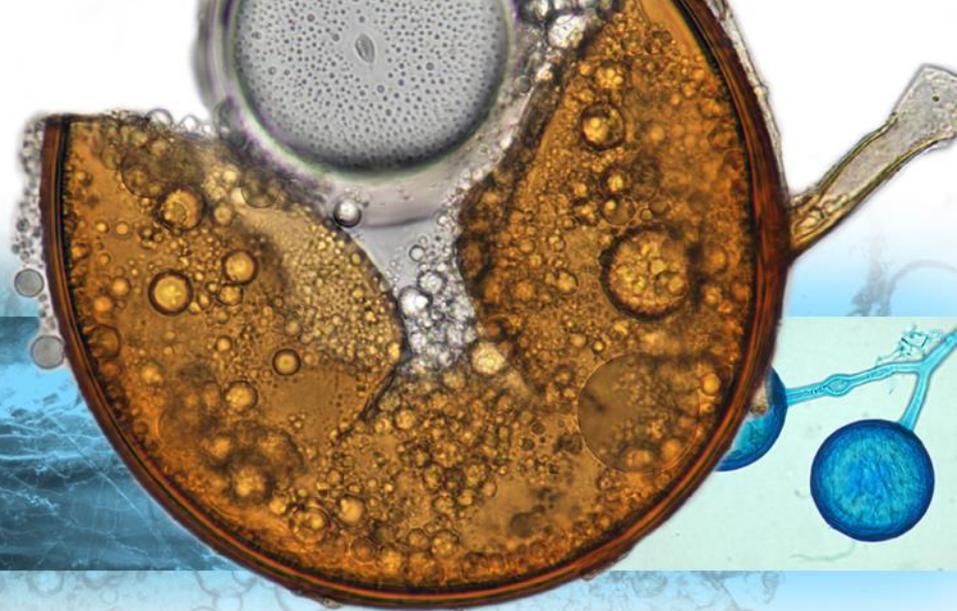




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# Caractérisation des mycorhizes arbusculaires par séquençage à haut débit

Franck Stefani  
Centre de Recherche et Développement Ottawa

Mycorhizes 2019

Canada 



## Identification taxonomique

## Analyses fonctionnelles

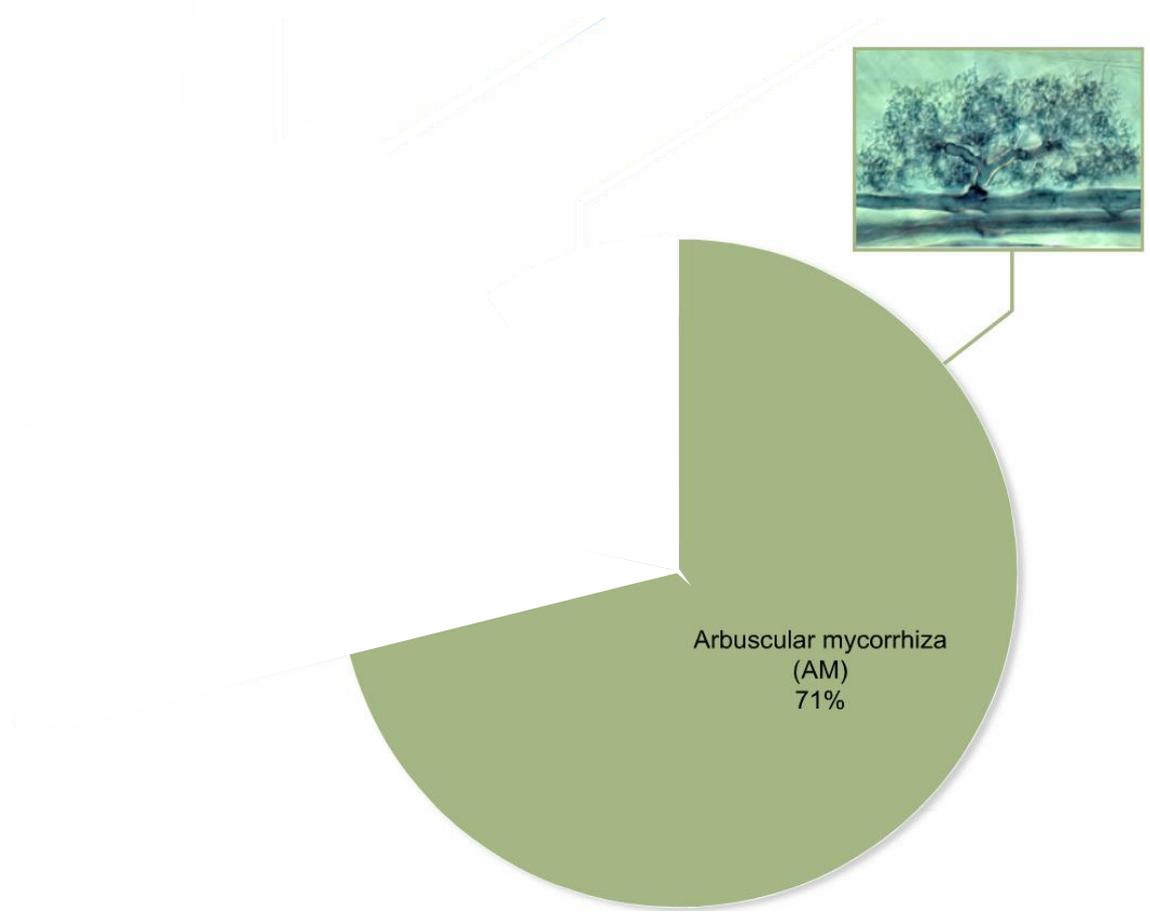


1. Quel marqueur moléculaire ?

2. Quel seuil de similitude ?

3. Quelle stratégie d'identification taxonomique?





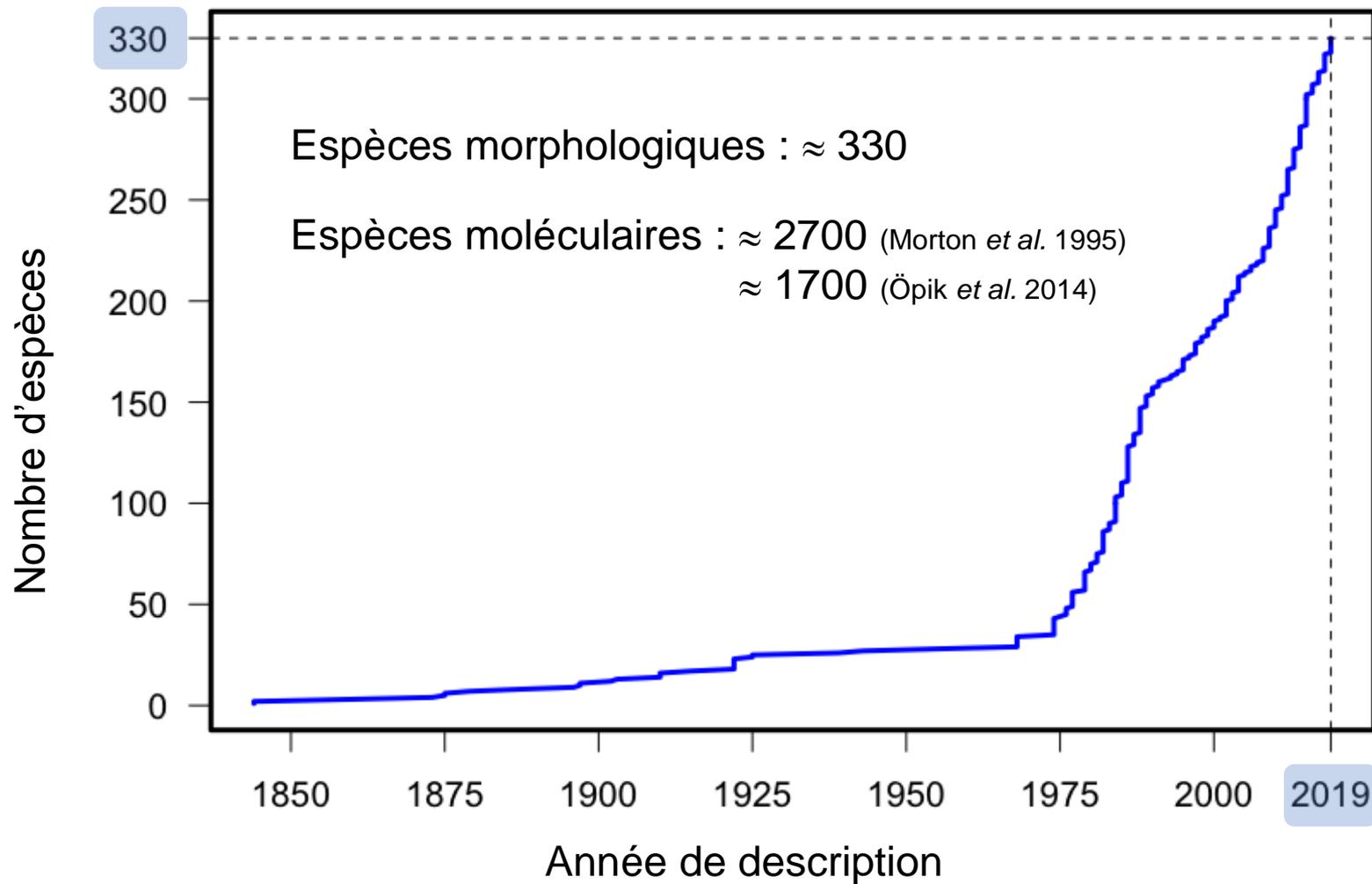
Brundrett & Tedersoo (2018) New Phytologist 220: 1108–1115



