Wnt2
GGTGGAA TGCAGTGCACCCGAT//TGCAAATTCCTGGTGGTGGC Wnt4
TGTGGCTTCAAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt5a
CACCGAGTGCAGTGCACCCGAT//TGCCTTCCACTGGTGGTGGC Wnt6
GCTGGAA TGAAGTGCACCCGAT//TGTAACTTCCACTGGTGGTGGC Wnt7a
GCTGGAGTCAAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt7b
AAGGAC TGCAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt8a
AGCCAGTGCAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt8b
GACCAC TCAAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt9a
GACCACTTCAAGTGCACCCGAT//TGCAGTTCCTGGTGGTGGC Wnt9b
GGGAA TGAAGTGCACCCGAT//TGTAACTTCCACTGGTGGTGGC Wnt11
AATGAAGTGAAGTGCACCCGAT//TGTAACTTCCACTGGTGGTGGC Wnt11
    
```

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UPPER PRIMER                               LOWER PRIMER
5'-GGGGAATTCANCGATGTAARTGCAY-3'          3'-KNSCRBTGGTGGCAGATCTTTT-5'
  Bc o r XI                                  Bg I II
    
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V=ACG, N=ACGT, R=AG, M=AC, Y=CT, B=ACT, W=AT, S=CG, X=TG, B=CCT

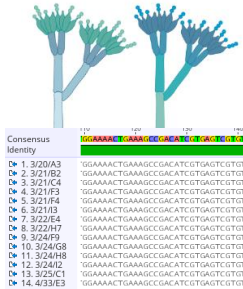


What more can HTS do for you, dear mycologist?



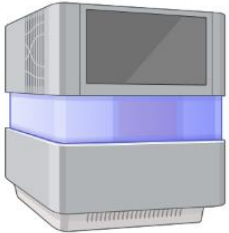
The use of HTS for comparative genomics

Use of HTS to design taxon-specific markers



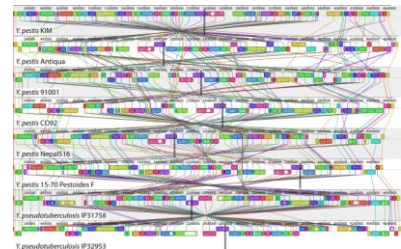
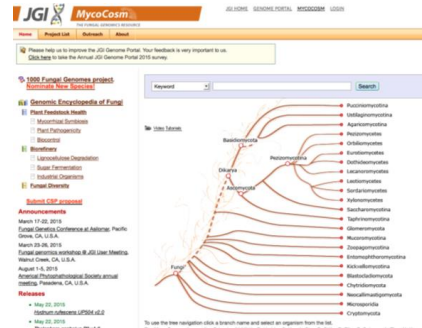
In case of cryptic species, or at subspecies levels, finding taxon-specific regions in the genome is challenging

Phylogenetic markers do not always show a sufficient level of polymorphism



Sequencing of entire fungal genomes is nowadays easy and affordable

Compare genomes to screen regions unique or sufficiently polymorphic and design specific oligonucleotides for molecular assays



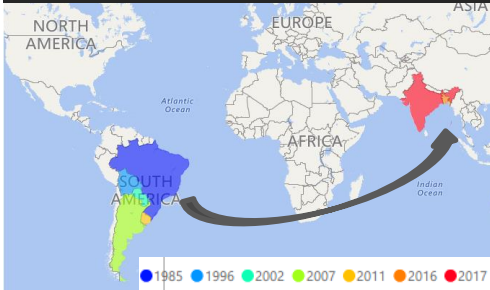
Case study 1 about wheat blast : an emerging and threatening disease

