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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

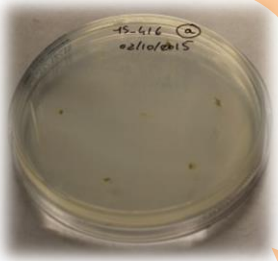
EURL for plant pathogenic fungi and oomycetes

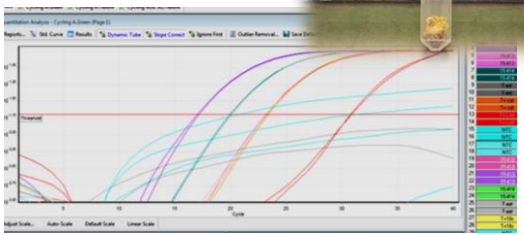
Nancy, France

HTS? Handle, **T**aste, **S**mell

A "low-tech" way to assess the fungal community…

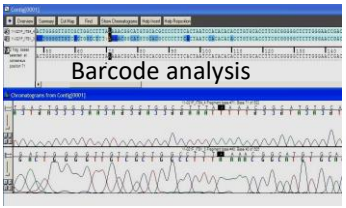
How to accurately detect a fungal plant pathogen?





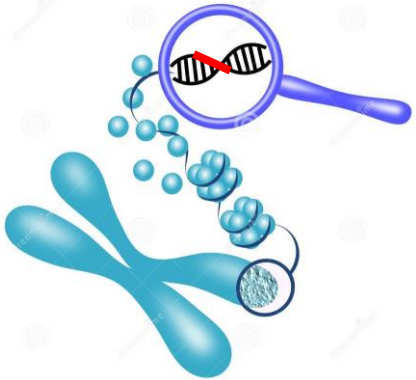
taxon-specific Real-time PCR

Morphological features

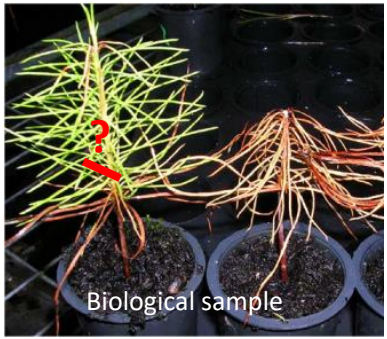


Barcode analysis

Development of specific detection assays targeting DNA

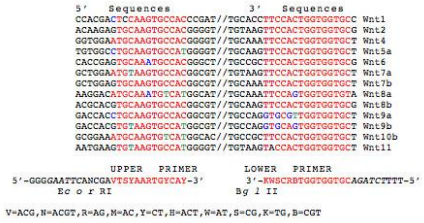


Principle: to detect exclusive DNA traces of a pest ( ) in a complex mixture and make them observable by PCR, qPCR, LAMP, …



Search for hallmarks: targeted or random

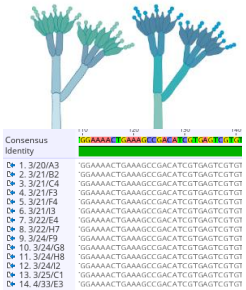
- Phylogenetic markers (housekeeping genes, rDNA, etc.)
- Fingerprints (microsat., SCAR, …)
- Genes related to pathogenicity



What more can HTS do for you, dear mycologist?

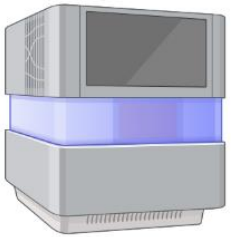
The use of HTS for comparative genomics

Use of HTS to design taxon-specific markers



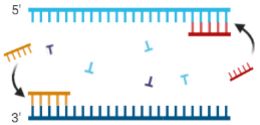
In case of cryptic species, or at subspecies levels, finding taxon-specific regions in the genome is challenging

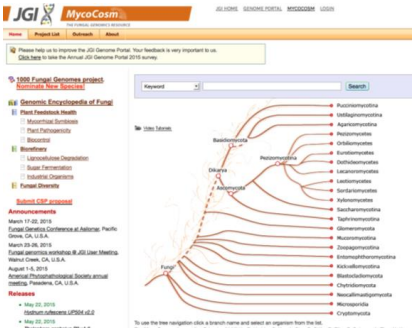
Phylogenetic markers do not always show a sufficient level of polymorphism

