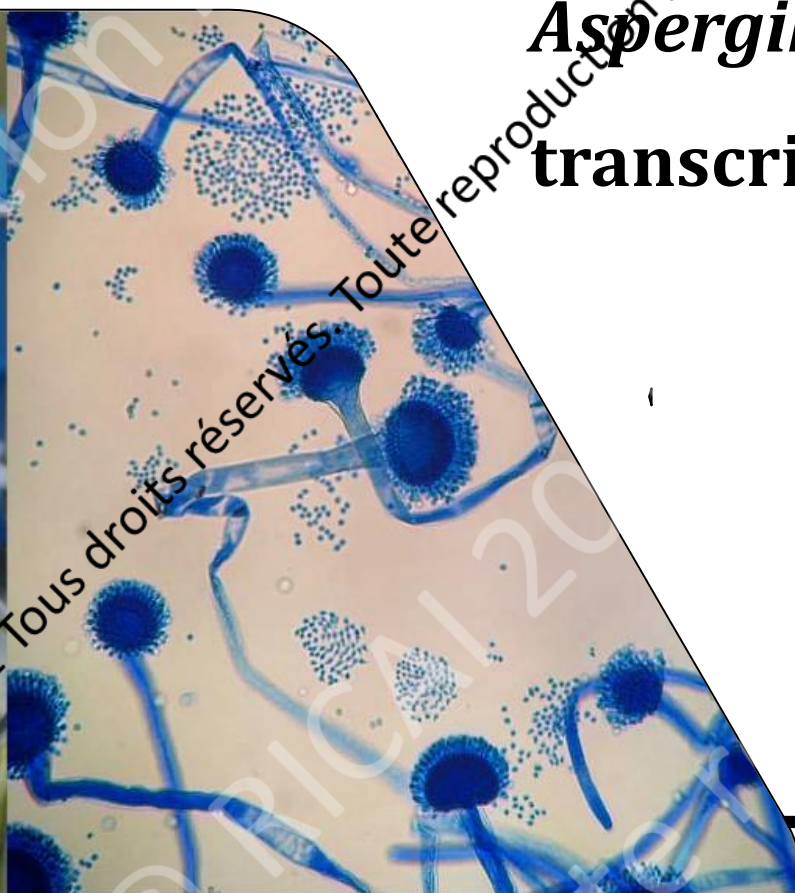
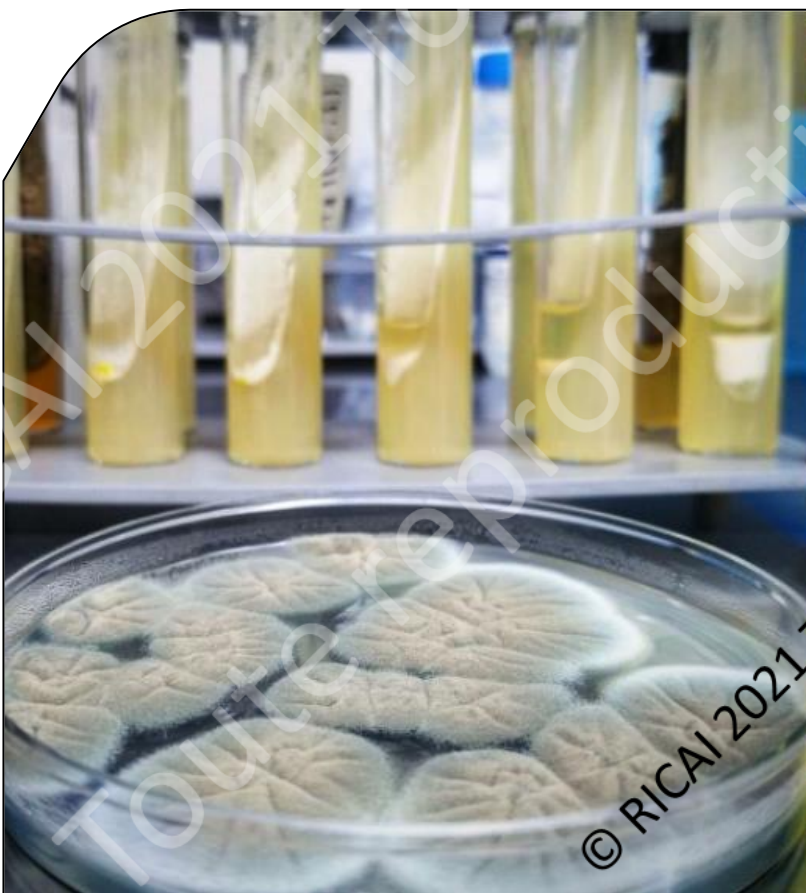


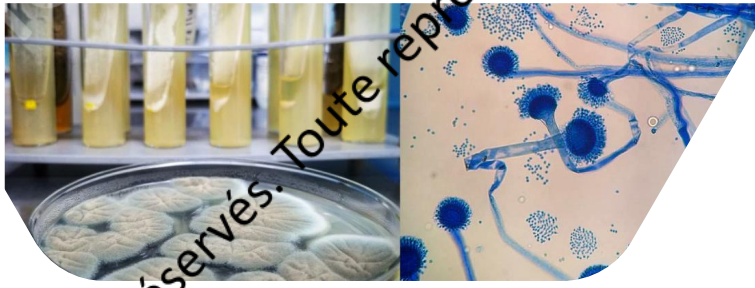
# Detection of mycovirus in *Aspergillus fumigatus* by *in silico* transcriptome-mining approach



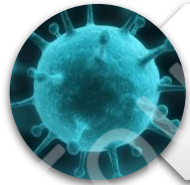
Ghelfenstein-Ferreira, Théo  
Molecular Mycology Unit- CNRS UMR2000  
Supervisor: Stéphane Bretagne  
13/12/2021



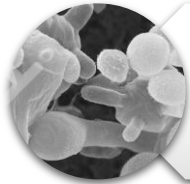
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# Why focus on mycovirus?



Infecting fungi – obligate parasite



Phenotypic host change



Agriculture field  
(*Cryphonectria parasitica*)



Medical field

**ss+RNA**

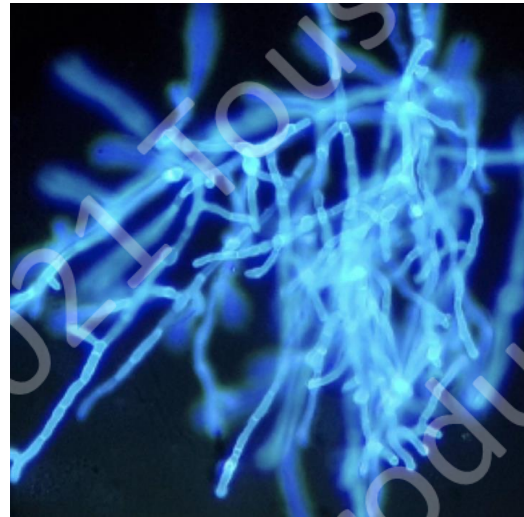
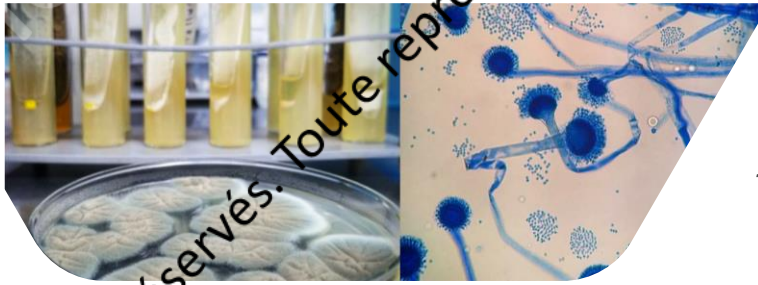
**dsRNA**

**ss-RNA**

**ssDNA**

**dsDNA**

# *Aspergillus fumigatus* & Mycovirus



Filamentous fungi  
Aspergillosis  
30 Mb genome

366 and 86 strains (AGE)  
6,6% to 18,6%

9/11 strains  
(mRNA sequencing)

Partitivirus (AfuPV-1)

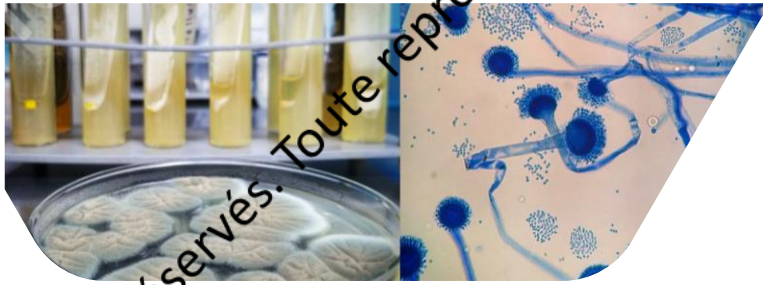
Chrysovirus (AfuCV-1,  
AthCV1, AfuCV41362)

Polymycovirus (AfuPmV-1)

Partitivirus (AfuPV-2)

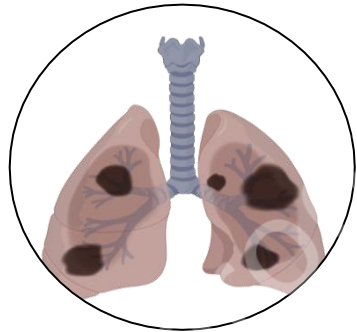
Narnavirus (AfuNV-1, 2)

Mitovirus (AfuMV-1)

