

A JOURNEY THROUGH THE BIOLOGICAL
COMPLEXITY OF THE CAPE FLORISTIC REGION,
SOUTH AFRICA

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*A JOURNEY THROUGH THE BIOLOGICAL COMPLEXITY OF
THE CAPE FLORISTIC REGION, SOUTH AFRICA*

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ABOUT THE AUTHOR

Léanne Dreyer's fascination with nature started in her pre-school years, and has increased steadily throughout her life. She was attracted by the different smells, colours, textures and shapes nature offered, and by the true beauty often revealed in the smallest details. This led her to enrol for a BSc degree at Stellenbosch University, majoring in Botany and Genetics. Her honours and MSc degrees in Botany at the same university focused on the systematics of *Pelargonium*, a large genus within the biologically rich Cape Floristic Region (CFR). After completing her MSc degree she was appointed as scientist by the South African National Biodiversity Institute (SANBI) in Pretoria, and she enrolled for a PhD at the University of Pretoria under the supervision of Prof AE van Wyk. Her dissertation focused on the palynological diversity of *Oxalis*, another of the large CFR plant genera. She was appointed by SANBI as South African Botanical Liaison Officer at the Royal Botanical Gardens, Kew (London) during the first year of her PhD studies. After completion of her PhD she was appointed as Postdoctoral Fellow in the laboratory of Prof Peter Linder at the University of Cape Town, and then as lecturer at Stellenbosch University in 1998. Her subsequent research career focused on the diversity and evolution of CFR biota, with a special focus on *Oxalis*. In 2005 she became a core team member of the NRF/DST Centre of Excellence in Tree Health Biotechnology. This led to an expansion of her research focus to include the fascinating plant-fungus-arthropod interactions prevalent within the CFR.

A JOURNEY THROUGH THE BIOLOGICAL COMPLEXITY OF THE CAPE FLORISTIC REGION, SOUTH AFRICA

My research focuses on the diversity, evolution and ecology of the biota of the Cape Floristic Region (CFR) of South Africa (SA). Two central themes of this work are (1) *Oxalis* systematics and (2) plant-fungal-arthropod interactions within this region. These two sub-foci are elaborated on below under these two subheadings.

I. OXALIS SYSTEMATICS

I.1 *Oxalis* in the context of the Cape Flora

The southern tip of the African continent is characterised by a highly unique and exceptionally rich flora. It includes both the CFR and the Succulent Karoo Region (Van Wyk & Smith 2001), each characterised by a specialised flora rich in species and endemics. The CFR is the richer of the two regions, including about 9 000 species in an area of 90 000 km² (Goldblatt & Manning 2000). It displays a species-level endemism of 68.8%, while genus- and family-level endemism is so high that it has been described as one of six global Floral Kingdoms (Good 1947; Takhtajan 1986), and it has been listed as one of 25 global biodiversity hotspots (Myers *et al.* 2000). The succulent Karoo Biome is the only arid region identified as a biodiversity hotspot. An intriguing aspect of the CFR is the relatively small number of taxa that have diversified into extraordinary species-rich lineages (e.g. the genus *Erica* includes 658 species within the CFR). In fact, Linder (2002) indicated that 50% of the species diversity of the CFR can be ascribed to only 33 lineages (ancestral species).

Oxalis represents the seventh largest genus within the CFR, and yet it was not included as one of the 33 Cape floral clades (*sensu* Linder 2002). The reason for this was that *Oxalis* is one of the more difficult lineages to separate from the Succulent Cape flora, as it shows remarkable species richness both within the CFR and the Succulent Karoo Region. Linder (2002) suggested that a critical evaluation of the floristic support for expanding the delimitation of the CFR to include the entire winter rainfall area would be interesting. Such a study was undertaken by Born *et al.* (2006), and they concluded that although the CFR constitutes a valid Floristic Region, the total endemism levels increase when the CFR and Succulent Karoo Region are combined into a

Greater CFR (entire winter rainfall region). Oberlander *et al.* (2011) reconstructed and dated the molecular phylogeny of southern African *Oxalis*, and convincingly showed that it should also be added as a true Cape clade. *Oxalis* could, in fact, be viewed as a true representative of such a Greater CFR, and therefore supports the expansion of the current delimitation of the CFR. Verboom *et al.* (2009) elegantly showed Succulent Karoo plant lineages to be much younger than Fynbos lineages in the CFR. In support of the findings of Verboom *et al.* (2009), *Oxalis* lineages in the Succulent Karoo were found to be much younger than *Oxalis* lineages in the Fynbos.

