

Fungi identification in large palaeovertebrate tunnels

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Some mammals of the Pleistocene megafauna of South America excavated tunnels whose lengths may reach up to 100 meters; maximum observed diameters are of more than 2 meters. These tunnels survived to present days in several stages of preservation, most of them completely filled with sediments by geologic processes. However, around 40% are open to some degree and can be entered for research purposes. Open tunnels usually show white fungi at the walls and at the roof. Hyphae density varies widely: during rainy periods, the mycelia are usually abundant. In extreme situations, the walls and roof of the tunnels are completely white and seem frosted. During the dry periods of the year, the hyphae may disappear almost completely, but the fungal spores probably are still present. Palaeovertebrate tunnels constitute a most unusual case of fungi environment. The host rock is not limestone as in common caves, but weathered rocks or sandstones with variable degrees of clay and silt. Humidity fluctuations inside the tunnels vary in a wide range. In rainy seasons, flowing water on the floor and water dripping from the roof creates a very humid stagnant atmosphere. In the summer, the sediments dry out to dust. We carried out a pilot DNA analysis of fungi of two tunnels located in the cities of Estância Velha and Novo Hamburgo (state of Rio Grande do Sul, Brazil). The ITS1 and ITS2 regions of the rDNA were amplified using the primers sequences TW81 5'-GTTTCCGTAGGTGAACCTGC -3' and AB28 5'-ATATGCTTAAGTTCAGCGGGT -3'. PCR products were purified, precipitated, and sequenced with the DYEnamic ET Dye Terminator kit in the MegaBace 1000 System (Amersham Biosciences). Contigs were assembled with the Staden package program and NCBI Blast was inquired. One species of fungi was prevalent and identified with 98% nucleotide identity as *Trichoderma atroviride*, a filamentous fungus capable of causing opportunistic infections in humans. This first fungi identification opens several research possibilities like comparing fungi in tunnels excavated in the same host rock, fungi in tunnels of different sizes, and fungi in tunnels with different air circulation patterns. This identification also improves personal safety conditions of in-tunnel research.