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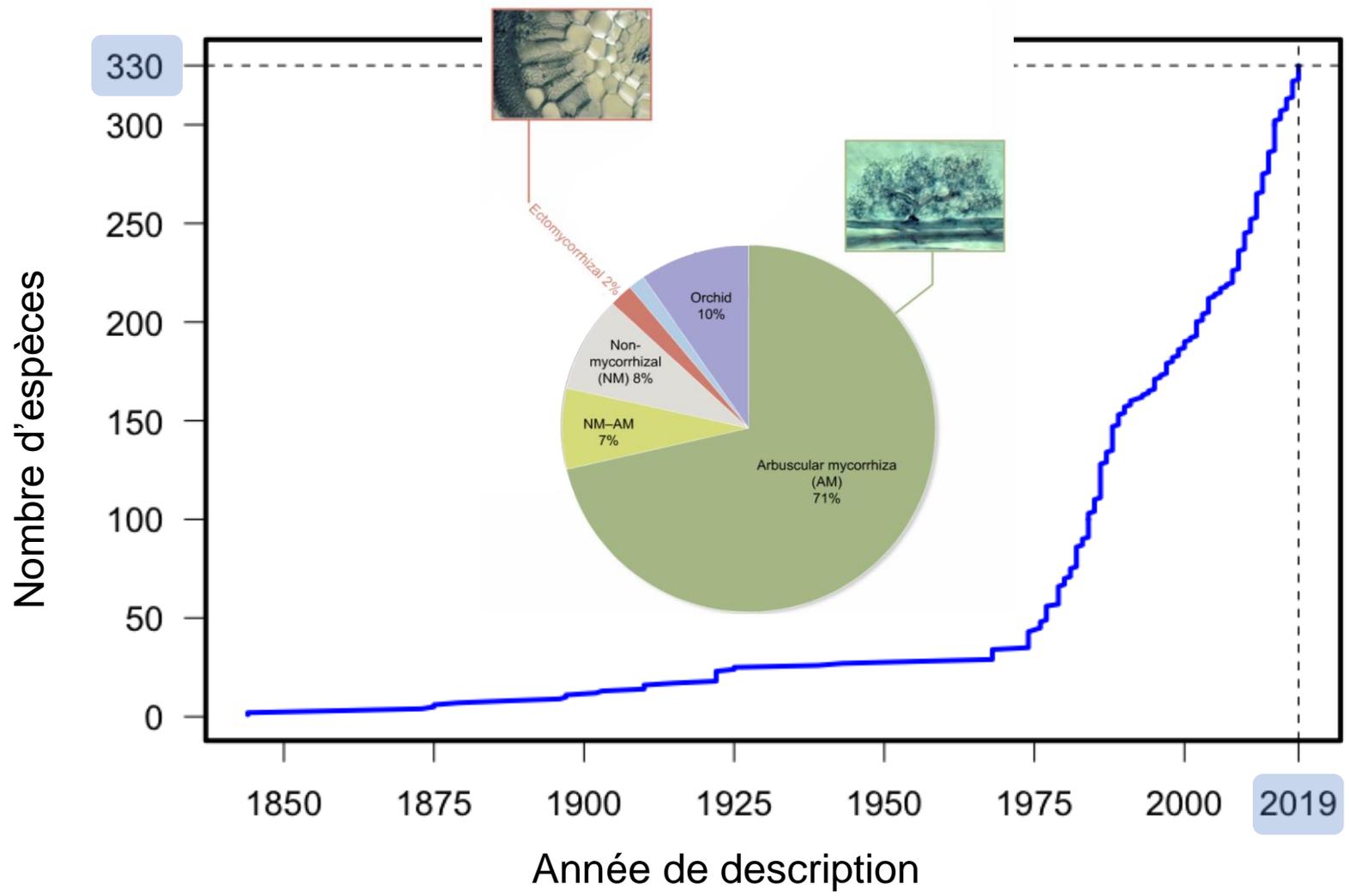
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Modifié d'après Opik *et al.* (2016) *Fungal Ecology* 24: 106-113 et Stürmer (2012) *Mycorrhiza* 22: 247-258.





Modifié d'après Opik et al. (2016) Fungal Ecology 24: 106-113 et Stürmer (2012) Mycorrhiza 22: 247-258.





# 1. Quel marqueur moléculaire ?

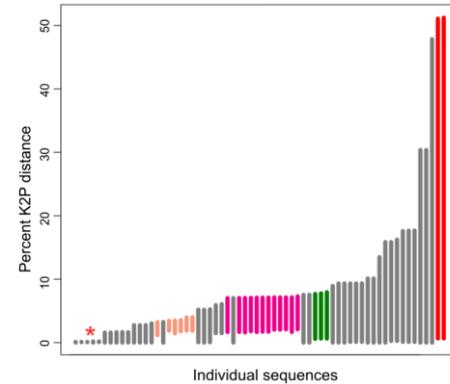
Gènes codants nucléaires

- Beta-tubuline : 50 isolats, 45 espèces → résolution semblable à SSU

Msiska & Morton. 2008. Mycorrhiza

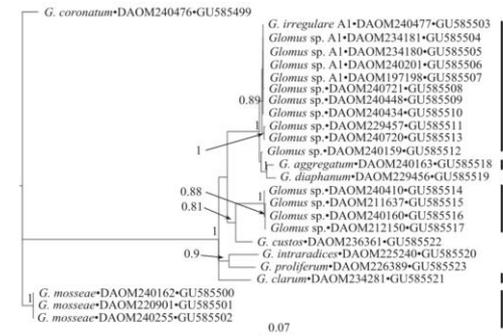
- Gène de la grande sous-unité de l'ARN polymérase II (RPB1)

Stockinger et al. (2014) PLoS ONE 9, e107783.



- Gène transporteur transmembranaire du phosphate (PTG)

Sokolski et al. 2011. AEM 77, 1888–1891.



