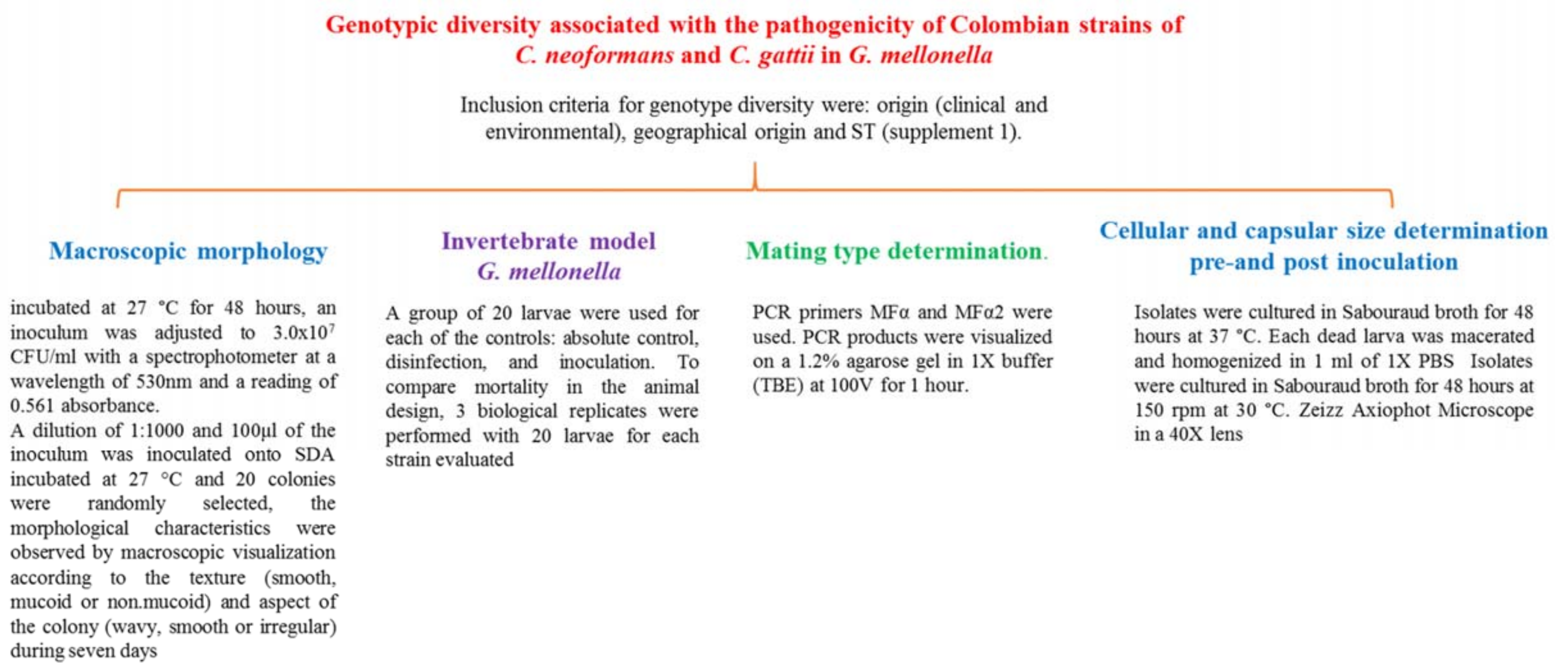
**Table S1**. General information of *Cryptococcus neoformans* and *C. gattii* clinical and environmental isolates according to species complex, sequence types (STs), macroscopic morphology, cellular and capsular size determination pre- and post-inoculation, mating type determination and mean survival time (days) in *G. mellonella.*

