**MID-TERM REPORT**

## Introduction

The Umtamvuna Nature Reserve (UNR) is one of the major conservation areas of the Pondoland Centre (PC) which is part of the Maputaland-Pondoland-Albany (MPA) biodiversity hotspot. It is located on the east coast of South Africa at Port Edward, bordering the KwaZulu-Natal and the Eastern Cape Provinces (GPS: approximately 30.1°E, 31°S).

The PC contains approximately 30 endemic trees and shrubs, of which at least 24 have been recorded in the UNR (Abbott & Van Wyk 2000). The forest flora of the UNR is the richest of 14 South African forests or forest complexes (Geldenhuys 1992) and is diverse with more than 500 tree species.

Despite of its floral diversity and high endemism, no investigation has been so far attempted on the occurrence of fungal diseases in the region. Thus, the primary aim of this study is to do a preliminary survey on any fungal diseases occurring on indigenous trees in the UNR. The study entails identification and morphological description, molecular characterization (DNA fingerprinting) and *in vitro* preservation of causative fungi; compilation and digitalization of all the data generated; and communication with a nature conservation organization.

## Materials and Methods

A permit (March-December 2008) to work in the Nature Reserve was obtained from Ezemvelo KZN Wildlife (KwaZulu-Natal, South Africa).

In May 2008 a five-day trip was made to the Umtamvuna Nature Reserve. The living leaves and twigs of 31 tree species (15 families, 24 genera) showing any disease symptoms such as leaf spots, necrosis or die-back were collected (Table 1). The names of plants were confirmed by Mr. Abbott who is an expert on the flora of the UNR.

Table. 1. List of host plants collected from Umtamvuna Nature Reserve.

| **Family** | **Genus** | **Species** | **Location** |
|---|---|---|---|
| Anacardiaceae | *Protorhus* | *longifolia* | Porcupine trail off-road |
| Asclepiadaceae | *Cryptolepis* | *capensis* | Fish Eagle trail |
| Bruniaceae | *Raspalia* | *trigyna* | Porcupine trail |
| Celastraceae | *Lydenburgia* | *abbottii* | Porcupine, Abbott's garden |

| | | | |
|---|---|---|---|
| | *Maytenus* | *abbottii* | Abbott's garden |
| | *Pseudosalacia* | *streyi* | Porcupine trail |
| | *Salacia* | *gerrardii* | Fish Eagle trail |
| Erythroxylaceae | *Nectaropetalum* | *zuluense* | Fish Eagle trail |
| Icacinaceae | *Apodytes* | *abbottii* | Porcupine trail |
| | | *dimidiata* | Fish Eagle trail |
| Lauraceae | *Dahlgrenodendron* | *natalense* | Porcupine trail |
| Melastomataceae | *Memecylon* | *bachmannii* | Fish Eagle trail |
| Meliaceae | *Turraea* | *floribunda* | Abbott's garden |
| Moraceae | *Ficus* | *sur* | Fish Eagle trail |
| Myrtaceae | *Eugenia* | *erythrophylla* | Porcupine trail off-road |
| | | *natalitia* | Fish Eagle trail |
| | | *simii* | Fish Eagle trail |
| | | *umtamvunensis* | Porcupine trail off-road |
| | *Heteropyxis* | *natalensis* | Abbott's garden |
| | *Syzygium* | *cordatum* | Fish Eagle trail, Porcupine trail |
| | | *pondoense* | Fish Eagle trail |
| | | sp. (*van wyk*) | Abbott's garden |
| Proteaceae | *Faurea* | *macnaughtonii* | Porcupine trail off-road |
| | *Leucadendron* | *pondoense* | Abbott's garden |
| | | *spissifolium natalense* | Porcupine trail |
| | *Protea* | *caffra* | Porcupine trail |
| Rubiaceae | *Psychotria* | *capensis* | Porcupine trail off-road |
| Rutaceae | *Oricia* | *bachmannii* | Fish Eagle trail |
| Sapotaceae | *Englerophytum* | *natalense* | Fish Eagle trail |
| | *Manilkara* | *nicholsonii* | Porcupine trail off-road |
| | *Vitellariopsis* | *marginata* | Fish Eagle trail |
| **15 families** | **24 genera** | **31 species** | **3 locations** |