- H<sup>+</sup>-ATPase / Glomaline



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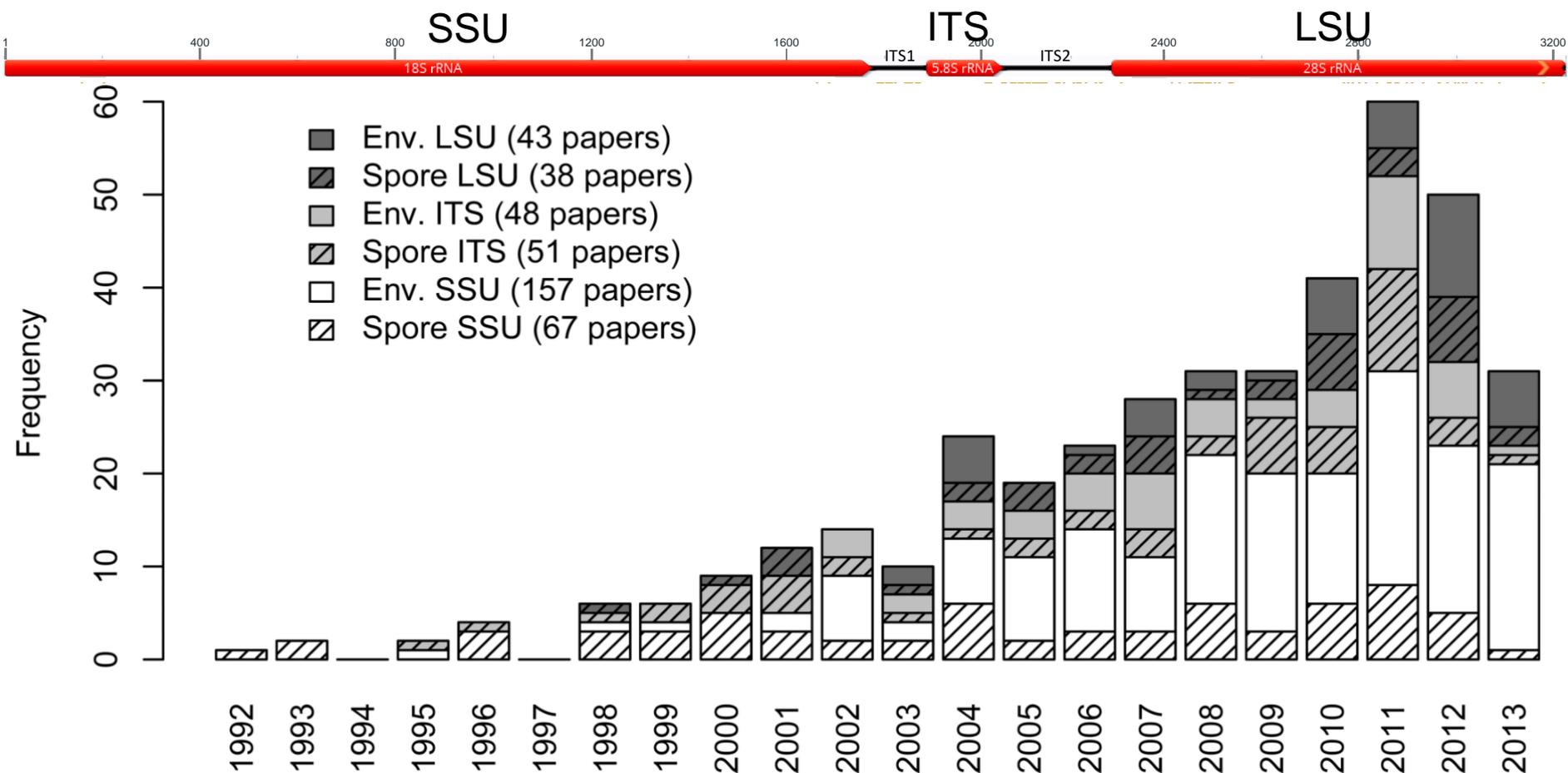
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# 1. Quel marqueur moléculaire ?

ADN ribosomal nucléaire



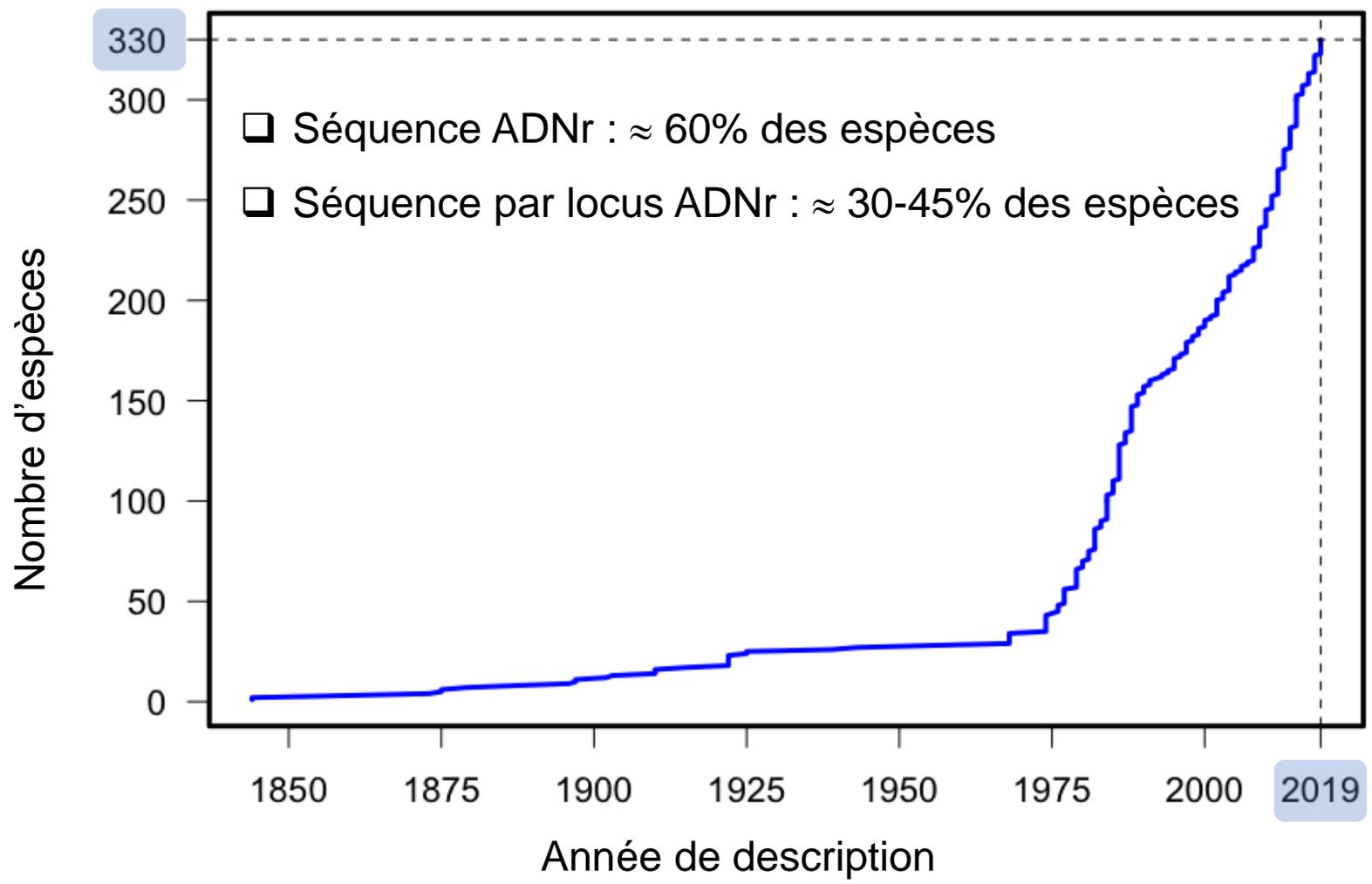
Öpik et al. 2014. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. Botany 92, 135-147.



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Modifié d'après Opik et al. (2016) *Fungal Ecology* 24: 106-113 et Stürmer (2012) *Mycorrhiza* 22: 247-258.