| | | | Macroscopic morphology | | | Pre-inoculation | | Post-inoculation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species complex | ST | Strain (H0058-I-) | Texture | Aspect | Diameter (mm) | Cellular (µm) | Capsular (µm) | Cellular (µm) | Capsular (µm) | Mating type | Mean survival in *G. mellonella* (days) |
| *C. neoformans* | Clinical | | | | | | | | | | |
| | | | | | | | | | | | Suppl. Fig 1a |
| | 2 | 3746 | Mucoid | Smooth | 3.70 | 7.53 | 0.21 | 4.49 | 0.52 | α | 5.60 |
| | 2 | 3852 | | | 4.10 | 5.34 | 0.48 | 6.98 | 1.60 | | 6.00 |
| | 5 | 2881 | | | 3.10 | 5.57 | 0.52 | 4.58 | 1.76 | | 2.80 |
| | 6 | 3463 | | | 3.20 | 5.68 | 0.50 | 4.05 | 2.88 | | 4.90 |
| | 25 | 3104 | | | 6.90 | 5.49 | 0.75 | 6.14 | 0.49 | | 3.90 |
| | 32 | 2340 | Non-mucoid | | 3.60 | 5.87 | 0.48 | 5.42 | 0.56 | | 4.00 |
| | 40 | 3589 | Mucoid | | 3.20 | 6.20 | 0.48 | 4.40 | 1.63 | | 9.90 |
| | 63 | 2503 | | | 5.60 | 5.75 | 0.52 | 4.48 | 0.87 | | 9.20 |
| | 69 | 3099 | | | 3.90 | 5.87 | 0.45 | 5.96 | 1.81 | | 6.40 |
| | 71 | 3489 | | | 3.00 | 3.30 | 0.34 | 5.22 | 1.60 | | 3.90 |
| | 77 | 708 | | | 6.30 | 6.71 | 1.14 | 6.40 | 1.03 | | 4.60 |
| | 77 | 3845 | | | 4.40 | 5.72 | 0.67 | 4.35 | 1.60 | | 9.70 |
| | 93 | 995 | | | 6.00 | 5.54 | 0.59 | 9.18 | 3.08 | | 5.60 |
| | 93 | 1226 | | | 5.40 | 5.99 | 0.57 | 6.39 | 0.86 | | 3.60 |
| | 93 | 2073 | | | 4.40 | 4.73 | 1.22 | 7.98 | 1.02 | | 3.90 |
| | 93 | 2356 | Non-mucoid | | 3.64 | 6.09 | 0.55 | 6.84 | 1.10 | | 4.10 |
| | 93 | 2624 | | Rugose | 4.37 | 6.53 | 0.45 | 11.88 | 1.61 | | 5.90 |
| | 93 | 3189 | Mucoid | Smooth | 4.07 | 6.72 | 0.59 | 11.51 | 1.25 | | 5.80 |
| | 93 | 3938 | | | 3.79 | 6.15 | 0.48 | 4.40 | 0.9 | | 10.80 |
| | 199 | 714 | | | 5.10 | 5.9 | 0.93 | 9.78 | 3.42 | | 5.70 |
| | 307 | 707 | | | 4.90 | 5.85 | 0.92 | 7.97 | 2.55 | | 4.10 |
| | 307 | 727 | | | 4.30 | 5.44 | 0.83 | 8.00 | 1.32 | | 6.20 |
| | 307 | 2087 | | | 4.20 | 6.19 | 0.85 | 10.33 | 3.24 | | 5.00 |
| | 307 | 2274 | | | 5.90 | 6.12 | 0.89 | 9.16 | 2.25 | | 4.00 |
| | **Environmental** | | | | | | | | | | |
| | | | | | | | | | | | Suppl. Fig 2a |
| | 15 | 4419 | Mucoid | Smooth | 3.10 | 5.69 | 0.65 | 6.04 | 1.12 | α | 7.80 |
| | 23 | 4706 | | | 3.40 | 4.29 | 0.25 | 6.80 | 1.34 | | 7.10 |
| | 56 | 4630 | | | 4.70 | 5.33 | 0.36 | 4.85 | 2.56 | | 10.80 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 77 | 4013 | | | 3.40 | 5.74 | 0.67 | 4.37 | 2.05 | | 9.05 |
| | 93 | 3877 | | | 4.10 | 6.12 | 0.50 | 4.23 | 0.91 | | 6.35 |
| | 93 | 4711 | | | 4.40 | 6.39 | 0.59 | 4.35 | 0.91 | | 4.75 |
| | 226 | 5353 | | | 3.20 | 1.70 | 0.29 | 4.35 | 0.91 | | 6.15 |
| ***C. gattii*** | **Clinical** | | | | | | | | | | |
| | | | | | | | | | | | Suppl. Fig 1b |
| | 25 | 212 | Non-mucoid | Smooth | 4.50 | 4.49 | 0.68 | 11.1 | 2.25 | a | 8.00 |
| | 25 | 2877 | Mucoid | | 3.90 | 5.74 | 0.46 | 5.50 | 1.20 | | 6.00 |
| | 47 | 255 | | | 5.40 | 5.40 | 2.88 | 9.40 | 1.85 | α | 4.20 |
| | 51 | 3286 | | | 4.10 | 5.64 | 0.33 | 4.30 | 2.10 | | 7.90 |
| | 58 | 3031 | | | 4.40 | 4.98 | 0.47 | 6.10 | 1.10 | | 5.00 |
| | 85 | 792 | | | 3.60 | 3.57 | 0.66 | 8.90 | 2.21 | | 8.00 |
| | 106 | 1510 | | | 6.90 | 6.93 | 2.76 | 7.60 | 0.90 | | 4.30 |
| | 323 | 3146 | | | 4.80 | 6.45 | 0.83 | 6.70 | 6.10 | a | 9.40 |
| | 324 | 3407 | | | 3.50 | 4.40 | 1.39 | 4.30 | 1.40 | α | 7.90 |
| | **Environmental** | | | | | | | | | | |
| | | | | | | | | | | | Suppl. Fig 2b |
| | 25 | 3526 | Mucoid | Smooth | 3.80 | 3.08 | 0.25 | 5.70 | 1.20 | a | 8.00 |
| | 75 | 3593 | | | 2.30 | 2.45 | 0.65 | 5.20 | 1.90 | | 5.40 |
| | 79 | 3080 | | | 3.80 | 1.89 | 0.25 | 4.30 | 1.60 | α | 10.60 |
| | 79 | 3874 | | | 3.20 | 3.09 | 0.29 | 4.30 | 1.50 | | 9.60 |
| | 79 | 4064 | | | 3.90 | 1.86 | 0.29 | 4.30 | 1.60 | | 8.70 |

