

**Positive selection of  
*Exophiala dermatitidis*  
genotype A in human  
environment**

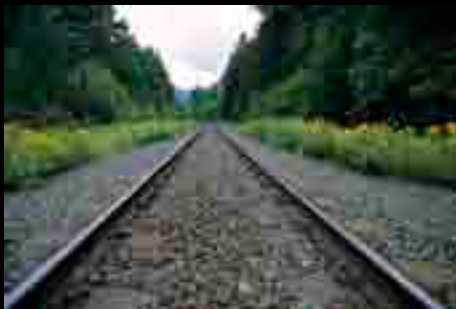
Pathogenic potential of black yeasts

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Montarop Sudhadham**

**ISHAM Working groups on Black Yeasts and Chromoblastomycosis  
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Ljubljana, Slovenia**



***Exophiala dermatitidis***



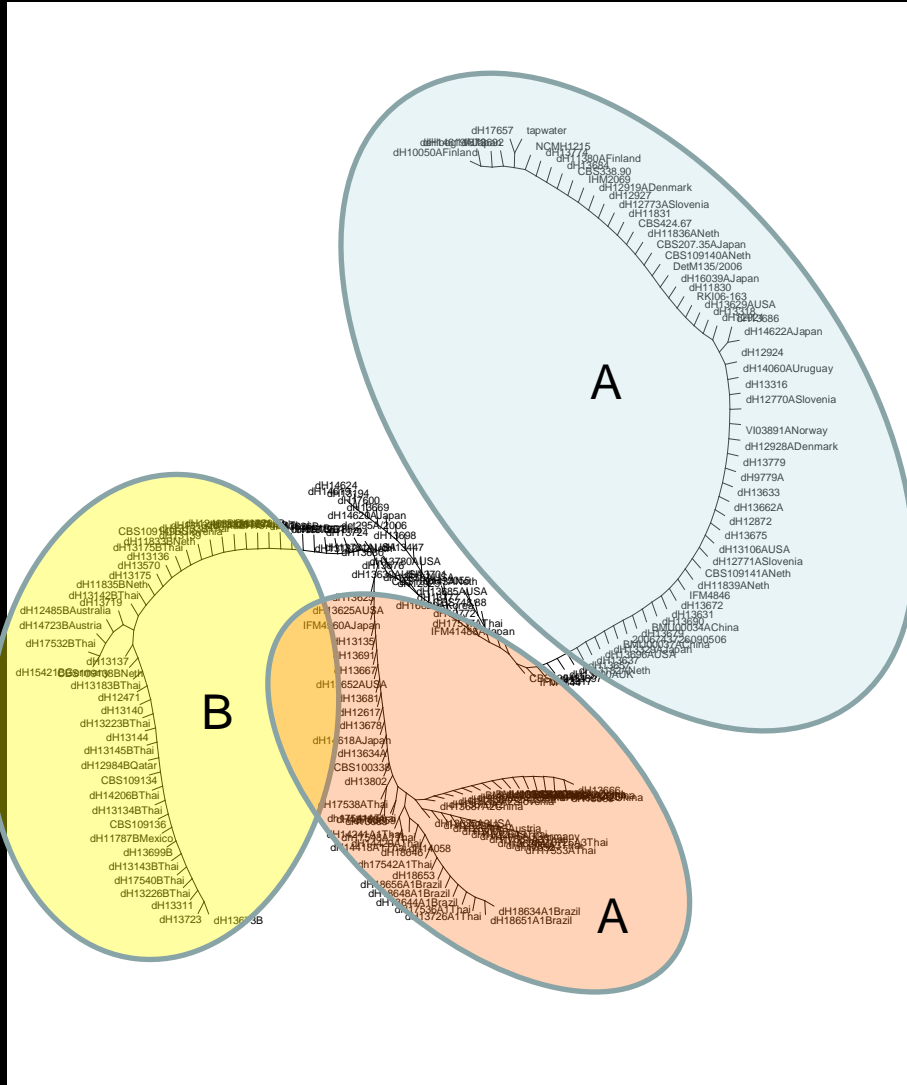


## Markers used for research

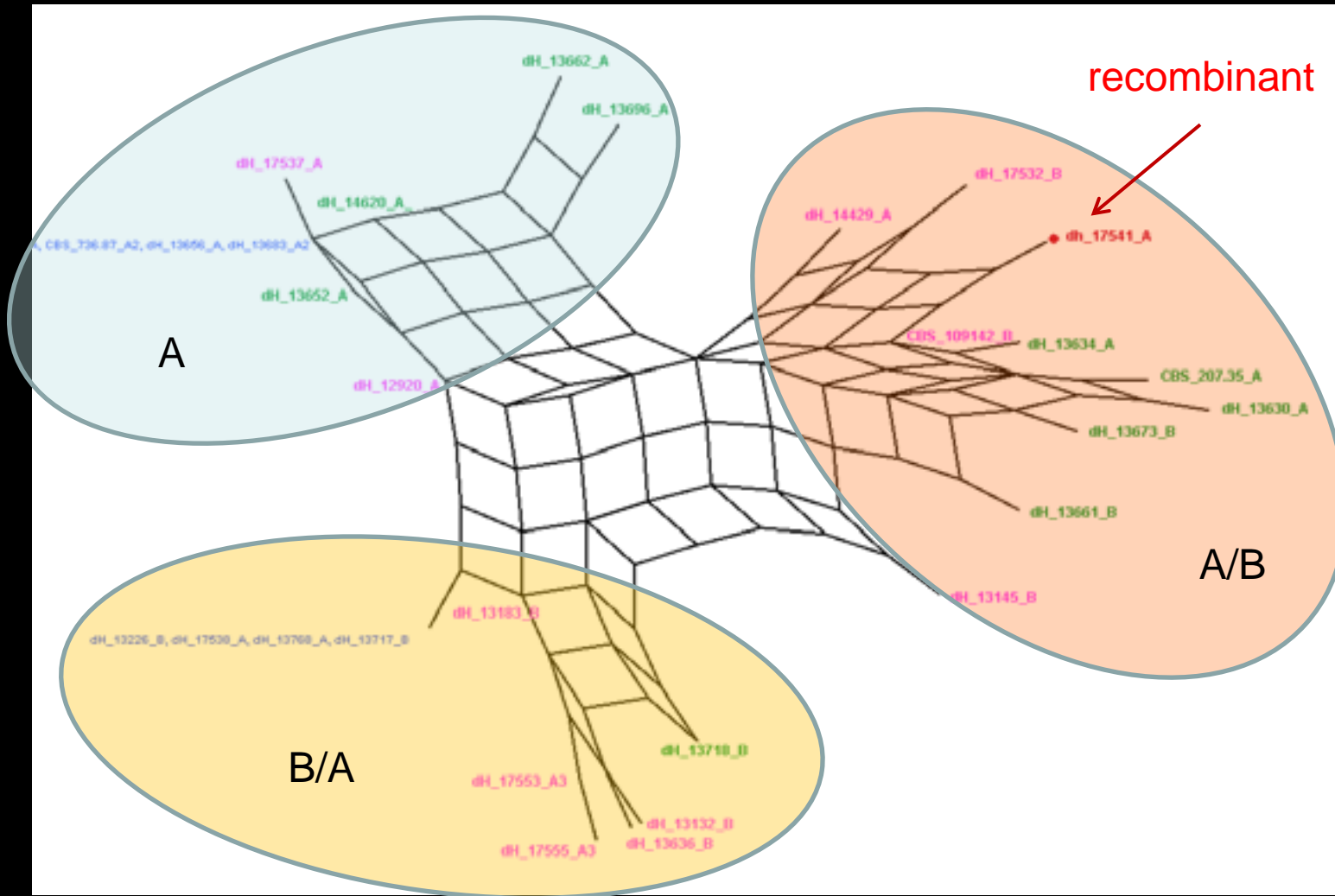
- internal transcribed spacers (ITS)
- $\beta$ -tubulin (BT2)
- elongation factor 1 (EF1)
- cell division cycle (CDC42)