ARTICLE

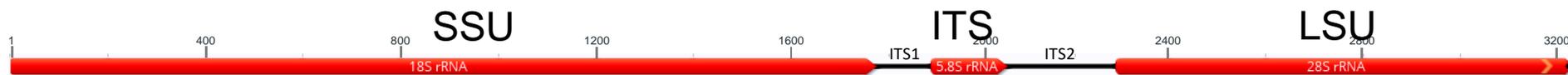
DOI: 10.1038/s42003-018-0094-7

OPEN

# Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*

Maeda *et al.* Communications Biology (2018) 1:87

| Species  | # Polymorphic sites | # of units in genome | # of polymorphic sites/100 bases |
|--|---------------------|----------------------|----------------------------------|
| <i>Rhizophagus irregularis</i>                       | 238                 | 10                   | 4.07                             |
| <i>Ashbya gossypii</i> <sup>30</sup>                 | 3                   | 50                   | 0.04                             |
| <i>Saccharomyces paradoxus</i> <sup>30</sup>         | 13                  | 180                  | 0.14                             |
| <i>Saccharomyces cerevisiae</i> <sup>30</sup>        | 4                   | 150                  | 0.04                             |
| <i>Aspergillus nidulans</i> <sup>30</sup>            | 11                  | 45                   | 0.14                             |
| <i>Cryptococcus neoformans</i> <sup>30</sup>         | 37                  | 55                   | 0.46                             |
| <i>Phoma exigua</i> var. <i>exigua</i> <sup>93</sup> | 27                  | -                    | 1.61                             |
| <i>Mycosphaerella punctiformis</i> <sup>93</sup>     | 26                  | -                    | 1.56                             |
| <i>Teratosphaeria microspora</i> <sup>93</sup>       | 16                  | -                    | 0.96                             |
| <i>Davidiella tassiana</i> <sup>93</sup>             | 33                  | -                    | 1.97                             |



10 copies

Pas d'organisation en tandem

90-99% de similitude



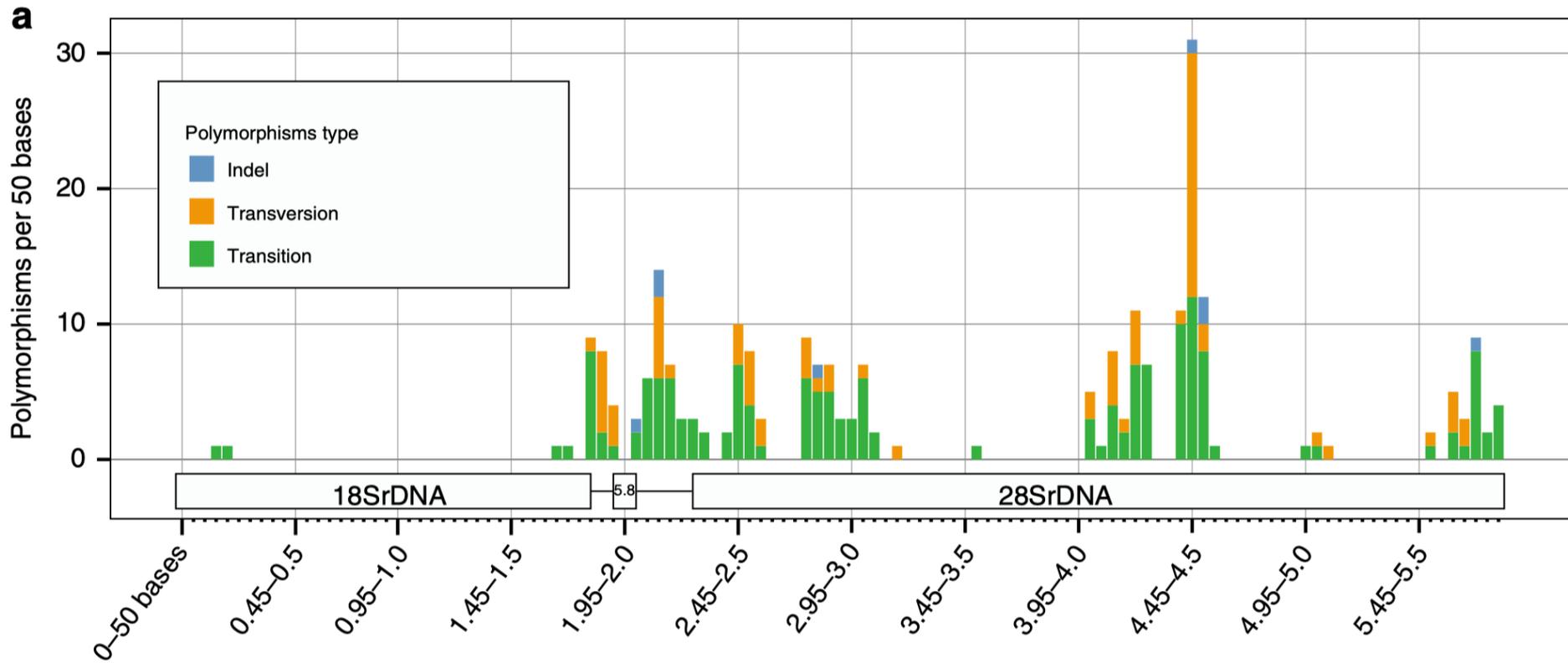
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# Polymorphisme de l'ADNr – *R. irregularis*



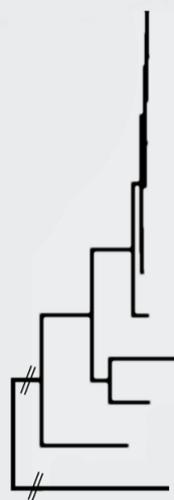
Maeda *et al.* Communications Biology (2018) 1:87



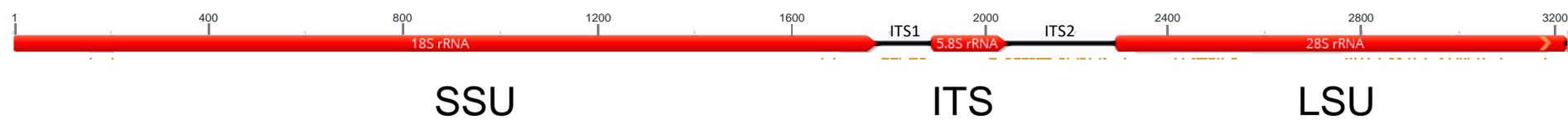
# Polymorphisme de l'ADNr – autres souches / espèces?

Stefani et al. (non publié)

| Strain ID | DAOM   | Identification   | Region / Country                         |
|-----------|--------|--|--|
| 4341      | 234179 | <i>Rhizophagus irregularis</i> (Blaszk., Wubet, Renker & Buscot) | Buckingham, QC, Canada                   |
| 4348      | 234180 | <i>Rhizophagus irregularis</i> (Blaszk., Wubet, Renker & Buscot) | Ripon, QC, Canada                        |
| M+        | 197198 | <i>Rhizophagus irregularis</i> (Blaszk., Wubet, Renker & Buscot) | Pont Rouge, QC, Canada                   |
| 9A2       | 212349 | <i>Rhizophagus</i> sp.   | Wasaga Beach Provincial Park, ON, Canada |
| 4375      | 234181 | <i>Rhizophagus irregularis</i> (Blaszk., Wubet, Renker & Buscot) | Îles-de-la-Madeleine, QC, Canada         |
| T3-1D     | 229455 | <i>Rhizophagus</i> sp.   | Terrebonne, QC, Canada                   |
| CC4-4A    | 229457 | <i>Rhizophagus irregularis</i> (Blaszk., Wubet, Renker & Buscot) | Clarence Creek, ON, Canada               |
| SD-1      | 229456 | <i>Rhizophagus</i> sp.   |  |
| GC-5      | 234281 | <i>Rhizophagus clarus</i> (T.H. Nicolson & N.C. Schenck)         | La Palma, Pinar del Rio, Cuba            |
| SD-5      | 226389 | <i>Rhizophagus proliferus</i> (Dalpé & Declerck)                 | Guadeloupe, France                       |
| JS-1      | 227022 | <i>Rhizophagus diaphanus</i> (J.B. Morton & C. Walker)           | Lotbinière, QC, Canada                   |
| GC-1      | 234280 | <i>Claroideoglomus claroideum</i> (N.C. Schenck & G.S. Sm.)      | Hoho, Laukaa, Finland                    |