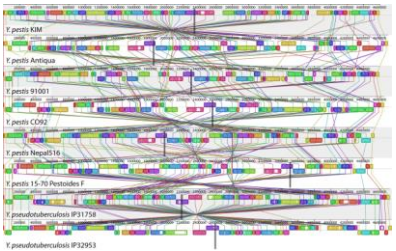


Sequencing of entire fungal genomes is nowadays easy and affordable

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





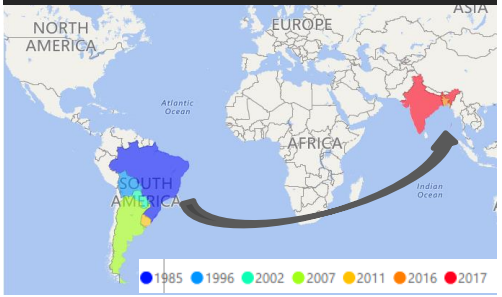


Case study 1 about wheat blast : an emerging and threatening disease

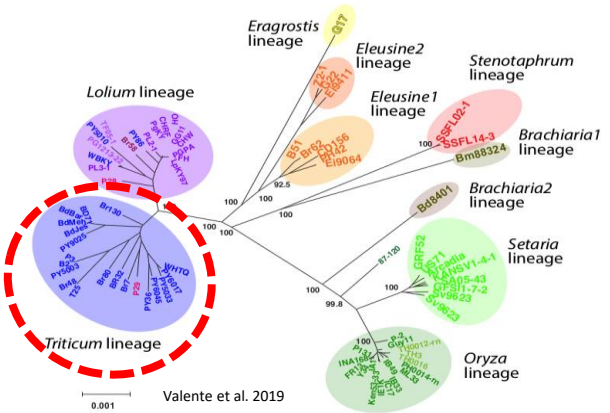
1-SYMPTOMS

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

2-SPREAD



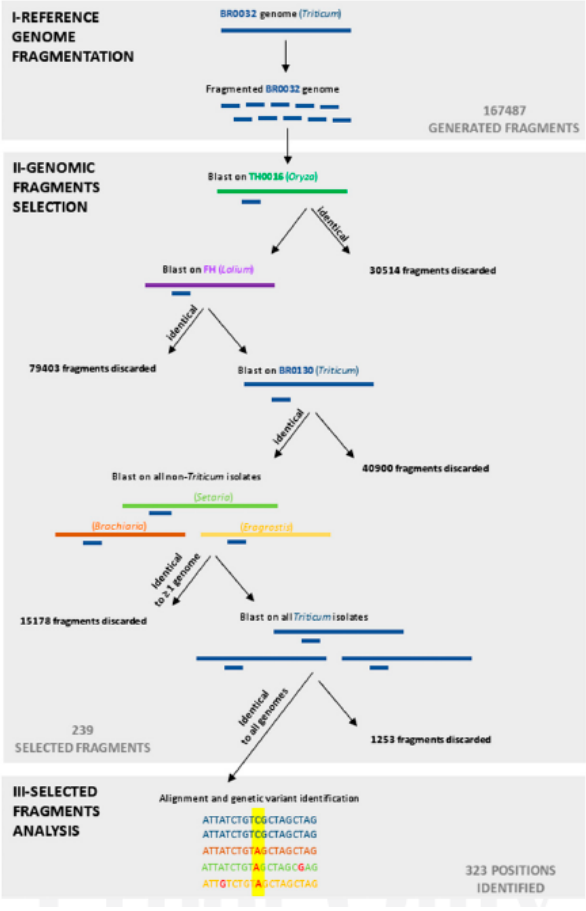
3-CAUSAL AGENT : *Pyricularia oryzae*



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 Ioos[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

plants MDPI

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 Ioos [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).