1-SYMPTOMS

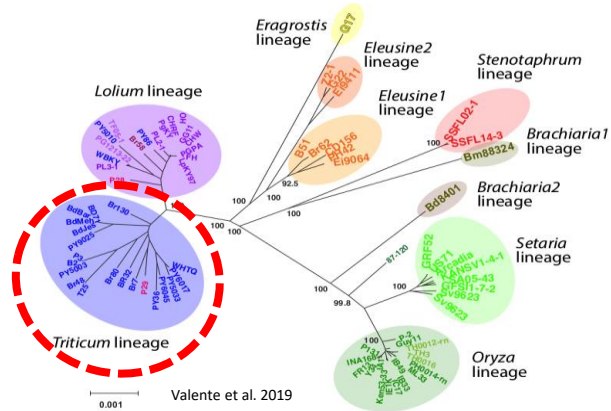


Wheat blast disease: danger on the move, Cruz & al., 2017

2-SPREAD



3-CAUSAL AGENT : *Pyricularia oryzae*



Valente et al. 2019

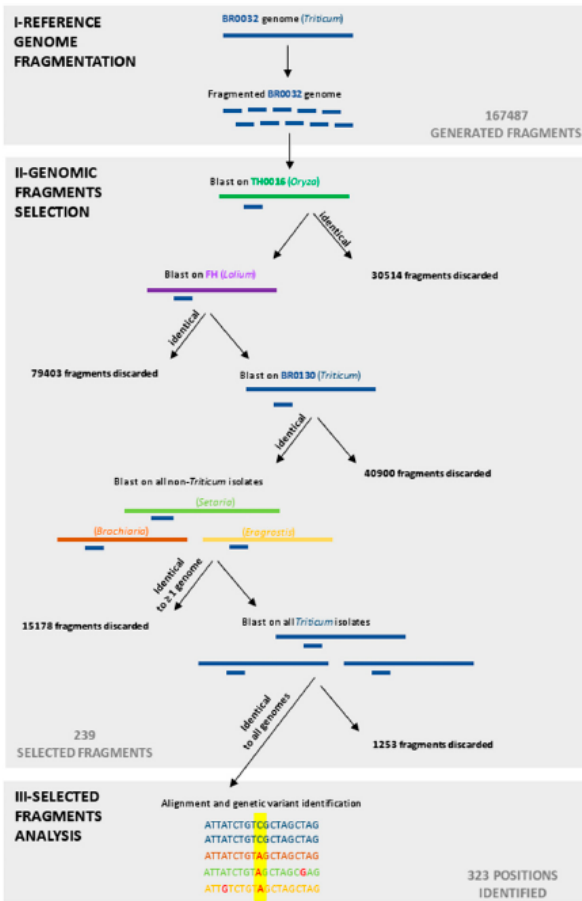


Challenge:

Prevent the spread of the disease via seeds and its introduction into Europe

=> Development of a detection assay in seeds, and **target a genetic lineage (sub specific level)**

The use of comparative genomics helps finding regions exclusive for *Triticum* lineage



1- Mass search for *Triticum* lineage hallmarks by comparing the whole genomes (unique/polymorphic)

2- Screening, selection and validation of detection tools

3- Obtaining tools of different technical levels that can be used in different types of laboratories:

- PCR tests (local laboratory application)
- Real-time PCR test (official laboratory application)
- LAMP test (field application)

plant disease

A Genomic Approach to Develop a New qPCR Test Enabling Detection of the *Pyricularia oryzae* Lineage Causing Wheat Blast

Maud Thierry,^{1,2} Pierre Gladieux,¹ Elisabeth Fournier,¹ Didier Tharreau,^{1,2} and Renaud Ios^{3,*}

¹ UMR BGPI, Montpellier University, INRA, CIRAD, Montpellier SupAgro, Montpellier, France

² CIRAD, UMR BGPI, F-34398 Montpellier, France

³ ANSES Plant Health Laboratory, Mycology Unit, Domaine de Pixérécourt, Bâtiment E, F-54220 Malzéville, France



Article

A PCR, qPCR, and LAMP Toolkit for the Detection of the Wheat Blast Pathogen in Seeds

Maud Thierry ^{1,2,3,*}, Axel Chatet ^{1,*}, Elisabeth Fournier ², Didier Tharreau ^{2,3} and Renaud Ios ^{1,*}



Maud Thierry

Case study 2 about *Phyllosticta citricarpa*, distinction from a new cryptic species



available online at www.studiesinmycology.org

STUDIES IN MYCOLOGY 87: 161–185 (2017).



