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Fungal Isolation - *Schwanniomyces capriottii*

Summary
Fungi exist all throughout our environment and play an important role in our ecosystem. Within the course “Diversity and Evolution of Microbial Eukaryotes”, a study was conducted where the goal was to identify fungi sampled from our environment. A phylogenetic tree was constructed to reveal the relations between the identified fungal species *Schwanniomyces capriottii* and other lineages within the fungal kingdom.

Sample collection
A soil sample was collected on the 7th of November 2023 at the position 59,84536°N, 17,59855°E in Ekeby, Uppsala. This is within close proximity to the pond, Ekebypölen. The soil was covered in plant material such as wilted leaves and longer blades of grass, this was removed upon sampling to reveal the underlying soil (Figure 1).

Figure 1: Location of where the sample was collected.

Isolation procedure
Two different types of agar plates and a liquid culture were used: yeast extract peptone dextrose (YEPD) agar with chloramphenicol and potato dextrose agar (PDA) with chloramphenicol. The liquid culture consisted of liquid YEPD medium with

chloramphenicol. Using a sterile inoculation technique, a small soil particle was plated on the respective petri-dishes and in the liquid culture. The plates were incubated for 3 days at 30°C. One plate was chosen for further restreaking in order to achieve a pure culture of fungi (Figure 2). In this case, the PDA plate with chloramphenicol was chosen. While both filamentous fungi and yeast grew in the first inoculation, only the yeast survived the restreakings and were therefore utilized for the upcoming identification process. The DNA extracted from the pure culture was prepared for PCR. The primers used were ITS-1 forward primer and ITS-4 reverse primer. These are specific for identifying fungi, as the primers amplify the ITS region. The PCR products with the amplified DNA were analyzed using agarose gel electrophoresis, separating the DNA by molecular size. A successful analysis indicates that the DNA samples can be purified and sent for sequencing.

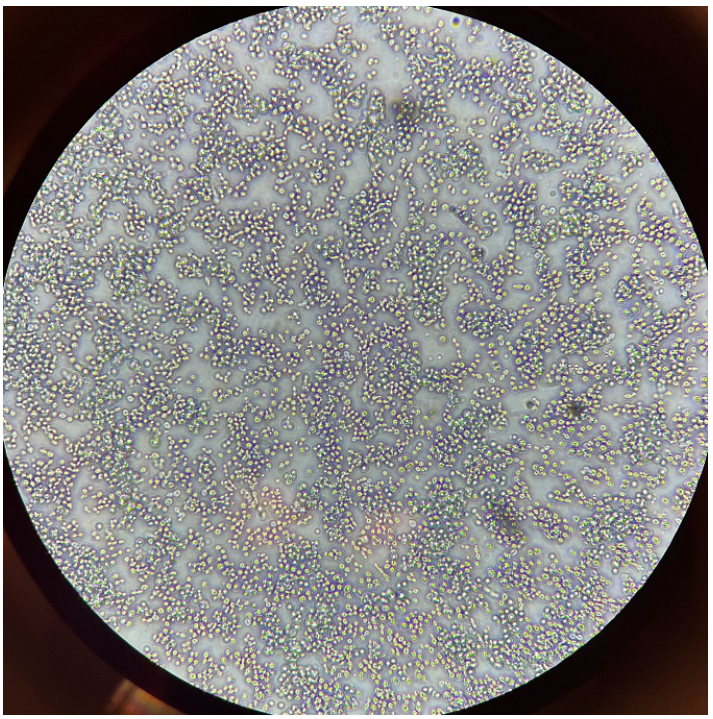

Figure 2: Pure culture of yeast under a 40x magnification. The yeast is milky white in color, and the cells small in size, but abundant on the prepared slide.

Yeast Identification

The identification process started with a chromatogram analysis of the forward and the reverse sequences, using the application SnapGene. The sequences were trimmed for quality and assembled into a consensus. Using the BLAST function in NCBI (Altschul *et al.* 1990), the consensus sequence was compared to a database to match with the most similar sequences. The assembled fungi sample had a 99.50% similarity to the species *Debaryomyces castellii* (also known as *Schwanniomyces capriottii*), a budding yeast within the Saccharomycetes class. When investigating the alignments, the species can be identified with 100% certainty and the spaces within the sequence can be disregarded. The top 25 species were downloaded as a FASTA file. Using a text editor application – the assembled fungi sequence, the FASTA file with the 25 most similar species and a retrieved reference-database-ITS file were placed together. The sequences were aligned using the software MAFFT (Katoh *et al.* 2019). The alignment file was saved and used as an input for IQTREE to generate a phylogenetic tree (Minh *et al.* 2020). Visualization of the tree was