### Statistics:

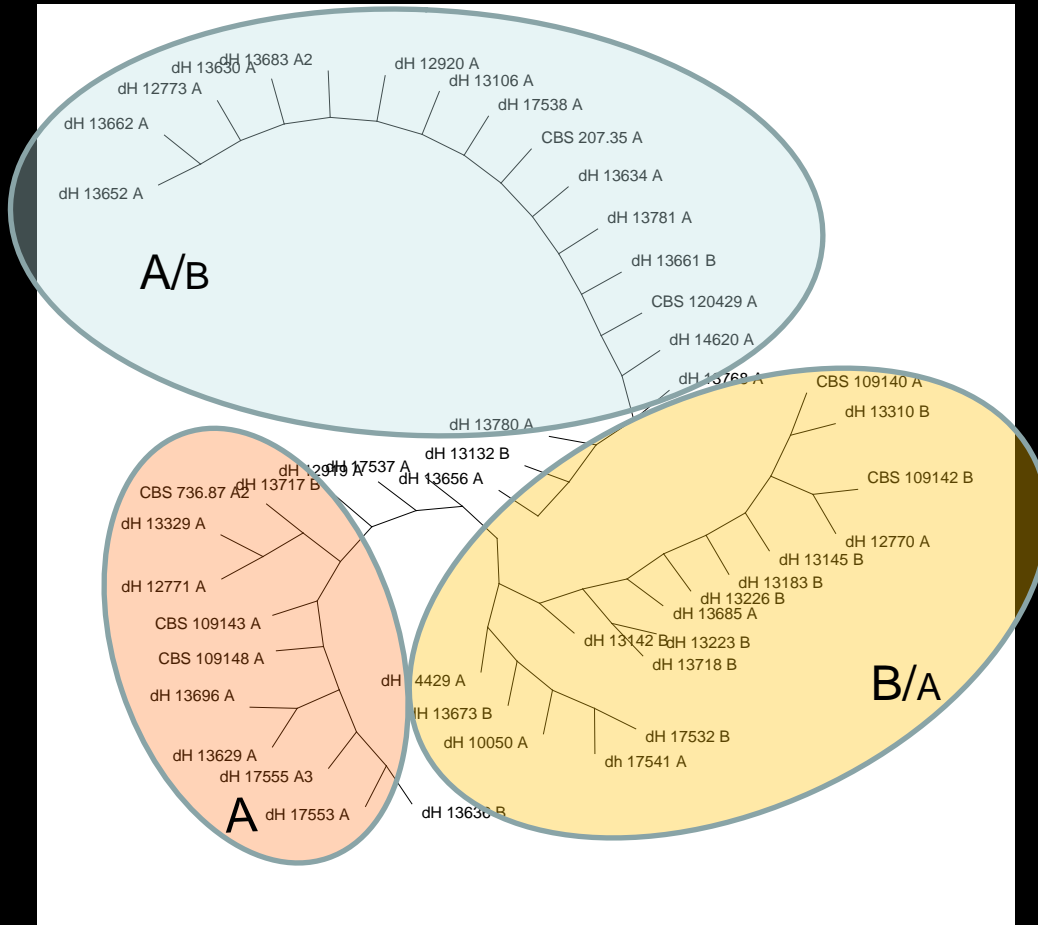
- Tajima's D
- Fu & Li's  $D^*$  and  $F^*$
- $F_{ST}$
- dN/dS



ITS – tree  
(substitution model HKY+G)



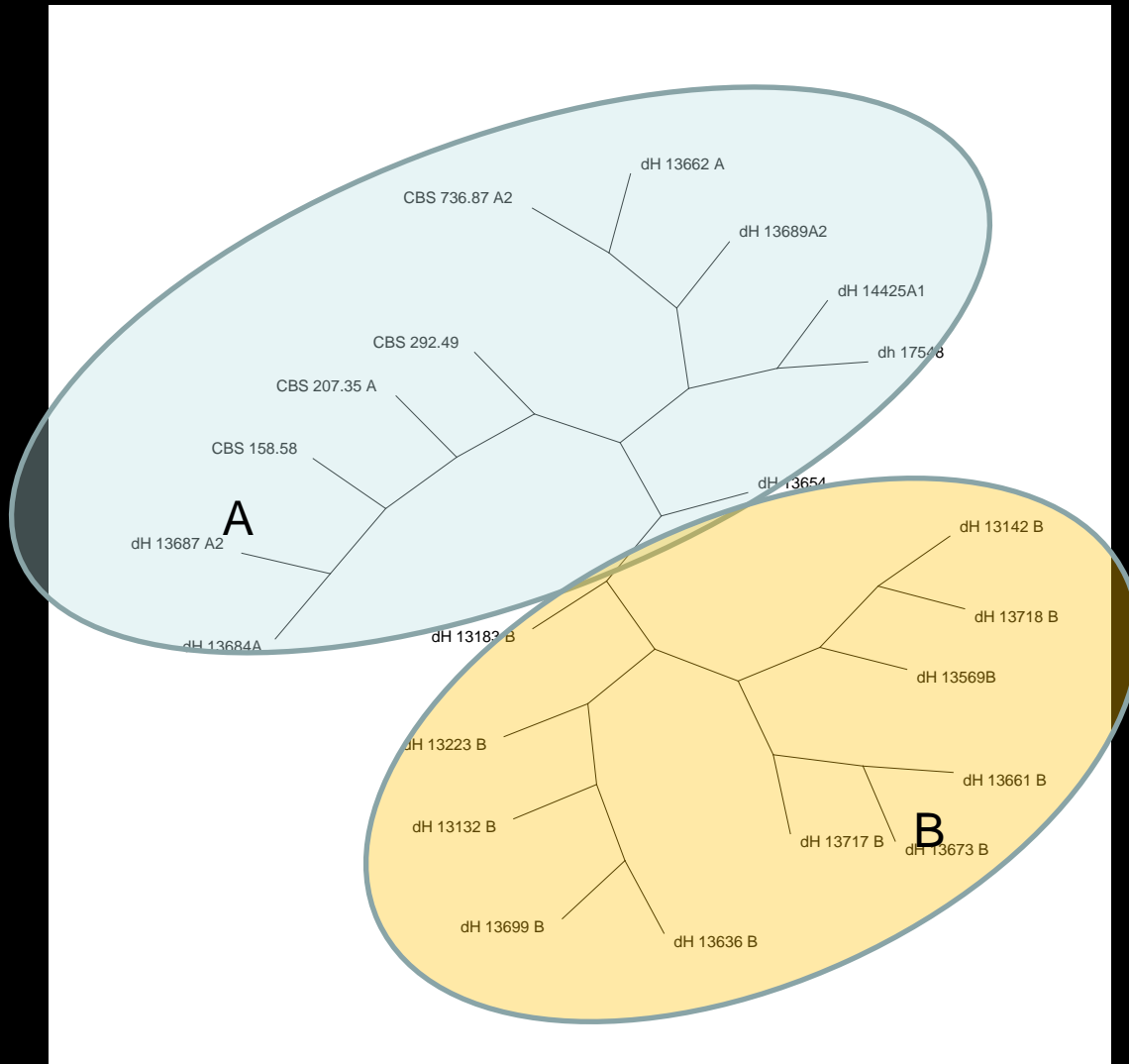
ITS – tree  
NeighborNet with SplitsTree 4