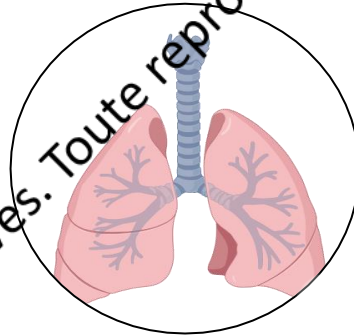


# Aim of the study

## Presence of RNA mycovirus in *Aspergillus fumigatus* by mRNA analysis



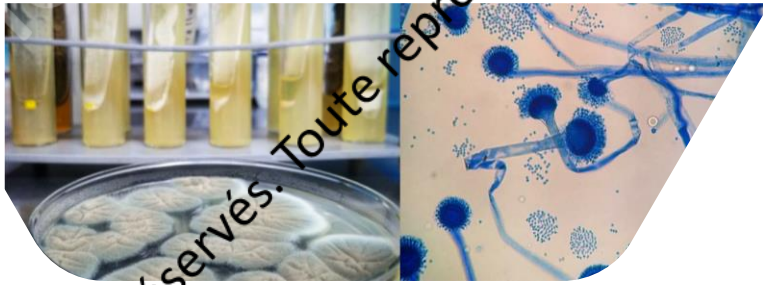
30 strains of *Aspergillus fumigatus* from **Invasive pulmonary aspergillosis**



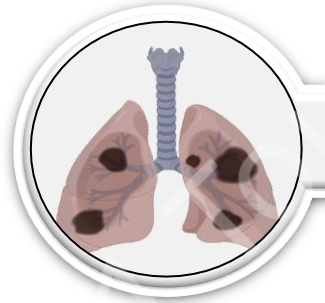
30 strains from **pulmonary colonization** by *Aspergillus fumigatus*  
COPD, Bronchiectasis, Cystic fibrosis,  
Respiratory diseases



30 strains from Saint-Louis hospital **environment**  
(air / surface)

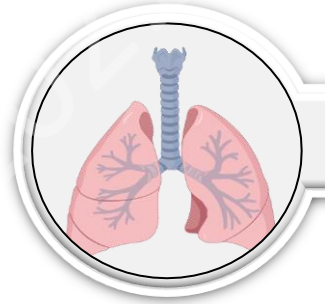


# *Aspergillus fumigatus*



Infection group : **30 isolates**

$\beta$ tub-seq *Aspergillus fumigatus*  
72h MIC : 1 pan-azole resistant isolate



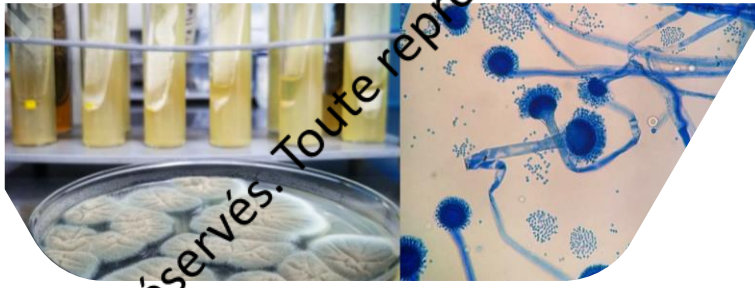
Colonization group : **30 isolates**

$\beta$ tub-seq *Aspergillus fumigatus*  
72h MIC : 1 azole resistant isolate (VRC)

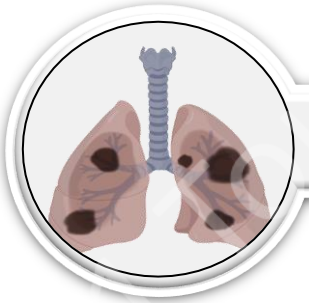


Environmental group : **30 isolates**

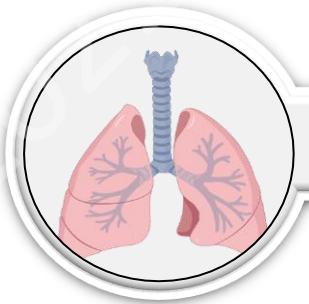
$\beta$ tub-seq *Aspergillus fumigatus*  
72h MIC : 1 azole resistant isolate (VRC)  
1 pan-azole resistant isolate



# Poly-A selection and mRNA sequencing



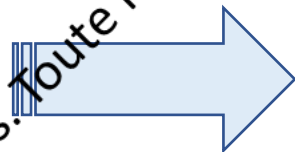
Infection group : 30 isolates



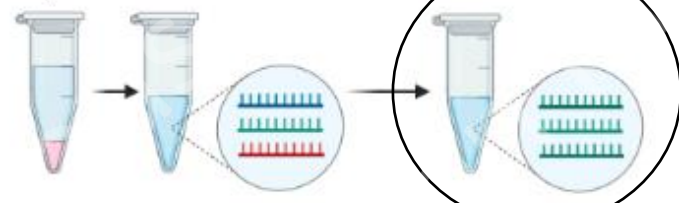
Colonization group : 30 isolates



Environmental group : 30 isolates



20 hours shaking culture in liquid minimal media at 37°C



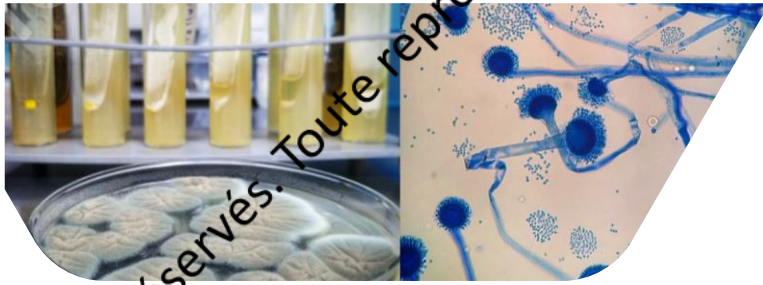
Total RNA extraction by Phenol/Chloroform

Poly-A selection with Illumina Stranded mRNA Prep Kit

mRNA

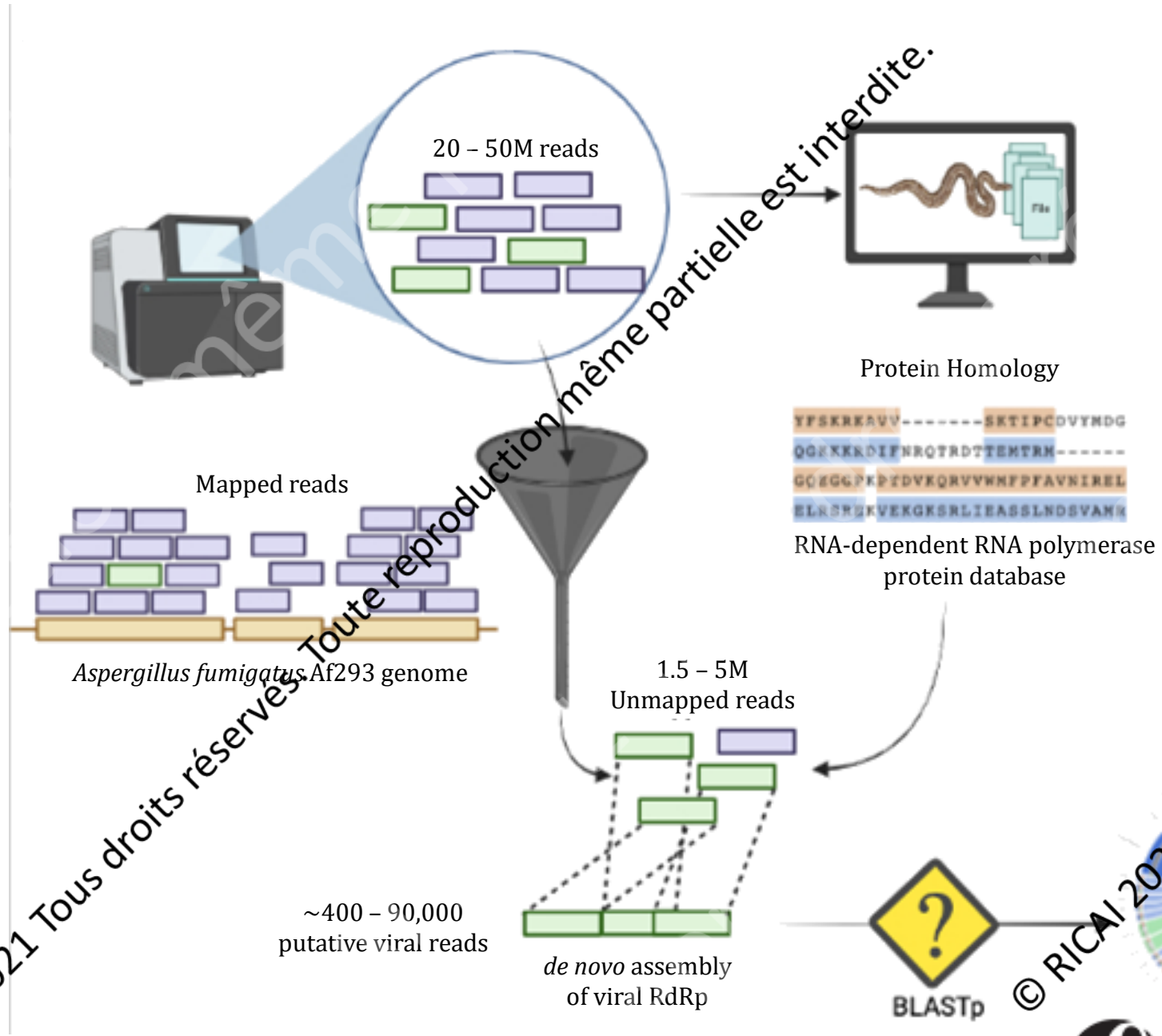


mRNA sequencing (NextSeq 2000 Illumina platform)