Leaf lesions or twigs with visible fungal structures (symptomatic specimens) were studied under a dissecting microscope. When a mature fungal structure was found on the specimens, it was rehydrated with a drop of sterile water directly on it. Then, the gelatinous inner part (containing spores and other filamentous structures that were expanded due to moist) was scooped out onto Petri dishes containing 2 % malt extract agar (MEA), supplemented with streptomycin. The spore mass was teased

with needles to separate them. The MEA plates were kept at 25 °C and regularly checked for germination. When spores started to germinate, a single spore was picked and transferred to a fresh MEA plate. Up to ten single spore cultures were established for each fungus. When the colony was fully grown, some were cut into pieces and placed in the bottles of water and paraffin oil for long-term *in vitro* preservation. The remaining cultures were freeze-dried for DNA extraction and air-dried for herbarium deposition.

Branches or twigs showing die-back symptoms were cut into small pieces and surface-sterilized with 5 % peroxide. The pieces were then placed onto the 2 % MEA, supplemented with streptomycin. Any hyphae growing out of the pieces were transferred to fresh plates.

Asymptomatic specimens were re-hydrated in moisture chambers for between 3–7 days to investigate any fungi "on" or "in" plant tissue that did not cause any visible symptoms at the time of collection. The same procedure mentioned above was applied for single spore isolation.

Morphological characteristics were studied under a light microscope as explained in Marincowitz *et al.* (2008). Microscopic observations, measurements and photography of characteristic structures were made. Measurements were made of at least 30 structures, whenever this was possible.

Herbarium specimens were air-dried, pressed and placed in an envelope written with the name of fungus, collection site, date and collector. A unique number (S.L.) was assigned to each fungus based on their isolation either from different hosts and different locations or from the same hosts and different locations. Microscopic slides were also prepared and semi-preserved with its corresponding numbers.

For DNA fingerprinting, single spore fungal colonies were established on MEA plates. Aerial hyphae were harvested and freeze-dried for isolation of genomic DNA. The primer pairs of either V9G and LR5 were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rDNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rDNA gene, the second ITS region (ITS2) and the 5'end of the 28S rDNA gene (LSU) (De Hoog & Gerrits van den Ende 1998, Vilgalys & Hester 1990).

**Results, Discussion and Future Works**

A total of approximately 110 fungi were isolated, which belong to 44 fungal genera and 54 species (Table 2). They were identified based on morphological characteristics. Sixty-nine isolates were germinated on the artificial media (MEA). DNA fingerprinting of the internal transcribed spacer regions (ITS1&2) was so far done on 24 isolates.

- More morphological studies including thin (10–12 µm) sectioning of fruiting structure and cultural characteristics need to be done to complete description and identification.
- For some isolates, in addition to the ITS1&2 regions, the partial sequences of the translation elongation factor 1-alpha gene and/or the β-tubulin gene will be determined for the correct species identification (Crous *et al*. 2006, Lee *et al*. 2004).
- New fungi to science and new reports on new host plants will be published in peer-reviewed journals.
- All the descriptive information of disease symptoms and their possible causative fungi will be digitally assembled, which will be supplied to a nature conservation body and can serve as a basic datum for disease monitoring in conservation management.
- This is the first database of its kind in the Umtamvuna Nature Reserve and will serve as biological figures in addition to fauna and flora.