EF1 – tree  
(substitution model HKY+G)







CDC42 – tree  
(substitution model HKY)





# Statistical calculations (DnaSP 5.1)

## Tajima's D:

Comparison of estimates of the number of segregating sites and the mean pairwise differences between sequences

## Fu & Li's D\* and F\*:

Comparison of the number of derived singleton mutations and the total number of derived nucleotide variants

## dN/dS:

Ratio of non-synonymous and synonymous mutations

## F<sub>ST</sub>:

Fixed differences among Subpopulations in comparison with the Total population

# Statistical calculations (DnaSP 5.1)



| <i>Exophiala dermatitidis</i> |     |         |    |                    |    |     |        |             |             |             |     |           |
|-------------------------------|-----|---------|----|--------------------|----|-----|--------|-------------|-------------|-------------|-----|-----------|
|                               | N   | $\pi$   | H  | Hd (SD)            | S  | Eta | Eta(s) | Fu & Li's D | Fu & Li's F | Tajima's D  | n   | n (-gaps) |
| ITS                           | 240 | 0.00679 | 23 | 0.344<br>(0.00161) | 61 | 79  | 33     | -3.99951**  | -3.97182**  | -2.52111*** | 681 | 316       |
| CDC42                         | 31  | 0.09138 | 13 | 0.806<br>(0.00418) | 46 | 66  | 11     | 0.66759     | 0.23325     | -0.74288    | 203 | 145       |
| EF1                           | 47  | 0.02007 | 8  | 0.380<br>(0.00804) | 20 | 24  | 9      | -1.08395    | -1.57834    | -1.79345    | 201 | 123       |

|           |  |
|-----------|--|
| *         | P<0.05                                     |
| **        | P<0.02                                     |
| ***       | P<0.001                                    |
| N         | no. of taxa                                |
| $\pi$     | nucleotide diversity                       |
| H         | no. of haplotypes                          |
| Hd        | haplotype diversity and standard deviation |
| S         | polymorphic (segregating) sites            |
| Eta       | Total no. of mutations                     |
| Eta(s)    | Total no. of singleton mutations           |
| n         | total nucleotides                          |
| n (-gaps) | nucleotides excluding gaps/missing data    |