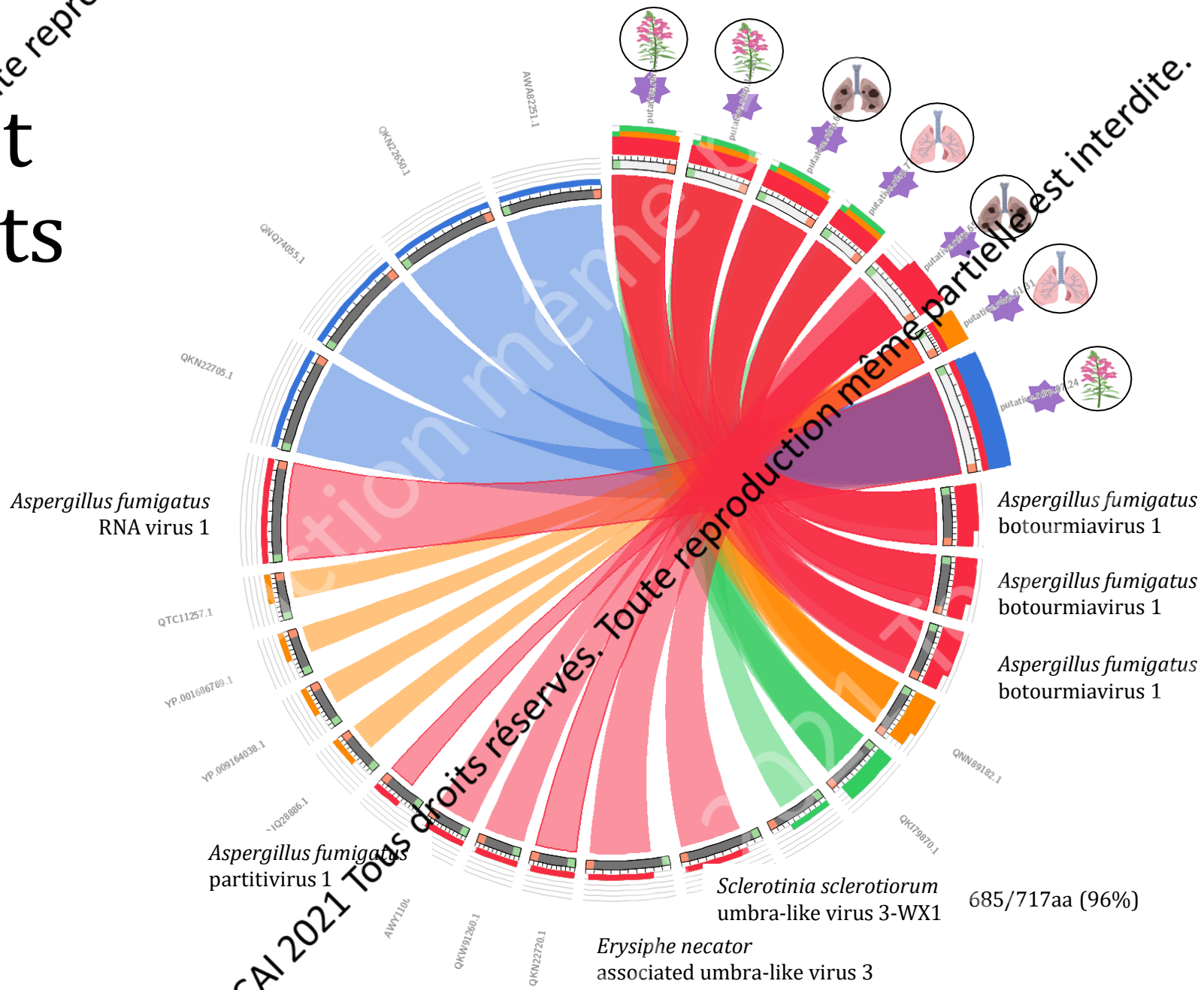


# Algorithm Analysis

1. Reads mapped against reference
2. Unmapped reads *de novo* assembly
3. Contigs homologous to RdRp family proteins
4. Contig Extender
5. BLASTp (protein database)



# Blast results



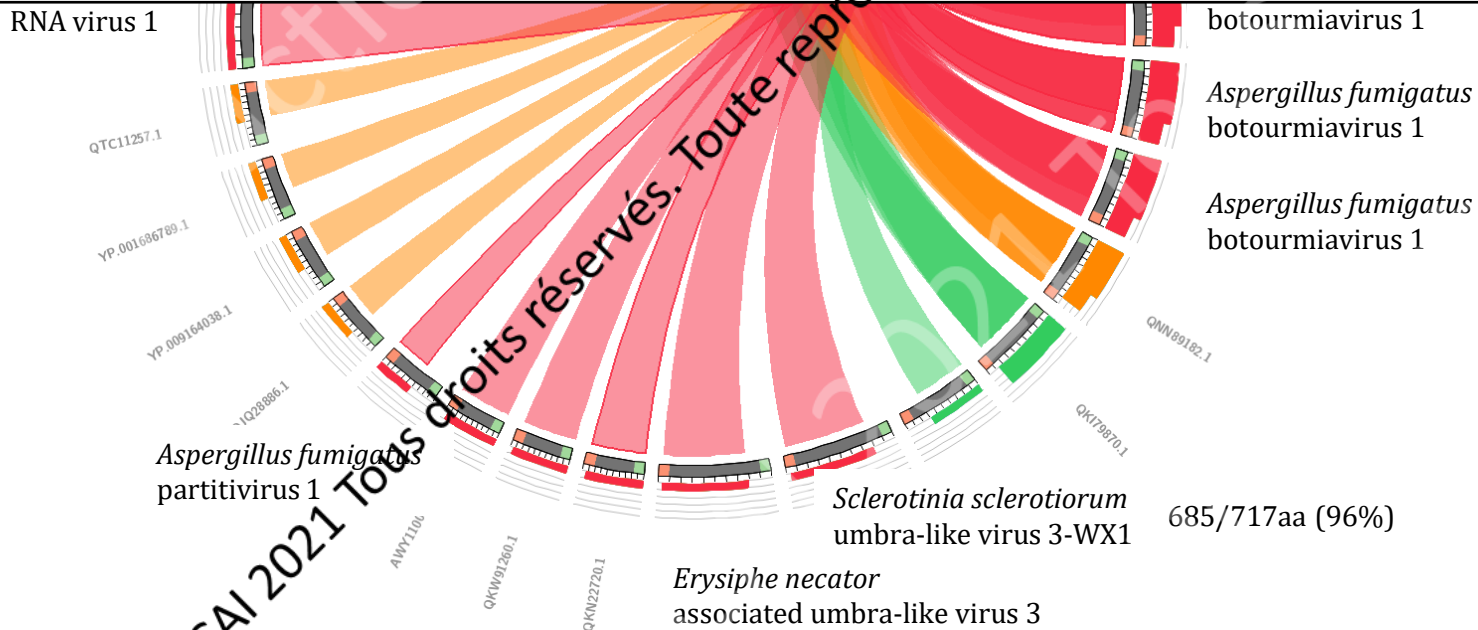


# Blast results



Environmental group : **30 isolates**

- 3 mycoviruses
- *Af botourmiavirus* (x2)
- *Af RNA virus* 1



★ Query sequence

% identity ratio:  
Score-min/max-min



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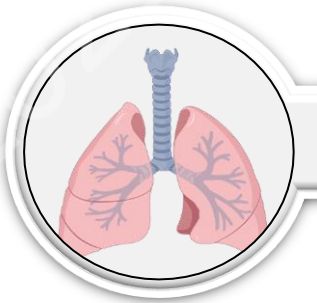
# Blast results



Environmental group : **30 isolates**

3 mycoviruses

- *Af botourmiavirus* (x2)
- *Af RNA virus 1*



Colonization group : **30 isolates**

2 mycoviruses

- *Af botourmiavirus*
- *Af partitivirus 1*

✦ Query sequence

% identity ratio:  
Score-min/max- $\mu$ m



*Aspergillus fumigatus*  
partitivirus 1

*Sclerotinia sclerotiorum*  
umbra-like virus 3-WX1 685/717aa (96%)