**Table S2**. Control strains of *Cryptococcus neoformans* and *C. gattii* used in the study of virulence in the invertebrate model of *Galleria mellonella* and in phenotypic assays.

| Collection ID | Species | Assay involved | Characteristics (Reference) |
|---|---|---|---|
| JEC20 | *C. neoformans* var. *neoformans* | In vivo study<br>Mating type | Low virulence in *G. mellonella* (10)<br>Mating type **a** (17) |
| JEC21 | | Mating type | Mating type α (17) |
| H99 | *C. neoformans* var. *grubii* | In vivo study | High virulence in *G. mellonella* (10) |
| H0058-I-580 | | Cellular and capsular size | Capsular size 0.59-0.85 μm (15) |
| H0058-I-755 | | | Capsular size 0.44-0.6 μm (15) |
| H0058-I-737 | | | Capsular size 0.33-0.85 μm (15) |
| H0058-I-1580 | | Colony morphology | Non-mucoid, rough border (15) |
| H0058-I-1580 | | | Non-mucoid, smooth (15) |
| H0058-I-1320 /B-3506 | *C. gattii* | Cellular and capsular size | Capsular size 0.75-0.95 μm (15) |
| | | Colony morphology | Mucoid, smooth (15) |
| H0058-I-2508 /CDC2272 | | In vivo study | High virulence in *G. mellonella* |



**Genotypic diversity associated with the pathogenicity of Colombian strains of *C. neoformans* and *C. gattii* in *G. mellonella***

Inclusion criteria for genotype diversity were: origin (clinical and environmental), geographical origin and ST (supplement 1).

**Macroscopic morphology**

incubated at 27 °C for 48 hours, an inoculum was adjusted to $3.0 \times 10^7$ CFU/ml with a spectrophotometer at a wavelength of 530nm and a reading of 0.561 absorbance.

A dilution of 1:1000 and 100µl of the inoculum was inoculated onto SDA incubated at 27 °C and 20 colonies were randomly selected, the morphological characteristics were observed by macroscopic visualization according to the texture (smooth, mucoid or non.mucoid) and aspect of the colony (wavy, smooth or irregular) during seven days

**Invertebrate model *G. mellonella***

A group of 20 larvae were used for each of the controls: absolute control, disinfection, and inoculation. To compare mortality in the animal design, 3 biological replicates were performed with 20 larvae for each strain evaluated

**Mating type determination.**

PCR primers MFα and MFα2 were used. PCR products were visualized on a 1.2% agarose gel in 1X buffer (TBE) at 100V for 1 hour.