An intriguing attribute of the combined CFR and the Succulent Karoo Region is that it harbours the greatest concentration of geophytic species in the world. Geophytes contribute 17% of the species within the CFR and 20% of the species within the Greater CFR (Proches *et al.* 2006a & b). The Oxalidaceae is one of the six largest geophytic lineages within the Greater CFR, and *Oxalis* is by far the largest geophytic genus in this region. Geophytism is not restricted to the southern African members of *Oxalis*, but it is much more prevalent here than in the other centre of *Oxalis* diversity in South-Central America.

An international collaborative study on the causes of species diversification within the CFR/Greater CFR has been active over the past decade. This collaborative effort aims to understand the patterns and processes of radiation in this botanically spectacular region. Only once we understand and combine insights into the evolutionary patterns and processes that have been operational in the diverse lineages within the GCFR will we truly be in a position to conserve this biodiversity, and to extrapolate from these insights into global biodiversity conservation. Maintenance of biodiversity may be achieved, in part, by directing conservation resources to species and ecosystems most at risk, and/or

by controlling or reversing the processes that place them at risk. However, in order to do this we need to understand something about what these processes are. A review of recent literature revealed a paucity of research pertaining to the reproductive systems of geophytes in the Cape Region. There is a generally consistent view held amongst terrestrial ecologists that geophytes are the floral element least affected by the typical fragmentation processes of the Succulent Karoo, Renosterveld and Fynbos biomes. This view can be attributed to the typical vegetative propagation capabilities of most geophytes. However, there are no data available to support this.

The large number of included species, its presence in both the CFR and Succulent Karoo regions, the evolution of geophytism, and many other biological attributes render the inclusion of *Oxalis* in the collaborative research focused on the radiation of the Cape Flora crucial. Despite its obvious biological importance, the systematics of the genus is still very poorly understood. The most recent revision (Salter 1944) is restricted to a macro-morphological species comparison, without any indication of true phylogenetic affinities. Many of the species delimitations are highly questionable, and the numerous large species complexes are still completely unresolved. The revision by Salter (1944) provides limited insight into the evolutionary radiation of this massive group, or into the evolution of key characters that may have enabled this massive local radiation (e.g. geophytism).

It is within this void that I have developed the main focus of my research career over the past twenty years. Modern systematic biology places strong emphasis on phylogenetic inferences obtained from DNA sequence data, and in the process has revolutionised how we think about organismal evolution. Newer and better techniques (both practical and theoretical) are being developed at an encouragingly fast rate, and the exciting new inferences to be made from such DNA-based studies are quite staggering. There is, however, an ever-increasing incentive to relate insights obtained through molecular phylogenetic systematics back to organismal morphology, and to the intricate interplay between this morphology and the ecological environment such organisms occupy. In a sense, systematic biologists have come full circle, and we are now challenged to use molecular phylogenetic reconstructions to understand and interpret the morphological and ecological determinants of species diversity and the speciation process itself (Linder & Hardy 2005). This, in itself, is very exciting, but demands a sound understanding of the diverse biological attributes of the organisms under study. I thus followed a multidisciplinary approach in my *Oxalis* systematic research, placing strong emphasis on molecular phylogenetics, but also considered macro-morphology, palynology, cytology, reproductive

biology and ecology. Through this total evidence approach we have incrementally gained considerable insight into the diversity and evolution of southern African *Oxalis*, which is outlined in some detail below.

1.2 Palynology

Pollen grains represent miniature male gametophyte plants that are produced in the anthers of a given flower, and are preferably transported to the stigma of another flower of the same species, mostly through the action of pollinators. In many ways, pollen thus represents the weakest link in the life cycle of a flowering plant, as the vulnerable male gametophyte plant inside the pollen grain is released and transported through a potentially desiccating external environment. In addition, it needs to find and then be recognised as own pollen by the stigma upon which it ultimately lands in order for successful fertilisation to occur. For these reasons there is very strong selection against change in pollen morphology. This renders pollen grain traits conservative and of considerable importance in plant systematic classification.