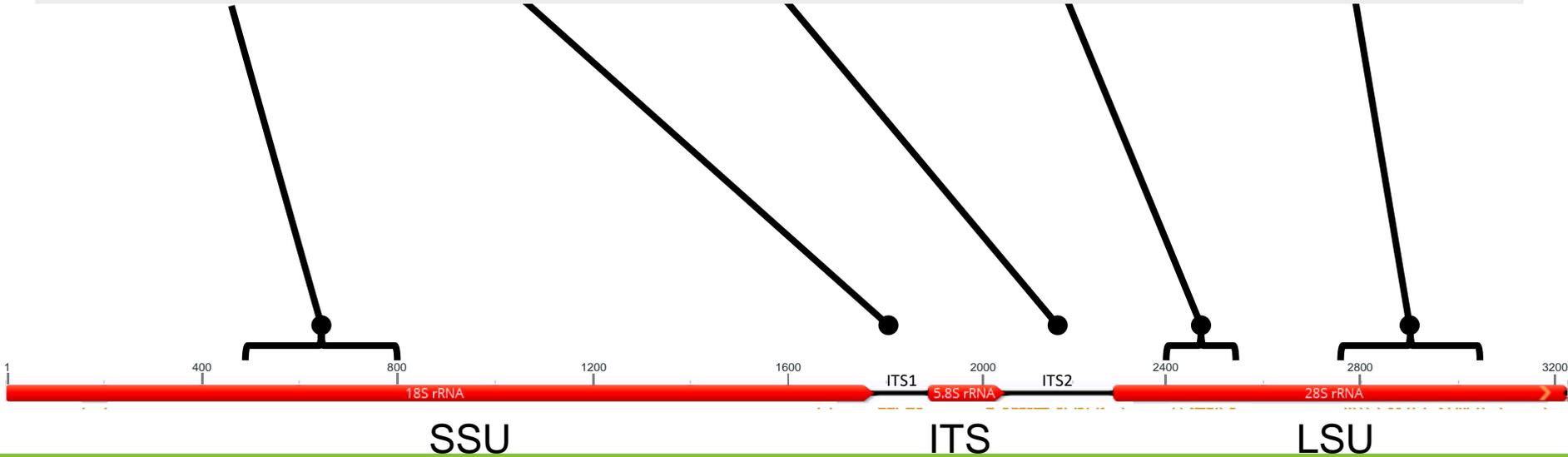
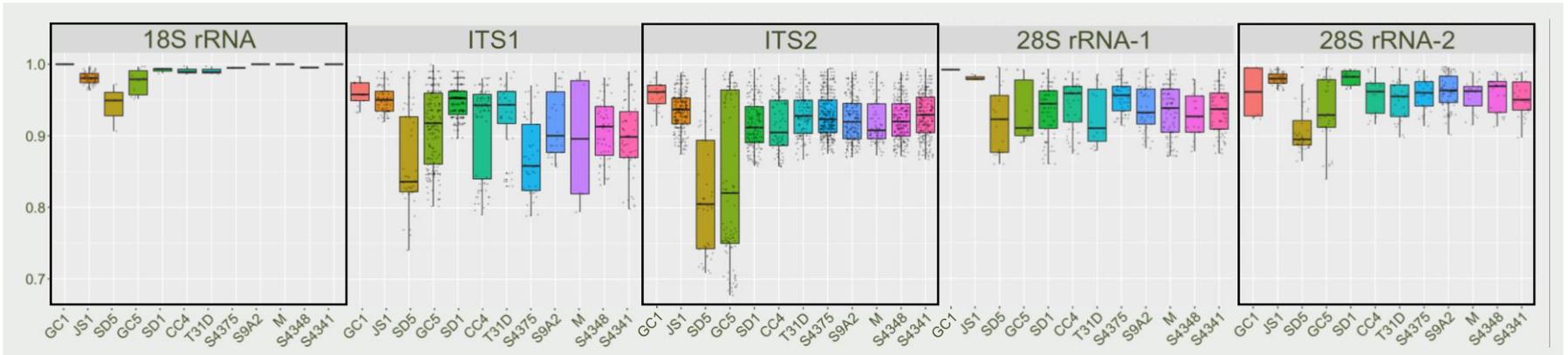
NS1/LSUmAr3 3.2 kb Fragments 550 pb MiSeq





# Polymorphisme de l'ADNr – autres souches / espèces?

Stefani et al. (non publié)



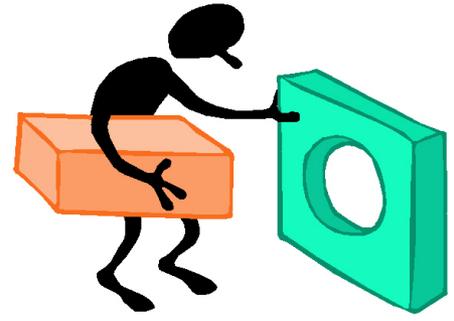


Lee et al. (2008) FEMS Microbiol Ecol 65: 339-349

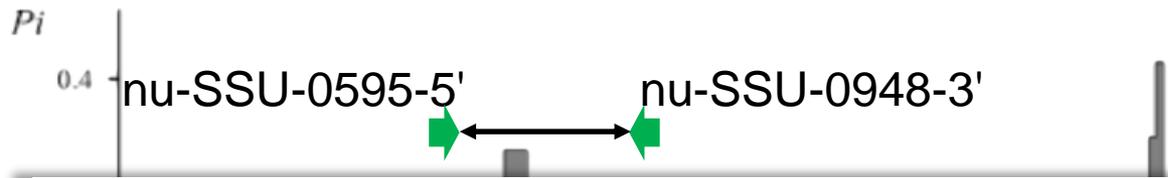
Stefani et al. (non publié)

Franck\_F / Franck\_R

AML1/AML2  
800 pb

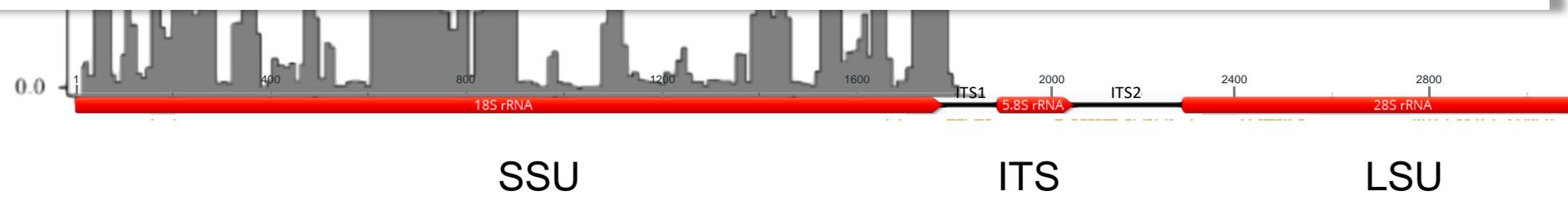


MiSeq  
2 x 250 pb  
2 x 300 pb



*Mycologia*, 88(5), 1996, pp. 745-748.  
© 1996 by The New York Botanical Garden, Bronx, NY 10458-5126