First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe

V. Guarnaccia^{1*}, J.Z. Groenewald¹, H. Li², C. Glienke³, E. Carstens^{4,5}, V. Hattingh^{4,6}, P.H. Fourie^{4,5}, and P.W. Crous^{1,7*}

¹Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT, Utrecht, the Netherlands; ²Institute of Biotechnology, Zhejiang University, Hangzhou, 310058, China; ³Federal University of Paraná, Department of Genetics, Curitiba, Paraná, Brazil; ⁴Citrus Research International, P.O. Box 28, Nelspruit, 1200, South Africa; ⁵Department of Plant Pathology, Stellenbosch University, P. Bag X1, Stellenbosch, 7602, South Africa; ⁶Department of Horticultural Science, Stellenbosch University, P. Bag X1, Stellenbosch, 7602, South Africa; ⁷Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, P. Bag X20, Pretoria 0028, South Africa

Almost all current methods for the detection of *P. citricarpa* are based on the ITS region

They cross-react with DNA of *P. paracitricarpa*

Identical ITS, *actA*, *gapdh* & *rpb2*
Only 5 SNPs in *tef-1α* & 2 SNPs in LSU



Are they really separate species?

Case study 2 about *Phyllosticta citricarpa*, distinction from a new cryptic species

1- Genome comparison to assess species delineation

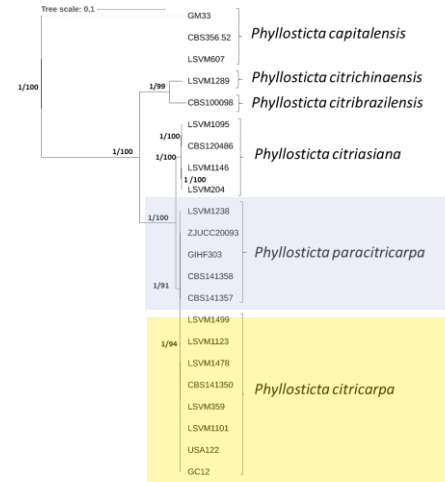
Funyorange database which includes 246 families of orthologues single copy genes extracted from 21 fungal genomes (Aguileta et al. 2008).

→ **Constructs a robust and well supported phylogenetic tree using 64 genes**

2- Genome comparison to find suitable genes to design primers & probes

GEDI pipeline (Genome-Enhanced Detection and Identification of plant pathogens, Feau et al. 2018)

→ **identifies polymorphic genomic regions between/within taxa., and design sets of specific oligonucleotides**



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LSVM1238 P. paracitricarpa TTGGAAGCAAGGCAAGTTTTCGGAAGTAAACGTTTCCGGAGCATTCGAGCGAGTGCCTACTGTCAAGCTCTGTTACGAGCGAGACTGGCTCA
LSVM204 P. citriasiana TTGGAAGCAAGGCAAGTTTTCGGAAGTAAACGTTTCCGGAGCATTCGAGCGAGTGCCTACTGTCAAGCTCTGTTACGAGCGAGACTGGCTCA
LSVM1101 P. citricarpa TTGGAAGCAAGGCAAGTTTTCGGAAGTAAACGTTTCCGGAGCATTCGAGCGAGTGCCTACTGTCAAGCTCTGTTACGAGCGAGACTGGCTCA

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LSVM1238 P. paracitricarpa CTCTGAATCCTCGCATCTCGAGATGTTTCCACGAAACATGAGC
LSVM204 P. citriasiana CTCTGAATCCTCGCATCTCGAGATGTTTCCACGAAACATGAGC
LSVM1101 P. citricarpa CTCTGAATCCTCGCATCTCGAGATGTTTCCACGAAACATGAGC

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Jaime Aguayo
(MS submitted)

Other ongoing projects using comparative genomics



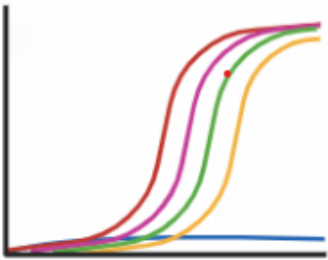
Seven species of *Armillaria* spp. are described in Europe.

Difficult to identify, requires very fresh samples

Alternative ? To develop multiplex qPCR identification tools

=>Genome comparison to find suitable genes to design primers & probes

Shaneyya Miriyagalla
Erasmus mundus Master student



A. mellea
A. cepistipes
A. ostoyae
A. gallica
A. tabescens
A. borealis
A. ectypa
Armillaria spp.