Despite this selection against change in pollen morphology, I found *Oxalis* to be palynologically extremely diverse (Dreyer 1996). I identified four main pollen types and 19 subtypes among southern African *Oxalis* species (Figure 1). A very unusual supra-areolate pollen type, which is unique among flowering plants, was identified in different sections and subsections of *Oxalis*. This seriously questioned the morphology-based taxonomic classification of southern African *Oxalis* by Salter (1944).

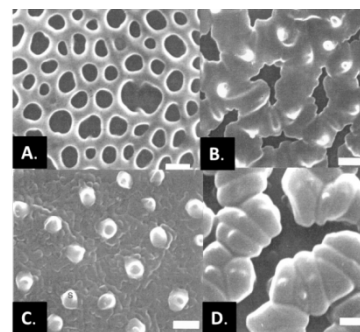


Figure 1. Pollen types among southern African members of *Oxalis*. A. Reticulate; B. Rugulate-reticulate; C. Micro-rugulate spinate; D. Supra-areolate.

1.3 Molecular Systematics

The conflict between the morphological and palynological classifications of southern African *Oxalis* species emphasised the need for a DNA sequence data-based molecular phylogenetic reconstruction of relationships in this genus. The first attempt was published

by Oberlander et al. (2004), and convincingly showed pollen and molecular characters to be congruent, but in conflict with the Salter (1944) morphological classification. Molecular systematic research thus formed a core focus in our subsequent research, and recently resulted in the publication of a near complete species-level phylogeny of southern African members of *Oxalis* (Oberlander et al. 2011). This phylogeny corresponds very well with the palynological classification, but has forced us to reconsider the interpretation of morphological traits and to search for synapomorphic characters from diverse other disciplines, including anatomy, karyology, reproductive biology and ecology. In all of these subsequent studies, the molecular phylogeny has been (and will continue to be) the basic framework upon which the evolution of other traits is evaluated.

In order to place the southern African radiation of *Oxalis* in biogeographic context in terms of the global radiation of the genus, we collaborated with Prof Eve Emschwiller at the University of Wisconsin-Madison, USA. Combined molecular phylogenetic analyses of southern African and American *Oxalis* species convincingly showed the southern African radiation to be monophyletic. Although geophytism has arisen among American members of the genus too, the unique bulb structure present in all southern African species has been shown to be synapomorphic for the southern African clade (Oberlander et al. 2009b). Recently, the combined molecular systematic research on African and American *Oxalis* taxa finally culminated in the reconstruction of an extensive, species-rich global phylogeny for the genus (Gardner et al., submitted).

Molecular tools have also been employed to answer a number of more specific research questions. *Oxalis* includes five aquatic species that are confined to vernal pools scattered across the Greater CFR. Salter (1944) placed them together in section *Campanulatae*, but our molecular reconstruction provides strong evidence that vernal pool species constitute at least two, and possibly three, distinct lineages across the phylogeny of southern African *Oxalis*. We could further show that *Oxalis* has invaded this habitat type multiple times, and that vernal pools are surprisingly ancient and have been extremely persistent in the Greater CFR (Oberlander et al. submitted).

In a second study on these aquatics we focused on the sister species, *Oxalis dines* and *O. disticha*, both of which are confined to a few small vernal pools in the Greater CFR. Due to extensive farming activity in their native range, all known populations are at risk of extinction. To identify priority sites for focused conservation management, the chloroplast intergenic spacer regions *psbA-trnH* and *trnS-trnG* were used to determine population structure and genetic diversity in this lineage across its distribution range

(Oberlander et al. 2012). Analyses of molecular variance indicated an extremely high level of inter-population differentiation across the entire aquatic lineage, as well as within *O. disticha* and *O. dines*. Low haplotypic diversity in local populations and differentiation between populations are consistent with low gene flow and sporadic founder effects. Based on these results, we could advise that conservation efforts should be focused on preserving as many pools as possible. Small, genetically distinct populations are of the most concern, and should be prioritised.



