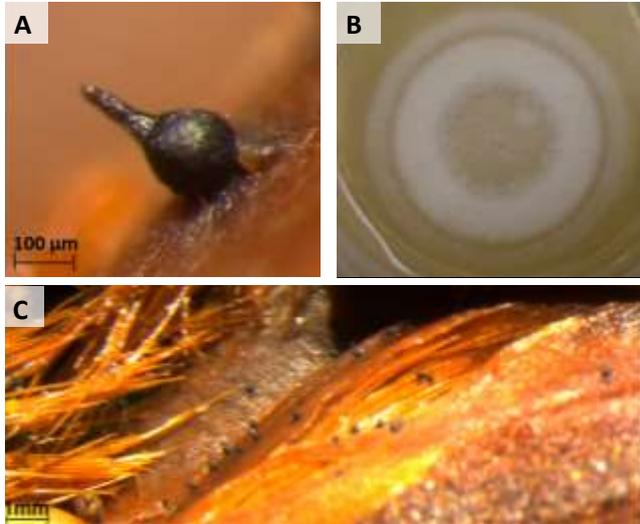


# POPULATION GENETIC STUDIES IN *PROTEA*-ASSOCIATED *SPOROTHRIX* SPECIES

Prepared by MSc student Nombuso Ngubane, University of Stellenbosch



We investigated the dispersal ecology of the South African fungal species in the *Sporothrix splendens* complex. Initially, we intended to use microsatellites as markers, however, these markers showed unusual levels of hyper-variability within and around the microsatellite regions. Thus, slower evolving options were explored and this is how we selected the beta-tubulin and one of the microsatellite markers as ideal markers of choice.

Primers targeting these two markers were selected and a collection of sequence data was built for the species under investigation (*Sporothrix splendens*, *S. protearum* and *S. africana*). These data were collected from 20 populations, 8 of *S. splendens* and 12 of *S. protearum* and *S. africana*.

First we explored the population genetics of *S. splendens* across the distribution range of *P. repens*, one of the most widespread *Protea* species in the Core Cape Subregion (CCR) of South Africa. We were interested in whether populations of *S. splendens* were structured according to geography or if, like *Knoxdaviesia proteae*, they were in near panmixia. Our analyses revealed that, like in *K. proteae*, populations of this species were characterised by high levels of migration supported by very low population differentiation scores. This species was also characterised by very high genetic diversity. The low nucleotide diversity uncovered pointed at a recent expansion in this species.

All of the available studies looking at the population genetics of ophiostomatoid fungi associated with *Protea* species have, up to this point, focused on fungal species in the CCR. As a second aim we thus wanted to extend this research focus to include species outside the Cape, and selected *S. africana* and *S. protearum*, two relatives of *S. splendens*. These fungi display interesting host associations with *S. africana* thought to be a generalist encountered in *Protea caffra*, *P. dracomontana* and *P. gagedi*, while *S. protearum* has only been recorded from *P. caffra*. In addition to investigating the dispersal patterns of *S.*



*africana* and *S. protearum*, we were also interested in evaluating their validity as standalone taxonomic entities. The results revealed that there was frequent long range dispersal of *S. africana* and *S. protearum* propagules leading to poorly differentiated populations. Host identity and geography could not explain the patterns uncovered in the populations of these species. However, these factors did play a minor role in structuring populations of *S. africana* and *S. protearum*. The results of this chapter strongly supported the notion that these two taxa represent an incredibly genetically diverse single species with low nucleotide diversity, like *S. splendens*. If we were to combine *S. africana* and *S. protearum*, the older name (*S. protearum*) would take precedence.

Two new *Protea*-associated *Sporothrix* taxa were also discovered and described. The first new species, *S. nsini* sp. nov. (toothless, origin: IsiZulu), belongs in the *S. splendens* clade. It is unusual in that, unlike the other taxa in this clade, it lacks denticles, with conidia often arising directly from hyphal tips. The second new species resides in the *S. bragantina* clade, however, depending on the molecular marker used it may move to the *S. curviconia* clade. This species was comparably rarer than *S. nsini*. We found it only in two locations (Drakensberg and Vryheid, KwaZulu-Natal), only from *P. dracomontana* and *P. gagedi* and only three isolates were found. Its rarity in this niche suggests that *Protea* may not be its preferred host; given that it groups with taxa from other niches it is quite possible that, like its sister *S. bragantina*, it may be a soil associate. This is the most phylogenetically unique *Sporothrix* species found in *Protea*. Its morphology is also quite unique, resembling taxa in the *S. bragantina* clade instead of the other *Protea*-associated *Sporothrix* species. Due to the unexpected discovery of a species so unique, we named it *S. smangaliso* (surprise or miracle, origin: isiZulu).

The outcomes of this study highlight how much still remains unknown about *Protea*-associated *Sporothrix* species. Factors shaping their populations are still largely unknown. However, previous studies and this work suggest that host chemistry, host identity and geography may

be contributing factors. Long distance vectors seem to play a crucial role in the distribution of alleles across the landscape. These vectors are suspected to be birds that frequent *Protea* species, such as the Malachite sunbirds and Gurney's sugarbirds. It still remains a mystery why species such as *S. nsini* and *S. protearum* remain separate taxa despite their shared ecology and their close phylogenetic relatedness.

It is quite clear that *Sporothrix* fungi have a vested interest in the *Protea* niche. This is evident in their phylogeny which shows that the niche has been colonised at least five times with some clades (*S. gemella* and the *S. splendens* clades) experiencing species radiations. Reasons for this are thought to include the availability of nutrients from the dead floral parts coupled with the diversity and abundance of available vectors, especially mites.