## A nomenclature for fungal PCR primers with examples from intron-containing SSU rDNA



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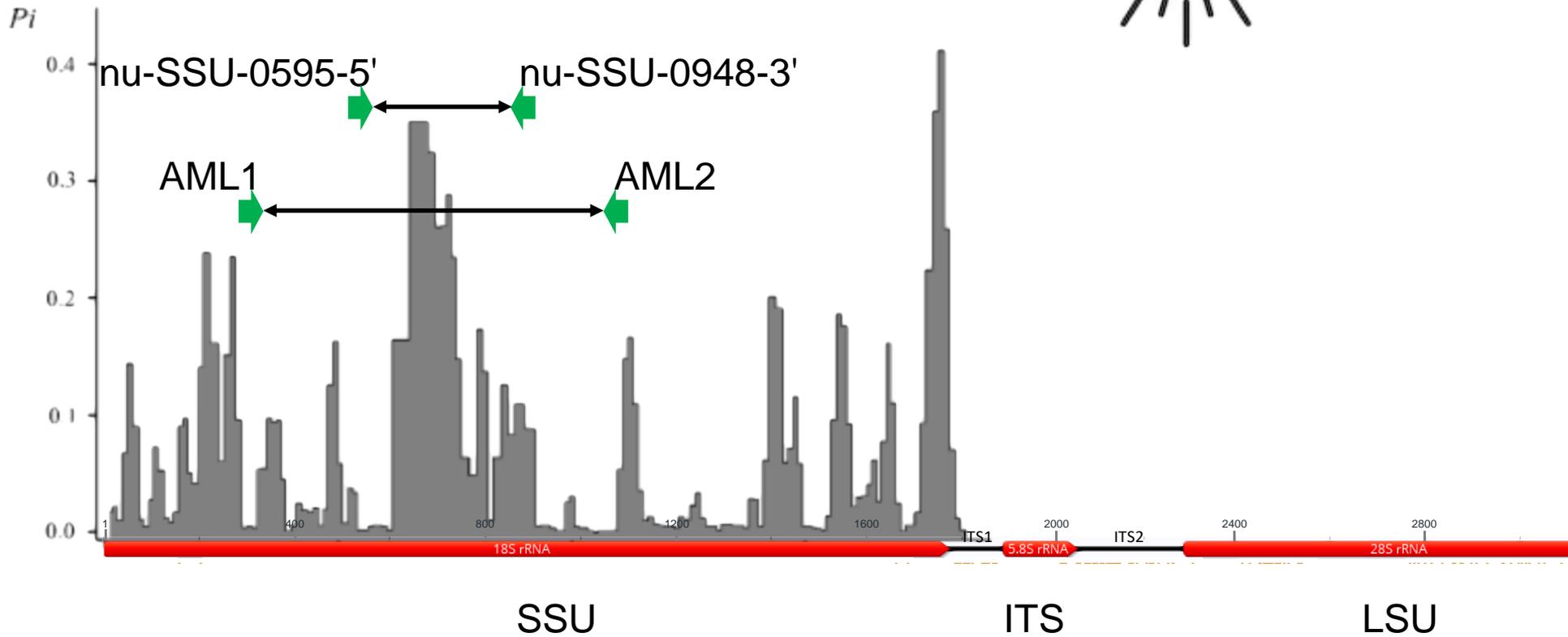
Lee et al. (2008) FEMS Microbiol Ecol 65: 339-349

Stefani et al. (non publié)

nu-SSU-0595-5' / nu-SSU-0948-3'  
400 pb



MiSeq  
2 x 250 pb  
2 x 300 pb





Lee et al. (2008) FEMS Microbiol Ecol 65: 339-349

Stefani et al. (non publié)

nu-SSU-0595-5' / nu-SSU-0948-3'

400 pb



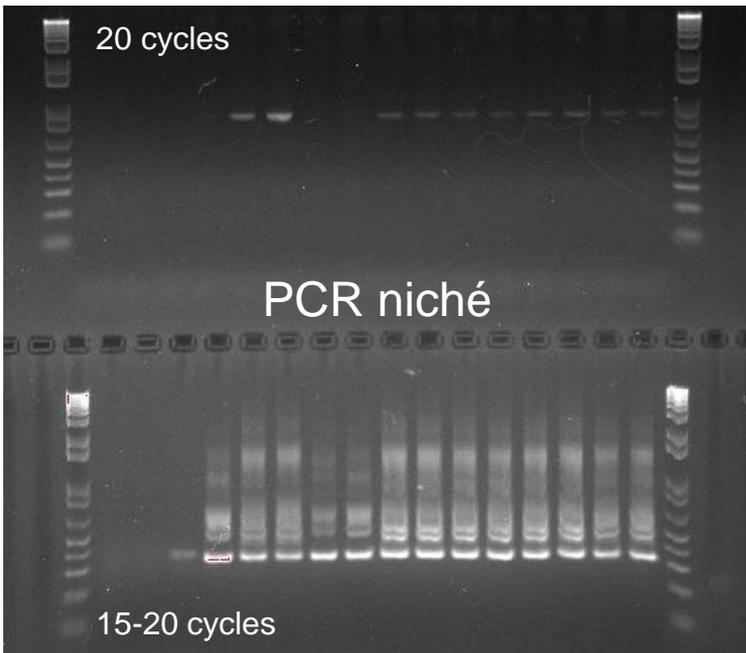
MiSeq

2 x 250 pb

2 x 300 pb



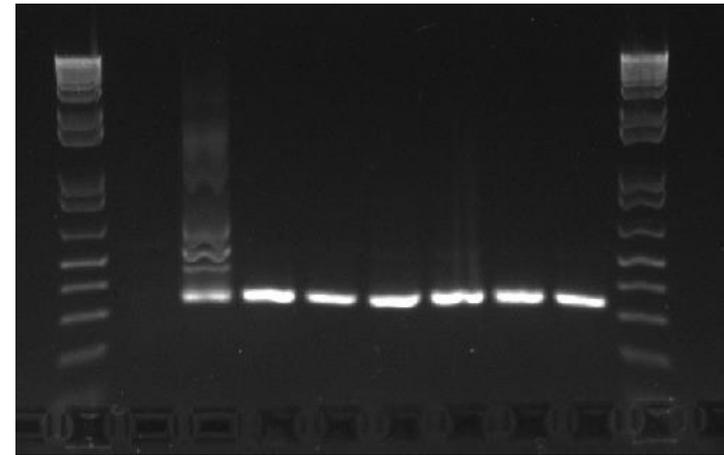
Amorces universelles



AML1 / AML2

Purification

nu-SSU-0595-5' / nu-SSU-0948-3





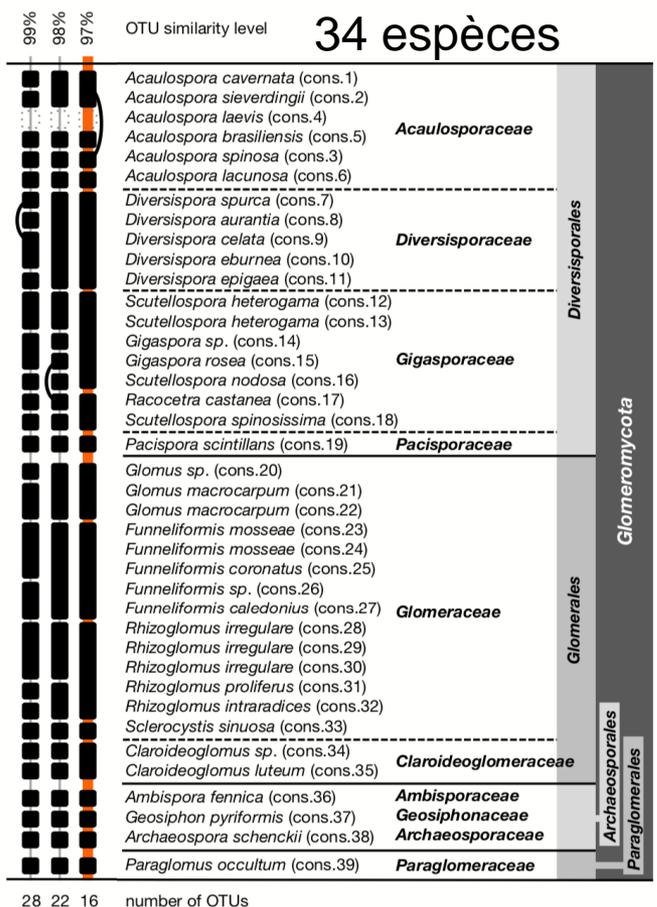
Identification  
taxonomique



## 2. Quel seuil de similitude ?

Schlaeppli et al. New Phytologist (2016) 212: 780–791

NS31-AML2 PCR primer combination  
(480 bp) (minimal length of common sequence fragment)



SSU = regroupement à 100%



ASVs, ESVs, z- radius OTUs, sub-OTUs



blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query.