Figure 2. *Oxalis disticha* in its aquatic habitat.

More than a third of southern African *Oxalis* species are listed as rare or threatened in the most recent Red List of South African plants (Raimondo et al. 2009). In order to better understand the dynamics in populations of some of the naturally rare (range-restricted) species, we conducted a population-level molecular analysis in which the degree of genetic differentiation between the rare and highly localised species, *O. oligophylla*, and two widespread species (*O. tomentosa* and *O. purpurea*) was compared (Zietsman et al. 2009). The results revealed low genetic diversity in *O. oligophylla* and *O. tomentosa* compared to the widespread *O. purpurea*. These results have important conservation implications, as the observed low genetic diversity in *O. oligophylla* may explain its rarity, while it ultimately may cause *O. tomentosa* also to become rare.

Over the past 20 years we have conducted extensive fieldwork across the entire range of southern African *Oxalis* in order to obtain material for DNA extraction, reference herbarium specimens and an extensive living collection currently housed in the Botanical Gardens of Stellenbosch University. This has led to the discovery of almost 25 new species, some of which have been described and others that are still in the process of being formally described. Given the artificial nature of the Salter (1944) taxonomy, we have relied extensively on the molecular phylogenetic placement of the new taxa to determine their phylogenetic affinities in all descriptions published to date (Dreyer et al. 2009; Oberlander et al. 2009a; Dreyer et al. 2010; Roets et al. 2013; Dreyer et al. 2013; Suda et al. 2013; Dreyer et al. in press).

1.4 Karyology

Cytogenetic data, including chromosome number, ploidy level and genome size, may help elucidate several challenging questions of contemporary biosystematics and evolutionary biology. While chromosome numbers represent the major traditional source of cytogenetic data, genome size (C-value data) is today increasingly used in systematic treatments due to the ease and speed with which vast amounts of data can be generated.

The interest in C-value data has been driven by the knowledge that genome size is involved in the scaling of living organisms and that it affects many characters at sub-cellular, cellular, tissue and organismal levels. These factors influence how, when and where a plant may grow, and its chances of survival in a changing world. In addition, variation in plant phenology, sensitivity to frost, and ecological sorting of species according to water availability may all be related to genome size (Leitch & Bennett 2007). Strong correlations between genome size data and a range of ecological agents of change (including global warming and elevated levels of CO₂) have also been reported (Jasienski & Bazzaz 1995).

Over the past number of years, Prof Jan Suda (Charles University, Prague, Czech Republic) and I have been involved in a collaborative project to measure the C-values of southern African members of *Oxalis*. The results of this research have been fantastically interesting. Although only two basic chromosome numbers ($X = 7$ and $X = 5$) have been confirmed among SA species, almost half of the species are polyploidy or include polyploidy representatives. Many species display large polyploid series, and yet very few species are exclusively polyploid.

Although this research is on-going, we have published a number of specific case studies thus far. *Oxalis pes-caprae* is native to South Africa, but a pentaploid form of it has become an aggressive invasive weed globally. We used DNA flow cytometry to screen the ploidy levels of this species across its native range, and interestingly only found di-, (very rare) tri- and tetraploids (dominant) (Krejčíková et al. 2013c). Our results thus challenged the conventional scenario that the pentaploid weed was introduced directly from the CFR. The origin of this weedy form remains unclear, and would need extensive DNA screening to identify.

Oxalis obtusa is a widespread and morphologically extremely diverse southern African species. Krejčíková et al. (2013a) found this species to display higher ploidy diversity than has been recorded in any other Cape plant species. We identified three majority (2x, 4x, 6x) and three minority (3x, 5x, 8x) cytotypes across the native range of this species.

Although most populations included only one cytotype, 12 populations displayed mixed ploidy. We were able to show some ecological segregation of ploidy level, with hexaploids mostly confined to Fynbos, while tetraploids dominated in the Succulent Karoo. Precipitation was the most important climatic variable associated with cytotype distribution. This study was followed by another that focused specifically on the contact zones in which mixed *O. obtusa* cytotypes co-occurred sympatrically (Krejčíková et al. 2013c). We found different cytotypes to display some spatio-ecological segregation, especially in terms of precipitation, but also in terms of altitudinal gradients.