# Statistical calculations (DnaSP 5.1)



## *Exophiala dermatitidis* A- and B-type

|          | N  | $\pi$   | H  | Hd (SD)       | S  | Eta | Fu & Li's D | Fu & Li's F | Tajima's D | n    | n (-gaps) | Gene flow estimates         |
|----------|----|---------|----|---------------|----|-----|-------------|-------------|------------|------|-----------|-----------------------------|
| ITS      | 22 | 0.02360 | 6  | 0.745 (0.073) | 29 | 30  | -2.98260*   | -3.10426*   | -1.91247*  | 574  | 175       | Fst: 0.43305<br>Nm: 0.65    |
| CDC42    | 22 | 0.01279 | 3  | 0.589 (0.066) | 5  | 5   | 1.17564     | 1.59854     | 1.90580    | 495  | 176       | Fst: 0.96552<br>Nm: 0.02    |
| EF1      | 22 | 0.0658  | 8  | 0.545 (0.128) | 49 | 55  | -2.68745*   | -2.95008*   | -2.14724*  | 232  | 105       | Fst: -0.02560<br>Nm: -20.03 |
| combined | 22 | 0.03321 | 10 | 0.840 (0.062) | 96 | 111 | -3.07461**  | -3.23420**  | -2.06245*  | 1383 | 449       | Fst: 0.53522<br>Nm: 0.53    |

CDC42:  $d_N/d_S \gg 1$  à **positive selection**

|           |  |
|-----------|--|
| *         | P<0.05                                     |
| **        | P<0.02                                     |
| ***       | P<0.001                                    |
| N         | no. of taxa                                |
| $\pi$     | nucleotide diversity                       |
| H         | no. of haplotypes                          |
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| S         | polymorphic (segregating) sites            |
| Eta       | Total no. of mutations                     |
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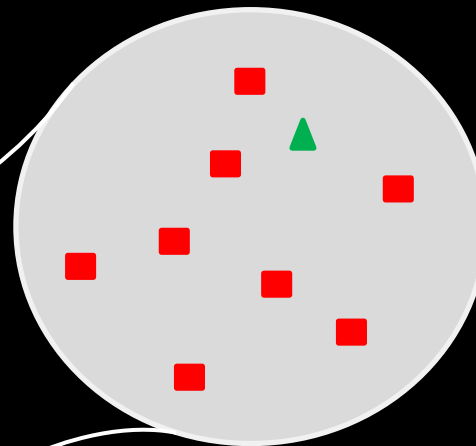
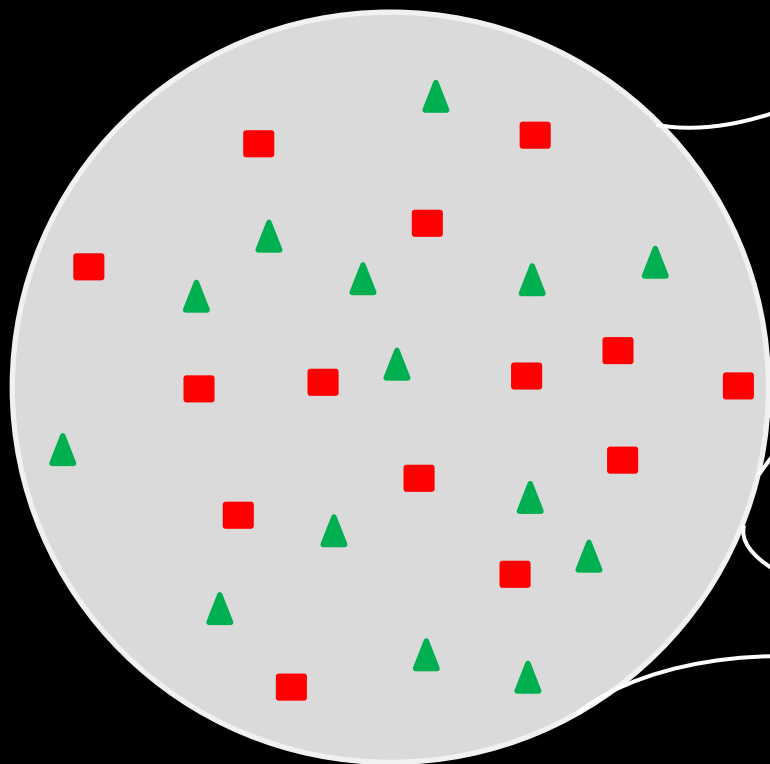
## Number of samples and types found per site