Table 2. List of microfungi isolated during a field trip to Umtamvuna Nature Reserve in May 2008.

| **Genus**[(1)] | **Species**[(2)] | **Host plants** | **Herbarium no.** | **Live cultures** (3) | **DNA fingerprinting** (4) |
|---|---|---|---|---|---|
| *Asteroma?* | sp | *Psychotria capensis* | SL1433 | No | n/a |
| *Bartalinia* | *bischofiae?* | *Maytenus abbottii* | SL1423(1) | Yes | Yes |
| *Belaina* | *umtamvuna* sp. nov. | *Nectaropetalum zuluense* | SL1428(1) | Yes | Yes |
| *Blennoria?* | sp | *Turraea floribunda* | SL1414(1) | Yes | |
| *Botryosphaeria* | sp | *Apodytes abbottii* | SL1407E | Yes | Yes |
| *Fusicoccum* | sp | *Lydenburgia abbottii* | SL1411A | Yes | Yes |
| *Fusicoccum?* | sp | *Syzygium cordatum* | SL1406A (2) | Yes | Yes |
| | sp | *Syzygium cordatum* | SL1427A (2) | Yes | |
| | sp | *Apodytes dimidiata* | SL1446A (=SL1446(2)) | Yes | |
| | sp | *Apodytes dimidiata* | SL1446(2') | No | n/a |

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|---|---|---|---|---|---|
| | sp | *Memecylon bachmanii* | SL1452(1) | Yes | |
| *Chaetospermum* | *camelliae* | *Syzygium cordatum* | SL1424(1) | No | n/a |
| *Circinotrichum* | sp | *Pseudosalacia streyi* | SL1413C | No | n/a |
| *Cladosporium* | sp | *Nectaropetalum zuluense* | SL1428(4) | Yes | |
| *Coleroa* | *senniana* | *Protea caffra* | SL1409A | No | n/a |
| *Colletotrichum* | sp | *Lydenburgia abbottii* | SL1411(4') | Yes | Yes |
| | sp | *Apodytes dimidiata* | SL1446(1) | Yes | |
| *Coniella* | sp | *Heteropyxis natalensis* | SL1418(2) | Yes | Yes |
| *Cryptosporiopsis?* | sp | *Eugenia erythrophylla* | SL1432B | Yes | |
| *Cystospora?* | sp | *Manilkara nicholsonii* | SL1403B | Yes | |
| *Didymosphaeria?* | sp | *Eugenia erythrophylla* | SL1432C | Yes | |
| *Discosporium?* | sp | *Apodytes abbottii* | SL1407C | Yes | Yes |
| *Ellisembia* | sp | *Eugenia simii* | SL1466B(1) | Yes | |
| *Endoxyla?* | sp | *Ficus sur* | SL1450 | Yes | |
| *Gliocladium* | sp | *Oricia bachmanii* | SL1425(1) | Yes | |
| *Glonium?* | sp | *Faurea macnaughtonii* | SL1443 (=SL1429A) | Yes | |
| *Gnomoniella?* | sp | *Syzygium cordatum* | SL1427B | Yes | |
| *Guignardia* | *mangiferae* | *Protea caffra* | SL1409(3) | No | n/a |
| *Leptodothiorella* | sp | *Turraea floribunda* | SL1414B | Yes | |
| *Phyllosticta* | sp | *Turraea floribunda* | SL1414C | No | n/a |
| | *capitalensis* | *Faurea macnughtonii* | SL1443(1) | Yes | |
| *Libertella?* | sp | *Eugenia natalitia* | SL1398(2), SL1447(2) | Yes | |
| *Lophium?* | sp | *Protorhus longifolia* | SL1442C | No | n/a |