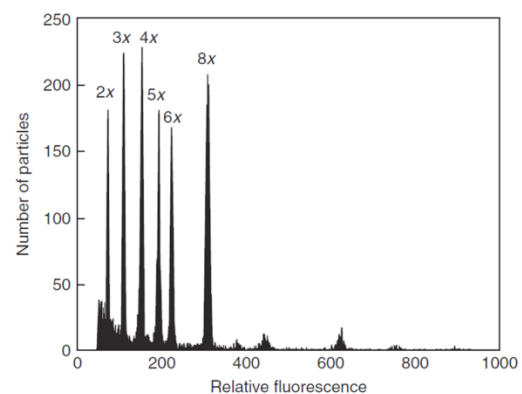


Figure 3. Fluorescence histogram of simultaneous analysis of six different ploidy levels of *Oxalis obtusa* collected in the Greater Cape Floristic Region. Nuclei from all samples were isolated simultaneously, stained and analysed (from Krejčíková et al. 2013a).

The evolutionary consequence of polyploidy among southern African *Oxalis* species forms the research focus of the current postdoctoral study of Dr Kenneth Oberlander. His preliminary results have confirmed that *Oxalis* has undergone exceptional polyploidisation, but that this seems to have had a surprisingly limited effect on the long-term evolution of the lineage.

1.5 Morphology/anatomy

The taxonomic classification of southern African members of *Oxalis* (Salter 1944) remains problematic. This is further compounded by the considerable morphological plasticity displayed by some species, and the presence of species complexes that defy classification by conventional means. Our reconstruction of a species-level molecular phylogeny of southern African *Oxalis* species has helped to provide new insight into species delimitations and the systematic relationships between these species. However, it has also emphasised the need for a critical re-evaluation of morphological characters in *Oxalis*, as only one of the nine sections proposed by

Salter (1944) was retrieved as a monophyletic lineage. Our preliminary investigations of selected morphological characters highlighted the systematic significance of a range of vegetative and reproductive characters. These include the morphology and anatomy of bulbs, bulb fleshy leaves, bulb tunics, leaves, fruit, seeds and seedlings.

1.6 Reproductive biology

Heterostyly is a breeding system that prevents inbreeding through the reciprocal arrangement of reproductive organs in different floral morphs, a physiological self-incompatibility system and a suite of ancillary morphological traits (Barrett 1990). Tristyly represents a complex form of heterostyly, in which three floral morphs (short, mid and long according to stigma height) are found within a population. Each plant possesses flowers with two anther levels that correspond to the stigma levels in the remaining two morphs. Seed production is normally restricted to pollination events between anthers and stigmas of equivalent height (legitimate pollinations), while pollinations between anthers and stigmas of different heights (illegitimate) usually fail to produce any seeds (Barrett 1988).

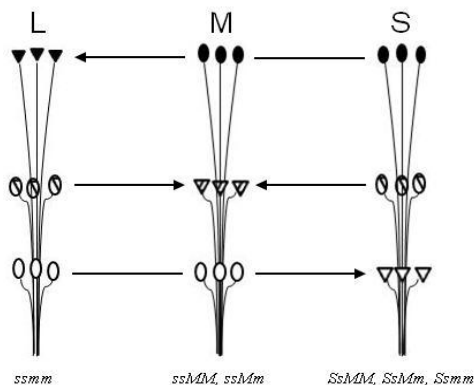


Figure 4. Diagrammatic illustration of tristyly. L, M and S represent long, mid and short morphs respectively. Triangles represent styles, while ovals represent anthers. Horizontal arrows indicate legitimate cross-pollinations (from Barrett & Shore 2008).