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Clear

Query subrange

GGACTGTTACCTTGAAAAATTAGAGTGTTTAAAGCAGGCTCACGCTTGAATACATTAGCATGG
ATAATGAAATAGGACATCCGATTCTATTTTGTGGTTTCTAGGATCGATGTAATGATTAATAGG
GATAGTTGGGGGCATTAGTATTCAATTGTCAGAGGTGAAATCTTGGATTTATTGAAGACTAAC
TACTGCGAAAGCATTGCGCAAGGATGTTTTTATTCAATCAAGAACGAAAGTTAGGGGATCGAAG
ACGATCAGATACCGTCGTAGTC

From

To

Or, upload file

Choisir le fichier aucun fichier sélectionné

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database

Human genomic + transcript Mouse genomic + transcript Others (nr etc.):

Nucleotide collection (nr/nt)

Organism Optional

Enter organism name or id--completions will be suggested exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude Optional

Models (XM/XP) Uncultured/environmental sample sequences

Limit to Optional

Sequences from type material

Entrez Query Optional

Enter an Entrez query to limit search YouTube Create custom database

Program Selection

Optimize for

Highly similar sequences (megablast)

More dissimilar sequences (discontiguous megablast)

Somewhat similar sequences (blastn)

Choose a BLAST algorithm

3. Q

Séq

OPEN A

PHY

Funç

Mahé et a

9120 S

Identification taxonomique



DS ONE



da



Fig. 2.

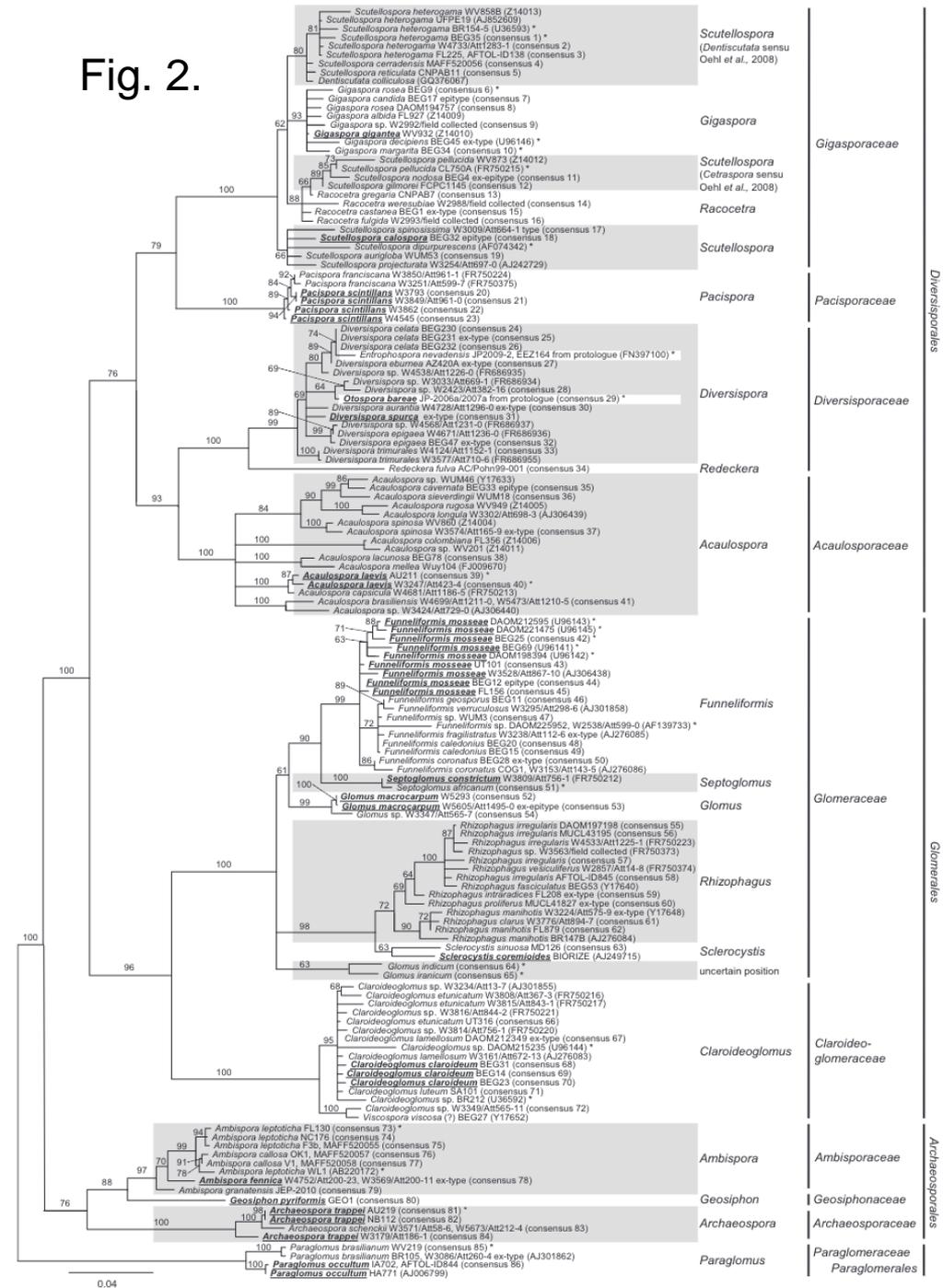
### 3. Quelle stratégie d'identification

#### 3.1 SSU Regroupement 100

#### 3.2. ASVs MA → Phylogénie

Phylogenetic reference data of arbuscular mycorrhizal fungi

Krüger et al. New Phytologist (2012) 193: 970–98





Stefani, Bencherif, et al. non publié

☐ *Tamarix articulata*

21 échantillons de racines

4 échantillons de sol

☐ PCR niché

1. AML1/AML2

2. nu-SSU-0595-5' / nu-SSU-0948-3'

☐ Séquençage

MiSeq Nano kit → 2 M séquences

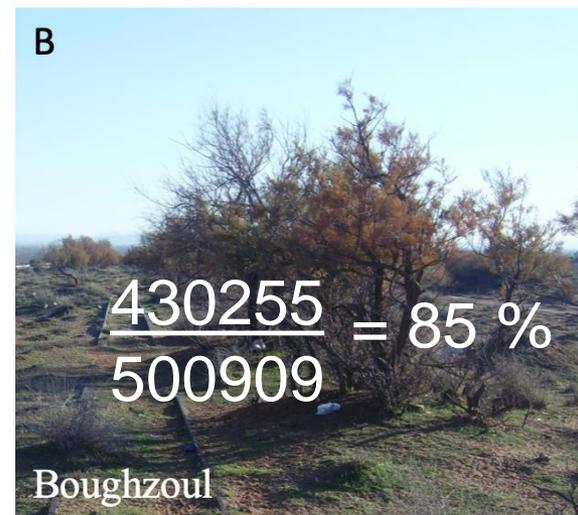
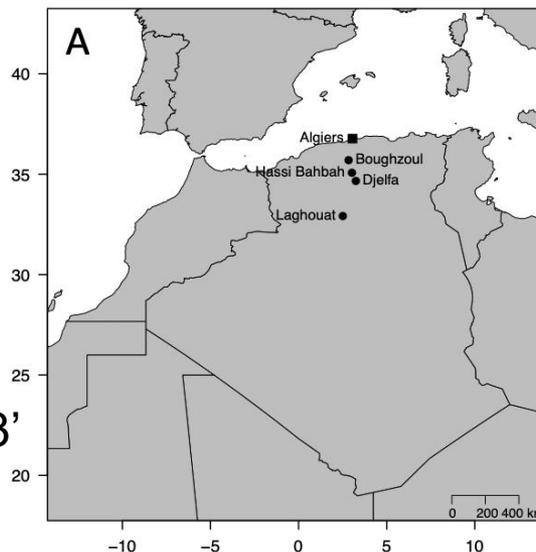
2 × 250 pb