*Erysiphe necator*  
associated umbra-like virus 3

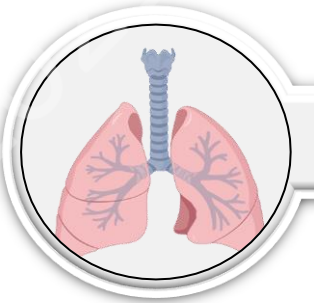
# Blast results



Environmental group : **30 isolates**

3 mycoviruses

- *Af botourmiavirus* (x2)
- *Af RNA virus 1*



Colonization group : **30 isolates**

2 mycoviruses

- *Af botourmiavirus*
- *Af partitivirus 1*



Infection group : **30 isolates**

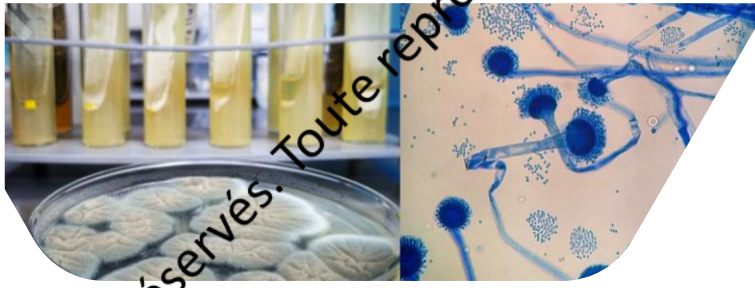
2 mycoviruses

- *Af botourmiavirus*
- Putative new mycovirus

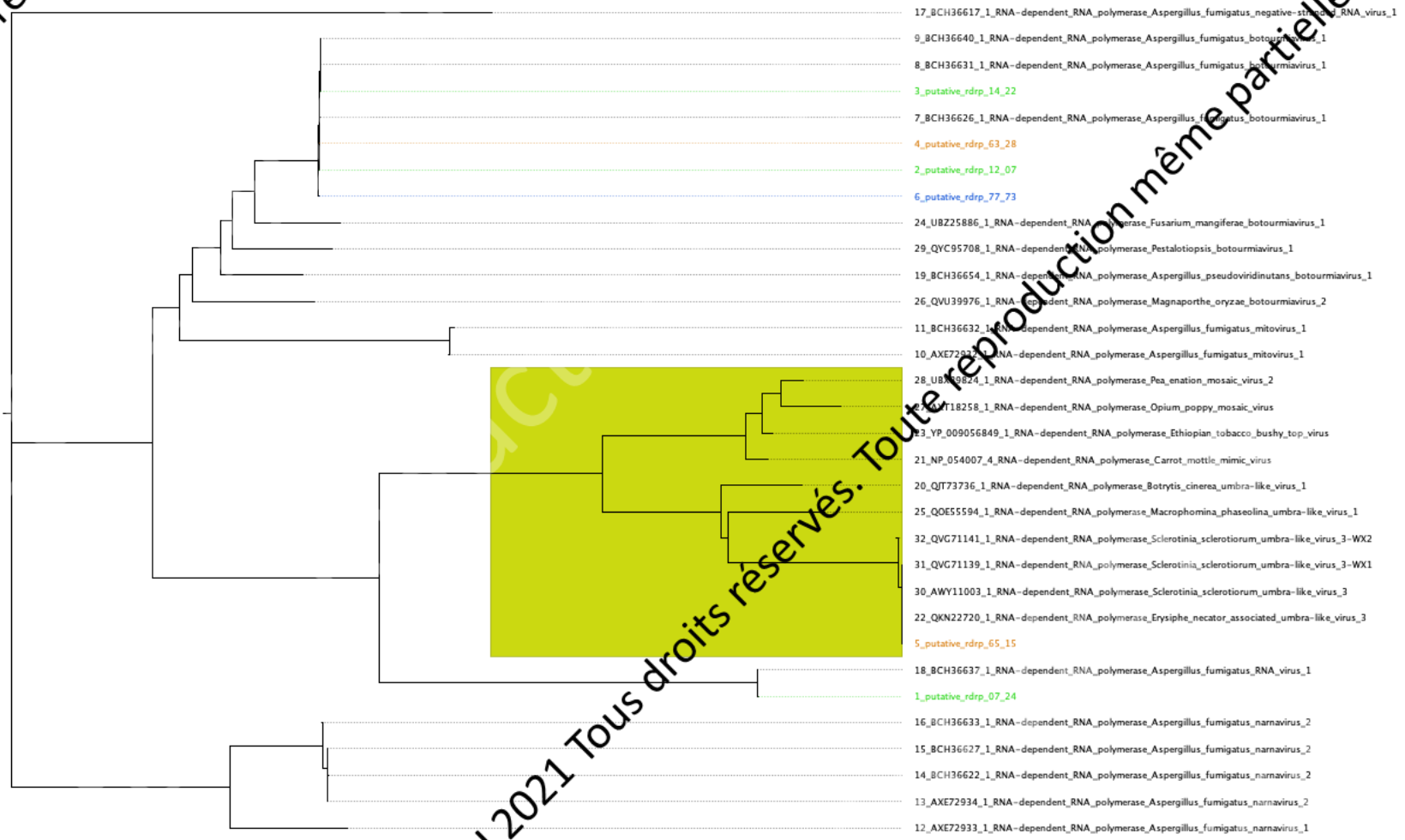
✦ Query sequence

% identity ratio:  
Score-min/max- $\mu$ m

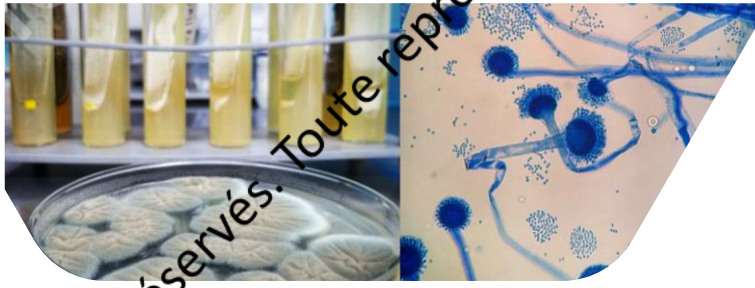




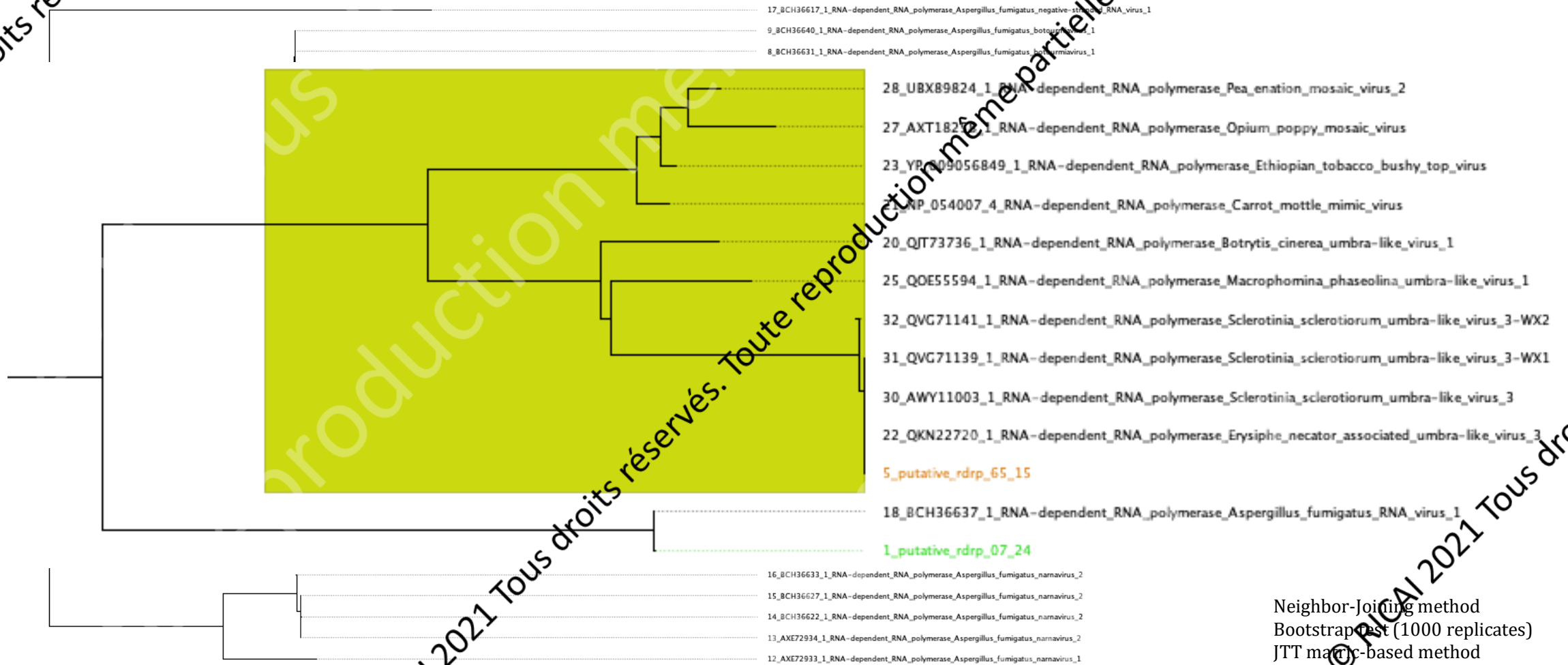
# Phylogenetic analysis of the putative RdRp virus in Family *Tombusviridae*



Neighbor-Joining method  
 Bootstrap test (1000 replicates)  
 JTT matrix-based method



# Phylogenetic analysis of the putative RdRp virus in Family *Tombusviridae*



Neighbor-Joining method  
 Bootstrap test (1000 replicates)  
 JTT matrix-based method

# Conclusion



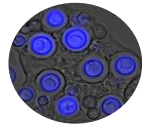
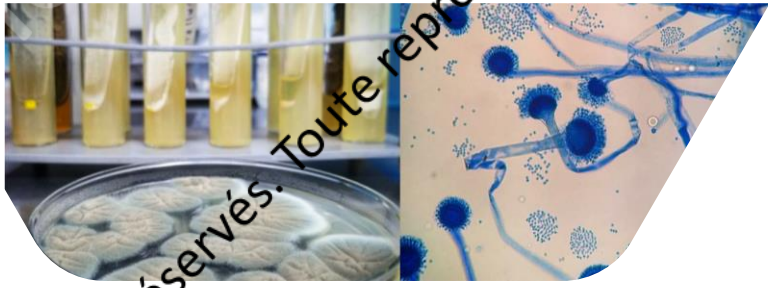
Development of an analysis pipeline for virus detection in RNA sequencing data

90 isolates successfully sequenced

- 7 viral sequences found (prevalence 7.8%)
  - Each group showed the presence of viral RNA sequence
  - No infected resistant isolates



1 putative new mycovirus related to *Umbra*-like virus  
(Invasive pulmonary aspergillosis Group)



## Molecular Mycology Unit

- Stéphane Bretagne (M2 supervisor)
- Vishukumar Aamanianda for his supervision

Françoise Dromer

Alexandre Alanio  
 Raffael Araujo de Castro  
 Christèle Auguste  
 Agathe Bertho  
 Karine Boukris-Sitbon  
 Sarah Dellière  
 Marie Desnos-Ollivier  
 Émilie Fruquière  
 Dea Garcia-Hernando  
 Fanny Lantermier  
 Olivier Lortholary  
 Aude Sporny-Leclere  
 Sarah Wong  
 Cécile Gautier



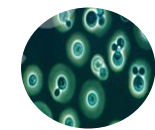
## Biomix platform

Directly involved:

- Thomas Cokelaer
- Juliana Pipoli Da Fonseca
- Valérie Briolat

For help, and welcome:

- Marc Monot
- Laurence Ma
- Etienne Kornobis
- Dimitri Desvillechabrol



## RNA Biology of fungal pathogens Unit

Isabelle Mouyna

Many thanks for the funding to:



Parasitology and Mycology Laboratory:

- Dr Samia Hamane
- Laboratory technician team

Virology Laboratory:

- Maud Salmona

# Acknowledgements

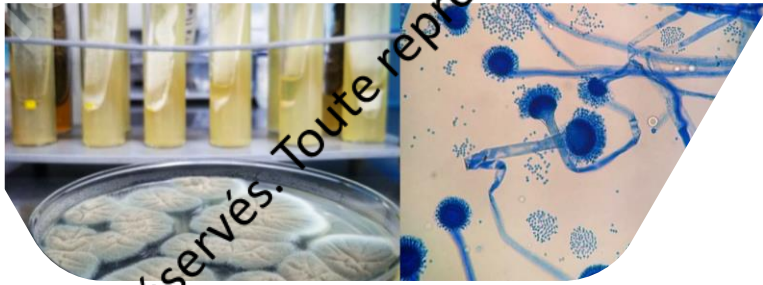


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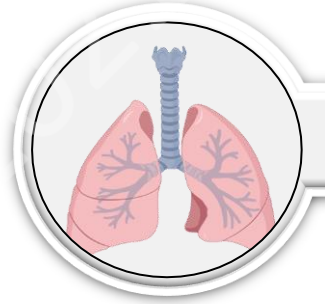
# Mycoviruses



Infection group : **30 isolates**

2 mycoviruses

- *Af* Botourmiavirus
- Putative new mycovirus



Colonization group : **30 isolates**

2 mycoviruses

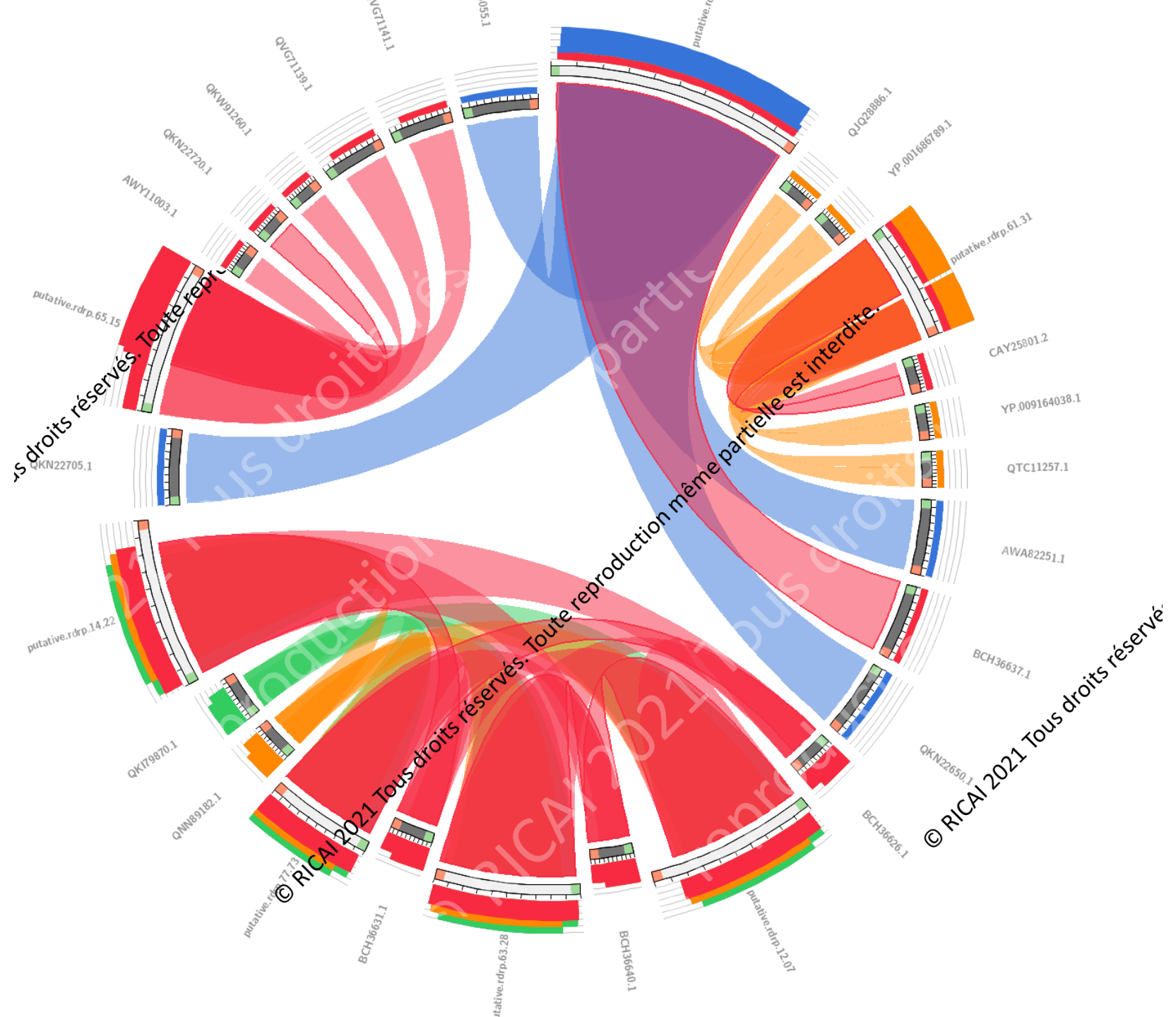
- *Af* Botourmiavirus
- *Af* partitivirus 1