The tristylous breeding system is generally documented well in only three flowering plant families, the Pontederiaceae, Oxalidaceae and Lythraceae, in which it has evolved independently (Ornduff 1974; Barrett 1979; Charlesworth 1979). A system with two loci, S and M, each with two alleles and S epistatic to M, has been reported for the genetic control of tristyly. With this genetic control, a 1:1:1 ratio of floral morphs will succeed in large tristylous populations at equilibrium, when all morphs have equal fitness. When such a ratio occurs, the population is said to be at isoplethic equilibrium. Despite the complexity of the tristylous breeding system, it does appear to be very

unstable. It has been shown that this system often breaks down among American members of *Oxalis*. The most common pathway involves the relaxation and eventual loss of self-incompatibility, followed by modifications in the relative heights of stigmas and anthers (Ornduff 1972; Barrett 1979).

Turketti (2010) assessed the extent of tristyly expression among southern African members of *Oxalis*. She found tristyly to be intact in most of the species and populations studied. There were interesting exceptions, however, with some populations deviating significantly from isoplethy in terms of morph frequency ratios, while others had lost one or even two floral morphs completely. The aim of her work was to study the broad-scale expression of tristyly among southern African species. More detailed, case-specific studies are now required to investigate the species and/or populations in which interesting deviations have been identified. Turketti *et al.* (2012) represent the first such specific study, in which a totally new variation of tristyly, three-dimensional reciprocity, is described for the first time.

Such an intricate reproductive syndrome, closely linked to clonal growth and further complicated by potentially varying stages of tristyly breakdown, should significantly influence the successful reproduction of *Oxalis* species in highly fragmented landscapes such as the remaining patches of Renosterveld. The small remaining fragments are highly susceptible to boundary effects and genetic drift, which could result in a loss of species integrity on the one hand, and possibilities for speciation on the other. An understanding of the state of the reproductive systems of this taxon thus is critical for management practices, or at least to understand the mechanism behind species loss under fragmentation. The breakdown of one or more components of tristyly, which may restrict sexual recruitment in small populations, may significantly alter the reproductive assurance and thus survival of a species, especially in fragmented landscapes. Zietsman *et al.* (2008) investigated the extent to which the reproductive successes of selected rare/endangered *Oxalis* species are limited by their level of tristyly expression. Interesting species-specific responses were observed, in which rarity appeared to be a unique response of each of the studied species to their unique environmental conditions. Highly specialised habitat preferences appear to be the main reason for rarity, and it is of great importance that the habitats of these species should be protected.

Recalcitrant seeds are seeds that undergo no maturation drying. They are shed while metabolically active and tolerate very little post-shedding desiccation (Berjak *et al.* 1989). This means that they germinate immediately after release. This recruitment strategy has been confirmed in 64 plant

families and 130 (mostly tree) species (Farnsworth 2000) thus far, but this probably represents an underestimate of the extent of the expression of this trait. Almost 80% of recalcitrant species are native to the tropics and produce seeds that mature during the tropical monsoons and rainy seasons.

Salter (1944) described the two very different seeding strategies expressed among southern African *Oxalis* as endospermy and exendospermy. Endospermous seeds contain a poorly developed embryo, surrounded by copious amounts of endosperm and by a hard, brown testa. They are produced in the winter after flowering, and then lie dormant through the following dry summer, only to germinate a year later. Exendospermous seeds contain a fully developed embryo, no endosperm and a thin testa, which is explosively dehisced shortly after the seeds are released from the capsule. To test whether exendospermous species are recalcitrant, we monitored seed germination in many phylogenetically unrelated species, and found that exendospermous seeds always germinate within a week (mostly much less) after release from the capsule. We also tested their ability to resist drying by drying them for one to ten days after release, after which they were placed in germination chambers. Most were unable to germinate after five to seven days of drying, rendering them truly recalcitrant. Dormant and recalcitrant species display significantly different flowering distributions, with recalcitrant species flowering during an early peak (May to June), while dormant species flower over a more extended period (mid-autumn to mid-spring) (Dreyer *et al.* 2006). It is unrealistic to expect that such massive changes in germination strategy have not had a major influence on the evolution of *Oxalis* in South Africa. Based on the phylogeny of Gardner *et al.* (submitted), seed dormancy is the ancestral state in *Oxalis*, and all non-SA species have dormant seeds. Recalcitrance evolved independently at least three times among SA species. When we tested for correlated evolution of seed germination strategy and biogeographic distribution (summer or winter rainfall distribution), the winter rainfall region was convincingly shown to be a sink for recalcitrant species. More research is needed to understand the evolution and ecological implications of this unusual reproductive trait.