WESTERDIJK FUNGAL BIODIVERSITY INSTITUTE

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. Groenewald[1], H. Li[2], C. Glienke[3], E. Carstens[4,5], V. Hattingh[4,6], P.H. Fourie[4,5], and P.W. Crous[1,7*]

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

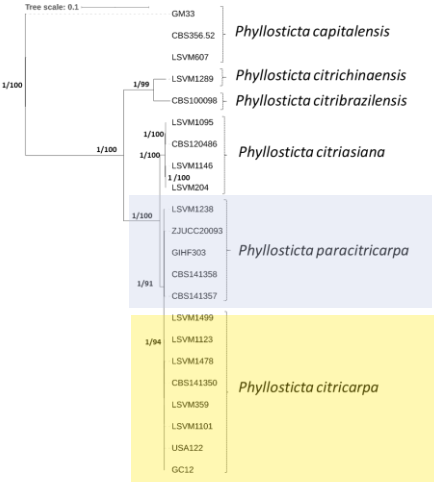
Are they really separat species?

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

1- Genome comparison to assess species delineation

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

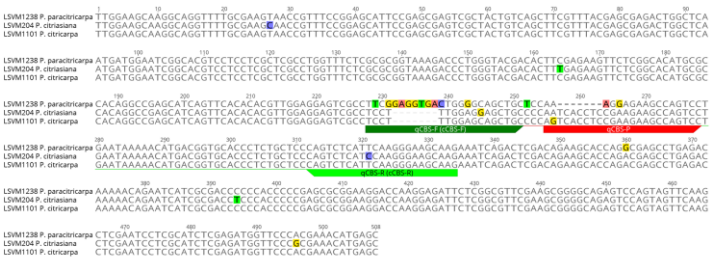
→ ***Constructs a robust and well supported phylogenetic tree using 64 genes***



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***



Jaime Aguayo
(MS submitted)

Other ongoing projects using comparative genomics

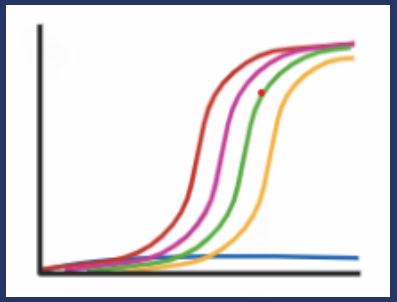
Seven species of *Armillaria* spp. are described in Europe.

Difficult to identify, requires very fresh samples

Alternative ? To develop multiplex qPCR identification tools

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

Shaneya 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.

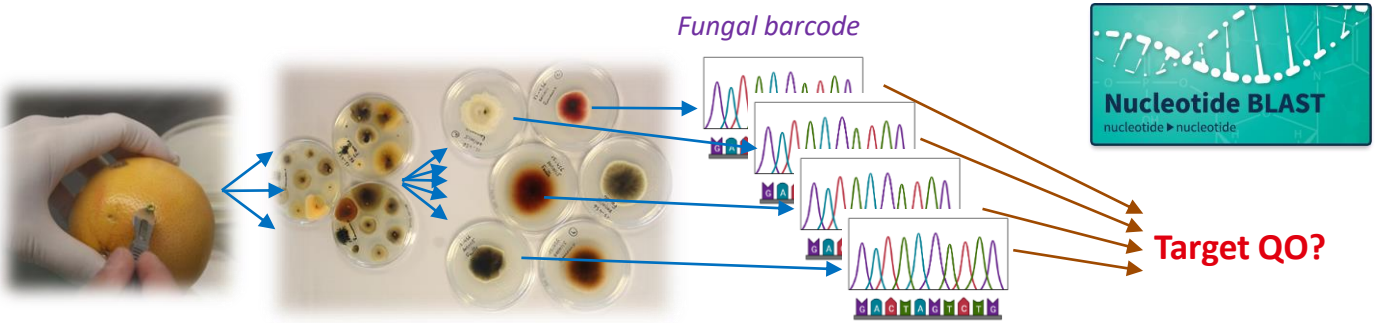
Alternative? To develop species-specific qPCR on Pyrus fruits.