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|---|---|---|---|---|---|
| *Melanospora* | sp | *Oricia bachmanii* | SL1426(1) | No | n/a |
| *Mycosphaerella* | sp | *Syzygium pondoense* | SL1397A (3) | Yes | Yes |
| | sp | *Apodytes abbottii* | SL1407A | No | n/a |
| | sp | *Syzygium cordatum* | SL1427A (5) | No | n/a |
| *Batchelormyces* | *leucadendri* | *Leucadendron spissifolium natalense* | SL1408A | Yes | |
| | *leucadendri* | *Leucadendron pondoense* | SL1465 | Yes | |
| *Pseudocercospora* | sp | *Heteropyxis natalensis* | SL1418(1) | Yes | Yes |
| | sp | *Syzygium cordatum* | SL1424A | Yes | |
| | sp | *Syzygium cordatum* | SL1427A | No | n/a |
| *Mycohyphallage* | *congesta* | *Syzygium cordatum* | SL1412A | No | n/a |
| | *magii* sp. nov. | *Syzygium cordatum* | SL1406A | No | n/a |
| | *magii* sp. nov. | *Syzygium van wyk* | SL1407C | Yes | |
| *Mycotribulus* | *mirabilis* | *Apodytes abbottii* | SL1407D | No | n/a |
| *Myrotheciu m* | sp | *Nectaropetalum zuluense* | SL1428(2) | Yes | |
| | sp | *Englerophytum natalense* | SL1467(1) | Yes | |
| *Paradiscula?* | sp | *Eugenia simii* | SL1464D (3) | Yes | |
| *Patellaria?* | sp | *Eugenia simii* | SL1464B (=SL1466B) | Yes | |
| *Pestalosphaeria* | *hansenii* | *Syzygium pondoense* | SL1397B (1') | No | n/a |
| | *hansenii* | *Eugenia natalitia* | SL1398(3) | Yes | |
| | *hansenii* | *Syzygium cordatum* | SL1406A (3), SL1427A(4) | No | n/a |
| | *hansenii* | *Protea caffra* | SL1409(2'/2) | Yes | |
| | *hansenii* | *Pseudosalacia streyi* | SL1413B | Yes | |

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|---|---|---|---|---|---|
| | *hansenii* | *Syzygium van wyk* | SL1417(1') | Yes | |
| | *hansenii* | *Heteropyxis natalensis* | SL1418(3') | Yes | |
| | *hansenii* | *Eugenia erythrophylla* | SL1432D | No | n/a |
| | *hansenii* | *Protorhus longifolia* | SL1442(3) | Yes | |
| | *hansenii* | *Eugenia simii* | SL1464D (4) | Yes | |
| *Pestalotiopsis* | *foedans* | *Syzygium beacon hill* | SL1391(1) | No | n/a |
| | *foedans* | *Syzygium pondoense* | SL1397A (2), SL1397B(1) | Yes | Yes |
| | *foedans* | *Eugenia natalatia* | SL1398(1) | Yes | Yes |
| | *foedans* | *Manilkara nicholsonii* | SL1403A (1) | Yes | |
| | *foedans* | *Pseudosalacia streyi* | SL1413A-5(1) | Yes | |
| | *foedans* | *Maytenus abbottii* | SL1423(2) | Yes | |
| | *foedans* | *Nectaropetalum zuluense* | SL1428(3) | Yes | |
| | *foedans* | *Englerophytum natalense* | SL1451(1) | Yes | |
| | sp | *Manilkara nicholsonii* | SL1403A | Yes | |
| | sp | *Syzygium cordatum* | SL1412B (1) | Yes | |
| | sp | *Syzygium van wyk* | SL1417(1) | Yes | |
| | sp | *Heteropyxis natalensis* | SL1418(3) | Yes | |
| | sp | *Lydenburgia abbottii* | SL1420(2) | Yes | |
| | sp | *Vitellariopsis marginata* | SL1455(1) | Yes | |
| | sp | *Eugenia simii* | SL1464D (1) | Yes | |
| | sp | *Eugenia simii* | SL1464D (2) | Yes | |
| | sp | *Eugenia natalatia* | SL1447(1) | Yes | |
| *Phacidiopycnis?* | sp | *Manilkara nicholsonii* | SL1403C | Yes | |