Venturia nashicola, QO for EU

Difficult to isolate/grow.

Alternative? To develop species-specific qPCR on *Pyrus* fruits.

=>Genome comparison to find suitable genes to design primers & probe

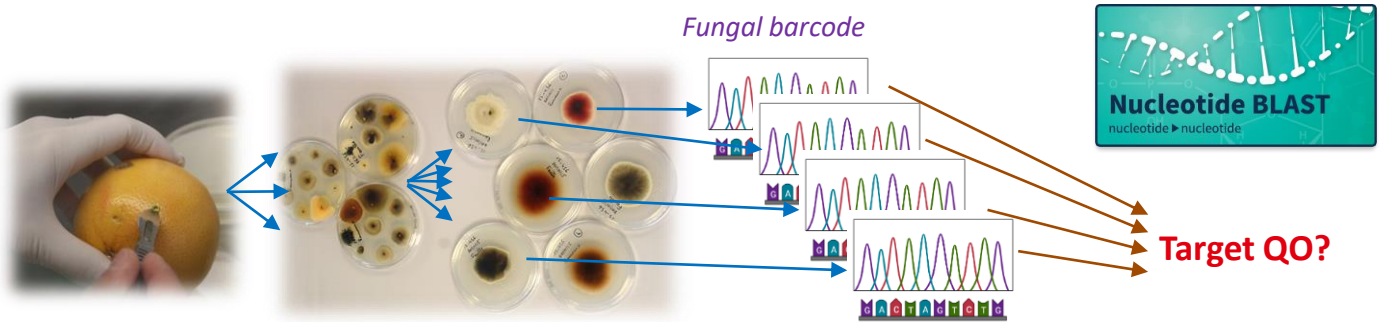


Cécile Guinet

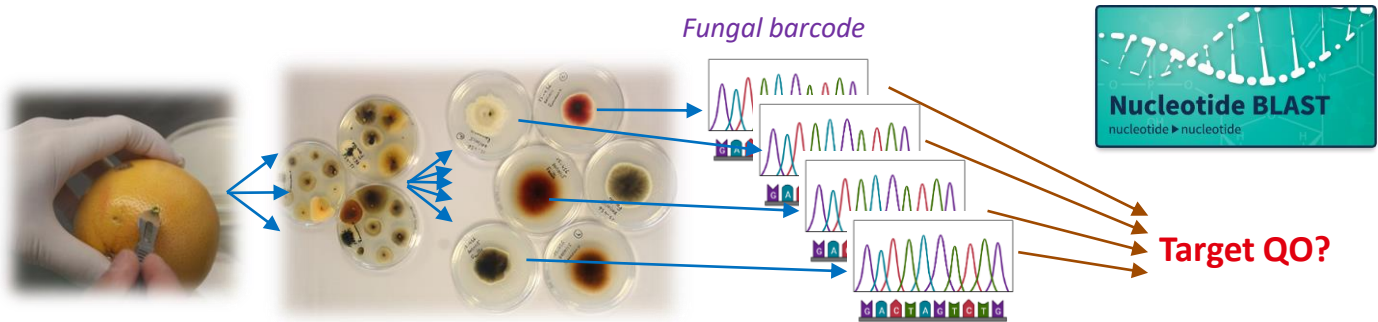


The use of HTS for fungal metabarcoding

Assessment of fungal community by classical means

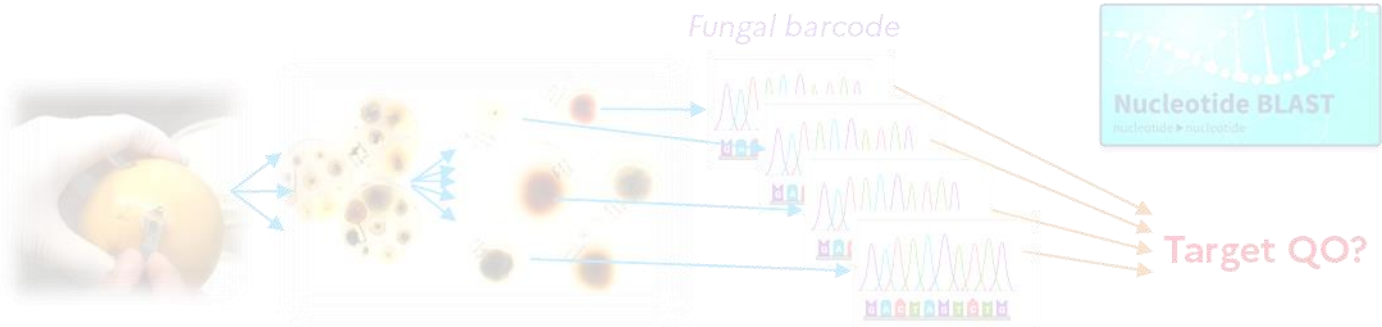


Assessment of fungal community by classical means

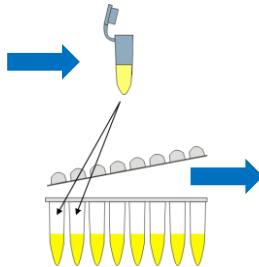


Leverage the power of HTS to mass-sequence phylogenetically relevant genes (barcodes) in environmental samples = **METAbarcoding**

Mass assessment of fungal community ... helps to find a needle in a haystack



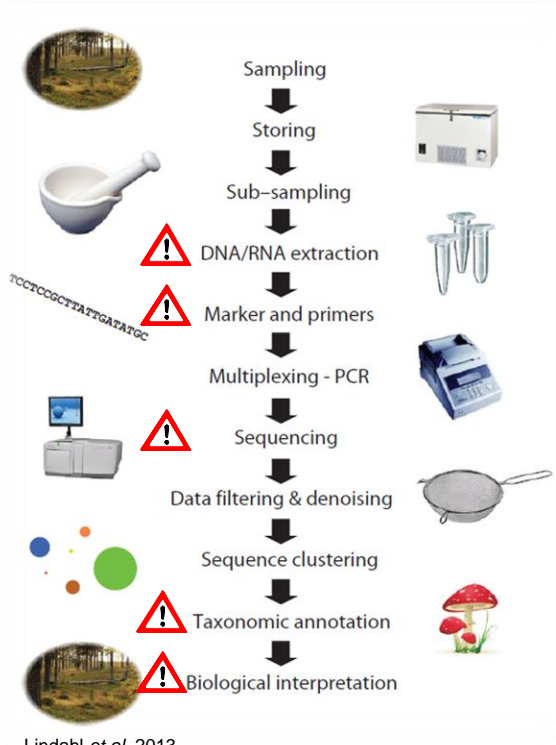
Leverage the power of HTS to mass-sequence phylogenetically relevant genes (barcodes) in environmental samples = **METAbarcoding**



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→ Target QO!