$n_{\text{tot}} = 214$

|                      | Eucalyptus | railway   | steambath, sauna | blood, brain | lungs     | (sub-) cutaneous | human faeces | fruit, plant | flying foxes bat, birds | dishwasher, carwash |
|----------------------|------------|-----------|------------------|--------------|-----------|------------------|--------------|--------------|-------------------------|---------------------|
| $n_A = 157$ <b>A</b> | 5          | <b>19</b> | <b>16</b>        | <b>14</b>    | <b>23</b> | <b>25</b>        | 8            | 10           | 1                       | <b>36</b>           |
| $n_B = 57$ <b>B</b>  | 0          | 3         | <b>31</b>        | 0            | 2         | 5                | 1            | 3            | 7                       | 5                   |
| total                | 5          | 22        | 47               | 14           | 25        | 30               | 9            | 13           | 8                       | 41                  |
| %                    | 2.3        | 10.3      | 22               | 6.5          | 11.7      | 14               | 4.2          | 6.1          | 3.7                     | 19.2                |

**Pearson chi-square test:** significant difference ( $p < 0.05$ ) between type A and B compared to human-dominant and environmental habitats

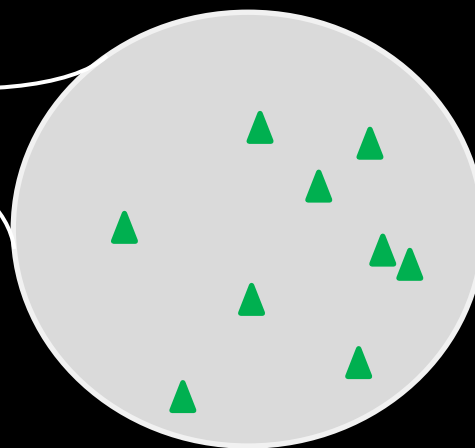
# Possible explanation



Complex A  
+ positive selection



brain infections  
lung colonization  
blood  
human faeces  
(sub)cutaneous  
dish washers  
steambaths  
railway ties



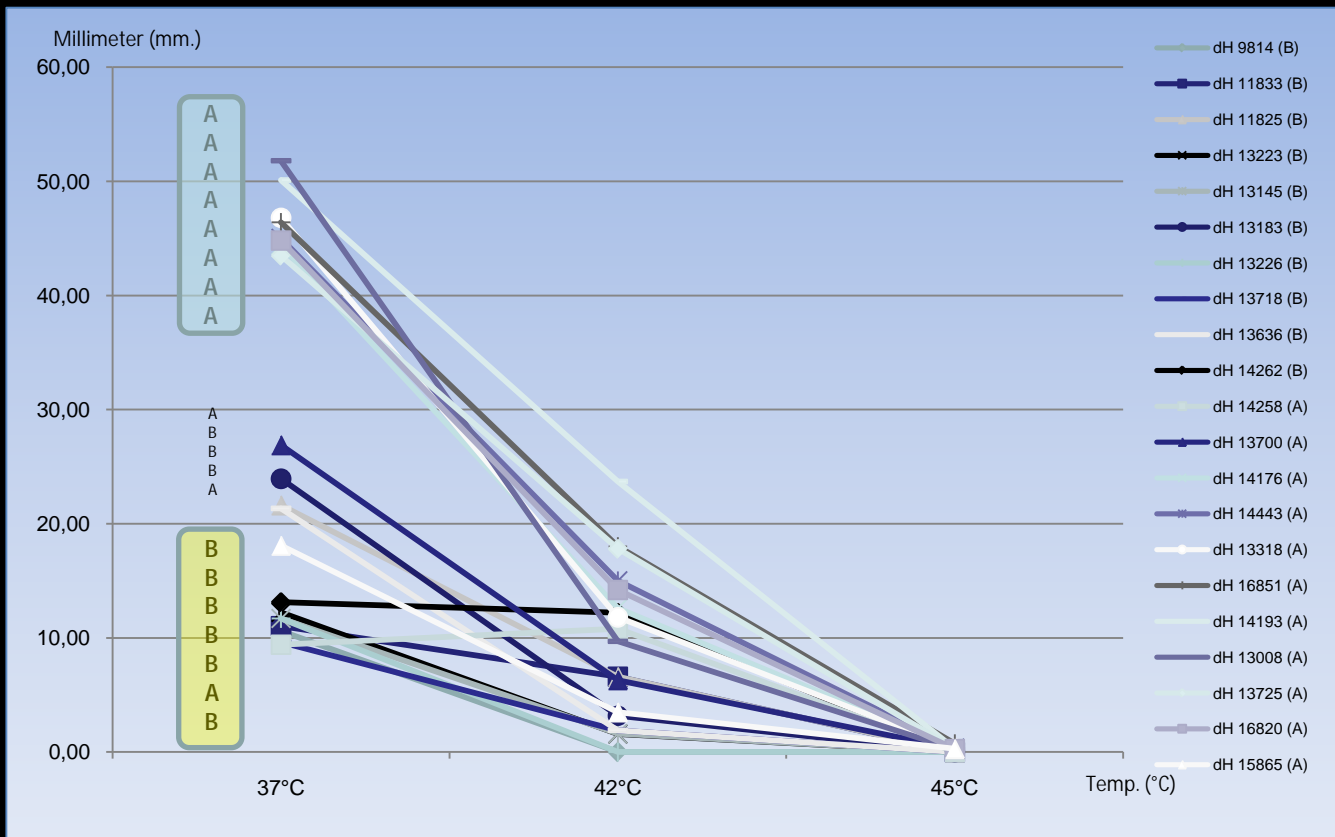
Complex B  
flying foxes  
fruit  
steambaths  
(sub)cutaneous