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

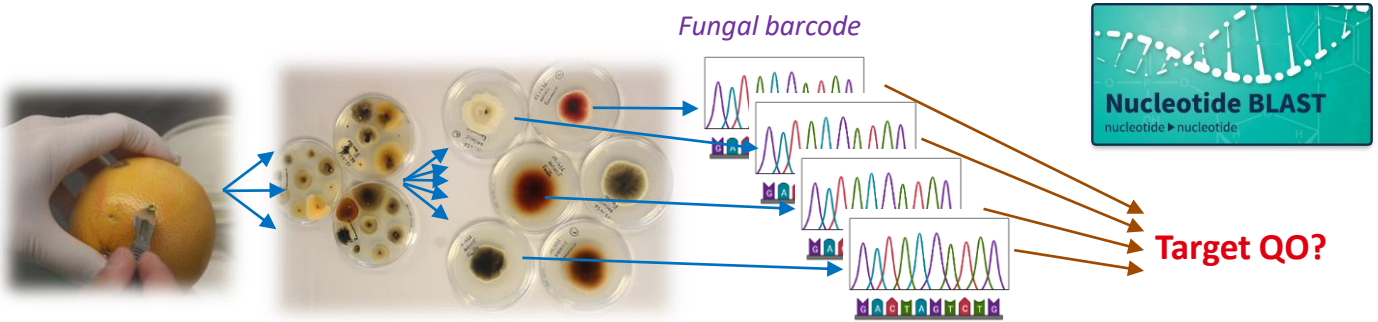
Cécile Guinet

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 phylogentically relevant genes (barcodes) in environmental samples = METAbarcoding

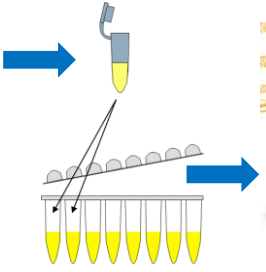
Mass assessment of fungal community
… helps to find a needle in a haystack

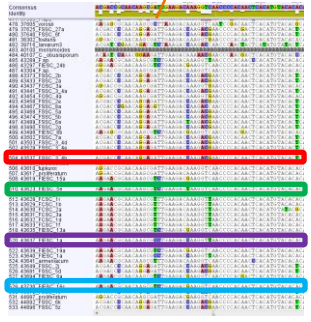
Leverage the power of HTS to mass-sequence phylogentically relevant genes (barcodes) in environmental samples = METAbarcoding

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

More complex environmental samples

+ non cultivable fungi
+ undescribed taxa

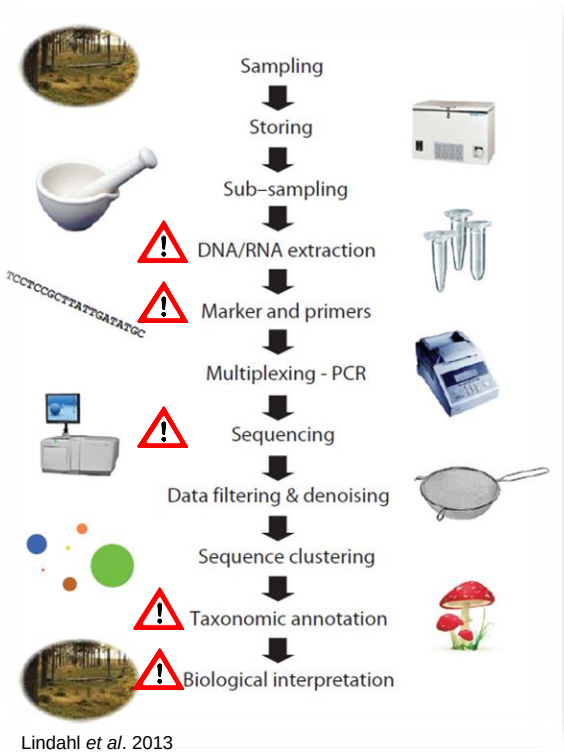




Target QO

Target QOs!