Environmental group : **30 isolates**

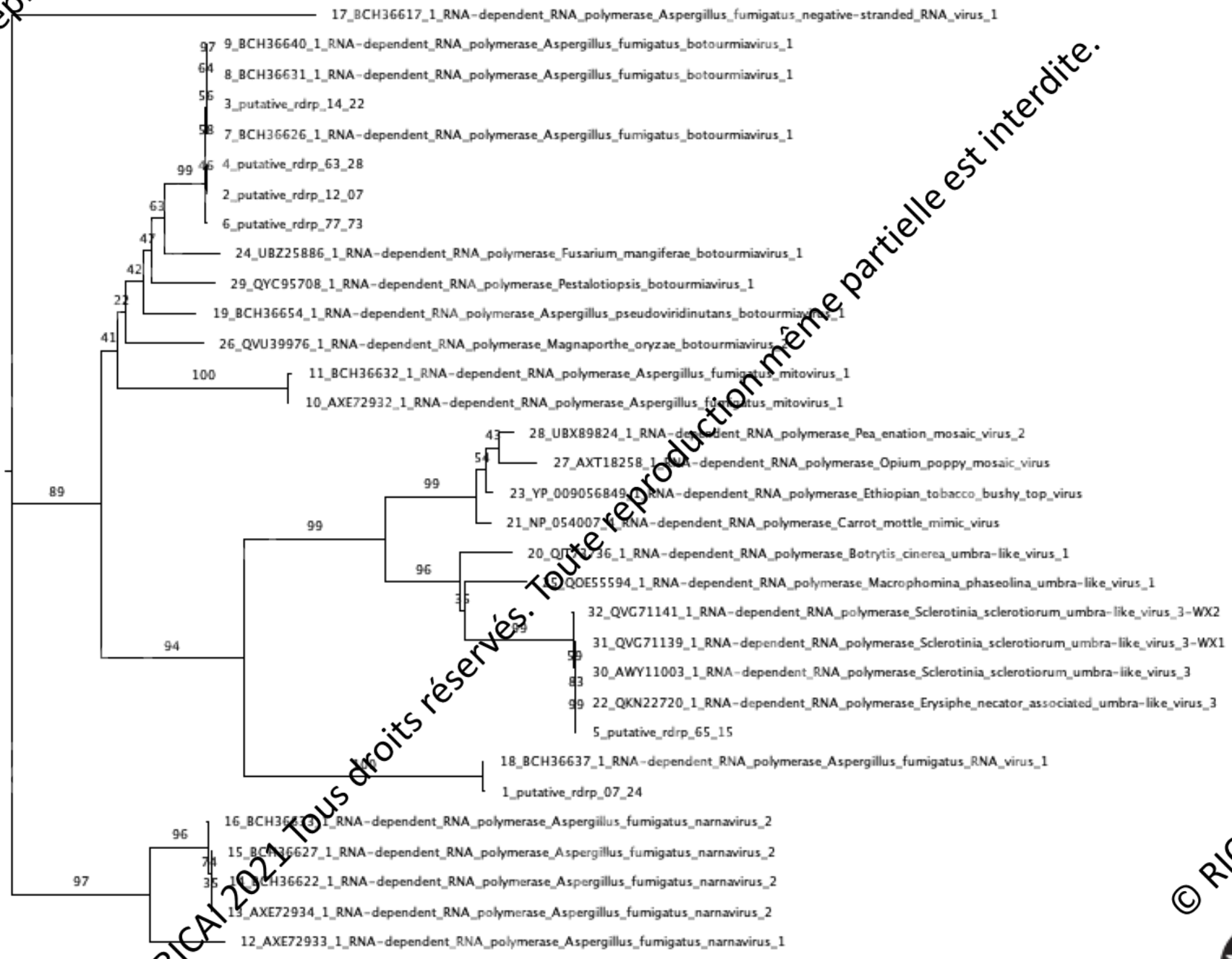
3 mycoviruses

- *Af* Botourmiavirus (x2)
- *Af* RNA virus 1



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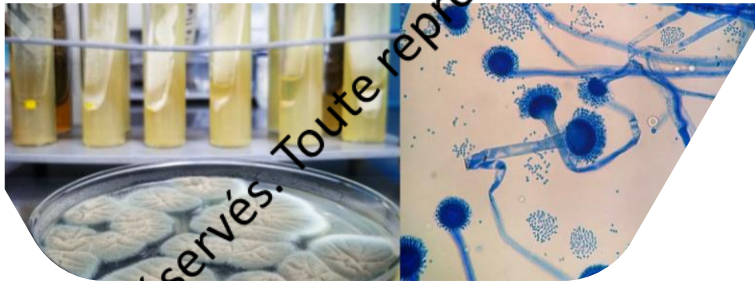
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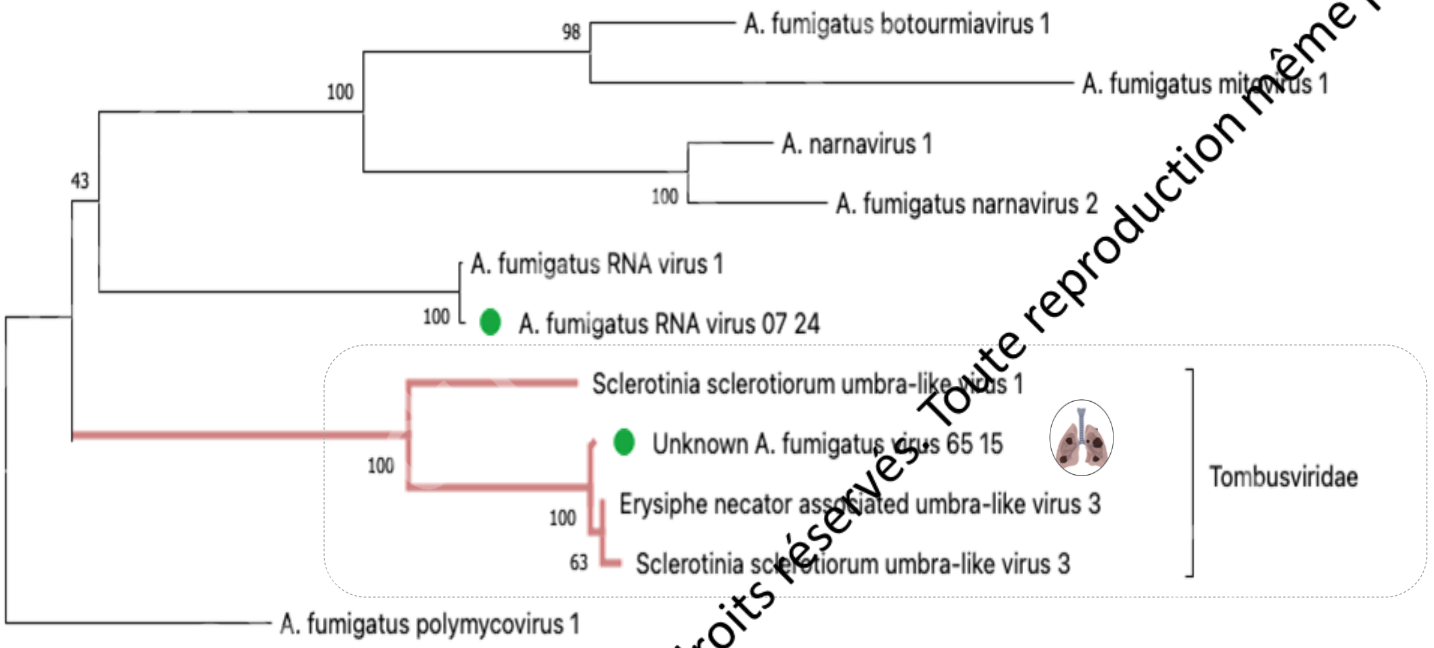
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# Phylogenetic analysis of the putative RdRp virus in Family *Tombusviridae*



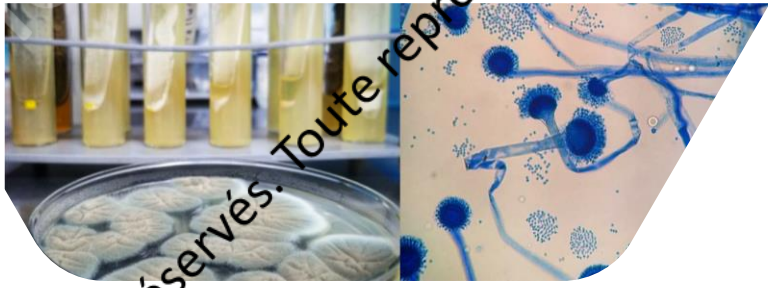
0.50

Neighbor-Joining method  
 Bootstrap test (500 replicates)  
 JTT matrix-based method (gamma = 11.78)

● RdRp sequence from the current study

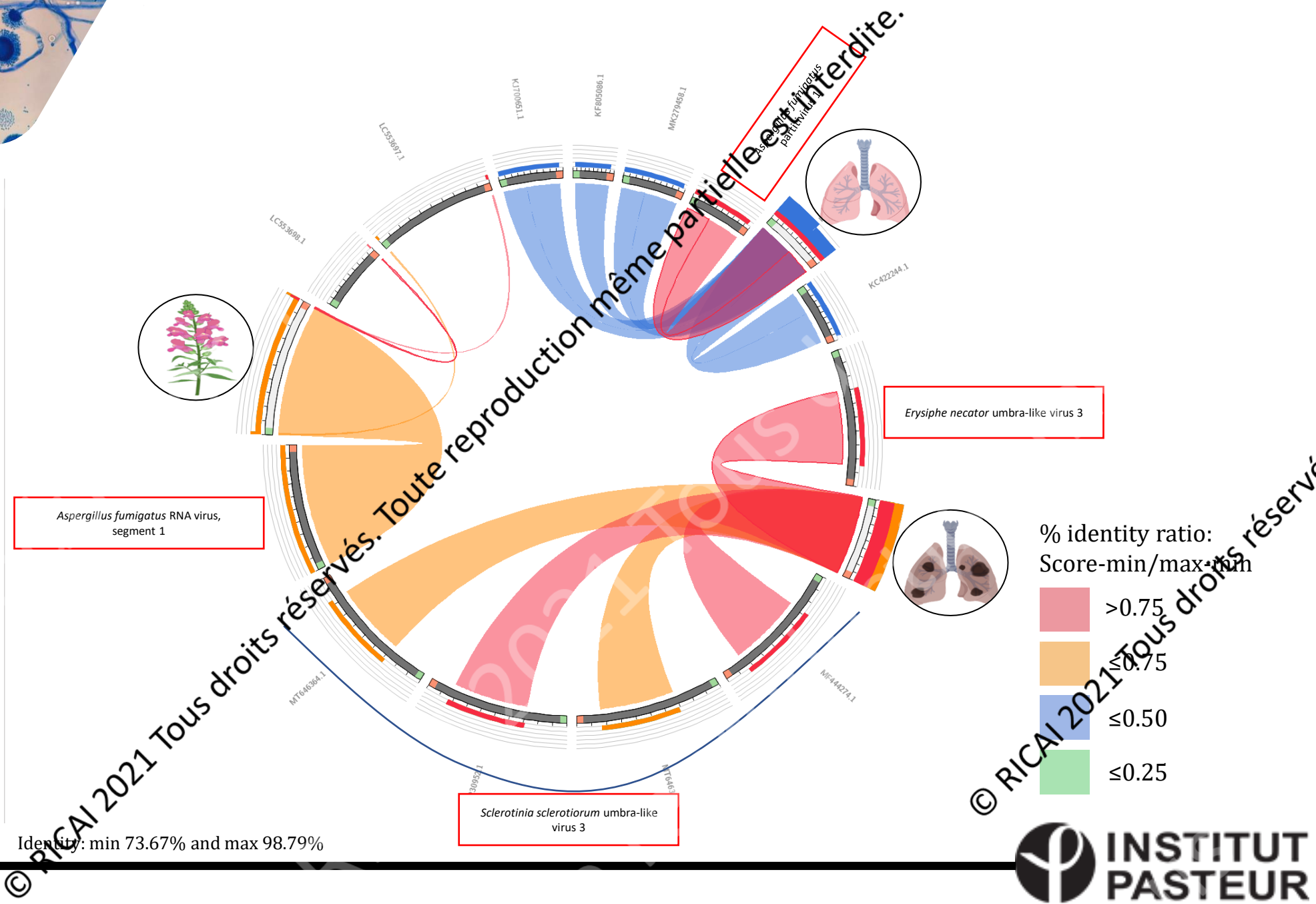
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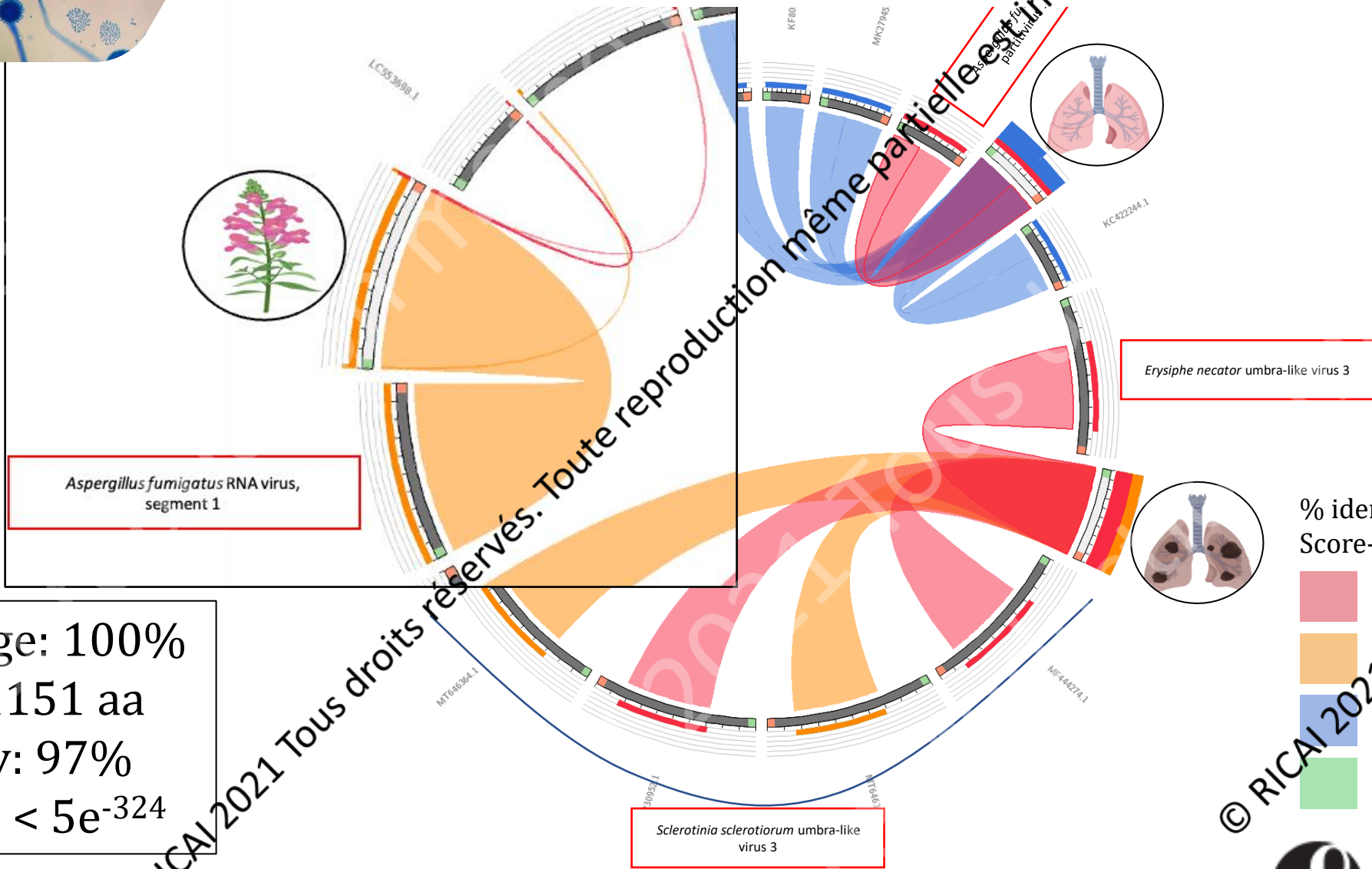
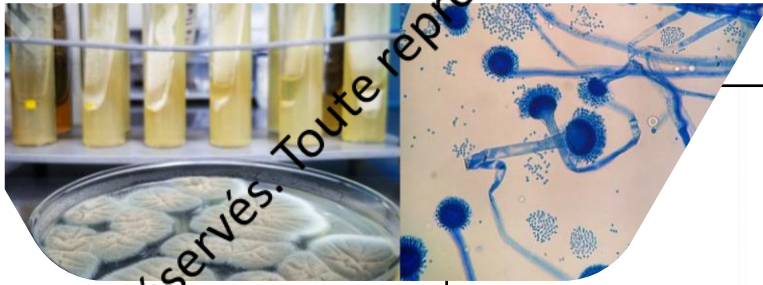