low nucleotide variability  
expanding population  
recombinational events  
fixed mutations  
positive selection

- A-type
- ▲ B-type



# Growth rate of A- and B-type at 37°, 42° and 45°C



Other phenotypic differences:

- adhesion
- Ca<sup>2+</sup>-dependant morphology
- pH tolerance
- melanin

Graph showing maximum growth of *Exophiala dermatitidis* at three temperatures; 37°C, 42°C and 45°C, at day 27.



## Emerging Potential of Black Yeasts



### Acknowledgements

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Sun Jiufeng (PR China)

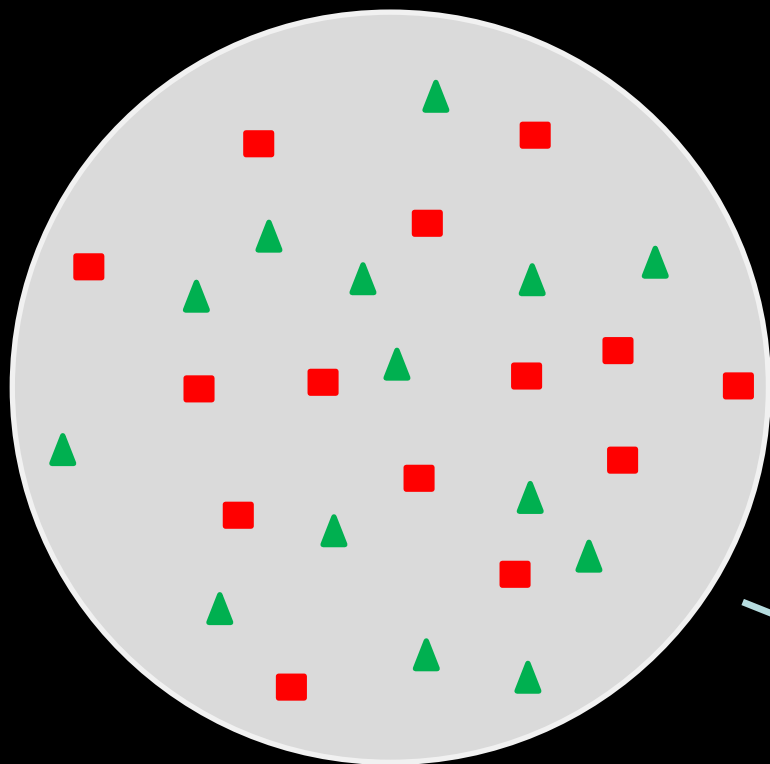
All others for sampling and obtaining sequence information