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performed using iTOL (Letunic & Bork 2021). The identified fungi species shows a phylogenetic relationship to species within the *Schwanniomyces* and *Debaryomyces* genera. This supports the identification of the sample being *Schwanniomyces capriottii.* It is also worth mentioning that the *Schwanniomyces* genus was emended, meaning that the nomenclature was changed and certain species previously clustered with *Debaryomyces* have been altered to *Schwanniomyces* (Kurtzman & Suzuki 2010). The phylogenetic tree has a variety of different bootstrap values, some branch nodes having better support than others. Bootstrap values above 90, indicate that the branches are strongly supported and the placement in the tree is recurring during the iterations of the replicates. Lower values, for instance those belonging to the node of the assembled fungi Sequence, could imply that the placement of the branch nodes has low support and the relationships are uncertain. With regards to the results from the BLAST as well as the positioning in the phylogenetic tree (Figure 3), the species is identified as *Schwanniomyces capriottii*.

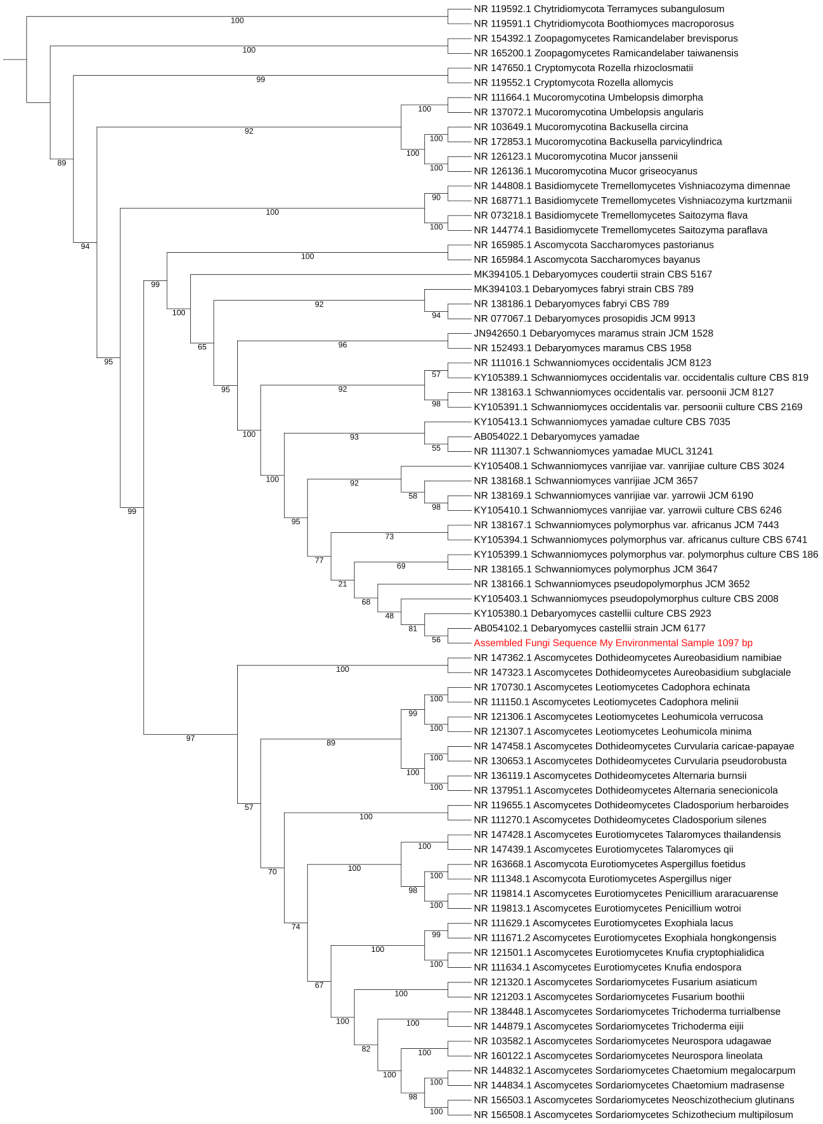

Figure 3: Phylogenetic tree including the "Assembled Fungi Sequence My Environmental Sample 1097 bp" marked in red, the top 25 species from the BLAST and the reference database with ITS.