**Cellular and capsular size determination pre-and post inoculation**

Isolates were cultured in Sabouraud broth for 48 hours at 37 °C. Each dead larva was macerated and homogenized in 1 ml of 1X PBS Isolates were cultured in Sabouraud broth for 48 hours at 150 rpm at 30 °C. Zeizz Axiophot Microscope in a 40X lens

**Figure S1**. Flowchart of the methodology used. Inclusion criteria, macroscopic morphology, mating type determination, invertebrate model in *Galleria mellonella* and cellular and capsular size determination pre- and post-inoculation.

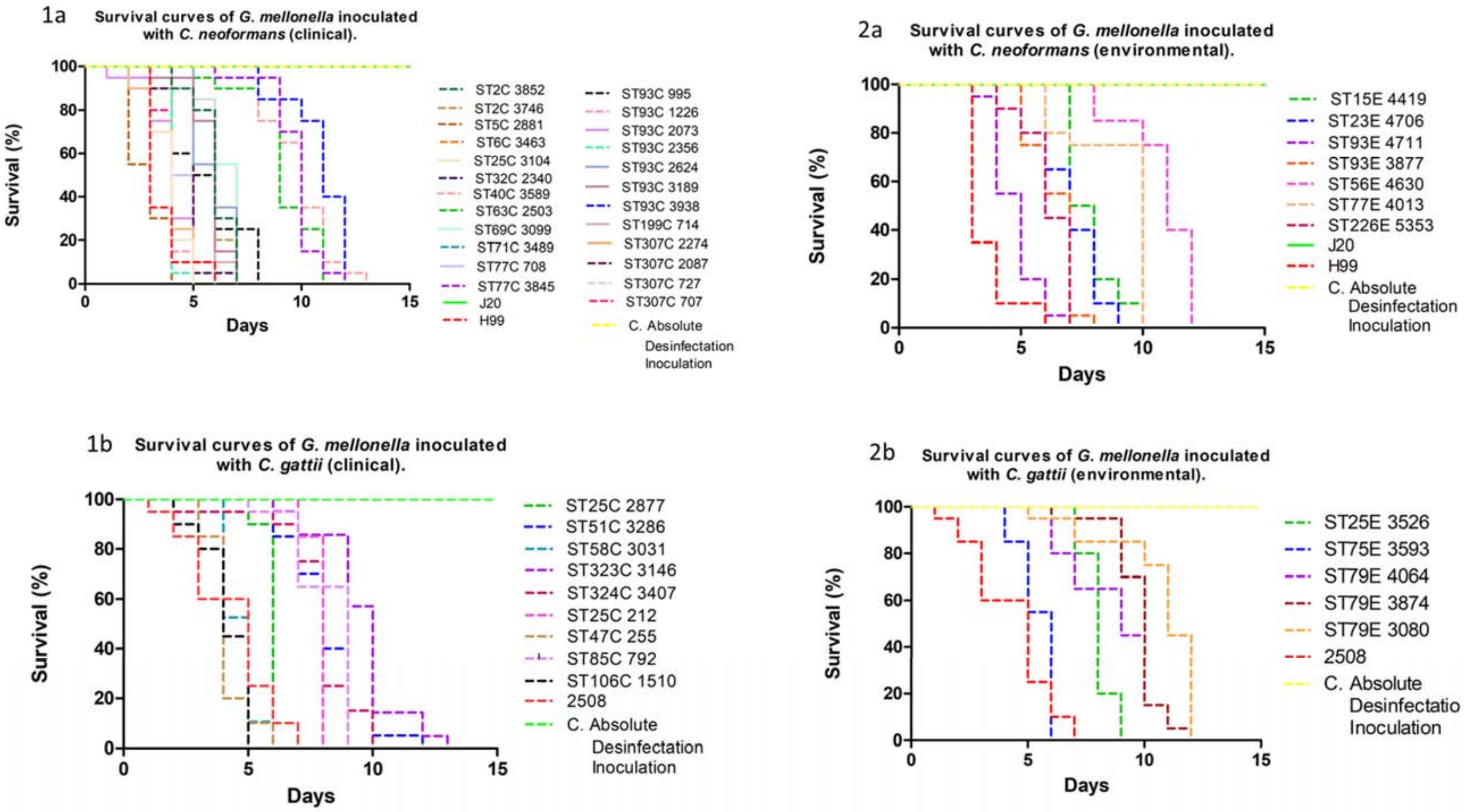


**Figure S2.** Survival curves of *G. mellonella* inoculated with *C. neoformans* (1a clinical isolates -2a environmental isolates). Survival curves of *G. mellonella* inoculated with *C. gattii* (1b clinical isolates, 2b environmental isolates).