Thank you very much for listening

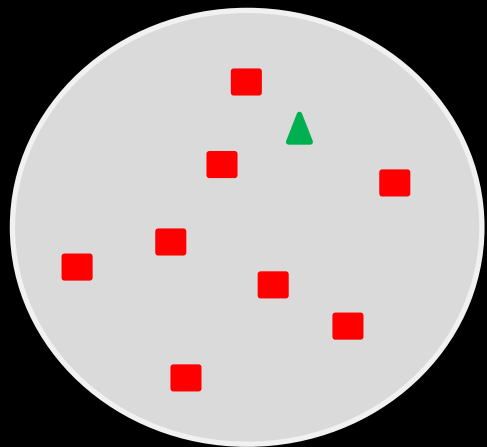




# Possible explanation



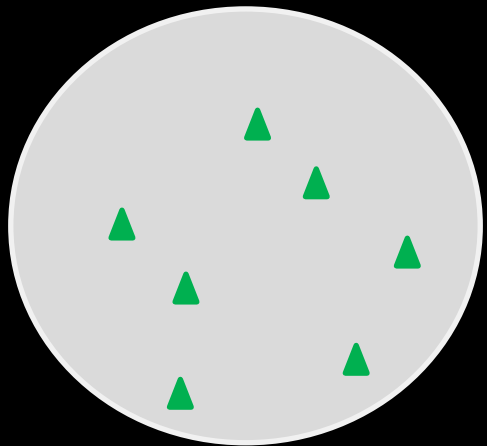
Founder effect



predominantly A-type

+ positive selection

Founder effect



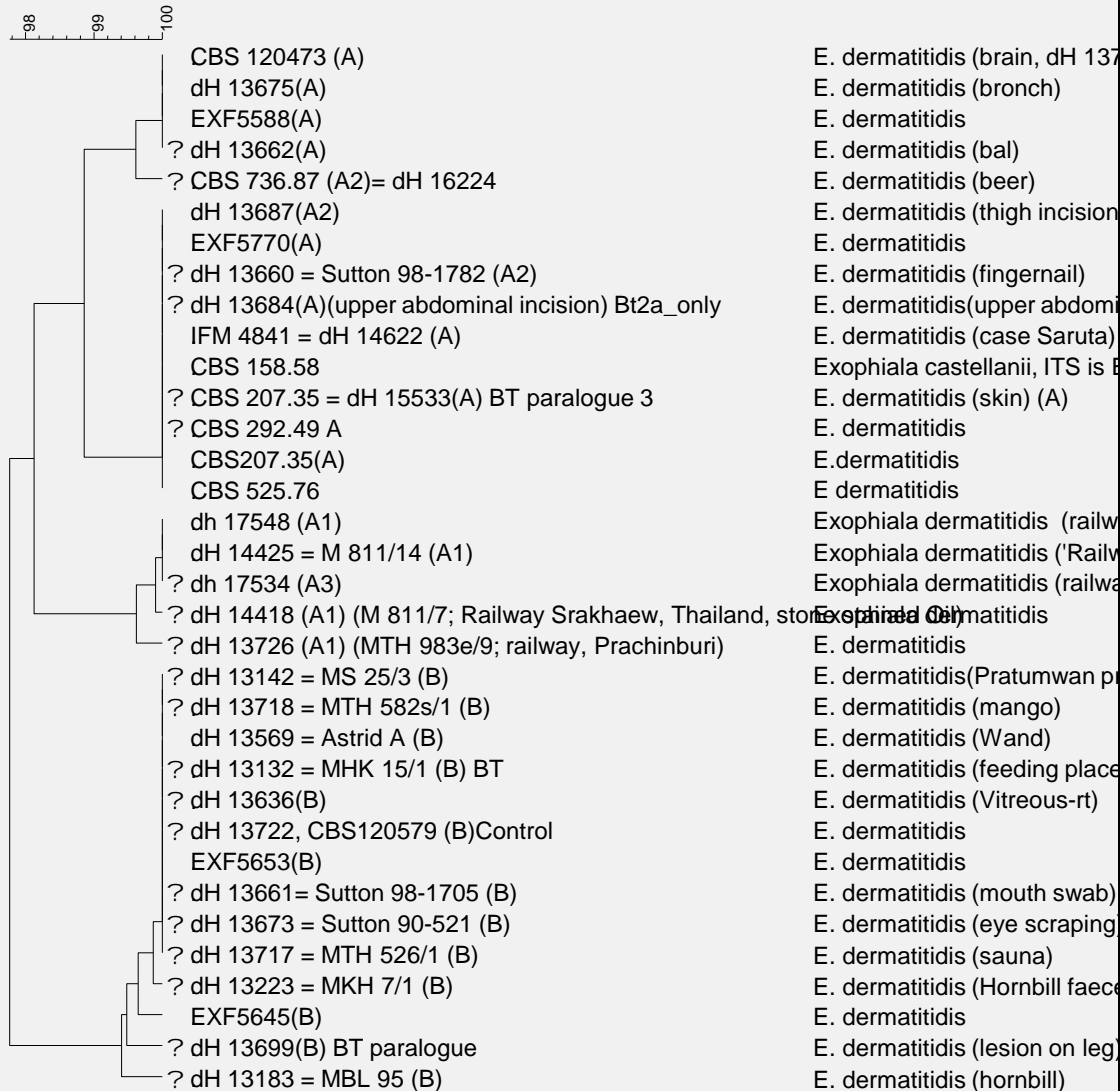
predominantly B-type

- A-type
- ▲ B-type

low nucleotide variability  
expanding population  
recombinational events  
positive selection  
fixed mutations

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0%

### CDC42



### CDC42