Several critical steps, but proofs of concept



Assessment of Passive Traps Combined with High-Throughput Sequencing To Study Airborne Fungal Communities

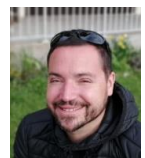
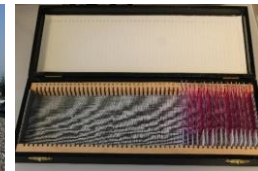
Jaime Aguayo,^a Céline Fourier-Jeandel,^a Claude Husson,^b Renaud loos^b

ORIGINAL ARTICLE

Plant Pathology WILEY

Combining permanent aerobiological networks and molecular analyses for large-scale surveillance of forest fungal pathogens: A proof-of-concept

Jaime Aguayo¹ | Claude Husson^{2,3} | Emilie Chancerel^{4,5} | Olivier Fabreguettes^{4,5} | Anne Chandelier⁶ | Céline Fourier-Jeandel¹ | Nadine Dupuy⁷ | Cyril Dutech^{4,5} | Renaud loos¹ | Cécile Robin^{4,5} | Michel Thibaudon⁷ | Benoit Marçais^{3,8} | Marie-Laure Desprez-Loustau^{4,5}



Jaime Aguayo

Future directions for accurate detection with metabarcoding?



Current limits...

For closely related species, cryptic species : need for several barcodes for accurate identification : « meta MLSA » ?