Several critical steps, but proofs of concept



Lindahl *et al*. 2013

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METHODS

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

Jaime Aguayo,[a] Céline Fourrier-Jeandel,[a] Claude Husson,[b] Renaud Ioos[a]

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 Aguayo[1] | Claude Husson[2,3] | Emilie Chancerel[4,5] | Olivier Fabreguettes[4,5] | Anne Chandelier[6] | Céline Fourrier-Jeandel[1] | Nadine Dupuy[7] | Cyril Dutech[4,5] | Renaud Ioos[1] | Cécile Robin[4,5] | Michel Thibaudon[7] | 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 » ?

PLOS ONE

RESEARCH ARTICLE

Identification of fungi in shotgun metagenomics datasets

Paul D. Donovan[1], Gabriel Gonzalez[2], Desmond G. Higgins[3], Geraldine Butler[1☯]*, 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

Check for updates

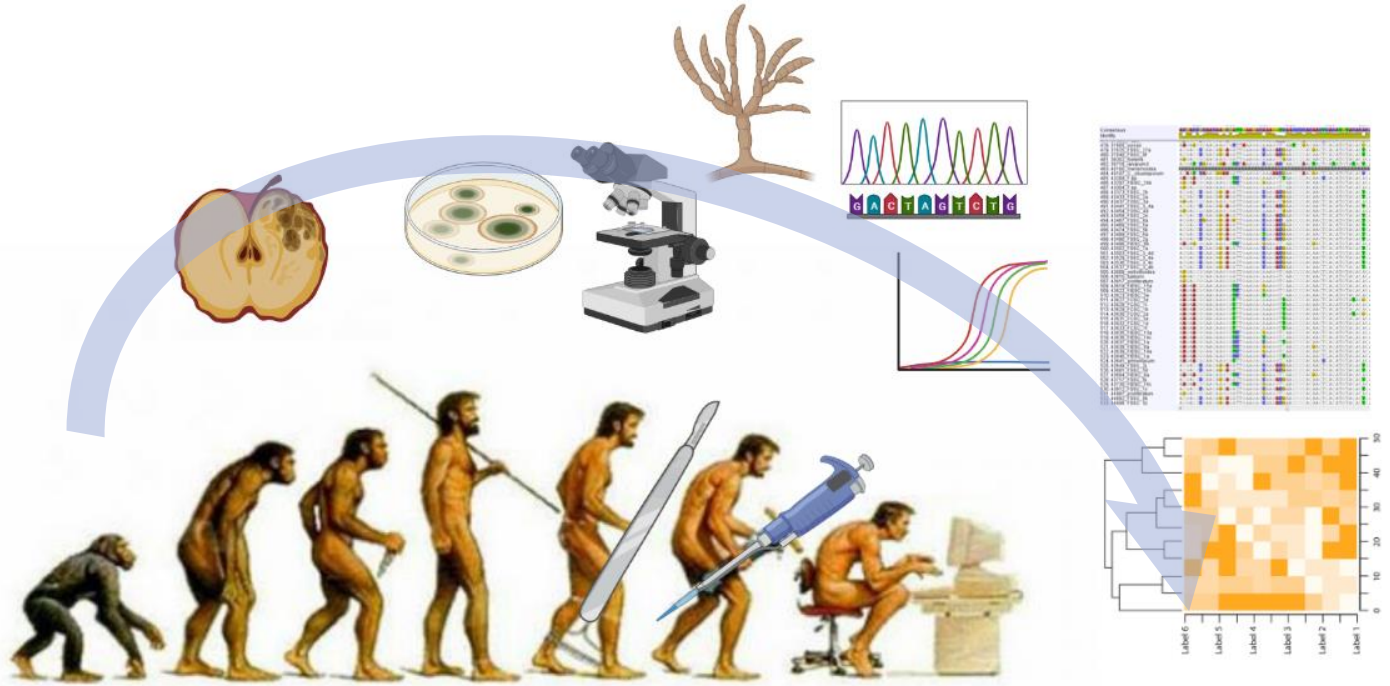
=>A pipeline for identifying fungal species in shotgun metagenomics datasets.

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 !