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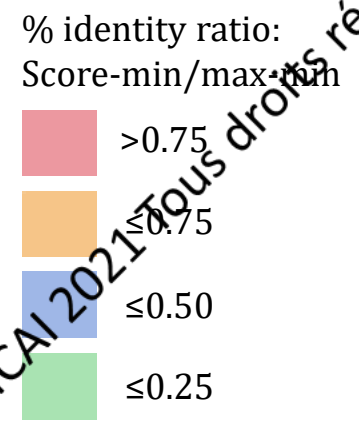
# Blast results

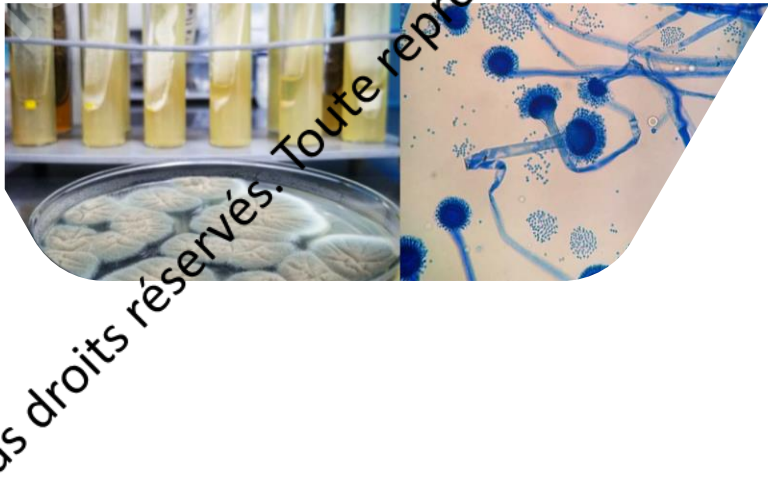


# Aspergillus fumigatus RNA virus 1



- Coverage: 100%
- 1151/1151 aa
- Identity: 97%
- E-value <  $5e^{-324}$

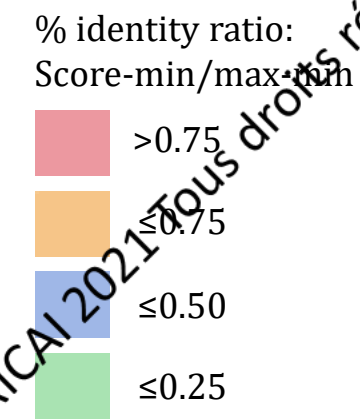
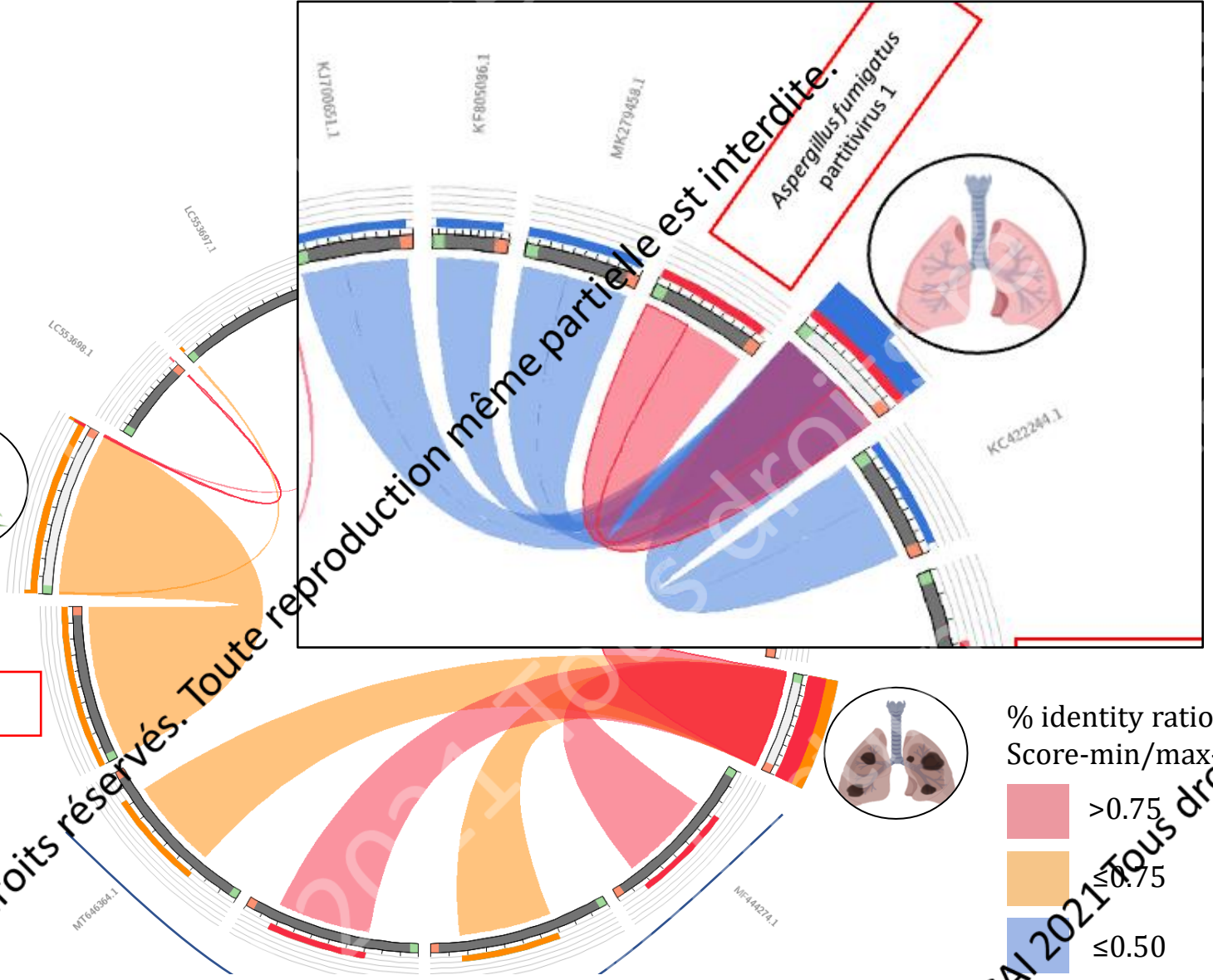