RESEARCH ARTICLE

Identification of fungi in shotgun metagenomics datasets

Paul D. Donovan¹, Gabriel Gonzalez², Desmond G. Higgins³, Geraldine Butler^{1,2,*}, Kimihito Ito^{2,4}

1 School of Biomedical and Biomolecular Science and UCD Conway Institute of Biomolecular and Biomedical Research, Conway Institute, University College Dublin, Belfield, Dublin, Ireland, **2** Division of Bioinformatics, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido, Japan, **3** School of Medicine and UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, Ireland, **4** Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education, Hokkaido University, Sapporo, Hokkaido, Japan

* These authors contributed equally to this work.

* gbutler@ucd.ie

=>A pipeline for identifying fungal species in shotgun metagenomics datasets.

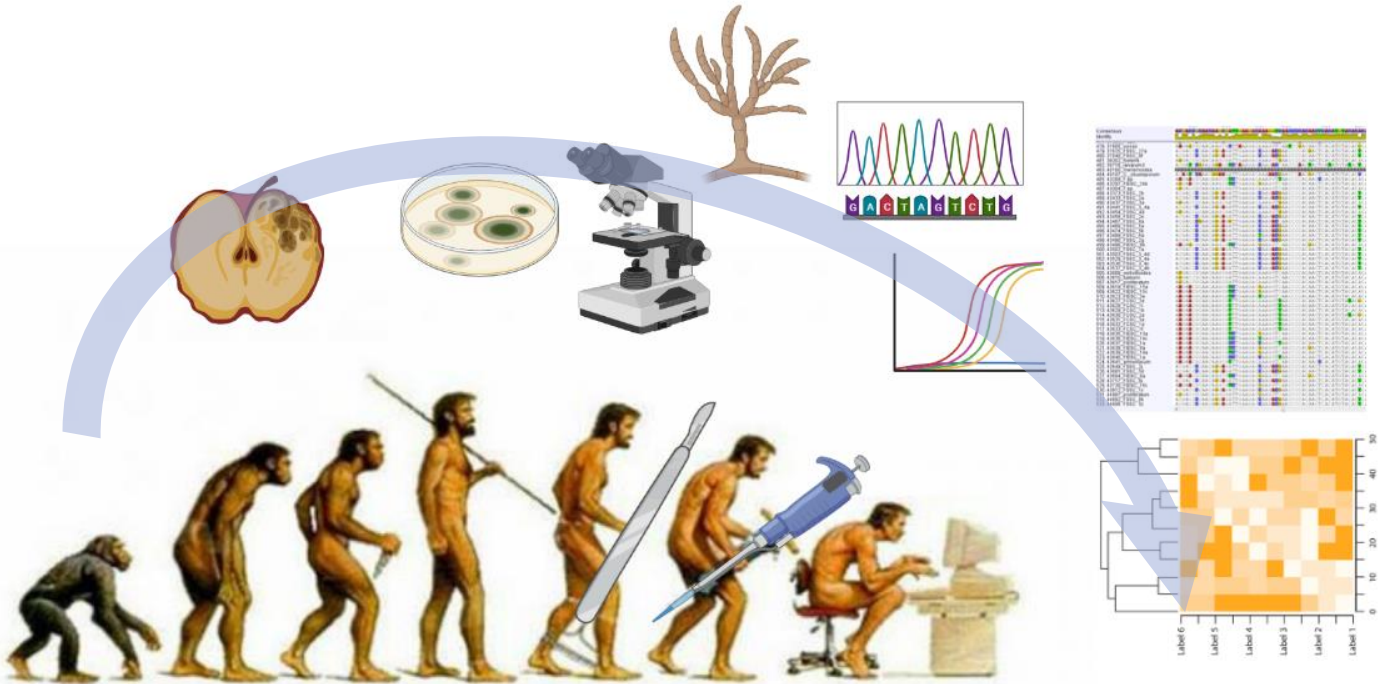
Current problems:

- ☹ contaminated data sets,
- ☹ availability/coverage of fungal diversity in public data sets,
- ☹ bias in genome amplification...

But « let's shoot for the moon ... » !



Inevitable evolution of mycologists?



We still need mycologists !