2. PLANT-FUNGUS-ARTHROPOD INTERACTIONS IN THE CFR

The ophiostomatoid fungi are a polyphyletic assemblage of fungal genera that have been grouped together artificially based on similarities in their sexual stages. They typically produce long-necked ascomata. Ascospores are produced in their bases, pushed through the neck, and accumulate in sticky masses at the tips of these structures. From here they are easily transported by diverse arthropods, including mites and Scolytine beetles.

Although the ophiostomatoid fungi include virulent pathogens that have caused devastation to trees in the Northern Hemisphere, very little is known about their African members. This void is currently being addressed by the DST/NRF Centre of Excellence (CoE) in Tree Health Biotechnology. Dr Francois Roets (Department of Conservation Ecology and Entomology, Stellenbosch University) and I are core team members of this CoE, and our main research aim is to study the ophiostomatoid fungi and their arthropod associates within the Cape Floristic Region of South Africa. Currently we focus on CFR ophiostomatoid fungi in two systems, namely those associated with *Protea* infructescence, and those associated with native Afri-montane trees.

2.1 Ophiostomatoid fungi-*Protea*-arthropod interactions

After flowering, the inflorescences of many native *Protea* species close to form woody, seed-bearing infructescences. These structures are retained on the plant, and only open to release their contained seeds after fire. Species of two ophiostomatoid fungal genera, *Ophiostoma* and *Knoxdaviesia*, have been observed growing on the dry floral remains within *Protea* infructescences in the CFR. Their presence in these structures is interesting, as they do not harm the *Protea* seeds, but, when present, appear to inhibit any other fungal growth (of potentially harmful fungi) in the infructescences.

Roets (2006) assessed the diversity, specificity and general ecology of different *Ophiostoma* and *Knoxdaviesia* species associated with different *Protea* infructescences. He identified mites as the primary vectors of the spores of these fungi, and showed the association between mites and the ophiostomatoid fungi to be mutualistic. Many mite species have specialised structures within which the fungal spores are collected and transported, and they can survive exclusively on a diet of these fungi. He further identified beetles as secondary vectors of the fungi, as the spore-carrying mites were shown to be phoretic on beetles visiting *Protea* flowers. In subsequent and on-going studies we have tried to unravel these very intricate inter-organismal associations in more detail in order to gain insight into the ecology and evolution of these systems. For example, Aylward (2014) recently showed the genotypic diversity of *Knoxdaviesia proteae* associated with *Protea repens* to be exceptionally high. She found no significant population structure in populations of this fungus, showing them to be in panmixia. In another, on-going PhD study, the diversity and evolution of mites associated with this system are being explored in more detail, and the role of birds as secondary fungal vectors is also being investigated.

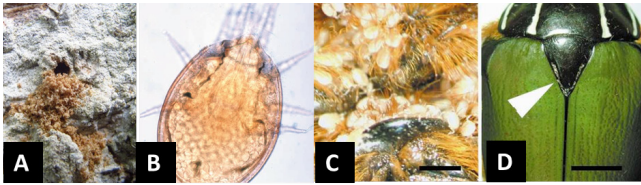


Figure 5. A. Bark beetle entrance hole on native Afromontane tree species. B. Mite associated with ophiostomatoid fungi in *Protea infructescens*. C and D. Spore-carrying mites accumulated in hairy cavities on the surface of *Protea* beetles.

2.1 Ophiostomatoid fungi associated with Afromontane forest trees in the CFR

A recently completed PhD study explored the diversity of ophiostomatoid fungi associated with native Afromontane forest tree species in forest patches across the CFR (Musvuugwa, 2014). A large number of fungi were isolated from this niche, the bulk of which were new to science. In many cases the fungi were also associated with various mite and Scolytine beetle species. Many of these fungi are pathogenic to their host trees. Future research will focus on assessing the host specificity and pathogenicity of these fungi on diverse hosts. We also still need to study the complex inter-organismal interactions in each of these systems in much more detail.

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