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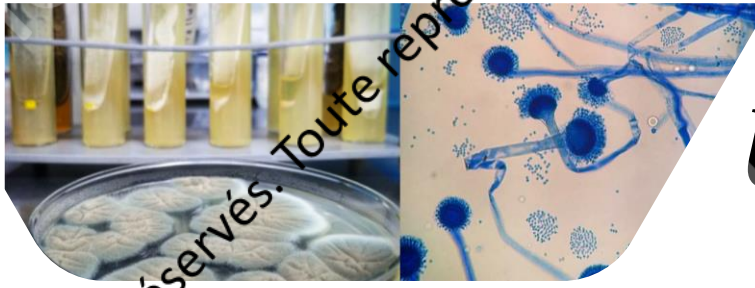
- Coverage: 100%
- 542/542 aa
- Identity: >99%
- E-value < 5e<sup>-324</sup>

*fumigatus* RNA virus, segment 1



# *Aspergillus fumigatus* partitivirus 1

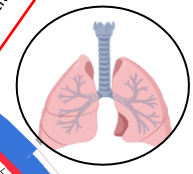
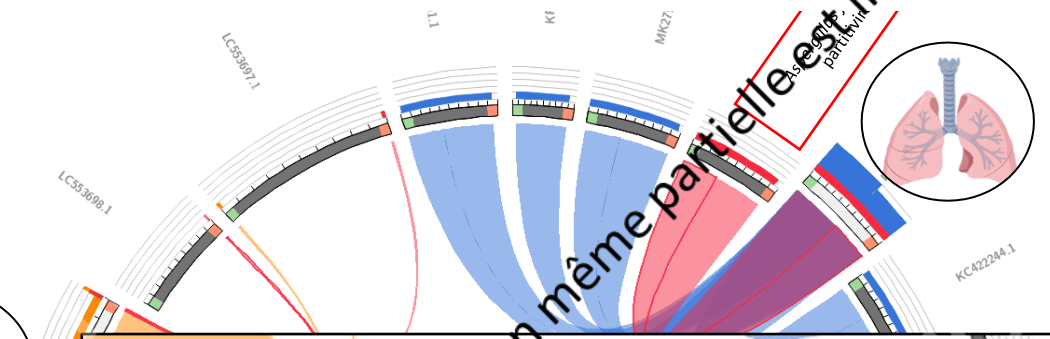
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# Undescribed *A. fumigatus* virus

- Coverage: 99%
- 636/953 aa
- Identity: 96%
- E-value <  $5e^{-324}$

*Aspergillus fumigatus* RNA virus, segment 1



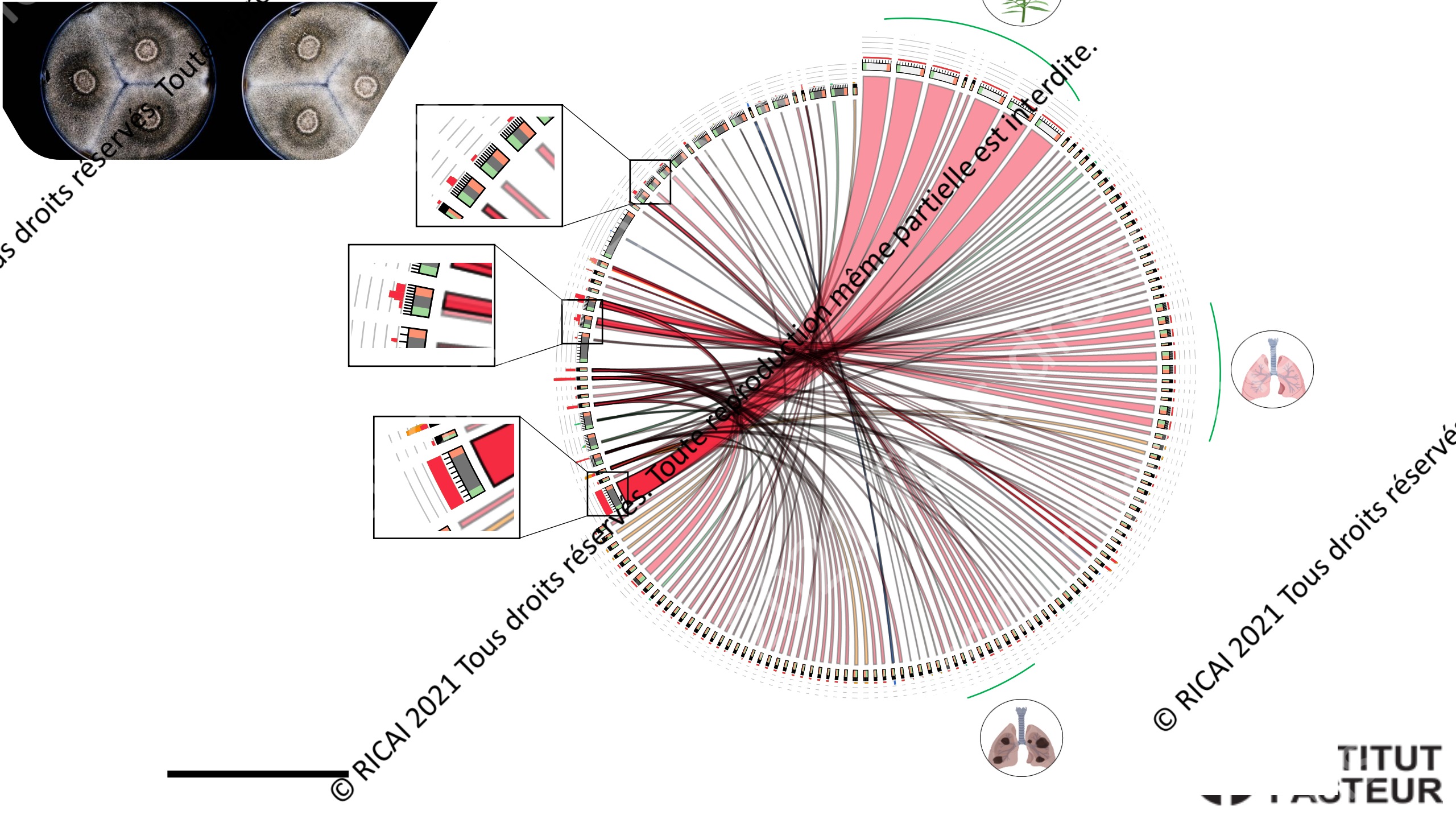
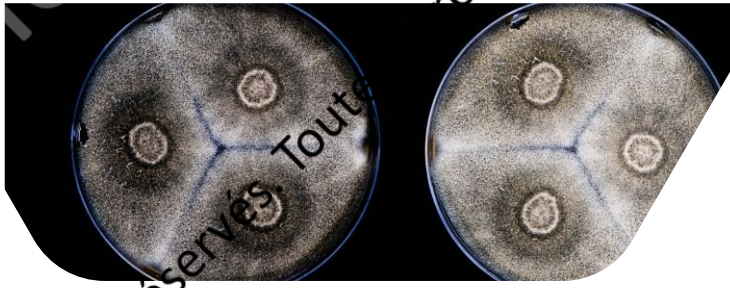
*Erysiphe necator* umbra-like virus 3



*Sclerotinia sclerotiorum* umbra-like virus 3

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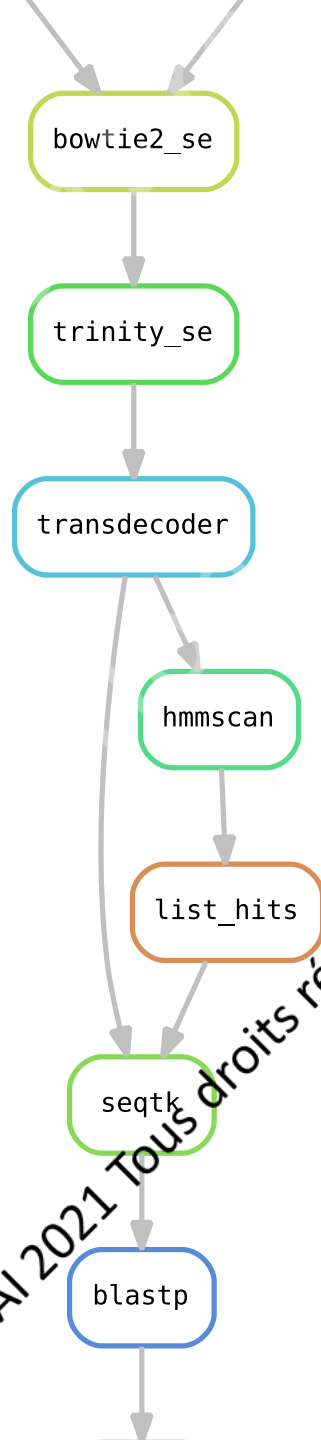




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<u>Scientific Name</u>	<u>Max Score</u>	<u>Total Score</u>	<u>Query Cover</u>	<u>E value</u>	<u>Per. Ident</u>	<u>Acc. Len</u>	Accession			
Select seq dbj BCH36637.1	RNA- depen t R pol e [As s fun RN 1]	Select seq gb QVG71 141.1	RNA- dependent RNA polymeras e [Sclerotinia sclerotioru m umbra- like virus 3- WX2]	1286	1286	99%	0.0	96.11%	2159 nt →953	<a href="#">QVG71141</a> .1

Select seq emb CAY2 5801.2	RNA- depen RNA polym [Aspe fumigatus partitivirus 1]	Select seq gb QVG71 139.1	RNA- dependent RNA polymeras e [Sclerotinia sclerotioru m umbra- like virus 3- WX1]	1274	1274	99%	0.0	95.54%	953	<a href="#">QVG71139</a> .1
----------------------------------	---	---------------------------------	--	------	------	-----	-----	--------	-----	--------------------------------

- L'idée de voir TOUS les isolats cliniques se différencier des autres, peu probable.
- Question de la persistance du virus avec repiquage, subculture ...
- « New » mycovirus, c'est simple pour une publi spécialisé, mais signification ? prévoir des modèles de tests in vitro ? éventuellement sur des plantes ?
- Perspectives du travail : quoi faire du reste des séquences ?
- Quid virus ADN