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|---|---|---|---|---|---|
| | sp | *Eugenia umtamvunensis* | SL1430B | No | n/a |
| *Phoma?* | sp | *Maytenus abbottii* | SL1423B | Yes | |
| *Phomopsis* | sp | *Syzygium pondoense* (β) | SL1397A (1) | No | n/a |
| | sp | *Protea caffra* (β) | SL1409(1) | No | n/a |
| | sp | *Turraea floribunda* (α&β) | SL1414(2) | Yes | |
| | sp | *Oricia bachmanii* (β) | SL1426(2) | No | n/a |
| | sp | *Syzygium cordatum* (β) | SL1427A (3) | No | n/a |
| | sp | *Eugenia erythrophylla* | SL1432(2) | No | n/a |
| | sp | *Lydenburgia abbottii* (α) | SL1420 (=SL1420(1)) | Yes | |
| *Phyllachora?* | sp | *Salacia gerrardii* | SL1449 | No | n/a |
| *Pithomyces* | *chartarum* | *Apodytes dimidiata* | SL1446(3') | Yes | |
| *Pseudophacidium?* | sp | *Eugenia simii* | SL1464A (=SL1466A) | Yes | |
| *Pleosporales* | sp | *Eugenia simii* | SL1466C | Yes | |
| *Pleosporales* | sp | *Eugenia simii* | SL1466D | Yes | |
| *Splanchnonema?* | sp | *Eugenia simii* | SL1464C | No | n/a |
| *Tubakia?* | sp | *Syzygium beacon hill* | SL1391(2) | No | n/a |
| | sp | *Syzygium pondoense* | SL1397A (4) | Yes | Yes |
| | sp | *Syzygium cordatum* | SL1412B (2) | Yes | Yes |

1. Asexual fungus with known sexual stage is placed under its sexual stage with indent, for example *Fusicoccum* under *Botryosphaeria*.
2. Unidentified species (sp) and genera with question marks will be correctly named after further investigation.
3. Fungi did not grow on the artificial media (MEA) even after 3 attempts are indicated as No.
4. DNA fingerprinting was attempted only for the fungi growing on MEA.

Approximately 20 lichens were collected. All of them were, however, remained unidentified because no specialist was available for this task.

- The specimens were dried and kept in envelopes, and images were taken and recorded.

The investigation of *Phytophthora* infestation on an endangered species, *Raspalia trigyna* (Bruniaceae), a so-called ghostbush, could not be concluded during the term of this study.

- The bush showed a die-back symptom in the field. The dead and dying branches were brought into the laboratory. The primary isolation from dying branches yielded a number of fungi which were, however, mostly either endophytes which grow asymptomatically inside plant tissue or saprophytes. There were no known pathogenic fungi isolated.
- The possibility of *Phytophthora* infestation, of which the symptoms are similar to that of die-back, was suggested and strongly supported by two facts: the history of sudden disappearance of the bushes in the 1960s which is often the case with *Phytophthora* epidemic, and the family (Bruniaceae) it belongs to is well known for its susceptibility to *Phytophthora*.
- A molecular technique using *Phytophthora*-specific primers was used to detect *Phytophthora* in the diseased tissues. The result was negative. This could mean that either there was no *Phytophthora* or there might be a flaw in the specimens or sampling. Because the field trip was not initially intended for *Phytophthora* detection, the standard collection protocol was not followed. A field *in situ* isolation is known to be crucially important for *Phytophthora* to be detected from diseased plants.
- Due to time constraints, the second visit could not be made. However, the disease occurrence was reported and attracted attention. A follow-up investigation is planned and will be carried out independently by a post-graduate student at FABI (Forest and Agriculture Biotechnology Institute, University of Pretoria, Pretoria, South Africa) where the primary investigator is based.

Young shoots of *Dahlgrenodendron* with a die-back symptom did not produce any suspicious fungi during the primary isolation. It may be caused by a small or minute insect that was mistakenly regarded as a fungus-causing disease.

- A joint excursion with entomologists is desired in the future due to the fact that insect damage such as insect galls, leaf rolling, and chewing was commonly observed during the field trip.

The isolated cultures and dried herbarium specimens will be deposited for future reference at the FABI culture collection centre and at the National Collection of Fungi in South Africa (PREM).

## References


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