☐ Regroupement 100%

☐ Identification taxonomique

1. Blast

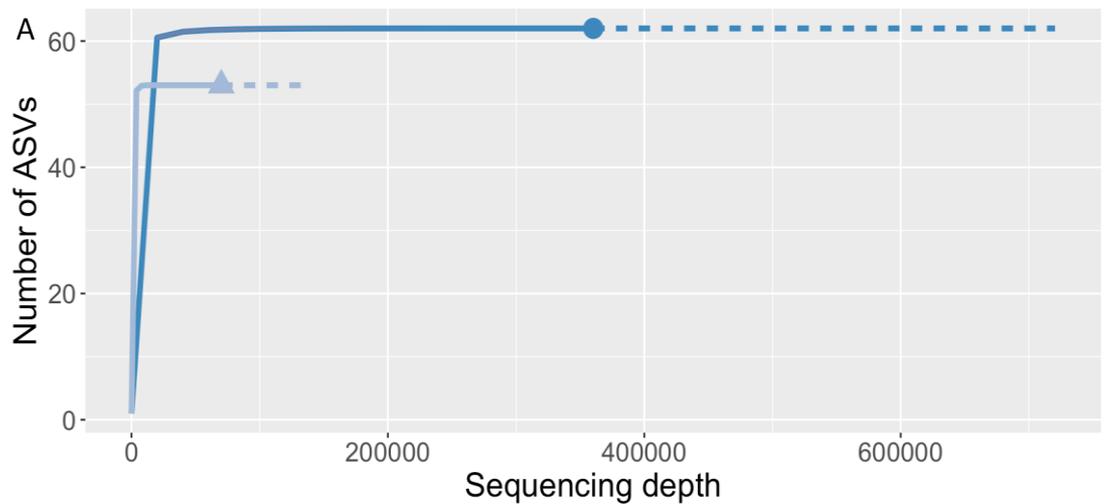
2. Phylogénie



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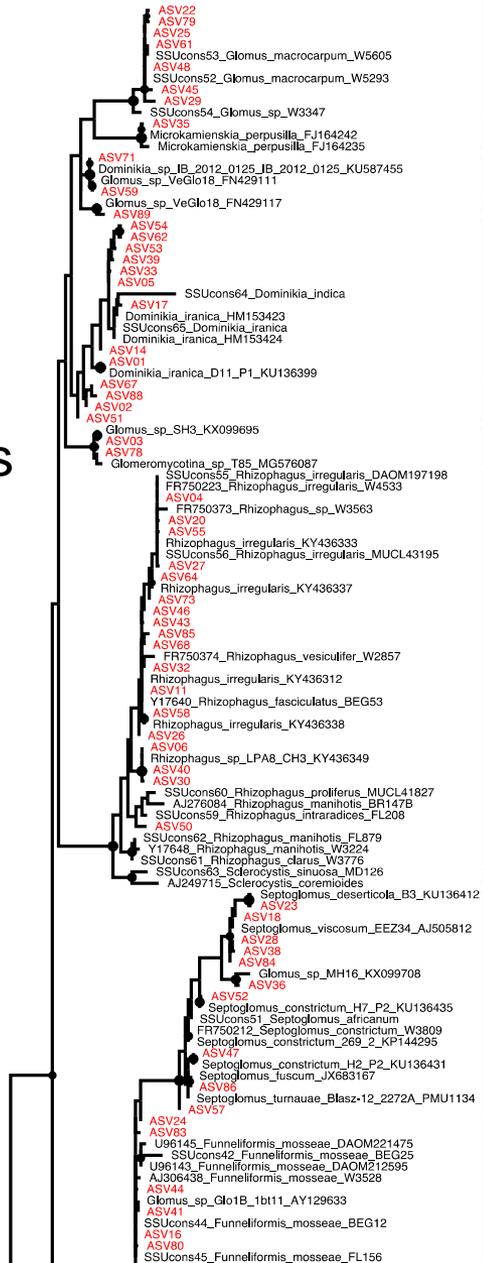




BLAST



25 ASVs identifiés  
comme Glomus



Glomus-1

Microkamienskia  
Glomus-2  
Glomus-3

Dominikia

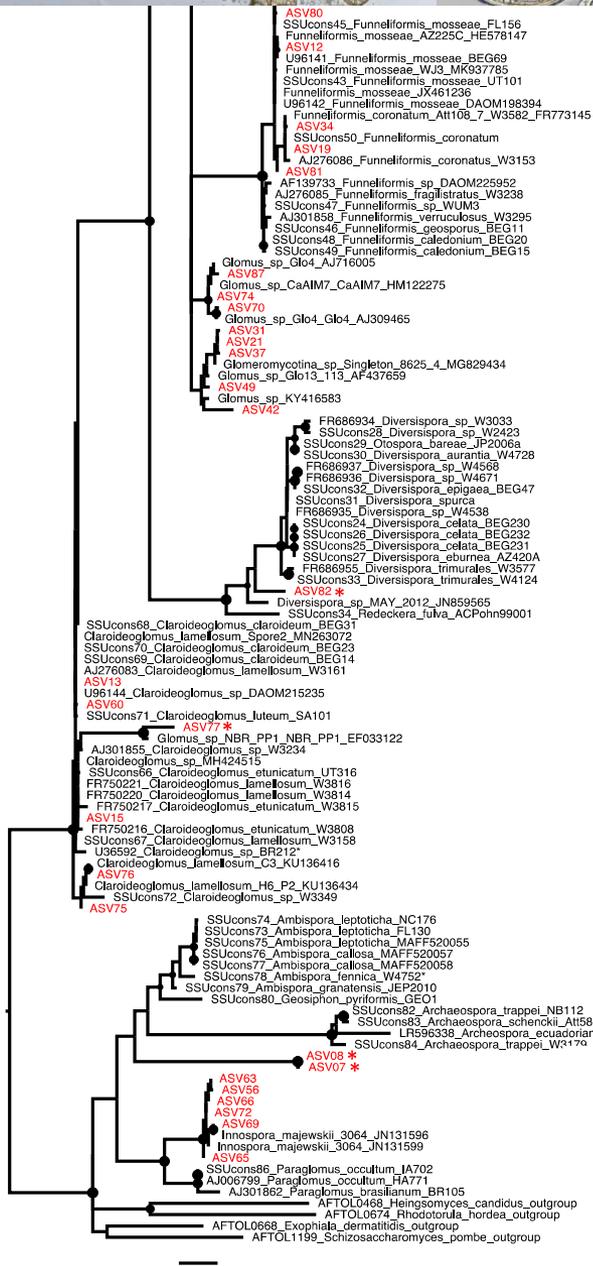
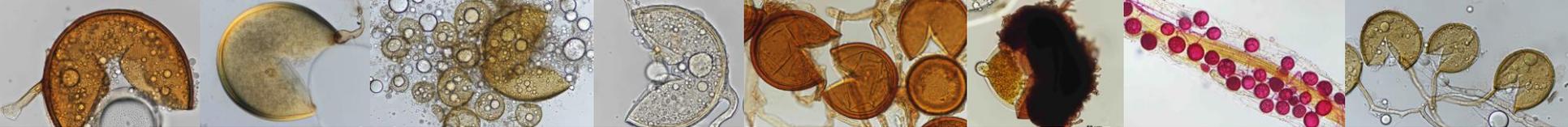
Glomus-4  
Glomus-5

Rhizophagus

Sclerocystis

Septogloimus

Glomeraceae



Funneliformis

Glomus-6

Glomus-7

Diversispora

Redeckera

Claroideoglomerus

Ambispora

Geosiphon  
Archaeospora  
Unknown

Innospora

Paraglomerus

Diversisporaceae

Claroideoglomeraceae

Ambisporaceae

Geosiphonaceae  
Archaeosporaceae  
Glomeromycota

Paraglomeraceae



## Conclusion

❑ PCR niché → pas de biais taxonomique

AML1/AML2 + nu-SSU-0595-5'/ nu-SSU-0948-3'

❑ Locus : 18S et regroupement 100%

❑ Identification des ASVs → [séquences références \(Collections\)](#)

❑ Direction future :

- 1) Séquençage de longues séquences (45S partiel, 3.2 kb)
- 2) Caractérisation de la variabilité de l'ADNr
- 3) Nouveau marqueur moléculaire?





# Remerciements



Sylvie Séguin



Claudia Banchini



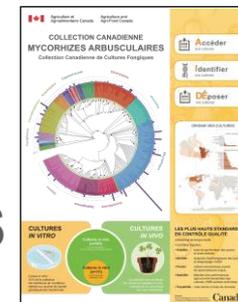
Yolande Dalpé

## COLLECTION CANADIENNE MYCORHIZES ARBUSCULAIRES

Collection Canadienne de Cultures Fongiques

### *ELAPHOMYCES* LA FAUSSE TRUFFE - THE FALSE TRUFFLE

- Karima Bencherif (U de Djelfa, Algérie)
- Stéphanie Sabourin (U Sherbrooke)
- Laboratoire des Technologies Moléculaires (LTM-AAC Ottawa)





?