Schwanniomyces capriottii

Schwanniomyces is a genus which belongs to the Saccharomycetes class within the Ascomycota phylum. Similar to the sister-group *Debaryomyces*, *Schwanniomyces* is a part of the Debaryomycetaceae family (Schoch *et al.* 2020). Isolations from *Schwanniomyces capriotti* specifically, have been retrieved from soil (MycoBank, 2010). Other species from the Schwanniomyces genus have been found in soil (Yurkov 2018), however not much information is found on other environments they may have been sampled from. As mentioned earlier, *Schwanniomyces capriottii* is a budding yeast. Reproduction by the species within *Schwanniomyces* occurs asexually, by multilateral budding off the mother cell (Suzuki & Kurtzman 2011). Species of the *Schwanniomyces* genus typically form spheroidal ascospores with a prominent equatorial ring (Kurtzman 1994). Under a magnification of 100x, the yeasts have a diameter between 3 μm and 6 μm (figure 4).

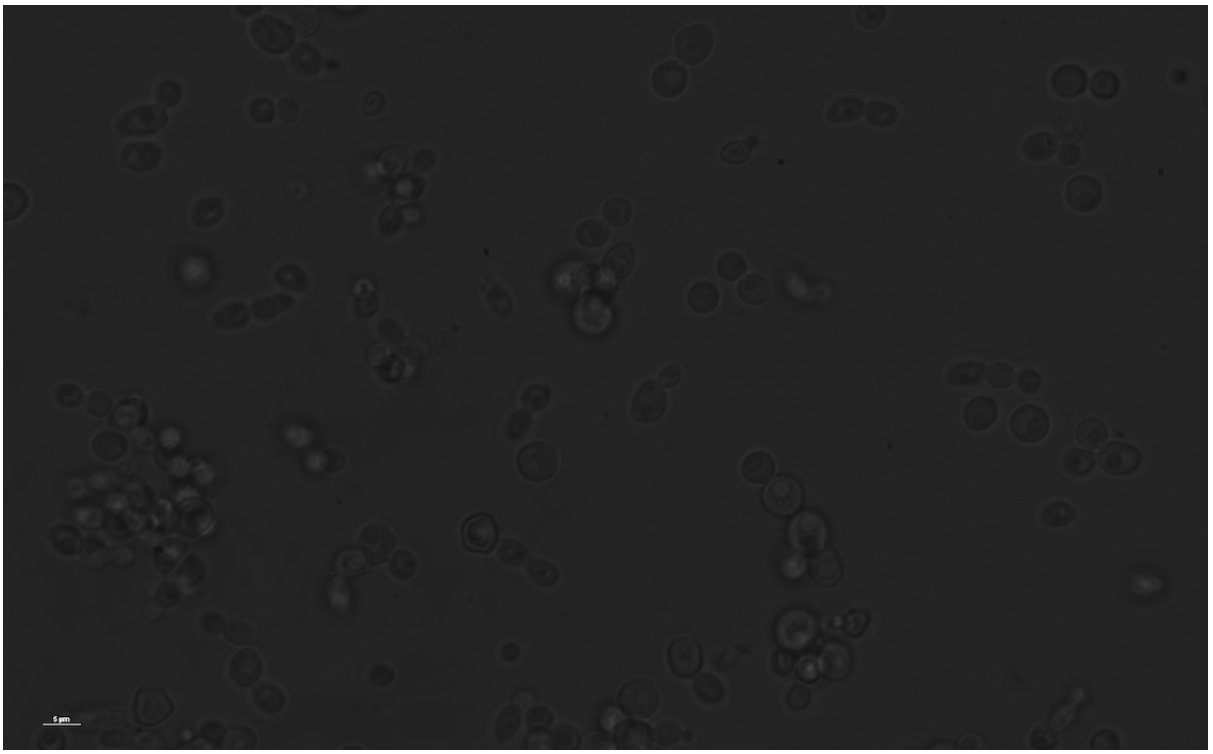

Figure 4: Slide of Schwanniomyces capriotti under 100x magnification. Phase contrast RGB. Size reference is 5 μm. Some cells are undergoing budding. Within yeast cells, the nuclei are visible. The vacuoles are also visible in the cells.

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